J Autism Dev Disord (2011) 41:395–404
DOI 10.1007/s10803-011-1184-2

ORIGINAL PAPER

Progress in Understanding Autism: 2007–2010

Michael L. Rutter

Published online: 12 February 2011

Abstract Scientific progress is discussed in relation to clinical issues; genetic issues; environmental issues; and the state of play on psychological treatments. It is concluded that substantial gains in knowledge have been achieved during the last 3 years, and there have been some unexpected findings, but major puzzles remain. We should be hopeful of ever greater gains in the years ahead, but both prevention and cure remain elusive.

Keywords Scientific advances · Research challenges

In this article, scientific progress will be discussed in relation to advances in our understanding of clinical features, advances in genetics, progress in environmental research, and the state of play on psychological treatments. Basic science, including animal models, is not reviewed here, although the findings on mirror neurons and immunology are obviously potentially very important. The emphasis is placed particularly on advances during the last 3 years, but attention is paid to earlier findings where they are relevant to the contemporary issues.

Paper based on a state-of-the-art lecture in the 9th International Congress of Autism Europe in Catania, Sicily 8th–10th October 2010.

M. L. Rutter (✉)
MRC Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, PO Box 80, De Crespigny Park, Denmark Hill, London SE5 8AF, UK
e-mail: camilla.azis@kcl.ac.uk

Clinical Features

Given that there has been a huge investment in clinical research that goes back for well over half a century, it might be supposed that all that needed to be known is already well established and free of controversies. However, that is far from the case.

Developmental Regression

Despite the fact that the phenomenon of temporary developmental regression (especially of language and language-related skills) is noted from the very first reports of autism, until recently there had been surprisingly little systematic research into the phenomenon. That situation is now changing. At first, some people were sceptical of the reality of regression but carefully conducted studies of home videos (Werner and Dawson 2005) confirmed the validity of the phenomenon. The next question that needed tackling was whether such regression occurred in all neurodevelopmental disorders or whether it was in some way particularly characteristic of autism. Findings from studies by Baird et al. (2008a) and Pickles et al. (2009) showed that the period of regression was distinctly rare in other neurodevelopmental disorders, but seemed to be quite strongly associated with autism. Findings also suggested that it was misleading to think of regression as a categorical present/absent phenomenon; rather, even minor degrees of regression were pointers towards autism. Parr et al. (in press), using data from affected sibling pairs, found that the concordance rate of 18.9% was not significantly above that of 13.5% expected under independence. The overall rate of regression found (24%), was closely comparable to that reported in singleton and epidemiological samples. Several questions derive out of these findings. First, if even

minor degrees of regression are of diagnostic importance, what criteria should be used to identify it? Second, what neural processes underlie the occurrence of regression? The findings suggest that it is unlikely that regression is due to some factors that are exogenous to autism but, nevertheless, what neural processes are giving rise to the regression?

Savant Skills

The situation with respect to savant skills is somewhat comparable to that already noted for regression. That is, such skills have been observed from the very outset (Asperger 1944; Frith 1989; Kanner 1971; Treffert 2010). Our understanding of savant skills was greatly increased by the pioneering studies undertaken by O'Connor and Hermelin (1988; Hermelin 2001). Through innovative experimental designs and use of appropriate comparison groups they showed that savant skills represented real abilities and not simply tricks. It has usually been supposed that savant skills are quite uncommon in autism, but Howlin et al.'s systematic study (2010) showed that about a third of individuals with autism had either a savant skill based on parental report or an exceptional cognitive skill. Strikingly, however, no individual with a non-verbal IQ below 50 met the criteria for a savant skill and it is evident that the traditional general term of "Idiot Savant" is misleading and should be abandoned. Savant skills may sometimes occur in individuals with a very low non verbal IQ but this is not the usual situation. Numerous studies have shown that the range of skills is very large, spanning splinter skills at one end, prodigious savants at the other end, and talented savants in the middle. Systematic comparative studies between autism and other disorders have yet to be undertaken, but it would appear that savant skills are particularly commonly associated with autism. Psychological studies have suggested that a detail-focused cognitive style may predispose to talent in savant domains; others have argued that the excellent attention to detail has its origin in sensory hypersensitivity. The term "talent" would seem to imply an in-built capacity but it is also apparent from a range of different research designs that intensive prolonged practice is also involved. It is striking that autism is associated with both intellectual disability and superior talents, and the question is—what sort of neural functioning could account for both?

Epilepsy and New Psychiatric Disorders

It has long been recognized that about a quarter of individuals with autism develop epilepsy (Volkmar and Nelson 1990). However, Rutter's (1970) early follow-up study was striking in showing that in many cases epileptic attacks did not begin until adolescence. The recent larger-scale follow-up into adult life undertaken by Bolton et al. (in press) had findings that were important in several different respects. First, the rate of epilepsy (22%) in individuals with autism was substantially higher than the general population rate of 0.63% at the same age. Second, the proportion of autistic individuals with epilepsy who developed seizures for the first time after the age of ten (58%) was significantly higher than that in either a national general population study or that in a Scottish cohort of children with idiopathic mental retardation (Goulden et al. 1991). Epilepsy was significantly more common in individuals with either very limited language or a low non-verbal IQ but epilepsy occurred in autistic individuals at all levels of intelligence. Epilepsy was not related to the severity of the autism; nor was it associated with a family history of epilepsy. On the other hand, epilepsy was associated with the likelihood of a relative having the broader autism phenotype—suggesting that the epilepsy was associated with an overall familial liability to autism. It was not associated with regression and it was also not associated with the development of new medical conditions. The unusual late onset of epilepsy must have some neuropathological meaning, but what that might be remains obscure.

A separate study based on the same sample (Hutton et al. 2008) showed that about a fifth of autistic individuals developed a new psychiatric disorder by adult life. This was unassociated with the presence of epilepsy or with the timing of the onset of epileptic attacks. The most common disorders were affective in type but the presence of obsessive–compulsive behavior and of catatonia (which seemed usually to stem from obsessive–compulsive symptoms) seemed to be particularly characteristic of individuals with autism. Although individuals with autism may sometimes develop new disorders that appear to be closely related to their autism, many new disorders seem to arise relatively independently, albeit possibly precipitated by major life changes.

Increased Brain Size

In his first paper describing autism, Kanner (1971) noted that four out of the eleven individuals studied had an unusually large head. Little notice was taken of head size for many years. However, during the 1990s, there were several reports on different studies noting that increased head size was common in individuals with autism (Woodhouse et al. 1996). At about the same time, the first structural brain imaging studies similarly showed an increased brain size (Piven et al. 1995). Since then, numerous imaging studies have shown increased brain size in a substantial minority of individuals with autism (Palmen and van Engeland 2004). The findings mainly

suggest a global increased brain growth. The findings on the pattern of brain growth are inconclusive and it is not known whether it reflects an excess of neurons, and/or reduced synaptic pruning (Keller and Persico 2003). The most important recent finding is that the brain size is normal at birth but increases markedly during the early years—a time period that parallels that of the first obvious manifestations of autism (Courchesne et al. 2003, 2007; Redcay and Courchesne 2005). On the whole, the evidence suggests that increasing brain growth plateaus during middle childhood, but there are some reports that, possibly to a lesser degree, brain size can remain enlarged during adolescence and adult life. The major importance of the early increase in brain growth finding is that it indicates some kind of neural process that only comes on line in the toddler age period, even though the genetic liability has presumably been present from before birth. Quite what this process comprises remains unknown but, although adequate comparative studies have not yet been undertaken, it does appear that this increase may be peculiar to autism. The challenge that remains is to determine what that neural process might be.

Dimension or a Diagnostic Category

Throughout the whole of medicine, including the field of mental disorders, it has become evident that most conditions have a dimensional liability (Rutter 2003). The concept of a broader phenotype of autism (see below) implies that dimensional approaches are relevant for autism, as well as with most other multifactorial conditions. However, in recent years, there has been the additional claim that autism may not constitute a cohesive syndrome. Rather, the individual components of autism may not only be more separate than usually appreciated, but also they may reflect different genetic influences (Happé and Ronald 2008; Ronald et al. 2005). This suggestion is a reasonable one but the evidence so far is contradictory and inconclusive. What is needed in order to test the proposition properly, is a general population study in which the different components of autism are individually, adequately and independently measured (in a way that does not necessitate the diagnostic concepts), in order to answer the question as to what extent the three main domains of impairment co-occur. In tackling this question, it would be important to recognize that it cannot be assumed that there are three such domains. For example, much evidence suggests that the distinction between social reciprocity and social communication is artificial and that these two domains would be better combined (see Gotham et al. 2007). On the other hand, there is more uncertainty as to quite how to deal with the abnormal language features such as stereotyped utterances, verbal rituals, inappropriate questions, neologisms, and pronominal reversal. The factor analysis of the social communication questionnaire suggested that these needed to be dealt with as a separate domain (Rutter et al. 2003; Berument et al. 1999). Somewhat similar questions have been posed with respect to restricted repetitive behaviors. For example, Lam et al. (2008) suggested that the evidence indicated that circumscribed interests needed to be differentiated from repetitive motor behaviors. What all of this means is that an open mind must be maintained on the cohesiveness, or otherwise, of the various features of autism spectrum disorders.

Broader Phenotype

Folstein and Rutter's twin study provided the first clear-cut evidence that the genetic liability for autism extended beyond the traditional diagnosis (Folstein and Rutter 1977; Le Couteur et al. 1996). Since then, many studies confirmed the extension of the traditional diagnosis to a broader phenotype, using data from family studies as well as twin studies (Bailey et al. 1998; Bailey and Parr 2003). In recent years there have been various attempts to develop measures of this broad autism phenotype. Thus, Losh et al. (2008) used a mixture of measures to assess broader phenotype features. The findings showed that the features were significantly more common in multiple incidence autism families than in single incidence autism families and both of these had higher rates than Down syndrome families. The findings were surprising in that three-quarters of the individuals in multiplex families showed at least one such feature, half of those in single incidence families also showed the same, but even in the Down syndrome families the rate was 22%. If the assumption is that the Down syndrome families involved no predisposition to show the broader phenotype, the implication is that the false positive rate in the general population would be very high. Dawson et al. (2007) developed a new instrument that combined interview and observational measures and for which systematic training of the professionals using this measure was provided. The findings showed reasonable inter-rater reliability and internal consistency but only moderate correlations between the observational and interview measures. There was no measure of test–retest reliability to assess the temporal stability of the measures.

It may be concluded that some limited progress has been made in the measurement of the broader phenotype using informant report, self-report and observation but there is no agreed set of measures as yet. The available evidence suggests that broader phenotype differs from traditional autism in that it is not associated with either intellectual disability or with epilepsy. The existence of the broader phenotype raises the query of how it becomes transformed into 'autism proper'. Is this simply a measure of the

severity of the genetic liability or is there some kind of two-hit mechanism and, if there is, what is the other influence? We do not as yet know.

Prodromal Features in Infancy

About a third to a half of parents of a child with an autism spectrum disorder recall abnormalities dating back to the first year and, similarly, early home videos have also identified early manifestations of autism by 12–18 months although the indications are often quite subtle (Rutter 2005a; Yirmiya and Charman 2010). Screening questionnaires for autism work reasonably well at 18 months and above, but are not particularly useful below that age when parents have no clinical concerns (Dietz et al. 2006). It became clear that if there was to be early detection of autism in the infancy period, much more detailed observational measures would be required. The way forward arose from the recognition that siblings of a child with autism have a much increased risk of developing autism. This has led to multiple international 'baby-sibling' studies in which siblings are studied prospectively from early in life to identify and delineate precursors of autism (Bryson et al. 2007; Landa et al. 2007; Zwaigenbaum et al. 2005). In the best of these studies observational and clinical measures are being combined with biological assessments (Elsabbagh and Johnson 2010). Preliminary data suggests that these studies are going to yield important findings because differences have been found between the siblings of children with autism and controls but it remains uncertain how far the findings can be used for individual predictions. Possible preventive interventions have been suggested but, necessarily, they remain speculative at the moment.

Adult Functioning

Long term follow-up studies have all shown substantial variability in outcome among individuals with autism (Howlin et al. 2004). Two factors that have been consistently associated with prognosis are language development and IQ. Very few children who have not developed some useful communicative speech by the age of 5–6 years have a positive outcome and, conversely, individuals who were either cognitively untestable as children or who had non-verbal scores below 50 were almost invariably reported as highly dependent. Best outcomes have been found for individuals with an IQ of at least 70 in childhood. Nevertheless, even in this higher-functioning group (of whom a third had a good or very good outcome in the Howlin et al. (2004) study but just over two-fifths had a 'poor' or 'very poor' outcome) it remains quite unclear why that was the case. Did it reflect the inadequacy of services in childhood, the inadequacy of services in adult life, or did it reflect a basic biological handicap? We do not know. The other query with respect to adult outcome concerns the functioning of individuals with the Asperger syndrome or the broader phenotype. Howlin and her colleagues have a current ongoing study to investigate this further but the small number of participants with a 'broader phenotype' is likely to mean that we will still lack adequate understanding of how they fare in adult life.

Cognitive Patterns

There are well replicated findings on impairments in theory of mind (Frith 2003); joint attention (Mundy and Burnette 2005); central coherence (Happé 2005); and executive functions (Ozonoff et al. 2005). A range of queries remains (Rutter and Bailey 1993; Happé 2003). There is now no doubt that specific cognitive deficits play a major role in the liability to autism. Progress has come particularly from a greater use of experimental designs, the application of eye-tracking methodology (Klin et al. 2005), the use of functional brain imaging (Frith and Frith 2008) and the baby-sibling prospective studies. The hope had been that it would be possible to identify a single modular cognitive deficit that fully accounted for autism, but that now seems less likely. Rather, the imaging studies suggest atypical connectivity as the basic feature, although there is inconsistency across studies in the details (Frith and Frith 2008). It has to be added, too, that it is not yet clear what atypical connectivity means in terms of neural functioning.

Subclassification

Both DSM IV and ICD 10 subdivided autism spectrum disorders (previously called pervasive developmental disorders) into several subcategories. The DSM V Child and Adolescent Psychiatry Working Party has recently suggested that all subdivisions be removed, leaving a single broad category of autism spectrum disorders (see Rutter, in press). They rightly argued that the sub-classification has not worked in practice. However, the removal of all subcategories presents difficulties. First, no one doubts that Rett syndrome constitutes a distinct condition as a virtue of both its progressive course and its origin in a single genetic mutation. Although not clearly spelled out, it seems that what is supposed to happen is that the overall undivided ASD category should be used for the period when children with Rett syndrome show features similar to autism. The recognition of Rett syndrome as a specific cause would be picked up by its diagnosis as a type of neurological disorder.

There are two problems with that. First, it was included as a sub-category of ASD purely because the neurological

section of ICD 10 did not make any mention of Rett syndrome. It is not known what is happening with ICD 11. As far as DSM V is concerned, the difficulty is that, unlike ICD, it does not form part of an overall medical classification and hence the designation of Rett syndrome is more problematic. Second, there is the category of disintegrative disorder. The problem here is that it has been subject to so little research that we simply do not know whether it constitutes an unusual variant of autism or something quite different. It would seem important to keep it in the classification somewhere in order that it may be subject to further research. Third, there is the uncertainty as to whether Asperger syndrome does, or does not, differ meaningfully from high-functioning autism. The published studies comparing the two are quite unhelpful because Asperger syndrome has been dealt with in such varied ways. When, however, there has been a focus on the one key feature of the presence or absence of competence in language structure, developmental trajectory, although similar in shape to that found with autism, is different in being associated with a better outcome (Szatmari et al. 2009). There may be a dispute on whether or not that is most appropriately equated with the syndrome as outlined by Asperger but the distinction does seem worthwhile. It seems that the DSM V working party envisage the distinction being picked up by dimensional codings and, if those can be made to work, that may well be a suitable solution. All that can be concluded firmly at the moment is that it is highly likely that there are meaningful sub-categories of autism spectrum disorders but that these are not well identified by the behavioral diagnoses in the existing classification systems.

Quasi-Autism

The UK study of English and Romanian adoptees (Rutter and Sonuga-Barke 2010) showed that the profound institutional deprivation that lasted beyond the child's age of 6 months was associated in about one in six children with a clinical picture that was similar to autism, but atypical in some features. The mechanism is not well understood, but the implication is that autism may develop on the basis of an external environmentally imposed restriction of stimuli, as well as an internal genetically influenced impairment in the processing of stimuli. It remains to be determined whether abuse and neglect in the family can have the same effect, but the limited available evidence suggests not.

Lack of a Marked Response to Medication

Numerous studies have documented that autism stands out from almost all other psychiatric disorders in showing no marked benefits of psychotropic medication on core symptoms (such as impaired social reciprocity and social communication). (Buitelaar 2003; Scahill and Martin 2005). Why? One possible implication is that the basic deficit does not involve neurotransmitters; if not, what does it involve? It is important to pose the question, not so much because of the implications for treatment today, but rather because a satisfactory answer could have important implications for the neural basis of autism. For the moment, medication is of some value for associated problems, but the enigma is why that seems to be all.

Genetic Findings

Twin and family studies undertaken over a period of several decades have been consistent in showing that ASDs have an overall heritability of about 90% (Rutter 2005b). The falloff rate from MZ to DZ twins, together with that from first degree to second degree relatives, was used by Pickles et al. (1995) to estimate the number of genes that were likely to be involved (Pickles et al. 2000). The findings indicated that there were likely to be at least three or four genes involved in susceptibility to autism but the number could be substantially greater than that. On the other hand, a single-gene Mendelian disorder would not account for the bulk of the findings. The third important finding was that the genetic liability for autism extended to include a broader phenotype (Bailey et al. 1995; Le Couteur et al. 1996). Fourthly, examination of MZ pairs concordant for autism showed that there was enormous clinical heterogeneity even when pairs shared exactly the same segregating genetic alleles (Le Couteur et al. 1996). Over the same period of time, numerous studies showed that Autism Spectrum Disorders were associated with chromosomal abnormalities or genetically determined medical conditions in at least 10% of cases (Rutter et al. 1994). All of these findings still stand today but, during the last decade, there has been no particular progress with respect to these aspects of genetics. Rather, attention has shifted to molecular genetic studies; see Abrahams and Geschwind (2008), Geschwind and Levitt (2007), Folstein and Rosen-Sheidley (2001) and Bacchelli and Maestrini (2006) for reviews of findings.

Rare Pathogenic Gene Mutations

There are multiple replicated findings that autism is associated with rare pathogenic gene mutations such as neuroligins, neurexin and SHANK 3 (Persico and Bugeron 2006; Durand et al. 2007; Bourgeron 2007; Geschwind and Levitt 2007; Jamain et al. 2008). They account for a tiny proportion of cases (circa 1%) but it has been claimed that they are, nevertheless, true 'causes' of autism. The

dilemma is that, although the clinical picture associated with these genes includes autistic features, intellectual disability often dominates. Of course, genes do not code for specific psychiatric categories and pleiotropic effects are to be expected. Nevertheless, the lack of specificity raises doubts on the extent to which the findings are informative with respect to most cases of Autism Spectrum Disorder.

Copy Number Variations (CNVs)

It is now possible to detect tiny sub-microscopic chromosomal deletions or duplications (known as copy number variations). Several studies have shown that CNVs, especially those involving chromosomal deletions, are found in some 5% of cases of autism—a rate significantly higher than that in controls (Cook and Scherer 2008; Szatmari et al. 2007; Sebat et al. 2007; Marshall et al. 2008). The evidence indicates a causal role for CNVs in both autism and schizophrenia (International Schizophrenia Consortium 2008), and also ADHD (Williams et al. 2010) but important queries remain. Most CNVs arise de novo and, therefore, cannot account for familiality. Also, when inherited, the CNVs may be present in family members who are unaffected by autism; thus the causal effect is not necessarily determinative. It is also striking that the relevant CNVs seem frequently to be different in different families (Pinto et al. 2010). Also, it is necessary to ask what causes the raised frequency of CNVs; one possibility is raised parental age (see below). The same question applies to major chromosome anomalies, which are also more frequent in individuals with autism than in the general population.

Genome-Wide Association Studies (GWAS)

It is now possible to undertake genome-wide association studies instead of relying on candidate genes to direct the search for susceptibility genes. GWAS require huge samples and, inevitability, will give rise to many false positives (Dodge and Rutter, in press). GWAS have the important advantage of being able to detect novel genetic associations but the findings so far have been unimpressive in the field of multifactorial mental disorders. Moreover, because the identified susceptibility genes have been found to have very weak effects, it remains uncertain whether the findings will be very informative on biological causal pathways.

Epigenetics

There is now great interest in the possibility that many genetic effects derive from epigenetics rather than changes in gene sequence. Epigenetics refers to neuro-chemical changes that influence gene expression (Meaney 2010). Expression is both tissue-specific and developmental phase-specific. It involves multiple DNA elements, chance effects, and environmental influences. There is limited evidence that epigenetic mechanisms might be involved in autism but so far their role remains uncertain (Gregory et al. 2009).

Why Doesn't Autism Become Extinct?

It is known that both autism and schizophrenia are associated with a markedly reduced fecundity (ability to reproduce). Accordingly, why doesn't autism die out? What enables it to persist in the population? (Uher 2009). No convincing answer is available, but the findings suggest that the genetic influences on autism and on schizophrenia may well involve mechanisms that are different from those that apply to other mental disorders (possibly involving a greater role for rare pathogenic gene mutations or CNVs—see above).

Why Haven't the Susceptibility Genes for Autism Been Identified?

As noted above, twin and family studies have been consistent in indicating that autism has a very high heritability (circa 90%); why, therefore, has it proved so difficult to find the specific genes responsible? We do not really know what the answer should be but, in addition to the likelihood of genetic heterogeneity and the very small effects of individual genes, the explanation may lie in epigenetics, or in gene environment correlations and interactions, or in synergistic effects among genes.

Environmental Findings

MMR and Thimerosal

Claims have been made that either the Measles, Mumps, Rubella (MMR) vaccine or Thimerosal (a mercury preservative used in some vaccines), or both, were responsible for an epidemic of autism. So far as MMR is concerned, epidemiological research has been consistently negative with respect to that claim (Rutter 2008); most decisively, the evidence from Japan that when MMR was totally withdrawn, there was no effect on the overall rise in the rate of diagnosed autism (Honda et al. 2005). Moreover, well conducted studies have also shown that the measles virus in tissue claims were mistaken (Baird et al. 2008b; Hornig et al. 2008; D'Souza et al. 2006; Afzal et al. 2006). The situation with respect to Thimerosal is somewhat more complicated in that there is no doubt that mercury is a proven neurotoxin. Nevertheless, the same types of

epidemiological research have also failed to support the claim that this has led to an epidemic of autism. In particular, the withdrawal of Thimerosal from all vaccines in Scandinavia, at a time when such use was continuing in the rest of the world, provides convincing evidence against the 'epidemic' notion (Atladóttir et al. 2007). A broader range of research has examined the effects of mercury toxicity in humans and has also examined putative Thimerosal effects in various different ways. Findings are rather consistently negative so far as Thimerosal is concerned (particularly as a risk factor for autism) but it is clear that, in moderately raised dosages, there can be significant effects from mercury toxicity.

Raised Parental Age

There are now replicated findings that children born to older fathers have an increased rate of autism (Reichenberg et al. 2006; Croen et al. 2007; Cantor et al. 2007). Less certainly, this may also apply to older mothers. The likely explanation is that the older father effect reflects the increased likelihood of genetic mutations with an increasing number of cell divisions, but the association has been too little investigated in humans to be certain about this mechanism. Little is known on the effects of older fathers on the risk for other mental disorders, apart from schizophrenia for which a meta-analysis suggested that late fatherhood increased the risk (Wohl and Gorwood 2007).

Maternal Immigration

There are now several studies (Keen et al. 2010 for example), showing that maternal immigration is associated with an increased risk of autism in the children. Earlier studies had examined the possibility that rates of autism might be higher among immigrants (see Fombonne 2005) with results that were inconsistent, but largely unsupportive of the suggestion. Newer findings refer specifically to immigration of the mother and these appear to be sound. Nevertheless, the evidence remains scanty, the effect is weak, and it remains uncertain whether this association represents a causal effect and, if it does, the mechanism remains obscure.

Other Pre-Natal and Early Post-Natal Influences

The evidence that autism spectrum disorders (ASD) are multifactorial in nature means that some environmental factors are likely to be implicated in causation. Increase over time in the rate of diagnosis of ASD, if it reflects a true rise in incidence (which remains uncertain), would also point to some environmental effect. Prospective longitudinal studies of very large samples starting during pregnancy, and including good biological measures, are needed to test the possibility. The Norwegian mother and baby study (MoBa) of some 100,000 children is one such investigation (Magnus et al. 2006; Rønningen et al. 2006; Stoltenberg et al. 2010).

Psychological Treatments

There have been no major developments during the last 3 years in our understanding of autism derived from psychological treatments, but there is continuing controversial discussion over claims that very intense, very early behavioral treatment can lead to 'recovery'. That such treatment can bring worthwhile benefits is not in doubt (Medical Research Council 2001; National Research Council 2001). Equally, it is known that, even in the absence of such early treatment, huge gains in functioning can sometimes occur. Whether or not there is complete recovery is much less certain (Helt et al. 2008). Also, the claims of the necessity for very early treatment and high intensity for some 40 h a week for at least 2 years remain very questionable (Howlin 2003, 2005). What is new is the accumulation of better evidence deriving from well planned randomized controlled trials.

An important new randomized controlled trial of early intensive behavioural intervention provides possibly the best evidence to date (Dawson et al. 2009). Findings showed that there was a significant increase in IQ (albeit of modest size) in the treated group but not in controls. However, what this means is uncertain because there was no increase in social functioning as measured by the Vineland scale at 12 months. Also, the results showed that there was no effect of the treatment on core features of autism as assessed by the autism diagnostic observation schedule. Accordingly, the study certainly does not support the Lovaas claims (Lovaas 1987; McEachin et al. 1993) about the huge benefits of early treatment leading to recovery.

What is also very new is the introduction of methods of treatment focused on improving parental sensitivity and responsiveness. The randomized controlled trial undertaken by Green et al. (2010) provide an excellent model of how RCTs should be undertaken. The findings are encouraging in showing substantial significant positive changes in parental sensitivity/responsivity but disappointing in that there was only a very small improvement (relative to the control group) in the children's autistic features.

Conclusions

Looking back over the last 50 years (Feinstein 2010) it is clear that the understanding of autism has been transformed

in numerous different ways. The substantial gains in knowledge during the last few years have been equally impressive. There have been many important findings, some of which have been rather unexpected, but major puzzles remain. We should be hopeful of even greater gains in the years ahead, but both prevention and cure remain elusive.

References

Abrahams, B. S., & Geschwind, D. H. (2008). Advances in autism genetics: On the threshold of a new neurobiology. *Nature Review: Genetics, 9*, 341–355.

Afzal, M. A., Ozoemena, L. C., O'Hare, A., Kidger, K. A., Bentley, M. L., & Minor, P. D. (2006). Absence of detectable measles virus genome sequence in blood of autistic children who have had their MMR vaccination during the routine childhood immunization schedule of UK. *Journal of Medical Virology, 78*, 623–630.

Asperger, H. (1944). Die 'Autistischen Psychopathen' im Kindesalter [Trans. 'Autistic psychopathy' in childhood]. *Archiv fur Psychiatrie und Nervenkrankheiten, 117*, 76–136.

Atladóttir, H. O., Parner, E. T., Schendel, D., Dalsgaard, S., Thomsen, P. H., & Thorsen, P. (2007). Time trends in reported diagnoses of childhood neuropsychiatric disorders: A Danish cohort study. *Archives of Pediatric and Adolescent Medicine, 161*, 193–198.

Bacchelli, A., & Maestrini, E. (2006). Autism spectrum disorders: Molecular genetic advances. *American Journal of Medical Genetics C. Seminars in Medical Genetics, 142*, 13–23.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, F. Y., et al. (1995). Autism as a strongly genetic disorder: Evidence from a British twin study. *Psychological Medicine, 25*, 63–77.

Bailey, A., Palferman, S., Heavey, L., & Le Couteur, A. (1998). Autism: The phenotype in relatives. *Journal of Autism and Developmental Disorders, 28*, 369–392.

Bailey, A., & Parr, J. (2003). Implications of the broader phenotype for concepts of autism. In G. Bock & J. Goode (Eds.), *Autism: Neural basis and treatment possibilities (pp 26–36)*. Chichester, UK: Wiley.

Baird, G., Charman, T., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., et al. (2008a). Regression, developmental trajectory and associated problems in disorders in the autism spectrum. *The SNAP Study, Journal of Autism& Developmental Disorders, 38*, 1827–1836.

Baird, G., Pickles, A., Simonoff, E., Charman, T., Sullivan, P., Chandler, S., et al. (2008b). Measles vaccination and antibody response in autism spectrum disorders. *Archives of Disease in Childhood, 93*, 832–837.

Berument, S. K., Rutter, M., Lord, C., Pickles, A., & Bailey, A. (1999). Autism screening questionnaire: Diagnostic validity. *British Journal of Psychiatry, 175*, 444–445.

Bolton, P. F., Carcani-Rathwell, I., Hutton, J., Goode, S., Howlin, P., & Rutter, M. L. (in press). Features and correlates of epilepsy in autism. *British Journal of Psychiatry*.

Bourgeron, T. (2007). The possible interplay of synaptic and clock genes in autism spectrum disorders. *Cold Spring Harbor Symposia on Quantitative Biology, 72*, 645–654.

Bryson, S. E., Zwaigenbaum, L., Brian, J., Roberts, W., Szatmari, P., Rombough, V., et al. (2007). A prospective cases series of high-risk infants who developed autism. *Journal of Autism and Developmental Disorders, 37*, 12–24.

Buitelaar, J. K. (2003). Why have drug treatments been so disappointing? In G. Bock & J. Goode (Eds.), *Autism: Neural basis and treatment possibilities* (pp. 235–249). Chichester, UK: Wiley.

Cantor, R. M., Yoon, J. L., Fuur, J., & Lajonchere, C. M. (2007). Paternal age and autism are associated in a family-based sample. *Molecular Psychiatry, 12*, 419–423.

Cook, E. H., & Scherer, S. W. (2008). Copy-number variations associated with neuropsychiatric conditions. *Nature, 455*(7215), 919–923.

Courchesne, E., Carper, R., & Akshoomoff, N. (2003). Evidence of brain overgrowth in the first year of life in autism. *Journal of the American Medical Association, 290*, 337–344.

Courchesne, E., Pierce, K., Schumann, C., Redcay, E., Buckwalter, J., Kennedy, D., et al. (2007). Mapping early brain development in autism. *Neuron, 56*, 399–413.

Croen, L. A., Najjar, D. V., Fireman, B., & Grether, J. K. (2007). Maternal and paternal age and risk of autism spectrum disorders. *Archives of Pediatric and Adolescent Medicine, 161*, 334–340.

D'Souza, Y., Fombonne, E., & Ward, B. J. (2006). No evidence of persisting measles virus in peripheral blood mononuclear cells from children with autism spectrum disorder. *Pediatrics, 118*, 1664–2608.

Dawson, G., Estes, A., Munson, J., Schellenberg, G., Bernier, R., Abott, R., et al. (2007). Quantitative assessment of autism symptom-related traits in probands and parents: Broader phenotype autism symptom scale. *Journal of Autism and Developmental Disorders, 37*, 523–536.

Dawson, G., Rogers, S., Munson, J., Smith, M., Winter, J., Greenson, J., et al. (2009). Randomized, controlled trial of an intervention for toddlers with autism: The early start Denver model. *Pediatrics, 125*, 17–23.

Dietz, C., Swinkels, S., van Daalen, E., van Engeland, H., & Buitelaar, J. K. (2006). Screening for autistic spectrum disorder in children aged 14–15 months. II. Population screening with the early screening of autistic traits questionnaire (ESAT): Design and general findings. *Journal of Autism and Developmental Disorders, 36*, 713–722.

Dodge, K. A., & Rutter, M. (Eds.). (in press). *Gene–environment interactions in developmental psychopathology: So what?* New York: Guilford Press.

Durand, C. M., Betancur, C., Boeckers, T. M., Bockman, J., Chaste, P., Faucherau, F., et al. (2007). Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nature Genetics, 39*, 25–27.

Elsabbagh, M., & Johnson, M. H. (2010). Getting answers from babies about autism. *Trends Cognitive Science, 4*, 81–87.

Feinstein, A. (2010). *A history of autism: Conversations with the pioneers*. Chichester, UK: Wiley Blackwell.

Folstein, S. E., & Rosen-Sheidley, B. (2001). Genetics of autism: Complex aetiology for a heterogeneous disorder. *Nature Reviews: Genetics, 2*, 943–955.

Folstein, S., & Rutter, M. (1977). Infantile autism: A genetic study of 21 twin pairs. *Journal of Child Psychology and Psychiatry, 18*, 297–321.

Fombonne, E. (2005). Epidemiological studies of pervasive developmental disorders. In F. Volkmar, R. Paul, A. Klin, & D. Cohen (Eds.), *Handbook of autism and pervasive developmental disorders (pp 62–63)*. Hoboken, NJ: Wiley.

Frith, U. (1989). *Autism: Explaining the enigma*. Oxford, UK: Blackwell Publishing.

Frith, U. (2003). *Autism: Explaining the enigma* (2nd ed.). Oxford: Blackwell.

Frith, C., & Frith, U. (2008). What can we learn from structural and functional brain imaging? In M. Rutter, D. Bishop, D. Pine, S. Scott, J. Stevenson, E. Taylor, & A. Thapar (Eds.), *Rutter's child*

and adolescent psychiatry (5th ed., pp. 134–144). Massachusetts, USA: Blackwell Publishing.

Geschwind, D. H., & Levitt, P. (2007). Autism spectrum disorders: Developmental disconnection syndromes. *Current Opinion in Neurobiology, 17*, 103–111.

Gotham, K., Risi, S., Pickles, A., & Lord, C. (2007). The autism diagnostic observation schedule: Revised algorithms for improved diagnostic validity. *Journal of Autism and Developmental Disorders, 37*, 613–627.

Goulden, K. J., Shinnar, S., Koller, H., Katz, M., & Richardson, S. A. (1991). Epilepsy in children with mental retardation: A cohort study. *Epilepsia, 32*, 690–697.

Green, J., Charman, T., McConachie, H., Aldred, C., Slonims, V., Howlin, P., et al. (2010). Parent-mediated communication-focused treatment in children with autism (PACT): A randomized controlled trial. *The Lancet, 375*, 2152–2160.

Gregory, S. G., Connelly, J. J., Towers, A. J., Johnson, J., Bisocho, D., Markunas, C. A., et al. (2009). Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Medicine, 7*, 62. doi:10.1186/1741-7015-7-62.

Happé, F. (2003). Cognition in autism: One deficit or many? Autism: Neural basis and treatment possibilities. *Novartis Foundation Symposium, 251*, 198–212.

Happé, F. (2005). The weak central coherence account of autism. In F. Volkmar, R. Paul, A. Klin, & D. Cohen (Eds.), *Handbook of autism and pervasive developmental disorders* (pp. 640–649). Hoboken, NJ: Wiley.

Happé, F., & Ronald, A. (2008). The 'fractionable autism triad': A review of evidence from behavioural, genetic, cognitive and neural research. *Neuropsychological Review, 18*(4), 287–304.

Helt, M., Kelley, E., Kinsbourne, M., Pandey, J., Boorstein, H., Herbert, M., et al. (2008). Can children with autism recover? If so, how? *Neuropsychology Review, 18*, 339–366.

Hermelin, B. (2001). *Bright splinters of the mind*. London, UK: Jessica Kingsley Publishers.

Honda, H., Shimizu, Y., & Rutter, M. (2005). No effect of MMR withdrawal on the incidence of autism: A total population study. *Journal of Child Psychology and Psychiatry, 46*, 572–579.

Hornig, M., Brieses, T., Buie, T., Mauman, M. L., Lauwers, G., Siemetzki, U., et al. (2008). Lack of association between measles virus vaccine and autism with enteropathy: A case-control study. *PLoS One, 3*, e3140.

Howlin, P. (2003). Can early interventions alter the course of autism? In G. Bock & J. Goode (Eds.), *Autism: Neural basis and treatment possibilities* (pp. 250–265). Chichester, UK: Wiley.

Howlin, P. (2005). The effectiveness of interventions for children with autism. *Journal of Neural Transmission (Suppementum), 69*, 101–119.

Howlin, P., Goode, S., Hutton, J., & Rutter, M. (2004). Adult outcomes for children with autism. *Journal of Child Psychology and Psychiatry, 45*, 212–229.

Howlin, P., Goode, S., Hutton, J., & Rutter, M. (2010). Savant skills in autism: Psychometric approaches and parental reports. In F. Happé & U. Frith (Eds.), *Autism and talent* (pp. 13–24). Oxford: University Press.

Hutton, J., Goode, S., Murphy, M., Le Couteur, A., & Rutter, M. (2008). New-onset psychiatric disorders in individuals with autism. *Autism, 12*(4), 373–390.

International Schizophrenia Consortium. (2008). Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature, 455*, 237–241.

Jamain, S., Radyushkin, K., Hammerschmidt, K., Granon, S., Boretius, S., Varoqueaux, F., et al. (2008). Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. *Proceedings of the National Academy of Sciences of the USA, 5*, 1710–1715.

Kanner, L. (1971). Follow-up study of eleven autistic children originally reported in 1943. *Journal of Autism and Childhood Schizophenia, 1*, 119–145.

Keen, D. V., Reid, F. D., & Arnone, D. (2010). Autism, ethnicity and maternal immigration. *British Journal of Psychiatry, 196*, 274–281.

Keller, F., & Persico, A. M. (2003). The neurobiological context of autism. *Molecular Neurobiology, 28*, 1–22.

Klin, A., Jones, W., Schultz, R. T., & Volkmar, F. (2005). The enactive mind—from actions to cognition: Lessons from autism. In F. Volkmar, R. Paul, A. Klin, & D. Cohen (Eds.), *Handbook of autism and pervasive developmental disorders* (pp. 682–703). Hoboken, NJ: Wiley.

Lam, K. S. L., Bodfish, J. W., & Piven, J. (2008). Evidence for three subtypes of repetitive behaviour in autism that differ in familiality and association with other symptoms. *Journal of Child Psychology and Psychiatry, 49*, 1193–1200.

Landa, R., Holman, K. C., & Garrett-Mayer, E. (2007). Social and communication development in toddlers with early and later diagnosis of autism spectrum disorders. *Archives of General Psychiatry, 64*, 853–864.

Le Couteur, A., Bailey, A. J., Goode, S., Pickles, A., Robertson, S., Gottesman, I., et al. (1996). A broader phenotype of autism: The clinical spectrum in twins. *Journal of Child Psychology and Psychiatry, 37*, 785–801.

Losh, M., Childress, D., Lam, K., & Piven, J. (2008). Defining key features of the broad autism phenotype: A comparison across parents of multiple- and single-incidence autism families. *American Journal of Medical Genetics B: Neuropsychiatric Genetics, 147B*, 424–433.

Lovaas, O. I. (1987). Behavioral treatment and normal educational and intellectual functioning in young autistic children. *Journal of Consulting and Clinical Psychology, 55*, 3–9.

Magnus, P., Irgens, L. M., Haug, K., Nystad, W., Skjaerven, R., Stoltenberg, C., et al. (2006). Cohort profile: The Norwegian mother and child cohort study (MoBa). *International Journal of Epidemiology, 35*, 1146–1150.

Marshall, C. R., Nooer, A., Vincent, J. B., Lionel, A. C., Feuk, L., Skaug, J., et al. (2008). Structural variations of chromosomes in autism spectrum disorder. *American Journal of Human Genetics, 82*(2), 477–488.

McEachin, J. J., Smith, T., & Lovaas, O. I. (1993). Long-term outcome for children with autism who received early intensive behavioral treatment. *American Journal of Mental Retardation, 97*, 359–372.

Meaney, M. J. (2010). Epigenetics and the biological definition of gene × environment interactions. *Child Development, 81*, 41–79.

Medical Research Council. (2001). *MRC review of autism research: Epidemiology and causes*. London: MRC.

Mundy, P., & Burnette, C. (2005). Joint attention and neurodevelopmental models of autism. In F. Volkmar, R. Paul, A. Klin, & D. Cohen (Eds.), *Handbook of autism and pervasive developmental disorders* (pp. 650–681). Hoboken, NJ: Wiley.

National Research Council. (2001). *Educating children with autism. Committee on educational interventions for children with autism*. Washington, DC: National Academy Press.

O'Connor, N., & Hermelin, B. (1998). Annotation: Low intelligence and special abilities. *Journal of Child Psychology and Psychiatry, 29*, 391–396.

Ozonoff, S., South, M., & Provencal, S. (2005). Executive functions. In F. Volkmar, R. Paul, A. Klin, & D. Cohen (Eds.), *Handbook of autism and pervasive developmental disorders* (pp. 606–627). Hoboken, NJ: Wiley.

Palmen, S. J., & van Engeland, H. (2004). Review on structural neuroimaging findings in autism. *Journal of Neural Transmission, 111*, 903–929.

Parr, J. R., Le Couteur, A., Baird, G., Rutter, M., Pickles, A., Fombonne, E., et al. (in press). Early developmental regression in autism spectrum disorder: Evidence from an international multiplex sample. *Journal of Autism and Developmental Disorders*. doi:10.1007/s10803-010-1055-2

Persico, A., & Bugeron, T. (2006). Searching for ways out of the autism maze: Genetic, epigenetic and environmental clues. *Trends in Neuroscience, 29*, 349–358.

Pickles, A., Bolton, P., Macdonald, H., Bailey, A., Le Couteur, A., Sim, C. H., et al. (1995). Latent class analysis of recurrence risks for complex phenotypes with selection and measurement error: A family history study of autism. *American Journal of Human Genetics, 57*, 717–726.

Pickles, A., Simonoff, E., Conti-Ramsden, G., Falcaro, M., Simkin, Z., Charman, T., et al. (2009). Loss of language in early development of autism and specific language impairment. *Journal of Child Psychology and Psychiatry, 50*, 843–852.

Pickles, A., Starr, E., Kazak, S., Bolton, P., Papanikolaou, K., Bailey, A., et al. (2000). Variable expression of the autism broader phenotype: Findings from extended pedigrees. *Journal of Child Psychology and Psychiatry, 41*, 491–502.

Pinto, D., Pagnamenta, A. T., Klei, L., Anney, R., Merico, D., Regan, R., et al. (2010). Functional impact of global rare copy number variation in autism spectrum disorders. *Nature, 466*, 368–372.

Piven, J., Arndt, S., Bailey, J., Haveramp, S., Andreasen, N. C., & Palmer, P. (1995). An MRI study of brain size in autism. *American Journal of Psychiatry, 152*, 1145–1149.

Redcay, E., & Courchesne, E. (2005). When is the brain enlarged in autism? A meta-analysis of all brain size reports. *Biological Psychiatry, 58*, 1–9.

Reichenberg, A., Gross, R., Weiser, M., Bresnahan, M., Silverman, J., Harlap, S., et al. (2006). Advancing paternal age and autism. *Archives of General Psychiatry, 63*, 1026–1032.

Ronald, A., Happé, F., & Plomin, R. (2005). The genetic relationship between individual differences in social and nonsocial behaviors characteristic of autism. *Developmental Science, 8*, 444–458.

Rønningen, A., Paltiel, L., Meltzer, H. M., Nordhagen, R., Lie, K. K., Hovengen, R., et al. (2006). The biobank of the Norwegian mother and child cohort study: A resource for the next 100 years. *European Journal of Epidemiology, 21*, 619–625.

Rutter, M. (1970). Autistic children: Infancy to adulthood. *Seminars in Psychiatry, 2*, 435–450.

Rutter, M. (2003). *Roots of mental illness* (pp. 11–21). New York: The New York Academy of Sciences.

Rutter, M. (2005a). Incidence of autism spectrum disorders: Changes over time and their meaning. *Acta Paediatrica, 94*, 2–15.

Rutter, M. (2005b). Genetic influences and autism. In F. Volkmar, R. Paul, A. Klin, & D. Cohen (Eds.), *Handbook of autism and pervasive developmental disorders* (pp. 425–452). Hoboken, NJ: Wiley.

Rutter, M. (2008). *Thimerosal vaccine litigation*. Report to US vaccine court 2008.

Rutter, M. (in press). Child psychiatric diagnosis and classification: Concept, findings, challenges & potential. *Journal of Child Psychology and Psychiatry*.

Rutter, M., & Bailey, A. (1993). Thinking and relationships: Mind and brain. In S. Baron-Cohen, H. Tager-Flusberg, & D. Cohen (Eds.), *Understanding other minds: Perspectives from autism* (pp. 481–505). New York: Oxford University Press.

Rutter, M., Bailey, A., Bolton, P., & Le Couture, A. (1994). Autism and known medical conditions: Myth and substance. *Journal of Child Psychology and Psychiatry, 35*, 311–322.

Rutter, M., Bailey, A., & Lord, C. (2003). *The social communication questionnaire. Manual*. Los Angeles, CA: Western Psychological Services.

Rutter, M., & Sonuga-Barke, E. J. (Eds.). (2010). Deprivation-specific psychological patterns: Effects of institutional deprivation. *Monographs of the Society for Research in Child Development*, 75:1. Serial no. 295.

Scahill, L., & Martin, A. (2005). Psychopharmacology. In F. Volkmar, R. Paul, A. Klin, & D. Cohen (Eds.), *Handbook of autism and pervasive developmental disorders* (pp. 1102–1117). Hoboken, NJ: Wiley.

Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., et al. (2007). Strong association of de novo copy number mutations with autism. *Science, 316*(58223), 445–449.

Stoltenberg, C., Schjølberg, S., Bresnahan, M., Hornig, M., Hirtz, C., et al. (2010). The autism birth cohort: A paradigm for gene–environment-timing research. *Molecular Psychiatry, 15*, 676–680.

Szatmari, P., Bryson, S., Duku, E., Vaccarella, L., Zwaigenbaum, L., Bennett, T., et al. (2009). Similar developmental trajectory in autism and Asperger syndrome: From early childhood to adolescence. *Journal of Child Psychology and Psychiatry, 50*, 1459–1467.

Szatmari, P., Paterson, A. D., Zwaigenbaum, L., Roberts, W., Brian, J., Liu, X.-Q., et al. (2007). Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nature Genetics, 39*(3), 319–328.

Treffert, D. A. (2010). The savant syndrome: An extraordinary condition. A synopsis: Past, present, future. In F. Happé & U. Frith (Eds.), *Autism and talent* (pp. 1–12). New York: Oxford University Press.

Uher, R. (2009). The role of genetic variation in the causation of mental illness: An evolution-informed framework. *Molecular Psychiatry, 14*, 1072–1082.

Volkmar, F. R., & Nelson, D. S. (1990). Seizure disorders in autism. *Journal of the American Academy of Child and Adolescent Psychiatry, 29*(1), 127–129.

Werner, E., & Dawson, G. (2005). Validation of the phenomenon of autistic regression using home videotapes. *Archives of General Psychiatry, 62*, 889–895.

Williams, N. M., Zaharieva, I., Martin, A., Langley, K., Mantripragada, K., Fossdal, R., et al. (2010). Rare chromosomal deletions and duplications in attention-deficit hyperactivity disorder: A genome-wide analysis. *Lancet, 376*, 1401–1408.

Wohl, M., & Gorwood, P. (2007). Paternal ages below or above 35 years old are associated with a different risk of schizophrenia in the offspring. *European Psychiatry, 22*, 22–26.

Woodhouse, W., Bailey, A., Rutter, M., Bolton, P., Baird, G., & Le Couteur, A. (1996). Head circumference in autism and other pervasive developmental disorders. *Journal of Child Psychology and Psychiatry, 37*, 785–801.

Yirmiya, N., & Charman, T. (2010). The prodrome of autism: Early behavioural and biological signs, regression, peri- and post-natal development and genetics. *Journal of Child Psychology and Psychiatry, 5*, 432–458.

Zwaigenbaum, L., Bryson, S., Rogers, T., Roberts, W., Brian, J., & Szatmari, P. (2005). Behavioral manifestations of autism in the first year of life. *International Journal of Developmental Neuroscience, 23*, 143–152.

THE JOURNAL OF CHILD PSYCHOLOGY AND PSYCHIATRY
ACAMH THE ASSOCIATION FOR CHILD AND ADOLESCENT MENTAL HEALTH
Journal of Child Psychology and Psychiatry **:* (2016), pp **–** doi:10.1111/jcpp.12530

Behavioral outcomes of picky eating in childhood: a prospective study in the general population

Sebastian Cardona Cano,[1,2] Hans W. Hoek,[2,3,4] Daphne van Hoeken,[2] Lisanne M. de Barse,[1,5] Vincent W.V. Jaddoe,[1,5,6] Frank C. Verhulst,[1,7] and Henning Tiemeier[5,7,8]

[1]The Generation R Study Group, Erasmus University Medical Center, Rotterdam; [2]Parnassia Psychiatric Institute, The Hague, The Netherlands; [3]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA; [4]Department of Psychiatry, University Medical Center Groningen, University of Groningen, Groningen; [5]Department of Epidemiology, Erasmus University Medical Center, Rotterdam; [6]Department of Pediatrics, Erasmus University Medical Center – Sophia Children's Hospital, Rotterdam; [7]Department of Child and Adolescent Psychiatry/Psychology, Erasmus University Medical Center – Sophia Children's Hospital, Rotterdam; [8]Department of Psychiatry, Erasmus University Medical Center, Rotterdam, The Netherlands

Background: Picky eaters in the general population form a heterogeneous group. It is important to differentiate between children with transient picky eating (PE) and persistent PE behavior when adverse outcomes are studied. We analyzed four PE trajectories to determine the associations with child mental health prospectively. **Methods:** From a population-based cohort, 3,748 participants were assessed for PE at 1.5, 3, and 6 years of age using maternal reports. Four trajectories were defined: persistent (PE at all ages); remitting (PE before 6 years only); late-onset (PE at 6 years only); and never (no PE at any assessment). Child's problem behaviors were assessed with the Teacher's Report Form at 7 years of age. We examined associations between picky eating trajectories and emotional problems, behavioral problems and pervasive developmental problems using logistic regressions. Analyses were adjusted for child, parental, and socioeconomic confounders. We also adjusted for maternal-reported baseline problem behavior at age 1.5 years; the never picky eating group was used as reference. **Results:** Persisting PE predicted pervasive developmental problems at age 7 years (OR = 2.00, 95% CI: 1.10–3.63). The association remained when adjusted for baseline pervasive developmental problems at 1.5 years (OR = 1.96, 95% CI: 1.10–3.51). Persistent PE was not associated with behavioral (OR = 0.92, 95% CI: 0.53–1.60) or emotional problems (OR = 1.24, 95% CI: 0.74–2.07). Other PE trajectories were not related to child behavioral or emotional problems. **Conclusions:** Persistent PE may be a symptom or sign of pervasive developmental problems, but is not predictive of other behavioral problems. Remitting PE was not associated with adverse mental health outcomes, which further indicates that it may be part of normal development. **Keywords:** Picky eating; emotional problems; behavioral problems; pervasive developmental problems.

Introduction

Picky eating is a frequent eating problem in early childhood, characterized by food refusal, eating a limited variety of food, an unwillingness to try new food (food neophobia) (Dovey, Staples, Gibson, & Halford, 2008) and aberrant eating behaviors, such as low enjoyment of food, slowness in eating and higher satiety responsiveness (Cardona Cano, Hoek, & Bryant-Waugh, 2015a). The prevalence of picky eating is highest (14–50%) in preschool children (Carruth, Ziegler, Gordon, & Barr, 2004; Dovey et al., 2008; Mascola, Bryson, & Agras, 2010), and declines (7–27%) in later childhood (Mascola et al., 2010; Micali et al., 2011a). Incidence also declines after preschool age (Mascola et al., 2010). The high prevalence and incidence are an indication that picky eating in the preschool age is often part of normal development (Mascola et al., 2010). Indeed many health professionals tend to regard picky eating as a normal phase which eventually passes (Nicholls, Christie, Randall, & Lask, 2001). However, this is in contrast to how many parents experience picky eating, that is, as a major cause of concern (Cerro, Zeunert, Simmer, & Daniels, 2002; Goh & Jacob, 2012; Mascola et al., 2010; Wright, Parkinson, Shipton, & Drewett, 2007). Parents often seek medical help for their child's picky eating (Wright et al., 2007), and express frustration with physicians for dismissing their concerns (McKee, Maher, Deen, & Blank, 2010). Our previous report within the Generation R Study on picky eating trajectories, confirmed that the majority of children's picky eating problems in the preschool age remitted before the age of 6 years (Cardona Cano et al., 2015b). However, we also found a small group of children with persisting picky eating problems who had a lower birth weight, were more often male and from a non-Dutch and low socioeconomic background, compared with nonpicky eaters (Cardona Cano et al., 2015b).

In previous studies, picky eating was associated with higher levels of behavioral, emotional, and pervasive developmental problems in childhood (Jacobi, Schmitz, & Agras, 2008; Micali et al., 2011a; Nicholls et al., 2001) and was suggested to be a precursor for anorexia nervosa (Marchi & Cohen, 1990). The most recent studies concluded

Conflict of interest statement: No conflicts declared.

Published by John Wiley & Sons Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK and 350 Main St, Malden, MA 02148, USA

that picky eating in children of school age must be seen as a risk factor or marker for general psychopathology, rather than a precursor of eating disorders (Jacobi et al., 2008; Micali et al., 2011a). However, picky eating problems are also specifically associated with pervasive developmental disorders (Bandini et al., 2010). The prevalence of picky eating in children with autism was found to be as high as 90% (Sharp et al., 2013) and often present from early age onwards (Emond, Emmett, Steer, & Golding, 2010). In addition, feeding problems and eating disorders are associated with anxiety problems (Galloway, Lee, & Birch, 2003; Swinbourne & Touyz, 2007), and distorted child–parent interactions are suggested to play an important role in feeding problems (Davies et al., 2006).

However, most picky eating studies have some important limitations. First, most studies were limited by their cross-sectional design. Second, they did not differentiate between different trajectories, clustering remitting and persistent picky eaters. Third, a lack of correction for baseline differences makes temporal inferences difficult. Also, most studies did not adjust or only poorly adjusted for confounders. Only a few studies included child, parental, and socioeconomic characteristics (Cardona Cano et al., 2015b; Hafstad, Abebe, Torgersen, & von Soest, 2013; Jacobi et al., 2008). Gender, weight at birth, parental income, maternal ethnicity and age, birth order, higher levels of child emotionality and maternal negative affectivity were found to predict picky eating at later age (Cardona Cano et al., 2015b; Hafstad et al., 2013). Lastly, the majority of studies in the field of eating disorders research rely on one informant to report both the determinant and outcome. However, this practice of using a single informant can lead to spurious associations, that is, information bias (shared method variance) (Ringoot et al., 2015). Typically, mothers' reports are used to assess picky eating as well as emotional and behavioral problems, possibly introducing this type of bias.

It is important to study the course and outcome of picky eating in the general population to determine which children are at high risk for adverse mental health outcomes. Furthermore, this should be evaluated in the context of the child's age. First, we hypothesize that remitting picky eating problems in the preschool age (0–4 years) are part of normal development and are not associated with an increased risk of any adverse mental health problems. Second, we hypothesize that children with persisting picky eating problems have a higher risk for adverse mental health outcomes. In particular, we expect that persistent picky eating is associated with more pervasive developmental problems and anxiety problems. Third, we will test whether late-onset picky eating is associated with emotional or behavioral problems; however, there are insufficient studies to date to formulate a specific hypothesis for this item.

Methods

Study design and population

This study was embedded within the Generation R Study (Jaddoe et al., 2012). The Generation R Study is a prospective population-based cohort in Rotterdam (the Netherlands), that aims to identify environmental and genetic causes of normal and abnormal growth, development, and health from fetal life onward. Pregnant women residing in Rotterdam with an expected delivery date between April 2002 and January 2006 were invited to participate. Written informed consent was obtained from all participants. The Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, approved the study. Information about child and family characteristics was obtained by postal questionnaires filled out by parents, and from the medical records of hospitals, midwives, and community Child Health Centers.

Picky eating was assessed by parental report questionnaires when children were 1.5, 3, and 6 years old. Children who were not assessed for picky eating at any of these time points, or with an inconsistent picky eating pattern, were excluded from the study. Behavioral outcomes were determined using the Teacher Report Form (TRF) when the child was 7 years old (mean 6.7, SD 1.3 years; $N = 4{,}696$). A total of 3,748 (78.8%) children were included in the present study.

Measures

Trajectories of picky eating. Picky eating was assessed with two questions of the Child Behavioral Checklist (CBCL) at age 1.5, 3, and 6 years. The detailed methodology is described elsewhere (Cardona Cano et al., 2015b). In short: at each assessment wave, mothers indicated whether their child 'did not eat well' and 'refused to eat' on a 3-point Likert scale. Based on the sum score of these two items (sum range 2–6) children with a score of sometimes and/or often (score of ≥4) were identified as a picky eater. This method approximates the concept of picky eating as defined by Dovey et al. (2008), with reduced caloric intake, lower variety of foods, higher food fussiness, less enjoyment of food, higher satiety response, and slowness in eating (Cardona Cano et al., 2015b). It is important to note that our method aims to determine picky eating problems in the general population, including (but not limited to) clinically significant 'picky eating disorders'. Four main picky eating trajectory groups were created: never picky eaters – those who were never identified as picky eaters; remitting picky eaters – those who were picky eaters at 1.5 and/or 3 years, but not at 6 years of age; late-onset picky eaters – those who were picky eaters at 6 years of age only; and persistent picky eaters – those who were picky eaters during all assessment waves (1.5, 3, and 6 years). The remaining 242 children with an inconsistent pattern (i.e., children assessed as picky eaters at 1.5 and 6 years, but not at 3 years, and children that were picky eaters at 3 and 6 years, but not at 1.5 years) were excluded from further analysis for two main reasons: the accurate categorization would depend strongly on future follow-up of picky eating status with the possibility that these children would then be categorized into remitting, late-onset, or persistent picky eaters; and analyses of this group revealed a different pattern compared to the other trajectories and did not differ from the never picky eaters (results not shown).

Child's problem behavior. To determine children's problem behavior the Dutch translation of the TRF (Verhulst, van Ende, & Koot, 1997) was used. The TRF is the teacher version of the CBCL, comprising 120 problem items that can be scored on a 3-point Likert scale (i.e., not true, sometimes true, or often true). The TRF has the following six DSM-Oriented Scales: affective problems, anxiety problems, pervasive

developmental problems, attention deficit hyperactivity problems, oppositional defiant problems, and conduct problems. The DSM-Oriented Scale problems were defined using the established borderline clinical cut-offs (Achenbach & Rescorla, 2001); however, linear regression analyses with continuously modeled outcomes are also presented to demonstrate that our findings do not depend on choice of cut-off (see Table S1). Three main groups of problems were formed in line with Micali et al. (2011a): 'emotional problems' consisting of the summed anxiety and affective problems; 'behavioral problems' consisting of attention hyperactivity and oppositional defiant problems; and pervasive developmental problems. Conduct problems were excluded from the behavioral problem group since, at a young age, the diagnosis of conduct disorder has a low prevalence (Ford, Goodman, & Meltzer, 2003). The Dutch TRF has good reliability and validity (Verhulst et al., 1997).

Baseline problem level was assessed by the mother using the CBCL/1.5–5 when the child was 1.5 years of age. The CBCL (Tick, van der Ende, & Verhulst, 2007) is a 99-item parent report questionnaire that assesses child emotional and behavioral problems in a manner similar to the TRF. The Dutch CBCL is reported to have good reliability and validity (Tick et al., 2007). Three main problem groups (as described above) were formed. However, in contrast to the TRF, the CBCL scale scores were used continuously with a higher score indicating more problems. One of the items used to assess picky eating was also present in the emotional problems scale. To avoid bias, this item was excluded from the emotional problems scale score.

Child, parental, and sociodemographic information. Based on previous studies (Cardona Cano et al., 2015b; Dubois, Farmer, Girard, Peterson, & Tatone-Tokuda, 2007; Hafstad et al., 2013; Micali, Simonoff, Stahl, & Treasure, 2011b), we defined several child, family, and socioeconomic characteristics as confounders. Information about child gender, birth weight, and gestational age at birth was obtained from midwife and hospital registries. Maternal ethnicity, family income, and child's birth order were assessed by postal questionnaire. Maternal educational level was coded as high (some college or university education), middle (secondary education), or low (primary education or none). Family income per month was coded as high or middle (above median income >2200 euro), low (1200–2200 euro), or very low (<1200 euro). Birth order was defined as firstborn or later born. Maternal ethnicity was coded as Dutch, Moroccan, Turkish, a combined code (Sur/Ant/Cape) for mothers with a Surinamese, Dutch Antillean or Cape Verdian ethnicity, other Western, and other non-Western. Birth weight is given in grams, gestational age at birth in weeks, and BMI in bodyweight/height^2 (kg/m^2).

Maternal psychiatric symptoms were assessed with the Brief Symptom Inventory (BSI) during pregnancy. The BSI is a validated self-report questionnaire (de Beurs & Zitman, 2006; Derogatis & Melisaratos, 1983) that consists of 53 items scored on a 5-point Likert scale. It assesses a spectrum of psychiatric problems such as anxiety, depression, somatization, and hostility problems. The global severity index is the mean of all subscales and is an appropriate measure of general psychopathology (Derogatis & Melisaratos, 1983; Skeem et al., 2006); that overall mean score was used as a continuous measure, with higher scores indicating more problems.

Statistical analysis

To examine the relationship between picky eating and child's behavioral problems separate logistic regressions were carried out with emotional problems, behavioral problems, and pervasive developmental problems as outcome, and the trajectories of picky eating as the independent variable. The never picky eating group was used as the reference group. First (Model 1), a univariate logistic regression was performed. In the second analysis (Model 2) many of the confounder variables found to predict picky eating (Cardona Cano et al., 2015b; Hafstad et al., 2013) were added. These included gender, weight at birth, maternal ethnicity and income, birth order and maternal psychopathology. Because maternal age did not predict picky eating in our earlier study (Cardona Cano et al., 2015b), it was not included as a confounder in the present study. Finally (Model 3), to address the temporal sequence of the relation, we corrected for baseline child behavioral problems. For this, we also adjusted for maternal-reported child behavioral problem using the CBCL at age 1.5 years. In addition, we reran all analyses using maternal-reported emotional, behavioral, and pervasive developmental outcomes using the CBCL at 6 years of age, to enable comparison with other studies. These additional analyses are presented in Table S2 and are contrasted with analyses using teacher reports in the same sample and also highlight possible informant bias. Except for the dependent variables, missing values were estimated using multiple imputation techniques. As the CBCL data included some missing values (<30% per assessment wave), proportions of trajectories of picky eating were based on multiple imputation if one or more scores were obtained. The pervasive developmental problems group was the only dependent variable (outcome) with missing data (N = 3734, missings n = 14). The presented results are based on pooled estimates of five imputed datasets. Analyses were performed using STATA/SE 12.0.

Results

Study population

General child and family characteristics of the study population are presented in Table 1. The amount of boys and girls was almost equal. The majority of the mothers were of Dutch ethnicity (57.9%), and from a higher socioeconomic status (56.5% higher education). The majority of the children never had picky eating problems (51.4%; n = 1926). Approximately 5.5% (n = 206) were persistent picky eaters, while 31.9% (n = 1197) were remitting picky eaters. These numbers are best estimates (variation <4% of sample) as they are based on imputed data.

Picky eating trajectories and associations with child behavioral problems

No associations were found between remitting, late-onset, persistent picky eaters and never picky eaters, and behavioral problems (Table 2).

In the unadjusted analyses (Model 1), remitting and persistent picky eaters showed more emotional problems than the reference group (Table 3). No difference in emotional problems was found between late-onset picky eaters and never picky eaters. After adjusting for child, family, and sociodemographic variables (Model 2), no differences in emotional problems were found.

Persistent picky eating was associated with more pervasive developmental problems, unadjusted (Model 1; OR = 2.41, 9% CI: 1.37–4.22), and after adjusting for confounders (Model 2; adjusted OR = 2.00, 95% CI: 1.10–3.63) (Table 4). After

Table 1 Population characteristics

	N = 3,748
Child characteristic	
Gender	
Boy, %	50.6
Girl, %	49.4
Birth weight	
Normal weight, %	88.6
Underweight, %	4.3
Overweight, %	7.2
Gestational age at birth	
Aterm, %	87.5
Preterm, %	4.9
Postterm, %	7.6
Parental characteristic	
Age mother [a], in years Mean (SD)	30.9 (5.0)
Age father[a], in years Mean (SD)	33.7 (5.7)
Maternal ethnicity	
Dutch, %	57.9
Moroccan, %	5.5
Turkish, %	8.5
Sur/Ant/Cape[b], %	12.3
Other Western, %	8.1
Other non-Western, %	7.7
Maternal educational level[c]	
High[d], %	56.5
Middle, %	38.8
Low, %	4.7
Family income	
High or middle, %	59.6
Low, %	15.3
Very low, %	25.1
Birth order	
Firstborn, %	55.15
Later born, %	44.85
Smoking during pregnancy	
No, %	75.7
Stopped at pregnancy, %	8.8
Yes, %	15.5
Picky Eating Trajectories[e]	
Never	1,926
Remitting	1,197
Late-onset	177
Persistent	206

[a]At intake
[b]Suriname / Antillean / Cape Verdean
[c]Highest followed
[d]Low = none or primary, middle = secondary school, high = higher vocational education/ university
[e]*n* is based on imputed trajectory groups. The inconsistent group (*n* = 242) was excluded.

additionally adjusting for baseline pervasive developmental problems at 1.5 years, persistent picky eating remained associated with a higher risk of pervasive developmental problems (Model 3; adjusted OR = 1.96, 95% CI: 1.10–3.51; data not in table). None of the other picky eating trajectories were associated with pervasive developmental problems.

Discussion

In this population-based study, we found that persistent picky eating was longitudinally associated with pervasive developmental problems at age 7 years as reported by teachers, even after adjustment of baseline pervasive developmental problems at 1.5 years. Remitting and late-onset picky eating were not associated with adverse mental health outcomes.

In line with our first hypothesis, this study demonstrates that remitting picky eating was not prospectively associated with adverse mental health outcomes. This suggests that remitting picky eating in preschool children can be seen as part of normal development in the general population (Nicholls et al., 2001); a behavior that might be considered as age-appropriate and will eventually remit without behavioral or emotional consequences. This is further strengthened by the fact that our findings are based on a longitudinal design. However, in the present study we did not include somatic health measures and other adverse outcomes cannot be ruled out (Drewett, Corbett, & Wright, 2006; Wright et al., 2007).

Table 2 Longitudinal association[a] between trajectories of picky eaters and borderline behavioral problems

		Behavioral problems	
Picky eating trajectories	*N*[b]	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Never	1,926	Reference	Reference
Remitting	1,197	1.07 (0.74–1.55)	1.03 (0.66–1.61)
Late-onset	177	1.42 (0.64–3.13)	1.05 (0.42–2.61)
Persistent	206	0.92 (0.53–1.60)	0.66 (0.38–1.15)

Behavioral problems; attention-deficit hyperactivity and oppositional defiant problems.
Model 2: adjusted gender, weight at birth, gestational age at birth, maternal ethnicity, household income, birth order, and maternal psychopathology.
[a]Logistic regression.
[b]N is based on imputed trajectory groups. The inconsistent group (*n* = 242) was excluded.

Table 3 Longitudinal association[a] between trajectories of picky eaters and borderline emotional problems

		Emotional problems	
Picky eating trajectories	*N*[b]	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Never	1,926	Reference	Reference
Remitting	1,197	**1.54* (1.01–2.36)**	1.47 (0.92–2.33)
Late-onset	177	1.53 (0.67–3.51)	1.21 (0.56–2.64)
Persistent	206	**1.71* (1.01–2.91)**	1.24 (0.74–2.07)

Emotional problems; anxiety and affective problems.
Model 2: adjusted gender, weight at birth, gestational age at birth, maternal ethnicity, household income, birth order, and maternal psychopathology.
The bold values are given to accentuate that these values are significant findings.
[a]Logistic regression.
[b]*N* is based on imputed trajectory groups. The inconsistent group (*n* = 242) was excluded.
*$p < .05$.

Table 4 Longitudinal association[a] between trajectories of picky eaters and borderline pervasive developmental problems

Picky eating trajectories	N[b]	Pervasive developmental problems	
		Model 1 OR (95% CI)	Model 2 OR (95% CI)
Never	1,920	Ref	Ref
Remitting	1,192	1.02 (0.64–1.61)	0.97 (0.59–1.59)
Late-onset	176	0.70 (0.21–2.24)	0.57 (0.16–2.05)
Persistent	205	**2.41** (1.37–4.22)**	**2.00* (1.10–3.63)**

Model 2: adjusted gender, weight at birth, gestational age at birth, maternal ethnicity, household income, birth order, and maternal psychopathology.
The bold values are given to accentuate that these values are significant findings.
[a]Logistic Regression.
[b]N is based on imputed trajectory groups. The inconsistent group (n = 241) was excluded.
*p < .05; **p < .01.

Second, we hypothesized that persistent picky eating would be prospectively associated with pervasive developmental problems. In line with clinical studies reporting more picky eating problems among children with autism spectrum disorders (ASD) (Bandini et al., 2010), our study suggests that persistent picky eating is also more common in children from the general population with elevated pervasive developmental problems. Importantly, when we corrected for baseline pervasive developmental problems, persistent picky eating remained related to pervasive developmental problems at age 7 years. Thus, the finding cannot be explained by developmental problems early in life. This assessment was based on maternal reports, as parents usually recognize signs of autism in an early stage (Ozonoff et al., 2009). Potentially, picky eating can help to detect pervasive developmental problems earlier, as picky eating in young children is easily noticed by parents. In the study of Emond et al. (2010), parents reported that difficulty in eating is often present in children with autism from infancy (6 months) onwards and persists throughout early childhood; our finding that persistent picky eating can be an early symptom or sign for pervasive developmental problems extends this observation and suggests that in the general population picky eating can precede other pervasive developmental problems symptoms.

However, the median age of the first ASD diagnosis remains older than age 4 years (Centers for Disease Control and Prevention, 2014; Maenner et al., 2013). Persistent picky eating trajectories in our study are based on assessments from 1.5 to 6 years of age, thus a majority of children with ASD would already be diagnosed before persistent picky eating can be defined. However, as the age at which ASD is diagnosed is inversely associated with the number of symptoms observed (Maenner et al., 2013), persisting picky eating can be used to detect ASD in a minority of children in those with less severe or clear symptoms. Future studies are needed to evaluate if a persistent picky eating trajectory can be delineated earlier, that is at age 4–5 years. Since parents often seek medical help for their child's eating behavior (Wright et al., 2007), clinicians should pay attention to children who persist in having picky eating behavior, as these children are at higher risk of pervasive developmental disorders. Some caution is warranted as the CBCL assesses pervasive developmental problems and is not a diagnostic instrument; however, several studies have demonstrated that the CBCL pervasive developmental problem scale can be used to screen for ASD, but has a particularly high specificity in the assessment of pervasive developmental disorders (Ooi, Rescorla, Ang, Woo, & Fung, 2011; Sikora, Hall, Hartley, Gerrard-Morris, & Cagle, 2008; So et al., 2013).

We did not confirm our hypothesis that persistent picky eating was also prospectively associated with anxiety problems. Rather, persistent picky eating was not associated with problems other than pervasive developmental problems. This is in contrast with an earlier report of the ALSPAC study that found strong associations of picky eating with behavioral and emotional problems (Micali et al., 2011b). In our study, the existing association between persistent picky eating and emotional problems disappeared when confounders were controlled for. Also, the present study found lower odds ratios compared with Micali et al. in the UK (2011b). The differences between the two studies might be explained by the design of our study (cross-sectional vs. longitudinal and repeated measures design) and, most importantly by a different informant (a teacher report vs. a mother report) as a measure for outcome. An earlier study showed that, when maternal reports are used for both the determinant and the outcome measure, the associations were strongly inflated (Ringoot et al., 2015). Thus, when mothers report both picky eating and problem behavior of the child, any observed association of picky eating with behavior and emotional problems is prone to reporter bias. Furthermore, mothers who are overconcerned about their child's wellbeing might rate their child's behavior as problematic in general. Our results suggest that the associations of picky eating with emotional and behavior problems may be inflated when mothers' reports of emotional and behavior problems are used (see Table S2). However, others may argue that teachers underreport (which would probably reduce precision) or more often incorrectly report, which would reduce the estimated effect of the associations.

Some caution is required when interpreting these results. First, because the concept of picky eating has not been fully operationalized (Dovey et al., 2008) and the boundaries between picky eating, food neophobia and eating disorders are not yet well defined. Also, in the present study, we defined trajectories of picky eating to differentiate subgroups across time that

might have distinct outcomes. Although we found no association between picky eating and emotional problems, picky eating persisting from early to late childhood might predict eating disorders in adolescence (Marchi & Cohen, 1990) or might be a risk factor for other severe psychopathology. However, this was beyond the scope of the present study and more research is required on this topic.

Our results emphasize the importance of differentiating between trajectories of picky eaters, as picky eating comprises distinct groups with different symptom clusters ranging from mild symptoms to clinical disorders such as Avoidant/Restrictive Food Intake Disorder (ARFID). ARFID can be considered an extreme form of picky eating and is associated with more pervasive developmental disorders compared with other eating disorders in a clinical setting (Nicely, Lane-Loney, Masciulli, Hollenbeak, & Ornstein, 2014). Therefore, we cautiously speculate that persistent picky eaters are at a higher risk for the development of ARFID.

Finally, we tested whether late-onset picky eating was associated with emotional or behavioral problems. Although late-onset picky eating was not longitudinally associated with any adverse mental health outcome, a study by Micali et al. (2011a) found more emotional, behavioral, and pervasive problems, as described above. It is possible that our study was underpowered to detect minor differences, given the relatively small group of late-onset picky eating to find differences when comparing them to children without picky eating problems. In the present study, late-onset picky eaters tended to have more emotional and behavioral problems, but only in the unadjusted models; after correcting for confounders the odds ratios were strongly attenuated. This implies that the observed effect of picky eating behavior in early childhood is partially explained by socioeconomic differences between groups.

Strengths and limitations

This study had several strengths including the large sample size, its population-based longitudinal design and inclusion of a large amount of confounders. Additional strengths are the use of the teacher report (as an independent measurement for child psychopathology) and correction for baseline problems.

Some limitations should also be discussed. First, the TRF reports on the DSM-Oriented Scales and is not equivalent to a DSM diagnosis. Thus, some caution is necessary interpreting these results, more so as the borderline clinical cut-off was used. Second, we had no measurements at 4 and 5 years of age in order to better determine picky eaters with a persistent pattern or late-onset. Also, parents might have adjusted eating regimes to compensate for their child's pickiness (Farrow & Blissett, 2006), resulting in a misclassification of the remitting group. However, maternal reports for the assessment of picky eating have been validated (Carruth & Skinner, 2000). We used 'my child refuses to eat' and 'my child doesn't eat well' to assess picky eating status. However, previous analyses (Cardona Cano et al., 2015b) found that this method correlates well with measures of picky eating, including a lower variety of food, lower caloric intake, more food fussiness, slowness in eating, and lower enjoyment of food. This indicates that our definition is a valid approximation of the concept. Lastly, a small group of picky eaters was excluded from further analysis due to having an inconsistent picky eating pattern. Follow-up of this group is needed to determine whether children in this group should be classified as remitting or persistent picky eaters, and whether picky eating is associated with adverse mental health outcomes.

Clinical implications

Persistent picky eating was found to be an early symptom for pervasive developmental problems, whereas remitting picky eating was not associated with adverse mental health outcomes. We cautiously propose to regard remitting picky eating as part of normal development and, in line with consensus-based professional health guidelines, suggest a watchful waiting approach to picky eating problems in preschool age. However, health professionals should be aware of the possible mental health implications of persisting picky eating and, if necessary, perform additional testing.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Associations between trajectories of picky eaters and continuously modeled child's behavioral problems.

Table S2. Possible informant bias in the association between trajectories of picky eating and problem behavior: using teacher versus mother-reported outcome.

Acknowledgements

The Generation R Study is conducted by the Erasmus University Medical Center Rotterdam in close collaboration with the Erasmus University Rotterdam, School of Law and Faculty of Social Sciences; the Municipal Health Service Rotterdam area, Rotterdam; the Rotterdam Homecare Foundation, Rotterdam; and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR), Rotterdam. We gratefully acknowledge the contribution of the participating pregnant women and their partners, general practitioners, hospitals, midwives, and pharmacies in Rotterdam.

The authors declare that they have no conflicts of interest in relation to this work; L.B. works in ErasmusAGE, a center for aging research across the

life course funded by Nestlé Nutrition (Nestec Ltd.), Metagenics Inc. and AXA. The funders had no role in the design or conduct of this study; collection, management, analysis, or interpretation of the data; and preparation, review, or approval of the manuscript.

Correspondence

Henning Tiemeier, Department of Child and Adolescent Psychiatry and Psychology, Erasmus Medical Center, P.O. Box 2060, 3000 CB Rotterdam, The Netherlands; Email: h.tiemeier@erasmusmc.nl

Key points

- Picky eating is a major concern for many parents, although picky eating problems between 1 and 4 years generally remit. Child outcome studies are needed to evaluate the prognosis of persistent and remitting picky eaters.
- Previous studies associated picky eating with more behavioral, emotional, and pervasive developmental problems in childhood, and characterized it as a symptom for general psychopathology.
- In this study, persistent picky eating, but not late-onset or remitting picky eating, was an early sign for pervasive developmental problems.
- Remitting picky eating was not associated with child behavior problems, suggesting that remitting picky eating is part of normal development.

References

Achenbach, T.M., & Rescorla, L.A. (2001). *Manual for the ASEBA school-age forms & profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.

Bandini, L.G., Anderson, S.E., Curtin, C., Cermak, S., Evans, E.W., Scampini, R., ... & Must, A. (2010). Food selectivity in children with autism spectrum disorders and typically developing children. *Journal of Pediatrics*, *157*, 259–264.

de Beurs, E., & Zitman, F.G. (2006). The Brief Symptom Inventory (BSI): The reliability and validity of a brief alternative of the SCL-90. *Maandblad Geestelijke Volksgezondheid*, *61*, 120–137.

Cardona Cano, S., Hoek, H.W., & Bryant-Waugh, R. (2015a). Picky eating: The current state of research. *Current Opinion in Psychiatry*, *28*, 448–454.

Cardona Cano, S., Tiemeier, H., van Hoeken, D., Tharner, A., Jaddoe, V.W., Hofman, A., ... & Hoek, H.W. (2015b). Trajectories of picky eating during childhood: A general population study. *International Journal of Eating Disorders*, *48*, 570–579.

Carruth, B.R., & Skinner, J.D. (2000). Revisiting the picky eater phenomenon: Neophobic behaviors of young children. *Journal of the American College of Nutrition*, *19*, 771–780.

Carruth, B.R., Ziegler, P.J., Gordon, A., & Barr, S.I. (2004). Prevalence of picky eaters among infants and toddlers and their caregivers' decisions about offering a new food. *Journal of the American Dietetic Association*, *104*, s57–s64.

Centers for Disease Control and Prevention. (2014). Prevalence of autism spectrum disorder among children aged 8 years – Autism and developmental disabilities monitoring network. 11 Sites. United States, 2010. *MMWR Surveillance Summary*, *63*(SS02), 1–21.

Cerro, N., Zeunert, S., Simmer, K.N., & Daniels, L.A. (2002). Eating behaviour of children 1.5–3.5 years born preterm: Parents' perceptions. *Journal of Paediatrics and Child Health*, *38*, 72–78.

Davies, W.H., Satter, E., Berlin, K.S., Sato, A.F., Silverman, A.H., Fischer, E.A., ... & Rudolph, C.D. (2006). Reconceptualizing feeding and feeding disorders in interpersonal context: The case for relational disorder. *Journal of Family Psychology*, *20*, 409–417.

Derogatis, L.R., & Melisaratos, N. (1983). The Brief Symptom Inventory: An introductory report. *Psychological Medicine*, *13*, 595–605.

Dovey, T.M., Staples, P.A., Gibson, E.L., & Halford, J.C. (2008). Food neophobia and 'picky/fussy' eating in children: A review. *Appetite*, *50*, 181–193.

Drewett, R.F., Corbett, S.S., & Wright, C.M. (2006). Physical and emotional development, appetite and body image in adolescents who failed to thrive as infants. *Journal of Child Psychology and Psychiatry*, *47*, 524–531.

Dubois, L., Farmer, A., Girard, M., Peterson, K., & Tatone-Tokuda, F. (2007). Problem eating behaviors related to social factors and body weight in preschool children: A longitudinal study. *International Journal of Behavioral Nutrition and Physical Activity*, *4*, 9.

Emond, A., Emmett, P., Steer, C., & Golding, J. (2010). Feeding symptoms, dietary patterns, and growth in young children with autism spectrum disorders. *Pediatrics*, *126*, e337–e342.

Farrow, C., & Blissett, J. (2006). Maternal cognitions, psychopathologic symptoms, and infant temperament as predictors of early infant feeding problems: A longitudinal study. *International Journal of Eating Disorders*, *39*, 128–134.

Ford, T., Goodman, R., & Meltzer, H. (2003). The British Child and Adolescent Mental Health Survey 1999: The prevalence of DSM-IV disorders. *Journal of the American Academy of Child and Adolescent Psychiatry*, *42*, 1203–1211.

Galloway, A.T., Lee, Y., & Birch, L.L. (2003). Predictors and consequences of food neophobia and pickiness in young girls. *Journal of the American Dietetic Association*, *103*, 692–698.

Goh, D.Y., & Jacob, A. (2012). Perception of picky eating among children in Singapore and its impact on caregivers: A questionnaire survey. *Asia Pacific Family Medicine*, *11*, 5.

Hafstad, G.S., Abebe, D.S., Torgersen, L., & von Soest, T. (2013). Picky eating in preschool children: The predictive role of the child's temperament and mother's negative affectivity. *Eating Behaviors*, *14*, 274–277.

Jacobi, C., Schmitz, G., & Agras, W.S. (2008). Is picky eating an eating disorder? *International Journal of Eating Disorders*, *41*, 626–634.

Jaddoe, V.W., van Duijn, C.M., Franco, O.H., van der Heijden, A.J., van IJzendoorn, M.H., de Jongste, J.C., ... & Hofman, A. (2012). The Generation R Study: Design and cohort update 2012. *European Journal of Epidemiology*, *27*, 739–756.

Maenner, M.J., Schieve, L.A., Rice, C.E., Cunnif, C., Giarelli, E., Kirby, R.S., ... & Durkin, M.S. (2013). Frequency and pattern of documented diagnostic features and the age of autism identification. *Journal of the American Academy of Child & Adolescent Psychiatry*, *52*, 401–413.

Marchi, M., & Cohen, P. (1990). Early childhood eating behaviors and adolescent eating disorders. *Journal of the American Academy of Child and Adolescent Psychiatry*, *29*, 112–117.

Mascola, A.J., Bryson, S.W., & Agras, W.S. (2010). Picky eating during childhood: A longitudinal study to age 11 years. *Eating Behaviors*, *11*, 253–257.

McKee, M.D., Maher, S., Deen, D., & Blank, A.E. (2010). Counseling to prevent obesity among preschool children: Acceptability of a pilot urban primary care intervention. *Annals of Family Medicine*, *8*, 249–255.

Micali, N., Simonoff, E., Elberling, H., Rask, C.U., Olsen, E.M., & Skovgaard, A.M. (2011a). Eating patterns in a population-based sample of children aged 5 to 7 years: Association with psychopathology and parentally perceived impairment. *Journal of Developmental and Behavioral Pediatrics*, *32*, 572–580.

Micali, N., Simonoff, E., Stahl, D., & Treasure, J. (2011b). Maternal eating disorders and infant feeding difficulties: Maternal and child mediators in a longitudinal general population study. *Journal of Child Psychology and Psychiatry*, *52*, 800–807.

Nicely, T.A., Lane-Loney, S., Masciulli, E., Hollenbeak, C.S., & Ornstein, R.M. (2014). Prevalence and characteristics of avoidant/restrictive food intake disorder in a cohort of young patients in day treatment for eating disorders. *Journal of Eating Disorders*, *2*, 21.

Nicholls, D., Christie, D., Randall, L., & Lask, B. (2001). Selective eating: Symptom, disorder or normal variant. *Clinical Child Psychology and Psychiatry*, *6*, 257–270.

Ooi, Y.P., Rescorla, L., Ang, R.P., Woo, B., & Fung, D.S. (2011). Identification of autism spectrum disorders using the Child Behavior Checklist in Singapore. *Journal of Autism and Developmental Disorders*, *41*, 1147–1156.

Ozonoff, S., Young, G.S., Steinfeld, M.B., Hill, M.M., Cook, I., Hutman, T., ... & Sigman, M. (2009). How early do parent concerns predict later autism diagnosis? *Journal of Developmental and Behavioral Pediatrics*, *30*, 367–375.

Ringoot, A.P., Tiemeier, H., Jaddoe, V.W., So, P., Hofman, A., Verhulst, F.C., & Jansen, P.W. (2015). Parental depression and child well-being: Young children's self-reports helped adressing biases in parent reports. *Journal of Clinical Epidemiology*, *62*, 928–939.

Sharp, W.G., Berry, R.C., McCracken, C., Nuhu, N.N., Marvel, E., Saulnier, C.A., ... & Jaquess, D.L. (2013). Feeding problems and nutrient intake in children with autism spectrum disorders: A meta-analysis and comprehensive review of the literature. *Journal of Autism and Developmental Disorders*, *43*, 2159–2173.

Sikora, D.M., Hall, T.A., Hartley, S.L., Gerrard-Morris, A.E., & Cagle, S. (2008). Does parent report of behavior differ across ADOS-G classifications: Analysis of scores from the CBCL and GARS. *Journal of Autism and Developmental Disorders*, *38*, 440–448.

Skeem, J.L., Schubert, C., Odgers, C., Mulvey, E.P., Gardner, W., & Lidz, C. (2006). Psychiatric symptoms and community violence among high-risk patients: A test of the relationship at the weekly level. *Journal of Consulting and Clinical Psychology*, *74*, 967–979.

So, P., Greaves-Lord, K., van der Ende, J., Verhulst, F.C., Rescorla, L., & de Nijs, P.F. (2013). Using the Child Behavior Checklist and the Teacher's Report Form for identification of children with autism spectrum disorders. *Autism: the International Journal of Research and Practice*, *17*, 595–607.

Swinbourne, J.M., & Touyz, S.W. (2007). The co-morbidity of eating disorders and anxiety disorders: A review. *European Eating Disorders Review*, *15*, 253–274.

Tick, N.T., van der Ende, J., & Verhulst, F.C. (2007). Twenty-year trends in emotional and behavioral problems in Dutch children in a changing society. *Acta Psychiatrica Scandinavica*, *116*, 473–482.

Verhulst, F.C., dervan Ende, J., & Koot, H.M. (1997). *Dutch manual for the Teacher's Report Form (TRF)*. Rotterdam: Department of Child and Adolescent Psychiatry, Erasmus University Medical Center – Sophia Children's Hospital.

Wright, C.M., Parkinson, K.N., Shipton, D., & Drewett, R.F. (2007). How do toddler eating problems relate to their eating behavior, food preferences, and growth? *Pediatrics*, *120*, e1069–e1075.

Accepted for publication: 7 December 2015

***Streptococcus pyogenes*: Basic Biology to Clinical Manifestations**
Editors: Joseph J. Ferretti, Dennis L. Stevens, and Vincent A. Fischetti.
Oklahoma City (OK): University of Oklahoma Health Sciences Center; 2016-.
National Center for Biotechnology Information

Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS)

Graziella Orefici, PhD
Istituto Superiore di Sanità, Rome; and Department of Pediatrics,Child and Adolescent Neuropsychiatry, University La Sapienza-Rome
orefici@tiscali.it
Corresponding author.

Francesco Cardona, MD
Department of Pediatrics and Child & Adolescent Neuropsychiatry, University of Rome, 'La Sapienza,' Rome
Francesco.Cardona@uniroma1.it

Carol J. Cox, PhD
Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Biomedical Research Center, Oklahoma City, OK 73104
Carol-Cox@ouhsc.edu

Madeleine W. Cunningham, PhD
Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Biomedical Research Center, Oklahoma City, OK 73104
Madeleine-Cunningham@ouhsc.edu
Corresponding author.

Created: February 10, 2016.

Foreword

The inclusion of a chapter on pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (or PANDAS) is essential to provide a history of the disease and provide current information about its association with *Streptococcus pyogenes* (group A streptococci), tics, obsessive compulsive disorder (OCD) and its relationship to Sydenham chorea (SC), which is the neurologic manifestation of acute rheumatic fever. PANDAS has been misunderstood and confusing to doctors since its discovery, but the original group of the first 50 cases as described by Dr Susan Swedo (Swedo, et al., 1998) has a similarity to Sydenham chorea that distinguishes this initial group from tic and OCD cases. As this chapter will examine, the acute onset is an important feature of these disorders, as are their piano-playing choreiform movements, enuresis, night-time fears, separation anxiety, learning regression, and handwriting disabilities.

The most current literature, which has been recently published in the *Journal of Child and Adolescent Psychopharmacology* (Murphy, et al., 2015b; Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a; Toufexis, et al., 2015; Gerardi, Casadonte, Patel, & Murphy, 2015; Chang, et al., 2015), provides new insight into the clinical phenotype of PANDAS; namely, a subgroup of pediatric acute-onset neuropsychiatric syndrome (PANS), which has been proposed to have multiple etiologies, including those that are genetic and immunologic, and that present either with or without preceding infections, such as with *Streptococcus pyogenes* (Toufexis, et al., 2015). PANS is a subtype of obsessive compulsive disorder (OCD) that presents with an abrupt onset or exacerbation of neuropsychiatric symptoms (Murphy, et al., 2015b), including moderate or severe OCD. Elevated anti-streptococcal antibody titers tended to have higher OCD severity and the

symptoms tended to lead to sudden and severe impairment, due to comorbidities, such as anxiety, behavioral regression, depression, and suicidality. Comorbid tics in PANS were associated with decline in school performance, visuomotor impairment, eating disorders, deterioration of handwriting skills, and lower quality of life, as compared to children without tics (Murphy, et al., 2015b). In addition, clinical evaluation of youth with PANS and PANDAS and recommendations for diagnosis were reported from the 2013 PANS conference held at Stanford University where a group of clinicians and researchers who were academicians with clinical and research interest in PANDAS and PANS (Chang, et al., 2015). PANDAS is clearly a subtype of PANS (Murphy, et al., 2015b; Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a; Chang, et al., 2015) and not all PANS cases have an underlying streptococcal infection—but all PANDAS cases are associated with streptococcal infections, at least temporally.

When these diseases appear, treatment with antibiotics can be successful, and a treatment trial of cefdinir by Murphy and colleagues indicated that therapy with cefdinir, a β lactam antibiotic, provided notable improvements in tic symptoms rated by the Yale Global Tic Severity Scale (YGTSS) and OCD symptoms rated by the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS). However, the differences within the groups as a whole were not significant. β-lactam antibiotics have been proposed to be neuroprotective above and beyond their antibiotic efficacy (Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a). Anti-neuronal autoantibodies against the brain in SC and PANDAS react with brain antigens including dopamine receptors (Cox, et al., 2013; Brimberg, et al., 2012), lysoganglioside (Kirvan, Swedo, Heuser, & Cunningham, 2003; Kirvan, Swedo, Snider, & Cunningham, 2006a), and tubulin (Kirvan, Cox, Swedo, & Cunningham, 2007), as well as the activation of the calcium calmodulin-dependent protein kinase II (CaM KII) in human neuronal cells (Kirvan, Swedo, Heuser, & Cunningham, 2003). Human anti-brain antibodies expressed in Tg mice targeted dopaminergic neurons and signaled the dopamine D2 receptor (D2R) (Cox, et al., 2013). Evidence strongly suggests that human anti-brain autoantibodies induced by *Streptococcus pyogenes* infections target the dopamine receptors (Cox, et al., 2013; Brimberg, et al., 2012) and that animal models immunized with the *S. pyogenes* antigen develop obsessive behaviors and movement problems, along with antibodies that react with the dopamine receptors and signal the CaMKII, similar to antibodies found in humans with SC and PANDAS (Brimberg, et al., 2012; Lotan, et al., 2014a).

Introduction and Background

In the last half of the 1990s, a group of clinical researchers, including Swedo et al. (Swedo, et al., 1998; Snider, et al., 2002) at the National Institutes of Mental Health (NIMH) described a subgroup of children who presented with obsessive-compulsive disorder (OCD) and/or tic disorders following an infectious illness, in particular after streptococcal infections, and proposed the term pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) for this subgroup (Swedo, et al., 1998).

The background information for this proposal stems from different sources: some anecdotal reports on the relationship between OCD or tic symptoms and infectious illness (Selling, 1929; von Economo, 1931; Kondo & Kabasawa, 1978; Kiessling, Marcotte, & Culpepper, 1993); the observation of OCD, as well as tic symptoms, in patients with Sydenham chorea (SC) (Langlois & Force, 1965; Kerbeshian, Burd, & Pettit, 1990; Swedo, et al., 1989); and the observation of a fluctuating, infectious-related course of OCD in some patients without choreoathetoid movements of SC (Allen, Leonard, & Swedo, 1995; Swedo, 1994).

The original working criteria established by the NIMH group for the diagnosis of PANDAS included: 1) the presence of OCD and/or a tic disorder; 2) a pediatric onset; 3) an episodic course of symptom severity; 4) an association with streptococcal infections; 5) an association with neurological abnormalities, including piano-playing choreiform movements

of the fingers and toes, which suggests that PANDAS may be similar to SC. Moreover, besides these core features, the first 50 cases described in the original series showed emotional lability (66%), deteriorated school performance (60%), personality changes (54%), separation anxiety (46%), nightmares (18%), bedtime rituals (50%), deterioration in handwriting (36%), oppositional behaviors (32%), and motoric hyperactivity (50%), as seen in Table 1.

During the following years, the concept of PANDAS has become very popular and at the same time has sparked a heated debate among researchers and clinicians. To date, a large number of studies on different aspects of PANDAS have been published, as well as some comprehensive and recent reviews (Murphy, 2013; Macerollo & Martino, 2013).

Several researchers have examined two main critical aspects of PANDAS: the difficulty in establishing a tight link between the inciting streptococcal infection/exposure and the onset/recrudescence of OCD or tic symptoms, and the lack of reliable biological markers. These difficulties have led to a recent revision of the diagnostic criteria and to the proposal of a new clinical entity, the pediatric acute-onset neuropsychiatric syndromes (PANS), in which the key clinical feature is ‘‘acute and dramatic symptom onset.’’ There are some changes in the presenting symptoms (with special relevance to OCD and anorexia, and loss of prominence of tics) without any reference to their relationship with streptococcal infections (Swedo, Leckman, & Rose, 2012), although PANDAS would be included under a broader PANS group (Figure 2).

In this chapter, we will review some clinical, microbiological, and immunological aspects of PANDAS. Because tics and OCD symptoms are the main clinical features of PANDAS, a short review on their prevalence, appearance, and natural history will be provided first.

A brief review of tic disorders

Tics are rapid, recurrent, non-rhythmic, and stereotyped movements or vocalizations that can be simple or complex; they are usually suggestible, may be preceded by premonitory urges, and may be suppressed voluntarily. The last edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) categorizes tic disorders in three main groups, based on the length of the disorder (a duration of more or less than 1 year) and on the signs of symptoms (motor or phonic): “provisional tic disorder,” with motor or phonic tics that last less than 1 year); “persistent tic disorder,” with motor or phonic tics that last more than 1 year; and “Tourette’s disorder” (or TS), with motor and phonic tics that last more than 1 year.

Tics are considered the most prevalent movement disorder in childhood, even if their exact prevalence in the general population is unknown. In fact, many cases of tics don’t come to clinical attention, probably because of mild symptoms, they cause little functional or psychosocial impairment, or that the tics don’t cause parental concern in parents. This is true for all the clinical forms of tics, including the chronic ones. Moreover, the epidemiological studies on the incidence and the prevalence of tic disorders are biased by different factors, such as sampling methods, sample sizes, rate of subject participation, assessment methods, and diagnostic thresholds used to define cases. With these cautions, the prevalence of transient tics in school-age children is estimated to be from 11 to 20% (Snider, et al., 2002; Cubo, et al., 2011; Kurlan, et al., 2001; Linazasoro, Van Blercom, & de Zárate, 2006), while the prevalence of TS in school-age children is likely to fall somewhere between 5 and 7 cases per 1,000. For all tic disorders, there is a clear male prevalence, with the male-to-female ratio ranging from 2 to 1 to as high as 3.5 to 1.

Tic disorders are mainly a childhood and adolescent disturbance. In persistent or chronic forms, the onset of tics occurs between 2 and 7 years; the worst period of tic expression usually peaks around pre-adolescence (9–12 years); then there is a phase of stabilization and attenuation of symptoms during adolescence and early adulthood. Some studies report that more than around 40% of the TS children have no more tics during adult life; another 40% have minimal or mild tics that cause no interference in their lives; and only 20% continue to show moderate or even severe symptoms.

From a symptomatic point of view, in the majority of cases, the first tics are motor tics, eye tics (Martino, Cavanna, Robertson, & Orth, 2012) or facial movements. However, in some cases, vocal tics (shouts or vocalizations) and other motor tics (arm jerking, trunk spasms or other more complex movements) can be the first sign of the disorder, and can often pose problems of differential diagnosis with other movement disorders, particularly if their appearance is abrupt.

Typically, the onset of symptoms is sub-acute: the tics tend to slowly increase in frequency and intensity during months or years, and parents often have difficulties in recalling a precise date when tics began. The tics rarely appear acutely (seemingly overnight), though when this occurs, their intensity and frequency are very high from the beginning. This can often cause a great deal of anxiety in parents, who turn to emergency departments for consultations.

The severity of tic symptoms generally varies over time, and the waxing and waning course of chronic tic disorders is an universally recognized feature. Beside the natural course of chronic tic disorders, as described before, this variation of severity occurs over a period of weeks to months, and can even occur over the same day. A relationship between tics and environmental contingencies or emotional factors has been proposed: in particular, psycho-social stress (Lin, et al., 2010) or abnormalities in the cortisol circadian rhythm (Corbett, Mendoza, Baym, Bunge, & Levine, 2008) have been reported to affect the modulation of tic severity, but for many tics, it is difficult to establish a link between these fluctuations and a specific situation or environmental cause.

However, in the absence of a general agreement on the cut-off that defines a true exacerbation from the “normal” fluctuation of symptoms, the notion of tic exacerbation is quite vague. In a few studies, including those on some PANDAS patients (Lin, et al., 2002; Luo, et al., 2004; Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008; Leckman, et al., 2011; Martino, et al., 2011), tic exacerbation thresholds that incorporated the change score from the previous month and the current symptom score were estimated by using state-of-the-art bootstrap methods. Such methods agreed with the judgement of clinical experts. A seven-points increase of the global score (without impairment score) at the Yale Global Tic Severity Scale (YGTSS) has been considered in some studies to be a reliable cut-off that defines an exacerbation. Unfortunately, in an attempt to provide such a crucial definition, little help has come from the psychopharmacological studies on anti-tic medications, in which the definition of responsiveness or refractoriness to a single drug is quite vague.

Brief Review of OCD

OCD is a disorder with a lifetime prevalence of 1–3% in the general population. The disorder is characterized by the presence of obsession (i.e., recurrent and persistent thoughts, urges, or images that are experienced as intrusive and unwanted) and/or compulsions (i.e., repetitive behaviors or mental acts that an individual feels driven to perform in response to an obsession or according to rules that must be rigidly applied).

In children and adolescents, OCD shows a bimodal age of onset: the first one peaks around 8-12 years, which is the so-called “early onset OCD” that is characterized by a frequent comorbidity with tics/TS, a male prevalence (nearly 25% of males with the disorder have an onset before 10 years), a different content of obsessions/compulsions or the presence of compulsions without obsessions, a different and reduced response to the pharmacological treatment, and a poorer prognosis. The second one peaks after puberty and is characterized by a slight female prevalence, a content of obsessions/compulsions similar to those seen in adulthood, a good response to treatment, and a better prognosis.

The bimodal age of the onset of OCD suggests some different etiological factors. In particular, patients with an early onset are likely to have a stronger genetic or biological component than patients with a late onset. In particular, family studies revealed higher familial aggregation among relatives of early-onset subjects (Geller, 2006).

The abrupt overnight onset of initial OCD symptoms reported in cases of PANDAS or PANS is characteristic of these disorders. However, in typical OCD, the onset of symptoms is more gradual. OCD is described as a chronic disorder with a fluctuation of symptoms (waxing and waning), even if an episodic course is described in some cases. Notably, in some longitudinal studies when both tic and OCD symptoms were present, there was a significant degree of covariation (Lin, et al., 2010; Luo, et al., 2004; Leckman, et al., 2011).

Pathophysiology of tics, TS, and OCD

In the last twenty years, a growing number of studies have investigated the neural and pathophysiological underpinnings of tics, TS, and OCD. Given the known role of the basal ganglia in motor control and in other movement disorders, these structures have been the primary focus of many studies that have investigated the neurobiology of these disorders.

The basal ganglia comprise a set of subcortical nuclei that include the caudate nucleus, putamen, globus pallidus, subthalamic nucleus, and substantia nigra. Their functional connections to several cortical regions have led to the conceptualization of the corticostriatal-thalamo-cortical (CSTC) circuits; namely, multiple parallel, segregated feedback circuits with outputs from striatum that target primary motor areas, and specific pre-motor and prefrontal cortical areas. The primary function of the CSTC circuits is to control and select goal-directed motor, cognitive and motivational behavior. Further, CSTC circuits are involved in inhibitory control (Aron, Behrens, Smith, Frank, & Poldrack, 2007) and habit formation (Graybiel, 2008).

Even if a clear explanation for the occurrence of tics hasn’t yet emerged, the most compelling finding so far is increased supplementary motor area (SMA) activity just prior to tic onset (Hampson, Tokoglu, King, Constable, & Leckman, 2009), which suggests that the SMA plays a role in the sensory phenomena that precede the execution of tics (premonitory urges).

Structural MRI studies have revealed reduced caudate volumes in children and adults with TS (Peterson, et al., 1993), with a negative correlation between caudate volume in childhood and the severity of symptoms later in life (Bloch, Leckman, Zhu, & Peterson, 2005).

The sensorimotor cortices are intuitive candidate cortical areas for investigation in TS due to the motor nature of tics and the sensory disturbances that frequently accompany them. MRI studies that have measured cortical thickness and grey matter volume in sensorimotor cortices in TS are limited in number, but they have provided consistent results. In particular, Sowell et al. (2008) found cortical thinning in sensorimotor cortex, along with

other regions (ventral frontal cortex, dorsal parietal cortex), in children with pure TS (Sowell, et al., 2008).

To date, neuroimaging studies of TS (and especially functional MRI studies) are limited and many study results are inconsistent. These inconsistencies could be due to the large heterogeneities in the samples that have been studied.

With regard to OCD, in recent years, a growing number of studies have identified the CSTC circuits as centrally implicated in the pathophysiology of the disorder (Saxena & Rauch, 2000). Most especially, the limbic or orbitofrontal circuit (orbitofrontal cortex, anterior cingulate cortex, and caudate nucleus) has consistently been shown to be involved in OCD symptoms. Imaging research (which concerns structural, functional, and connectivity investigations) have shown a particularly high degree of concordance across the studies and have led to the conceptualization of the CSTC model of OCD. This model has received further support through neuropsychological and treatment studies (Menzies, et al., 2008).

PANDAS phenotype

Most of the studies published on PANDAS investigated a possible relationship between the onset or recrudescence of symptoms—mainly tics—and clinical or biological signs of *S. pyogenes* infections in different populations (such as tics or TS patients) observed in cross-sectional or longitudinal ways; furthermore, other research was conducted to search for possible markers of the proposed autoimmune process. In most of these studies, the definition of PANDAS cases was made after a retrospective review of clinical records. As a matter of fact, up until now, little attention has been paid to the clinical signs that could differentiate PANDAS from tics, TS, or OCD patients, besides the inclusion criteria. To date, four studies reported data that was useful for a comparison.

In 2008, Kurlan et al. compared 40 PANDAS patients with 40 OCD or chronic tic disorder matched subjects, followed for a period of 24 months. From a clinical point of view, the groups were comparable, with the exceptions that the PANDAS case subjects seemed to more often have a psychiatric diagnosis other than tic disorder or OCD (Leckman, et al., 2011).

In 2010, Bernstein et al. compared 21 PANDAS children with a control group of 19 children with non-PANDAS OCD, with respect to ancillary symptoms, types of obsessions and compulsions, symptom severity, and co-morbid DSM-IV diagnoses. Both groups were retrospectively defined by reviewing their medical records. PANDAS children were significantly more likely to present with separation anxiety, urinary urgency, hyperactivity, impulsivity, deterioration in handwriting, and decline in school performance during their initial episode of neuropsychiatric illness, as compared with children with non-PANDAS OCD. The total number of tics was higher and the vocal tics were more severe in PANDAS children. Separation anxiety disorder and social phobia were more prevalent in non-PANDAS OCD children, and children with non-PANDAS OCD were significantly more likely to include others in their rituals (Bernstein, Victor, Pipal, & Williams, 2010).

In 2011, Leckman et al. conducted a multi-centric longitudinal study that compared 31 children who met the criteria for PANDAS with 53 TS or OCD non-PANDAS subjects. Both groups showed a similar severity of symptoms and a similar rate of tic or OCD symptom exacerbations. Only a quarter of exacerbations identified in the PANDAS group were associated with a simultaneous sudden acute onset with increase in anxiety, depression, and/or attention deficit and hyperactivity disorder (ADHD) symptoms (Martino, et al., 2011).

Finally, in 2012, Murphy et al. examined 109 children showing tics, TS, or OCD; the assignment to the PANDAS (41 subjects) or the non-PANDAS (68 subjects) group was based on the presence of PANDAS operational criteria, as developed by Swedo et al. in 1998. The clinical assessment didn't show any clinical difference between groups. Children classified as PANDAS had a high rate of dramatic symptoms onset and clumsiness; however, it should be noted that these are two of five criteria for PANDAS (Murphy, Storch, Lewin, Edge, & Goodman, 2012). (A subset of the non-PANDAS group would have met the criteria for PANS, but did not have the temporal association normally found with streptococcal infections).

Although these studies did not provide overwhelming evidence for the existence of ''PANDAS-specific'' phenomenological features, PANDAS is similar to SC and shows the choreiform movements of fingers and toes that may not have been observed in earlier comparative studies. The presence of these movements may constitute a red flag that signals a PANDAS diagnosis. However, a lack of choreiform movements does not exclude PANDAS, and these signs have to be regarded with caution, as they are also present in typically developing children and in children with other childhood disorders, such as ADHD and developmental coordination disorders.

PANDAS vs. Sydenham chorea

Sydenham chorea (SC) has provided a model for the conceptualization of PANDAS, and Swedo and the NIH group have shown that PANDAS is similar to SC and is characterized by choreiform piano-playing movements of the fingers and toes (Snider & Swedo, 2004). Both PANDAS and SC have immunological similarities, as a later section will show. Human sera studies in immunoassays suggest that human dopamine D2 receptor (D2R) is the target of autoantibodies that are produced in both SC and PANDAS (Cox, et al., 2013). Tics or OCD symptoms are often present in the early prodromal phases of SC, with choreoathetoid movements following. Deterioration in handwriting and irritability, often seen in early phases of SC, are also accompanying symptoms of PANDAS. However, the clinical course of PANDAS vs. SC is different: SC often is a monophasic illness, even if recurrent or persistent cases have been described (Cardoso, Vargas, Oliveira, Guerra, & Amaral, 1999). In contrast, the recurrence of tics or OCD symptoms after streptococcal (or other) infections has been one of the basic criteria for a PANDAS diagnosis. Finally, echocardiographic abnormalities (valvular incompetencies) are present in nearly 80% of SC patients, and they constitute a feature that often leads to the right diagnosis in the anamnestic or clinical doubtful cases. Conversely, PANDAS patients don't generally show signs of cardiac involvement (Snider, Sachdev, MacKaronis, St Peter, & Swedo, 2004); however, minimal echocardiographic abnormalities have been described in some patients with *S. pyogenes* related tics disorders (Cardona, et al., 2007) and in some children with PANDAS (Segarra & Murphy, 2008).

Association of *S. pyogenes* infections with tics/OCD

To fully understand the different studies published on the relationship between *S. pyogenes* and some neuropsychiatric disorders (such as tics, TS, or OCD) is not an easy task and has often raised more questions than answers. Some of these studies strongly support this association (Lin, et al., 2010; Murphy, Storch, Lewin, Edge, & Goodman, 2012; Cardona & Orefici, 2001; Leslie, et al., 2008; Mell, Davis, & Owens, 2005; Murphy & Pichichero, 2002) while others firmly deny it (Macerollo & Martino, 2013; Luo, et al., 2004; Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008; Leckman, et al., 2011), but all agree on the necessity of more research for a definitive demonstration of the existence/absence of this relationship and of the basic cellular and immune mechanisms involved. Table 2 provides a list of these studies.

The difficulty of having definitive results partially reflects the complexity and the possibly multifactorial nature of neuropsychiatric disorders. Animal models used (generally mice and rats) are not completely satisfactory and should be interpreted with caution; since humans are the only natural reservoir for *S. pyogenes* , these animal models may not exactly reproduce a human disorder. However, animal models in mice and rats have been very instructive in PANDAS and SC, with several studies indicating that immunization of rats and mice leads to behavioral alterations similar to SC and PANDAS (Brimberg, et al., 2012; Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004). In addition, passive transfer of anti-streptococcal antibodies to naïve rats or mice led to behavioral changes (Lotan, et al., 2014a; Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004).

After reviewing human studies, it is clear that many are looking at heterogeneous human populations; as a result, it may not be appropriate to analyze these data, which can be misleading when considered together. In some cases, the population studied is not in prepubertal age (Bencivenga, Johnson, & Kaplan, 2009; Schrag, et al., 2009; Morshed, et al., 2001); in others, there is a larger than usual presence of females; other studies are enhanced with more tic spectrum patients than acute onset OCD patients (Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008; Leckman, et al., 2011), which indicates some bias in the selection of patients; in others the original population with tics/OCD described by Swedo for PANDAS (Garvey, Giedd, & Swedo, 1998) is enlarged by the inclusion of ADHD cases (Swedo, et al., 1998). In some studies, the involvement of *S. pyogenes* is investigated only through the detection of antibodies against one or two *S. pyogenes* antigens, without looking for the presence of the bacterium. It is important to note these differences when considering the results.

Streptococcus pyogenes is known to be a complex organism with a vast repertoire of virulence factors produced for bacterial adhesion and invasion, for evasion of phagocytosis, or for modulating host defenses (Sjöholm, Karlsson, Linder, & Malmström, 2014). It is able to change over time by the acquisition of new mechanisms and structures to avoid host defenses (Bryant, et al., 2014; Hertzén E. , et al., 2012) or to have long intracellular persistence (Wang, Li, Southern, & Cleary, 2006; Hertzén E. , et al., 2010; Kaplan, Gastanaduy, & Huwe, 1981; Kaplan, Chhatwal, & Rohde, 2006). The same strain may cause suppurative diseases, non-suppurative sequelae, toxic shock, or may colonize carriers without provoking an infection.

Many human studies have focused more on clinical findings or on host immune responses to specific antigens than on the bacterium itself, and as a result, only the presence or absence of *S. pyogenes* is reported. Microbiologists often act as simple blinded operators, and no information is reported on the methods used for taking specimens or isolating the strain. This result is evident from differences in the percentage of *S. pyogenes* positive samples found in different studies, or in multicenter studies, by the different microbiology results between participating centers.

Unfortunately, while a *S. pyogenes* -positive sample demonstrates the real presence of the bacterium in the infected or carrier host throat, a negative swab is not really informative; in particular, a swab that contains just a few colonies may be not detected as positive by routine laboratory methods. Since a true infection may be accompanied by very few colonies in the throat swab (Johnson, Kurlan, Leckman, & Kaplan, 2010), the percentage of positive samples found largely depends on the methods used to detect them. In our experience, at the time of their first visit for tics, children rarely present clinical signs of pharyngitis, and a percentage of them often carry even fewer than 10 colonies/plate in their throat swabs. That requires great care in taking the swab, to avoid *S. pyogenes* being covered by a too large amount of saprophytic flora present in the sample, and the use of selective media and careful

methods in processing the swab, even different from those routinely in use for pharyngitis. If samples of children with tics but without clinical signs of pharyngitis are processed in the same way as those of children with sore throats, there are strong possibilities that the result will be below the threshold of detection and that the culture will be considered negative. *S. pyogenes* colonizing tonsillar criptae have been found in greater than 30% of children undergoing tonsillectomy for recurrent *S. pyogenes* tonsillopharyngitis and in children with no history of previous frequent infections who underwent surgery for different reasons, who were selected as healthy controls (Roberts, et al., 2012). These percentages are higher than all those found in PANDAS studies or in carriers, which indicates that even with accurate methods, many positive subjects are not detected; but, in the studies on the association between *S. pyogenes* infection/exposure and neuropsychiatric symptoms, false negative results can result in the true differences in positivity between cases and controls being entirely undetectable.

A second important point to consider is that *S. pyogenes* is not only an extracellular pathogen, but can survive to phagocytosis inside the cells. M-protein–expressing *S. pyogenes* strains can survive after phagocytosis by human neutrophils (Staali, Mörgelin, Björck, & Tapper, 2003) and the surface M-anchored protein has been identified as the pivotal factor that affects the phagosomal maturation in macrophages (Hertzén, et al., 2012; Hertzén, et al., 2010). During the intracellular phase, the expression of many genes—namely, the majority of those involved in cell wall synthesis and energy production—is significantly altered; after a replicative phase, *S. pyogenes* egress after having destroyed the host cells, and are fit to infect new cells and may persist in the throat for a long time, releasing any type of streptococcal antigens and causing the permanence of high-antibody titers even in the absence of overt disease. This may also account for the intermittent presence of the same serotype in the throat of tic patients and for the high percentage of carriers seen in some studies after treatment (Pichichero, et al., 1999). Host cells are a useful niche to escape many antibiotic drugs used against *S. pyogenes* (such as penicillin, for instance), and microorganisms during carriage or infection are selected on the basis of their capacity to enter and survive the treatment (Kaplan, Gastanaduy, & Huwe, 1981; Sela, Neeman, Keller, & Barzilai, 2000; Park, Francis, Yu, & Cleary, 2003).

The last point to consider concerns the level of antibodies (Anti Streptolysin O, or ASLO, and Anti DnaseB, or ADB) used to indicate the infection. In several cases (Johnson, Kurlan, Leckman, & Kaplan, 2010), a true infection causes a moderate increase in this level, though it remains below the threshold considered the upper limit of normal titers (ULN) and, in the absence of an accurate monitoring of the subject, it is disregarded. On the other hand, choosing a too low level of antibodies as the ULN may result in undetectable differences between cases and controls.

All these considerations show the difficulties in studying the association between *S. pyogenes* infection/exposure and neuropsychiatric symptoms and in explaining why, despite the large number of studies published, the demonstration of PANDAS following the original definition given by Swedo et al. (Garvey, Giedd, & Swedo, 1998) is debated. Initially, studies of the association of SC with streptococcal infection determined that chorea can occur anywhere from several weeks to nine months following a streptococcal infection (Cardoso, Vargas, Oliveira, Guerra, & Amaral, 1999).

Epidemiologic evidence for some *S. pyogenes* involvement in tic disorders comes from administrative data from a health maintenance organization in the Seattle area where 144 new cases of TS and OCD/tics were matched with 609 controls (Mell, Davis, & Owens, 2005). A significant association was found (13-fold more for TS) with prior *S. pyogenes* infections diagnosed either 3 months or 1 year before the onset of disturbance. The presence

of multiple *S. pyogenes* infections in the previous 12 months significantly increased the risk of TS, which indicates a sort of threshold of anti-streptococcal antibodies to be reached before the onset of the manifestation.

A strong association with a prior *S. pyogenes* infection was also found in an USA national health insurance study where 479 cases of OCD, tics, and TS were matched with 3647 controls, but the results of the study did not include a rigorous ascertainment of tic or OCD from consistent diagnostic criteria (Leslie, et al., 2008).

In a retrospective study, 80 consecutive children (15–17 years) were investigated through a structured clinical interview to establish if infection and an abrupt onset of symptoms could be identified; 53% of the patients reported such an abrupt onset and 21% of this subset had it within 6 weeks of infection (Singer, Giuliano, Zimmerman, & Walkup, 2000). It was suggested that in some cases, the abrupt onset might have been exaggerated by a biased memory of parents.

In the UK, Schrag et al. (Schrag, et al., 2009), on the contrary, were unable to support an association with *S. pyogenes* infection through a database analysis. However, in this case, both the mean age of their patients (16 years instead of prepubertal age) and the lack of established analysis parameters to determine the time between streptococcal infection and onset of tics/OCD (up to 3 years) could have accounted for the temporal association and thus the different results.

The age of the population studied is very important: in a large study that included 3006 school children, the percentage with motor or vocal tics was 22.3% for preschool children, 7.8% for elementary school, and 3.4% for secondary school, with the male/female ratio of 3.8/1 in the elementary school group and 6.1/1 for the secondary school group (Gadow, Nolan, Sprafkin, & Schwartz, 2002). Therefore, results using patients not in prepubertal age or with a too low rate of male/female subjects need to be carefully evaluated, since the population may be different from that of other studies.

Many of the studies performed on tic disorders are cross-sectional: clinical, serological, and microbiological data are collected at the time of the neuropsychiatric manifestation, onset, or increase of tics /OCD, but are not monitored over the time. This type of study may give useful insights (and they often do) to demonstrate that patients with tics differ from healthy normal people for a higher exposure to *S. pyogenes* antigens, but these studies are inadequate to demonstrate the overall PANDAS concept (*S. pyogenes* clinical infection with the subsequent or antecedent rise of antibodies associated with onset /recrudescence of symptoms in a pediatric population), which can be only assessed through sequential observations.

In a case-control study performed between March 1996 and November 1998, 150 children were examined for sudden onset, recrudescence, or protracted duration of their tic disorders (Cardona & Orefici, 2001). The controls were 150 healthy children without tics. In this study, 38% of the cases (in comparison with 2% of the controls had ASLO titers higher than 500 IU with a mean ASLO titer of 434 IU, in comparison with 155 IU in controls ($p < 0.01$). Moreover, 58% had a family history of tics and frequent upper respiratory tract infections and 17% had a throat swab positive for *S. pyogenes* . At the time of the visit, none of the patients had clinical evidence of pharyngitis and, if analyzed by standard methods, the rate of *S. pyogenes* positive specimens was very low, with only a few colonies per plate; as a result, an old pour plate method was used (Taranta & Moody, 1971) that gave better results than the one routinely used for pharyngitis (Johnson, et al., 1997), which made it easier to detect and isolate every single colony (see Figures 1A and B).

In several studies, the increase of the immune response to those streptococcal antigens (ASLO and ADB) generally used as indicators of *S. pyogenes* infection is considered to be evidence of infection. It is interesting that when studying more or less the same matter (i.e., the possible involvement of *S. pyogenes* in tic disorders), the results and the conclusions produced by different groups were different. Loiselle et al. (Loiselle, Wendlandt, Rohde, & Singer, 2003) were unable to confirm differences in ASLO, anti-DNase B, and anti-basal ganglia (ABGA) titers in 41 children with TS and ADHD and 38 controls, even if ASLO titers were significantly higher in children with ADHD, as compared to the non-ADHD group.

In another study, sera from 30 children with PANDAS, 30 with TS, and 30 controls were examined for ABGA. Though more antibody positivity and a higher immunofluorescence against human striatum samples were found in samples from children with PANDAS and TS in comparison with controls, no statistically significant association was found between immunofluorescent reactivity and the diagnosis (Morris, Pardo-Villamizar, Gause, & Singer, 2009).

In a cross-sectional study on 100 British patients with TS (50% children), Church et al. (Church, Dale, Lees, Giovannoni, & Robertson, 2003) found that 64% of children and 68% of adults had a significantly higher ASLO titer, as compared to 15% of children with recent uncomplicated streptococcal pharyngitis, but no attempts to study the presence of *S. pyogenes* was reported.

A higher ASLO titer in comparison with controls was also found in an American study on 81 patients with TS (Morshed, et al., 2001); antistreptococcal higher titers (ASLO, ADB, anti-M12 and anti-M19) were also found in a sample of German patients (Corbett, Mendoza, Baym, Bunge, & Levine, 2008).

In a cross-sectional case-control study on 69 Italian TS children, Martino and Rizzo et al. found that 59% of the patients had significantly high ASLO titers (> 400 IU), in comparison with 19% of the controls (Martino, et al., 2011); a high percentage of children with high ABGA was also found. Finally in another cross-sectional study (Geller, 2006), the mean ASLO titers (246 IU vs 125 IU, $p>0.01$), the number of positive *S. pyogenes* throat cultures (8%, compared with 2%; $p=0.009$) and the positivity of anti-basal ganglia antibodies (ABGA) (23% in comparison with 8%; $p >0.001$) were significantly higher in TS patients than in controls, but no difference in ASLO titers was detected between ABGA-positive and ABGA-negative children

These are just some examples that show the elusiveness of the matter and the difficulty in reaching firm conclusions from published studies. Retrospective studies from health maintenance data may be a good source of information, even if they are not wholly collected with an ad hoc questionnaire.

Longitudinal studies to demonstrate the relationship between new infections and recrudescences of symptoms: the problem of carriers

The main difficulty in demonstrating the concept of PANDAS is showing the association of a new *S. pyogenes* infection with a sudden exacerbation of tics or OCD. In fact, this association can't be demonstrated by cross-sectional studies and requires monitoring the microbiological and serological parameters of patients and controls for a long time, both to detect infections and to establish that these infections are new and are caused by *S. pyogenes* types that were not previously present. Longitudinal studies (in particular, those that follow patients from the initial tic manifestation) are informative, but are much more difficult to perform, and only a limited number of patients can be followed.

A prospective microbiology study analyzed the data of 160 children with tics enrolled in two clinical studies with identical protocols (Johnson, Kurlan, Leckman, & Kaplan, 2010). The goal of the study was to provide a description of the long-term kinetics of the immune response in patients with tics, either with *S. pyogenes* infections or who were carriers. The presence of *S. pyogenes* in the throat samples, M types, and the variation of antibody responses to ASLO and ADB were reported without specific attempts to correlate *S. pyogenes* infection with the concurrent recrudescences of tics. Pharyngeal swabs were taken every month for 120 weeks, and blood was examined approximately every three months. The study was very accurate, and shows that a true rise in anti-streptococcal antibodies may occur at levels below the ULN used in many studies. It stresses the need to use at least two antibodies (ASLO and ADB) for the diagnosis of new infection (since sometimes only a single antibody titer increases) and clarifies that a true infection with a significant antibody response may be associated with cultures with <10 colonies per plate, which demonstrates that many infections would be unidentified without a longitudinal observation. Obviously, only a limited number of example cases were reported; therefore, not much detailed information is available on the total population studied (number of *S. pyogenes* positive children, total number of infections seen during the entire study, characteristics of all *S. pyogenes* isolated) to enable comparisons to other large studies.

The conclusions of this research are convincing. Unfortunately, the same accuracy is difficult to reach in clinical practice, where the clinician is not necessarily informed on the basal level of anti-streptococcal antibody titers before the onset of the clinical manifestation or on the previous microbiology of *S. pyogenes* in the throat.

S. pyogenes throat infections and carriage are rather common in school-age children: several previous studies (Kaplan E. L., 1980) and in a recent meta-analysis (Shaikh, Leonard, & Martin, 2010) found 37% *S. pyogenes* positivity in children with sore throat and a 12% positivity in healthy children. In the Johnson study (Johnson, Kurlan, Leckman, & Kaplan, 2010) and in other studies, patients bearing the same *emm* type for a long time without changes in antibody levels were considered “carriers,” since the presence of *S. pyogenes* in these cases could not be considered a new infection. Nevertheless, the status of a “carrier” is difficult to define: historically, *carriage* was defined as the prolonged permanence of *S. pyogenes* in the pharynx without evidence of immune or inflammatory response (Kaplan, 1980; Tanz & Shulman, 2007). Carriage is a very complex phenomenon, in which the *S. pyogenes* strain, the host immune response, and environmental factors all play roles, as demonstrated by the fact that the same strain can provoke pharyngitis, invasive disease, or simply be carried by a healthy population. We agree with Kaplan (Kaplan, 1980) in that “antibody titers remain elevated as long as the organism is present in the upper respiratory tract,” and that “in many so-called prolonged carriers, there was a continual anti-streptococcal immune response.”

In Johnson’s study and other examples, there are some cases where the same strain was repeatedly isolated and the antibody titers remained high for a long time (therefore, correctly defined as “no new infection”), which made it difficult to differentiate a true carriage from a persistent infection. In these cases, it would be interesting to see if these “carriers” are more frequent in the tic population, and to characterize these strains to evaluate if this long presence of *S. pyogenes* (either simply as carriage or as a persistent infection), together with other environmental factors, may be associated with a higher frequency of tic recrudescences. The model of colonization followed by a long “carriage” with a rise in antibody titers without evidence of clinical infection has been described by Ashbaugh in baboons (Ashbaugh, et al., 2000), and had already been described by Kaplan in humans (Kaplan, 1980). In any case, patients with tics and long-lasting antibody titers should be carefully studied, even if the patients have negative swabs. If that does not change anything

for the clinical treatment of a single patient, it may nevertheless give new insights into the role played by *S. pyogenes* in these movement, tic, and neuropsychiatric disorders. In 2007, Murphy et al., a school study showed that those with repeated *S. pyogenes* infections had higher rates of behavioral and movement findings. Although the strains were not characterized, the findings suggest that a carrier state could contribute to neuropsychiatric symptoms (Murphy, et al., 2007).

In other longitudinal studies, patients were followed for a long time for clinical and immunological findings to verify if recrudescences may be temporally associated with a *S. pyogenes* infection, as described in the PANDAS definition. In a 3-year prospective study, 12 children with new-onset PANDAS were followed (Murphy & Pichichero, 2002). All tested throat-positive for *S. pyogenes* , and had neuropsychiatric symptoms along with signs of tonsillo-pharyngitis with rises in antibody titers. Antibiotics were effective in both eradicating *S. pyogenes* and suppressing tics at the first episode and at recurrences.

In another prospective longitudinal study on 47 patients and 19 controls, *S. pyogenes* infection rate in children with TS and/or OCD was 0.42 per year, as compared with 0.28 per year in non-tic patients**,** but the association of symptom exacerbations and new *S. pyogenes* infections was not higher than those on the basis of chance. Therefore, the study suggested no clear relationship between exacerbations and new *S. pyogenes* infections (Luo, et al., 2004). Since school-aged children may have a higher rate of *S. pyogenes* positivity or carriage due to classroom exposures, it may be difficult to demonstrate new infection and its association with exacerbation in some studies.

Murphy et al. (Murphy, et al., 2004) investigated the relationship between *S. pyogenes* infections and symptom fluctuations in 25 children followed for 9–22 months with visits every 6.2 weeks on average for each subject. Authors reported that a part of their patients named ESC (episodic/sawtooth course) closely approximated the criteria described for PANDAS. In this study, beside ASLO and ADB titers, antibodies against the streptococcal capsular polysaccharide (ACHO) were measured. The typical pattern reported for one of these patients showed that, unlike ASLO and ADB, ACHO antibodies that target the group A streptococcus carbohydrate after a rapid increase remain elevated for a long time; moreover, a positive correlation between YGTSS and ACHO (p=0.063) or CYBOCS and ACHO (p=0.013) was found. This observation is interesting since in tic, TS, OCD or PANDAS studies, only the variation of antibody titers against protein antigens (ASLO and ADB) is usually considered, while ACHO is known from studies in SC to be a key antigen in rheumatic disease (Martins, et al., 2008; Cunningham, 2012). Moreover, it has been observed that for many patients with PANDAS, symptoms appeared only after repeated *S. pyogenes* infections.

Mell also reported this higher risk of tic development in children with frequent infections (Mell, Davis, & Owens, 2005), who noticed that for some PANDAS patients, symptoms emerged only after repeated *S. pyogenes* infections and that increases in behavioral and motor symptoms were found in children with repeated *S. pyogenes* infections (Murphy, et al., 2007); these findings suggest that a threshold of antibodies is needed to trigger symptoms. It is still unclear and unproven whether a true *S. pyogenes* clinical infection is really needed to develop symptoms, or whether repeated exposure to *S. pyogenes* antigens (maybe together with other external agents such as stress or a concomitant viral infection) may stimulate recrudescences on its own.

An accurate analysis was used to validate the PANDAS entity in a longitudinal study (Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008) which followed a group of 40 PANDAS cases, matched to 40 controls with TS without any documented

association of recrudescences with *S. pyogenes* infection, for two years. Results showed that even if not statistically significant, the group of PANDAS had more exacerbations than controls (65 clinical exacerbations in total: 40 in PANDAS, 25 in the control group). Moreover, among the 43 definite or probable *S. pyogenes* infections, 31 were in 22 PANDAS cases and 12 were in 9 subjects of the control group. The number of exacerbations associated with *S. pyogenes* infections (defined as hits) was outside the 95% confidence limit for the mean number of hits, which suggests that PANDAS exacerbations are significantly associated with an antecedent *S. pyogenes* infection. On the other hand, 75% of exacerbations had no observed temporal relationship with *S. pyogenes* infection. After noting that the number of recrudescences was lower and milder than expected, the authors concluded that the vast majority of PANDAS clinical recrudescences could not be linked to *S. pyogenes* infections, and that children with PANDAS represented a subgroup of patients with TS or OCD who are susceptible to *S. pyogenes* infections as part of their initial symptoms. An interesting note is that 22.5% of the PANDAS cases (compared with 5.3% of the controls) have a family history of rheumatic fever, which might indicate a special genetic predisposition in PANDAS cases that perhaps makes them more prone to develop *S. pyogenes* infections. Similar data were also reported by other authors (Cardona & Orefici, 2001).

Following the same study design and the same protocol, Leckman et al. (Leckman, et al., 2011) did not find an increase in exacerbations in the group of PANDAS, but on the contrary, a higher number was detected in the non-PANDAS group. As in the Kurlan study (Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008), the total number of recrudescences and *S. pyogenes* infections reported was lower than previously estimated, which raises suspicions that the study was underpowered; in contrast to what had been described in the definition of PANDAS, only a small number of recrudescences was associated with a sudden increase of tic /OCD severity. Again, 20 out of 31 children in the PANDAS group (in comparison to 8 of 53 in the non-PANDAS group) had a family history positive for rheumatic fever, which suggests some genetic predisposition and susceptibility to *S. pyogenes* sequelae. It should be noted that in both these latter studies, patients could continue to have their usual tic medications and that based on laboratory results, physicians were free to prescribe antibiotics. In particular, 28% of the controls (in comparison with 60% of the PANDAS group) were treated with antibiotics. This might partially account for the results achieved, and could be an unintentional indirect support for antibiotic treatment to suppress recrudescences.

In another multicenter longitudinal study on TS patients (Martino, et al., 2011), *S. pyogenes* infections ASLO titers, ADB, and anti-basal ganglia antibodies (ABGA) antibodies were compared among 168 children with TS, and 177 matched controls with epileptic or sleep disorders without tics. Seven definite (2%) and 32 possible (10%) infections were reported with a rise in ASLO titers in 26 (18%) of the subjects and in 11 (8%) of the ADB titers; 14% of patients had ABGA test persistently positive over at least 2 consecutive visits, and 20% became ABGA positive throughout the study. Nevertheless, it was not possible to correlate *S. pyogenes* infections with exacerbations, and the occurrence of a new identification of ABGA did not predict the occurrence of an exacerbation. Authors concluded that children and adolescents with TS show an increased exposure to and immune response against *S. pyogenes* and increased expression of antineuronal antibodies. This supports the view that patients with TS, independently of the PANDAS definition, may be more prone to *S. pyogenes* infections and may develop stronger immune responses against streptococcus, possibly as a result of immune dysregulation.

Other infections

S. pyogenes is not the only pathogen thought to be associated with the onset or recrudescence of tics. Other pathogens, such as intracellular microorganisms with the capacity of living and replicating inside host cells (Riedel, Straube, Schwatz, Wilske, & Müller, 1998; Müller, et al., 2004; Krause, et al., 2010), have been described as possibly being involved in these movement disorders, and particularly associated with recrudescences, but no strict observance of common parameters have been reported. Hoekstra (Hoekstra, Manson, Steenhuis, Kallenberg, & Minderaa, 2005) found a recrudescence association with the common cold, but it is difficult to evaluate this report because of the vague assessment of "common cold" and of the low isolation of *S. pyogenes* from patients.

Since the involvement of *S. pyogenes* in PANDAS has never been completely demonstrated, and there is no complete evidence of which *S. pyogenes* antigen(s) (if any) could be associated with tics, it is also impossible to define the way in which these other microorganisms are related to tics: could they be the causes, or could they collaborate with *S. pyogenes* in triggering symptoms? Are some of their antigenic determinants common with *S. pyogenes* ? Or are none of them really associated with tics? In any case, even if some reports have been published, they could support the hypothesis that the genetic background of these patients (including any immunodeficiencies) may generally make them more susceptible to certain infectious organisms and more prone to develop antibodies against microbial and brain antigens.

Plurality of antigens involved

This last hypothesis stated above seems to be supported by the results of a study (Bombaci, et al., 2009) that used a protein array to test the antibody responses of children with tics to a panel of more than 100 recombinant *S. pyogenes* antigens. These patients had chronic tic disorders, but no overt pharyngitis and no previous rheumatic diseases; their results were compared with those of healthy control children without tics and with children with microbiologically demonstrated *S. pyogenes* pharyngitis. The results showed that a group of 25 antigens were recognized by sera of all three groups; 21 antigens reacted with sera of tic and pharyngitis patients, but poorly with control sera; and 5 antigens were preferentially recognized by sera from children with chronic tics. Moreover, the overall response to the tested antigens appeared to be stronger in tic patients than in pharyngitis cases. What is most interesting is that this strong response to streptococcal antigens in the absence of clinical evidence of pharyngitis was independent of ASLO titers or an *S. pyogenes* positive throat culture. The results of the study indicated that a subgroup of tic patients show a typical profile of subjects who mount a broad, specific immune response to *S. pyogenes* antigens in the absence of clinical pharyngitis, and suggests that in genetically predisposed patients, a strong anti- *S. pyogenes* response due to a lengthy exposure to streptococcal antigens (like in long-term carriers or children with frequent pharyngitis) may produce a cumulative threshold of antibodies that are needed to produce recrudescences. The effect of other environmental factors (stress or other concomitant infections) may help in triggering the disorder in the absence of overt infection. The results show how ASLO, ADB, and the positive *S. pyogenes* throat swab may be sufficient to predict children who are at risk of developing neuropsychiatric movement or tic-like symptoms.

The high number of proteins tested in the study, and the strong preferential response by the tic sera to five of them, does not necessarily mean that these antigens are involved in tics, since only protein antigens have been examined, and because of the broad plasticity demonstrated by *S. pyogenes* in switching on or off the genes (Hertzén, et al., 2012) on the basis of the intracellular or extracellular environment. However, the stronger response in the

children with tics suggests that they responded more strongly to streptococcal antigens than did children with pharyngitis or children in the control group. This could be due to repeated streptococcal infections, as is believed to occur with rheumatic fever.

Characterization of the strains

No attempts to characterize the strains from tic patients for their possible specific virulence factors or antibiotic resistance have been made, nor have studies been attempted of M proteins (the fundamental antigens of *S. pyogenes*), which are involved in pathogenicity and used in characterization of the strains. Could M proteins play any role in the development of tics, or are specific M types more frequently found in these movement disorders? In one study (Müller, et al., 2001), Mueller detected antibodies against M1 and M 23, and in the Johnson study (Johnson, Kurlan, Leckman, & Kaplan, 2010) some M types of the strains isolated from swabs are reported, but not many studies that specifically examine the M types of the *S. pyogenes* isolated in these patients have been published.

Creti et al. (Creti, et al., 2004) examined 100 strains collected from 368 children with tics during years 1996-2001. Strains were typed by M protein agglutination and *emm* molecular typing. Sixty-seven children (18%), 53 males and 14 females had one or more throat swab test positive for *S. pyogenes* . Notably, while no problems were found with the molecular typing, 35% of the isolates resulted in being non-typable by anti M-protein sera even after repeating the typing in 3 centers (Rome, Prague, and Minneapolis), which indicates a very scarce presence of M protein on the surface.

No specific *emm* types associated with these patients were found, but 5 types, namely M12 (11.40%), M22 (11.40%), M5 (8.86%), M3 (6.32%), and M89 (6.32%) accounted for 44.3% of the strains, while M4, M2, and M1 accounted for 5.06% each. A large number of different types was found, and in some cases, a type was represented by only one or two isolates. M3 and M5 were generally associated with ASLO titers higher than 407 IU, but the numbers were too small to make a comparison to other M types. It is interesting that, even if the rank order appeared different from that of strains from pharyngitis (Dicuonzo, et al., 2001) or invasive disease (Creti, et al., 2007) isolated from the same area in the same period, the same M types presented the same “virulence and antibiotic string” (*spe* A, *spe* C, *mef* A, *erm* A, *erm* B), independent from the source of isolation (tic, pharyngitis or invasive disease). In Italy, M12 is the M type most frequently isolated from carriers, and M4 is frequently associated with scarlet fever epidemics; the M types typical for rheumatic disease, like M1, M3, M5, and M18 (Shulman, Stollerman, Beall, Dale, & Tanz, 2006), when found, were never related to previous histories of rheumatic fever.

Even when taken with caution (due to the small amount of data available), the M types found represent the serotypes present in a “normal” population with no specific M types or particular virulence factors. In this sense, the observation of the scarce typability by anti-M protein sera, the fact that different *emm* types were isolated in strains in the following years (2002–2007)—as happens in the natural selection of the strains—and that strongly mucoid strains, which are traditionally “rheumatogenic,” were never found, all support the hypothesis that the strains found were those that circulate in the normal population. On the other hand, changes in the rank of frequency can be expected when a limited number of strains are studied; this possibly reflects the normal epidemiological relationship between circulating types and the population immunological condition and dynamics (when antibodies have been raised against one serotype among the population, this type decreases its frequency). Nevertheless, this finding could be important, along with immunologic and genetic study of these patients, to determine why individuals are more prone to streptococcal infections, to improve the knowledge about these isolated strains, and to detect if there is

any specific reason that facilitates their colonization or their long-term residence in the throat.

The effect of a genetic predisposition for rheumatic disease has already been described by Bryant et al. (Bryant, et al., 2014), who investigated whether any difference in immune response detectable by gene expression can be found between individuals susceptible to ARF and those who are not. The authors found that 34 genes were significantly and differentially expressed between ARF-susceptible and ARF-resistant subjects, 7 of which were involved in immune response genes, chemotaxis, and apoptosis.

Kotb et al. (Kotb, et al., 2008), used *S. pyogenes* as a model to demonstrate in cell cultures and in animal models (including transgenic mice) how the host's genetically determined response may be modulated by environmental factors and how the response to some streptococcal superantigens may account for the different severity of the invasive disease determined. The same type of study might be useful for PANDAS and for tics in general.

The role of stress

Studies on TS/OCD report the relevance of psychosocial stress, which suggests that these disorders are sensitive to stress and show a high stress response (Corbett, Mendoza, Baym, Bunge, & Levine, 2008; Chappell, et al., 1994; Buse, Kirschbaum, Leckman, Münchau, & Roessner, 2014); these findings offer further evidence that many different factors contribute to these movement disorders. A cohort of 86 children diagnosed with TS/OCD and 41 matched controls were followed in a longitudinal study to verify if TS/OCD patients showed higher levels of psychosocial stress, as compared to the healthy population (Lin, et al., 2007). Notably, while levels of psychosocial stress were modest but were significant predictors of future tic symptom severity, current tic severity was not a significant predictor of psychosocial stress.

The same group in a longitudinal study monitored 45 cases (with 11 defined as PANDAS) and 41 healthy controls for 2 years with thrice-yearly visits and monthly telephone conversations to examine the impact of new *S. pyogenes* infections and psychosocial stress on future fluctuations of tic/OCD and the severity of depressive symptoms. PANDAS cases had higher (even if not significant) number of *S. pyogenes* infections compared to normal controls or non-PANDAS cases. Psychosocial stress and newly defined (or possible diagnosed) *S. pyogenes* infections were significant predictors of future symptom severity: newly diagnosed *S. pyogenes* infections increased by a factor of more than three, which indicates the power of psychosocial stress to predict future symptom severity. The study suggests that a minority of children with tics or OCD are sensitive to antecedent *S. pyogenes* infections, and that psychosocial stress is a potent factor associated with future worsening of tics (Lin, et al., 2010).

Clinical trial in PANDAS

Another source of information on the relationship between *S. pyogenes* and neuropsychiatric symptoms comes from clinical trials. Based on experience with acute rheumatic fever (ARF), in which secondary prophylaxis with penicillin reduced recurrences of ARF or SC by preventing *S. pyogenes* infections, some studies were conducted on patients with PANDAS.

The first one was an 8 month, double-blind, balanced cross-over study (Garvey, et al., 1999). Thirty-seven children who met the five classical criteria for PANDAS, were randomized to receive either 4 months of the active compound (twice daily oral 250 mg penicillin V) followed by 4 months of a placebo, or a placebo followed by penicillin V. Subjects were evaluated monthly for eight consecutive visits in order to assess the clinical

features (ratings of tics, obsessive compulsive symptomatology, anxiety, and depression) as well as undergo laboratory evaluation (including serum titers of antistreptolysin-O (ASLO), anti-deoxyribonuclease B (anti-DNaseB) and throat cultures). These results showed no significant difference between the two phases in the number of both streptococcal infections and symptom exacerbations. The authors reported a lack of compliance by 26 of the children, which they attributed to an overall failure to achieve the aims of the study.

Some years later, the same group conducted another double-blind, randomized controlled trial (Snider, Lougee, Slattery, Grant, & Swedo, 2005); in this study, 23 subjects with PANDAS received an antibiotic prophylaxis with penicillin or azithromycin for 12 months. In particular, subjects were randomized in a double-blind fashion to receive either penicillin V-K 250 mg two times a day, or azithromycin 250 mg capsules two times a day, on one day of the week and placebo capsules taken two times a day on the other six days. The rate of streptococcal infections and symptom exacerbations were assessed during the study year by monthly visits and laboratory evaluation (ASLO and Anti-DNase B titers) and were then compared with those of a baseline year (for which subjects/parents were asked to retroactively recall the number of clinical exacerbations and streptococcal infections; medical records were also reviewed). Results showed a significant reduction (96%) of the rate of streptococcal infections, as well as of neuropsychiatric symptoms (64%) in both groups.

The authors concluded that both penicillin and azithromycin are effective in preventing *S. pyogenes* infections, and that both penicillin and azithromycin may be effective in preventing *S. pyogenes* -triggered neuropsychiatric exacerbations in children in the PANDAS subgroup. However, they also suggest great caution when interpreting the data, due to the small number of patients and the lack of a placebo. It should be mentioned that this study received several criticisms, including from the Tourette's Syndrome Study group (Budman, et al., 2005), about many of the study's methodological aspects. Most recently, a report published by Murphy et al. suggested that a reduction of symptoms using the β-lactam antibiotic cefdinir was observed in PANS (Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a), which supports the potential usefulness of antibiotics in these diseases.

To our knowledge, apart from the above-mentioned studies and a case series report (Murphy & Pichichero, 2002), no other data have been published to establish a treatment protocol for antibiotic therapy or prophylaxis in PANDAS subjects. Caution is advised against the over-use of antibiotic treatments of PANDAS or PANS patients, as well as of subjects that show only a single exacerbation of neuropsychiatric (tics or OCD) symptoms, which has been reported in the US (Gabbay, et al., 2008) and in other countries.

Anti-Neuronal Autoantibodies in Sydenham Chorea and Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococci (PANDAS)

Sydenham chorea (SC) is well established as the neurologic manifestation of acute rheumatic fever (Taranta & Stollerman, 1956), and is characterized by antibodies found in the cytoplasm of neurons in the caudate and putamen regions of the human brain (Husby, van de Rijn, Zabriskie, Abdin, & Williams, Jr., 1976). Little was known about the antibodies and how they affected the brain until human mAbs were derived from SC (Kirvan, Swedo, Heuser, & Cunningham, 2003) and were found to react with the group A streptococcal carbohydrate epitope N-acetyl-beta-D-glucosamine and brain antigens lysoganglioside (Kirvan, Swedo, Heuser, & Cunningham, 2003) and tubulin (Kirvan, Cox, Swedo, & Cunningham, 2007). Evidence from studies of human chorea-derived mAbs strongly suggests that autoantibody crossreactivity between streptococci and brain is an important feature in Sydenham chorea (Kirvan, Swedo, Heuser, & Cunningham, 2003; Kirvan, Cox, Swedo, & Cunningham, 2007; Kirvan, Swedo, Kurahara, & Cunningham, 2006b). Human

mAbs and antibodies in sera or cerebrospinal fluid from the SC-activated calcium calmodulin dependent protein kinase II (CaMKII) in human neuronal cells (Kirvan, Swedo, Heuser, & Cunningham, 2003) and led to an increase in dopamine release from the human neuronal cell line using tritiated dopamine assays (Kirvan, Swedo, Kurahara, & Cunningham, 2006b). Further study indicated that chorea-derived mAb (24.3.1) induced tyrosine hydroxylase activity in dopaminergic neurons after the intrathecal transfer of purified human mAb 24.3.1 (Kirvan, Swedo, Heuser, & Cunningham, 2003) into a Lewis rat brain (Kirvan, Swedo, Kurahara, & Cunningham, 2006b). The removal of IgG from serum caused a loss of neuronal cell-signaling activity (Brimberg, et al., 2012; Kirvan, Swedo, Heuser, & Cunningham, 2003), and plasmaphoresis was found to improve chorea symptoms (Perlmutter, et al., 1999; Garvey, Snider, Leitman, Werden, & Swedo, 2005). Therefore, antibody-mediated neuronal cell signaling was induced by IgG antibodies in serum or cerebrospinal fluid from SC, and the presence of these signaling autoantibodies were associated with symptoms (Kirvan, Swedo, Heuser, & Cunningham, 2003; Ben-Pazi, Stoner, & Cunningham, 2013). Antibody-mediated neuronal cell signaling in SC is a novel pathogenic mechanism which is important in the movement and neuropsychiatric disorder of acute rheumatic fever (Kirvan, Swedo, Heuser, & Cunningham, 2003). SC may be a model for other movement and neuropsychiatric disorders associated with infections, such as PANDAS (Swedo, et al., 1998).

To further the studies of the antibodies in SC and related diseases, a novel transgenic mouse model expressing an SC-derived brain autoantibody was developed to gain insight into *in vivo* functional antibody targets that may be involved in the mechanisms of SC, and to test the hypothesis that autoantibodies from movement and behavioral disorders target neurons and possibly the dopamine D2 receptor (D2R) in the brain (Cox, et al., 2013). Transgenic mice expressed chorea-derived, human mAb 24.3.1, heavy and light chain variable region (V_H and V_L) genes as part of a chimeric (human V gene/mouse constant region) $IgG1^a$ antibody construct (Figure 3). Mice transgenic for mAb 24.3.1 V genes were validated by characteristic cross-reactive anti-neuronal antibody specificities in serum, and of mAbs produced from lymphocytes from spleens of transgenic mice. In our SC transgenic mouse model, chimeric 24.3.1 antibody expressed in mouse B cells and serum penetrated the brain and dopaminergic neurons in the basal ganglia of transgenic mice. Expression of the V genes of SC mAb 24.3.1 (Cox, et al., 2013) in transgenic mice demonstrated that the SC antibody V gene expression in the serum of transgenic mice targeted dopaminergic tyrosine hydroxylase positive neurons in the basal ganglia of the transgenic mice (Cox, et al., 2013), as shown in Figure 2. These results were consistent with evidence seen in human SC (Husby, van de Rijn, Zabriskie, Abdin, & Williams, Jr., 1976). In addition, human mAb 24.3.1 from SC was shown to react with and signal the human dopamine D2 receptor expressed in transfected cell lines (Cox, et al., 2013). Evidence using a flag-tagged D2 receptor, as well as signaling of the human D2 receptor in transfected cell lines, demonstrated that human mAb, as well as human SC sera IgG, targeted the dopamine D2 receptor (Cox, et al., 2013). In addition, antibodies (IgG) were also present in serum against the human D1 receptor, and further studies suggested that the ratio of the anti-D1R/D2R antibodies correlated with symptoms (Ben-Pazi, Stoner, & Cunningham, 2013). The studies also showed that anti-D1 receptor and anti-D2 receptor antibodies (IgG) were significantly elevated in serum from SC, as well as PANDAS, as described by Cox et al. (Cox, et al., 2013).

PANDAS shares similar antibodies against the dopamine receptors, as does SC (Cox, et al., 2013). The symptoms of PANDAS, as originally reported, appear as small choreiform piano-playing movements of the fingers and toes which were reported in the first 50 cases by Swedo et al. (Swedo, et al., 1998). PANDAS is characterized by tics and OCD; which, in addition to the fine choreiform movements, are not as obvious as those movements seen in

SC (Garvey, Snider, Leitman, Werden, & Swedo, 2005; Garvey & Swedo, 1997). The fine choreiform movements of lower amplitude than chorea may go unnoticed in PANDAS and can lead to poor handwriting associated with learning and behavioral regression, enuresis, separation anxiety and night-time fears, and anorexia in approximately 17 percent of cases (Swedo, et al., 1998). The appearance of PANDAS is very striking because the onset is very sudden, such as overnight behavioral changes.

For years the focus of research on SC was primarily on the chorea and involuntary movements, with little attention given to the neuropsychiatric obsessive-compulsive symptoms which predate the chorea and characterize the neurological manifestations of acute rheumatic fever (Ben-Pazi, Stoner, & Cunningham, 2013). These manifestations may be seen in other types of infections, and in these cases, is termed pediatric acute onset neuropsychiatric syndrome or PANS (Swedo, Leckman, & Rose, 2012). There have been many questions about PANDAS/PANS, which current research is attempting to answer. Clearly, the original PANDAS group has many similarities to SC, including a previous *S. pyogenes* infection; however, unlike SC, PANDAS has a male predominance (Swedo, et al., 1998; Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a; Swedo, et al., 1989; Swedo, 1994; Swedo, Leckman, & Rose, 2012; Snider & Swedo, 2004). PANS and more chronic types of tics and OCD are not always associated with *S. pyogenes* infections. More chronic tics and OCD may not display the small choreiform piano-playing movements of the fingers and toes, and are not similar to SC in their anti-neuronal antibody patterns of antibodies against the dopamine D2 receptor (Cox, et al., 2015; Singer, et al., 2015). More chronic forms of tics and OCD do not have the IgG antibodies against the D2 receptor (Cox, et al., 2015; Singer, et al., 2015; Morris-Berry, et al., 2013). PANDAS with small choreiform piano-playing movements of the fingers and toes (Swedo, et al., 1998) share the antibodies against both D1 and D2 receptors with SC (Cox, et al., 2013; Brimberg, et al., 2012; Ben-Pazi, Stoner, & Cunningham, 2013) and also have elevated antibodies against tubulin and lysoganglioside (Cox, et al., 2013; Brimberg, et al., 2012; Kirvan, Cox, Swedo, & Cunningham, 2007; Ben-Pazi, Stoner, & Cunningham, 2013). Both tics and OCD, including the original PANDAS (Swedo, et al., 1998) and the more chronic tics and OCD are both temporally associated with *S. pyogenes* infection and have a significantly elevated abnormal CaMKII (Kirvan, Swedo, Snider, & Cunningham, 2006a; Cox, et al., 2015; Singer, et al., 2015). More studies of PANS are required to study children who have OCD and tics that are not associated with *S. pyogenes* infection.

Animal models of movement and obsessive compulsive symptoms have been studied in a mouse model and Lewis rat model, where both models show positive evidence that symptoms are associated with anti-streptococcal antibodies. Immunization of a mouse model (Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004) with streptococcal components in Freund's complete adjuvant led to behavioral alterations and compulsions, and a subset of mice with antibody deposits in several brain regions, including deep cerebellar nuclei (DCN), globus pallidus, and the thalamus (Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004). Group A streptococcal immunized mice with increased deposits of IgG in the deep cerebellar nuclei exhibited increased rearing behavior, as compared to controls. These data suggested that immune responses against *S. pyogenes* were associated with motoric and behavioral disturbances, and suggested anti- *S. pyogenes* antibodies that cross-react with brain components may lead to symptomatology (Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004). Passive transfer of anti-streptococcal antibodies from the immunized mice into naïve mice led to autoantibody deposits in the brain, as well as behavior changes (Yaddanapudi, et al., 2010).

Another animal model of SC, and potentially PANDAS, was created in the Lewis rat (Brimberg, et al., 2012), which demonstrated that exposure to group A streptococcal

antigens during immunization led to behaviors characteristic of SC and PANDAS. After at least two immunizations, rats were not able to hold a food pellet as well as control rats, and also could not traverse a narrow beam as well as control rats (Brimberg, et al., 2012). In addition, the rats demonstrated a compulsive grooming behavior. Antibody IgG deposits were observed in the Lewis rat striatum, thalamus, and frontal cortex, and concomitant alterations in dopamine and glutamate levels in the cortex and basal ganglia were observed, which were consistent with SC and its related neuropsychiatric disorder. In the rat model, serum from group A streptococcal immunized rats activated CaMKII in SKNSH neuronal cells (Brimberg, et al., 2012) like that observed for sera from acute SC (Kirvan, Swedo, Heuser, & Cunningham, 2003). The expression of SC mAb V genes in transgenic mice demonstrated that antibody in SC most likely targets the dopamine receptors on dopaminergic neurons, since the antibody was observed in the cytoplasm of dopaminergic neurons in the basal ganglia (Cox, et al., 2013) and was found to signal the dopamine D2 receptor, as well as associate with the flag-tagged D2 receptor on transfected cells (Cox, et al., 2013). The reactivity of chorea-derived mAb 24.3.1 or SC IgG with D2R was also confirmed by the blocking of Ab reactivity by an extracellular D2R peptide (Cox, et al., 2013).

To summarize, the anti-neuronal antibodies present in SC and PANDAS with fine choreiform piano-playing movements include anti-lysoganglioside (Kirvan, Swedo, Snider, & Cunningham, 2006a), anti-tubulin (Kirvan, Cox, Swedo, & Cunningham, 2007), anti-dopamine D2 receptor (D2R) (Cox, et al., 2013; Brimberg, et al., 2012; Ben-Pazi, Stoner, & Cunningham, 2013), and anti-dopamine D1 receptor (D1R) (Ben-Pazi, Stoner, & Cunningham, 2013) antibodies. In SC, the ratio of the anti-dopamine D2 receptor / anti-dopamine D1 receptor antibodies correlated with the UFMG-Sydenham's-Chorea-Rating-Scale (USCRS) clinical rating scale of neuropsychiatric symptoms (Ben-Pazi, Stoner, & Cunningham, 2013). Most importantly, these antibodies in both SC and PANDAS signaled the SKNSH human neuronal cell line and activated calcium calmodulin-dependent protein kinase II (CaMKII) (Kirvan, Swedo, Heuser, & Cunningham, 2003; Kirvan, Swedo, Snider, & Cunningham, 2006a), which may have led to excess dopamine release (Kirvan, Swedo, Kurahara, & Cunningham, 2006b). Figure 4 shows a model diagram from a recent review (Cunningham, 2012).

In our most recent studies of tics and OCD, anti-neuronal autoantibodies were investigated as well as antibody-mediated neuronal cell signaling activity as previously reported for SC and PANDAS to determine immunological profiles for a large cohort (n=742) of children with tics and/or OCD (Cox, et al., 2015). The goal of this study was to expand upon these earlier observations and to investigate whether sera from patients with OCD, tics, or both resulted in higher CaMKII induction, as compared to healthy controls, and also to see if sera from patients with OCD, tics, or both showed elevated reactivity to previously tested neuronal antigens, tubulin and lysoganglioside, and to dopamine D1 and D2 receptors, which appeared to be targets of autoantibodies in an animal model as well as human sera IgG from PANDAS sera (Brimberg, et al., 2012). In addition, the link between streptococcal infection and OCD, tics, or both was investigated. The study focused on 311 of the 742 participants who had a history of neuropsychiatric illness with streptococcal infections or not, and tics, OCD, or both. Not all 311 subjects fell into every category studied, which resulted in only 261 of the 311 participants with confirmed tics, OCD, or both. Of the 311 individuals, 222 (71%) had evidence of a confirmed group A streptococcal infection, which was associated with tics and/or OCD status (p=0.0087) (Cox, et al., 2015). In our study, the presence of OCD and/or tics was associated with positive streptococcal infection status (p = 0.0087). It was also found that subjects who were positive for streptococcal infection were more likely to have both OCD and tics (51%), as opposed to those who were negative for

streptococcal infection (30%), while there was no significant association when tics or OCD were considered alone, relative to streptococcal infection.

Individuals with tics and/or OCD (n=261) had evidence of elevated serum IgG antibodies against human D1R ($p<0.0001$) and lysoganglioside ($p=0.0001$), and higher activation of CaMKII activity ($p<0.0001$) in a human neuronal cell line, as compared to healthy controls (n=16). Furthermore, children with tics and/or OCD had significantly increased activation of CaMKII activity, as compared to children with only tics or only OCD ($p<0.033$ for each) (Cox, et al., 2015).

Our new study also revealed two important correlations that involved CaM kinase II activation: one, the presence of OCD and/or tics was positively associated with CaM kinase II activation ($n = 261$, $p = 0.0008$); and two, CaM kinase II activation was elevated for children with OCD and/or tics (n=261), with the median percentile of CaMKII increased values ranging from 149 to 162 percentile units above the baseline enzyme activity, while CaMKII activation remained unaffected in healthy controls, with a median of 94 (equivalent to baseline CaMKII activity at approximately 100) ($n = 16$, $p < 0.0001$) (Cox, et al., 2015). The difference in the median value for CaMK II activation between patient samples and healthy controls is similar to what was found for PANDAS sera and non-PANDAS sera in previous studies (Kirvan, Swedo, Snider, & Cunningham, 2006a).

Our study showed that sera IgG from cases with OCD, tics, or both reacted more significantly with human D1 receptor antigen, as compared to healthy controls in direct ELISA ($p \leq 0.0001$) (Cox, et al., 2015). Clearly, serum IgG from our tics and OCD cohort did not react significantly above normal values with the human D2 receptor and were determined to be more chronic, since the symptoms were present for >1 year or longer in our cohort. Reactivity of the original acute onset PANDAS and SC sera IgG as tested in direct ELISA reacted more significantly with the dopamine D2 receptor antigen, as compared to healthy controls, while and PANDAS sera reacted more significantly with both the D1 and D2 receptor antigens when compared with healthy controls. Additionally, when the sera of 261 patients diagnosed with OCD, tics, or both was found to react in a direct ELISA with lysoganglioside as the antigen, sera IgG had statistically significant higher titers than healthy controls ($p = 0.0001$) (Cox, et al., 2015). The direct ELISA with tubulin as the antigen did not show a statistically significant difference between sera from tics, OCD, or both, versus healthy controls.

To summarize this study, the presence of OCD and/or tics was associated with positive streptococcal infection status ($p = 0.0087$). It was also found that subjects who tested positive for streptococcal infection were more likely to have both OCD and tics (51%) versus those who tested negative for streptococcal infection (30%), while there was no significant association when tics or OCD were considered alone, relative to streptococcal infection. As a result, it is possible that subjects that present with both OCD and tics are more likely to have had streptococcal infections. Presentations of OCD and tics alone are potentially manifestations of disorders not associated with *S. pyogenes* . The study also suggested a significant correlation of streptococcal associated tics and OCD with elevated anti-D1R and anti-lysoganglioside anti-neuronal antibodies concomitant with the higher activation of CaMKII in human neuronal cells. The statistically significant correlation between a history of chronic tics/OCD with anti-neuronal antibodies against the D1R and lysoganglioside and functional activation of CaMKII suggests that at least some pediatric neuropsychiatric disorders may be associated with autoimmunity against the brain. The functional activity of the autoantibodies which signal CaMKII in human neuronal cells suggests that antibodies could target receptors in the brain and alter dopamine neurotransmission, which could lead to neuropsychiatric symptoms.

The mechanisms and effects of anti-neuronal antibodies on the brain include alterations in dopamine transmission, including the release of excess dopamine from neuronal cells. Excess dopamine was released from the SKNSH cell line when treated with a human mAb from SC (Kirvan, Swedo, Kurahara, & Cunningham, 2006b) and human mAb from PANDAS was found to cause alterations in the sensitivity of the receptors to dopamine (Zuccolo, 2015). Evidence in animal models and humans strongly suggest that antibodies mediate inflammatory consequences in SC, PANDAS, and PANS (Brimberg, et al., 2012; Lotan, et al., 2014a; Perlmutter, et al., 1999; Lotan, Cunningham, & Joel, 2014b). There may be other brain antigens targeted by autoantibodies in PANDAS/PANS and related autoimmune diseases that may affect memory and behavior (Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004; Yaddanapudi, et al., 2010; Huerta, Kowal, DeGiorgio, Volpe, & Diamond, 2006; Kowal, et al., 2004; DeGiorgio, et al., 2001).

Finally, molecular mimicry between *S. pyogenes* and the brain is supported by evidence from studies of human mAbs and serum IgG antibodies from rheumatic fever (Kirvan, Swedo, Heuser, & Cunningham, 2003; Galvin, Hemric, Ward, & Cunningham, 2000). The investigation of human mAbs from SC has supported the hypothesis that antibodies against the *S. pyogenes* carbohydrate epitope GlcNAc (Kirvan, Swedo, Heuser, & Cunningham, 2003) recognize crossreactive structures on neuronal cells in the brain, which may lead to the onset of SC. In the brain, antibody-mediated neuronal cell signaling may be a mechanism of antibody pathogenesis in SC. The emerging theme in mimicry suggests that crossreactive autoantibodies target intracellular antigens—but for disease pathogenesis, the antibodies must target the surface of neuronal cells by affecting the signaling pathways in neurons. These mechanisms of molecular mimicry lead to the effects seen in acute rheumatic fever and related autoimmune sequelae associated with *S. pyogenes* infections.

References

Allen A. J. Leonard H. L. Swedo S. E. Case study: a new infection-triggered, autoimmune subtype of pediatric OCD and Tourette's syndrome. Journal of the American Academy of Child and Adolescent Psychiatry 1995;34(3):307–311. [PubMed: 7896671]

Aron A. R. Behrens T. E. Smith S. Frank M. J. Poldrack R. A. Triangulating a cognitive control network using diffusion-weighted magnetic resonance imaging (MRI) and functional MRI. The Journal of Neuroscience 2007;27(14):3743–3752. [PubMed: 17409238]

Ashbaugh C. D. Moser T. J. Shearer M. H. White G. L. Kennedy R. C. Wessels M. R. Bacterial determinants of persistent throat colonization and the associated immune response in a primate model of human group A streptococcal pharyngeal infection. Cellular Microbiology 2000;2(4):283–292. [PubMed: 11207585]

Bencivenga J. F. Johnson D. R. Kaplan E. L. Determination of Group A Streptococcal Anti-M Type-Specific Antibody in Sera of Rheumatic Fever Patients after 45 Years. Clinical Infectious Diseases 2009;49(8):1237–1239. [PubMed: 19761409]

Ben-Pazi H. Stoner J. A. Cunningham M. W. Dopamine Receptor Autoantibodies Correlate with Symptoms in Sydenham's Chorea. PLoS One 2013;8(9):e73516. [PubMed: 24073196]

Bernstein G. A. Victor A. M. Pipal A. J. Williams K. A. Comparison of clinical characteristics of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections and childhood obsessive-compulsive disorder. Journal of Child and Adolescent Psychopharmacology 2010;20(4):333–340. [PubMed: 20807071]

Bloch M. H. Leckman J. F. Zhu H. Peterson B. S. Caudate volumes in childhood predict symptom severity in adults with Tourette syndrome. Neurology 2005;65(8):1253–1258. [PubMed: 16247053]

Bombaci M. Grifantini R. Mora M. Reguzzi V. Petracca R. Meoni E. et al. Protein array profiling of tic patient sera reveals a broad range and enhanced immune response against Group A Streptococcus antigens. PLoS One 2009;4(7):e6332. [PubMed: 19623252]

Brimberg L. Benhar I. Mascaro-Blanco A. Alvarez K. Lotan D. Winter C. et al. Behavioral, pharmacological, and immunological abnormalities after streptococcal exposure: a novel rat model

of Sydenham chorea and related neuropsychiatric disorders. Neuropsychopharmacology 2012;37(9):2076–2087. [PubMed: 22534626]

Bryant P. A. Smyth G. K. Gooding T. Oshlack A. Harrington Z. Currie B. et al. Susceptibility to acute rheumatic fever based on differential expression of genes involved in cytotoxicity, chemotaxis, and apoptosis. Infection and Immunity 2014;82(2):753–761. [PubMed: 24478089]

Budman C. Coffey B. Dure L. Gilbert D. L. Juncos J. Kaplan E. Regarding "Antibiotic prophylaxis with azithromycin or penicillin for childhood-onset neuropsychiatric disorders". Biological Psychiatry 2005;58(11):917–919. [PubMed: 16242121]

Buse J. Kirschbaum C. Leckman J. F. Münchau A. Roessner V. The Modulating Role of Stress in the Onset and Course of Tourette's Syndrome: A Review. Behavior Modification 2014;38(2):184–216. [PubMed: 24516255]

Cardona F. Orefici G. Group A streptococcal infections and tic disorders in an Italian pediatric population. The Journal of Pediatrics 2001;138(1):71–75. [PubMed: 11148515]

Cardona F. Ventriglia F. Cipolla O. Romano A. Creti R. Orefici G. A post-streptococcal pathogenesis in children with tic disorders is suggested by a color Doppler echocardiographic study. European Journal of Paediatric Neurology 2007;11(5):270–276. [PubMed: 17403609]

Cardoso F. Vargas A. P. Oliveira L. D. Guerra A. A. Amaral S. V. Persistent Sydenham's chorea. Movement Disorders 1999;14(5):805–807. [PubMed: 10495042]

Chang K. Frankovich J. Cooperstock M. Cunningham M. W. Latimer M. E. Murphy T. K. et al. Clinical evaluation of youth with pediatric acute-onset neuropsychiatric syndrome (PANS): recommendations from the 2013 PANS Consensus Conference. Journal of Child and Adolescent Psychopharmacology 2015;25(1):3–13. [PubMed: 25325534]

Chappell P. Riddle M. Anderson G. Scahill L. Hardin M. Walker D. et al. Enhanced stress responsivity of Tourette syndrome patients undergoing lumbar puncture. Biological Psychiatry 1994;36(1):35–43. [PubMed: 8080901]

Church A. J. Dale R. C. Lees A. J. Giovannoni G. Robertson M. M. Tourette's syndrome: a cross sectional study to examine the PANDAS hypothesis. Journal of Neurology, Neurosurgery & Psychiatry 2003;74(5):602–607. [PubMed: 12700302]

Corbett B. A. Mendoza S. P. Baym C. L. Bunge S. A. Levine S. Examining cortisol rhythmicity and responsivity to stress in children with Tourette syndrome. Psychoneuroendocrinology 2008;33(6):810–820. [PubMed: 18487023]

Cox C. J. Sharma M. Leckman J. F. Zuccolo J. Zuccolo A. Kovoor A. et al. Brain human monoclonal autoantibody from Sydenham chorea targets dopaminergic neurons in transgenic mice and signals dopamine D2 receptor: Implications in human disease. The Journal of Immunology 2013;191(11):5524–5541. [PubMed: 24184556]

Cox C. J. Zuccolo A. J. Edwards E. V. Mascaro-Blanco A. Alvarez K. Stoner J. et al. Antineuronal antibodies in a heterogeneous group of youth and young adults with tics and obsessive-compulsive disorder. Journal of Child and Adolescent Psychopharmacology 2015;25(1):76–85. [PubMed: 25658702]

Creti R. Cardona F. Pataracchia M. Hunolstein C. V. Cundari G. Romano A. et al. Characterisation of group A streptococcal (GAS) isolates from children with tic disorders. Indian Journal of Medical Research 2004;119:174–178. [PubMed: 15232189]

Creti R. Imperi M. Baldassari L. Pataracchia M. Recchia S. Alfarone G. et al. emm types, virulence factors, and antibiotic resistance of invasive Streptococcus pyogenes isolates from Italy: what has changed in 11 years? Journal of Clinical Microbiology 2007;45(7):2249–2256. [PubMed: 17494723]

Cubo E. Gabriel y Galán J. M. Villaverde V. A. Velasco S. S. Benito V. D. Macarrón J. V. et al. Prevalence of tics in schoolchildren in central Spain: a population-based study. Pediatric Neurology 2011;45(2):100–108. [PubMed: 21763950]

Cunningham M. W. Streptococcus and rheumatic fever. Current Opinion in Rheumatology 2012;24(4):408–416. [PubMed: 22617826]

Dale R. C. Church A. J. Cardoso F. Goddard E. Cox T. C. Chong W. K. et al. Poststreptococcal acute disseminated encephalomyelitis with basal ganglia involvement and auto-reactive antibasl ganglia antibodies. Annals of Neurology 2001;50(5):588–595. [PubMed: 11706964]

Dale R. C. Merheb V. Pillai S. Wang D. Cantrill L. Murphy T. K. et al. Antibodies to surface dopamine-2 receptor in autoimmune movement and psychiatric disorders. Brain 2012;135(Pt 11): 3453–3468. [PubMed: 23065479]

DeGiorgio L. A. Konstantinov K. N. Lee S. C. Hardin J. A. Volpe B. T. Diamond B. A subset of lupus anti-DNA antibodies cross-reacts with theNR2 glutamate receptor in systemic lupus erythematosus. Nature Medicine 2001;7(11):1189–1193. [PubMed: 11689882]

Dicuonzo G. Gherardi G. Lorino G. Angeletti S. De Cesaris M. Fiscarelli E. et al. Group A streptococcal genotypes from pediatric throat isolates in Rome, Italy. Journal of Clinical Microbiology 2001;39(5):1687–1690. [PubMed: 11325974]

Gabbay V. Coffey B. J. Babb J. S. Meyer L. Wachtel C. Anam S. et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcus: Comparison of diagnosis and treatment in the community and at a Specialty clinic. Pediatrics 2008;122(2):273–278. [PubMed: 18676543]

Gadow K. D. Nolan E. E. Sprafkin J. Schwartz J. Tics and psychiatric comorbidity in children and adolescents. Developmental Medicine & Child Neurology 2002;44(5):330–338. [PubMed: 12033719]

Galvin J. E. Hemric M. E. Ward K. Cunningham M. W. Cytotoxic monoclonal antibody from rheumatic carditis reacts with human endothelium: implications in rheumatic heart disease. The Journal of Clinical Investigation 2000;106(2):217–224. [PubMed: 10903337]

Garvey M. A. Swedo S. E. Sydenham's chorea. Clinical and therapeutic update. Advances in Experimental Medicine and Biology 1997;418:115–120. [PubMed: 9331612]

Garvey M. A. Giedd J. Swedo S. E. PANDAS: the search for environmental triggers of pediatric neuropsychiatric disorders. Lessons from rheumatic fever. Journal of Child Neurology 1998;13(9): 413–423. [PubMed: 9733286]

Garvey M. A. Perlmutter S. J. Allen A. J. Hamburger S. Lougee L. Leonard H. L. et al. A pilot study of penicillin prophylaxis for neuropsychiatric exacerbations triggered by streptococcal infections. Biological Psychiatry 1999;45(12):1564–1571. [PubMed: 10376116]

Garvey M. A. Snider L. A. Leitman S. F. Werden R. Swedo S. E. Treatment of Sydenham's chorea with intravenous immunoglobulin, plasma exchange, or prednisone. Journal of Child Neurology 2005;20(5):424–429. [PubMed: 15968928]

Geller D. A. Obsessive compulsive and spectrum disorders in children and adolescents. Psychiatric Clinics of North America 2006;29(2):353–370. [PubMed: 16650713]

Gerardi D. M. Casadonte J. Patel P. Murphy T. K. PANDAS and comorbid Kleine-Levin syndrome. Journal of Child and Adolescent Psychopharmacology 2015;25(1):93–98. [PubMed: 25329605]

Graybiel A. M. Habits, rituals, and the evaluative brain. Annual Review of Neuroscience 2008;31:359–387. [PubMed: 18558860]

Hampson M. Tokoglu F. King R. A. Constable R. T. Leckman J. F. Brain areas coactivating with motor cortex during chronic motor tics and intentional movements. Biological Psychiatry 2009;65(7):594–599. [PubMed: 19111281]

Hertzén E. Johansson L. Kansal R. Hecht A. Dahesh S. Janos M. et al. Intracellular Streptococcus pyogenes in Human Macrophages Display an Altered Gene Expression Profile. PLoS One 2012;7(4):e35218. [PubMed: 22511985]

Hertzén E. Johansson L. Wallin R. Schmidt H. Kroll M. Rehn A. P. et al. M1 protein-dependent intracellular trafficking promotes persistence and replication of Streptococcus pyogenes in macrophages. Journal of Innate Immunity 2010;2(6):534–545. [PubMed: 20798480]

Hoekstra P. J. Manson W. L. Steenhuis M. P. Kallenberg C. G. Minderaa R. B. Association of common cold with exacerbations in pediatric but not adult patients with tic disorder: A prospective longitudinal study. Journal of Child and Adolescent Psychopharmacology 2005;15(2):285–292. [PubMed: 15910212]

Hoffman K. L. Hornig M. Yaddanapudi K. Jabado O. Lipkin W. I. A murine model for neuropsychiatric disorders associated with group A beta-hemolytic streptococcal infection. The Journal of Neuroscience 2004;24(7):1780–1791. [PubMed: 14973249]

Huerta P. T. Kowal C. DeGiorgio L. A. Volpe B. T. Diamond B. Immunity and behavior: Antibodies alter emotion. Proceedings of the National Academy of Sciences of the United States of America 2006;103(3):678–683. [PubMed: 16407105]

Husby G. van de Rijn I. Zabriskie J. B. Abdin Z. H. Williams R. C. Jr. Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. The Journal of Experimental Medicine 1976;144(4):1094–1110. [PubMed: 789810]

Johnson, D. R., Kaplan, E. L., Sramek, J., Bicova, R., Havlicek, J., Havlickova, H., et al. (1997). *Laboratory diagnosis of group A streptococcal infections.* Geneva: World Health Organization.

Johnson D. R. Kurlan R. Leckman J. Kaplan E. L. The Human Immune Response to Streptococcal Extracellular Antigens: Clinical, Diagnostic, and Potential Pathogenetic Implications. Clinical Infectious Diseases 2010;50(4):481–490. [PubMed: 20067422]

Kaplan E. L. The group A streptococcal upper respiratory tract carrier state: an enigma. The Journal of Pediatrics 1980;97(3):337–345. [PubMed: 6997450]

Kaplan E. L. Chhatwal G. S. Rohde M. Reduced ability of penicillin to eradicate ingested group A streptococci from epithelial cells: Clinical and pathogenetic implications. Clinical Infectious Diseases 2006;43(11):1398–1406. [PubMed: 17083011]

Kaplan E. L. Gastanaduy A. S. Huwe B. B. The role of the carrier in treatment failures after antibiotic for group A streptococci in the upper respiratory tract. Journal of Laboratory and Clinical Medicine 1981;98(3):326–335. [PubMed: 7021717]

Kerbeshian J. Burd L. Pettit R. A possible post-streptococcal movement disorder with chorea and tics. Developmental Medicine & Child Neurology 1990;32(7):642–644. [PubMed: 2391015]

Kiessling L. S. Marcotte A. C. Culpepper L. Anti-neuronal antibodies in movement disorders. Pediatrics 1993;92(1):39–43. [PubMed: 8516083]

Kirvan C. A. Cox C. J. Swedo S. E. Cunningham M. W. Tubulin is a neuronal target of autoantibodies in Sydenham's chorea. The Journal of Immunology 2007;178(11):7412–7421. [PubMed: 17513792]

Kirvan C. A. Swedo S. E. Heuser J. S. Cunningham M. W. Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. Nature Medicine 2003;9(7):914–920. [PubMed: 12819778]

Kirvan C. A. Swedo S. E. Kurahara D. Cunningham M. W. Streptococcal mimicry and antibody-mediated cell signaling in the pathogenesis of Sydenham's chorea. Autoimmunity 2006b;39(1):21–29. [PubMed: 16455579]

Kirvan C. A. Swedo S. E. Snider L. A. Cunningham M. W. Antibody-mediated neuronal cell signaling in behavior and movement disorders. Journal of Neuroimmunology 2006a;179(1-2):173–179. [PubMed: 16875742]

Kondo K. Kabasawa T. Improvement in Gilles de la Tourette syndrome after corticosteroid therapy. Annals of Neurology 1978;4(4):387. [PubMed: 281893]

Kotb M. Fathey N. Aziz R. Rowe S. Williams R. W. Lu L. Unbiased forward genetics and systems biology approaches to understanding how gene-environment interactions work to predict susceptibility and outcomes of infections. Novartis Foundation Symposia 2008;293:156–165, 181-183. [PubMed: 18972751]

Kowal C. DeGiorgio L. A. Nakaoka T. Hetherington H. Huerta P. T. Diamond B. et al. Cognition and immunity: antibody impairs memory. Immunity 2004;21(2):179–188. [PubMed: 15308099]

Krause D. Matz J. Weidinger E. Wagner J. Wildenauer A. Obermeier M. et al. Association between intracellular infectious agents and Tourette's syndrome. European Archives of Psychiatry and Clinical Neuroscience 2010;260(4):359–363. [PubMed: 19890596]

Kurlan R. Johnson D. Kaplan E. L. Tourette Syndrome Study Group. Streptococcal infection and exacerbations of childhood tics and obsessive-compulsive symptoms: a prospective blinded cohort study. Pediatrics 2008;121(6):1188–1197. [PubMed: 18519489]

Kurlan R. McDermott M. P. Deeley C. Como P. G. Brower C. Eapen S. et al. Prevalence of tics in schoolchildren and association with placement in special education. Neurology 2001;57(8):1383–1388. [PubMed: 11673576]

Langlois M. Force L. Revue Neurologique 1965;113(6):641–645. [PubMed: 4379216][Nosologic and clinical revision of Gilles de la Tourette disease evoked by the action of certain neuroleptics on its course]

Leckman J. F. King R. A. Gilbert D. L. Coffey B. J. Singer H. S. Dure L. S. et al. Streptococcal upper respiratory tract infections and exacerbations of tic and obsessive-compulsive symptoms: a

prospective longitudinal study. Journal of the American Academy of Child and Adolescent Psychiatry 2011;50(2):108–118.e3. [PubMed: 21241948]

Leslie D. L. Kozma L. Martin A. Landeros A. Katsovich L. King R. A. et al. Neuropsychiatric disorders associated with streptococcal infection: A case-control study among privately insured children. Journal of the American Academy of Child and Adolescent Psychiatry 2008;47(10): 1166–1172. [PubMed: 18724258]

Lin H. Katsovich L. Ghebremichael M. Findley D. B. Grantz H. Lombroso P. J. et al. Psychosocial stress predicts future symptom severities in children and adolescents with Tourette syndrome and/or obsessive-compulsive disorder. Journal of Child Psychology and Psychiatry 2007;48(2): 157–166. [PubMed: 17300554]

Lin H. Williams K. A. Katsovich L. Findley D. B. Grantz H. Lombroso P. J. et al. Streptococcal upper respiratory tract infections and psychosocial stress predict future tic and obsessive-compulsive symptom severity in children and adolescents with Tourette syndrome and obsessive-compulsive disorder. Biological Psychiatry 2010;67(7):684–691. [PubMed: 19833320]

Lin H. Yeh C. B. Peterson B. S. Scahill L. Grantz H. Findley D. B. et al. Assessment of symptom exacerbations in a longitudinal study of children with Tourette's syndrome or obsessive-compulsive disorder. Journal of the American Academy of Child and Adolescent Psychiatry 2002;41(9):1070–1077. [PubMed: 12218428]

Linazasoro G. Van Blercom N. de Zárate C. O. Prevalence of tic disorder in two schools in the Basque country: Results and methodological caveats. Movement Disorders 2006;21(12):2106–2109. [PubMed: 17013915]

Loiselle C. R. Wendlandt J. T. Rohde C. A. Singer H. S. Antistreptococcal, neuronal, and nuclear antibodies in Tourette syndrome. Pediatric Neurology 2003;28(2):119–125. [PubMed: 12699862]

Lotan D. Benhar I. Alvarez K. Mascaro-Blanco A. Brimberg L. Frenkel D. et al. Behavioral and neural effects of intra-striatal infusion of anti-streptococcal antibodies in rats. Brain, Behavior, and Immunity 2014a;38:249–262. [PubMed: 24561489]

Lotan D. Cunningham M. W. Joel D. Antibiotic treatment attenuates behavioral and neurochemical changes induced by exposure of rats to group a streptococcal antigen. PLoS One 2014b; 9(6):e101257. [PubMed: 24979049]

Luo F. Leckman J. F. Katsovich L. Findley D. Grantz H. Tucker D. M. et al. Prospective longitudinal study of children with tic disorders and/or obsessive-compulsive disorder: relationship of symptom exacerbations to newly acquired streptococcal infections. Pediatrics 2004;113(6):e578–e585. [PubMed: 15173540]

Macerollo A. Martino D. Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS): An Evolving Concept. Tremor and Other Hyperkinetic Movements 2013;3:3. [PubMed: 24106651]

Martino D. Cavanna A. E. Robertson M. M. Orth M. Prevalence and phenomenology of eye tics in Gilles de la Tourette syndrome. Journal of Neurology 2012;259(10):2137–2140. [PubMed: 22434162]

Martino D. Chiarotti F. Buttglione M. Cardona F. Creti R. Nardocci N. et al. The relationship between group A streptococcal infections and Tourette syndrome: a study on a large service-based cohort. Developmental Medicine & Child Neurology 2011;53(10):951–957. [PubMed: 21679362]

Martins T. B. Hoffman J. L. Augustine N. H. Phansalkar A. R. Fischetti V. A. Zabriskie J. B. et al. Comprehensive analysis of antibody responses to streptococcal and tissue antigens in patients with acute rheumatic fever. International Immunology 2008;20(3):445–452. [PubMed: 18245783]

Mell L. K. Davis R. L. Owens D. Association between streptococcal infection and obsessive-compulsive disorder, Tourette's syndrome, and tic disorder. Pediatrics 2005;116(1):56–60. [PubMed: 15995031]

Menzies L. Chamberlain S. R. Laird A. R. Thelen S. M. Sahakian B. J. Bullmore E. T. Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: The orbitofronto-striatal model revisited. Neuroscience & Biobehavioral Reviews 2008;32(3): 525–549. [PubMed: 18061263]

Morris C. M. Pardo-Villamizar C. Gause C. D. Singer H. S. Serum autoantibodies measured by immunofluorescence confirm a failure to differentiate PANDAS and Tourette syndrome from controls. Journal of the Neurological Sciences 2009;276(1-2):45–48. [PubMed: 18823914]

Morris-BerryC. M.PollardM.GaoS.ThompsonC.Tourette Syndrome Study GroupSinger, H. S2013Anti-streptococcal, tubulin, and dopamine receptor 2 antibodies in children with PANDAS and Tourette syndrome: Single-point and longitundinal assessments. Journal of Neuroimmunology2641-2106113 [PubMed: 24080310]

Morshed S. A. Parveen S. Leckman J. F. Mercadante M. T. Bittencourt Kiss M. H. Miguel E. C. et al. Antibodies against neural, nuclear, cytoskeletal, and streptococcal epitopes in children and adults with Tourette's syndrome, Sydenham's chorea, and autoimmune disorders. Biological Psychiatry 2001;50(8):566–577. [PubMed: 11690591]

Müller N. Kroll B. Schwarz M. J. Riedel M. Straube A. Lütticken R. et al. Increased titers of antibodies against streptococcal M12 and M19 proteins in patients with Tourette's syndrome. Psychiatry Research 2001;101(2):187–193. [PubMed: 11286821]

Müller N. Riedel M. Blendinger C. Oberle K. Jacobs E. Abele-Horn M. Mycoplasma pneumoniae infection and Tourette's syndrome. Psychiatry Research 2004;129(2):119–125. [PubMed: 15590039]

Murphy M. L. Pichichero M. E. Prospective identification and treatment of children with pediatric autoimmune neuropsychiatric disorder associated with group A streptococcal infection (PANDAS). Archives of Pediatrics and Adolescent Medicine 2002;156(4):356–361. [PubMed: 11929370]

Murphy, T. K. (2013). Infections and tic disorders. In D. Martino, & J. F. Leckman (Eds.), *Tourette Syndrome* (pp. 168-201). New York: Oxford University Press.

Murphy T. K. Parker-Athill E. C. Lewin A. B. Storch E. A. Mutch P. J. Cefdinir for recent-onset pediatric neuropsychiatric disorders: a pilot randomized trial. Journal of Child and Adolescent Psychopharmacology 2015a;25(1):57–64. [PubMed: 25299463]

Murphy T. K. Patel P. D. McGuire J. F. Kennel A. Mutch P. J. Parker-Athill E. C. et al. Characterization of the pediatric acute-onset neuropsychiatric syndrome phenotype. Journal of Child and Adolescent Psychopharmacology 2015b;25(1):14–25. [PubMed: 25314221]

Murphy T. K. Sajid M. Soto O. Shapira N. Edge P. Yang M. et al. Detecting pediatric autoimmune neuropsychiatric disorders associated with streptococcus in children with obsessive-compulsive disorder and tics. Biological Psychiatry 2004;55(1):61–68. [PubMed: 14706426]

Murphy T. K. Snider L. A. Mutch P. J. Harden E. Zaytoun A. Edge P. J. et al. Relationship of movements and behaviors to Group A Streptococcus infections in elementary school children. Biological Psychiatry 2007;61(3):279–284. [PubMed: 17126304]

Murphy T. K. Storch E. A. Lewin A. B. Edge P. J. Goodman W. K. Clinical factors associated with Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections. Journal of Pediatrics. The Journal of Pediatrics 2012;160(2):314–319. [PubMed: 21868033]

Park H. S. Francis K. P. Yu J. Cleary P. P. Membranous cells in nasal-associated lymphoid tissue: A portal of entry for the respiratory mucosal pathogen group A streptococcus. The Journal of Immunology 2003;171(5):2532–2537. [PubMed: 12928403]

Perlmutter S. J. Leitman S. F. Garvey M. A. Hamburger S. Feldman E. Leonard H. L. et al. Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. Lancet 1999;354(9185):1153–1158. [PubMed: 10513708]

Peterson B. Riddle M. A. Cohen D. J. Katz L. D. Smith J. C. Hardin M. T. et al. Reduced basal ganglia volumes in Tourette's syndrome using three-dimensional reconstruction techniques from magnetic resonance images. Neurology 1993;43(5):941–949. [PubMed: 8492950]

Pichichero M. E. Marsocci S. M. Murphy M. L. Hoeger W. Green J. L. Sorrento A. Incidence of streptococcal carriers in private pediatric practice. Archives of Pediatric and Adolescent Medicine 1999;153(6):624–628. [PubMed: 10357305]

Riedel M. Straube A. Schwatz M. J. Wilske B. Müller N. Lyme disease presenting as Tourette's syndrome. Lancet 1998;351(9100):418–419. [PubMed: 9482302]

Roberts A. L. Connolly K. L. Kirse D. J. Evans A. K. Poehling K. A. Peters T. R. et al. Detection of group A Streptococcus in tonsils from pediatric patients reveals high rate of asymptomatic streptococcal carriage. BMC Pediatrics 2012;12:3. [PubMed: 22230361]

Saxena S. Rauch S. L. Functional neuroimaging and the neuroanatomy of obsessive-compulsive disorder. Psychiatric Clinics of North America 2000;23(3):563–586. [PubMed: 10986728]

Schrag A. Gilbert R. Giovannoni G. Robertson M. M. Metcalfe C. Ben-Shlomo Y. Streptococcal infection, Tourette syndrome, and OCD: is there a connection? Neurology 2009;73(16):1256–1263. [PubMed: 19794128]

Segarra A. R. Murphy T. K. Cardiac involvement in children with PANDAS. Journal of the American Academy of Child and Adolescent Psychiatry 2008;47(5):603–604. [PubMed: 18438188]

Sela S. Neeman R. Keller N. Barzilai A. Relationship between asymptomatic carriage of Streptococcus pyogenes and the ability of the strains to adhere to and be internalised by cultured epithelial cells. Journal of Medical Microbiology 2000;49(6):499–502. [PubMed: 10847202]

Selling L. The role of infection in the etiology of tics. Archives of Neurology & Psychiatry 1929;22:1163–1171.

Shaikh N. Leonard E. Martin J. M. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-anaylsis. Pediatrics 2010;126(3):e557–e564. [PubMed: 20696723]

Shulman S. T. Stollerman G. Beall B. Dale J. B. Tanz R. R. Temporal changes in streptococcal M protein types and the near-disappearance of acute rheumatic fever in the United States. Clinical Infectious Diseases 2006;42(4):441–447. [PubMed: 16421785]

Singer H. S. Giuliano J. D. Zimmerman A. M. Walkup J. T. Infection: a stimulus for tic disorders. Pediatric Neurology 2000;22(5):380–383. [PubMed: 10913730]

Singer H. S. Mascaro-Blanco A. Alvarez K. Morris-Berry C. Kawikova I. Ben-Pazi H. et al. Neuronal antibody biomarkers for Sydenham's chorea identify a new group of children with chronic recurrent episodic acute exacerbations of tic and obsessive compulsive symptoms following a streptococcal infection. PLoS One 2015;10(3):e0120499. [PubMed: 25793715]

Sjöholm K. Karlsson C. Linder A. Malmström J. A comprehensive analysis of the Streptococcus pyogenes and human plasma protein interaction network. Molecular BioSystems 2014;10(7):1698–1708. [PubMed: 24525632]

Snider L. A. Swedo S. E. PANDAS: current status and directions for research. Molecular Psychiatry 2004;9(10):900–907. [PubMed: 15241433]

Snider L. A. Lougee L. Slattery M. Grant P. Swedo S. E. Antibiotic prophylaxis with azithromycin or penicillin for childhood-onset neuropsychiatric disorders. Biological Psychiatry 2005;57(7):788–792. [PubMed: 15820236]

Snider L. A. Sachdev V. MacKaronis J. E. St Peter M. Swedo S. E. Echocardiographic findings in the PANDAS subgroup. Pediatrics 2004;114(6):e748–e751. [PubMed: 15545618]

Snider L. A. Seligman L. D. Ketchen B. R. Levitt S. J. Bates L. R. Garvey M. A. et al. Tics and problem behaviors in schoolchildren: prevalence, characterization, and associations. Pediatrics 2002;110(2 Pt 1):331–336. [PubMed: 12165586]

Sowell E. R. Kan E. Yoshii J. Thompson P. M. Bansal R. Xu D. et al. Thinning of sensorimotor cortices in children with Tourette syndrome. Nature Neuroscience 2008;11(6):637–639. [PubMed: 18488025]

Staali L. Mörgelin M. Björck L. Tapper H. Streptococcus pyogenes expressing M and M-like surface proteins are phagocytosed but survive inside human neutrophils. Cellular Microbiology 2003;5(4):253–265. [PubMed: 12675683]

Swedo S. E. Sydenham's chorea: A model for childhood autoimmune neuropsychiatric disorders. JAMA 1994;272(22):1788–1791. [PubMed: 7661914]

Swedo S. E. Leckman J. F. Rose N. R. From research subgroup to clinical syndrome: modifying the PANDAS criteria to describe PANS (pediatric acute-onset neuropsychiatric syndrome). Pediatrics & Therapeutics 2012;2:113.

Swedo S. E. Leonard H. L. Garvey M. Mittleman B. Allen A. J. Perlmutter S. et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. American Journal of Psychiatry 1998;155(2):264–271. [PubMed: 9464208]

Swedo S. E. Rapoport J. L. Cheslow D. L. Leonard H. L. Ayoub E. M. Hosier D. M. et al. High prevalence of obsessive-compulsive symptoms in patients with Sydenham's chorea. The American Journal of Psychiatry 1989;146(2):246–249. [PubMed: 2912267]

Tanz R. R. Shulman S. T. Chronic pharyngeal carriage of group A streptococci. The Pediatric Infectious Disease Journal 2007;26(2):175–176. [PubMed: 17259882]

Taranta A. Moody M. D. Diagnosis of streptococcal pharyngitis and rheumatic fever. Pediatric Clinics of North America 1971;18(1):125–143. [PubMed: 25868179]viii.

Taranta A. Stollerman G. H. The relationship of Sydenham's chorea to infection with group A streptococci. The American Journal of Medicine 1956;20(2):170–175. [PubMed: 13282936]

Toufexis M. D. Hommer R. Gerardi D. M. Grant P. Rothschild L. D'Souza P. et al. Disordered eating and food restrictions in children with PANDAS/PANS. Journal of Child and Adolescent Psychopharmacology 2015;25(1):48–56. [PubMed: 25329522]

von Economo, C. (1931). *Encephalitis lethargica, its sequelae and treatment.* Oxford: Oxford University Press.

Wang B. Li S. Southern P. J. Cleary P. P. Streptococcal modulation of cellular invasion via TGF-beta1 signaling. Proceedings of the National Academy of Sciences of the United States of America 2006;103(7):2380–2385. [PubMed: 16467160]

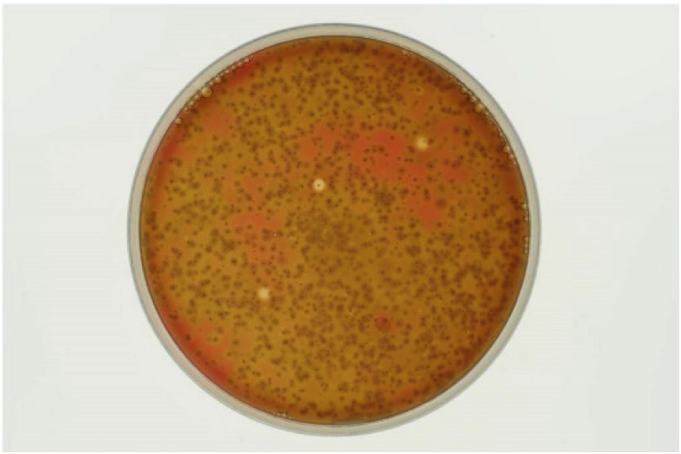
Yaddanapudi K. Hornig M. Serge R. De Miranda J. Baghban A. Villar G. et al. Passive transfer of streptococcus-induced antibodies reproduces behavioral disturbances in a mouse model of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection. Molecular Psychiatry 2010;15(7):712–726. [PubMed: 19668249]

Zuccolo. (2015). Manuscript in preparation.

Streptococcus pyogenes

Streptococcus pyogenes

A.



B.

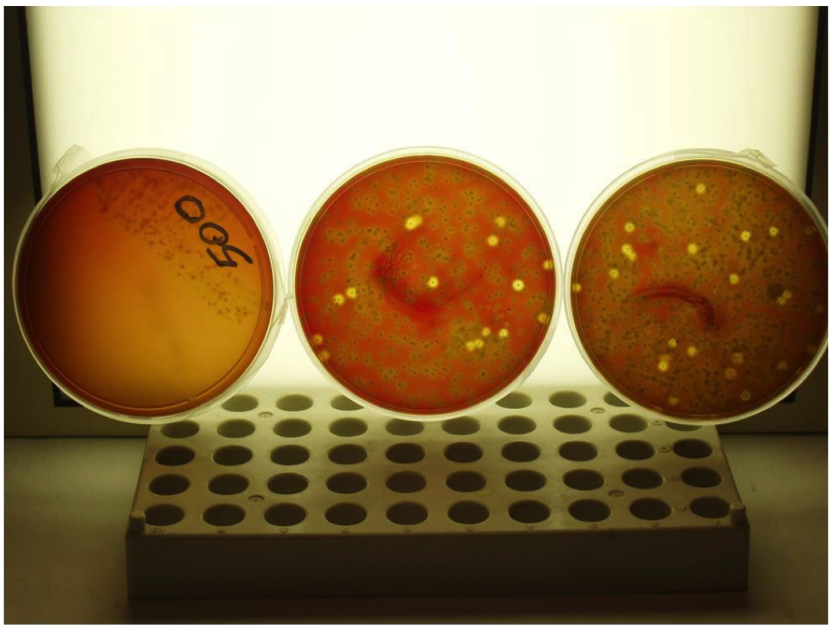


Figure 1.

A. 300 *S. pyogenes* colony-forming units (CFUs) were suspended in 5 mL Todd Hewitt broth in an 0.1mL plated (pour plate) in blood Columbia agar. The recovery was about 50%. **B.** 500 *S. pyogenes* CFUs put in 5 mL Todd Hewitt Broth. 0.1mL and 0.2 ml were pour plated in blood Columbia agar and compared to 0.1 mL directly streaked on the surface.

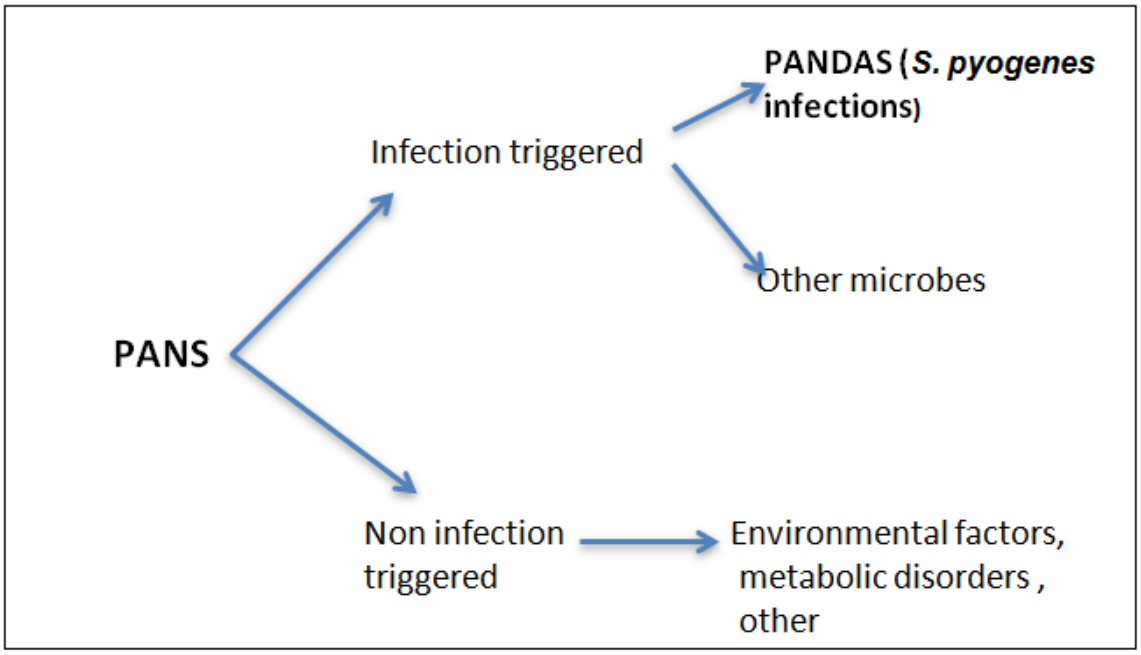


Figure 2.
Evolution criteria of PANDAS and PANS (modified from (Swedo, Leckman, & Rose, 2012)

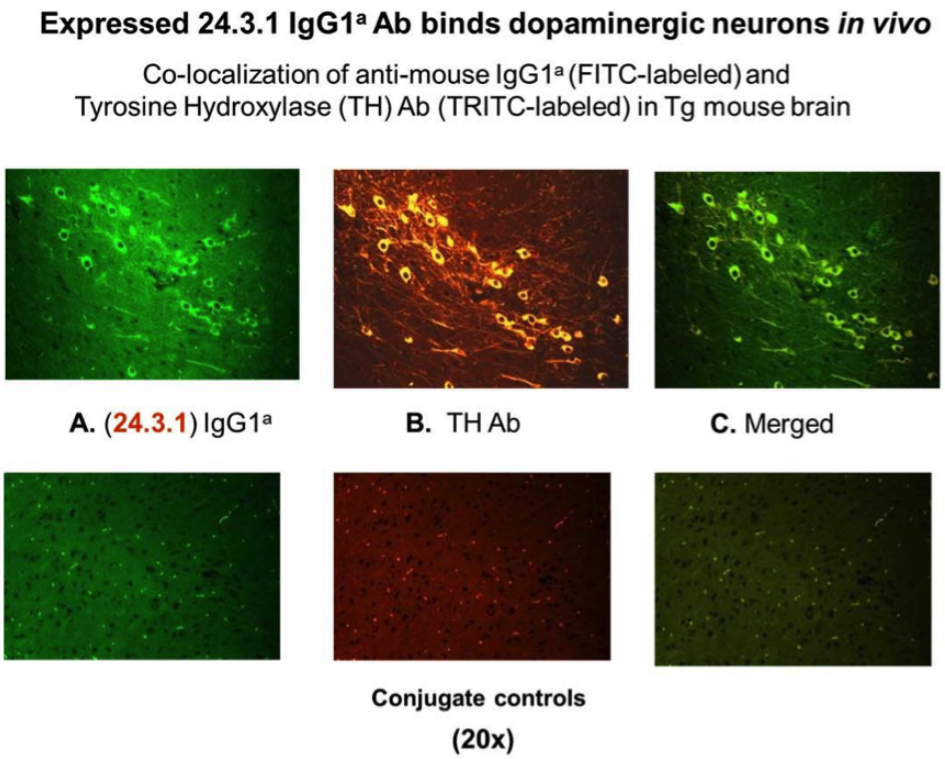


Figure 3.
Human Sydenham chorea 24.3.1 V gene expressed as a human V gene-mouse IgG1a constant region in Transgenic(Tg) mice targets dopaminergic neurons in the basal ganglia (most likely substantia nigra, based on location). Chimeric Tg24.3.1 VH

IgG1a Ab expressed in Tg mouse sera penetrated dopaminergic neurons in Tg mouse brain in vivo. Colocalization of Tg 24.3.1 IgG1a (anti-IgG1a Ab, green Left Panel) and Tyrosine Hydroxylase Antibody (anti-TH Ab, yellow Middle Panel). TH is a marker for dopaminergic neurons. Left panel shows IgG1a (FITC labeled), center panel shows TH Ab (TRITC labeled), and right panel is merged image (FITC-TRITC). Brain sections (basal ganglia) of VH24.3.1 Tg mouse (original magnification 320), showing FITC labeled anti-mouse IgG1a (A), TRITC-labeled anti-TH Ab(B), and merged image (C). Controls treated with secondary antibody are negative. Figure 3 is similar to the figure shown in Cox et al. (Cox, et al., 2013).

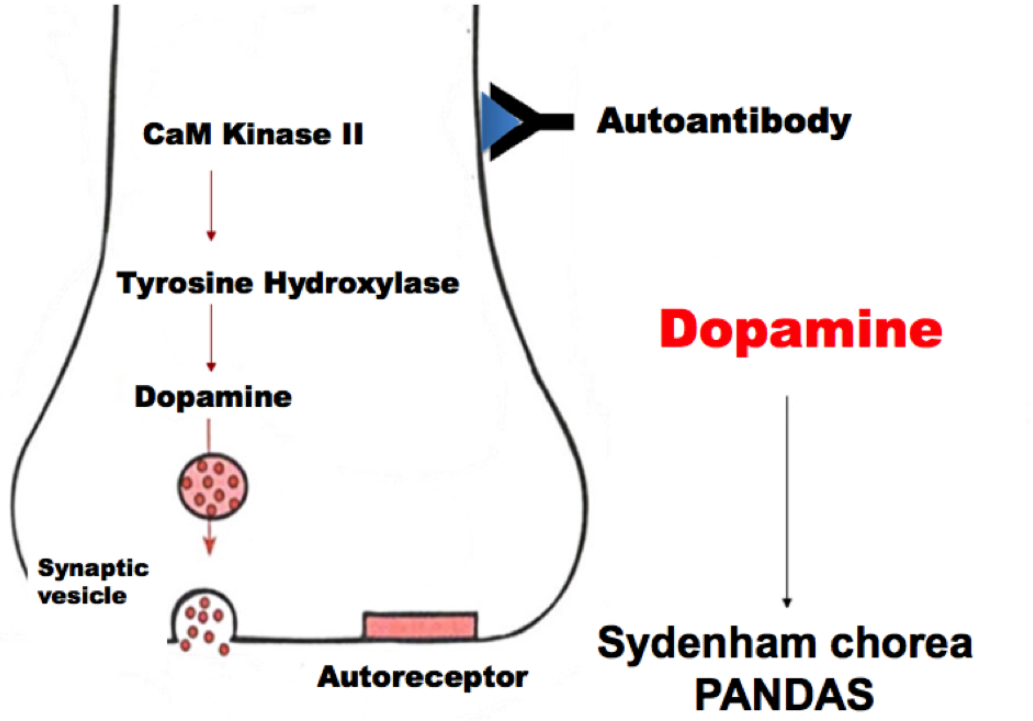


Figure 4.

Simplified illustration of a potential pathogenic mechanism of antibody mediated neuronal cell signaling in Sydenham chorea and PANDAS. Antineuronal antibody (IgG) may bind to receptors (blue triangle) on the surface of neuronal cells and trigger the signaling cascade of CaMKII, tyrosine hydroxylase, and dopamine release, which may potentially lead to excess dopamine and the manifestations of Sydenham chorea. Similar to figure shown in Cunningham 2012 review (Cunningham, 2012).

Table 1:

Historical PANDAS diagnostic criteria (from (Swedo, et al., 1998))

All five criteria must be met	
	Presence of obsessive-compulsive disorder (OCD) and/or a tic disorder
	Prepubertal symptom onset
	Acute symptom onset and episodic (relapsing remitting) course
	Temporal association between S. pyogenes infection and symptom onset /exacerbation
	Associated with neurologic abnormalities (choreiform movements, motoric hyperactivity)

Streptococcus pyogenes

Streptococcus pyogenes

Table 2:

Studies to establish the association of *S. pyogenes* and other infections or stress situation with some neuropsychiatric disorder (tics/OCD, TS)

	Pros/Conclusive	**Cons/Inconclusive**
Anti-*S. pyogenes* antibodies and neuropsychiatric behaviors in animal models of group A streptococcal immunization and passive antibody transfer	(Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004) (Yaddanapudi, et al., 2010) (Brimberg, et al., 2012) (Lotan, et al., 2014a) (Lotan, Cunningham, & Joel, 2014b)	
Retrospective studies associate *S. pyogenes* with tics and OCD	(Mell, Davis, & Owens, 2005) (Singer, Giuliano, Zimmerman, & Walkup, 2000) (Cox, et al., 2015)	(Schrag, et al., 2009)
Cross sectional studies associate *S. pyogenes* with tics and OCD	(Swedo, et al., 1998) (Cardona & Orefici, Group A streptococcal infections and tic disorders in an Italian pediatric population, 2001) (Cardona, et al., 2007) (Garvey, Giedd, & Swedo, 1998) (Macerollo & Martino, 2013)	
Longitudinal studies associate *S. pyogenes* with tics and OCD	(Murphy & Pichichero, 2002) (Murphy, et al., 2004) (Martino, et al., 2011) (Murphy, et al., 2007) (Singer, et al., 2015)	(Luo, et al., 2004) (Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008) (Leckman, et al., 2011) (Morris-Berry, et al., 2013)
Antibodies against streptococcal antigens in tics and OCD	(Morshed, et al., 2001) (Müller, et al., 2001) (Church, Dale, Lees, Giovannoni, & Robertson, 2003) (Lin, et al., 2010) (Martino, et al., 2011) (Bombaci, et al., 2009)	(Loiselle, Wendlandt, Rohde, & Singer, 2003)
Antibodies against anti-basal ganglia in *S. pyogenes* sequelae, tics and OCD	(Dale, et al., 2001) (Kirvan, Swedo, Heuser, & Cunningham, 2003) (Kirvan, Swedo, Snider, & Cunningham, 2006a) (Martino, et al., 2011) (Dale, et al., 2012) (Cox, et al., 2013)	(Loiselle, Wendlandt, Rohde, & Singer, 2003) (Morris, Pardo-Villamizar, Gause, & Singer, 2009)
Other pathogens associated with tics and OCD	(Riedel, Straube, Schwatz, Wilske, & Müller, 1998) (Müller, et al., 2004) (Hoekstra, Manson, Steenhuis, Kallenberg, & Minderaa, 2005) (Krause, et al., 2010)	
Involvement of psychosocial stress in tics and OCD	(Chappell, et al., 1994) (Lin, et al., 2007) (Corbett, Mendoza, Baym, Bunge, & Levine, 2008) (Buse, Kirschbaum, Leckman, Münchau, & Roessner, 2014)	

Published in final edited form as:
JAMA. 2009 September 9; 302(10): 1084–1091. doi:10.1001/jama.2009.1308.

Evaluating Dopamine Reward Pathway in ADHD:

Clinical Implications

Dr. Nora D. Volkow, MD, **Dr. Gene-Jack Wang, MD**, **Dr. Scott H. Kollins, PhD**, **Dr. Tim L. Wigal, PhD**, **Dr. Jeffrey H. Newcorn, MD**, **Dr. Frank Telang, MD**, **Dr. Joanna S. Fowler, PhD**, **Dr. Wei Zhu, PhD**, **Dr. Jean Logan, PhD**, **Dr. Yeming Ma, PhD**, **Dr. Kith Pradhan, MS**, **Dr. Christopher Wong, MS**, and **Dr. James M. Swanson, PhD**

National Institute on Drug Abuse (Dr Volkow) and Laboratory of Neuroimaging, National Institute on Alcohol Abuse and Alcoholism (Drs Volkow, Telang, and Ma), Bethesda, Maryland; Medical and Chemistry Departments, Brookhaven National Laboratory, Upton, New York (Drs Wang, Fowler, and Logan, Messrs Pradhan and Wong); Department of Psychiatry, Mount Sinai Medical Center, New York, New York (Drs Wang, Newcorn, and Fowler); Department of Psychiatry, Duke University Medical Center, Durham, North Carolina (Dr Kollins); Child Development Center, University of California, Irvine (Drs Wigal and Swanson); Department of Applied Mathematics and Statistics, State University of New York at Stony Brook, Stony Brook (Dr Zhu)

Abstract

Context—Attention-deficit/hyperactivity disorder (ADHD)—characterized by symptoms of inattention and hyperactivity-impulsivity—is the most prevalent childhood psychiatric disorder that

Corresponding Author: Nora D. Volkow, MD, National Institute on Drug Abuse, 6001 Executive Blvd, Room 5274, MSC 9581, Bethesda, MD 20892 (nvolkow@nida.nih.gov).

Author Contributions: Dr Volkow had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Volkow, Wang, Wigal, Newcorn, Swanson.
Acquisition of data: Wang, Kollins, Wigal, Newcorn, Telang, Fowler, Pradhan.
Analysis and interpretation of data: Volkow, Wang, Kollins, Wigal, Newcorn, Zhu, Logan, Ma, Wong, Swanson.
Drafting of the manuscript: Volkow, Wang, Fowler.
Critical revision of the manuscript for important intellectual content: Volkow, Wang, Kollins, Wigal, Newcorn, Telang, Zhu, Logan, Ma, Pradhan, Wong, Swanson.
Statistical analysis: Zhu, Wong, Swanson.
Obtained funding: Volkow, Wang, Newcorn.
Administrative, technical, or material support: Wang, Kollins, Wigal, Telang, Fowler, Ma, Swanson.
Study supervision: Wang, Kollins, Wigal, Fowler.

Financial Disclosures: Dr Kollins reported receiving research support, consulting fees, or both from the following sources: Addrenex Pharmaceuticals, Otsuka Pharmaceuticals, Shire Pharmaceuticals, NIDA, NIMH, NINDS, NIEHS, EPA. Dr Newcorn reported being a recipient of research support from Eli Lilly and Ortho-McNeil Janssen, serves as a consultant, advisor, or both for Astra Zeneca, BioBehavioral Diagnostics, Eli Lilly, Novartis, Ortho-McNeil Janssen, and Shire and as a speaker for Ortho-McNeil Janssen. Dr Swanson reported receiving support from Alza, Richwood, Shire, Celgene, Novartis, Celltech, Gliatech, Cephalon, Watson, CIBA, Janssen, and McNeil; has been on advisory boards of Alza, Richwood, Shire, Celgene, Novartis, Celltech, UCB, Gliatech, Cephalon, McNeil, and Eli Lilly; has been on the speakers bureaus of Alza, Shire, Novartis, Cellthech, UCB, Cephalon, CIBA, Janssen, and McNeil; and has consulted to Alza, Richwood, Shire, Clegene, Novarits, Celltech, UCB, Gliatech, Cephalon, Watson, CIBA, Jansen, McNeil and Eli Lilly. Dr Wigal reported receiving support from Eli Lilly, McNeil, Novartis, and Shire. No other financial disclosures were reported.

Additional information: The eTable is available at http://www.jama.com.

Additional Contributions: We thank the following BNL employees: Donald Warner for PET operations; David Schlyer and Michael Schueller for cyclotron operations; Pauline Carter, Millard Jayne, and Barbara Hubbard for nursing care; Payton King for plasma analysis; and Lisa Muench, Youwen Xu, and Colleen Shea for radiotracer preparation; and Karen Appelskog-Torres for protocol coordination. We also thank Duke employees Joseph English and Allan Chrisman for participant recruitment and evaluation; and NIH employee Linda Thomas for editorial assistance. We also thank the individuals who volunteered for these studies. None of the authors or the individuals acknowledged was compensated for their contributions other than their salaries.

frequently persists into adulthood, and there is increasing evidence of reward-motivation deficits in this disorder.

Objective—To evaluate biological bases that might underlie a reward/motivation deficit by imaging key components of the brain dopamine reward pathway (mesoaccumbens).

Design, Setting, and Participants—We used positron emission tomography to measure dopamine synaptic markers (transporters and D_2/D_3 receptors) in 53 nonmedicated adults with ADHD and 44 healthy controls between 2001–2009 at Brookhaven National Laboratory.

Main Outcome Measures—We measured specific binding of positron emission tomographic radioligands for dopamine transporters (DAT) using [^{11}C]cocaine and for D_2/D_3 receptors using [^{11}C]raclopride, quantified as binding potential (distribution volume ratio −1).

Results—For both ligands, statistical parametric mapping showed that specific binding was lower in ADHD than in controls (threshold for significance set at $P<.005$) in regions of the dopamine reward pathway in the left side of the brain. Region-of-interest analyses corroborated these findings. The mean (95% confidence interval [CI] of mean difference) for DAT in the nucleus accumbens for controls was 0.71 vs 0.63 for those with ADHD (95% CI, 0.03–0.13, P=.004) and in the midbrain for controls was 0.16 vs 0.09 for those with ADHD (95% CI, 0.03–0.12; $P \leq .001$); for D_2/D_3 receptors, the mean accumbens for controls was 2.85 vs 2.68 for those with ADHD (95% CI, 0.06–0.30, P=.004); and in the midbrain, it was for controls 0.28 vs 0.18 for those with ADHD (95% CI, 0.02–0.17, P=.01). The analysis also corroborated differences in the left caudate: the mean DAT for controls was 0.66 vs 0.53 for those with ADHD (95% CI, 0.04–0.22; P=.003) and the mean D_2/D_3 for controls was 2.80 vs 2.47 for those with ADHD (95% CI, 0.10–0.56; P=.005) and differences in D_2/D_3 in the hypothalamic region, with controls having a mean of 0.12 vs 0.05 for those with ADHD (95% CI, 0.02–0.12; P=.004). Ratings of attention correlated with D_2/D_3 in the accumbens (r =0.35; 95% CI, 0.15–0.52; P =.001), midbrain (r=0.35; 95% CI, 0.14–0.52; P=.001), caudate (r=0.32; 95% CI, 0.11–0.50; P=.003), and hypothalamic (r=0.31; CI, 0.10–0.49; P=.003) regions and with DAT in the midbrain (r=0.37; 95% CI, 0.16–0.53; $P \leq .001$).

Conclusion—A reduction in dopamine synaptic markers associated with symptoms of inattention was shown in the dopamine reward pathway of participants with ADHD.

Attention-deficit/hyperactivity disorder (ADHD) is characterized by symptoms of inattention, hyperactivity, or impulsivity that produce impairment across cognitive, behavioral, and interpersonal domains.[1] Although for many years it was believed to be a disorder of childhood and adolescence, it is now recognized to also occur in adulthood. It is estimated that ADHD affects 3% to 5% of the US adult population,[2] which makes it one of the most prevalent of all psychiatric disorders.

Genetic and environmental etiologies that implicate the neurotransmitter dopamine have been proposed for ADHD.[3] Genetic studies have identified a few genes with polymorphisms associated with ADHD, with the most replicated being 2 dopamine genes (eg, *DRD4* and *DAT 1* genes),[3] and environmental studies have identified important non-genetic risk factors (eg, maternal smoking during pregnancy and lead levels) that also may affect the dopamine systems of the brain.[4] Evidence from brain imaging studies have shown that brain dopamine neurotransmission is disrupted in ADHD[5–9] and that these deficits may underlie core symptoms of inattention[8] and impulsivity.[9]

There is also increased awareness that patients with ADHD may have reward and motivation deficits.[10–12] Although defined in different way sacross studies, this reward-motivation deficitis typically characterized by abnormal behavior change following conditions of reward and punishment. For example, compared with nondiagnosed children, those with ADHD do not modify their behavior in the face of changing reward conditions.[13] The mesoaccumbens dopamine pathway, which projects from the ventral tegmental area (VTA) in the midbrain to

the nucleus accumbens is critically involved in reward and motivation[14] and has been hypothesized to underlie the reward and motivational deficits observed in ADHD.[11,15] Indeed recent functional magnetic resonance imaging (fMRI) studies showed decreased nucleus accumbens activation with processing of reward in participants with ADHD.[16,17] However, to our knowledge no study has directly measured synaptic dopamine markers in the accumbens region of individuals with ADHD.

Based on this, we hypothesized abnormalities in the mesoaccumbens dopamine pathway (composed of dopamine cells in the midbrain and their projections to the accumbens) in ADHD. To test this hypothesis, we evaluated dopamine D_2/D_3 receptor(dopamine postsynaptic marker) and DAT(dopamine presynaptic marker) availability in these brain regions in 53 adult participants with ADHD (never medicated) and 44 non-ADHD controls using positron emission tomography (PET) and both [^{11}C]raclopride and [^{11}C]cocaine (D_2/D_3 receptor and DAT radioligands respectively).[18,19]

METHODS

Participants

The PET imaging was carried out at Brookhaven National Laboratory and patient recruitment and evaluation occurred at Duke University, Mount Sinai Medical Center, and University of California, Irvine, from 2001–2009. Institutional review board approval was obtained from all participating institutions. Written informed consent was obtained from all participants after the study had been fully explained to them. Participants were paid for their participation. We studied 53 never-medicated ADHD patients (including 20 described in a prior report of striatal DAT and dopamine release[6,8]) and 44 healthy controls. Participants with ADHD were recruited from clinical referrals to the ADHD programs at each institution.

To minimize confounding from prior drug exposures or comorbidity, participants were excluded if they had a prior history of substance abuse (other than nicotine) or with positive urine drug screen results, prior or current treatment with psychotropic medications (including stimulants), psychiatric comorbidities (axis I or II diagnosis other than ADHD), neurological disease, medical conditions that may alter cerebral function (ie, cardiovascular, endocrinological, oncological, or autoimmune diseases), or head trauma with loss of consciousness (>30 minutes). These rigorous exclusion criteria contributed to the length of the study (from 2001 to 2009).

Two clinicians interviewed the patients to ensure that *Diagnostic and Statistical Manual of Mental Disorders*(Fourth Edition) (*DSM-IV*) diagnostic criteria were met, including the presence of at least 6 of 9 inattention symptoms (with or without 6 of 9 hyperactive or impulsive symptoms) as ascertained with a semi-structured psychiatric interview using modifications for adult prompts of ADHD behaviors. The Clinical Global Impressions Severity scale[20] was used to assess over all impairment. For diagnosis, ADHD participants were required to have atleast a moderate severity level of 4 or greater. In addition, evidence was required from each participant's history that some symptoms of ADHD started before age 7 years. Controls were recruited from advertisements in the local newspapers and met the same exclusion criteria but not the inclusion criteria for diagnosis of ADHD. Controls were excluded if they described symptoms of inattention or hyperactivity that interfered with everyday activities. Table 1 provides demographic and clinical characteristics of the participants.

Clinical Scales

The *DSM-IV* ADHD items were assessed using the Strengths and Weaknesses of ADHD-symptoms and Normal-behavior (SWAN) rating scale, which uses a positive scale for

symptoms (1 to 3) and a negative scale for the opposite of the symptoms (−1 to −3) ranging from far below average to far above average.[21] This allows one to assess the full range of functioning in the 2 domains of ADHD defined as dimensions in the population (ie, attention and activity or reflectivity) to be assessed rather than the severity of psychopathology related to presence of inattention and hyperactivity-impulsivity symptoms in those with ADHD. The range for the scores of the SWANis-3 to 3. The psychometric properties of the SWAN rating scale are superior to those of truncated symptom-severity ratings scales.[22] Ratings on the SWAN were completed on 46 ADHD participants and 38 controls and were used to assess the correlations between these dimensions across all participants and the PET dopamine measures (Table 1).

Also obtained was the Conners Adult ADHD Rating Scale long version, which provides self-assessment of severity of ADHD symptoms on a 4-point scale (not at all, 0; just a little, 1; pretty much, 2; and very much, 3). Eight scores are provided (range of possible scores): A, inattention/memory problems (0–36); B, hyperactivity/restlessness (0–36); C, impulsivity/emotional lability (0–36); D, problems with self-concept (0–18); E, *DSM-IV* inattentive symptoms (0–27); F, *DSM-IV* hyperactive-impulsive symptoms (0–27); G, *DSM-IV* symptom total (0–54); and H, ADHD index (0–36).[23] This rating system has been widely used in clinical and research settings and has well-established factor structure, reliability, and validity (Table 1).[24]

PET Scans

A Siemens HR^+ tomograph was used (Siemens/CTIKnoxville, Tennessee; resolution 4.5 × 4.5 × 4.5 mm full width half-maximum). Dynamic scans were started immediately after injection of 4 to 10 mCi of [^{11}C]raclopride (specific activity 0.5–1.5 Ci/μM at end of bombardment) and after injection of 4 to 8 mCi of [^{11}C]cocaine (specific activity >0.53 Ci/μmol at end of bombardment) and were obtained for a total of 60 minutes as previously described.[18,19] Arterial blood was obtained to measure the concentration of unchanged [^{11}C]raclopride[18] and [^{11}C]cocaine[19] inplasma. Forth is study,[^{11}C]cocaine was chosen as the DAT radioligand because its specific binding is selective for DAT (its binding is inhibited by drugs that block the DAT but not by drugs the block the norepinephrine or the serotonin transporters)[25]; it provides with reproducible measures when participants are tested on separate occasions[19] and its kinetics are ideal for in vivo quantification.[26] Moreover, its synthesis is very reliable, which is important when conducting complex multitracer studies like those performed in this study.

Image Analysis and Statistics

The[^{11}C]raclopride and the[^{11}C]cocaine images were transformed into distribution volume ratio images by computing the total distribution volume in each pixel and then dividing it by the distribution volume in the cerebellum. To obtain the distribution volume, circular regions in the cerebellar hemispheres were extracted in 2 planes located at −28 mm and −36 mm from the intercommissural plane. The cerebellar regions were then projected to the dynamic scans to obtain concentrations of ^{11} C vs time, which along with the concentration of unchanged tracer in plasma were used to calculate the distribution volume in the cerebellum, using a graphical analysis technique for reversible systems.[26] B_{max}/K_d (distribution volume ratio −1, for which K_d and B_{max} are the effective in vivo constants in the presence of endogenous neurotransmitter and nonspecific binding) was used as the measure of D_2/D_3 receptor and DAT availability.[26] The ratio B_{max}/K_d measured in this way is referred to as the binding potential, BP_{ND}. Also measured was the plasma-to-tissue transfer constant (K_1) in striatum and cerebellum for both radioligands using the graphical analysis technique.[26]

Statistical parametric mapping [27] was used to assess the differences in the distribution volume ratio images (for both [^{11}C]raclopride and [^{11}C]cocaine images) between controls and

participants with ADHD without an a priori selection of anatomical brain regions. For this purpose the distribution volume ratio images were spatially normalized using the Montreal Neurological Institute template provided in the statistical parametric mapping 99 package (Wellcome Trust Centre for Neuroimaging, London, England) and subsequently smoothed with a 16-mm isotropic Gaussian kernel. Independent samples *t* tests were performed to compare the differences between groups. Significance was set at $P<.005$ (cluster corrected > 100 voxels) and statistical maps were overlaid on an MRI structural image.

Significance detected by statistical parametric mapping was corroborated with independently drawn region-of-interest analyses using templates from the Talairach Daemon database.[28] Figure 1 shows the location of the region of interest used for this analysis. Differences in D_2/D_3 receptor and DAT availability were assessed with independent samples *t* tests (2 tailed).

Pearson product-moment correlations were used to assess the relationship between the DAT and D_2/D_3 receptors and the 2 dimensions of the SWAN rating score (attention and activity or reflectivity).

Definitions for significant difference for the outcome measures[1] were that statistical parametric mapping comparisons for the DAT and the D_2/D_3 images had to be significant at $P<.005$ (cluster corrected > 100 voxels) and the regional findings had to be corroborated by independently drawn region of interests[2]; comparisons for these corroborative measures had to be significant at $P<.05$[3]; correlations analyses had to be significant at $P<.006$, which was chosen to maintain an overall significance level of $P<.05$ based on a Bonferroni correction for 4 regions and 2 clinical measures (attention and activity or reflectivity). The statistical package used was Statview, version 5.0.1 (Abacus Concepts, Berkeley, California).

Sample-size calculation for this study was based on our preliminary studies (with smaller sample sizes) on DAT[6] and D_2/D_3 receptors,[8] which revealed a difference in caudate between groups at an effect size (ratio between the mean difference and the pooled standard deviation) between 0.65 and 0.80. For such effect sizes, to achieve a power of at least 80% using the independent samples *t* test with a significance level of .05 (2 sided), we needed to recruit at least 40 participants per group. The eventual sample sizes of 53 in the ADHD and 44 in the control groups allowed the detection of the estimated mean differences with a power between 88% and 97% via the independent samples *t* test at the significance level of .05 (2 sided).

RESULTS

Dopamine D_2/D_3 Receptors

Statistical parametric mapping analysis of the [^{11}C]raclopride distribution volume ratio images revealed 1 cluster with lower D_2/D_3 availability in ADHD participants than controls in the left hemisphere. This cluster included brain regions of the dopamine reward pathway–ventral caudate, accumbens, and midbrain regions, as well as the hypothalamic region (Figure 2 and the eTable available at http://www.jama.com). These findings were confirmed by independently drawn region of interest, which also showed ADHD-control differences in left accumbens, midbrain, caudate, and in hypothalamic regions(Table 2). There were no regions that were higher in ADHD participants than in controls. In contrast the K_1 measures for [^{11}C] raclopride (transport of radioligand from plasma to tissue) did not differ either in left caudate with the both groups having a mean 0.11 (95% confidence interval [CI], −0.01 to 0.006 mean difference) or in left accumbens region with the controls having a mean of 0.12 vs a mean of 0.11 for those with ADHD (95% CI, −0.01 to 0.005).

Dopamine Transporters

Statistical parametric mapping analysis of the [^{11}C]cocaine distribution volume ratio images revealed a cluster in the same location as manifested in the [^{11}C]raclopride images. This cluster included the left ventral caudate, accumbal, midbrain, and hypothalamic regions, and in these regions the mean DAT availability was lower in ADHD participants than controls (Figure 2 and eTable). There were no regions that were higher in ADHD participants than in controls. Independently drawn region of interest corroborated significantly lower DAT availability in left accumbens, midbrain, and caudate among participants with ADHD than among controls, but the reductions in left hypothalamic region were not significantly different (Table 2). The mean (95% CI for mean difference) of the K_1 measures for [^{11}C]cocaine did not differ in the left caudate with 0.49 among the controls vs 0.48 among those with ADHD (95% CI, −0.05 to 0.03) or in left accumbens region with a respective difference of 0.49 vs 0.51 among those with ADHD (95% CI, −0.02 to 0.07).

Correlation With ADHD Symptoms Dimensions

The dimension of attention (from the SWAN) was negatively correlated with D_2/D_3 receptor availability in the left accumbens region (r=0.35; 95% CI, 0.15–0.52; P=*.001*), left midbrain (r = 0.35; 95% CI, 0.14–0.52; P = *.001*), left caudate (r = 0.32; 95% CI, 0.11–0.50; P=*.003*), and left hypothalamic region (r=0.31; 95% CI, 0.10–0.49; P=*.003*) and with DAT availability in left midbrain (r=0.37; CI, 0.16, 0.53; P<.001; Figure 3). Because the SWAN scale rates symptoms with a positive scale (from 1 to 3) and the opposite of symptoms with a negative scales (from −1 to −3) the negative correlation indicates that the lower the dopamine measures, the greater the symptoms of inattention. None of the correlations with the dimension of activity or reflectivity was significant.

COMMENT

This study provides evidence in favor of the predicted disruption in the mesoaccumbens dopamine pathway in ADHD. With PET imaging, lower D_2/D_3 receptor and DAT availability in those with ADHD than in the control group was documented in 2 key brain regions for reward and motivation (accumbens and midbrain).[29] It also corroborates disruption of synaptic dopamine markers in caudate in adults with ADHD and provides preliminary evidence that the hypothalamus may also be affected.

The lower than normal D_2/D_3 receptor and DAT availability in the accumbens and midbrain regions supports the hypothesis of an impairment of the dopamine reward pathway in ADHD.[30] Because measures of reward sensitivity were not measured, we can only infer that the impairment in the dopamine reward pathway could underlie the clinical evidence of abnormal responses to reward in ADHD. The reward deficits in ADHD are characterized by a failure to delay gratification, impaired response to partial schedules of reinforcement, and preference for small immediate rewards over larger delayed rewards.[31] Consistent with this important clinical feature of the ADHD syndrome, a recent fMRI study reported decreased activation of the ventral striatum (wherein nucleus accumbens is located) for both immediate and delayed rewards in adult participants with ADHD compared with controls.[17]

In our study, the D_2/D_3 receptor measures in accumbens were correlated with the dimension of attention, which would implicate the dopamine reward pathway in the symptoms of inattention in ADHD. This could provide an explanation of why the attentional deficits in individuals with ADHD are most evident in tasks that are considered boring, repetitive, and uninteresting (ie, tasks or assignments that are not intrinsically rewarding).[32] Finally, because a low number of dopamine D_2/D_3 receptors in the nucleus accumbens have been associated with a greater risk for drug abuse,[33] future work should determine if the lower than normal

D_2/D_3 receptor availability in the accumbens region in ADHD underlies the higher vulnerability for substance abuse in this population.[34]

The lower D_2/D_3 receptor and DAT availability in the midbrain, which contains most of the dopamine neurons in the brain, is consistent with findings from prior imaging studies of children and adolescents with ADHD documenting midbrain abnormalities.[5,35] This could underlie the decreased dopamine release reported in adults with ADHD[8] because firing of dopamine neurons in the midbrain is responsible for release of dopamine in striatum. Moreover, the negative correlation between dopamine markers in the midbrain and the dimension of attention (DAT and D_2 receptors) suggests that impaired signaling from dopamine cells may contribute to severity of symptoms of inattention in ADHD.

Lower than normal D_2/D_3 receptors and DAT availability in ADHD in the caudate was also demonstrated. Prior imaging studies had reported smaller caudate volumes[36–40] and caudate functional under activation[41,42] in ADHD participants compared with controls. In contrast, DAT findings in striatum (including caudate) have been inconsistent in studies of participants with ADHD vs controls, with some studies reporting high,[43] others low,[6] and others no differences.[44] Reason(s) for the discrepancies have been outlined else where[6] and could reflect differences in radiotracers, the methods used (radiotracers; PET vs single photon emission computed tomography), differences in patients characteristics (including prior medication histories; comorbidities, and age of participants), and sample sizes, which vary from 6 to 53 (in this study). These findings differ from those reported in adolescents with ADHD, which showed higher D_2/D_3 receptor availability in the left striatum (including caudate) than in young adults, that was interpreted to reflect deficient dopamine occupancy of these receptors.[7] In these adolescents with ADHD, the largest increases in striatal D_2/D_3 receptor availability were seen in those patients who at birth had the lowest cerebral blood flow measures, which was interpreted to reflect the adverse consequences of neonatal distress on dopamine brain function.[9]

The preliminary finding reported herein of lower than normal dopamine D_2/D_3 receptor availability in the hypothalamic region of ADHD participants is intriguing because if replicated, it could hypothetically provide a neurobiological basis for the high co-morbidity of ADHD with signs and symptoms suggestive of hypothalamic pathology[45] such as sleep disturbances,[46] overweight or obesity,[47] and abnormal responses to stress.[48] Multiple hypothalamic nuclei express dopamine D_2 receptors,[49] but the limited spatial resolution of a PET scan does not allow for localizing where the differences between the groups occurred. Relevant to the role of the hypothalamus in ADHD is the association of a mutation in the melanocortin-4-receptor (*MC4R*) gene, expressed in several hypothalamic nuclei that results in obesity, with ADHD.[50]

Our findings of an association of the mesoaccumbens dopamine pathway with ADHD inattention symptoms may have clinical relevance. This pathway plays a key role in reinforcement-motivation and in learning stimuli-reward associations,[51] and its involvement in ADHD supports the use of interventions to enhance the saliency of school and work tasks to improve performance. Both motivational interventions and contingency management have been shown to improve performance in ADHD patients.[52] Also stimulant medications have been shown to increase the saliency of a cognitive task (motivation, interest) in proportion to the drug-induced dopamine increases in striatum.[53]

Limitations

[^{11}C] Raclopride measures are influenced by extracellular dopamine (the higher the extracellular dopamine, the less the binding of [^{11}C]raclopride to D_2/D_3 receptors), and thus low-binding potential could reflect low D_2/D_3 receptor levels or increased dopamine release.

[54] However, the latter is unlikely since we had previously reported that dopamine release in a sub-group of our ADHD participants was lower than in controls.[8] Also although [^{11}C]cocaine's binding to DATs is minimally affected by competition with endogenous dopamine,[55] DAT availability reflects not only the density of dopamine terminals but also synaptic dopamine tone, because DAT up-regulates when synaptic dopamine is high and down-regulates when dopamine is low.[56] Thus low DAT availability could reflect fewer dopamine terminals or decreased DAT expression per dopamine terminal.

The relatively low affinity of [^{11}C]raclopride and [^{11}C]cocaine for their targets makes them better suited to measure regions with high D_2/D_3 receptor or DAT density (ie, caudate, putamen, and accumbens) and less sensitive to regions with lower levels such as the hypothalamus and midbrain. However, despite this limitation, significant differences in the latter regions between controls and participants with ADHD was shown.

Another study limitation was that measures of reward sensitivity were not performed. Thus, we can only infer that the decreases in the dopamine markers in the accumbens region could underlie the reward deficits that have been reported in patients with ADHD.

Morphological MRI images were not obtained and thus whether volumetric differences in striatum in those with ADHD that could account for these findings could not be ascertained since volumetric differences in striatum have been reported in ADHD.[36–40] However, that there were no group differences in measures of K_1 (transport of radiotracer from plasma to tissue) in striatum, which would have also been affected by volumetric changes, indicates that these findings reflect decreased availability of DAT and D_2/D_3 receptors rather than decreases secondary to partial volume effects.

The correlations with reflectivity or impulsivity and the PET dopamine measures were not significant, which could reflect that the scores were low and thus the sensitivity to observe such a correlation was lacking. Alternatively it could reflect the involvement of frontal regions in impulsivity,[57] which could not be measured with current PET radioligands; D_2/D_3 receptors and DAT levels in frontal regions are very low.

Although the significant findings in this study are restricted to the left hemisphere, low statistical power may have contributed to the lack of significant ADHD-normal differences in the right brain regions. Moreover, because an a priori laterality hypothesis was lacking and, to our knowledge, no solid evidence exists in the literature to support laterality for reward, the laterality effects should be interpreted as preliminary and in need of replication.

This study was not initially designed to evaluate hypothalamic dopamine involvement in ADHD. Thus, this finding is preliminary and in need of replication. Moreover, future studies designed to evaluate hypothalamic pathology in ADHD and its potential clinical significance should assess sleep pathology and should not exclude obese participants, as was the case for the current study.

In conclusion, these findings show a reduction in dopamine synaptic markers in the dopamine reward pathway midbrain and accumbens region of participants with ADHD that were associated with measures of attention. It also provides preliminary evidence of hypothalamic involvement in ADHD (lower than normal D_2/D_3 receptor availability).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support: This research was carried out at Brookhaven National Laboratory (BNL) and was supported in part by grant MH66961-02 from the Intramural Research Program of the National Institutes of Health (NIH), the National Institute of Mental Health and infrastructure support from the Department of Energy.

Role of the Sponsor: The funding agencies did not participate in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

References

1. National Institutes of Health Consensus Development Conference Statement. J Am Acad Child Adolesc Psychiatry 2000;39(2):182–193. [PubMed: 10673829]
2. Dopheide JA, Pliszka SR. Attention-deficit-hyperactivity disorder: an update. Pharmacotherapy 2009;29(6):656–679. [PubMed: 19476419]
3. Swanson JM, Kinsbourne M, Nigg J, et al. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. Neuropsychol Rev 2007;17(1):39–59. [PubMed: 17318414]
4. Braun JM, Kahn RS, Froehlich T, Auinger P, Lanphear BP. Exposures to environmental toxicants and attention deficit hyperactivity disorder in US children. Environ Health Perspect 2006;114(12):1904–1909. [PubMed: 17185283]
5. Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Jons PH, Cohen RM. High midbrain [18F]DOPA accumulation in children with attention deficit hyper-activity disorder. Am J Psychiatry 1999;156(8):1209–1215. [PubMed: 10450262]
6. Volkow ND, Wang GJ, Newcorn J, et al. Brain dopamine transporter levels in treatment and drug naïve adults with ADHD. Neuroimage 2007;34(3):1182–1190. [PubMed: 17126039]
7. Lou HC, Rosa P, Pryds O, et al. ADHD: increased dopamine receptor availability linked to attention deficit and low neonatal cerebral blood flow. Dev Med Child Neurol 2004;46(3):179–183. [PubMed: 14995087]
8. Volkow ND, Wang GJ, Newcorn J, et al. Depressed dopamine activity in caudate and preliminary evidence of limbic involvement in adults with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 2007;64(8):932–940. [PubMed: 17679638]
9. Rosa Neto P, Lou H, Cumming P, Pryds O, Gjedde A. Methylphenidate-evoked potentiation of extracellular dopamine in the brain of adolescents with premature birth. Ann N Y Acad Sci 2002;965:434–439. [PubMed: 12105118]
10. Luman M, Oosterlaan J, Sergeant JA. The impact of reinforcement contingencies on AD/HD. Clin Psychol Rev 2005;25(2):183–213. [PubMed: 15642646]
11. Johansen EB, Killeen PR, Russell VA, et al. Origins of altered reinforcement effects in ADHD. Behav Brain Funct 2009;5:7. [PubMed: 19226460]
12. Haenlein M, Caul WF. Attention deficit disorder with hyperactivity. J Am Acad Child Adolesc Psychiatry 1987;26(3):356–362. [PubMed: 3597291]
13. Kollins SH, Lane SD, Shapiro SK. The experimental analysis of childhood psychopathology. Psychol Rec 1997;47(1):25–44.
14. Wise RA. Brain reward circuitry. Neuron 2002;36(2):229–240. [PubMed: 12383779]
15. Sonuga-Barke EJ. Causal models of attention-deficit/hyperactivity disorder. Biol Psychiatry 2005;57(11):1231–1238. [PubMed: 15949993]
16. Ströhle A, Stoy M, Wrase J, et al. Reward anticipation and outcomes in adult males with attention-deficit/hyperactivity disorder. Neuroimage 2008;39(3):966–972. [PubMed: 17996464]
17. Plichta MM, Vasic N, Wolf RC, et al. Neural hyporesponsiveness and hyperresponsiveness during immediate and delayed reward processing in adult attention-deficit/hyperactivity disorder. Biol Psychiatry 2009;65(1):7–14. [PubMed: 18718573]
18. Volkow ND, Fowler JS, Wang GJ, et al. Reproducibility of repeated measures of carbon-11-raclopride binding in the human brain. J Nucl Med 1993;34(4):609–613. [PubMed: 8455077]
19. Fowler JS, Volkow ND, Wolf AP, et al. Mapping cocaine binding sites in human and baboon brain in vivo. Synapse 1989;4(4):371–377. [PubMed: 2557686]

20. Guy, W. Clinical Global Impression (CGI) scale. In: Rush, AJ.; First, MB.; Blacker, D., editors. Handbook of Psychiatric Measures. Washington, DC: American Psychiatric Publishing; 2000.
21. Swanson JM, Deutsch C, Cantwell D, et al. Genes and attention-deficit hyperactivity disorder. Clin Neurosci Res 2001;1:207–216.
22. Young DJ, Levy F, Martin NC, Hay DA. Attention deficit hyperactivity disorder: a Rasch analysis of the SWAN rating scale [published online May 20, 2009]. Child Psychiatry Hum Dev. 10.1007/s10578–009–0143-z
23. Conners CK. Rating scales in attention-deficit/hyperactivity disorder. J Clin Psychiatry 1998;59(suppl 7):24–30. [PubMed: 9680050]
24. Conners, CK.; Erhardt, D.; Sparrow, E. Adult ADHD Rating Scales: Technical Manual. North Tonawanda, NY: Multi-Health Systems Inc; 1999.
25. Volkow ND, Fowler JS, Logan J, et al. Carbon-11-cocaine binding compared at subpharmacological and pharmacological doses. J Nucl Med 1995;36(7):1289–1297. [PubMed: 7790958]
26. Logan J, Fowler JS, Volkow ND, et al. Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl]-(−)-cocaine PET studies in human subjects. J Cereb Blood Flow Metab 1990;10(5):740–747. [PubMed: 2384545]
27. Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging. Hum Brain Mapp 1995;2:189–210.
28. Lancaster JL, Woldorff MG, Parsons LM, et al. Automated Talairach atlas labels for functional brain mapping. Hum Brain Mapp 2000;10(3):120–131. [PubMed: 10912591]
29. Wise RA, Rompre PP. Brain dopamine and reward. Annu Rev Psychol 1989;40:191–225. [PubMed: 2648975]
30. Sonuga-Barke EJ. The dual pathway model of AD/HD. Neurosci Biobehav Rev 2003;27(7):593–604. [PubMed: 14624804]
31. Tripp G, Wickens JR. Dopamine transfer deficit. J Child Psychol Psychiatry 2008;49(7):691–704. [PubMed: 18081766]
32. Barkley, RA. Attention Deficit Hyperactivity Disorder: A Handbook for Diagnosis and Treatment. New York, NY: The Guilford Press; 1990.
33. Dalley JW, Fryer TD, Brichard L, et al. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science 2007;315(5816):1267–1270. [PubMed: 17332411]
34. Elkins IJ, McGue M, Iacono WG. Prospective effects of attention-deficit/hyperactivity disorder, conduct disorder, and sex on adolescent substance use and abuse. Arch Gen Psychiatry 2007;64(10):1145–1152. [PubMed: 17909126]
35. Jucaite A, Fernell E, Halldin C, Forssberg H, Farde L. Reduced midbrain dopamine transporter binding in male adolescents with attention-deficit/hyperactivity disorder. Biol Psychiatry 2005;57(3):229–238. [PubMed: 15691523]
36. Castellanos FX, Giedd JN, Marsh WL, et al. Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. Arch Gen Psychiatry 1996;53(7):607–616. [PubMed: 8660127]
37. Filipek PA, Semrud-Clikeman M, Steingard RJ, Renshaw PF, Kennedy DN, Biederman J. Volumetric MRI analysis comparing subjects having attention-deficit hyperactivity disorder with normal controls. Neurology 1997;48(3):589–601. [PubMed: 9065532]
38. Castellanos FX, Giedd JN, Berquin PC, et al. Quantitative brain magnetic resonance imaging in girls with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 2001;58(3):289–295. [PubMed: 11231836]
39. Lopez-Larson M, Michael ES, Terry JE, et al. Subcortical differences among youths with attention-deficit/hyperactivity disorder compared to those with bipolar disorder with and without attention-deficit/hyperactivity disorder. J Child Adolesc Psychopharmacol 2009;19(1):31–39. [PubMed: 19232021]
40. Qiu A, Crocetti D, Adler M, et al. Basal ganglia volume and shape in children with attention deficit hyperactivity disorder. Am J Psychiatry 2009;166 (1):74–82. [PubMed: 19015232]
41. Vaidya CJ, Bunge SA, Dudukovic NM, Zalecki CA, Elliott GR, Gabrieli JD. Altered neural substrates of cognitive control in childhood ADHD. Am J Psychiatry 2005;162(9):1605–1613. [PubMed: 16135618]

42. Booth JR, Burman DD, Meyer JR, et al. Larger deficits in brain networks for response inhibition than for visual selective attention in attention deficit hyperactivity disorder (ADHD). J Child Psychol Psychiatry 2005;46(1):94–111. [PubMed: 15660647]
43. Spencer TJ, Biederman J, Madras BK, et al. Further evidence of dopamine transporter dysregulation in ADHD: a controlled PET imaging study using altropane. Biol Psychiatry 2007;62(9):1059–1061. [PubMed: 17511972]
44. van Dyck CH, Quinlan DM, Cretella LM, et al. Unaltered dopamine transporter availability in adult attention deficit hyperactivity disorder. Am J Psychiatry 2002;159(2):309–312. [PubMed: 11823278]
45. Cortese S, Konofal E, Lecendreux M. Alertness and feeding behaviors in ADHD. Med Hypotheses 2008;71(5):770–775. [PubMed: 18678446]
46. Cortese S, Konofal E, Yateman N, Mouren MC, Lecendreux M. Sleep and alertness in children with attention-deficit/hyperactivity disorder. Sleep 2006;29(4):504–511. [PubMed: 16676784]
47. Waring ME, Lapane KL. Overweight in children and adolescents in relation to attention-deficit/hyperactivity disorder. Pediatrics 2008;122(1):e1–e6. [PubMed: 18595954]
48. King JA, Barkley RA, Barrett S. Attention-deficit hyperactivity disorder and the stress response. Biol Psychiatry 1998;44(1):72–74. [PubMed: 9646887]
49. Gurevich EV, Joyce JN. Distribution of dopamine D3 receptor expressing neurons in the human forebrain. Neuropsychopharmacology 1999;20(1):60–80. [PubMed: 9885786]
50. Agranat-Meged A, Ghanadri Y, Eisenberg I, Ben Neriah Z, Kieselstein-Gross E, Mitrani-Rosenbaum S. Attention deficit hyperactivity disorder in obese melanocortin-4-receptor (*MC4R*) deficient subjects. Am J Med Genet B Neuropsychiatr Genet 2008;147B (8):1547–1553. [PubMed: 18777518]
51. Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci 2007;10(8):1020–1028. [PubMed: 17603481]
52. Barkley RA. Adolescents with attention-deficit/hyperactivity disorder. J Psychiatr Pract 2004;10(1): 39–56. [PubMed: 15334986]
53. Volkow ND, Wang GJ, Fowler JS, et al. Evidence that methylphenidate enhances the saliency of a mathematical task by increasing dopamine in the human brain. Am J Psychiatry 2004;161(7):1173–1180. [PubMed: 15229048]
54. Gjedde A, Wong DF, Rosa-Neto P, Cumming P. Mapping neuroreceptors at work: on the definition and interpretation of binding potentials after 20 years of progress. Int Rev Neurobiol 2005;63:1–20. [PubMed: 15797463]
55. Gatley SJ, Volkow ND, Fowler JS, Dewey SL, Logan J. Sensitivity of striatal [11C]cocaine binding to decreases in synaptic dopamine. Synapse 1995;20 (2):137–144. [PubMed: 7570343]
56. Zahniser NR, Doolen S. Chronic and acute regulation of Na+/Cl–dependent neurotransmitter transporters. Pharmacol Ther 2001;92(1):21–55. [PubMed: 11750035]
57. Winstanley CA, Eagle DM, Robbins TW. Behavioral models of impulsivity in relation to ADHD: translation between clinical and preclinical studies. Clin Psychol Rev 2006;26(4):379–395. [PubMed: 16504359]

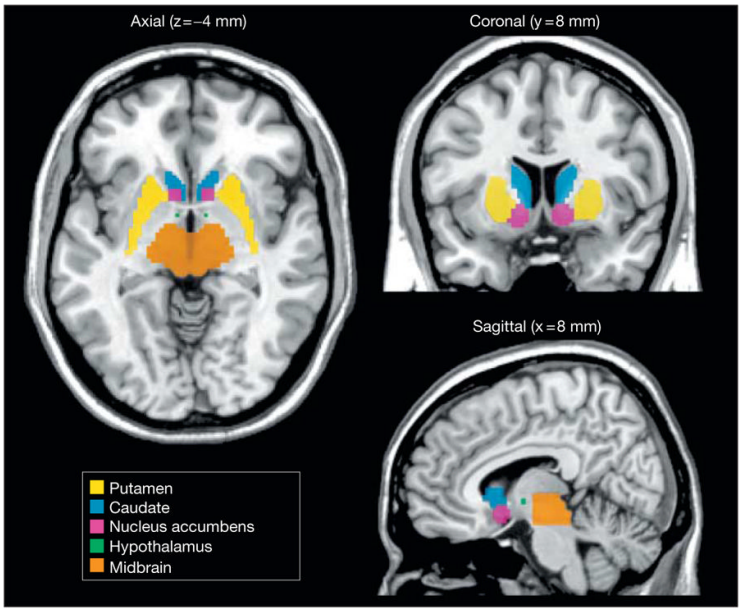


Figure 1. Regions of Interest Used to Extract the D_2/D_3 Receptor and Dopamine Transporter Measures

The regions of interest for the midbrain are obtained in several planes, and the shadow is projected to the axial image shown in the figure, which explains why the third ventricle is covered by the region. The x coordinate maps the left-right position; they coordinate, the anterior-posterior position; and the z coordinate, the superior-inferior position.

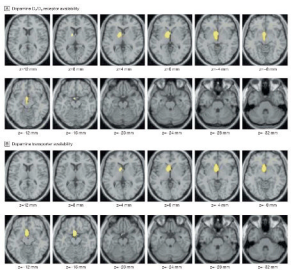


Figure 2. Regions in the Brain in Which Dopamine Measures Were Lower in Participants With ADHD Than in Controls

A, Regions showed significantly lower dopamine D_2/D_3 receptor availability in participants with attention-deficit/hyperactivity disorder (ADHD) than in controls (obtained from [^{11}C] raclopride images). B, Regions showed significantly lower dopamine transporter availability in the participants with ADHD than in controls (obtained from [^{11}C]cocaine images). Significance corresponds to $P<.005$, cluster >100 voxels. The yellow regions identify the areas in the brain for which the measures differed between controls and participants with ADHD. The location of the region that differed was similar for the dopamine D_2/D_3 receptor and for the dopamine transporter and included the locations of the left ventral striatum (including accumbens and ventral caudate), left midbrain, and left hypothalamus. The z coordinate maps the superior-inferior position.

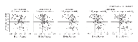


Figure 3. Regression Slopes Between Dopamine D_2/D_3 Receptor and Dopamine Transporter Availability and Scores on Attention

The Dimension of the Strengths and Weaknesses of Attention-Deficit/Hyperactivity Disorder (ADHD)–symptoms and Normal-behavior (SWAN) rating scale uses a positive scale for symptoms (1 to 3) and a negative scale for the opposite of the symptoms (−1 to −3) ranging from "far below average" to "far above average." The negative numbers in some of the regions show that the ratio of the specific to nonspecific binding of the radioligand is very low for these regions. The solid line in each scatter plot corresponds to the regression line (line of best fit).

Table 1

Demographic and Clinical Characteristics of Participants

	Controls (n = 44)	ADHD (n = 53)
Age, mean (SD), y	31 (6)	32 (8)
Body mass index	25 (5)	25 (3)
Sex, No. (%)		
Men	30 (68)	27 (51)
Women	14 (32)	26 (49)
Education, mean (SD), y	15 (2)	15 (4)
Smoking status, No. (%)		
Current	1 (2)	4 (7)
Past[a]	4 (9)	1 (2)
CGI-severity, mean (SD)	NA	5 (1)
ADHD subtype, No. (%)	NA	
Inattentive		30 (57)
Hyperactive		4 (7)
Combined		19 (36)
CAARS, mean (SD), score		
Inattention	5 (4)	25 (5)
Hyperactivity	7 (4)	23 (8)
Impulsivity	4 (3)	19 (7)
Self-concept	3 (3)	9 (4)
DSM inattentive	3 (3)	20 (4)
DSM hyperactive	3 (3)	15 (6)
Total symptoms	6 (5)	36 (7)
ADHD index	4 (3)	22 (5)
SWAN, mean (SD), score		
Attention	−1.5 (1)	1.6 (1)
Hyperactivity	−1.2 (1)	0.6 (1)

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; CAARS, Conners Adult ADHD Rating Scale; CGI, Clinical Global Impressions Severity; *DSM*, *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition); SWAN, Strengths and Weaknesses of ADHD-symptoms and Normal-behavior.

[a]Two participants had quit smoking in the past year, whereas the others had quit more than 2 years before study start.

Table 2

Measures of Dopamine D_2/D_3 Receptor and Dopamine Transporter Availability[a]

	Availability, Mean (SD)				
Left Hemisphere	**Controls**	**ADHD**	**Effect Size[b]**	**95% Confidence Interval[b]**	***P* Value[c]**
Dopamine D_2/D_3 receptor					
Accumbens region	2.85 (0.31)	2.68 (0.28)	0.61	0.06 to 0.30	.004
Caudate	2.80 (0.49)	2.47 (0.61)	0.60	0.10 to 0.56	.005
Midbrain	0.28 (0.14)	0.18 (0.19)	0.57	0.02 to 0.17	.01
Hypothalamic region	0.12 (0.13)	0.04 (0.12)	0.61	0.02 to 0.12	.004
Dopamine transporter					
Accumbens region	0.71 (0.16)	0.63 (0.11)	0.59	0.03 to 0.13	.004
Caudate	0.66 (0.23)	0.53 (0.19)	0.62	0.04 to 0.22	.003
Midbrain	0.16 (0.10)	0.09 (0.11)	0.66	0.03 to 0.12	<.001
Hypothalamic region	−0.01 (0.10)	−0.05 (0.12)	0.36	−0.01 to 0.09	.08

[a] Measures of receptor and transporter availability (BP_{ND}=DVR −1) obtained using an independent region-of-interest analysis to corroborate the statistical parametric mapping findings.

[b] Mean differences and effect sizes for the comparisons between controls and participants with attention-deficit/hyperactivity disorder.

[c] Comparisons correspond to independent samples 2-tailed *t* tests.

Original Investigation

Developmentally Stable Whole-Brain Volume Reductions and Developmentally Sensitive Caudate and Putamen Volume Alterations in Those With Attention-Deficit/Hyperactivity Disorder and Their Unaffected Siblings

Corina U. Greven, PhD; Janita Bralten, Msc; Maarten Mennes, PhD; Laurence O'Dwyer, PhD; Kimm J. E. van Hulzen, PhD; Nanda Rommelse, PhD; Lizanne J. S. Schweren, MSc; Pieter J. Hoekstra, MD, PhD; Catharina A. Hartman, PhD; Dirk Heslenfeld, PhD; Jaap Oosterlaan, PhD; Stephen V. Faraone, PhD; Barbara Franke, PhD; Marcel P. Zwiers, PhD; Alejandro Arias-Vasquez, PhD; Jan K. Buitelaar, MD, PhD

IMPORTANCE Attention-deficit/hyperactivity disorder (ADHD) is a heritable neurodevelopmental disorder. It has been linked to reductions in total brain volume and subcortical abnormalities. However, owing to heterogeneity within and between studies and limited sample sizes, findings on the neuroanatomical substrates of ADHD have shown considerable variability. Moreover, it remains unclear whether neuroanatomical alterations linked to ADHD are also present in the unaffected siblings of those with ADHD.

OBJECTIVE To examine whether ADHD is linked to alterations in whole-brain and subcortical volumes and to study familial underpinnings of brain volumetric alterations in ADHD.

DESIGN, SETTING, AND PARTICIPANTS In this cross-sectional study, we included participants from the large and carefully phenotyped Dutch NeuroIMAGE sample (collected from September 2009-December 2012) consisting of 307 participants with ADHD, 169 of their unaffected siblings, and 196 typically developing control individuals (mean age, 17.21 years; age range, 8-30 years).

MAIN OUTCOMES AND MEASURES Whole-brain volumes (total brain and gray and white matter volumes) and volumes of subcortical regions (nucleus accumbens, amygdala, caudate nucleus, globus pallidus, hippocampus, putamen, thalamus, and brainstem) were derived from structural magnetic resonance imaging scans using automated tissue segmentation.

RESULTS Regression analyses revealed that relative to control individuals, participants with ADHD had a 2.5% smaller total brain (β = −31.92; 95% CI, −52.69 to −11.16; P = .0027) and a 3% smaller total gray matter volume (β = −22.51; 95% CI, −35.07 to −9.96; P = .0005), while total white matter volume was unaltered (β = −10.10; 95% CI, −20.73 to 0.53; P = .06). Unaffected siblings had total brain and total gray matter volumes intermediate to participants with ADHD and control individuals. Significant age-by-diagnosis interactions showed that older age was linked to smaller caudate (P < .001) and putamen (P = .01) volumes (both corrected for total brain volume) in control individuals, whereas age was unrelated to these volumes in participants with ADHD and their unaffected siblings. Attention-deficit/hyperactivity disorder was not significantly related to the other subcortical volumes.

CONCLUSIONS AND RELEVANCE Global differences in gray matter volume may be due to alterations in the general mechanisms underlying normal brain development in ADHD. The age-by-diagnosis interaction in the caudate and putamen supports the relevance of different brain developmental trajectories in participants with ADHD vs control individuals and supports the role of subcortical basal ganglia alterations in the pathophysiology of ADHD. Alterations in total gray matter and caudate and putamen volumes in unaffected siblings suggest that these volumes are linked to familial risk for ADHD.

JAMA Psychiatry. 2015;72(5):490-499. doi:10.1001/jamapsychiatry.2014.3162
Published online March 18, 2015.

Supplemental content at jamapsychiatry.com

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Corina U. Greven, PhD, Donders Institute for Brain, Cognition, and Behaviour, Department of Cognitive Neuroscience, Radboud University Medical Center, Internal Post 204, Nijmegen, the Netherlands (corina.greven@donders.ru.nl).

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder characterized by symptoms of inattention and hyperactivity/impulsivity.[1] Anatomical magnetic resonance imaging (MRI) studies have associated ADHD with a 3% to 5% smaller total brain size compared with control individuals.[2-4] Meta-analyses have further documented smaller volumes in ADHD across several specific brain regions, most consistently in basal ganglia volumes: right globus pallidus and right putamen and caudate.[5-8] These abnormalities are in accordance with a neurodevelopmental theory that hypothesizes ADHD to be caused by early-emerging persistent subcortical abnormalities.[9]

Existing brain volumetric studies in ADHD face some limitations. First, the child and adult literature have largely been kept separate, ignoring the transition from adolescence into early adulthood in studying age effects. Hyperactive/impulsive more than inattentive symptoms of ADHD tend to decrease with age.[10-12] Moreover, cross-sectional evidence suggests that some brain volumetric alterations observed in childhood may normalize with age.[7,8] Second, findings on brain volumetric alterations in ADHD have been variable (eg, one meta-analysis reported that only 25% to 50% of included studies revealed similar results).[8] Heterogeneity in sample composition within and between studies and small samples sizes may explain inconsistencies; individual studies included in meta-analyses ranged from 17 to 291 participants with ADHD and control individuals, with only 4 studies exceeding a sample size of 100.[5-8] The use of a large well-defined sample has added value over and extends existing meta-analyses, as meta-analyses are limited by shortcomings of the individual studies. Third, ADHD is more common in boys than girls,[1,13] and brain volumetric alterations in ADHD may be hemisphere specific.[5-8] However, most studies used small samples to study sex and lateralization effects or revealed inconsistent findings.[5,7,8,14] Finally, ADHD runs in families[15-17] and is heritable (76%[18]), and brain volumes are also heritable (total brain volume, 66%-97%[19]; subcortical volumes, 44%-88%[20]). Two studies reported alterations in prefrontal gray matter and occipital gray and white matter,[4] as well as inferior frontal gyrus gray matter and inferior fronto-occipital fasciculus white matter[21] not only in participants with ADHD, but also in their unaffected siblings relative to control individuals. Because first-degree relatives on average share 50% of their genetic information, as well as all familywide environmental influences, these findings suggest familial (ie, shared genetic and/or shared environmental) underpinnings to associations between ADHD and brain volumes. However, whether ADHD-brain associations are influenced by familial factors remains widely unexplored.

The present cross-sectional study addresses these shortcomings through studying the brain volumetric correlates of ADHD in a large well-characterized sample of adolescents and young adults with ADHD, their unaffected siblings, and typically developing control individuals. First, we investigated whether ADHD was linked to whole-brain and subcortical volumes and whether results were influenced by age, sex, or lateralization. Second, familial underpinnings of brain volumetric alterations in ADHD were examined.

Methods

Sample

Participants came from the NeuroIMAGE project,[22] a follow-up of the Dutch part of the International Multicenter ADHD Genetics (IMAGE) Study performed between 2003 and 2006.[23-26] The IMAGE Study recruited ADHD families with at least 1 child with combined subtype ADHD and at least 1 biological sibling (regardless of ADHD diagnosis) and control families with at least 1 child and 1 biological sibling with no formal or suspected ADHD diagnosis in first-degree family members. Inclusion criteria were age between 8 and 30 years; European white descent; intelligence quotient (IQ) greater than or equal to 70; and no diagnosis of autism, epilepsy, general learning difficulties, brain disorders, and known genetic disorders. Dutch participants of the IMAGE Study were invited for follow-up measurement and (re) assessed in the NeuroIMAGE Study (follow-up rate: ADHD families, 75.6%; control families, 75.1%; mean [SD] time between measurements, 5.9 [0.74] years). Inclusion criteria were largely consistent with the IMAGE Study, except that inclusion of any ADHD subtype was allowed. Following exclusion for contraindications to MRI scanning and quality control of MRI scans (described further on), the final sample consisted of 307 participants with ADHD, 169 unaffected siblings, and 196 control participants (siblings from 389 families) (**Table 1**). The group with ADHD (n = 307) included 128 siblings (only siblings from ADHD families where each sibling had an ADHD diagnosis), the unaffected siblings group (n = 169) included 53 siblings (only siblings from ADHD families without an ADHD diagnosis), and the control group (n = 196) included 142 siblings (only siblings from control families). Ethical approval for the current study was obtained from CMO Regio Arnhem-Nijmegen, and all participants provided written informed consent. For details on diagnostic assessment, see eAppendix 1 in the Supplement.

Image Acquisition and Segmentation

Imaging was conducted at 2 locations (VU University Amsterdam, Amsterdam, and Radboud University Medical Center, Nijmegen) using 2 comparable 1.5-T scanners and scan protocols (Sonata and Avanto; Siemens) with the same product 8-channel head-coil and closely matched scan protocols.[22] The protocol included 2 high-resolution T1-weighted magnetization-prepared rapid acquisition gradient echo anatomical scans (176 sagittal slices; repetition time = 2730 milliseconds; echo time = 2.95 milliseconds; inversion time = 1000 milliseconds; flip angle = 7°; GRAPPA (generalized autocalibrating partial parallel acquisition) 2; voxel size = 1.0 × 1.0 × 1.0 mm; field of view = 256 mm). Magnetic resonance imaging scans that yielded relevant incidental findings or in which manual ratings revealed poor quality or motion artifacts were excluded.[28] In participants with 2 good-quality scans, volume estimates were averaged across scans (eTable 1 in the Supplement).

Whole-Brain Volumes

Normalization, bias correction, and segmentation into gray matter, white matter, and cerebrospinal fluid volumes were

Table 1. Participant Characteristics

Characteristic	ADHD (n = 307)	Siblings[a] (n = 169)	Controls (n = 196)
Male, %	68	43	49
Age, mean (SD), y	17.06 (3.42)	17.52 (4.11)	16.66 (3.07)
Total ADHD, mean (SD)[b]	69.99 (12.88)	47.60 (6.58)	45.74 (5.24)
Median	70.00	46.00	45.00
Inattentive Behavior Scale, mean (SD)[b]	66.10 (11.16)	47.72 (6.56)	46.27 (5.61)
Median	66.00	45.00	45.00
Hyperactive-Impulsive Behavior Scale, mean (SD)[b]	69.85 (14.48)	48.29 (7.11)	46.27 (4.93)
Median	68.00	46.00	44.00
IQ, mean (SD)	97.08 (15.18)	102.19 (14.54)	106.61 (13.70)

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; IQ, intelligence quotient.

[a] Unaffected siblings of participants with ADHD.

[b] Refers to *t* scores on the *DSM* Total, Inattentive Behavior, and Hyperactive-Impulsive Behavior scales of the Conners' Parent Rating Scale–Revised[27] (scales N, L, and M). *T* scores ≥63 are considered clinically elevated.

performed using the unified procedure of the VBM 8.1 toolbox (http://dbm.neuro.uni-jena.de/vbm/) in SPM (default settings). Total gray and white matter volumes were calculated by summation of their tissue probability maps. Total brain volume was the sum of total gray and white matter volumes.

Subcortical Volumes

Automated FIRST (FMRIB's Integrated Registration and Segmentation Tool) subcortical segmentation was applied to estimate total, left, and right volumes of the amygdala, caudate nucleus, hippocampus, nucleus accumbens, globus pallidus, putamen, thalamus, and brainstem. Previous research has shown these volumes to be heritable.[20] FIRST is part of FMRIB's Software Library and performs registration and shape modeling of the just-mentioned regions in Montreal Neurological Institute 152 standard space.[29] FIRST-based segmentation includes the hippocampus as a subcortical region, although it is not usually considered subcortical.[29]

Assessment of IQ and Medication Use

Participants' full-scale IQ was estimated using the Vocabulary and Block Design subtests of the Wechsler Intelligence Scale for Children III or the Wechsler Adult Intelligence Scale III (for participants aged ≥17 years).[22] Cumulative medication intake, calculated as treatment duration corrected for age multiplied by the mean daily dose in milligrams, was gathered from pharmacy transcripts and questionnaire reports (eAppendix 1 in the Supplement).

Analyses

Comparisons Between Participants With ADHD and Control Individuals

Brain volumetric measures were normally distributed and outliers more than 3 SDs greater than or less than the mean were removed. Overall, there were few outliers (1-5 individuals per volume). Associations between ADHD and brain volumes were examined using regression analyses that included brain volumes 1 by 1 as outcome measures, with scanner location (Amsterdam or Nijmegen), age, age squared, and sex as covariates.[20,30,31] Total brain volume was included as an additional covariate for regressions of subcortical volumes to enable inferences about subcortical alterations unconfounded by total brain volume.[32] The regression model included binary ADHD diagnosis (ADHD and control) as a main effect, as well as the 2-way interaction effects between binary ADHD diagnosis and age, age squared, and sex (ADHD by age; ADHD by age squared; and ADHD by sex). Interaction effects not reaching nominal significance (.05) were dropped from the final model. Centering of variables was used[33] before creating interaction terms, and multicollinearity statistics were examined. To correct for the nonindependence of the data of siblings in the ADHD and control groups (see Sample description), the correlation structure of the data was accounted for by calculating robust standard errors using the cluster command in Stata (StataCorp).[34,35] This was merely a correction to account for the underlying assumption in regression that observations are independent and does not preclude examination of familiality effects underlying brain-ADHD associations.

Correction for Multiple Testing

A multiple-testing correction was applied, which adjusts correlated tests based on an effective number of independent comparisons[36] derived from the eigenvalues of a correlation matrix between the included outcome measures adjusted for covariates. The multiple-testing adjusted *P* value was determined to be .003 (eTable 2 in the Supplement) and applied to analyses comparing participants with ADHD and control individuals. Any follow-up analyses of volumes surviving multiple-testing correction used the nominal significance level (.05) and should be seen as exploratory.

Comparisons of Unaffected Siblings With Participants With ADHD and Control Individuals

For volumes surviving multiple-testing correction, the previously mentioned regression models were repeated, replacing binary ADHD diagnosis with a 3-level group membership variable (ADHD, unaffected sibling, and control individual). All other model parameters were kept the same as in the analyses comparing participants with ADHD and control individuals and the cluster command, and the same covariates were used. Familiality was considered present if the unaffected siblings differed in brain volumes from control individuals but not from participants with ADHD or differed from both groups showing intermediate brain volumes.

Table 2. Participants With ADHD, Unaffected Siblings, and Control Individuals

Region	Brain Volume, Mean (SE), mL[a]		
	ADHD (n = 307)	Siblings (n = 169)[b]	Controls (n = 196)
Total brain[c]	1230.59 (6.08)	1250.72 (8.27)	1262.31 (8.15)
Gray matter	724.49 (3.49)	737.32 (4.97)	746.82 (5.07)
White matter	505.59 (3.21)	513.11 (4.17)	515.52 (4.15)
Accumbens			
Total volume of left and right hemispheres summed	1.15 (0.01)	1.11 (0.01)	1.11 (0.01)
Volume of left hemisphere	0.61 (0.01)	0.60 (0.01)	0.59 (0.01)
Volume of right hemisphere	0.54 (0.01)	0.51 (0.01)	0.52 (0.01)
Amygdala			
Total volume of left and right hemispheres summed	2.67 (0.02)	2.67 (0.03)	2.63 (0.03)
Volume of left hemisphere	1.30 (0.01)	1.28 (0.02)	1.25 (0.02)
Volume of right hemisphere	1.36 (0.02)	1.38 (0.02)	1.37 (0.02)
Caudate			
Total volume of left and right hemispheres summed	8.11 (0.05)	7.99 (0.06)	8.09 (0.06)
Volume of left hemisphere	3.98 (0.02)	3.92 (0.03)	3.97 (0.03)
Volume of right hemisphere	4.13 (0.02)	4.07 (0.03)	4.12 (0.03)
Globus pallidus			
Total volume of left and right hemispheres summed	3.76 (0.02)	3.74 (0.02)	3.71 (0.02)
Volume of left hemisphere	1.87 (0.01)	1.85 (0.01)	1.83 (0.01)
Volume of right hemisphere	1.90 (0.01)	1.89 (0.01)	1.88 (0.01)
Hippocampus			
Total volume of left and right hemispheres summed	7.81 (0.04)	7.82 (0.05)	7.80 (0.06)
Volume of left hemisphere	3.84 (0.02)	3.83 (0.03)	3.81 (0.03)
Volume of right hemisphere	3.97 (0.02)	3.99 (0.03)	4.00 (0.03)
Putamen			
Total volume of left and right hemispheres summed	10.87 (0.06)	10.85 (0.07)	10.73 (0.07)
Volume of left hemisphere	5.45 (0.03)	5.44 (0.04)	5.38 (0.03)
Volume of right hemisphere	5.43 (0.03)	5.41 (0.03)	5.35 (0.04)
Thalamus			
Total volume of left and right hemispheres summed	16.88 (0.06)	16.79 (0.07)	16.80 (0.08)
Volume of left hemisphere	8.53 (0.03)	8.48 (0.04)	8.49 (0.05)
Volume of right hemisphere	8.34 (0.03)	8.31 (0.04)	8.30 (0.04)
Brainstem	22.10 (0.11)	22.18 (0.14)	22.03 (0.12)

Abbreviation: ADHD, attention-deficit/hyperactivity disorder.

[a] Means are based on estimated marginal means corrected for age, age squared, sex, and scanner location; for subcortical volumes, correction for total brain volume is also included.

[b] Unaffected siblings of participants with ADHD.

[c] Sum of total gray and white matter volumes.

Sensitivity Analyses

For volumes surviving multiple-testing correction, sensitivity analyses were conducted to examine potential effects of total brain volume, IQ, medication, scanner location, and sex distribution (eAppendix 2 in the Supplement).

Results

Descriptives

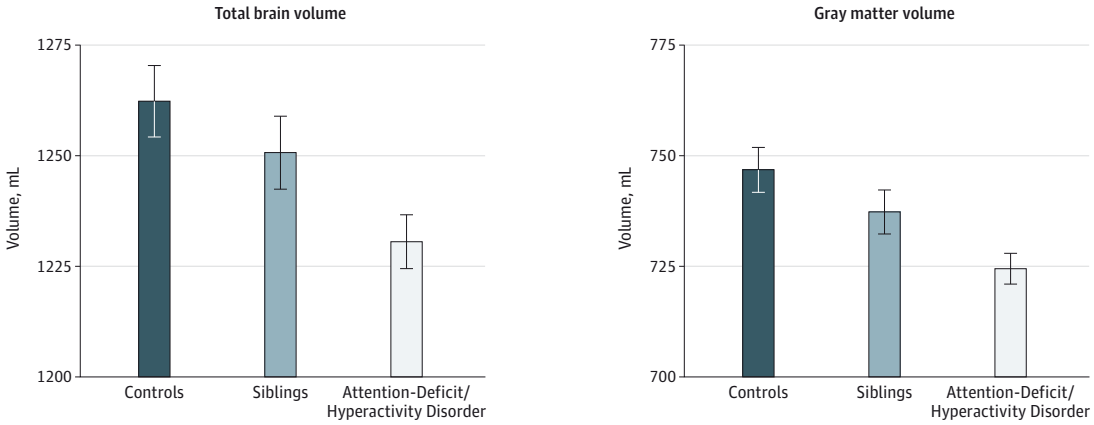
Mean brain volumes corrected for covariates and for raw data are shown in **Table 2** and eTable 3 in the Supplement, respectively. For mean volumes relating to the sensitivity analyses, see eTables 4 through 7 in the Supplement.

Comparisons Between Participants With ADHD and Control Individuals

Whole-Brain Volumes

Compared with control individuals, participants with ADHD had smaller total brain (β = −31.92; 95% CI, −52.69 to −11.16; P = .0027) and total gray matter (β = −22.51; 95% CI, −35.07 to −9.96; P = .0005) volumes. Total brain volume was 2.5% (32 mL) smaller and total gray matter volume was 3% (22 mL) smaller (**Figure 1**; **Table 3**). No significant group differences were found for total white matter volume (β = −10.10; 95% CI, −20.73 to 0.53; P = .06). None of the interactions between ADHD diagnosis and sex, age, and age squared on whole-brain volumes were significant (Table 3). Hence, differences between participants with ADHD and control individuals in total brain and gray matter volumes were stable across age and sex.

Figure 1. Mean Total Brain and Total Gray Matter Volumes in Participants With Attention-Deficit/Hyperactivity Disorder, Unaffected Siblings, and Control Individuals



Siblings indicate the unaffected siblings of participants with attention-deficit/hyperactivity disorder. The means are based on estimated marginal means corrected for age, age squared, sex, and scanner location. The error bars represent standard errors.

Subcortical Volumes

Main effects of binary ADHD diagnosis on subcortical volumes were nonsignificant (Table 3). However, the interaction between binary ADHD diagnosis and age on total, left, and right caudate and putamen volumes was significant (total: $P = .0004$; left: $P = .0003$; and right: $P = .0035$ for caudate) (total: $P = .0011$; left: $P < .0004$; and right: $P = .0018$ for putamen) (Table 3). Because results were similar for left, right, and total volumes, subsequent results are described only for total caudate and putamen volumes. Post hoc analyses revealed that older age was related to smaller total caudate ($P < .001$) and putamen ($P = .01$) volumes in control individuals but not in participants with ADHD (caudate: $P = .82$; putamen: $P = .16$). Dividing the sample into 3 roughly equal age bands (younger: ≤15 years; middle: >15-≤22 years, and older: >22 years) revealed that relative to control individuals, participants with ADHD had significantly smaller total caudate (0.33 mL; 3.9%; $P = .04$) and total putamen (0.36 mL; 3.3%; $P = .0061$) volumes in the younger group, had nonsignificantly larger total caudate volume (0.04 mL; 0.5%; $P = .67$) and significantly larger putamen volumes in the middle group (0.26 mL; 2.4%; $P = .02$), and had significantly larger total caudate (0.87 mL; 12.0%; $P = .0088$) and total putamen (0.89 mL; 8.6%; $P = .0038$) volumes in the older group (eTable 8 and the eFigure in the Supplement). No significant interaction effects were present for the other subcortical volumes.

Comparisons of Unaffected Siblings With Participants With ADHD and Control Individuals

Whole-Brain Volumes

Compared with participants with ADHD, unaffected siblings had a 1.6% (20 mL; $P = .04$) larger total brain volume and a 1.8% (13 mL; $P = .03$) larger total gray matter volume (Tables 2 and 3). Compared with control individuals, unaffected siblings had nonsignificantly smaller total brain (0.9%; 12 mL; $P = .32$) and total gray matter (1.3%; 10 mL; $P = .18$) volumes. Nonetheless, a linear trend was present in these volumes across the 3 groups (total brain: $P = .0018$; total gray matter: $P < .0003$; eAppendix 3 in the Supplement), indicating that unaffected siblings had total brain and gray matter volumes intermediate to participants with ADHD and control individuals.

Subcortical Volumes

The interaction between group membership unaffected siblings vs control individuals and age was a significant predictor of caudate and putamen volumes (total caudate: $P = .05$; total putamen: $P = .0086$); however, the interaction between group membership unaffected sibling vs ADHD and age was not significant (total caudate: $P = .15$; total putamen: $P = .48$) (**Figure 2**). This suggests that the unaffected siblings differed from control individuals but not from participants with ADHD. As was the case for participants with ADHD, age was not significantly related to caudate and putamen volumes in unaffected siblings (total caudate: $P = .09$; total putamen: $P = .47$).

Sensitivity Analyses

Findings were robust across sensitivity analyses (eAppendix 2 in the Supplement).

Discussion

We found that ADHD was linked to a 2.5% smaller total brain volume relative to control individuals, driven by a 3% smaller total gray matter volume. Moreover, ADHD was linked to alterations in caudate and putamen volumes, which were developmentally sensitive. Unaffected siblings showed a pattern of results intermediate to participants with ADHD and control individuals. In this large sample, neurobiological alterations associated with ADHD were neither sex nor hemisphere specific.

Globalized differences between participants with ADHD and control individuals in gray matter volume across the brain could indicate alterations to general mechanisms underlying

Table 3. Regression of Binary ADHD Diagnosis on Brain Volumes[a]

Region	ADHD		ADHD by Age		ADHD by Age Squared		ADHD by Sex	
	β (95% CI)[b]	*P* Value	β (95% CI)[b]	*P* Value	β (95% CI)[b]	*P* Value	β (95% CI)[b]	*P* Value
Total brain[c]	**−31.92 (−52.69 to −11.16)**	**.0027**[d]	NA	NA	NA	NA	NA	NA
Gray matter	**−22.51 (−35.07 to −9.96)**	**.0005**[d]	NA	NA	NA	NA	NA	NA
White matter	−10.10 (−20.73 to 0.53)	.06	NA	NA	NA	NA	NA	NA
Accumbens								
Total volume of left and right hemispheres summed	0.04 (0.10 to 0.07)	.0082	NA	NA	NA	NA	NA	NA
Volume of left hemisphere	0.01 (−0.01 to 0.03)	.45	0.01 (0.00 to 0.01)	.02	0.00 (0.00 to 0.00)	.03	NA	NA
Volume of right hemisphere	0.02 (0.01 to 0.04)	.0079	NA	NA	NA	NA	NA	NA
Amygdala								
Total volume of left and right hemispheres summed	−0.05 (−0.15 to 0.05)	.33	NA	NA	NA	NA	0.18 (0.04 to 0.32)	.01
Volume of left hemisphere	0.00 (−0.05 to 0.06)	.87	NA	NA	NA	NA	0.09 (0.01 to 0.17)	.04
Volume of right hemisphere	−0.06 (−0.12 to 0.01)	.08	NA	NA	NA	NA	0.03 (0.01 to 0.18)	.03
Caudate								
Total volume of left and right hemispheres summed	−0.10 (−0.29 to 0.09)	.31	**0.08 (0.03 to 0.12)**	**.0004**[d]	0.01 (0.00 to 0.02)	.03	NA	NA
Volume of left hemisphere	−0.05 (−0.14 to 0.05)	.33	**0.04 (0.02 to 0.06)**	**.0003**[d]	0.01 (0.00 to 0.01)	.04	NA	NA
Volume of right hemisphere	−0.04 (−0.14 to 0.06)	.45	**0.03 (0.01 to 0.06)**	**.0035**[e]	0.00 (0.00 to 0.01)	.05	NA	NA
Globus pallidus								
Total volume of left and right hemispheres summed	0.06 (0.00 to 0.12)	.05	0.02 (0.01 to 0.04)	.0073	NA	NA	NA	NA
Volume of left hemisphere	0.04 (0.01 to 0.07)	.01	0.01 (0.00 to 0.02)	.02	NA	NA	NA	NA
Volume of right hemisphere	0.02 (−0.01 to 0.05)	.17	0.01 (0.00 to 0.02)	.0081	NA	NA	NA	NA
Hippocampus								
Total volume of left and right hemispheres summed	0.00 (−0.15 to 0.14)	.96	NA	NA	NA	NA	NA	NA
Volume of left hemisphere	0.02 (−0.06 to 0.10)	.67	NA	NA	NA	NA	NA	NA
Volume of right hemisphere	−0.03 (−0.11 to 0.05)	.48	NA	NA	NA	NA	NA	NA
Putamen								
Total volume of left and right hemispheres summed	0.18 (0.00 to 0.36)	.04	**0.09 (0.04 to 0.15)**	**.0011**[d]	NA	NA	NA	NA
Volume of left hemisphere	0.09 (0.00 to 0.17)	.05	**0.05 (0.02 to 0.07)**	**.0004**[d]	NA	NA	NA	NA
Volume of right hemisphere	0.10 (0.00 to 0.20)	.06	**0.04 (0.02 to 0.07)**	**.0018**[d]	NA	NA	NA	NA
Thalamus								
Total volume of left and right hemispheres summed	0.08 (−0.13 to 0.29)	.44	NA	NA	NA	NA	NA	NA
Volume of left hemisphere	0.04 (−0.08 to 0.15)	.51	NA	NA	NA	NA	NA	NA
Volume of right hemisphere	0.04 (−0.06 to 0.14)	.45	NA	NA	NA	NA	NA	NA
Brainstem	0.11 (−0.23 to 0.44)	.54	NA	NA	NA	NA	NA	NA

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; NA, not applicable (interaction was dropped from the final model because it did not reach nominal significance [$P < .05$]).

[a] Results from the final regression model examining associations between binary ADHD diagnosis (ADHD vs control) and brain volumes. Boldface indicates results surviving multiple-testing correction.

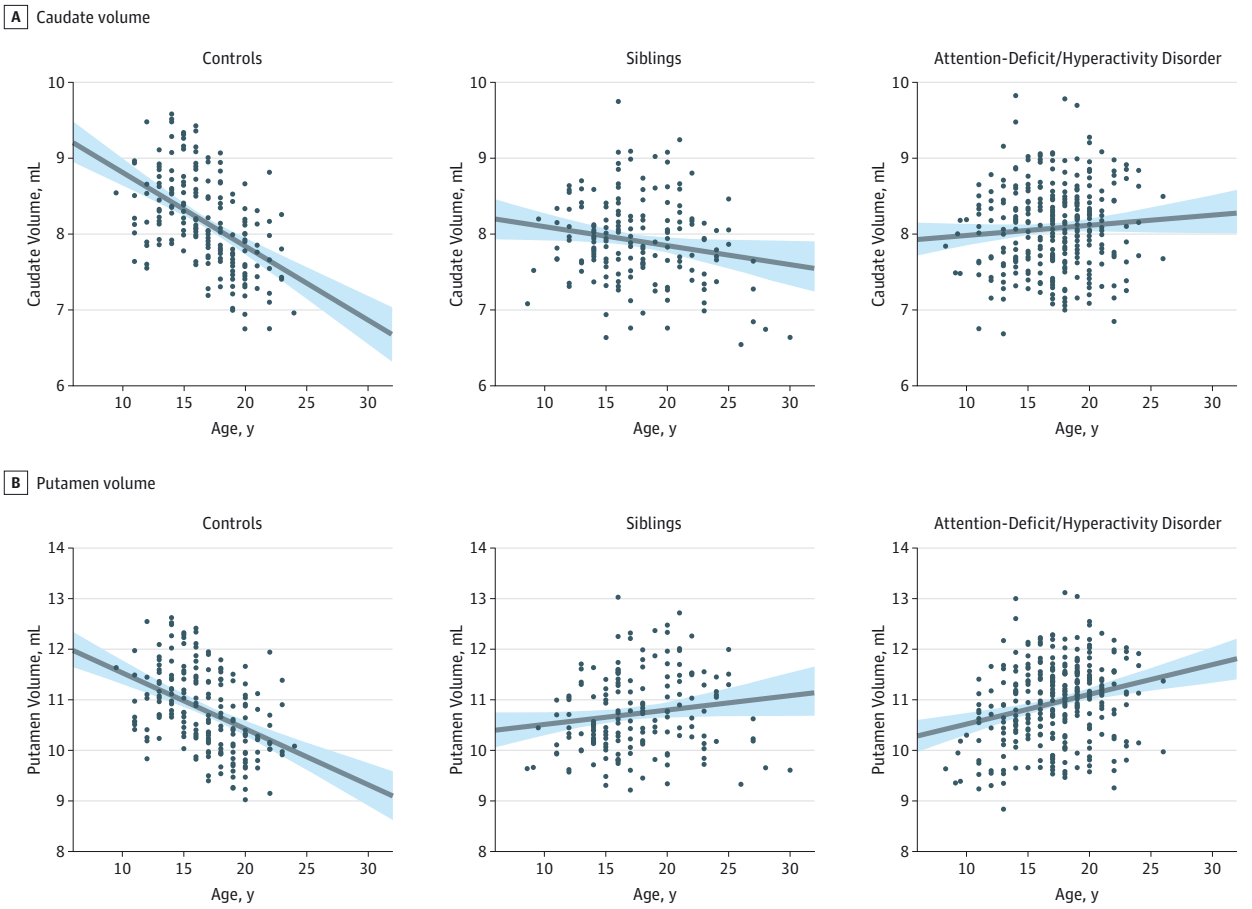
[b] For main effects, β (unstandardized regression coefficient) is equal to the difference in mean brain volumes (in milliliters) between the diagnostic groups adjusted for covariates in the model (eg, β = −31.92 denotes that participants with ADHD had a 31.9-mL-smaller total brain volume than control individuals). The main effect of ADHD was never dropped from the model. Included covariates were age, age squared, sex, and scanner location; for subcortical volumes, covariates also included total brain volume.

[c] Sum of total gray and white matter volumes.

[d] Indicates *P* value remains significant following multiple testing (effective number–adjusted *P* value threshold of .003).

[e] Although the ADHD-by-age interaction on right caudate volume just failed to meet the multiple-testing–corrected threshold, plotting the data revealed that the pattern of results was highly similar for left, right, and total caudate volumes.

Figure 2. Group-by-Age Interaction on Caudate Nucleus Volume (A) and Putamen Volume (B)



Siblings indicate the unaffected siblings of participants with attention-deficit/hyperactivity disorder. The same pattern of results was found for total, left, and right volumes. The lines represent the regression lines, with the shading representing the 95% CIs.

normal brain development such as those concerning neuron number (eg, neurogenesis and naturally occurring neuron death) and neuronal migration.[37,38] This is in line with results from genome-wide association and bioinformatics analyses that implicated abnormal neuronal migration and directed neurite outgrowth in ADHD.[39,40] Such mechanisms could be probed further through studying post-mortem brain tissue or animal models. Smaller total brain and gray matter volumes in participants with ADHD, relative to control individuals, were found at different ages, consistent with previous research that related smaller total brain size to ADHD in children and adolescents,[2,4] as well as in adults.[41]

The caudate and putamen play important roles in several basal ganglia-thalamocortical circuits[42] involved in motor control and learning, as well as selecting and enabling cognitive, executive, and emotional programs.[43] Several of these processes (eg, motor functions, reward processing, and cognitive and attentional control) are impaired in ADHD.[9,26] We found that older age was significantly related to smaller caudate and putamen volumes in control individuals, in line with previous studies in typically developing individuals reporting a decline in basal ganglia volumes across childhood development.[30,44] In contrast, in participants with ADHD, age was not significantly related to caudate and putamen volumes. As a result, participants with ADHD had smaller caudate and putamen volumes than control individuals in childhood and early adolescence (aged 8-15 years); these differences diminished in midadolescence to late adolescence. Previous studies also found smaller, or different-shaped, basal ganglia volumes in ADHD[5-8,45,46]; further, the different developmental trajectories in participants with ADHD and control individuals may provide a potential explanation for previous studies suggesting that alterations in basal ganglia volumes in ADHD normalize with age.[2,7,8] By early adulthood (aged 22-30 years), ADHD was related to larger caudate and putamen volumes relative to control individuals, inconsistent with previous studies in adults with ADHD showing smaller basal ganglia volumes[14,47,48] or no differences.[49-51] Most adult studies included older participants than the current study, potentially explaining this inconsistency. Delays in developmental trajectories of the caudate and putamen[30,44] in ADHD may potentially explain why larger volumes observed in the current study are no longer observed in studies on older samples. Alternatively, the larger volumes in early adulthood may repre-

sent overcompensatory mechanisms or could be related to reduced neuronal pruning. In a longitudinal study of children and adolescents (aged 4-19 years), participants with ADHD had smaller basal ganglia volumes and total surface areas compared with control participants; however, inconsistent with our results, these differences were fixed over time.[52] Only in a small region involved in reward processing, the ventral striatal surface area, did developmental trajectories differ between participants with ADHD and control participants.[52] Hence, a next step to extend the present findings would be to study the mechanisms underlying the age-by-diagnosis interaction effects in caudate and putamen volumes in a longitudinal design and to apply complementary methods in the same participants (eg, surface area and voxel-based morphometry) to reveal findings that may be undetectable by the volumetric technique used here. Overall, our results were consistent with the hypothesis that subcortical alterations are key in the pathophysiology of ADHD.[9]

Findings in the unaffected siblings provided evidence that total brain, gray matter, caudate, and putamen volumes are linked to familial risk for ADHD. As familial underpinnings of ADHD are thought to be largely genetic,[17,53] it is plausible that genetic mechanisms may underlie the reported ADHD-brain associations, creating possible new targets for molecular genetic research. Given the small percentage of volume difference and additional challenges added by age-dependent effects in the caudate and putamen, multisite international consortia comprising large data sets from different age groups are necessary in such gene-identification efforts.

Although effect sizes were small, effects were robust across sensitivity analyses. The only exception was that the intermediate position of unaffected siblings for total brain and gray matter volumes was carried by only 1 of our scanner locations (Nijmegen). Hence, familiality with regard to total brain and gray matter volumes should be interpreted with some caution. When IQ was included as a covariate, alterations in total brain and gray matter volumes were attenuated but remained. There is an active debate whether studies on ADHD should adjust for IQ group differences[54] because IQ is likely to share meaningful variance with ADHD.[55] Therefore, including IQ as a covariate may lead to overcorrection. Hence, we report findings with and without adjustment for IQ. Two cross-sectional meta-analyses and a review, based on binary medication use or percentage treated, concluded that ADHD medication may decrease structural brain alterations in participants with ADHD.[7,8,56] In contrast, a longitudinal study found no significant association between using/not using medication or the proportion of time taking medication and developmental trajectories of basal ganglia.[52] The present study is in line with the latter results, as we found no association between cumulative medication intake and brain volumes in participants with ADHD. Hence, our findings do not support a normalizing effect of stimulant medication on structural brain alterations. Nonetheless, medication effects need to be explored more fully longitudinally before drawing firm conclusions (eg, through studying age at medication initiation, long- vs short-acting stimulants, and stimulants vs nonstimulants).

A major innovation of this study was the inclusion of unaffected siblings, allowing the study of familial effects, the use of a large sample, and the use of a more precise measure of medication (cumulative medication intake) for which we had complete data. The present sample followed a naturalistic design, allowing greater generalization to families with ADHD as a whole, in contrast to the common approach to use a matched-sample design. Nonetheless, our findings were also robust when groups were matched for sex. Further, our sample included adolescents and young adults, which are an underrepresented age group because most studies have focused either on child or adult samples. Therefore, the current study contributes to fill several gaps in the literature and helps to investigate the influence of age on brain volumetric alterations in ADHD.

Naturally, this study also comes with limitations. First, the current study was cross sectional. Longitudinal follow-up is underway. Second, because most of this sample was adolescent, age-dependent results in early adulthood should be interpreted with some caution. Third, although we had clear reasons to focus on total and subcortical volumes, there are other relevant brain regions such as the cerebellum and frontal areas.[2,4,9] Finally, the FIRST algorithm used to segment the subcortical volumes comes with limitations[29]; nonetheless, it is more objective than manual segmentation methods.

Conclusions

Our cross-sectional findings point to the relevance of different trajectories of brain development in ADHD vs control participants that are influenced by familial factors. Our results also support the role of subcortical basal ganglia alterations as key to understanding the pathophysiology of ADHD.

ARTICLE INFORMATION

Submitted for Publication: June 7, 2014; final revision received December 10, 2014; accepted December 12, 2014.

Published Online: March 18, 2015. doi:10.1001/jamapsychiatry.2014.3162.

Author Affiliations: Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands (Greven, Bralten, Mennes, O'Dwyer, Zwiers, Arias-Vasquez, Buitelaar); Karakter Child and Adolescent Psychiatry University Center, Nijmegen, the Netherlands (Greven, Rommelse, Buitelaar); Social, Genetic, and Developmental Psychiatry Center, Institute of Psychiatry, King's College London, London, England (Greven); Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands (Bralten, van Hulzen, Franke, Arias-Vasquez); Department of Psychiatry, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands (Rommelse, Franke, Arias-Vasquez); Department of Psychiatry, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands (Schweren, Hoekstra, Hartman); Department of Clinical Neuropsychology, VU University Amsterdam, Amsterdam, the Netherlands (Heslenfeld, Oosterlaan); Department of Psychiatry, State University of New York Upstate Medical University, Syracuse (Faraone); Donders Institute for Brain, Cognition, and Behaviour, Radboud University, Nijmegen, the Netherlands (Zwiers).

Author Contributions: Dr Greven and Ms Bralten had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr Greven and Ms Bralten were joint first authors.
Study concept and design: Greven, Bralten, Rommelse, Hartman, Heslenfeld, Oosterlaan,

Faraone, Franke, Zwiers, Arias-Vasquez, Buitelaar.
Acquisition, analysis, or interpretation of data: Greven, Bralten, Mennes, O'Dwyer, van Hulzen, Rommelse, Schweren, Hoekstra, Hartman, Oosterlaan, Faraone, Franke, Zwiers, Arias-Vasquez, Buitelaar.
Drafting of the manuscript: Greven, Bralten, Mennes, Rommelse, Arias-Vasquez, Buitelaar.
Critical revision of the manuscript for important intellectual content: Greven, Bralten, Mennes, O'Dwyer, van Hulzen, Schweren, Hoekstra, Hartman, Heslenfeld, Oosterlaan, Faraone, Franke, Zwiers, Arias-Vasquez, Buitelaar.
Statistical analysis: Greven, Bralten, Mennes, van Hulzen, Schweren, Zwiers, Arias-Vasquez.
Obtained funding: Heslenfeld, Oosterlaan, Faraone, Franke, Buitelaar.
Administrative, technical, or material support: O'Dwyer, Rommelse, Heslenfeld, Oosterlaan, Zwiers.
Study supervision: Hoekstra, Oosterlaan, Faraone, Franke, Arias-Vasquez, Buitelaar.

Conflict of Interest Disclosures: Dr Hoekstra has received honoraria from Eli Lilly and Shire and an unrestricted research grant from Shire. Dr Oosterlaan has received an unrestricted grant from Shire. Dr Faraone has received consulting income and/or research support from Shire, Otsuka Pharmaceutical, and Alcobra Pharma. He has also received consulting fees from, was on advisory boards for, or participated in continuing medical education programs sponsored by Shire, Ortho-McNeil-Janssen Pharmaceuticals, Novartis, Pfizer, and Eli Lilly. Dr Faraone receives royalties from books published by Guilford Press, *Straight Talk About Your Child's Mental Health*, and Oxford University Press, *Schizophrenia: The Facts*. Dr Buitelaar has been a consultant to/member of advisory boards of and/or a speaker for Janssen-Cilag BV, Eli Lilly, Bristol-Myers Squibb, Schering Plough, UCB, Shire, Novartis, and Servier. No other disclosures were reported.

Funding/Support: This work was supported by National Institutes of Health grant R01MH62873 (Dr Faraone), NWO Large Investment grant 1750102007010 (Dr Buitelaar), NWO Brain & Cognition grant 433-09-242 (Dr Buitelaar), and grants from Radboud University Medical Center, University Medical Center Groningen, Accare, and VU University Amsterdam. Dr Faraone has received research support from the National Institutes of Health.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Previous Presentations: This study was presented at the 25th Annual Convention of the Association for Psychological Science, May 24, 2013, Washington, DC; the 23rd Eunethydis ADHD Network Meeting, October 6, 2013, Prague, Czech Republic; and the 3rd Eunethydis International Conference on ADHD, May 22, 2014, Istanbul, Turkey.

Additional Contributions: We acknowledge the Department of Pediatrics of the VU University Medical Center for the opportunity to use the mock scanner for preparation of our participants.

REFERENCES

1. Polanczyk G, Rohde LA. Epidemiology of attention-deficit/hyperactivity disorder across the lifespan. *Curr Opin Psychiatry*. 2007;20(4):386-392.

2. Castellanos FX, Lee PP, Sharp W, et al. Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *JAMA*. 2002;288(14):1740-1748.

3. Castellanos FX, Giedd JN, Marsh WL, et al. Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. *Arch Gen Psychiatry*. 1996;53(7):607-616.

4. Durston S, Hulshoff Pol HE, Schnack HG, et al. Magnetic resonance imaging of boys with attention-deficit/hyperactivity disorder and their unaffected siblings. *J Am Acad Child Adolesc Psychiatry*. 2004;43(3):332-340.

5. Valera EM, Faraone SV, Murray KE, Seidman LJ. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. *Biol Psychiatry*. 2007;61(12):1361-1369.

6. Ellison-Wright I, Ellison-Wright Z, Bullmore E. Structural brain change in attention deficit hyperactivity disorder identified by meta-analysis. *BMC Psychiatry*. 2008;8(51):51.

7. Nakao T, Radua J, Rubia K, Mataix-Cols D. Gray matter volume abnormalities in ADHD: voxel-based meta-analysis exploring the effects of age and stimulant medication. *Am J Psychiatry*. 2011;168(11):1154-1163.

8. Frodl T, Skokauskas N. Meta-analysis of structural MRI studies in children and adults with attention deficit hyperactivity disorder indicates treatment effects. *Acta Psychiatr Scand*. 2012;125(2):114-126.

9. Halperin JM, Schulz KP. Revisiting the role of the prefrontal cortex in the pathophysiology of attention-deficit/hyperactivity disorder. *Psychol Bull*. 2006;132(4):560-581.

10. Biederman J, Mick E, Faraone SV. Age-dependent decline of symptoms of attention deficit hyperactivity disorder: impact of remission definition and symptom type. *Am J Psychiatry*. 2000;157(5):816-818.

11. Greven CU, Asherson P, Rijsdijk FV, Plomin R. A longitudinal twin study on the association between inattentive and hyperactive-impulsive ADHD symptoms. *J Abnorm Child Psychol*. 2011;39(5):623-632.

12. Larsson H, Lichtenstein P, Larsson JO. Genetic contributions to the development of ADHD subtypes from childhood to adolescence. *J Am Acad Child Adolesc Psychiatry*. 2006;45(8):973-981.

13. Greven CU, Rijsdijk FV, Plomin R. A twin study of ADHD symptoms in early adolescence: hyperactivity-impulsivity and inattentiveness show substantial genetic overlap but also genetic specificity. *J Abnorm Child Psychol*. 2011;39(2):265-275.

14. Onnink AM, Zwiers MP, Hoogman M, et al. Brain alterations in adult ADHD: effects of gender, treatment and comorbid depression. *Eur Neuropsychopharmacol*. 2014;24(3):397-409.

15. Levy F, Hay DA, Bennett KS. Genetics of attention deficit hyperactivity disorder: a current review and future prospects. *Int J Disabil Dev Educ*. 2006;53(1):5-20.

16. Faraone SV, Biederman J, Mick E, et al. Family study of girls with attention deficit hyperactivity disorder. *Am J Psychiatry*. 2000;157(7):1077-1083.

17. Sprich S, Biederman J, Crawford MH, Mundy E, Faraone SV. Adoptive and biological families of children and adolescents with ADHD. *J Am Acad Child Adolesc Psychiatry*. 2000;39(11):1432-1437.

18. Faraone SV, Mick E. Molecular genetics of attention deficit hyperactivity disorder. *Psychiatr Clin North Am*. 2010;33(1):159-180.

19. Peper JS, Brouwer RM, Boomsma DI, Kahn RS, Hulshoff Pol HE. Genetic influences on human brain structure: a review of brain imaging studies in twins. *Hum Brain Mapp*. 2007;28(6):464-473.

20. den Braber A, Bohlken MM, Brouwer RM, et al. Heritability of subcortical brain measures: a perspective for future genome-wide association studies. *Neuroimage*. 2013;83:98-102.

21. Pironti VA, Lai MC, Müller U, et al. Neuroanatomical abnormalities and cognitive impairments are shared by adults with attention-deficit/hyperactivity disorder and their unaffected first-degree relatives. *Biol Psychiatry*. 2014;76(8):639-647.

22. von Rhein D, Mennes M, van Ewijk H, et al. The NeuroIMAGE Study: a prospective phenotypic, cognitive, genetic and MRI study in children with attention-deficit/hyperactivity disorder: design and descriptives [published online July 11, 2014]. *Eur Child Adolesc Psychiatry*.

23. Müller UC, Asherson P, Banaschewski T, et al. The impact of study design and diagnostic approach in a large multi-centre ADHD study, part 1: ADHD symptom patterns. *BMC Psychiatry*. 2011;11(54):54.

24. Müller UC, Asherson P, Banaschewski T, et al. The impact of study design and diagnostic approach in a large multi-centre ADHD study, part 2: dimensional measures of psychopathology and intelligence. *BMC Psychiatry*. 2011;11(55):55.

25. Nijmeijer JS, Hoekstra PJ, Minderaa RB, et al. PDD symptoms in ADHD: an independent familial trait? *J Abnorm Child Psychol*. 2009;37(3):443-453.

26. Rommelse NN, Altink ME, Martin NC, et al. Neuropsychological measures probably facilitate heritability research of ADHD. *Arch Clin Neuropsychol*. 2008;23(5):579-591.

27. Conners CK, Sitarenios G, Parker JD, Epstein JN. The revised Conners' Parent Rating Scale (CPRS-R): factor structure, reliability, and criterion validity. *J Abnorm Child Psychol*. 1998;26(4):257-268.

28. Blumenthal JD, Zijdenbos A, Molloy E, Giedd JN. Motion artifact in magnetic resonance imaging: implications for automated analysis. *Neuroimage*. 2002;16(1):89-92.

29. Patenaude B, Smith SM, Kennedy DN, Jenkinson M. A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage*. 2011;56(3):907-922.

30. Brain Development Cooperative Group. Total and regional brain volumes in a population-based normative sample from 4 to 18 years: the NIH MRI Study of Normal Brain Development. *Cereb Cortex*. 2012;22(1):1-12.

31. Reiss AL, Abrams MT, Singer HS, Ross JL, Denckla MB. Brain development, gender and IQ in children: a volumetric imaging study. *Brain*. 1996;119(pt 5):1763-1774.

32. Mechelli A, Price CJ, Friston KJ, Ashburner J. Voxel-based morphometry of the human brain: methods and applications. *Curr Med Imaging Rev*. 2005;1(2):105-113.

33. Bradley RA, Srivastava SS. Correlation in polynomial regression. *Am Stat*. 1979;33(1):11-14. doi:10.2307/2683059.

34. StataCorp. *Stata Statistical Software: Release 10*. College Station, TX: StataCorp; 2007.

35. Williams RL. A note on robust variance estimation for cluster-correlated data. *Biometrics*. 2000;56(2):645-646.

36. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity (Edinb)*. 2005;95(3):221-227.

37. Kwan KY, Sestan N, Anton ES. Transcriptional co-regulation of neuronal migration and laminar identity in the neocortex. *Development*. 2012;139(9):1535-1546.

38. Kuan CY, Roth KA, Flavell RA, Rakic P. Mechanisms of programmed cell death in the developing brain. *Trends Neurosci*. 2000;23(7):291-297.

39. Poelmans G, Pauls DL, Buitelaar JK, Franke B. Integrated genome-wide association study findings: identification of a neurodevelopmental network for attention deficit hyperactivity disorder. *Am J Psychiatry*. 2011;168(4):365-377.

40. Franke B, Neale BM, Faraone SV. Genome-wide association studies in ADHD. *Hum Genet*. 2009;126(1):13-50.

41. Hoogman M, Rijpkema M, Janss L, et al. Current self-reported symptoms of attention deficit/hyperactivity disorder are associated with total brain volume in healthy adults. *PLoS One*. 2012;7(2):e31273.

42. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci*. 1986;9:357-381.

43. Ring HA, Serra-Mestres J. Neuropsychiatry of the basal ganglia. *J Neurol Neurosurg Psychiatry*. 2002;72(1):12-21.

44. Goddings AL, Mills KL, Clasen LS, Giedd JN, Viner RM, Blakemore SJ. The influence of puberty on subcortical brain development. *Neuroimage*. 2014;88:242-251.

45. Qiu A, Crocetti D, Adler M, et al. Basal ganglia volume and shape in children with attention deficit hyperactivity disorder. *Am J Psychiatry*. 2009;166(1):74-82.

46. Sobel LJ, Bansal R, Maia TV, et al. Basal ganglia surface morphology and the effects of stimulant medications in youth with attention deficit hyperactivity disorder. *Am J Psychiatry*. 2010;167(8):977-986.

47. Almeida Montes LG, Ricardo-Garcell J, Barajas De La Torre LB, et al. Clinical correlations of grey matter reductions in the caudate nucleus of adults with attention deficit hyperactivity disorder. *J Psychiatry Neurosci*. 2010;35(4):238-246.

48. Seidman LJ, Biederman J, Liang L, et al. Gray matter alterations in adults with attention-deficit/hyperactivity disorder identified by voxel based morphometry. *Biol Psychiatry*. 2011;69(9):857-866.

49. Ahrendts J, Rüsch N, Wilke M, et al. Visual cortex abnormalities in adults with ADHD: a structural MRI study. *World J Biol Psychiatry*. 2011;12(4):260-270.

50. Depue BE, Burgess GC, Bidwell LC, Willcutt EG, Banich MT. Behavioral performance predicts grey matter reductions in the right inferior frontal gyrus in young adults with combined type ADHD. *Psychiatry Res*. 2010;182(3):231-237.

51. Seidman LJ, Valera EM, Makris N, et al. Dorsolateral prefrontal and anterior cingulate cortex volumetric abnormalities in adults with attention-deficit/hyperactivity disorder identified by magnetic resonance imaging. *Biol Psychiatry*. 2006;60(10):1071-1080.

52. Shaw P, De Rossi P, Watson B, et al. Mapping the development of the basal ganglia in children with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry*. 2014;53(7):780-789.e11.

53. Faraone SV, Perlis RH, Doyle AE, et al. Molecular genetics of attention-deficit /hyperactivity disorder. *Biol Psychiatry*. 2005;57(11):1313-1323.

54. Dennis M, Francis DJ, Cirino PT, Schachar R, Barnes MA, Fletcher JM. Why IQ is not a covariate in cognitive studies of neurodevelopmental disorders. *J Int Neuropsychol Soc*. 2009;15(3):331-343.

55. Rommelse NN, Altink ME, Oosterlaan J, Buschgens CJ, Buitelaar J, Sergeant JA. Support for an independent familial segregation of executive and intelligence endophenotypes in ADHD families. *Psychol Med*. 2008;38(11):1595-1606.

56. Spencer TJ, Brown A, Seidman LJ, et al. Effect of psychostimulants on brain structure and function in ADHD: a qualitative literature review of magnetic resonance imaging-based neuroimaging studies. *J Clin Psychiatry*. 2013;74(9):902-917.

J Autism Dev Disord (2008) 38:1827–1836
DOI 10.1007/s10803-008-0571-9

ORIGINAL PAPER

Regression, Developmental Trajectory and Associated Problems in Disorders in the Autism Spectrum: The SNAP Study

Gillian Baird · Tony Charman · Andrew Pickles · Susie Chandler · Tom Loucas · David Meldrum · Iris Carcani-Rathwell · Devanitha Serkana · Emily Simonoff

Published online: 1 May 2008

Abstract We report rates of regression and associated findings in a population derived group of 255 children aged 9–14 years, participating in a prevalence study of autism spectrum disorders (ASD); 53 with narrowly defined autism, 105 with broader ASD and 97 with non-ASD neurodevelopmental problems, drawn from those with special educational needs within a population of 56,946 children. Language regression was reported in 30% with narrowly defined autism, 8% with broader ASD and less than 3% with developmental problems without ASD. A smaller group of children were identified who underwent a less clear setback. Regression was associated with higher rates of autistic symptoms and a deviation in developmental trajectory. Regression was not associated with epilepsy or gastrointestinal problems.

Keywords SNAP · Autism · Regression · Outcome · Epilepsy · Gastro-intestinal problems

G. Baird (✉) · S. Chandler
Newcomen Centre, Guy's & St. Thomas' NHS Foundation Trust, St. Thomas's Street, London SE1 9RT, UK
e-mail: gillian.baird@gstt.nhs.uk

T. Charman
Behavioural & Brain Sciences Unit, UCL Institute of Child Health, London, UK

A. Pickles
Biostatistics, Health Methodology Research Group, University of Manchester, Manchester, UK

T. Loucas
School of Psychology and Clinical Language Sciences, University of Reading, Reading, UK

D. Meldrum
Chatswood Assessment Centre, Sydney, NSW, Australia

I. Carcani-Rathwell
Greenwich Neurodevelopmental CAMHS, Oxleas NHS Trust, London, UK

D. Serkana
Cheyne Child Development Centre, Chelsea & Westminster Hospital, London, UK

E. Simonoff
Department of Child and Adolescent Psychiatry, Institute of Psychiatry, King's College London, London, UK

Retrospective histories from parents and analysis of home videotapes have shown that for most children with autism, abnormalities in development become clear prior to 2 years of age (Adrien et al. 1991; Dahlgren and Gillberg 1989; Losche 1990; Osterling and Dawson 1994; Rogers and DiLalla 1990; Werner et al. 2005; Werner and Dawson 2005) and often within the first year of life. However, a feature of autistic development that remains a puzzle is that some children present with apparently normal development as perceived by parents followed by quite marked cessation of skill acquisition and frequent loss of, or failure to use, acquired language and social skills. Commonest in the second year of life, this has been termed 'autistic regression', and occurs in 15–40% of children with autism (Fombonne and Chakrabarti 2001; Kurita 1985; Lord et al. 2004; Luyster et al. 2005; Prizant 1996; Richler et al. 2006; Tuchman and Rapin 1997).

Some parents report that there is a very abrupt change in their child's development and behaviour, others report a much more gradual change lasting weeks. In some cases parents report that development has been normal prior to the regression (although more detailed examination and retrospective videos may indicate some subtle impairment),

others report that although there was some delay in acquiring skills they still note significant regression. Even in those children where there is no obvious loss of skills, stasis of cognitive and social development may be reported by parents and has now been found in studies of at risk infants—the siblings of children with autism (Landa and Garrett-Mayer 2006; Landa et al. 2007).

A common operational definition of regression is a loss of spoken language after the first 3–5 word stage of acquisition (LeCouteur et al. 1989; Lord et al. 1994, 2004; Tuchman and Rapin 1997). There is also usually loss of non-verbal communication (e.g. gestures such as waving bye-bye) frequently decreased use of eye gaze to regulate social interaction, some social withdrawal and lack of social interest, and sometimes a loss of play skills (Ozonoff et al. 2005; Werner et al. 2005). Gross motor development is not usually affected although some parents note a change in fine manipulative skills (Davidovitch et al. 2000). However, in children who have not reached the 3–5 word stage of language development, parents may note regression of babble and proto-words with or without regression in social interest, gestures etc. The period of developmental stasis or loss of skill-use is usually followed by a regaining of skills but at varying rates. Some children never regain lost skills (Evans-Jones and Rosenbloom 1978; Lord et al. 2004).

Several reports have suggested that the eventual outcome in children with regression is that of a lower language level, lower IQ and lower adaptive level compared with those who do not regress (Kurita 1985; Hoshino et al. 1987; Kobayashi and Murata 1998; Rogers and DiLalla 1990). However, other studies have found no difference in outcome (Chakrabarti and Fombonne 2001; Davidovitch et al. 2000; Lord et al. 2004) or mixed results with the regressed group showing a bimodal outcome in verbal IQ and social reciprocity (Richler et al. 2006) which may be a result of inclusion of differing groups within the autism spectrum.

Regression to autism in older children following a period of *clearly normal* development up to the age of at least 2 years is classified separately in ICD-10 (WHO 1993) and DSM-IV (APA 2000) as Developmental or Other Childhood Disintegrative Disorder (CDD). Previously titled Heller's syndrome, regression is in language, communication, social play, curiosity in the environment, sometimes loss of bowel/bladder function and the onset of stereotyped skills. This is a rare phenomenon. Fombonne (2002) estimates a prevalence of CDD of no more than 0.2/10,000. In several studies the CDD group have a poorer outcome in terms of cognitive and functional skills (Malhotra and Gupta 2002; Volkmar and Cohen 1989), though Kurita et al. (2004) found no such difference.

The process underlying regression and stasis is unknown. There has been speculation that the anatomical remodelling of the brain with synaptic growth and pruning during the second year of life is impaired in autism due to gene-based mechanisms (Carper and Courchesne 2005) resulting in variable effect on function. Also a suggestion that regression constitutes a genetically different disorder (Molloy et al. 2005), unconfirmed in the IMGSAC sample where in families in which more than one child has autism, regression occurred in one sib and not another (Parr et al. 2006 and in preparation), although newer genetic techniques may cast further light on possible genetic contributions (Marshall et al. 2008; Weiss et al. 2008). Whether the regressive process is influenced by environmental factors is also unknown. There has been concern that the number of children with regressive autism has increased but recent reviews (Fombonne and Chakrabarti 2001; Taylor et al. 2002) have shown no such increase. Considerable research has failed to support an association between one suggested environmental factor, MMR immunisation, postulated to be linked with enterocolitis and the risk of a regressive autistic disorder (Baird et al. 2008; DeStefano 2002; Honda et al. 2005; Richler et al. 2006). Other suggestions have included epilepsy as a causative factor in regression allied to Landau-Kleffner syndrome, an aphasia due to localised peri-sylvian epilepsy (Robinson et al. 2001), although most children with regression of language who have autism do not have epileptic seizures and language regression with autism is not more common in those with epilepsy than those without epilepsy (Shinnar et al. 2001; Tuchman and Rapin 1997).

As part of a prevalence study of autism and related pervasive developmental disorders (commonly called 'autism spectrum disorders'; ASDs) we assessed a group of 255 9-to-14 year old children with and without ASD drawn from a geographically defined population rather than a clinically referred group. A sample weighting procedure enabled us to estimate characteristics of the total population of children with autism and ASD. This study provided us with the opportunity to examine the following questions regarding the nature, timing, consequences and associated features of regression in children with autism, other ASDs and children without ASD with mental retardation, learning difficulties and behavioural disorders: (a) Does regression affect developmental trajectory and outcome? (b) Does regression occur in non-ASD cases? (c) Is there a greater prevalence of gastro-intestinal problems or epilepsy in the regressed versus non-regressed groups?

Methods

Participants

The population studied is a cohort of 56,946 children born between July 1st 1990 and December 31st 1991 from 12

districts in the South Thames region of the UK. Children with a statement of special educational needs (SEN) (1733; 218 of whom had a local ASD diagnosis) or a local diagnosis of ASD but no SEN statement (37) were screened using the Social Communication Questionnaire (SCQ; Rutter et al. 2003). The mean (SD) age at SCQ screening in the whole cohort was 10.3 (0.4) years.

A subset of children, stratified by local diagnosis and high, medium and low SCQ score (255) received an in-depth diagnostic assessment (see Fig. 1 in Baird et al. 2006). The diagnostic assessment included standardized clinical observation (Autism Diagnostic Observation Schedule—Generic (ADOS-G; Lord et al. 2000)) parent interview assessments of autism symptoms (Autism Diagnostic Interview-Revised (ADI-R; Lord et al. 1994)) and assessment of IQ. Children were classified using ICD-10 research criteria as ASD or no ASD by clinical consensus using all sources of information. The ASD group was divided into a 'broad ASD' (105) and 'narrow autism' (53) group, the latter defined as meeting autism criteria on the ADI-R, the ADOS-G and clinical consensus of ICD-10 childhood autism and the broad ASD defined as all other cases meeting clinical consensus of any ASD. The total number of ICD-10 autism symptoms was recorded. The 'no ASD' group (97) had a variety of diagnoses: intellectual disability (mental retardation), specific language or literacy impairments, ADHD/ODD, cerebral palsy, deafness and visual impairment. Collectively they form a control group for some of the analyses.

Measures

The ADI-R has specific questions (Items 11–15) about regression of language and other skills. Regression was defined in two ways. The first adopted the ADI-R definition of strict language regression as 'loss of 5 words used communicatively for 3 months before loss' with or without loss of skills in other areas, a group called 'definite language regression'. An additional group was defined where the parent described stasis or loss of words or babble, but where the child had not reached the 5-word stage or there was regression of skills other than language (Q20 in ADI 2000), called the 'lower level regression' group.

A systematic enquiry was additionally made of early development using items based on the Diagnostic Interview for Social and Communication Disorders (with permission) (DISCO; Wing et al. 2002) that supplemented the ADI items on language acquisition. The DISCO was developed for systematic enquiry about a range of normal and abnormal behaviours but does not have population 'norms'. 17 questions about the normality of development of sucking, babble, gesture, play and social responsiveness in the first year were used and scored as described by the authors yielding a range of scores from 0 to 34 to give a single figure as proxy for normal early development, a higher score indicating greater abnormality. Both the ADI-R and DISCO questions rely on retrospective memory. Contemporaneous child health records were available in the majority of cases (79%) and were systematically searched to look for age of concern, age of referral, medical problems and any contemporaneous note of developmental problems or regression to validate the parental history. No case met criteria for Rett syndrome or CDD.

Epilepsy was enquired about twice, once during the ADI-R and at subsequent interview about medical conditions. Seizures were classified as febrile only, epileptic past or current (on treatment) and non-epileptic (e.g. reflex anoxic seizures). Medical notes were checked for corroboration of epilepsy. Gastrointestinal (GI) symptoms, reflecting the presentation of GI symptoms in general clinical paediatric practice, for the last 3 months (current) and at any point in the past, were assessed using a questionnaire completed by the main caregiver (Circani-Rathwell et al. in preparation). Current symptoms elicited were of vomiting (occurring at least once per day or more than five times in a week); diarrhoea (defined as loose/watery stools three or more times a day >14 days); persistent abdominal pain (three or more episodes severe enough to interfere with activity) constipation (defined as a bowel action <3 times per week); weight loss; blood in stools and soiling. Past symptoms of vomiting, diarrhoea, abdominal pain (defined as above) and stool withholding were also elicited. The four symptoms of vomiting, diarrhoea, abdominal pain and constipation were summated to give a possible score of 0–4 either of past or current GI symptoms. A 'possible enterocolitis' group was constructed from the presence of 2 or more of the following 5 current gastro intestinal symptoms: persistent diarrhoea, persistent vomiting, weight loss, persistent abdominal pain; blood in stool; plus past diarrhoea >14 days duration and excluding current constipation. 87 children were screened for coeliac antibodies (in whom a sufficient blood sample was obtained) and the single child (from the control group) found to be positive, but asymptomatic, was excluded from the gastro-intestinal analysis together with eight children with cerebral palsy who might be expected to have motility or upper GI problems.

Measures used were IQ, adaptive behaviour on the Vineland Scales (Sparrow et al. 1984) and severity of autism symptoms using an ICD-10 ASD symptom score (0–12). IQ was measured in 209 children using the Wechsler Intelligence Scale for Children (WISC-III-UK; Wechsler 1992), Raven's Standard Progressive Matrices (SPM) or Coloured Progressive Matrices (CPM; Raven et al. 1990a, b), depending on the child's ability. For the 35 cases where SPM or CPM but not WISC full scale IQs

were available, imputed full-scale IQs were obtained using the regression relationship of full to SPM/CPM IQ within each diagnostic group (conversion to IQ from Catherine Lord, personal communication February 2008). For the 11 cases where no direct cognitive testing was possible all cases had Adaptive Behaviour Composite on the Vineland Adaptive Behaviour Scales (Sparrow et al. 1984) below 20 and these cases were assigned an IQ score of 19 to reflect their profound level of mental retardation.

Statistical Analysis

The text includes data on the exact numbers of children with and without regression seen in the study. All subsequent analyses presented in the tables and text are adjusted using stratification weights. Stratification of the ASD/SEN sample was based on whether or not a child had a locally recorded ASD diagnosis (yes/no) and 4 levels of SCQ score (low score (<8), moderately low score (8–14), moderately high score (15–21), high score (≥22; see Fig. 1 in Baird et al. 2006 for details). Use of weights allowed all statistics such as proportions, means, group differences and screen performance measures to be presented as target population estimates, taking account not only of the differences in sampling proportions according to SCQ score and local ASD diagnosis, but also the differential response to the SCQ associated with a prior local ASD diagnosis, health district and child's sex. Standard errors of simple means and regression, logistic regression and proportional hazards, regression coefficients and contrasts, Wald test statistics and *p*-values were calculated using the linearisation version of the robust parameter covariance matrix as implemented by the svy procedures of Stata 9 (Stata 2005). Confidence intervals for the rate of regression were estimated at the 2.5th and 97.5th percentiles from 1,000 bootstrap samples.

Results

In the 255 cases assessed, regression was reported in 38 cases, 28 with definite language regression and 10 with lower level regression who are reported separately. Table 1 shows the number of cases, weighted rates and 95% confidence intervals (CIs) according to regression category and diagnosis.

The Definite Language Regression Group

Of the 28 children who met criteria for 'definite language regression', 26 had ASD and 2 did not. The rate of definite language regression was significantly higher (30.2%) in the narrow autism group than in the broad ASD group (8%, $p = .01$) and the no ASD group (2.8%, $p = .003$). The rate of definite language regression did not significantly differ between the (combined) ASD group (12.6%) and the no ASD group ($p = .08$). 16.3% regressed in one or more area other than language: 5.5% were reported to have lost purposive hand movements (but did not follow a trajectory typical of Rett syndrome), 10.5% motor skills, 2.4% self-help skills, 19.4% constructive or imaginative play and 19.4% were reported to have regressed in the area of social engagement/responsiveness. Association with illness (regression reported by parents as occurring within 7 days of an illness) was reported in 11 children: non-encephalopathic illness in 8 with ASD (two parents reported that illness and regression followed within 2 weeks of the MMR); 1 ASD case presented aged 1 year with frequent epileptic seizures and had a left temporal tumour subsequently removed. Of the 2 remaining non-ASD cases, one had a definite encephalitis, the other was a child with Down's syndrome who developed leukaemia.

Table 1 Presence of regression according to diagnostic category

	No ASD	Broad ASD	Narrow autism
No regression			
N	94	93	30
Rate	.97	.89	.61
95% CIs	.91–1.00	.79–.97	.45–.76
Lower-level regression			
N	1	4	5
Rate	.00	.03	.08
95% CIs	0–.02	.01–.06	.02–.17
Language regression			
N	2	8	18
Rate	.03	.08	.30
95% CIs	0–.09	.02–.18	.18–.45

Rates and confidence intervals (CIs) are weighted. Rates are given for proportion of each regression classification within each diagnostic group

Age at Regression

The mean weighted age of regression was 25.0 (SE 1.5) months for the cases with definite language regression. For the two non-ASD cases with clear language regression the age of loss was 20 months and 48 months, respectively. The only other case with age of language loss greater than 33 months was one case with autism whose parents, on the ADI-R, reported plateauing of development at 24 months and then loss of language at 69 months of age, although contemporaneous health records indicated parental report of language loss at 24 months of age and they also reported that development prior to language loss was not normal.

Table 2 Outcome in IQ, Vineland adaptive behaviour scale and ICD-10 ASD symptom severity according to regression and diagnostic classification

		Mean score (95% confidence intervals)		
		No regression	Lower-level regression	Definite language regression
IQ	Combined ASD	70.3 (63.1–77.5)	60.6 (41.9–79.3)	65.0 (57.6–70.3)
	Broad ASD	72.8 (64.1–81.6)	72.2 (58.5–86.0)	64.1 (55.5–72.8)
	Narrow autism	55.2 (50.6–61.8)	43.9 (14.6–73.0)	63.8 (54.7–73.0)
Vineland	Combined ASD	46.7 (42.0–51.4)	37.5 (25.9–49.2)	42.3 (34.7–49.8)
	Broad ASD	49.1 (44.1–54.1)	45.1 (33.8–56.4)	47.7 (39.2–56.0)
	Narrow autism	38.5 (26.8–40.2)	26.5 (13.8–39.3)	37.7 (31.0–44.4)
ICD-10 symptom score	Combined ASD[a,b]	7.00 (6.41–7.52)	10.15 (9.14–11.17)	8.07 (5.53–10.61)
	Broad ASD [a,b,c]	6.31 (5.86–6.76)	9.69 (8.16–11.24)	5.69 (3.19–8.19)
	Narrow autism	10.55 (9.99–11.11)	10.81 (10.46–11.16)	10.12 (9.23–11.01)

F tests for significant differences within diagnostic category across regression groups, $p < .05$

[a] Overall test between all three groups

[b] No regression vs. lower level regression

[c] Lower-level regression vs. definite language regression

The pattern of development was thus not consistent with CDD.

Outcome at 9–14 Years of Definite Language Regression Compared with No Regression

Outcome was examined in terms of IQ, Vineland composite scores, and ICD-10 ASD symptom score. Table 2 shows mean differences and pair-wise comparisons. The effects of regression controlling for diagnosis were tested in multivariate regression models. With regard to IQ and Vineland scores, there was no significant difference between regression and non-regression once diagnostic category was taken into account. The ICD-10 ASD symptom score was significantly greater in the regression group than the no regression (non-standardized B = 3.25, $p < .001$). The effect remained significant when diagnosis (broad ASD or narrow autism) was accounted for. Age at regression was not significantly associated with outcome IQ, Vineland scores or ICD-10 ASD symptom score.

Validation of Parental History of Regression

Contemporaneous casenote information was available for 16 of the 28 cases with clear regression. Loss of skills or stasis/plateau was documented by paediatricians (from parental report) in 11/16 cases (69%). For these 11 cases the age of regression recorded in casenotes was 25.1 (SE = 1.9) months compared to 28.4 (4.7) reported by parents in the ADI-R, a difference that was not significant (paired t-test; t = 0.65, $p > .10$). The discrepancy between the reported ages was 12 months or less in N = 8 cases and greater than 12 months in N = 3 cases, including the case where contemporaneous casenotes indicated a parent-reported loss of language from more than 20 words at age 24 months but loss was subsequently reported at age 69 months in the ADI-R.

Language Development and Regression

Age of first words and age of first phrases (weighted) as reported by parents during the ADI-R are shown in

Table 3 Early development according to regression classification

Diagnoses combined	Mean score (95% confidence intervals)		
	No regression	Lower level regression	Definite language regression
Age of first words[a,b,c]	26.1 (22.0–30.2)	46.7 (17.5–75.8)	15.9 (13.6–19.0)
Age of phrase speech	43.5 (37.2–49.7)	51.6 (30.0–73.2)	49.7 (39.4–70.0)
Early development problems[a,b]	10.3 (8.6–12.1)	10.7 (4.8–16.5)	6.6 (4.8–8.3)

F tests for significant differences within diagnostic category across regression groups, $p < .05$

[a] Overall test between all three groups

[b] Definite language regression vs. no regression

[c] Definite language regression vs. lower-level regression

Table 3. At the time of our assessment, amongst those with autism or ASD, only 1 child with an ASD had not attained single words. The age of acquiring first words in the definite language loss group is significantly younger than the no regression group (B = −30.6, $p = .03$); these findings remained significant when diagnosis was accounted for. There was no significant difference between the definite language regression and no regression groups in age of acquiring phrase speech, either on its own, or with diagnosis added as a covariate.

We examined whether the failure to achieve phrase speech varied according to regression group. Twelve children with a ASD had not achieved phrase speech by the time of the assessment, of whom 2 had a ASD diagnosis and 10 an autism diagnosis representing 8% of the non-regression group, and 9% of the definite language regression group. A Cox proportional hazards model of the time to phrase speech that took into account the censored times from those who had not achieved phrase speech, confirmed the absence of significant regression group differences, particularly when controlling for delay in phrase acquisition due to diagnosis (hazard ratio 0.61, $p = .03$).

Early Developmental Skills, Developmental Trajectory and Regression

The definite language regression group had lower DISCO total scores (indicating less abnormality) than the no regression group (B = −3.7, $p = .003$) and remained so after diagnosis was accounted for (B = −4.4, $p < .001$).

The relationship between outcome (symptom severity), regression and early development, was then explored by predicting in a linear regression model symptom severity from early development score, level of regression and interaction between the two independent variables. A significant interaction (t = −2.17, $p = 0.03$) was found, such that the early development score was significantly positively related to later symptom severity in the no regression and lower level regression groups but unrelated in the definite regression group (Wald test $F(1,150) = 1.12$, $p = 0.29$).

'Lower Level' Regression in ASD

Parents of 10 children reported symptoms that met the criteria for 'lower level' regression. The rate of lower-level regression was significantly higher (8.4%) in the narrow autism group than in the broad ASD group (2.6%, $p = .04$) and the no ASD group (0.4%, $p = .002$). The (combined) ASD group was significantly more likely to show lower level regression ($p = .02$). Of the 10 children with lower-level regression, 9 had ASD. Regression was not associated with illness in the 9 with ASD; the one child without ASD regressed in motor skills only having had a cerebrovascular event complicating an ear infection and resulting in cerebral palsy. Of the 9 with ASD, two (20%) lost babble or words, 6 (60%) lost social engagement or play skills, 1 lost hand and self-help skills. Contemporaneous casenote information was available on 7/10 of the lower-level regression cases. Five of these 7 (71%) have a note of some type of regression or stasis. The age at regression was 25.0 (SE 3.3) months for cases with lower level regression (not different to definite language regression).

Prior to regression, the score for DISCO items in the lower level regression group (mean 10.6) was similar to that in the no regression group (mean 10.3). Hence, there was greater developmental impairment than in the definite regression group (mean 6.6), although this difference was not significant due to small sample size in the lower level regression group. The lower level regression group had a significantly higher ICD-10 ASD symptom score than the no regression group (B = 2.06, $p = .003$) and the definite language regression group (B = 2.31, $p = .01$). 36% of the low-level regression group had not developed phrase speech at the time of assessment (compared with 8 and 9% of the no regression and language regression groups, respectively).

Both Regression Groups Combined

The outcome of both definite language and lower level regression combined is similar to each in that the main effect is on increase in ICD10 symptoms and thus a diagnosis of autism rather than ASD.

Table 4 Rate of febrile convulsions and epilepsy according to regression group

	Rate (95% confidence intervals) and N affected		
	No regression	Lower-level regression	Definite language regression
Febrile convulsions	.03 (0–.07), N = 5	.07 (.01–.07), N = 1	.05 (0–.31), N = 1
Epilepsy ever	.12 (05–.21), N = 15	.33 (0–.71), N = 2	.07 (0–.25), N = 2
Current epilepsy	.07 (.02–.15), N = 8	.33 (0–.75), N = 2	0 (N/A), N = 0

Table 5 Mean number of gastrointestinal symptoms[a]

	Mean number of symptoms (95% confidence intervals), total subjects included		
	No regression	Lower level regression	Definite language regression
Current problems	.50 (321–.7890), N = 100	0 (N/A), N = 7	.13 (0–.341), N = 17
Past problems	.54 (.27–.80), N = 102	.21 (0–.61), N = 7	.81 (.51–1.11), N = 17

[a] Mean scores range from 0 to 4

Association of Regression with Epilepsy or Bowel Problems

The weighted rates of epilepsy are shown in Table 4. Eighteen percent had a past or current history of epilepsy. Eight percent have current epilepsy. There was no evidence suggestive of differential rates of febrile seizures, past or current epilepsy when comparing the definite and no regression groups. Although past and current epilepsy are highest in the group with lower level regression, the difference is not statistically significant due to the small sample size in this cell.

Table 5 shows the weighted mean symptom count for current and past gastrointestinal problems. Current symptoms varied across regression groups ($F(2,122) = 11.96$, $p = .001$), but the rate was higher in the no-regression group than the lower ($F(1,122) = 7.09$, $p = 0.0007$) or definite regression ($F(1,122) = 4.70$, $p = 0.03$) groups. No significant group difference was found in past gastrointestinal symptoms ($F(2,121) = 2.84$, $p = 0.6$). 'Possible enterocolitis', as defined above was reported in one child who did not have ASD, and also did not regress. No child had a previous diagnosis of inflammatory bowel disorder.

Regression in Non-ASD SEN Cases

Clear language regression occurred in 2 non-ASD cases at ages 20 months and 48 months, one had encephalitis, the other was a child with Down's syndrome severely ill with leukaemia. The one non-ASD case with lower level regression had a cerebral incident at age 9 months.

Discussion

In this study, regression is confirmed as a feature of ASD development. It is rare in children who do not have ASD and in these cases if it occurs is likely to be in association with a neurological illness. We have found that the main effect of a history of regression in autism is an outcome of increased symptom score and more severe autism as shown by diagnostic category. This is true for both definite language regression and 'lower level' regression. To investigate the important question of whether regression as a feature of autism presentation exerts an additional effect on potential developmental outcome, we have used a measure of development in the first year as a proxy for developmental competence and compared the predicted trajectory from the DISCO score in non-regressed and regressed groups. The accuracy of parental recall in the DISCO items is unknown. However, the early DISCO score does predict outcome in the non-regressed group and our results suggest that there is an expected continuity in development which is displaced by regression. Thus, despite the definite language regression group showing more typical development in infancy evidenced by earlier first words and less abnormal social communication in the first year (lower DISCO scores), that early trajectory is not maintained (cf. Landa et al. 2007; Pickles et al. under review). The neurodevelopmental abnormality that underlies this deviant developmental trajectory remains to the determined.

This study has used two levels of regression, one clearly defined, the other looser but based on clear parental report of stasis and loss of babble or 1–2 words plus or minus loss in other areas. It remains unclear whether the aetiological or pathological process differs between definite language regression and 'lower level' regression. Although reported here separately to enable comparison with other studies, our results show that the two regression groups show common trends in association with diagnostic group and effects on outcome.

Recent studies reporting the development of siblings of children with autism who go on to develop autism suggests that there is stasis and plateauing of the rate of development in the second year (Landa and Garrett-Mayer 2006; Landa et al. 2007). Thus, overt regression may lie on a continuum of no arrest in developmental progress through plateauing to frank Regression, and the manifestation of the regressive process appears to depend on the stage of brain maturation and of development the child has reached rather than their chronological age (Pickles et al. under review). No case in this study met full criteria for Rett syndrome or CDD. The one case that by parental report on the ADI-R regressed at 69 months was not totally normal prior to language loss, plateauing was reported at 24 months and indeed examination of contemporaneous health records indicated parent report of loss of language at 2 years of age.

Fombonne and Chakrabarti (2001), using a similar definition of 'clear language regression', found no differences in outcome between regressed and non-regressed. Richler et al. (2006) did find lower VIQ scores and higher (more impaired) social reciprocity ADI-R scores following regression but also found a bimodal distribution of VIQ scores in their regression group. That study had much larger numbers than ours (163 with regression and 188 without) and hence greater power to detect differences. However, there were also differences in methods: Richler et al. (2006) used a less stringent diagnostic criterion for autism and a broader definition of regression. Neither of these studies attempted to predict developmental trajectory from reported developmental status prior to regression.

We defined gastro-intestinal problems in a standard way. Several studies have found high reported rates of gastro-intestinal symptoms in ASD (CPEA study, Richler et al. 2006; Valicentini-McDermott et al. 2006). The choice of 14 days for diarrhoea symptoms chosen in this study may be regarded as too short and reflective of acute illness rather than chronic GI disorder, however we found no evidence of more current or past gastrointestinal problems in regressed versus non-regressed groups. This finding is in contrast with the larger CPEA study although there are differences in the questions asked. For example, the CPEA study enquired about 'change in stool frequency and consistency' rather than the specific stool frequency indicating constipation as in the present study. The conclusions in their paper point out that had corrections been made for multiple comparisons of data, the differences would have no longer been significant.

We found no evidence for excess epilepsy in regressed versus non-regressed groups past or current, which is consistent with Tuchman and Rapin (1997). Neither did regression signal increased epilepsy with age (to 11–14 years). No child had a diagnosis of Landau-Kleffner syndrome. The reported age of language regression in this study (25 months) is slightly later than in some other studies. There is variation from a mean 16 months in Lord et al. (2004) and Ozonoff et al. (2005) to around 20 months in many studies. Tuchman and Rapin (1997) noted the age of regression to be 12–48 months. Some variation in age of reported regression may be accounted for by age at reporting. The sample of Lord et al. (2004) was 4–5 years old at their most recent ADI-R but the large CPEA study (Luyster et al. 2005) were interviewed at a mean of 9 years and mean age of reported word loss was 20 months.

Importantly, regression of social, language or motor development rarely occurred in children with non-ASD neurodevelopmental problems (see also Pickles et al. under review who show that regression is rare in children with language disorder), and then in association with encephalopathic illness.

Strengths and Limitations of the Study

The present study reported on a carefully ascertained and assessed population derived sample and thus free of the bias usually associated with a clinically referred sample. It included non-ASD as well as narrow autism and broader ASD cases. The use of a statistical weighting procedure enables generalisation of the findings to the unselected population, though at the expense of precision (note the wide confidence intervals for some estimates). Another strength of the present study was to be able to corroborate parental reporting of regression and medical problems from contemporaneous health records although with only 57% of records available, and only 69% of those documenting regression, positive corroboration exists for <40% of the reported regression cases. The limitations are the relatively small number of cases with regression and in common with most other studies investigating regression in ASD, the reliance on parental report regarding the nature and timing of regression as well as pre-regression development.

Clinical Implications

In young children presenting to child health services with concerns about development, the spectrum of autistic disorders is among the commonest of the developmental disorders. One feature of the history that is particularly important to take note of is regression. In the absence of an acute neurological event or neurological signs including epilepsy, regression in a child of 1–3 years should be a 'red alert' for assessment of autism and signals an altered trajectory of development (Filipek et al. 2000).

Acknowledgments The study was funded by the Wellcome Trust and the Department of Health. We thank the expert group, Patrick Bolton, Antony Cox, Ann Gilchrist, Rebecca Landa, Ann Le Couteur, Catherine Lord, Lennart Pedersen and Michael Rutter. Thanks also to Greg Pasco, Samantha Ross, Vicky Slonims, Emma Rowley and Martha Turner for their help with assessments.

References

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders 4th Edn.—Test Revision (DSM-IV-TR.* Washington, DC: American Psychiatric Association.

Adrien, J. L., Faure, M., Perrot, A., Hameury, L., Garreau, B., Barthelemy, C., et al. (1991). Autism and family home movies—preliminary findings. *Journal of Autism and Developmental Disorders, 21*, 43–49.

Baird, G., Pickles, A., Simonoff, E., Charman, T., Chandler, S., Loucas, T., Meldrum, D., Afzal, M., Thomas, B., Jin, L. & Brown, D. (2008). The measles virus and antibody response to infection and vaccination in autistic spectrum disorder: A virological case control study. *Archives of Disease in Childhood*. doi:10.1136/adc.2007.122937.

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., & Charman, T. (2006). Prevalence of pervasive developmental disorders in a population cohort of children in South East Thames: The Special Needs and Autism Project (SNAP). *The Lancet, 368*, 210–215.

Carper, R. A., & Courchesne, E. (2005). Localized enlargement of the frontal cortex in early autism. *Biological Psychiatry, 57*, 126–133.

Chakrabarti, S., & Fombonne, E. (2001). Pervasive developmental disorders in preschool children. *Journal of the American Medical Association, 285*, 3093–3099.

Dahlgren, S. O., & Gillberg, C. (1989). Symptoms in the 1st 2 years of life—A preliminary population study of infantile-autism. *European Archives of Psychiatry and Clinical Neuroscience, 238*, 169–174.

Davidovitch, M., Glick, L., Holtzman, G., Tirosh, E., & Safir, M. P. (2000). Developmental regression in autism: Maternal perception. *Journal of Autism and Developmental Disorders, 30*, 113–119.

DeStefano, F. (2002). MMR vaccine and autism: A review of the evidence for a causal association. *Molecular Psychiatry, 7*, S51–S52.

Evans-Jones, L. G., & Rosenbloom, L. (1978). Disintegrative psychosis in childhood. *Developmental Medicine and Child Neurology, 20*, 462–470.

Filipek, P. A., Accardo, P. L., Ashwal, S., Baranek, G. T., Cook, E. H., et al. (2000). Practice parameters: Screening and diagnosis of autism. *Neurology*, *55*, 468–479.

Fombonne, E. (2002). Prevalence of childhood disintegrative disorder. *Autism, 6*, 149–157.

Fombonne, E., & Chakrabarti, S. (2001). No evidence for a new variant of measles-mumps-rubella-induced autism. *Pediatrics, 108*, e58.

Honda, H., Shimizu, Y., & Rutter, M. (2005). No effect of MMR withdrawal on the incidence of autism: A total population study. *Journal of Child Psychology and Psychiatry, 46*, 572–579.

Hoshino, Y., Kanako, M., Yashima, Y., Kumashiro, H., Volkmar, F., & Cohen, D. (1987). Clinical features of autistic children with setback course in their infancy. *Japanese Journal of Psychiatry and Neurology, 41*, 237–245.

Kobayashi, R., & Murata, T. (1998). Setback phenomenon in autism and long-term prognosis. *Acta Psychiatrica Scandinavica, 98*, 296–303.

Kurita, H. (1985). Infantile-autism with speech loss before the age of 30 months. *Journal of the American Academy of Child and Adolescent Psychiatry, 24*, 191–196.

Kurita, H., Koyama, T., Setoya, Y., Shimizu, K., & Osada, H. (2004). Validity of childhood disintegrative disorder apart from autistic disorder with speech loss. *European Child and Adolescent Psychiatry, 13*, 221–226.

Landa, R., & Garrett-Mayer, E. (2006). Development in infants with autism spectrum disorders: A prospective study. *Journal of Child Psychology and Psychiatry*, *47*, 629–638.

Landa, R. J., Holman, K. C., & Garrett-Mayer, E. (2007). Social and communication development in toddlers with early and later diagnosis of autism spectrum disorders. *Archives of General Psychiatry*, *64*, 853–864.

LeCouteur, A., Rutter, M., Lord, C., Rios, P., Robertson, S., Holdgrafer, M, et al. (1989). Autism Diagnostic Interview—A standardized investigator-based instrument. *Journal of Autism and Developmental Disorders, 19*, 363–387.

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Leventhal, B. L., DiLavore, P. C., et al. (2000). The Autism Diagnostic Observation Schedule-Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders, 30*, 205–223.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised—A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24*, 659–685.

Lord, C., Shulman, C., & DiLavore, P. (2004). Regression and word loss in autistic spectrum disorders. *Journal of Child Psychology and Psychiatry, 45*, 936–955.

Losche, G. (1990). Sensorimotor and action development in autistic children from infancy to early childhood. *Journal of Child Psychology and Psychiatry*, *31*, 749–761.

Luyster, R., Richler, J., Risi, S., Hsu, W. L., Dawson, G., Bernier, R., et al. (2005). Early regression in social communication in autism spectrum disorders: A CPEA study. *Developmental Neuropsychology, 27*, 311–336.

Malhotra, S., & Gupta, N. (2002). Childhood disintegrative disorder—Re-examination of the current concept. *European Child and Adolescent Psychiatry, 11*, 108–114.

Marshall, C. R., Noor, A., Vincent, J. B., Lionel, A. C., Feuk, L., et al. (2008). Structural variation of chromosomes in autism spectrum disorder. *American Journal of Human Genetics, 82*, 477–488.

Molloy, C. A., Keddache, M., & Martin, L. J. (2005). Evidence for linkage on 21q and 7q in a subset of autism characterized by developmental regression. *Molecular Psychiatry, 10*, 741–746.

Osterling, J., & Dawson, G. (1994). Early recognition of children with autism—A study of 1st birthday home videotapes. *Journal of Autism and Developmental Disorders, 24*, 247–257.

Ozonoff, S., Williams, B. J., & Landa, R. (2005). Parental report of the early development of children with regressive autism—The delays-plus-regression phenotype. *Autism*, *9*, 461–486.

Parr, J. R., Lamb, J. A., Bailey, A. J., Monaco, A. P., & The International Molecular Genetic Study of Autism Consortium (IMGSAC). (2006). Response to paper by Molloy et al: Linkage on 21q and 7q in autism subset with regression. *Molecular Psychiatry, 11*, 617–619.

Pickles, A., Simonoff, E., Conti-Ramsden, G., Falcaro, M., Simkin, Z., Charman, T., Chandler, S., Loucas, T., & Baird, G. (under review). Loss of language in early development of autism and specific language impairment.

Prizant, B. M. (1996). Brief report: Communication, language, social, and emotional development. *Journal of Autism and Developmental Disorders, 26*, 173–178.

Raven, J. C., Court, J. H., & Raven, J. (1990a). *Coloured progressive matrices*. Oxford: Oxford University Press.

Raven, J. C., Court, J. H., & Raven, J. (1990b). *Standard progressive matrices*. Oxford: Oxford University Press.

Richler, J., Luyster, R., Risi, S., Hsu, W. L., Dawson, G., & Bernier, R., et al. (2006). Is there a 'Regressive Phenotype' of autism spectrum disorder associated with the measles-mumps-rubella vaccine? A CPEA Study. *Journal of Autism and Developmental Disorders, 36*, 299–316.

Robinson, R. O., Baird, G., Robinson, G., & Simonoff, E. (2001). Landau-Kleffner syndrome: Course and correlates with outcome. *Developmental Medicine and Child Neurology, 48*, 243–247.

Rogers, S. J., & DiLalla, D. (1990). Age of symptom onset in young children with pervasive developmental disorders. *Journal of the American Academy of Child and Adolescent Psychiatry*, *29*, 863–872.

Rutter, M., Bailey, A., & Lord, C. (2003). *Social Communication Questionnaire (SCQ)*. Los Angeles: Western Psychological Services.

Shinnar, S., Rapin, I., Arnold, S., Tuchman, R. F., Shulman, L., Ballaban-Gil, K., et al. (2001). Language regression in childhood. *Pediatric Neurology, 24*, 183–189.

Sparrow, S., Balla, D., & Cichetti, D. (1984). *Vineland Adaptive Behaviour Scales*. Circle Pines, Minnesota: American Guidance Services.

Stata Statistical Software Release 9.0: Survey Data Manual. (2005). College Station, TX: Stata Corporation.

Taylor, B., Miller, E., Lingam, R., Andrews, N., Simmons, A., & Stowe, J. (2002). Measles, mumps, and rubella vaccination and bowel problems or developmental regression in children with autism: Population study. *British Medical Journal, 324*, 393–396.

Tuchman, R. F., & Rapin, I. (1997). Regression in pervasive developmental disorders: Seizures and epileptiform electroencephalogram correlates. *Pediatrics, 99*, 560–566.

Valicenti-McDermott, M., McVicar, K., Rapin, I., Wershil, B. K., Cohen, H., & Shinnar, S. (2006). Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. *Journal of Developmental Behavior and Pediatrics, 27*, S128–S136.

Volkmar, F. R., & Cohen, D. J. (1989). Disintegrative disorder or late onset autism. *Journal of Child Psychology and Psychiatry, 30*, 717–724.

Wechsler, D. (1992). *Wechsler Intelligence Scale for Children (III-UK Edition)*. London: The Psychological Corporation.

Weiss, L. A., Shen, Y., Korn, J. M., Arking, D. E., Miller, D. T., Fossdal, R., et al. (2008). Association between microdeletion and microduplication at 16p11.2 and autism. *New England Journal of Medicine*, eprint Jan 9 2008.

Werner, E., & Dawson, G. (2005). Validation of the phenomenon of autistic regression using home videotapes. *Archives of General Psychiatry*, *62*, 889–893.

Werner, E., Dawson, G., Munson, J., & Osterling, J. (2005). Variation in early developmental course in autism and its relation with behavioral outcome at 3–4 years of age. *Journal of Autism and Developmental Disorders, 35*, 337–350.

Wing, L., Leekam, S. R., Libby, S. J., Gould, J., & Larcombe, M. (2002). The Diagnostic Interview for Social and Communication Disorders: Background, inter-rater reliability and clinical use. *Journal of Child Psychology and Psychiatry, 43*, 307–325.

World Health Organisation. (1993). *Mental disorders: A glossary and guide to their classification in accordance with the 10th revision of the international classification of diseases: Research diagnostic criteria (ICD-10)*. Geneva: WHO.

www.nature.com/clinicalpractice/neuro

Diagnosis of autism spectrum disorders in the first 3 years of life

Rebecca J Landa

SUMMARY

Autism spectrum disorders (ASDs) are a class of neurodevelopmental disorders defined by qualitative impairments in social functioning and communication, often accompanied by repetitive and stereotyped patterns of behavior and interests. The term 'ASD' encompasses autism, pervasive developmental disorder not otherwise specified, and Asperger's syndrome. ASDs show etiologic heterogeneity, and there is no definitive medical test or cure for these conditions. Around 1 in 150 children have an ASD, with males being affected three to four times more frequently than females. The age at diagnosis of ASD ranges from 3 to 6 years, but there is increasing evidence that diagnosis in the second year of life is possible in some children. Early diagnosis will lead to earlier behavior-based intervention, which is associated with improvements in core areas, such as social functioning and communication. Early detection of—and intervention to treat—ASD is crucial because it is likely to lead to an improved outcome.

KEYWORDS autism, autism spectrum disorder, early detection, early diagnosis, early intervention

REVIEW CRITERIA

PubMed was searched for articles on early symptoms of autism and intervention studies published from January 1993 to August 2007. Search terms included "autism" and "autism spectrum disorders", in combination with "early detection", "early diagnosis", "early intervention", or "regression". Abstracts and relevant articles were reviewed and checked for new information. One intervention study published before 1993 was included in this Review. One review article published after August 2007 was also included.

CME

Medscape **Continuing Medical Education online**

Medscape, LLC is pleased to provide online continuing medical education (CME) for this journal article, allowing clinicians the opportunity to earn CME credit. Medscape, LLC is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide CME for physicians. Medscape, LLC designates this educational activity for a maximum of 1.0 AMA PRA Category 1 Credits™. Physicians should only claim credit commensurate with the extent of their participation in the activity. All other clinicians completing this activity will be issued a certificate of participation. To receive credit, please go to **http://www.medscape.com/cme/ncp** and complete the post-test.

Learning objectives

Upon completion of this activity, participants should be able to:

1 Describe the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*) criteria for the diagnosis of autism spectrum disorders (ASD).
2 Identify the proportion of parents who recognize patterns of developmental disruption in children who subsequently develop ASD.
3 Recognize types of impairment in infants that may predict a later diagnosis of ASD.
4 Describe the recommendations of the American Academy of Pediatrics for autism-specific screening in children.
5 List the recommendations for early intervention that are likely to improve the prognosis of ASD.

Competing interests

The author declared no competing interests. Désirée Lie, the CME questions author, declared no relevant financial relationships.

INTRODUCTION

The term 'autism spectrum disorder' (ASD) refers to a class of neurodevelopmental disorders characterized by qualitative impairments in the development of social and communication skills, often accompanied by stereotyped and restricted patterns of interests and behavior, with onset of impairment before 3 years of age (definition based on the criteria for pervasive developmental disorders in the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition [DSM-IV®; American Psychiatric Association, Washington, DC]).[1] ASDs include the diagnostic categories of autism, pervasive

RJ Landa is Associate Professor of Psychiatry at John Hopkins University, and Director of the Kennedy Krieger Institute's Center for Autism and Related Disorders, Baltimore, MD, USA.

Correspondence
Kennedy Krieger Institute, 3901 Greenspring Avenue, Baltimore, MD 21211, USA
landa@kennedykrieger.org

Received 29 June 2007 **Accepted** 15 November 2007 **Published online** 5 February 2008
www.nature.com/clinicalpractice
doi:10.1038/ncpneuro0731

Table 1 Social, communication, and other developmental disruptions reported before 24 months of age in retrospective and prospective studies of children later diagnosed with an autism spectrum disorder.

Behavioral characteristic	Disruptions first reported at 6–12 months	Disruptions first reported at 9–14 months	Disruptions first reported at 20–24 months
Social responsiveness	Infrequent look to others' faces or gaze aversion[14,101] Poor eye contact[101]	Abnormal orienting to name or others' voice[16–19,102] Infrequent monitoring of others' gaze[35,103] Infrequent response to others' nonverbal cues of shifted attention[35,104]	Lack of imitation[105] Lack of interest in other children[39] Infrequent social gaze in response to others' distress[105] Brief duration of gaze towards others[103]
Social initiation	Poor social initiative[15]	Infrequent initiation of joint attention through pointing or showing[18,35] Infrequent directing of play acts towards others[35] Infrequent initiation of communicative bids for social or regulatory purposes[35]	Infrequent seeking to share[39]
Social–emotional interaction	Absence of facial expression[14,15] Decreased frequency of smiling[101] Lack of emotional modulation[15]	Infrequent sharing of positive affect[35]	Limited range of facial expression[39] Infrequent offering of comfort[39]
Communication and play	Delay in babbling[26] Decreased frequency of vocalization[101]	Low diversity in consonants produced communicatively[35] Infrequent and low variety of conventional gestures[20,35,107] Delayed receptive and expressive language[25] Reduced variety of play acts[35] Reduced variety of action sequences in play[35]	Failure to integrate gaze with other communication behaviors[106] Reduced inventory of words produced[35,66] Abnormal prosody[106]
Sensory, motor or attention behavior	Abnormal postural control[24] Atypical movements[24] Low diversity of movements[24] Hypotonicity[15,24] Poor motor coordination[24,26] Hypoactivity and passivity, and decreased object exploration [15,24] Abnormal pattern and focus of attention[26]	Repetitive and perseverative actions[107,108] Difficulty with attention disengagement[16] Abnormalities in arousal or unusual sensory responses[14,16]	Repetitive behaviors and restricted interests[106]

developmental disorder not otherwise specified (PDD-NOS), and Asperger's syndrome, and they occur in about 1 in 150 children.[2]

The DSM-IV® diagnostic criteria for ASDs were established in children aged 3 years and older, and it is not yet clear whether they are appropriate for younger children. Nevertheless, an increasing emphasis is being placed on earlier detection of ASDs. There are two main reasons for this change: first, there is mounting evidence that developmental disruption is present before 3 years of age in children who are subsequently diagnosed with ASDs; and second, evidence from research on neuroplasticity[3] and intervention for ASDs indicates that early intervention is likely to optimize the outcome for children with ASDs.[4–6]

In this article, I review the evidence for early developmental disruption in children with ASDs and discuss the stability of early diagnosis of an ASD, early ASD-specific screening, and early intervention. Table 1 summarizes the signs of developmental disruption identified in retrospective video analysis studies and prospective studies of ASDs in the first 2 years of life. I shall focus only on autism and PDD-NOS, which are usually diagnosed between 3 and 6 years of age.[7] These conditions will be referred to collectively as ASDs, unless the cited literature specifies one condition or the other.

DEVELOPMENTAL DISRUPTION IN THE FIRST 2 YEARS OF LIFE

Parental concerns

Parental concerns that a child has an ASD can arise as early as the first year of life, but they are most likely to arise when a child who is later diagnosed with an ASD is at a mean age of 18 months.[8] Approximately 80% of parents of children with ASDs notice abnormalities in their child by 24 months of age, which usually involve delays in speech and language development[9] and, less often, social, play, sensory, motor,[10]

or medical problems, or regulatory problems related to sleep, eating and attention.[9] One study reported that, in cases in which parents became concerned in the child's first year of life, the diagnosis at 4 years of age was more likely to be autism than PDD-NOS.[11] There is an average delay of 13 months between the mean age at first evaluation and the initial diagnosis of ASD.[12] The age at first evaluation is related to the level of impairment, such that individuals with more-severe impairments tend to be diagnosed at a younger age.[12]

Retrospective studies involving home videos

Observation of early signs of ASDs by researchers was first made possible through analysis of home videos of infants and toddlers later diagnosed with ASDs.[13] Before 9 months of age, children with ASDs might show signs of developmental disruption in social behavior (e.g. gaze avoidance or looking at people infrequently, absence of emotional expression, and poor social initiative) and in motility (e.g. hypoactivity).[14,15] One group estimated that 87.5% of children with ASDs displayed such symptoms before their first birthday.[15] Some studies that used this retrospective approach also included a comparison group of children with typical development, and two studies included a group of children with non-ASD-related developmental delays.[16,17] Children with a later diagnosis of ASD could be differentiated from those with typical development or mental retardation at around the time of their first birthday by less frequently responding to their name[16] and less frequently looking at the faces of other individuals.[17] In addition, 12-month-old children who were later diagnosed with ASDs differed from those with typical development by less often using a pointing gesture to request or to share interest.[17–19] A limited variety of gestures in children aged between 9 and 12 months was reported to be associated with a subsequent diagnosis of ASD in one retrospective study.[20]

Prospective studies involving infant siblings of children with autism spectrum disorders

The earliest behavioral indicators of ASDs are being revealed in well-controlled prospective, longitudinal studies involving infant siblings of children with autism (hereafter referred to as 'SIBS-A'), who are at increased genetic risk of an ASD.[21–23]

This is a relatively new approach to the study of ASDs, and so far only two studies have reported on a sizeable number of 6-month-old SIBS-A who were given diagnostic classifications at 2–3 years of age[24,25]—the time at which the diagnosis of ASD becomes more stable (see below). The evidence so far indicates that 6-month-old children who later become diagnosed with an ASD are likely to score within normal limits on standardized tests of visual perception and language development,[25] but around a third of these infants score in the impaired range on standardized motor scales.[24] Motor abnormalities include one or more of the following: fine and gross motor delays, passivity, postural instability, hypotonia, and atypical motor behaviors.[24,26] Other studies, in which infant SIBS-A have not yet been assigned an outcome diagnosis, indicate that variations from typical development might appear by 6 months of age and might include difficulty shifting attention in novel contexts,[27,28] late onset of babbling and motor milestones,[29] and, possibly, decreased gaze towards faces and impaired affect regulation[30,31] (but see Merin *et al.*'s negative findings with 6-month-olds[32]). Research is underway in several laboratories to determine whether the presence of these and other characteristics during the first 6 months of life are predictive of ASDs or related, milder disorders.

The literature reviewed above and data from my own laboratory suggest that, during the first 9 months of life, signs of developmental disruption are variable across the child population and might even be absent altogether in some infants later diagnosed with an ASD.[24,26] Signs of abnormality might manifest even if the developmental quotient falls within normal limits[24,25] on standardized developmental tests such as the Mullen Scales of Early Learning.[33] The consistent presence, particularly in combination, of behaviors such as those described above and in Table 1, warrant referral and developmental surveillance, as they might be precursors to an ASD or to other non-ASD developmental disorders.

EARLY BEHAVIORAL INDICATORS OF AUTISM SPECTRUM DISORDERS

From the first birthday onwards, behavioral indicators of ASD become increasingly identifiable and can be used to differentiate toddlers with ASDs from those with other developmental delays or typical development. These indicators, as identified in the retrospective and prospective literature, are aligned with the three categories of behavior associated with

the diagnostic criteria for an ASD within the DSM-IV®:[1] qualitative impairment in social functioning, qualitative impairment in communication functioning, and the presence of stereotyped and repetitive patterns of behavior and interests (Table 1). Although repetitive and stereotyped patterns of behavior and interests occur in disorders other than ASDs, comparisons among 2-year-olds with ASDs, developmental delay, or typical development revealed that the presence of several different repetitive behaviors within the same child might be an indicator of an ASD, especially if social and communication skills are abnormal.[34] In this study, parents of children with ASDs did not, however, report more self-injurious behavior, sensitivity to noise, or resistance to trivial changes in the environment than did parents of children with developmental delay or typical development.[34]

Patterns of onset and developmental trajectory

Data from two prospective, longitudinal studies[25,35] suggest that there are several different onset patterns of ASD. In some children, multiple signs of ASD, particularly impairments in social functioning and communication, are present by 14 months of age to such a degree that an expert in early child development and autism might consider a diagnosis of ASD. Development in these children is slow, at least in the social domain.[35] Around a third of toddlers judged to have an ASD near the time of their first birthday are likely to exhibit instability with regard to the presence of ASD-related behavior, and diagnostic impressions might shift from an ASD at the time of the first birthday to a non-ASD by the third birthday.[36] Similarly, Turner and Stone's report from 2007[37] indicated that 68% of 2-year-olds who meet the diagnostic criteria for ASD fail to meet such criteria at 4 years of age. The majority of children with an unstable diagnosis of ASD were younger than 30 months of age at the time of the first diagnosis, had higher cognitive functioning than 2-year-olds with a stable diagnosis of ASD, or both.[37] Sutera and colleagues have reported similar findings.[38]

In other children, clear signs of ASDs are not present until later in the second year of life, or even until the third year.[19,35,39–41] These children might have mild signs of developmental disruption at 14 months of age or might even seem to be developing normally, but they gradually become less socially engaged after 14 months of age.[35] Regardless of the pattern of onset, any child with an ASD can show regression, in which existing skills, particularly spoken language[35,36,39,42] and social–emotional reciprocity,[35,42] are diminished or lost altogether, and atypical patterns of behaviors might emerge (e.g. temperamental and sensory dysregulation or repetitive and stereotyped patterns of behavior and interests).[26,35,36] Regression, as described in the retrospective literature, occurs in 10–50% of children with autism, at a mean age of 19 months,[42] and usually involves a loss of language skills.[42] Language regression in autism does not rule out the possibility of the acquisition of language skills later in life; nor does it predict a more severe impairment in language skills.[43]

The heterogeneous nature of ASDs means that multiple etiologic factors can be anticipated. Despite anecdotal reports to the contrary, a large, collaborative, retrospective study found no evidence that regression in ASD is associated with the measles–mumps–rubella (MMR) vaccine.[42] Importantly, variations in the timing of MMR vaccinations were not related to variations in the timing of regression.[42]

The evidence to date indicates that genetics have a major role in the etiology of ASDs, with an additional role for environmental influences that are yet to be defined.[44,45] Although the neurobiological basis of ASDs is still poorly understood, some intriguing recent findings have provided the impetus for the development of new etiologic hypotheses. One such finding is overgrowth of the brain in infants with ASDs (on the basis of group data), particularly between 6 and 12 months of age; this timing parallels the onset of clinical signs of developmental disruption in some children with ASDs. Although the head circumference of children with ASDs does not differ from the norm at birth,[46,47] brain growth accelerates abnormally in some children with ASDs, beginning some time between 6 and 12 months of age and leading to macrocephaly.[48–50] Hazlett and colleagues reported a generalized enlargement of the gray and white matter in the cerebrum in a group of 2-year-old children with ASDs compared with control children with typical development or developmental delay.[49] In a small autopsy study, evidence of neuroimmune activation, including activation of neuroglia and elevated levels of cytokines in brain tissue

and the cerebrospinal fluid, was reported in individuals with ASDs.[51,52] This neuroimmune activation is an endogenous process (occurring within the brain) that might result from disordered brain development and probably does not have an exogenous cause.[51,52] Such activation could have an important role in synaptic plasticity and modeling of neuronal networks that influence behavior and cognition.[53] The presence of an abnormal pattern of brain growth in ASDs, in addition to abnormalities in cortical and white matter cytoarchitecture,[54] suggests that late stages of neuronal organization are disrupted, although gross neuroanatomical abnormalities of the brain are rarely detected on clinical anatomical MRI.[55]

The findings reviewed above, combined with evidence from the retrospective literature on developmental regression in ASDs, support the recommendations put forward by the American Academy of Pediatrics.[56,57] These guidelines recommend developmental surveillance at every well-child preventative care visit, with developmental screening at 9, 18, and 30 months of age and autism-specific screening at 18 and 24 months of age. They also provide recommendations with regard to referral and evaluation of children suspected of having an ASD. In addition, a set of medical tests for children with suspected ASDs has been proposed by the American Academy of Neurology.[55]

CAVEATS OF EARLY DETECTION

Screening tools for ASDs, such as the Early Screening of Autistic Traits Questionnaire,[58] the First Year Inventory,[59] and the Modified Checklist for Autism in Toddlers (M-CHAT),[60] are available for use beginning at 12–16 months of age, but the validity of these instruments in children within this age range has not been established in large populations. Studies that used the Checklist for Autism in Toddlers (CHAT)[61] or the M-CHAT have focused on children aged 18–30 months.[62] Initial data on these instruments suggest that they have low sensitivity in the general population (many false-negatives) but good specificity. Recent studies of the M-CHAT, which takes 5–10 min for parents to complete and 5 min to score, estimated that the sensitivity falls within the 75–91% range.[62,63] The sensitivity of the M-CHAT is strongest if used in a clinical setting or with children referred owing to developmental concerns.[62]

Primary care providers must balance screening results against parents' perceptions of their child. In cases in which parental concerns are substantial, are perceived to interfere with the parents' interactions with their child, or are expressed about multiple aspects of development, referral to the local public early intervention program (e.g. Part C providers) is appropriate. Second-stage screening tools, such as the Screening Tool for Autism in Two-Year-Olds[64] or the Pervasive Developmental Disorders Screening Test-II,[65] are also available to help differentiate children with ASDs from those with other disorders; these tools are designed for children aged 24 months or older. The sensitivity and specificity of these measures are still under investigation.

Two issues that arise with ASD screening are false-positives and false-negatives. Strategies aimed at decreasing false-positives, such as conducting a brief interview with parents if their child's score on an ASD-specific screening tool raises suspicions about an ASD, are considerably improving the positive predictive value of ASD-specific screening tools.[60] False-negatives are more of a problem and can arise for numerous reasons. One reason is that many screening tools for ASDs use the terminology 'lack of' for behaviors, such as pointing, that are expected in typically developing toddlers, but are often abnormal in children with ASDs. Most toddlers with ASDs do, however, exhibit these behaviors, albeit less often, with less diversity and flexibility, less well coordinated with other behaviors, such as gaze, vocalization, or smiling, and for less duration within an interaction, than in typically developing children.[35,66] Parents, therefore, fail to endorse the item, and the child might pass the screening test, despite having abnormalities. Furthermore, parents might not report key autism-related behaviors in their infants and toddlers, particularly those involving subtle impairments in social functioning.[11,67] Another possible reason for false-negatives is the, often gradual, progressive nature of ASDs, which underlines the need for repeat screening in some cases.[35,56]

STABILITY OF EARLY DIAGNOSIS OF AN AUTISM SPECTRUM DISORDER

Although ASDs can be detected by 14 months of age in some children,[35] there is less stability of symptomatology, and hence in diagnosis, at this age than later in development. Nevertheless, children who met our preliminary criteria for

ASDs at 14 months of age, but not at 36 months of age, usually exhibited another type of impairment at 36 months of age, most often impairment in social communication.[36] Two reports indicate that by 20–21 months, the short-term stability of the diagnosis is high.[11,39] At least six studies have reported that, in most cases, the diagnosis of an ASD made during the third year of life (although most of the children studied were actually nearing their third birthday) remained stable during follow-up periods ranging from 1 to 7 years.[68–73] Two recent reports, however, provided more conservative estimates of the stability of the diagnosis of ASD in 2-year-olds.[37,38] These reports indicated that the greatest instability of the diagnosis was observed in children younger than 30 months of age at diagnosis and/or who had relatively high levels of cognitive functioning.[37] Diagnostic shifts within or off the autism spectrum over the course of 2–7 years occur more often in children diagnosed with PDD-NOS at 2 years of age than in those diagnosed with autism at 2 years of age.[38,70] Despite these reports, Sutera and colleagues[38] reported that nearly 22% of the 2-year-olds diagnosed with an ASD in their sample no longer had a diagnosis of an ASD at 4 years of age. Between the ages of 2 and 7 years, there is considerable variability in the severity and nature of symptoms in children with ASDs.[68] In addition, expert clinical judgment, whereby information from a variety of sources is considered, is more reliable than a diagnosis made only on the basis of a standardized assessment instrument for autism or by a less experienced clinician.[72,74]

Prediction of the outcome for young children with ASDs is not straightforward. Standard assessments of autism at the age of 2 years did not predict functioning at 7 years of age, even within the same domain of social functioning, communication, or repetitive and stereotyped patterns of behavior and interests, but assessments made at 3 years of age did predict behavior at the age of 7 years.[68] Children whose diagnosis moved off the autism spectrum between the ages of 2 and 4 years tended to show better motor skills, an increased ability to sit and listen to a story, and a greater desire to please their parents at the age of 2 years than those whose diagnosis remained within the autism spectrum; however, neither the symptom severity nor the cognitive level at 2 years of age was useful for prediction of who would fail to meet the criteria for an ASD at the age of 4 years.[38]

The literature reviewed above indicates that the prognosis is uncertain for children diagnosed with an ASD before 3 years of age. Rather than attempting prognostic statements for very young children who are thought to have an ASD, clinicians should, therefore, focus on instituting appropriate intervention and establishing systematic follow-up to evaluate developmental progress and assess etiologic bases as new information appears in the literature about diagnostically fruitful medical tests and treatment for ASDs.

EVIDENCE TO SUPPORT EARLY INTERVENTION

Most of the literature on the efficacy of intervention for ASDs in preschool-aged children focuses on behavior-based interventions. In general, preschool-aged children with ASDs have positive responses to intensive interventions (≥20 h per week) that target a wide range of skills. An average gain in IQ of 20 points has been noted following interventions involving applied behavior analysis (ABA) that are based on operant conditioning, in which discrete skills are taught by use of massed trials conducted outside the natural context.[75] Similar improvements have also been noted in developmentally based interventions, in which objects and activities of the child's interest form the basis of many teaching interactions and in which teaching often occurs within the natural context.[76,77] More-naturalistic applications of ABA, such as in Pivotal Response Training,[78] have also been shown to be effective for teaching important social and communication skills to young children with ASDs.[79,80] Such applications of ABA emphasize the use of child-selected activities to increase the child's motivation and generalization of skills, flexible teaching of multiple skills within a single activity, use of natural and meaningful rewards, and child initiation of play with toys and interaction with people. Other developmental approaches (e.g. the Floortime[81] or Social Communication, Emotional Regulation, and Transactional Support[82] models) are characterized by multimodal integration of sensory stimulation, child-selected play-based reciprocal interactions, use of visual teaching aids, and family involvement to emphasize goals of developing social communication, social reciprocity, and affective and self-regulation skills. Studies of the efficacy of these intervention approaches are in progress. In view of the fact that ASD is a heterogeneous disorder that affects

multiple systems, and that children with ASDs have different needs at different points in their development, it is unlikely that a single method of intervention will be optimally sufficient for all children with these disorders.[83,84]

Guidelines for early intervention[85] recommend that a combination of direct intervention and stimulation within natural routines should be administered on an intensive basis for children with ASDs. Although the term 'intensive' has not been empirically defined for toddlers with ASDs, the number of hours of speech–language therapy received between the ages of 2 and 4 years by children with ASDs is related to their development of spoken language.[86] In general, improvement might be proportional to the number of hours of intervention per week.[87,88] For preschool-aged children with ASDs, the National Research Council has recommended 25 h per week of therapy-based engagement, which can be delivered by therapists, family members, and other caregivers.[85] The literature has shown that caregivers who are trained to recognize signs of a child's comprehension and attempts to initiate interaction or communication can have a marked impact on the child's development,[89] and manualized approaches are available to teach caregivers such skills.[90]

A combination of home-based and center-based intervention is proving to have a beneficial impact on the development of 2-year-olds with ASDs. A randomized clinical trial showed that intensive engagement, involving classroom-based intervention for 10 h per week paired with caregiver training, was associated with robust improvement in cognitive, language and social functioning over a 6-month period.[91] A combination of intervention methods was used, including discrete-trial teaching based on operant conditioning to prime new skills, Pivotal Response Training[92] to stimulate concept-based functional skills in a natural context, input and output augmentation to assist learning through visual aids, sensory–social routines to heighten attention, environmental engineering to increase predictability and facilitate transitions, joint action routines to promote social engagement, and communication temptations to increase motivation to communicate.[91] Other studies, some involving parent-mediated intervention[93] and some involving interventions delivered by therapists,[80,94,95] also showed that imitation, joint attention, language, play, and affect sharing can be greatly improved in children with ASDs and these improvements might be sustained in the long term. Early intervention in young children with ASDs might reduce impairments in social and communicative skills that interfere with their ability to elicit and sustain social engagement with others and limit their moment-to-moment learning opportunities. Early intervention might, therefore, interrupt an otherwise possibly devastating cascade of events associated with social isolation and maladaptive behaviors. Early detection and appropriate intervention might prevent a decline in social functioning and decelerated language functioning between 14 and 24 months of age[35] and prevent development from reaching a plateau between 2 and 3 years of age.[11]

Although many children with ASDs will show a moderate to high improvement in response to intensive early intervention, other children might show a limited improvement. Factors such as the pretreatment IQ,[96–98] social interest,[75,83] language functioning,[75] age at entry into the intervention,[4,97,98] and the rate of initial learning[99,100] have been reported as predictors of outcome.

CONCLUSIONS

Much ongoing research is focused on early predictors and characteristics of ASDs. Current scientific thinking indicates that, in the majority of cases, behavior-based clinical signs of ASDs begin to emerge most clearly between the first and the second birthdays. These signs will involve abnormalities in social and communication responsiveness and initiative, which can occur with or without spoken language or mental retardation. Repetitive and stereotyped patterns of behavior can also occur, and might become increasingly intense or frequent, in the first 3 years of life. Some children with ASDs will have a period of development during which signs of developmental disruption will be subtle or absent. Screening for ASDs should, therefore, begin by 18 months of age and be repeated at 24 and 36 months of age. The diagnosis of ASD becomes increasingly stable over the first 3 years of life. Detection of an ASD-related behavioral profile is possible as early as the first birthday and warrants enrollment in an intervention. Early intervention should address a comprehensive range of skills and involve a mixture of parent-mediated and therapist-mediated interventions. An emphasis should be placed on development of social and communicative abilities within natural settings.

KEY POINTS

- Early signs of autism spectrum disorders (ASDs) include infrequent social orienting, infrequent initiation of social engagement, poorly sustained and coordinated engagement with others, a limited variety of gestures and other forms of communication, and repetitive motor behaviors
- Development of siblings of children with ASDs and other groups at high risk of ASDs should be followed closely from 6 months of age onwards
- Diagnosis of ASDs becomes possible at 14 months of age, but the diagnosis might be unstable in up to a third of children diagnosed before 30 months of age
- Young children with ASDs show improved social, communication, language, play and cognitive functioning when they are enrolled in a developmentally appropriate intensive intervention

References

1 American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders*, edn 4, text revision. Washington, DC: American Psychiatric Press
2 Rice C (2007) Prevalence of autism spectrum disorders—autism and developmental disabilities monitoring network, 14 sites, United States, 2002. *MMWR Morb Mortal Wkly Rep* **56:** 12–28
3 Martin KC and Kandel ER (1996) Cell adhesion molecules, CREB, and the formation of new synaptic connections. *Neuron* **17:** 567–570
4 Harris SL and Handleman JS (2000) Age and IQ at intake as predictors of placement for young children with autism: a four- to six-year follow-up. *J Autism Dev Disord* **30:** 137–142
5 McGee GG *et al*. (1999) An incidental teaching approach to early intervention for toddlers with autism. *J Assoc Pers Sev Handicaps* **24:** 133–146
6 Rogers SJ (1996) Brief report: early intervention in autism. *J Autism Dev Disord* **26:** 243–246
7 Mandell DS *et al*. (2005) Factors associated with age of diagnosis among children with autism spectrum disorders. *Pediatrics* **116:** 1480–1486
8 Howlin P and Asgharian A (1999) The diagnosis of autism and Asperger syndrome: findings from a survey of 770 families. *Dev Med Child Neurol* **41:** 834–839
9 De Giacomo A and Fombonne E (1998) Parental recognition of developmental abnormalities in autism. *Eur Child Adolesc Psychiatry* **7:** 131–136
10 Charman T *et al*. (2000) Testing joint attention, imitation, and play as infancy precursors to language and theory of mind. *Cogn Dev* **15:** 481–498
11 Chawarska K *et al*. (2007) Autism spectrum disorder in the second year of life: stability and change in syndrome expression. *J Child Psychol Psychiatry* **48:** 128–138
12 Wiggins LD *et al*. (2006) Examination of the time between first evaluation and first autism spectrum diagnosis in a population-based sample. *J Dev Behav Pediatr* **27 (Suppl):** S79–S87
13 Palomo R *et al*. (2006) Autism and family home movies: a comprehensive review. *J Dev Behav Pediatr* **2 (Suppl):** S59–S68
14 Adrien JL *et al*. (1993) Blind ratings of early symptoms of autism based upon family home movies. *J Am Acad Child Adolesc Psychiatry* **32:** 617–626
15 Maestro S *et al*. (2005) The course of autism signs in the first year of life. *Psychopathology* **1:** 26–31
16 Baranek GT (1999) Autism during infancy: a retrospective video analysis of sensory–motor and social behaviors at 9–12 months of age. *J Autism Dev Disord* **29:** 213–224
17 Osterling J *et al*. (2002) Early recognition of 1-year-old infants with autism spectrum disorders versus mental retardation. *Dev Psychopathol* **14:** 239–251
18 Osterling J and Dawson G (1994) Early recognition of children with autism: a study of first birthday home videotapes. *J Autism Dev Disord* **24:** 247–257
19 Werner E and Dawson G (2005) Validation of the phenomenon of autistic regression using home videotapes. *Arch Gen Psychiatry* **62:** 889–895
20 Colgan SE *et al*. (2006) Analysis of social interaction gestures in infants with autism. *Child Neuropsychol* **12:** 307–319
21 Folstein S and Rutter M (1977) Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry* **18:** 297–321
22 Landa R *et al*. (1992) Social language use in parents of autistic individuals. *Psychol Med* **22:** 245–254
23 Piven J (1997) The biological basis of autism. *Curr Opin Neurobiol* **7:** 708–712
24 Flanagan JE and Landa R (2007) Longitudinal study of motor development in infants at high and low risk for autism. Presented at the American Occupational Therapy Association Annual Conference: 2007 April 20–23, St Louis, MO, USA
25 Landa R and Garrett-Mayer E (2006) Development in infants with autism spectrum disorders: a prospective study. *J Child Psychol Psychiatry* **47:** 629–638
26 Bryson SE *et al*. (2007) A prospective case series of high-risk infants who developed autism. *J Autism Dev Disord* **37:** 12–24
27 Bhat AN *et al*. (2007) Learning, visual attention, affect, and coordination in young infants at risk for autism and typically developing. Presented at the Society for Research in Child Development Biennial Meeting: 2007 April 2–4, Boston, MA, USA
28 Zwaigenbaum L *et al*. (2005) Behavioral manifestations of autism in the first year of life. *Int J Dev Neurosci* **23:** 143–152
29 Iverson J and Wozniak RH (2007) Variation in vocal-motor development in infant siblings of children with autism. *J Autism Dev Disord* **37:** 158–170
30 Cassel TD *et al*. (2007) Early social and emotional communication in the infant siblings of children with autism spectrum disorders: an examination of the broad phenotype. *J Autism Dev Disord* **37:** 122–132
31 Yirmiya N *et al*. (2006) The development of siblings of children with autism at 4 and 14 months: social engagement, communication, and cognition. *J Child Psychol Psychiatry* **47:** 511–523
32 Merin N *et al*. (2007) Visual fixation patterns during reciprocal social interaction distinguish a subgroup of 6-month-old infants at-risk for autism from comparison infants. *J Autism Dev Disord* **37:** 108–121
33 Mullen EM (1995) *Mullen: Scales of Early Learning (AGS edition)*. Circle Pines: American Guidance Service
34 Richler J *et al*. (2007) Restricted and repetitive behaviors in young children with autism spectrum disorders. *J Autism Dev Disord* **37:** 73–85

35 Landa RJ *et al*. (2007) Early social and communication development associated with early and later onset of autism. *Arch Gen Psychiatry* **64:** 853–864
36 Landa RJ *et al*. (2005) Change in autism diagnostic classification and symptoms from 14 to 24 months of age. Presented at the Society for Research in Child Development Biennial Meeting: 2005 April 7–10, Atlanta, USA
37 Turner LM and Stone WL (2007) Variability in outcome for children with an ASD diagnosis at age 2. *J Child Psychol Psychiatry* **48:** 793–802
38 Sutera S *et al*. (2007) Predictors of optimal outcome in toddlers diagnosed with autism spectrum disorders. *J Autism Dev Disord* **37:** 98–107
39 Cox A *et al*. (1999) Autism spectrum disorders at 20 and 42 months of age: stability of clinical and ADI-R diagnosis. *J Child Psychol Psychiatry* **40:** 719–732
40 Receveur C *et al*. (2005) Interaction and imitation deficits from infancy to 4 years of age in children with autism: a pilot study based on videotapes. *Autism* **9:** 69–82
41 Werner E *et al*. (2005) Variation in early developmental course in autism and its relation with behavioral outcome at 3–4 years of age. *J Autism Dev Disord* **35:** 37–50
42 Luyster R *et al*. (2005) Early regression in social communication in autism spectrum disorders: a CPEA study. *Dev Neuropsychol* **27:** 311–336
43 Goldberg WA *et al*. (2003) Language and other regression: assessment and timing. *J Autism Dev Disord* **33:** 607–616
44 Jones M and Szatmari P (2002) A risk-factor model of epistatic interaction, focusing on autism. *Am J Med Genet* **114:** 558–565
45 Newschaffer CJ *et al*. (2007) The epidemiology of autism spectrum disorders. *Annu Rev Public Health* **28:** 235–258
46 Courchesne E *et al*. (2001) Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology* **57:** 245–254
47 Stevenson RE *et al*. (1997) Autism and macrocephaly. *Lancet* **349:** 1744–1745
48 Courchesne E and Pierce K (2005) Brain overgrowth in autism during a critical time in development: implications for frontal pyramidal neuron and interneuron development and connectivity. *Int J Dev Neurosci* **23:** 153–170
49 Hazlett HC *et al*. (2005) Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. *Arch Gen Psychiatry* **62:** 1366–1376
50 Herbert MR *et al*. (2005) Brain asymmetries in autism and developmental language disorder: a nested whole-brain analysis. *Brain* **128:** 213–226
51 Pardo CA *et al*. (2005) Immunity, neuroglia and neuroinflammation in autism. *Int Rev Psychiatry* **17:** 485–495
52 Zimmerman AW *et al*. (2005) Cerebrospinal fluid and serum markers of inflammation in autism. *Pediatr Neurol* **33:** 195–201
53 Fields RD and Stevens-Graham B (2002) New insights into neuron–glia communication. *Science* **298:** 556–562
54 DiCicco-Bloom E *et al*. (2006) The developmental neurobiology of autism spectrum disorder. *J Neurosci* **28:** 6897–6906
55 Filipek PA *et al*. (2000) Practice parameter: screening and diagnosis of autism: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Child Neurology Society. *Neurology* **55:** 468–479
56 Council on Children with Disabilities; Section on Developmental Behavioral Pediatrics; Bright Futures Steering Committee; Medical Home Initiatives for Children With Special Needs Project Advisory Committee (2006) Identifying infants and young children with developmental disorders in the medical home: an algorithm for developmental surveillance and screening. *Pediatrics* **118:** 405–420
57 Johnson CP *et al*. (2007) Identification and evaluation of children with autism spectrum disorders. *Pediatrics* **120:** 1183–1215
58 Dietz C *et al*. (2006) Screening for autistic spectrum disorder in children aged 14–15 months. II: population screening with the Early Screening of Autistic Traits Questionnaire (ESAT). Design and general findings. *J Autism Dev Disord* **36:** 713–722
59 Reznick JS *et al*. (2006) A parent-report instrument for identifying one-year-olds at risk for an eventual diagnosis of autism: the first year inventory. *J Autism Dev Disord* **37:** 1691–1710
60 Kleinman JM *et al*. (2007) The Modified Checklist for Autism in Toddlers: a follow-up investigating the early detection of autism spectrum disorders. *J Autism Dev Disord* [doi:10.1007/s10803-007-0450-9]
61 Baron-Cohen S *et al*. (1996) Psychological markers in the detection of autism in infancy in a large population. *Br J Psychiatry* **168:** 158–163
62 Robins DL *et al*. (2001) The Modified Checklist for Autism in Toddlers: an initial study investigating the early detection of autism and pervasive developmental disorders. *J Autism Dev Disord* **31:** 131–144
63 Eaves *et al*. (2006) Screening for autism: agreement with diagnosis. *Autism* **10:** 229–242
64 Stone WL *et al*. (1997) Motor imitation in young children with autism: what's the object? *J Abnorm Child Psychol* **25:** 475–485
65 Siegel B (2004) *Pervasive Developmental Disorders Screening Test-II (PDDST-II)*. San Antonio: Harcourt
66 Wetherby AM *et al*. (2007) Social communication profiles of children with autism spectrum disorders late in the second year of life. *J Autism Dev Disord* **37:** 960–975
67 Hess CR *et al*. (2007) Parent reported concern compared to standardized test results in young sibs of children with autism. Presented at the International Meeting for Autism Research: 2007 May 3–5, Seattle, WA, USA
68 Charman T *et al*. (2005) Outcome at 7 years of children diagnosed with autism at age 2: Predictive validity of assessments conducted at 2 and 3 years of age and pattern of symptom change over time. *J Child Psychol Psychiatry* **46:** 500–513
69 Lord C (1995) Follow-up of two-year-olds referred for possible autism. *J Child Psychol Psychiatry* **36:** 1365–1382
70 Lord C *et al*. (2006) Autism from 2 to 9 years of age. *Arch Gen Psychiatry* **63:** 694–701
71 Moore V and Goodson S (2003) How well does early diagnosis of autism stand the test of time? Follow-up study of children assessed for autism at age 2 and development of an early diagnostic service. *Autism* **7:** 47–63
72 Stone WL *et al*. (1999) Can autism be diagnosed accurately in children under 3 years? *J Child Psychol Psychiatry* **40:** 219–226
73 Turner LM *et al*. (2006) Follow-up of children with autism spectrum disorders from age 2 to age 9. *Autism* **10:** 243–265
74 Charman T and Baird G (2002) Practitioner review: diagnosis of autism spectrum disorder in 2- and 3-year-old children. *J Child Psychol Psychiatry* **43:** 289–305

75 Sallows GO and Graupner TD (2005) Intensive behavioral treatment for children with autism: four-year outcome and predictors. *Am J Ment Retard* **110:** 417–438
76 Rogers SJ (1998) Empirically supported comprehensive treatments for young children with autism. *J Clin Child Psychol* **27:** 168–179
77 Rogers SJ *et al*. (1986) An approach for enhancing the symbolic, communicative, and interpersonal functioning of young children with autism and severe emotional disorders. *J Div Early Childhood* **10:** 135–148
78 Koegel RL and Koegel LK (2006) *Pivotal Response Treaments for Autism: Communication, Social, and Academic Development*. Baltimore: Brookes
79 Ingersoll B and Schreibman L (2006) Teaching reciprocal imitation skills to young children with autism using a naturalistic behavioral approach: effects on language, pretend play, and joint attention. *J Autism Dev Disord* **36:** 487–505
80 Whalen C and Schreibman L (2003) Joint attention training for children with autism using behavior modification procedures. *J Child Psychol Psychiatry* **44:** 456–468
81 Greenspan SI and Wieder S (2006) *Engaging Autism: Using the Floortime Approach to Help Children Relate, Communicate, and Think*. Cambridge: Da Capo Press
82 Prizant *et al*. (2006) *SCERTS Model: a Comprehensive Approach for Children with Autism Spectrum Disorders*. Baltimore: Brookes
83 Beglinger L and Smith T (2005) Concurrent validity of social subtype and IQ after early intensive behavioral intervention in children with autism: a preliminary investigation. *J Autism Dev Disord* **35:** 295–303
84 Sherer MR and Schreibman L (2005) Individual behavioral profiles and predictors of treatment effectiveness for children with autism. *J Consult Clin Psychol* **73:** 525–538
85 National Research Council (2001) *Educating Children with Autism*. Washington, DC: National Academies Press
86 Stone WL and Yoder PJ (2001) Predicting spoken language level in children with autism spectrum disorders. *Autism* **5:** 341–361
87 Cohen H *et al*. (2006) Early intensive behavioral treatment: replication of the UCLA model in a community setting. *J Dev Behav Pediatr* **27 (Suppl):** S145–S155
88 Howard JS *et al*. (2005) A comparison of intensive behavior analytic and eclectic treatments for young children with autism. *Res Dev Disabil* **26:** 359–383
89 Schertz HH and Odom SL (2007) Promoting joint attention in toddlers with autism: a parent-mediated developmental model. *J Autism Dev Disord* **37:** 1562–1575
90 Sussman F (1999) *More than Words: Helping Parents Promote Communication and Social Skills in Children with Autism Spectrum Disorder*. Toronto: Hanen Centre
91 Holman K *et al*. (2007) Social and communication change in a 6-month toddler intervention program. Presented at the International Meeting for Autism Research: 2007 May 3–5, Seattle, WA, USA
92 Koegel RL and Koegel LK (1995) *Teaching Children with Autism*. Baltimore: Brookes
93 Schertz HH and Odom SL (2006) promoting joint attention in toddlers with autism: a parent-mediated developmental model. *J Autism Dev Disord* **37:** 1562–1575
94 Kasari C *et al*. (2006) Joint attention and symbolic play in young children with autism: a randomized controlled intervention study. *J Child Psychol Psychiatry* **47:** 611–620
95 Ingersoll B and Schreibman L (2006) Teaching reciprocal imitation skills to young children with autism using a naturalistic behavioral approach: effects on language, pretend play, and joint attention. *J Autism Dev Disord* **36:** 487–505
96 Bibby P *et al*. (2002) Progress and outcomes for children with autism receiving parent-managed intensive interventions. *Res Dev Disabil* **23:** 81–104
97 Eikeseth S *et al*. (2002) Intensive behavioral treatment at school for 4- to 7-year old children with autism: a one-year comparison controlled study. *Behav Modif* **26:** 49–68
98 Goldstein H (2002) Communication intervention for children with autism: a review of treatment efficacy. *J Autism Dev Disord* **32:** 373–396
99 Newsom C and Rincover A (1998) Autism. In *Treatment of Childhood Disorders*, 286–346 (Eds Mash EJ and Barkley RA) New York: Guilford
100 Weiss MJ (1999) Differential rates of skill acquisition and outcomes of early intensive behavioral intervention for autism. *Behav Interv* **14:** 3–22
101 Maestro *et al*. (2002) Attentional skills during the first 6 months of age in Autism Spetrum Disorder. *J Am Acad Child Adolesc Psychiatry* **41:** 1239–1245
102 Nadig AS *et al*. (2007) A prospective study of response to name in infants at risk for autism. *Arch Pediatr Adolesc Med* **161:** 378–383
103 Swettenham J *et al*. (1998) The frequency and distribution of spontaneous attention shifts between social and nonsocial stimuli in autistic, typically developing, and nonautistic developmentally delayed infants. *J Child Psychol Psychiatry* **39:** 747–753
104 Sullivan M *et al*. (2007) Response to joint attention in toddlers at risk for autism spectrum disorder: a prospective study. *J Autism Dev Disord* **37:** 37–48
105 Charman T *et al*. (1997) Infants with autism: an investigation of empathy, pretend play, joint attention, and imitation. *Dev Psychopathol* **33:** 781–789
106 Wetherby A *et al*. (2004) Early indicators of autism spectrum disorders in the second year of life. *J Autism Dev Disord* **34:** 473–493
107 Osterling J *et al*. (2002) Early recognition of 1-year-old infants with autism spectrum disorder versus mental retardation. *Dev Psychopathol* **14:** 239–251
108 Loh A *et al*. (2007) Stereotyped motor behaviors associated with autism in high-risk infants: a pilot videotape analysis of a sibling sample. *J Autism Dev Disord* **37:** 25–36

Acknowledgments
This work was supported by grants MH59630 and MH066417 from the NIH, Bethesda, MD, USA, which were awarded to the author. Désirée Lie, University of California, Irvine, CA, is the author of and is solely responsible for the content of the learning objectives, questions and answers of the Medscape-accredited continuing medical education activity associated with this article.

Competing interests
The author declared no competing interests.

Archival Report

Biological Psychiatry

Neural Connectivity Evidence for a Categorical-Dimensional Hybrid Model of Autism Spectrum Disorder

Amanda Elton, Adriana Di Martino, Heather Cody Hazlett, and Wei Gao

ABSTRACT

BACKGROUND: Autism spectrum disorder (ASD) encompasses a complex manifestation of symptoms that include deficits in social interaction and repetitive or stereotyped interests and behaviors. In keeping with the increasing recognition of the dimensional characteristics of ASD symptoms and the categorical nature of a diagnosis, we sought to delineate the neural mechanisms of ASD symptoms based on the functional connectivity of four known neural networks (i.e., default mode network, dorsal attention network, salience network, and executive control network).

METHODS: We leveraged an open data resource (Autism Brain Imaging Data Exchange) providing resting-state functional magnetic resonance imaging data sets from 90 boys with ASD and 95 typically developing boys. This data set also included the Social Responsiveness Scale as a dimensional measure of ASD traits. Seed-based functional connectivity was paired with linear regression to identify functional connectivity abnormalities associated with categorical effects of ASD diagnosis, dimensional effects of ASD-like behaviors, and their interaction.

RESULTS: Our results revealed the existence of dimensional mechanisms of ASD uniquely affecting each network based on the presence of connectivity-behavioral relationships; these were independent of diagnostic category. However, we also found evidence of categorical differences (i.e., diagnostic group differences) in connectivity strength for each network as well as categorical differences in connectivity-behavioral relationships (i.e., diagnosis-by-behavior interactions), supporting the coexistence of categorical mechanisms of ASD.

CONCLUSIONS: Our findings support a hybrid model for ASD characterization that includes a combination of categorical and dimensional brain mechanisms and provide a novel understanding of the neural underpinnings of ASD.

Keywords: Autism spectrum disorder, Default mode network, Dimensional measures, Functional connectivity, Resting-state fMRI, Social cognition

http://dx.doi.org/10.1016/j.biopsych.2015.10.020

Autism spectrum disorder (ASD) is characterized by poor social and reciprocal communication skills combined with repetitive or stereotyped interests and behaviors (1,2). However, a range of symptom severity and functional impairment exists within and across these disorders, in agreement with the notion that ASD represents a spectrum. Previous studies revealed that multiple subtypes of ASD exist along a continuum of the same disorder (3–5). Furthermore, children without a diagnosis of ASD may exhibit varying degrees of social impairment qualitatively similar to ASD without meeting diagnostic criteria, suggesting that the continuum of ASD symptoms may span beyond the categorical diagnosis of ASD (6,7). Therefore, a dimensional characterization of ASD has become increasingly favored within the clinical and research communities, prompting a revision to DSM-5 to include severity ratings for ASD rather than categorical subgroups. In parallel with this clinical evidence, more recent studies have identified dimensional brain-behavior relationships related to ASD (8,9). However, it is unknown whether behaviors observed in children with ASD are similarly represented in the brain as in typically developing children (TDC). Moreover, diagnoses ultimately remain categorical in nature, yet the particular contributions of categorical brain mechanisms, especially after controlling for dimensional relationships, are poorly defined. Studies that systematically examine both the categorical and the dimensional mechanisms of ASD are needed to disentangle the complex neural correlates of ASD.

It has been increasingly recognized that ASD is a disorder of disrupted neural interactions (10). The largest resting-state functional magnetic resonance imaging (fMRI) investigation of ASD to date provided convincing support for this notion (11), as have many other studies (12–17). An examination of functional connectivity measurements represents a promising direction for delineating the potential categorical and dimensional neural mechanisms of ASD. We (18) and others (19) have demonstrated the feasibility of such an endeavor in

ISSN: 0006-3223

studies of attention-deficit/hyperactivity disorder (ADHD). Specifically, we explored functional connectivity alterations associated with both categorical diagnosis and ADHD symptom severity in relation to four large-scale neural networks: the dorsal attention network (DAN) (20), the default mode network (DMN) (21), the salience network (SAL) (22), and the executive control network (ECN) (23). Findings demonstrated three distinct patterns of brain-behavioral relationships: 1) categorical differences in network-level functional connectivity strength between children with and without a diagnosis of ADHD, supporting the existence of categorically represented neural mechanisms; 2) quantitative relationships between network-level functional connectivity and behavioral measures that were independent of categorical diagnosis, indicating dimensional mechanisms; and 3) diagnostic group differences in the quantitative relationships between network-level functional connectivity and behavioral measures, suggesting qualitatively different behavioral representations in the brain and reinforcing the categorical differences. The demonstration of the presence of three categories of neural mechanisms in ADHD provides a compelling model for studies of other categorically defined disorders that are known to occur along a spectrum; ASD is the next natural candidate given the evidence that ASD symptoms exhibit categorical and dimensional qualities (24). Moreover, the same four networks previously investigated in ADHD are also involved in processes that are disrupted in ASD, including social processing [i.e., DMN (25) and SAL (26)], restricted and repetitive behaviors [i.e., SAL (27)], cognitive control [i.e., ECN and SAL (28)], and attention [i.e., DAN (29)]. A parallel investigation of these networks in ASD to examine the categorical or dimensional nature of this disorder may ultimately aid ASD diagnosis and characterization.

In this study, resting-state fMRI data from 107 TDC and 109 children with ASD selected from a large data repository, the Autism Brain Imaging Data Exchange (11), were analyzed. Functional connectivity measures, derived from four large-scale higher order cognitive networks (i.e., DAN, DMN, SAL, and ECN) were tested to identify three types of effects: 1) categorical differences between TDC and children with ASD in the magnitude of functional connectivity, 2) congruent dimensional relationships between symptom severity and functional connectivity existing across both TDC and children with ASD, and 3) categorical differences between TDC and children with ASD in the relationship between symptom severity and functional connectivity. Our results demonstrate evidence of all three categories of neural mechanisms of ASD.

METHODS AND MATERIALS

Subjects

Data were selected from the Autism Brain Imaging Data Exchange repository of resting-state fMRI scans of children, adolescents, and adults with and without ASD from multiple international sites (http://fcon_1000.projects.nitrc.org/indi/abide/). All sites provided ASD diagnostic status for each subject, and several sites offered various continuous measures of autism-related symptoms. For the present study, sites were selected based on their inclusion of magnetic resonance imaging data, categorical diagnosis, and Social Responsiveness Scale (SRS) scores (30) from TDC and children and adolescents with ASD (age range, 6.5–18.7 years). This limited age range was selected to ensure a similar age distribution across sites and to minimize potential developmental effects of ASD-related neural alterations (31). Because boys are most often affected by this disorder, not enough data sets from girls with ASD were available to draw meaningful estimates of sex effects (32), limiting our analyses to boys. The data sets were further limited to data sets passing the quality assessment protocol performed before release of the preprocessed Autism Brain Imaging Data Exchange data sets to the public. This selection process resulted in 185 subjects, including 95 TDC and 90 children with ASD across four sites (Katholieke Universiteit Leuven [sample 2], New York University, Utah School of Medicine, and Yale University) (Table 1).

The SRS total raw scores, indicating the severity of impairment related to ASD, provided our dimensional measure of ASD. The SRS is a 65-item quantitative assessment based on parent ratings of core deficits pertaining to autism. This assessment offers a continuous measure of ASD as an alternative to other categorically oriented diagnostic tools (30), providing a single score of symptom severity. Children with a categorical diagnosis of ASD and children not meeting ASD diagnostic criteria (TDC) fall somewhere along the continuum of behaviors measured by the SRS.

Table 1. Demographic and Symptom Severity Information of Included Subjects

	All Sites	KU Leuven	NYU	USM	Yale University
Number	185	25	101	25	34
TDC	95	14	51	11	19
ASD	90	11	50	14	15
Autism/Asperger/PDD-NOS	85/17/14	11/0/0	38/8/4	13/0/1	3/4/8
Age (Years)	13.2 (3.2)	14.1 (1.4)	12.0 (3.0)	16.3 (2.4)	12.5 (3.0)
TDC	13.2 (3.1)	14.5 (1.6)	12.5 (3.1)	15.8 (2.6)	12.3 (2.9)
ASD	13.1 (3.3)	13.6 (1.0)	11.5 (2.9)	16.7 (2.2)	12.8 (3.4)
SRS Score	56.4 (43.4)	49.8 (45.0)	57.5 (41.8)	62.0 (46.6)	52.0 (43.1)
TDC	19.1 (14.7)	17.6 (13.6)	22.4 (13.6)	14.6 (12.8)	20.4 (21.3)
ASD	93.0 (28.4)	91.0 (36.0)	91.9 (29.6)	99.3 (22.2)	95.7 (21.1)

Mean (SD) values are provided for each continuous measure.

ASD, autism spectrum disorder; KU Leuven, Katholieke Universiteit Leuven; NYU, New York University; PDD-NOS, pervasive developmental disorder not otherwise specified; SRS, Social Responsiveness Scale; TDC, typically developing children; USM, Utah School of Medicine.

Categorical diagnoses of ASD were determined by clinician evaluation at each site and were supported by additional ASD-related dimensional measures, which varied by site (see Supplement 1). The ASD subtypes described by DSM-IV-TR were included as a single ASD group (Table 1), consistent with emerging views that these subtypes represent different presentations of the same disorder (2,4). Detailed inclusion and exclusion criteria for each site are described in Supplement 1.

fMRI Acquisition

Resting-state fMRI scans and magnetization prepared rapid acquisition gradient-echo structural images were acquired on Philips Intera (Philips Healthcare, Best, The Netherlands; Katholieke Universiteit Leuven, Leuven, Belgium), Siemens Allegra (Siemens Healthcare GmbH, Erlangen, Germany; New York University), and Siemens Trio (Siemens Healthcare GmbH; Utah School of Medicine, Yale University) 3-Tesla magnetic resonance imaging scanners. Image acquisition parameters for each site are detailed in Table S1 in Supplement 1.

Preprocessing

Resting-state fMRI data sets were downloaded in their preprocessed form following the Configurable Pipeline for Analysis of Connectomes (http://fcp-indi.github.com) (33). Preprocessing steps using Analysis of Functional NeuroImages (AFNI) software (34) and custom scripts included slice time correction, motion correction, global mean intensity normalization, nuisance signal regression including 24 motion parameters (six directions head motion, motion from one time point prior, and their squares), the top five principal components from white matter and cerebrospinal fluid signals, linear and quadratic trends, and band-pass filtering (.01–.1 Hz). Registration to Montreal Neurological Institute (MNI) standard space included linear registration to anatomic images using FMRIB's Linear Image Registration Tool (35) and application of the nonlinear anatomic-to-MNI transformation calculated with Advanced Normalization Tools (36). Final voxel size was $3 \times 3 \times 3$ mm^3. To minimize effects of motion on our analyses further, only data sets with a framewise displacement across all volumes of no more than .2 mm were included. Finally, linear regression was performed, and no significant relationships between mean framewise displacement and categorical diagnosis ($t = 1.57$, $p = .12$) or SRS scores ($t = -.57$, $p = .57$) were detected, ensuring that the results would not be secondary to motion parameters.

Functional Connectivity

Functional connectivity was calculated using a seed-based approach by applying 3dfim+ in AFNI software. Consistent with our previous study in ADHD (18), we examined ASD-related functional connectivity associated with four well-described neural networks: DAN (20), DMN (21), SAL (22), and ECN (23). Each network was defined by the voxelwise Pearson correlation with a reference time series extracted as the simple average time series of all voxels within a 6-mm spherical seed at coordinates obtained from the literature (22,23,37). Specifically, the ECN was defined by a seed in the right dorsolateral prefrontal cortex (MNI: 44, 36, 20), and the SAL was defined by a seed in the right anterior insula (MNI: 38, 26, −10), based on Seeley *et al.* (22). Seeds for DMN and DAN were placed in the posterior cingulate cortex (MNI: 1, −55, 17) and bilateral intraparietal sulcus (MNI: −27, −52, 57; 24, −56, 55), respectively (23,37). Pearson correlation maps were normalized using a Fisher z-transform.

Statistical Models

To identify categorical effects of ASD diagnosis and dimensional effects of symptom severity on brain functional connectivity, hierarchical linear regression analyses were employed. This model was selected to account for the nested nature of our data because site-specific characteristics may influence categorical or dimensional effects of interest. We designed a linear mixed-effects model and added random intercepts and slopes (capturing potential site-specific categorical and dimensional effects) for each site to better account for the nested nature of the multisite data. To further minimize the effects of motion or other systematic differences (e.g., scanner, scanning parameters and procedures, data quality) across sites on global connectivity, we employed mean connectivity regression, a technique in which the mean value of each subject's functional connectivity map is entered as a covariate of no interest in the group analysis (38). The first model tested ASD diagnosis (1 or 0) and the SRS score as predictors of network functional connectivity, covarying for age, mean connectivity, and site effects. This model was designed to identify categorical effects associated with an ASD diagnosis that were not driven by differences in symptom severity scores, which we term "categorical effects" in functional connectivity magnitude. Significant effects of symptoms measured by the SRS that were not due to effects of categorical diagnosis were also explored. These effects are subsequently referred to as "congruent dimensional effects" because the dimensional relationships are congruent across the groups. A second analysis included the interaction of ASD diagnosis and the SRS score as a predictor in the model to test whether there are categorical effects in the relationship of ASD behaviors to functional connectivity. Such categorical-by-dimensional interactions are subsequently described as "incongruent dimensional effects." Results were cluster-level corrected for multiple comparisons using 3dClustSim in AFNI at $p < .05$ with a minimum cluster size of 66 voxels providing a corrected false-positive rate of .05. Finally, a composite map of regions showing dimensional relationships, categorical effects in magnitude, and categorical effects in brain-behavior relationships was calculated, identifying regions showing each of the three effects as well as the overlap of effects.

RESULTS

Demographic variables and clinical measures for the TDC and ASD groups are presented in Table 1. Mean functional connectivity maps for each of the four networks for TDC and children with ASD are presented in Figure 1A, B. Spatial maps of functional connectivity for each network in TDC largely resembled the networks reported in adult populations. For the DAN, functional connectivity was observed bilaterally in the frontal eye fields, intraparietal sulcus, and ventral visual association regions, including visual motion area MT+ (39).

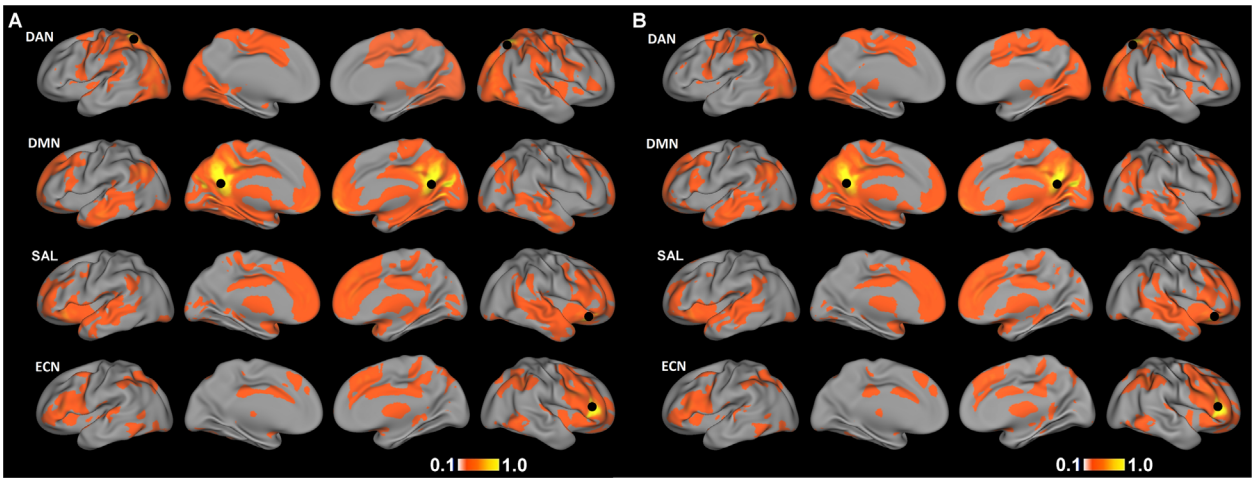


Figure 1. Mean functional connectivity maps for **(A)** typically developing children and (**B**) children with autism spectrum disorder for the dorsal attention network (DAN), default mode network (DMN), salience network (SAL), and executive control network (ECN). Black circles mark the location of seed regions used to define each network. Images are displayed at a threshold of $r > .1$.

For the DMN, functional connectivity was observed in the posterior cingulate cortex, precuneus, medial prefrontal cortex, and bilateral angular gyrus (21,40). The SAL consisted of the bilateral inferior frontal gyrus/anterior insula, anterior cingulate cortex, and bilateral middle temporal gyrus (22). The ECN connectivity included the bilateral middle and inferior frontal gyrus, dorsomedial prefrontal cortex, and bilateral parietal cortex (22,23).

Congruent Dimensional Effects

Dimensional brain-behavior relationships that were consistent across the TDC and ASD groups were observed in each of the four networks (Figure 2 and Table S2 in Supplement 1). For the DAN, higher scores on the SRS were associated with greater connectivity with the medial frontal gyrus and bilateral middle temporal gyrus across both groups, whereas negative relationships were observed in the thalamus and bilateral putamen. For the DMN, consistent positive relationships between SRS scores and connectivity were observed in precentral gyrus, right insula, and right inferior frontal gyrus. Significant positive brain-behavior relationships for SAL connectivity were detected in the precuneus, right lingual gyrus, and posterior cingulate cortex. For the ECN, significant positive dimensional relationships were present in the medial frontal gyrus, bilateral middle frontal gyrus, right lingual gyrus, and posterior cingulate cortex. Negative relationships were primarily observed across the right precentral, postcentral, and inferior frontal gyri. The exact coordinates and sizes of all detected regions showing congruent dimensional effects are listed in Table S2 in Supplement 1.

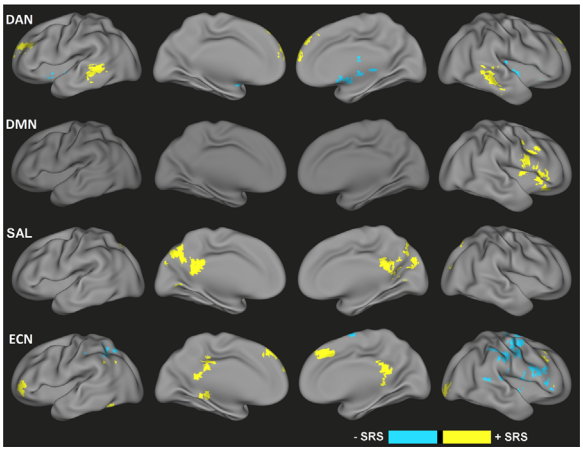


Figure 2. Congruent dimensional effects of autism spectrum disorder symptoms (i.e., Social Responsiveness Scale [SRS] score) across all subjects (i.e., independent of categorical diagnosis) for the dorsal attention network (DAN), default mode network (DMN), salience network (SAL), and executive control network (ECN) functional connectivity. Yellow represents positive relationships with SRS; blue represents negative relationships with SRS.

Categorical Effects in Magnitude

After controlling for dimensional effects, numerous brain regions demonstrated categorical differences in functional connectivity for each of the four networks (Figure 3 and Table S3 in Supplement 1). In particular, the ASD group demonstrated enhanced DAN functional connectivity in regions including the precuneus, cerebellum, and right precentral gyrus but decreased connectivity in the medial frontal gyrus and lateral temporal cortices. For the DMN, the ASD group was associated with increased connectivity in the bilateral middle frontal gyrus, bilateral inferior parietal lobules, and right insula. The SAL connectivity increases associated with a categorical ASD diagnosis were found in the dorsal anterior cingulate cortex, whereas decreases were noted along the medial frontal gyrus, left middle frontal gyrus, and left postcentral gyrus. For the ECN, greater connectivity for ASD was detected in the left cerebellum. Categorical ECN connectivity decreases for the ASD group were detected in the medial prefrontal cortex, right superior frontal gyrus, right precentral gyrus, left middle frontal gyrus, left postcentral gyrus, and medial frontal gyrus. Coordinates of all regions

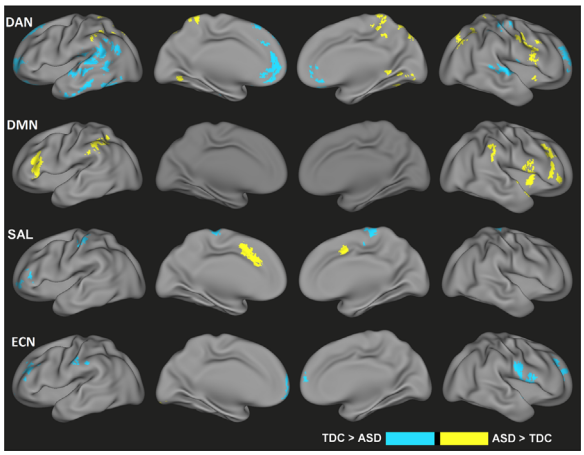


Figure 3. Categorical differences in functional connectivity values associated with an autism spectrum disorder (ASD) diagnosis but not explained by ASD-related symptom severity (i.e., Social Responsiveness Scale score) for the dorsal attention network (DAN), default mode network (DMN), salience network (SAL), and executive control network (ECN). Yellow indicates ASD > typically developing children (TDC); blue indicates TDC > ASD.

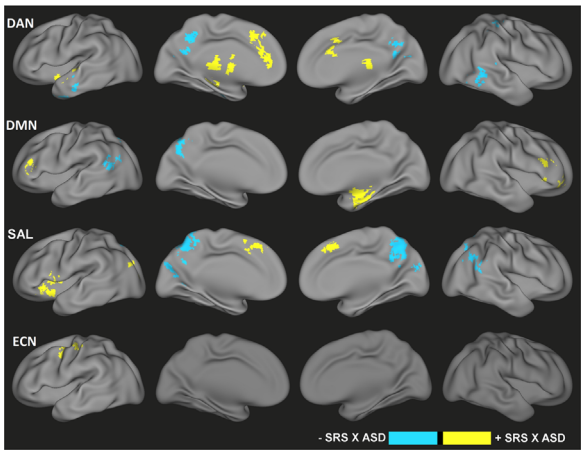


Figure 4. Significant categorical-by-dimensional interaction effects of autism spectrum disorder (ASD) for the dorsal attention network (DAN), default mode network (DMN), salience network (SAL), and executive control network (ECN). Yellow indicates regions for which the relationship between functional connectivity and SRS was increased in slope (i.e., either become more positive or change from negative to positive) for children with ASD vs. typically developing children, and blue indicates regions with a decrease in slope (i.e., either become more negative or change from positive to negative) relationship between functional connectivity and SRS for ASD vs. typically developing children. SRS, Social Responsiveness Scale.

demonstrating categorical effects on functional connectivity magnitude are listed in Table S3 in Supplement 1.

Incongruent Dimensional Effects

Tests of the interaction between categorical groups and dimensional relationships indicated differential brain-behavior relationships for children with an ASD diagnosis compared with TDC (Figure 4 and Table S4 in Supplement 1). For example, for the DAN, the ASD group demonstrated increased slopes in the brain-behavior relationships between the SRS and connectivity with the anterior cingulate cortex, thalamus, and left insula and decreased slopes in the brain-behavior relationships between the SRS and connectivity within the posterior cingulate cortex and bilateral middle temporal gyrus. Categorical differences in brain-behavior correlations were observed for the DMN in the right parahippocampal gyrus and bilateral middle frontal gyrus (ASD > TDC in slope) as well as precuneus and left superior temporal gyrus (TDC > ASD in slope). Increased slopes in brain-behavior relationships for the ASD group were also detected in left insula, bilateral superior frontal gyrus, and left middle occipital gyrus for the SAL, in addition to decreased slopes in the brain-behavior relationships in the precuneus and right angular gyrus. Finally, increased slopes in the brain-behavior relationships for the ASD group were found in the cerebellum and left middle/precentral gyrus for the ECN. Table S4 in Supplement 1 contains coordinates for all regions demonstrating incongruent dimensional effects.

Overlap of Categorical and Dimensional Effects

Categorical and dimensional effects largely affected distinct regions as shown by Figure 5. However, several regions also demonstrated a convergence of effects (Figure 5). For example, a diagnosis of ASD was associated with greater connectivity between the DMN and right inferior frontal gyrus (categorical effect), whereas this same region was also positively associated with ASD symptoms as measured by the SRS (congruent dimensional effect). There was also a small degree of overlap of categorical and incongruent

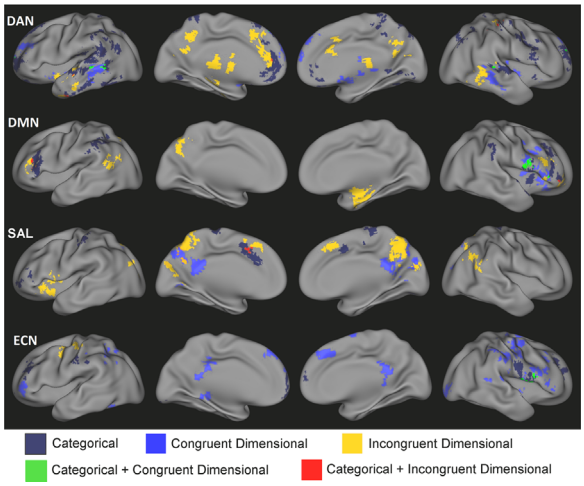


Figure 5. Composite maps of the dorsal attention network (DAN), default mode network (DMN), salience network (SAL), and executive control network (ECN) representing the regions demonstrating categorical effects of autism spectrum disorder on functional connectivity (white), consistent dimensional relationships for children with autism spectrum disorder and typically developing children (green), categorical differences in dimensional relationships between children with autism spectrum disorder and typically developing children (red), an overlap between categorical and congruent dimensional effects (blue), and an overlap between categorical and incongruent dimensional effects (yellow).

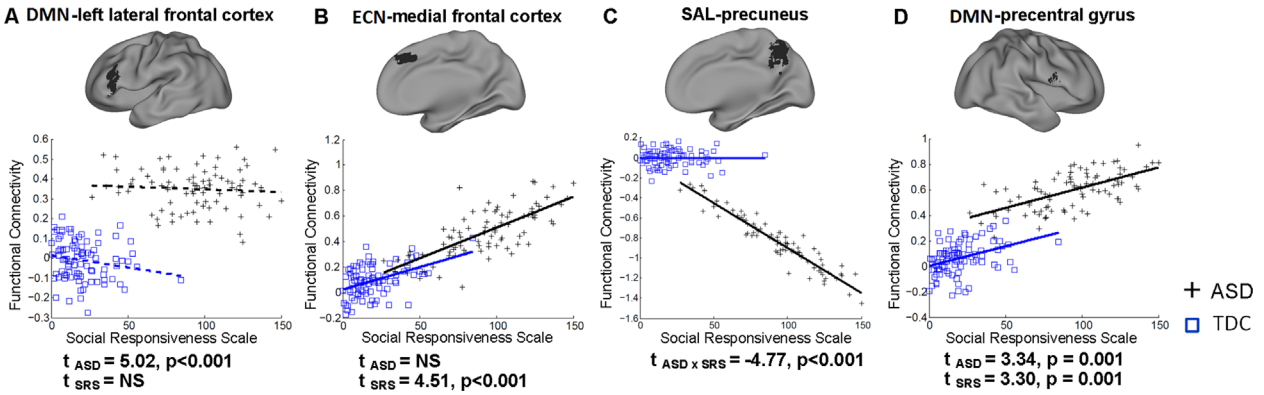


Figure 6. Scatter plots depicting the relationship between Social Responsiveness Scale (SRS) scores and functional connectivity for typically developing children (TDC) and children with autism spectrum disorder (ASD) for selected regions. Statistically significant linear relationships (solid lines) or nonsignificant linear relationships (dashed lines) are fitted to data points for TDC and children with ASD. *T* statistics for categorical (t_{ASD}) and dimensional effects (t_{SRS}) on regional connectivity are reported below each plot demonstrating **(A)** categorical effects only, **(B)** dimensional effects only, **(C)** an interaction of categorical and dimensional effects ($t_{ASD\ x\ SRS}$), and **(D)** dimensional and categorical effects. Plotted functional connectivity values represent residuals after removing nuisance effects. DMN, default mode network; ECN, executive control network; SAL, salience network.

dimensional effects for SAL connectivity with the supplementary motor area.

To further demonstrate the separability of categorical and dimensional effects of ASD, we produced scatter plots of the different types of relationships (Figure 6; see Figure S1 in Supplement 1 for separate plots from different sites). An example of a categorical effect without a significant dimensional effect for DMN connectivity is displayed in Figure 6A, and a dimensional effect of ECN connectivity in the absence of a significant categorical effect is displayed in Figure 6B. A significant diagnosis-by-behavior interaction demonstrates an incongruent dimensional effect for SAL connectivity (Figure 6C). An example of overlapping categorical and congruent dimensional effects on DMN connectivity is presented in Figure 6D.

DISCUSSION

Based on a large, multisite analysis of resting-state fMRI scans, we demonstrate functional connectivity abnormalities related to ASD that encompass categorical and dimensional brain-behavior relationships. The effects of the ASD group were not restricted to a particular brain region or network, but rather demonstrated extensive functional connectivity alterations across each of the four networks tested (i.e., DAN, DMN, SAL, and ECN). The functional connectivity variations associated with a continuous measure of ASD symptoms (i.e., the SRS) consistently across both ASD and TDC groups support the existence of dimensional brain mechanisms in ASD. However, we also found evidence to support categorical brain mechanisms. Numerous regions exhibited categorical differences in magnitude of functional connectivity, which could not be explained by quantitative relationships with ASD symptoms; another set of regions demonstrated categorical differences in their linear relationship with ASD symptoms. Therefore, consistent with previously reported findings in children with ADHD (18,19), the characterization of functional connectivity alterations in this sample points to combined categorical and dimensional brain mechanisms underlying ASD-related deficits.

The detection of brain regions exhibiting a consistent association with ASD-like behaviors across the TDC and ASD groups (Figures 2 and 6B, D) suggests that ASD impairments are represented in the brain—at least to some degree—as alterations in brain circuits supporting typical behaviors. In the present study, the SRS was used to characterize behavioral abnormalities associated with ASD. This instrument largely measures impairments in reciprocal social interactions (30), but some questions also tap into restricted, repetitive behaviors. Its scores capture the two major symptom categories required for a DSM-5 diagnosis of ASD (41) as a single measure of severity distributed continuously in the population (30,42). The regions in which functional connectivity was associated with SRS scores consistently across the two groups suggest that ASD symptoms partly stem from a single and continuously distributed factor. For example, an interpretation of the relationship between higher SRS scores and heightened connectivity of the SAL seed with the posterior cingulate cortex (Figure 2) would be that a greater connectivity between these regions is related to a greater severity of social impairment, regardless of diagnosis. The posterior cingulate cortex has been linked to social cognition (25) and is a key brain region of the DMN (Figure 1) (21). An inference of this finding is that greater connectivity between these regions at rest could signal impairment in normal interactions between the DMN and SAL in response to salient social stimuli, resulting in a poorer understanding of intentions and actions of others during social interactions. Overall, regions showing congruent brain-behavior relationships between the ASD and TDC groups may underlie the normal expression of social behaviors that form a continuum, on which children with ASD fall toward one end, and therefore provide support for the dimensional nature of ASD.

Functional connectivity of numerous other regions that exhibited categorical differences—either diagnostic group differences in functional connectivity or differences in the relationship between behavioral scores and functional

connectivity—suggests that ASD also represents a discrete syndrome. Although it is possible that categorical differences detected after controlling for symptoms may be due to the inability of the SRS to explain the entirety of ASD behavioral deficits (e.g., intellectual deficits, language deficits, comorbidities), this explanation seems unlikely to account for the extensive categorical effects we observed (Figure 3). Rather, we suggest that factors that contribute to ASD either are themselves categorical or affect the brain in a categorical manner (i.e., genetic polymorphisms, environmental insults). For example, the DMN demonstrated greater connectivity in children with ASD compared with TDC in the bilateral middle frontal gyrus, bilateral inferior parietal lobules, and right insula; this was not related to severity of social impairments (Figure 3). The impacted regions are closely related to the executive control network (Figure 1). Given the importance of the DMN for social cognition (25) and the role of ECN in attentional control (43,44) and coordination of activity in other networks (37,45), the abnormal connectivity between these sets of regions at rest suggests altered regulation of the DMN activity by the ECN, which may promote, in a categorical way (i.e., only in the group of children with ASD), an increased bias toward internal cognitive processes and reduced reaction to external (i.e., social) stimuli (37,45).

Differences between children with ASD and TDC in the relationship between functional connectivity and ASD symptom severity scores also support the existence of categorical brain mechanisms of ASD. Such findings imply that the brain representation of ASD symptom severity is qualitatively different from the brain representation of the normal spectrum of social behaviors in TDC (41,46). Regions demonstrating this type of effect included functional connectivity between the middle occipital gyrus and SAL (Figures 4 and 6C), for which there was a positive brain-behavior relationship for ASD but a negative relationship for TDC, suggesting a potential role of altered integration of visual processing with salience detection in the expression of social impairment within ASD. Another discrepancy between children with ASD and TDC in brain-behavior relationships was detected for DAN connectivity with the posterior cingulate cortex, a key region of the DMN. The opposing functions of these two networks has been well described (40) and seems to be important for behavior (47). Although lesser connectivity between these regions is associated with reduced social impairments in TDC, this relationship is altered in children with ASD, indicating that ASD-related impairments are associated with a categorical disruption in the intrinsic organization of these two opposing neural networks. These findings point to potential categorical mechanisms of ASD and provide support for the existence of a dual categorical-dimensional characterization of ASD.

Although dimensional and categorical effects were each identified while covarying for the other, there were a couple of regions in which both types of effects demonstrated overlap (Figure 5). An example of such a region was found for right precentral gyrus functional connectivity with the DMN (Figure 6D). Functional connectivity of this region exhibited a consistent positive dimensional relationship for the ASD and TDC groups; however, after controlling for differences in symptom severity, a significant categorical effect remained in which children with ASD exhibited hyperconnectivity of these regions. Although it is conceivable that such effects are unrelated, the possibility that categorical and dimensional mechanisms can work in tandem warrants further exploration.

This study has some limitations. Selecting an all-male sample may limit the extension of the study inferences to females. Future studies should consider sex differences in the brain representations of ASD to elucidate the neural mechanisms contributing to the strong male bias in the prevalence of this disorder. Additionally, we did not statistically control for medication use because information regarding psychoactive medication use was inconsistently available across sites, and sites further varied as to whether stimulants were withheld before scanning. Given the well-documented heterogeneity of ASD in terms of etiology and in terms in behavioral expression, it is unclear to what extent our findings are representative of these variations, particularly in behavioral domains not fully captured by the SRS. Moreover, previous work demonstrated that SRS scores may be biased by factors not related to ASD (48), including behavioral problems not related to ASD, age, language skills, and cognitive skills. As such, future studies should include other complementary measures of ASD severity that are currently not available across both groups of TDC and children with ASD. Finally, although we performed parallel examinations of ASD and ADHD, the lack of corresponding dimensional measures for these data sets precluded a formal statistical comparison of their functional connectivity alterations. Future studies that explore the potential overlapping and distinct neural mechanisms underlying these two neurodevelopmental disorders are highly desired (49–51).

In conclusion, based on combined analyses of functional connectivity of four large-scale neural networks, this study demonstrated the presence of distinct categorical and dimensional brain abnormalities associated with ASD. On one hand, the detection of shared brain-behavior relationships across both children with ASD and TDC supports a dimensional characterization of ASD. On the other hand, functional connectivity deficits associated with a categorical ASD diagnosis or diagnosis-by-behavior interaction suggest that children with ASD are also categorically distinct from TDC. Taken together, these findings shed light on the neural bases of ASD and support the use of a categorical-dimensional hybrid model for researchers and clinicians to conceptualize this disorder.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by funding provided to contributing research institutions by a multitude of sources, which are listed for each site at http://fcon_1000.projects.nitrc.org/indi/abide/, and included the Fund for Scientific Research-Flanders, Belgian Inter University Attraction Pole, Katholieke Universiteit Leuven Research Council, National Institutes of Health, Autism Speaks, Stavros Niarchos Foundation, Leon Levy Foundation, an endowment provided by Phyllis Green and Randolph Cowen, University of Utah Multidisciplinary Research Seed Grant, Ben B. and Iris M. Margolis Foundation, Simons Foundation, John Merck Scholars Fund, and Autism Science Foundation.

We thank Dr. Joseph Piven at the University of North Carolina at Chapel Hill for helpful comments and discussions during the preparation of this manuscript.

ADM is a coauthor of the Italian version of the Social Responsiveness Scale, for which she is entitled to royalties. All other authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Biomedical Research Imaging Center (AE), Department of Psychiatry and Carolina Institute for Developmental Disabilities (HCH), and Department of Radiology and Biomedical Research Imaging Center (WG), University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; Phyllis Green and Randolph Cowen Institute for Pediatric Neuroscience at the Child Study Center (ADM), New York University Langone Medical Center, New York, New York; and Biomedical Imaging Research Institute (WG), Department of Biomedical Sciences and Imaging, Cedars-Sinai Medical Center, Los Angeles, California.

Address correspondence to Wei Gao, Ph.D., Biomedical Imaging Research Institute, Department of Biomedical Sciences and Imaging, Cedars-Sinai Medical Center, 116 North Robertson Boulevard, PACT 800.7G, Los Angeles, CA 90048; E-mail: Wei.Gao@csmc.edu.

Received May 22, 2015; revised Oct 20, 2015; accepted Oct 24, 2015.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2015.10.020.

REFERENCES

1. Ousley O, Cermak T (2014): Autism spectrum disorder: Defining dimensions and subgroups. Curr Dev Disord Rep 1:20–28.
2. American Psychiatric Association. (2013): Diagnostic and Statistical Manual of Mental Disorders, *5th ed.* Washington, DC: American Psychiatric Publishing, Incorporated.
3. Wiggins L, Robins D, Adamson L, Bakeman R, Henrich C (2012): Support for a dimensional view of autism spectrum disorders in toddlers. J Autism Dev Disord 42:191–200.
4. Kamp-Becker I, Smidt J, Ghahreman M, Heinzel-Gutenbrunner M, Becker K, Remschmidt H (2010): Categorical and dimensional structure of autism spectrum disorders: The nosologic validity of Asperger syndrome. J Autism Dev Disord 40:921–929.
5. Grzadzinski R, Huerta M, Lord C (2013): DSM-5 and autism spectrum disorders (ASDs): An opportunity for identifying ASD subtypes. Mol Autism 4; 12–12.
6. Constantino JN, Przybeck T, Friesen D, Todd RD (2000): Reciprocal social behavior in children with and without pervasive developmental disorders. J Dev Behav Pediatr 21:2–11.
7. Grzadzinski R, Di Martino A, Brady E, Mairena MA, O'Neale M, Petkova E, *et al*. (2011): Examining autistic traits in children with ADHD: Does the autism spectrum extend to ADHD? J Autism Dev Disord 41:1178–1191.
8. Di Martino A, Shehzad Z, Kelly C, Roy A, Gee D, Uddin L, *et al*. (2009): Relationship between cingulo-insular functional connectivity and autistic traits in neurotypical adults. Am J Psychiatry 166: 891–899.
9. von dem Hagen EA, Nummenmaa L, Yu R, Engell AD, Ewbank MP, Calder AJ (2011): Autism spectrum traits in the typical population predict structure and function in the posterior superior temporal sulcus. Cerebral Cortex 21:493–500.
10. Misra V (2014): The social brain network and autism. Ann Neurosci 21:69.
11. Di Martino A, Yan C, Li Q, Denio E, Castellanos F, Alaerts K, *et al*. (2014): The autism brain imaging data exchange: Towards a large-scale evaluation of the intrinsic brain architecture in autism. Mol Psychiatry 19:659–667.
12. Cherkassky VL, Kana RK, Keller TA, Just MA (2006): Functional connectivity in a baseline resting-state network in autism. NeuroReport 17:1687–1690.
13. Weng S-J, Wiggins JL, Peltier SJ, Carrasco M, Risi S, Lord C, *et al*. (2010): Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. Brain Res 1313:202–214.
14. Paakki J-J, Rahko J, Long X, Moilanen I, Tervonen O, Nikkinen J, *et al*. (2010): Alterations in regional homogeneity of resting-state brain activity in autism spectrum disorders. Brain Res 1321:169–179.
15. Padmanabhan A, Lynn A, Foran W, Luna B, O'Hearn K (2013): Age related changes in striatal resting state functional connectivity in autism. Front Hum Neurosci 7:814.
16. Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ (2007): Functional and anatomical cortical underconnectivity in autism: Evidence from an fMRI study of an executive function task and corpus callosum morphometry. Cerebral Cortex 17:951–961.
17. Minshew NJ, Williams DL (2007): The new neurobiology of autism: Cortex, connectivity, and neuronal organization. Arch Neurol 64: 945–950.
18. Elton A, Alcauter S, Gao W (2014): Network connectivity abnormality profile supports a categorical-dimensional hybrid model of ADHD. Hum Brain Mapp 35:4531–4543.
19. Chabernaud C, Mennes M, Kelly C, Nooner K, Di Martino A, Castellanos FX, *et al*. (2012): Dimensional brain-behavior relationships in children with attention-deficit/hyperactivity disorder. Biol Psychiatry 71:434–442.
20. Corbetta M, Akbudak E, Conturo TE, Snyder AZ, Ollinger JM, Drury HA, *et al*. (1998): A common network of functional areas for attention and eye movements. Neuron 21:761–773.
21. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001): A default mode of brain function. Proc Natl Acad Sci U S A 98:676–682.
22. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, *et al*. (2007): Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci 27:2349–2356.
23. Vincent JL, Kahn I, Snyder AZ, Raichle ME, Buckner RL (2008): Evidence for a frontoparietal control system revealed by intrinsic functional connectivity. J Neurophysiol 100:3328–3342.
24. Frazier TW, Youngstrom EA, Speer L, Embacher R, Law P, Constantino J, *et al*. (2012): Validation of proposed DSM-5 criteria for autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 51 (28–40):e23.
25. Mars RB, Neubert F-X, Noonan MP, Sallet J, Toni I, Rushworth MFS (2012): On the relationship between the 'default mode network' and the 'social brain'. Front Hum Neurosci 6:189.
26. Eger E, Moretti L, Dehaene S, Sirigu A (2013): Decoding the representation of learned social roles in the human brain. Cortex 49: 2484–2493.
27. Uddin LQ, Supekar K, Lynch CJ, Khouzam A, Phillips J, Feinstein C, *et al*. (2013): Salience network–based classification and prediction of symptom severity in children with autism. JAMA Psychiatry 70: 869–879.
28. Solomon M, Ozonoff SJ, Ursu S, Ravizza S, Cummings N, Ly S, *et al*. (2009): The neural substrates of cognitive control deficits in autism spectrum disorders. Neuropsychologia 47:2515–2526.
29. Remington A, Swettenham J, Campbell R, Coleman M (2009): Selective attention and perceptual load in autism spectrum disorder. Psychol Sci 20:1388–1393.
30. Constantino JN, Davis SA, Todd RD, Schindler MK, Gross MM, Brophy SL, *et al*. (2003): Validation of a brief quantitative measure of autistic traits: Comparison of the Social Responsiveness Scale with the Autism Diagnostic Interview-Revised. J Autism Dev Disord 33: 427–433.
31. Dickstein DP, Pescosolido MF, Reidy BL, Galvan T, Kim KL, Seymour KE, *et al*. (2013): Developmental meta-analysis of the functional neural correlates of autism spectrum disorders. J Am Acad Child Adolesc Psychiatry 52(279–289):e216.
32. Werling DM, Geschwind DH (2013): Sex differences in autism spectrum disorders. Curr Opin Neurol 26:146–153.
33. Sikka S, Cheung B, Khanuja R, Ghosh S, Yan C, Li Q, *et al*. (2014): Towards automated analysis of connectomes: The configurable pipeline for the analysis of connectomes (C-PAC). Front Neuroinform Conference Abstract: 5th INCF Congress of Neuroinformatics. http://dx.doi.org/10.3389/conf.fninf.2014.08.00117. Available at: http://www.frontiersin.org/10.3389/conf.fninf.2014.08.00117/event_abstract. Accessed November 23, 2015.
34. Cox RW (1996): AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res 29: 162–173.
35. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM (2012): FSL. Neuroimage 62:782–790.

36. Tustison NJ, Cook PA, Klein A, Song G, Das SR, Duda JT, *et al*. (2014): Large-scale evaluation of ANTs and FreeSurfer cortical thickness measurements. Neuroimage 99:166–179.
37. Gao W, Lin W (2012): Frontal parietal control network regulates the anti-correlated default and dorsal attention networks. Hum Brain Mapp 33:192–202.
38. Yan CG, Craddock RC, Zuo XN, Zang YF, Milham MP (2013): Standardizing the intrinsic brain: Towards robust measurement of inter-individual variation in 1000 functional connectomes. Neuroimage 80:246–262.
39. Corbetta M, Shulman GL (2002): Control of goal-directed and stimulus-driven attention in the brain. Nat Rev Neurosci 3:201–215.
40. Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME (2005): The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 102: 9673–9678.
41. Frazier TW, Ratliff KR, Gruber C, Zhang Y, Law PA, Constantino JN (2014): Confirmatory factor analytic structure and measurement invariance of quantitative autistic traits measured by the Social Responsiveness Scale-2. Autism 18:31–34.
42. Constantino JN, Gruber CP, Davis S, Hayes S, Passanante N, Przybeck T (2004): The factor structure of autistic traits. J Child Psychol Psychiatry 45:719–726.
43. Dosenbach NUF, Fair DA, Miezin FM, Cohen AL, Wenger KK, Dosenbach RAT, *et al*. (2007): Distinct brain networks for adaptive and stable task control in humans. Proc Natl Acad Sci U S A 104: 11073–11078.
44. Dosenbach NUF, Fair DA, Cohen AL, Schlaggar BL, Petersen SE (2008): A dual-networks architecture of top-down control. Trends Cogn Sci 12:99–105.
45. Elton A, Gao W (2014): Divergent task-dependent functional connectivity of executive control and salience networks. Cortex 51:56–66.
46. Frazier TW, Youngstrom EA, Sinclair L, Kubu CS, Law P, Rezai A, *et al*. (2010): Autism spectrum disorders as a qualitatively distinct category from typical behavior in a large, clinically ascertained sample. Assessment 17:308–320.
47. Kelly AM, Uddin LQ, Biswal BB, Castellanos FX, Milham MP (2008): Competition between functional brain networks mediates behavioral variability. Neuroimage 39:527–537.
48. Hus V, Bishop S, Gotham K, Huerta M, Lord C (2013): Factors influencing scores on the social responsiveness scale. J Child Psychol Psychiatry 54:216–224.
49. Christakou A, Murphy CM, Chantiluke K, Cubillo AI, Smith AB, Giampietro V, *et al*. (2013): Disorder-specific functional abnormalities during sustained attention in youth with attention deficit hyperactivity disorder (ADHD) and with autism. Mol Psychiatry 18:236–244.
50. Di Martino A, Zuo X-N, Kelly C, Grzadzinski R, Mennes M, Schvarcz A, *et al*. (2013): Shared and distinct intrinsic functional network centrality in autism and attention-deficit/hyperactivity disorder. Biol Psychiatry 74:623–632.
51. Ray S, Miller M, Karalunas S, Robertson C, Grayson DS, Cary RP, *et al*. (2014): Structural and functional connectivity of the human brain in autism spectrum disorders and attention-deficit/hyperactivity disorder: A rich club-organization study. Hum Brain Mapp 35:6032–6048.

Published in final edited form as:
Neuroimage. 2010 October 15; 53(1): 247–256. doi:10.1016/j.neuroimage.2010.05.067.

Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients

Michal Assaf[a,b,*], **Kanchana Jagannathan**[a], **Vince D. Calhoun**[a,b,c,d], **Laura Miller**[a], **Michael C. Stevens**[a,b], **Robert Sahl**[e], **Jacqueline G. O'Boyle**[a], **Robert T. Schultz**[f,g], and **Godfrey D. Pearlson**[a,b]

[a]Olin Neuropsychiatry Research Center, Institute of Living, Hartford Hospital, Hartford, CT, USA

[b]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA

[c]The Mind Research Network, Albuquerque, NM, USA

[d]Department of Electrical and Computer Engineering, The University of New Mexico, Albuquerque, NM, USA

[e]Child and Adolescent Division, Institute of Living, Hartford Hospital, Hartford, CT, USA

[f]Center for Autism Research, Children's Hospital of Philadelphia, USA

[g]Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Abstract

Autism spectrum disorders (ASDs) are characterized by deficits in social and communication processes. Recent data suggest that altered functional connectivity (FC), i.e. synchronous brain activity, might contribute to these deficits. Of specific interest is the FC integrity of the default mode network (DMN), a network active during passive resting states and cognitive processes related to social deficits seen in ASD, e.g. Theory of Mind. We investigated the role of altered FC of default mode sub-networks (DM-SNs) in 16 patients with high-functioning ASD compared to 16 matched healthy controls of short resting fMRI scans using independent component analysis (ICA). ICA is a multivariate data-driven approach that identifies temporally coherent networks, providing a natural measure of FC. Results show that compared to controls, patients showed decreased FC between the precuneus and medial prefrontal cortex/anterior cingulate cortex, DMN core areas, and other DM-SNs areas. FC magnitude in these regions inversely correlated with the severity of patients' social and communication deficits as measured by the Autism Diagnostic Observational Schedule and the Social Responsiveness Scale. Importantly, supplemental analyses suggest that these results were independent of treatment status. These results support the hypothesis that DM-SNs under-connectivity contributes to the core deficits seen in ASD. Moreover, these data provide further support for the use of data-driven analysis with resting-state data for illuminating neural systems that differ between groups. This approach seems especially well suited for populations where compliance with and performance of active tasks might be a challenge, as it requires minimal cooperation.

*Corresponding author. Olin Neuropsychiatry Research Center, Institute of Living, 200 Retreat Ave., Hartford, CT 06106, USA. Fax: +1 860 545 7797. massaf@harthosp.org (M. Assaf).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2010.05.067.

Keywords

Independent component analysis; Functional MRI; Resting state; Default mode network; High-functioning autism

Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental conditions characterized by core deficits in social communication skills. One cognitive theory proposed to explain ASDs' deficits is that patients with ASD have difficulty with “Theory of Mind” (ToM) processes (Baron-Cohen, 1995; Hill and Frith, 2003). ToM is the ability to attribute states of mind (e.g. emotions, desires and goals) to other people and is a crucial component of social behavior. A distinct neural network normally mediates ToM, that includes medial prefrontal cortex (MPFC), temporoparietal junction, and temporal pole (Frith and Frith, 2003). A few studies have explored activation and functional connectivity of this network in individuals with ASDs using tasks of mental state attribution (Baron-Cohen et al., 1999; Castelli et al., 2002; Kana et al., 2009; Piggot et al., 2004) and narrative comprehension (Happé et al., 1996; Mason et al., 2008), with inconclusive results regarding the brain areas involved and the directionality of the impaired activation in relation to healthy controls.

Current research on social cognition in ASDs, including ToM, is limited to tasks that employ highly demanding cognitive procedures. This confines research to high-functioning and older subjects. Moreover, since patients' performance on these tasks is typically impaired, it is hard to conclude whether abnormal brain activations are primary, or secondary to patients' disengagement in the task. Studying brain networks that are activated during rest and overlap those related to social cognition, such as the default mode network (DMN), might overcome these limitations.

The DMN consists of the MPFC, posterior cingulate cortex/ retrosplenial cortex (PCC/Rsp) including the precuneus (PrC) and bilateral inferior parietal lobules (IPL) and systematically shows more prominent activity during passive resting conditions (e.g. Broyd et al., 2009; Buckner et al., 2008; Raichle et al., 2001). However, it is also active in tasks involving autobiographical memory, future prospection and ToM (for reviews see Broyd et al., 2009; Buckner et al., 2008). The close relationship between DMN and ToM network's brain areas makes it logical to explore whether abnormal DMN activity and/or functional connectivity might be related to ASD symptoms. The term “functional connectivity” (FC) refers to synchronous activation of spatially remote brain regions (Broyd et al., 2009; Friston et al., 1993; van de Ven et al., 2004). It has been suggested that functional under-connectivity among brain regions might explain ASD symptoms (Just et al., 2004). Several neuroimaging studies demonstrated reduced autism FC during the performance of different cognitive tasks (Castelli et al., 2002; Just et al., 2007, 2004; Kana et al., 2006, [2009], [2007]; Kleinhans et al., 2008; Koshino et al., 2005, 2008; Mason et al., 2008; Mostofsky et al., 2009; Solomon et al., 2009); although see other studies (Lee et al., 2008; Mizuno et al., 2006; Turner et al., 2006) for contradictory results.

Most studies investigating the DMN in individuals with ASDs analyzed data from resting blocks, comparing them to interleaved blocks of cognitive tasks, essentially measuring task induced deactiva-tions (TID) (Cherkassky et al., 2006; Kennedy and Courchesne, 2008a; Kennedy et al., 2006) and not FC per se (although Cherkassky et al. (2006) also performed a FC analysis, see below). Of those, Kennedy and Courchesne (2008a) and (Kennedy et al. 2006) showed abnormal DMN TID in patients. Measuring FC with a seed-voxel approach,Cherkassky et al. (2006) reported reduced anterior/posterior DMN connectivity in

ASD. However, resting block designs from cognitive tasks have limitations. First, patient cooperation and task understanding is required. Second, there is typically a correlation between DMN TID and task difficulty (e.g. McKiernan et al., 2003). Thus task difficulty in addition to task performance might influence group differences in DMN activations and connectivity.

To mitigate these drawbacks, subjects can be fMRI scanned during the resting state, where participants are instructed to lie still and awake in the scanner for few minutes, without performing any specific task. These scans are appealing to research involving patients because of their shortness and simplicity (Franco et al., 2009; Pearlson and Calhoun, 2009). While traditional analytical techniques such as the general linear model (GLM) using performance variables as regressors cannot be applied to resting data, it is possible to measure FC of different brain areas. To our knowledge, only two fMRI studies to date have tested resting state connectivity in ASD (Kennedy and Courchesne, 2008b; Monk et al., 2009). These calculated DMN connectivity within the network and with the rest of the brain, using a region of interest (ROI) seed-voxel correlation technique. *A priori* ROIs were defined based on previous reported DMN brain areas in healthy individuals. While both studies found decreased connectivity within the DMN, specifically involving the MPFC, Monk et al. (2009) also found increased connectivity between PCC and temporal regions in ASD. They further demonstrated that worse social functions in patients were associated with decreased connectivity between PCC and MPFC and that increased restrictive and repetitive behaviors were related to increased connectivity between PCC and parahippocampal gyrus. These results support impaired functional DMN connectivity as potentially underlying deficits seen in patients with ASD.

Group independent component analysis, ICA, has been recently used successfully to identify the DMN and other resting-state networks and to assess FC within these networks during resting fMRI scans (Calhoun et al., 2001b; McKeown and Sejnowski, 1998). ICA identifies spatially independent components of brain areas with hemodynamic timecourses that closely co-vary. Thus, the regions comprising each component are conceptualized as part of a specific network (Calhoun et al., 2001b) with highly synchronous time courses. ICA is a data-driven method, allowing data analysis without either regressors extracted from behavioral data (i.e. no task is needed) or *a priori* hypotheses of a specified relevant ROI. Thus, ICA identifies multiple integrated networks from fMRI timeseries data without being limited to *a priori* ROIs or specific cognitive processes (for review see Calhoun et al., 2009). It consistently identifies the DMN during scans of resting state and cognitive tasks in healthy individuals (e.g. Calhoun et al., 2008a; Stevens et al., 2009) and in patient groups (e.g. Garrity et al., 2007; Kim et al., 2009). Moreover, various ICA analyses suggest that resting fMRI data comprise several consistent synchronous networks, including fronto-parietal, motor, visual and auditory networks (Beckmann et al., 2005; Calhoun et al., 2008a; De Luca et al., 2006; Stevens et al., 2009; van de Ven et al., 2004). ICA has been used to identify sub-networks of the DMN as separate components, each with a distinctive timecourse. When detected in active tasks, all these sub-networks are negatively modulated by cognitive demands and include portions of typical DMN brain regions, namely MPFC and PCC/PrC (Beckmann et al., 2005; Calhoun et al., 2008a; Kim et al., 2009; Stevens et al., 2009). While seed-voxel correlation techniques can also detect DMN subnetworks (Uddin et al., 2009), ICA has the advantage of identifying sub-networks with subtly different spatiotemporal patterns, without specific ROI restriction. Thus, we used ICA to investigate the FC integrity of the default mode sub-networks (DM-SNs) during fMRI resting scans in high-functioning patients with ASD. We hypothesized that compared to matched healthy controls (HC), patients would show decreased strength of connectivity in DM-SNs, specifically in the PCC/PrC and MPFC (Broyd et al., 2009), and that social and communication skill deficits would be predicted by reduced connectivity within DM-SNs'.

Methods and materials

Participants

Sixteen high-functioning patients with ASDs (ages 11–20, 15 males) and 16 typically developing healthy controls (HC) (ages 13– 23, 14 males) were recruited. Full scale IQ was assessed with the Vocabulary and Block Design subsets of the Wechsler Adult Intelligence Scale (WAIS) or Wechsler Intelligence Scale for Children (WISC). Data from all 32 participants were used to run the ICA analysis and identify the DM-SNs. However, since IQ was missing for one patient and one control they were excluded from group analyses. Table 1 summarizes the demographic information for the remaining participants. Notably, there were no group differences on age, gender, race and full scale IQ. Patients' diagnosis was confirmed with the Autism Diagnostic Observational Schedule (ADOS, Lord et al., 2000) and the Autism Diagnostic Interview-Revised (ADI-R, Lord et al., 1994). ASD symptomatology was further assessed by the Social Responsiveness Scale (SRS, Constantino and Gruber, 2005), in the 13 patients younger than 18. Eight of the 15 patients were medicated when scanned (information was missing for 2 patients): 6 received CNS stimulants, 3 antipsychotic drugs, 4 SSRIs, 2 other antidepres-sants and one anti-epileptic treatment (note that 7 of the medicated patients were treated with more than one drug). ASD was ruled out in HCs using the ADOS (available for 14 of the 15 controls), the Social Communication Questionnaire (SCQ, Rutter et al., 2003) lifetime form (available for 9 controls, including the individual without ADOS) and a detailed health questionnaire. ADOS total scores ranged from 0 to 4 (mean=0.8±1.2) and SCQ scores ranged from 0 to 7 (mean=2.7± 2.9), well within the normal range. All participants provided written informed consent, approved by the Hartford Hospital Institution Review Committee, after complete description of the study and were paid for their time.

Scanning procedures

During fMRI, participants were instructed to lie still with their eyes open, fixating on a centrally presented cross for 5 minutes and 15 seconds. No cognitive task was performed during this time. Blood oxygenation level dependent (BOLD) signal was obtained with T2*-weighted echo planar imaging (EPI) sequence (TR/TE=1500/27 ms, Flip angle=70°, Field of view=22 cm with a 64×64 acquisition matrix) using a Siemens 3 T Allegra. We acquired 29 contiguous axial functional slices of 4 mm thickness with 1 mm gap, yielding 3.4×3.4×5 mm^3 voxels for 210 time points. The first 6 images of the scan were not included in the data analysis to allow global image intensity to reach equilibrium.

Image preprocessing

Data were preprocessed using SPM5 (Wellcome Department of Cognitive Neurology). Each individual's dataset was realigned to the first “non-dummy” image using the INRIAlign toolbox (A. Roche, INRIA Sophia Antipolis, EPIDAURE Group). The average translation motion parameter was 3.2 mm with no group differences ($t_{(1, 30)}$= 0.97, p=0.34). In addition to excluding components that represented movement artifacts (see below), all individual component maps were inspected to ensure that there were no obvious motion artifacts in the components of interest. Next, slice timing correction was applied to the data, adjusting slice timing based on the middle slice. Data were then spatially normalized to Montreal Neurological Institute (MNI) space (Friston et al., 1995), and smoothed with 9 mm^3 FWHM Gaussian kernel.

Component identification

Group spatial ICA was conducted for all 32 participants using the Infomax algorithm (Bell and Sejnowski, 1995) within the GIFT software (http://icatb.sourceforge.net/, version 1.3e).

Dimensionality estimation to determine the number of components was performed using the minimum description length criteria, modified to account for spatial correlation (Li et al., 2007). The mean dimension estimation was 28.9 (SD=13.4). We rounded this number slightly and estimated 28 components. Single subject time courses and spatial maps were then computed, during which the aggregate components and the results from data reduction were used to compute the individual subject components (i.e. back-reconstruction Calhoun et al., 2001a, 2009).

Selecting the components related to the DMN was done in 2 stages. First, a systematic process was used to inspect and select components whose patterns of correlated signal change were largely constrained to gray matter from the 28 estimated components (Stevens et al., 2007). To do so, the correlationsof each component's spatial map with *a priori* mask maps of gray matter, white matter, and cerebral spinal fluid (CSF) within standardized brain space provided in WFU Pickatlas (Maldjian et al., 2003) were computed. Components with high correlation to *a priori* localized CSF or white matter, or with low correlation to gray matter, were inferred to be likely artifactual. Visual inspection of discarded components suggested that they represented eye movements, head motion, or cardiac-induced pulsatile artifact at the base of the brain. Ten components were selected as of interest for further analysis to identify the DMN components.

Next, a spatial correlation analysis between the 10 chosen components and an a priori DMN binary mask of the DMN template provided in GIFT was performed. This template is based on DMN regions reported by Raichle et al. (2001), including the PCC/PrC, IPL and MPFC (Calhoun et al., 2008a,b; Garrity et al., 2007). This procedure was used only to select the components that corresponded to the DMN but not to modify them (i.e. no ROIs were included or excluded from the different components following this correlation analysis). Visual inspection confirmed that components that included all or part of the DMN regions ranked highest in this analysis.

For each subject, the chosen DMN components (3 components were chosen, see below), referred to here as the DM-SNs, were then converted to *z* values. For each component, individual maps of all subjects regardless of group were entered into random effect one-sample *t*-tests in SPM5 and thresholded at $p<0.05$ corrected for family-wise error, to create a sample-specific component map. These maps were used as a mask for group analyses within the corresponding component. Thus, results are not biased by components maps defined from healthy participants only.

Statistical analysis

Group comparison—Individual DM-SN components GIFT maps were entered into SPM5 for group analyses. The *z* values in these individual maps represent the fit of a specific voxel BOLD timecourse to the group averaged component's timecourse. Thus, group analyses test the connectivity strength (i.e. signal synchronization) of each voxel to the whole spatial component. For each component, random effects one-sample *t*-tests were performed for each group separately to assess the within group integrity of the component maps. Random effects two-sample *t*-tests examined group differences. The resulting statistical maps were masked with the study-specific general map of the relevant component (generated based on data from all participants) to explore results within this network only. All group tests were controlled for age and IQ (due to their wide range, see Table 1) and were thresholded at $p<0.05$ corrected for false discovery rate (FDR). Reported coordinates were converted from MNI space to standardized Talairach coordinates (Talairach and Tournoux, 1988) (http:// imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach).

To further assess the potential effect of drug treatment on results of group differences in functional connectivity, we recalculated the random effects two-sample *t*-test for each component, comparing only the 5 patients without current treatment to controls. In addition, for each component we performed random effects two-sample *t*-test comparing the patients without current treatment to the patients with current treatment (n=8; as mentioned above the treatment status of 2 patients was missing, therefore they were not included in these analyses). All group tests were controlled for age and IQ and were thresholded a $p<0.005$ uncorrected (due to the low number of subjects).

Correlation of components maps with symptom severity—For each DM-SN component, multiple regression analyses were performed to assess the relationship between individual patients' maps and their symptom severity as assessed by the ADOS total, communication and social scores and the SRS total and subscale scores. Again, analyses were controlled for age and IQ and thresholded at $p_{FDR}<0.05$, and the resulting statistical maps were masked with the general specific component's map, limiting the analysis to each component's ROIs.

Results

Component identification

Examination of the 10 component maps yielded 3 components that included brain areas previously reported to be part of the DMN (e.g. Broyd et al., 2009; Buckner et al., 2008; McKiernan et al., 2003; Raichle et al., 2001) (Fig. 1 and Table 2): (A) DMN component, including mainly the PCC and bilateral IPL, but also the PrC and MPFC (we refer to this network as the DMN since it is the only one that includes all suggested “classic” DMN regions); (B) Precuneus (PrC) component, which also included adjacent areas in the PCC, postcentral gyrus and paracentral lobule; and (C) MPFC component that also included a PCC/PrC cluster and small IPL clusters. Spatial correlation with the DMN *a priori* mask revealed that these components were the 3 highest ranking components of the 10 components of interest (r=0.30, 0.32 and 0.21 respectively), indicating that regions in these components closely resembled the regions in the mask. As evident in Fig. 1, some of the component maps partially overlap. One of the strengths of ICA is that a given voxel can contribute to multiple components. This provides for the case where a region can be a hub for more than one network (McKeown and Sejnowski, 1998).

One-sample *t*-tests that quantified conservation of spatial structure across participants in each component in each group separately demonstrated that the same brain regions were evident in the 3 selected ICA components in both groups (Table 2).

Group comparison

Although the DM-SNs' maps of patients and controls were similar overall, voxelwise two-sample *t*-tests revealed significant differences in regional FC strength for component A in the precuneus (x=21, y= –56, z=38; t=4.7, $p_{FDR}<0.05$), and for component C in the ACC (x= –9, y=47, z=–2; t=4.65, $p_{FDR}<0.05$). For both components patients showed decreased strength of connectivity compared to HC (Fig. 2).

Analyses including only patients with no current drug treatment replicated these results, showing stronger connectivity in HC in the precuneus (x=15, y=–53, z=38, t=3.86) for component A and in the ACC (χ=–9, y=49, z=–2, t=3.58) for component C. In our a priori threshold of p<0.005, additional group differences were found in the PCC (x=–3, y=–40, 13, t=3.43) in component A, and in the right ACC (χ=6, y=47, z=0, t=3.98) in component C (as in the original results, patients showed decreased strength of connectivity compared to HC).

In addition, comparing drug-treated patients to non-treated patients found no group differences in any of the 3 components.

Importantly, we used data from all participants regardless of diagnostic group to identify components, and to test for group differences in the second-level analyses, to avoid the problem of matching components between groups. This approach preserves group differences in the back-reconstructed maps (Calhoun et al., 2001a,b). We also performed a separate ICA for each group and identified the 3 DM-SNs components in both groups. Follow-up 2 sample *t*-tests found regional group differences similar to the group differences describe above (see Supplementary Materials).

Correlation of DM-SNs' connectivity maps with symptoms severity

Component A: No correlations were found with ADOS and SRS scores ($p_{FDR}<0.05$).

Component B: Significant negative correlations were found between the precuneus FC strength and ADOS total and social scale scores (x=0, y=−42, z=44, r=−0.90 and r=−0.89, respectively, $p_{FDR}<0.05$) and ADOS communication scale score (x=0, y=−41, z=63, r=−0.85, $p_{FDR}<0.05$) while controlling for age and IQ. The correlation pattern indicates that worse symptom severity was associated with lesser PrC connectivity (Fig. 3; Note that graphs depict participants with and without drug treatment at the time of the scan).

Component C: Significant negative correlations were found (a) between MPFC connectivity and SRS total score (x=6, y=48, z=28, r=0.91, $p_{FDR}<0.05$), and (b) between ACC connectivity and SRS total and autistic mannerisms subscale score (x=6, 38, 1, r=−0.85 and x=9, 38, −2, r=−0.93, respectively, $p_{FDR}<0.05$). All analyses were controlled for age and IQ; worse symptom severities were associated with decreased connectivity (Fig. 3).

Discussion

We investigated the integrity of FC within sub-networks of resting-state default mode areas in high-functioning patients with ASDs compared to matched healthy controls using ICA of relatively straightforward and short resting fMRI scans. Group ICA of fMRI data identifies components, each comprised of several brain regions with synchronous BOLD time courses. The regions in each component therefore have strong FC and can be considered as a specific neural network (for review see Calhoun et al., 2009). Of the 10 meaningful brain components we identified by ICA, 3 included brain regions most often associated with DMN (e.g. Broyd et al., 2009; Buckner et al., 2008; McKiernan et al., 2003; Raichle et al., 2001). *Component A* included all DMN areas, but mostly the PCC/PrC and bilateral IPL, *Component B* included mainly the PrC, and *Component C* included MPFC and ACC with a small cluster at the PCC. These 3 DM-SNs were previously identified in other ICA studies of resting state and cognitive fMRI studies in both non-clinical samples and schizophrenia patients (e.g. Assaf et al., 2009; Calhoun et al., 2008a; Damoiseaux et al., 2006; Esposito et al., 2006; Garrity et al., 2007; Jafri et al., 2008; Kim et al., 2009; Stevens et al., 2009). The heterogeneity of connectivity among regions indicates that spatiotemporal patterns of the MPFC/ACC and PrC are sufficiently different from the rest of the DMN (i.e. PCC and IPL); as such, they are identified by ICA as different components. Separation of DMN into posterior and anterior regions (corresponding to our components A and C) is the most consistent result in the above studies (Calhoun et al., 2008a; Damoiseaux et al., 2006; Esposito et al., 2006; Kim et al., 2009; Stevens et al., 2009). Working memory task load differentially influences these regions' temporal and spatial modulation (Esposito et al., 2006) and posterior regions show less activity modulation during resting scans (Damoiseaux

et al., 2006). To our knowledge, no study has investigated the temporospatial modulation of the PrC component, although its activity manifests task-related decreases (Calhoun et al., 2008a). Our results support the idea that the DMN has multiple interacting hubs or subsystems (Buckner et al., 2008).

Although all 3 DM-SNs were evident in both ASD and HC, direct group comparisons showed decreased connectivity strength in precuneus in component A and MPFC/ACC in component C (each with other brain areas in the corresponding component) in patients. There was no group difference in the connectivity of component B, representing local PrC FC. In addition, the connectivity integrity of different DMN areas negatively correlated with specific measures of patients' symptoms. Thus, decreased connectivity between DMN regions (a brain network associated with social cognitive functions impaired in individuals with ASD, see below) might underlie specific deficits in these patients, supporting the under-connectivity hypothesis of ASD (Just et al., 2004).

Our results mostly agree with other fMRI studies of DMN in ASD, generally showing decreased long-range (and not local) FC or activation of this network (Cherkassky et al., 2006; Kennedy and Courchesne, 2008a,b; Kennedy et al., 2006; Monk et al., 2009), although the impaired regions identified by these studies do not always overlap with ours. Methodological differences make direct comparisons between results somewhat challenging. Most previous studies used a task induced deactivation (TID) method in fMRI scans of cognitive tasks to explore the DMN (Cherkassky et al., 2006; Kennedy and Courchesne, 2008a; Kennedy et al., 2006). Of those, Kennedy and Courchesne (2008a) and Kennedy et al. (2006) linked ASD to abnormal deactivation of the DMN, but did not examine FC. Cherkassky et al. (2006), although not finding similar reduced TID, reported decreased connectivity between the ACC and precuneus in patients using a formal DMN connectivity analysis after combining data from different cognitive tasks. It is difficult to conclude whether group differences in these studies represent true abnormal DMN activity/ connectivity, or if they are driven by abnormal task-related performance (McKiernan et al., 2003).

In recent studies, Kennedy and Courchesne (2008b) and Monk et al. (2009) used resting fMRI scans similar to ours in individuals with ASD to explore FC among DMN brain regions, but reported conflicting results. Kennedy and Courchesne (2008b) found reduced connectivity in patients in the MPFC and left angular gyrus, but not in PCC/PrC. Monk et al. (2009) demonstrated *decreased* connectivity between PCC and frontal regions and *increased* connectivity between PCC and temporal regions. Importantly, these studies explored connectivity integrity by calculating correlations between time courses of pre-defined ROIs based on a previous study of DMN in healthy participants (Fox et al., 2005). This method could potentially bias results to specific areas while missing others. Conversely, ICA requires no *a priori* hypotheses regarding ROIs. Note that although we used a DMN mask to identify the most appropriate components, this procedure did not limit the ICA results to the regions included in this mask. Instead, this approach merely permitted us to empirically quantify how strongly any given component resembles the proposed structure of the DMN to choose what networks to examine. Since we used data from patients and controls to identify DM-SNs, and group differences were assessed by second-level analyses, our results are not biased by areas selected based on healthy participants data only.

Our results also show an association between connectivity strength of DMN regions and characteristic social, communication and behavioral deficits in patients. Areas in the PrC showed negative correlations with ADOS Total, Social and Communication scores (component B) while areas in the MPFC/ACC negatively correlated with SRS total score and the social mannerism subscale scores (component C). For all these correlations,

decreased functional connectivity was associated with more severe symptoms. The SRS is a parental report that assesses several dimensions of social, communication and repetitive/ stereotypic behaviors in ASDs. The Autistic Mannerisms subscale specifically measures stereotypical behaviors and highly restricted interests that characterize patients, including obsessive behaviors and thoughts (Constantino and Gruber, 2005). The ADOS is an observational tool, designed to elucidate and evaluate social and communication deficits specifically related to ASDs. Although SRS and ADOS scores are reported to correlate positively (Charman et al., 2007), they probably represent somewhat different aspects of patients' impairments. The SRS mannerism subscale in particular captures behavioral aspects not incorporated into the ADOS algorithm. Importantly, the association of the SRS with ACC FC corresponds to the documented involvement of the ACC in the pathopysiology of obsessive-compulsive disorder (for review see Maia et al., 2008; Rotge et al., 2009), suggesting that these symptoms in patients with ASD might have similar underlying neural cause (Hollander et al., 2009). On the other hand, the correlation between ADOS scores and the PrC FC corresponds to this region known involvement in high social cognitive processes, such as self-referential thinking (e.g. Cavanna and Trimble, 2006; Wolf et al., 2010) and ToM, which are known to be impaired in patients with ASD (Baron-Cohen, 1995; Frith and Frith, 2003; Hill and Frith, 2003). Related to our results in this region, Rojas et al. (2006) found negative correlation between PrC (among other regions) gray matter volume and ADI Social and Communication total score (although they did not find similar correlation with ADOS scores), further emphasizing the relationship between the PrC and social and communication deficits in patients with ASD.

While our correlation analysis results support an under-connec-tivity hypothesis of autism (Just et al., 2004), they are not in full agreement with other reports. To our knowledge only 3 studies to date have evaluated the correlations between FC and symptoms severity in ASD, two measured FC during cognitive tasks (Just et al., 2007; Kleinhans et al., 2008) and the third measured FC during a resting fMRI scan (Monk et al., 2009). Just et al. (2007) showed that frontal-parietal FC negatively correlated with ADOS total scores during an executive task. Using face processing task, Kleinhans et al. (2008) showed that FFA-amygdala FC negatively correlated with social impairments measured by ADI, but FFA-right IFG FC positively correlated with ADOS social impairments. Finally, Monk et al. (2009) showed negative correlations between social impairments (measured by ADI) and the FC between the PCC and MPFC, but positive correlations between restricted and repetitive behaviors and FC between PCC and parahippocampal region during resting scans similar to ours. Differences in results might arise from the methods used to measure FC (i.e. task vs. resting-state scans; ICA vs. voxel-based correlations) and symptom severity. Importantly, Components A–C depicted in our study did not include hippocampal/parahippo-campal regions, which have been suggested by some studies to be part of the DMN (for review see Buckner et al., 2008) and showed impaired connectivity in patients with ASD (Monk et al., 2009), and therefore we could not assess their connectivity to other DMN regions. Moreover, while the ADOS and SRS assess current symptoms the ADI assesses the most abnormal lifetime symptoms, also possibly contributing to seeming contradictions among existing results from different studies.

While DMN functions are still a matter of debate, a leading hypothesis suggests that the “resting brain” is engaged in self-referential mental representation and other high order social cognitive processes, such as ToM (for reviews see Broyd et al., 2009; Buckner et al., 2008), since although it deactivates during most cognitive tasks, it activates during tasks involving these processes. Thus, investigating the DMN FC from resting scans can potentially approximate FC of brain networks related to social cognition, allowing us to investigate brain networks hypothesized to be impaired in individuals with ASD (Baron-Cohen, 1995; Frith and Frith, 2003; Hill and Frith, 2003) without highly demanding

cognitive tasks (Franco et al., 2009; Pearlson and Calhoun, 2009). Importantly, this suggested functional significance of the DMN has been challenged by observations of its activity in unconscious states in both humans and monkeys (e.g. Boly et al., 2008; Horovitz et al., 2008; Vincent et al., 2007), implying that DMN activity might be related to intrinsic brain functional organization. Notably, these latter studies explored this activity in the "classic" DMN (mostly in PCC/PrC and IPL) but not in DM-SNs, such as the MPFC. Nevertheless, we do not argue that DM-SNs are exclusively involved in higher-order social processes. Rather we speculate that these sub-networks show coherent fluctuations during rest in different levels of consciousness and during social cognitive tasks, much like other brain networks, such as somatomotor and visual circuits (e.g. Vincent et al., 2007). Higher-order social cognitive processes are hypothesized to be impaired in patients with ASD and to underlie core social and communication clinical deficits (Baron-Cohen, 1995; Frith and Frith, 2003; Hill and Frith, 2003) and therefore it is logical to investigate patient DM-SNs activity and connectivity. Our results add to the growing evidence that regional DMN under-connectivity may underlie the etio-pathology of patients' clinical deficits. Furthermore, our findings suggest that assessing FC of fMRI data collected during a simple resting scan could serve as a biological marker for treatments designed to target specific deficits.

Limitations of the current study include that 8 of the 15 patients were medicated (data missing for 2 patients), making it difficult to determine whether some of our findings were secondary to possible effects of medication on FC. However, our analyses confirmed that the same group differences are evident when examining unmediated patients only and that medicated and unmediated patients showed no connectivity differences. In addition, as can be seen in Fig. 3, none of the 2 patients groups is driving the correlation of FC with symptoms severity. Albeit preliminary due to small sample size, these results suggest that connectivity impairments in patients with ASD are primarily due to their baseline illness and not drug treatment. Second, although resting-state fMRI's major advantage is its relative simplicity and lack of potential performance confounds, this can be also viewed as a disadvantage, since participants are not monitored through the scan and no cognitive data exists to interpret the results. For example, we did not objectively verify that participants had not fallen asleep during the scan. However, all patients responded to the MR technologist at the beginning and end of the scan and no subject indicated that they slept during the resting scan. It is also possible that group differences are due to different anxiety/arousal levels, which are more prevalent in individuals with ASD (Davism et al., 2008); however, we did not assess anxiety. Third, we did not evaluate mutual casual interactions among different ICA DM-SN components and their potential impairments in patients with ASD (Assaf et al., 2009;Jafri et al., 2008). Finally, our sample included only high-functioning patients with ASD, limiting generalizability. Given the simplicity of resting scans, applying our methods to lower-functioning patients is an obtainable goal.

In summary, using brief resting fMRI scans and ICA, we have shown that although high-functioning patients with ASD have similar DM-SNs to healthy controls, they have decreased connectivity in the MPFC/ACC and PrC regions. Strength of connectivity in these regions also negatively correlated with the severity of patients' social and communication deficits. These results are in accord with an ASD under-connectivity hypothesis, which suggests that a general decrease in cortico-cortical functional connectivity underlies patients' behavioral and cognitive impairments. Resting fMRI scans and ICA as a data analysis method provide us with the tools to probe this network easily without a cognitive task (that patients might find difficult to understand and/or perform) opening the way to explore functional connectivity in younger and low-functioning patients with ASD as well. If our results generalize to these patients, DMN connectivity promises to be a useful imaging biological marker and treatment target of ASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from Autism Speaks and Hartford Hospital (PI: M. Assaf), and from NIH 2R01 EB000840 (PI: V. D. Calhoun). Parts of these data were presented in the 2009 IMFAR annual meeting, Chicago, IL, May, 2009.

References

Assaf M, Jagannathan K, Calhoun V, Kraut M, Hart J Jr, Pearlson G. Temporal sequence of hemispheric network activation during semantic processing: a functional network connectivity analysis. Brain Cogn. 2009; 70:238–246. [PubMed: 19307050]

Baron-Cohen, S. Cambridge, Mass: MIT Press; 1995. Mindblindness : an essay on autism and theory of mind.

Baron-Cohen S, Ring HA, Wheelwright S, Bullmore ET, Brammer MJ, Simmons A, Williams SC. Social intelligence in the normal and autistic brain: an fMRI study. Eur. J. Neuro sci. 1999; 11:1891–1898.

Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. Philos. Trans. R. Soc. Lond. B Biol Sci. 2005; 360:1001–1013. [PubMed: 16087444]

Bell AJ, Sejnowski TJ. An information maximization approach to blind separation and blind deconvolution. Neural Comput. 1995; 7:1129–1159. [PubMed: 7584893]

Boly M, Phillips C, Tshibanda L, Vanhaudenhuyse A, Schabus M, Dang Vu TT, Moonen G, Hustinx R, Maquet P, Laureys S. Intrinsic brain activity in altered states of consciousness: how conscious is the default mode of brain function? Ann. N. Y. Acad Sci. 2008; 1129:119–129. [PubMed: 18591474]

Broyd SJ, Demanuele C, Debener S, Helps SK, James CJ, Sonuga Barke EJ. Default-mode brain dysfunction in mental disorders: a systematic review. Neurosci. Biobehav Rev. 2009; 33:279–296. [PubMed: 18824195]

Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. Ann. N. Y. Acad Sci. 2008; 1124:1–38. [PubMed: 18400922]

Calhoun VD, Adali T, Pearlson GD, Pekar JJ. A method for making group inferences from functional MRI data using independent component analysis. Hum Brain Mapp. 2001a; 14:140–151. [PubMed: 11559959]

Calhoun VD, Adali T, Pearlson GD, Pekar JJ. Spatial and temporal independent component analysis of functional MRI data containing a pair of task-related waveforms. Hum Brain Mapp. 2001b; 13:43–53. [PubMed: 11284046]

Calhoun VD, Kiehl KA, Pearlson GD. Modulation of temporally coherent brain networks estimated using ICA at rest and during cognitive tasks. Hum Brain Mapp. 2008a; 29:828–838. [PubMed: 18438867]

Calhoun VD, Maciejewski PK, Pearlson GD, Kiehl KA. Temporal lobe and “default” hemodynamic brain modes discriminate between schizophrenia and bipolar disorder. Hum Brain Mapp. 2008b; 29:1265–1275. [PubMed: 17894392]

Calhoun VD, Liu J, Adali T. A review of group ICA for fMRI data and ICA for joint inference of imaging, genetic, and ERP data. Neuroimage. 2009; 45:S163–S172. [PubMed: 19059344]

Castelli F, Frith C, Happe F, Frith U. Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. Brain. 2002; 125:1839–1849. [PubMed: 12135974]

Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. Brain. 2006; 129:564–583. [PubMed: 16399806]

Charman T, Baird G, Simonoff E, Loucas T, Chandler S, Meldrum D, Pickles A. Efficacy of three screening instruments in the identification of autistic-spectrum disorders. Br. J Psychiatry. 2007; 191:554–559. [PubMed: 18055961]

Cherkassky VL, Kana RK, Keller TA, Just MA. Functional connectivity in a baseline resting-state network in autism. Neuroreport. 2006; 17:1687–1690. [PubMed: 17047454]

Constantino, JN.; Gruber, CP. Western Psychological Services. Los Angeles, California: 2005. Social Responsiveness Scale (SRS).

Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF. Consistent resting-state networks across healthy subjects. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:13848–13853. [PubMed: 16945915]

Davism E, Saeed SA, Antonacci DJ. Anxiety disorders in persons with developmental disabilities: empirically informed diagnosis and treatment. Reviews literature on anxiety disorders in DD population with practical take-home messages for the clinician. Psychiatr Q. 2008; 79:249–263. [PubMed: 18726156]

De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM. fMRI resting state networks define distinct modes of long-distance interactions in the human brain. Neuroimage. 2006; 29:1359–1367. [PubMed: 16260155]

Esposito F, Bertolino A, Scarabino T, Latorre V, Blasi G, Popolizio T, Tedeschi G, Cirillo S, Goebel R, Di Salle F. Independent component model of the default-mode brain function: Assessing the impact of active thinking. Brain Res Bull. 2006; 70:263–269. [PubMed: 17027761]

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc. Natl. Acad. Sci. U. S. A. 2005; 102:9673–9678. [PubMed: 15976020]

Franco AR, Pritchard A, Calhoun VD, Mayer AR. Interrater and intermethod reliability of default mode network selection. Hum. Brain Mapp. 2009; 30:2293–2303. [PubMed: 19206103]

Friston KJ, Frith CD, Liddle PF, Frackowiak RS. Functional connectivity: the principal-component analysis of large (PET) data sets. J. Cereb Blood Flow Metab. 1993; 13:5–14. [PubMed: 8417010]

Friston KJ, Ashburner J, Frith CD, Poline J-B, Heather JD, Frackowiak RSJ. Spatial registration and normalization of images. Hum. Brain Mapp. 1995; 2:165–189.

Frith U, Frith CD. Development and neurophysiology of mentalizing. Philos. Trans. R. Soc. Lond. B Biol Sci. 2003; 358:459–473. [PubMed: 12689373]

Garrity AG, Pearlson GD, McKiernan K, Lloyd D, Kiehl KA, Calhoun VD. Aberrant “default mode” functional connectivity in schizophrenia. Am. J Psychiatry. 2007; 164:450–457. [PubMed: 17329470]

Happé F, Ehlers S, Fletcher P, Frith U, Johansson M, Gillberg C, Dolan R, Frackowiak R, Frith C. ‘Theory of mind' in the brain. Evidence from a PET scan study of Asperger syndrome. Neuroreport. 1996; 8:197–201. [PubMed: 9051780]

Hill EL, Frith U. Understanding autism: insights from mind and brain. Philos. Trans. R. Soc. Lond. B Biol Sci. 2009; 358:281–289. [PubMed: 12639326]

Hollander E, Kim S, Braun A, Simeon D, Zohar J. Cross-cutting issues and future directions for the OCD spectrum. Psychiatry Res. 2009; 170:3–6. [PubMed: 19811839]

Horovitz SG, Fukunaga M, de Zwart JA, van Gelderen P, Fulton SC, Balkin TJ, Duyn JH. Low frequency BOLD fluctuations during resting wakefulness and light sleep: a simultaneous EEG-fMRI study. Hum. Brain Mapp. 2008; 29:671–682. [PubMed: 17598166]

Jafri MJ, Pearlson GD, Stevens M, Calhoun VD. A method for functional network connectivity among spatially independent resting-state components in schizophrenia. Neuroimage. 2008; 39:1666–1681. [PubMed: 18082428]

Just MA, Cherkassky VL, Keller TA, Minshew NJ. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain. 2004; 127:1811–1821. [PubMed: 15215213]

Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. Cereb Cortex. 2007; 17:951–961. [PubMed: 16772313]

Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. Brain. 2006; 129:2484–2493. [PubMed: 16835247]

Kana RK, Keller TA, Minshew NJ, Just MA. Inhibitory control in high-functioning autism: decreased activation and underconnectivity in inhibition networks. Biol Psychiatry. 2007; 62:198–206. [PubMed: 17137558]

Kana RK, Keller TA, Cherkassky VL, Minshew NJ, M.A.J. Atypical frontal- posterior synchronization of Theory of Mind regions in autism during mental state attribution. Soc Neuro sci. 2009; 4:135–152.

Kennedy DP, Courchesne E. Functional abnormalities of the default network during self- and other-reflection in autism. Soc. Cogn. Affect Neuro sci. 2008a; 3:177–190.

Kennedy DP, Courchesne E. The intrinsic functional organization of the brain is altered in autism. Neuroimage. 2008b; 39:1877–1885. [PubMed: 18083565]

Kennedy DP, Redcay E, Courchesne E. Failing to deactivate: resting functional abnormalities in autism. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:8275–8280. [PubMed: 16702548]

Kim DI, Manoach DS, Mathalon DH, Turner JA, Mannell M, Brown GG, Ford JM, Gollub RL, White T, Wible C, Belger A, Bockholt HJ, Clark VP, Lauriello J, O'Leary D, Mueller BA, Lim KO, Andreasen N, Potkin SG, Calhoun VD. Dysregulation of working memory and default-mode networks in schizophrenia using independent component analysis, an fBIRN and MCIC study. Hum. Brain Mapp. 2009; 30(11):3795–3811. [PubMed: 19434601]

Kleinhans NM, Richards T, Sterling L, Stegbauer KC, Mahurin R, Johnson LC, Greenson J, Dawson G, Aylward E. Abnormal functional connectivity in autism spectrum disorders during face processing. Brain. 2008; 131:1000–1012. [PubMed: 18234695]

Koshino H, Carpenter PA, Minshew NJ, Cherkassky VL, Keller TA, Just MA. Functional connectivity in an fMRI working memory task in high-functioning autism. Neuroimage. 2005; 24:810–821. [PubMed: 15652316]

Koshino H, Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. fMRI investigation of working memory for faces in autism: visual coding and under-connectivity with frontal areas. Cereb Cortex. 2008; 18:289–300. [PubMed: 17517680]

Lee PS, Yerys BE, Della Rosa A, Foss-Feig J, Barnes KA, James JD, Vanmeter J, Vaidya CJ, Gaillard WD, Kenworthy LE. Functional Connectivity of the Inferior Frontal Cortex Changes with Age in Children with Autism Spectrum Disorders: A fcMRI Study of Response Inhibition. Cereb Cortex. 2008; 19:1787–1794. [PubMed: 19068486]

Li YO, Adali T, Calhoun VD. Estimating the number of independent components for functional magnetic resonance imaging data. Hum. Brain Mapp. 2007; 28:1251–1266. [PubMed: 17274023]

Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J. Autism Dev Disord. 1994; 24:659–685. [PubMed: 7814313]

Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, Pickles A, Rutter M. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J. Autism Dev Disord. 2000; 30:205–223. [PubMed: 11055457]

Maia TV, Cooney RE, Peterson BS. The neural bases of obsessive-compulsive disorder in children and adults. Dev Psychopathol. 2008; 20:1251–1283. [PubMed: 18838041]

Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage. 2003; 19:1233–1239. [PubMed: 12880848]

Mason RA, Williams DL, Kana RK, Minshew N, Just MA. Theory of Mind disruption and recruitment of the right hemisphere during narrative comprehension in autism. Neuropsychologia. 2008; 46:269–280. [PubMed: 17869314]

McKeown MJ, Sejnowski TJ. Independent component analysis of fMRI data: examining the assumptions. Hum. Brain Mapp. 1998; 6:368–372. [PubMed: 9788074]

McKiernan KA, Kaufman JN, Kucera-Thompson J, Binder JR. A parametric manipulation of factors affecting task-induced deactivation in functional neuroi-maging. J. Cogn Neurosci. 2003; 15:394–408. [PubMed: 12729491]

Mizuno A, Villalobos ME, Davies MM, Dahl BC, Muller RA. Partially enhanced thalamocortical functional connectivity in autism. Brain Res. 2006; 1104:160–174. [PubMed: 16828063]

Monk CS, Peltier SJ, Wiggins JL, Weng SJ, Carrasco M, Risi S, Lord C. Abnormalities of intrinsic functional connectivity in autism spectrum disorders. Neuroimage. 2009; 47:764–772. [PubMed: 19409498]

Mostofsky SH, Powell SK, Simmonds DJ, Goldberg MC, Caffo B, Pekar JJ. Decreased connectivity and cerebellar activity in autism during motor task performance. Brain. 2009; 132:2413–2425. [PubMed: 19389870]

Pearlson GD, Calhoun VD. Convergent approaches for defining functional imaging endophenotypes in schizophrenia. Front. Hum Neurosci. 2009; 3:37. [PubMed: 19956400]

Piggot J, Kwon H, Mobbs D, Blasey C, Lotspeich L, Menon V, Bookheimer S, Reiss AL. Emotional attribution in high-functioning individuals with autistic spectrum disorder: a functional imaging study. J. Am. Acad. Child Adolesc Psychiatry. 2004; 43:473–480.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc. Natl. Acad. Sci. U. S. A. 2001; 98:676–682. [PubMed: 11209064]

Rojas DC, Peterson E, Winterrowd E, Reite ML, Rogers SJ, Tregellas JR. Regional gray matter volumetric changes in autism associated with social and repetitive behavior symptoms. BMC Psychiatry. 2006; 6:56. [PubMed: 17166273]

Rotge JY, Guehl D, Dilharreguy B, Tignol J, Bioulac B, Allard M, Burbaud P, Aouizerate B. Meta-analysis of brain volume changes in obsessive- compulsive disorder. Biol Psychiatry. 2009; 65:75–83. [PubMed: 18718575]

Rutter, M.; Bailey, A.; Lord, C. Western Psychological Services. Los Angeles, California: 2003. Social Communication Questionnaire–WPS edition.

Solomon M, Ozonoff SJ, Ursu S, Ravizza S, Cummings N, Ly S, Carter CS. The neural substrates of cognitive control deficits in autism spectrum disorders. Neuropsychologia. 2009; 47:2515–2526. [PubMed: 19410583]

Stevens MC, Kiehl KA, Pearlson G, Calhoun VD. Functional neural circuits for mental timekeeping. Hum. Brain Mapp. 2007; 28:394–408. [PubMed: 16944489]

Stevens MC, Pearlson GD, Calhoun VD. Changes in the interaction of resting-state neural networks from adolescence to adulthood. Hum. Brain Mapp. 2009; 30:2356–2366. [PubMed: 19172655]

Talairach, J.; Tournoux, P. Thieme. New York: 1988. A co-planar stereotaxic atlas of a human brain.

Turner KC, Frost L, Linsenbardt D, McIlroy JR, Muller RA. Atypically diffuse functional connectivity between caudate nuclei and cerebral cortex in autism. Behav. Brain Funct. 2006; 2:34. [PubMed: 17042953]

Uddin LQ, Kelly AM, Biswal BB, Xavier Castellanos F, Milham MP. Functional connectivity of default mode network components: correlation, antic-orrelation, and causality. Hum. Brain Mapp. 2009; 30:625–637. [PubMed: 18219617]

van de Ven VG, Formisano E, Prvulovic D, Roeder CH, Linden DE. Functional connectivity as revealed by spatial independent component analysis of fMRI measurements during rest. Hum. Brain Mapp. 2004; 22:165–178. [PubMed: 15195284]

Vincent JL, Patel GH, Fox MD, Snyder AZ, Baker JT, Van Essen DC, Zempel JM, Snyder LH, Corbetta M, Raichle ME. Intrinsic functional architecture in the anaesthetized monkey brain. Nature. 2007; 447:83–86. [PubMed: 17476267]

Wolf I, Dziobek I, Heekeren HR. Neural correlates of social cognition in naturalistic settings: a model-free analysis approach. Neuroimage. 2010; 49:894–904. [PubMed: 19733672]

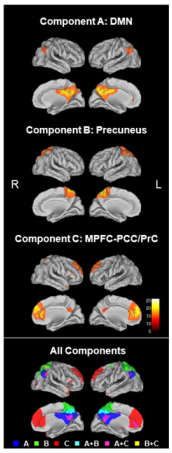


Fig. 1.

The upper panel depicts the 3 ICA components that represent the Default Mode sub-networks. These maps were identified by GIFT (http://icatb.sourceforge.net/, version 1.3e) using resting scans data from all 32 participants (patients and controls) and thresholded at $p < 0.05$ corrected for family-wise errors. The lower panel shows all 3 components concurrently.

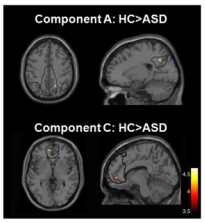


Fig. 2.

Group differences in the Default Mode sub-networks. Only the PrC in Component A and MPFC in component C showed significant group differences, such that patients had decreased strength of connectivity. Each map is masked with the corresponding component mask generated from all participants (black outline, see Fig. 1) and threshold at $p_{FDR} < 0.05$.

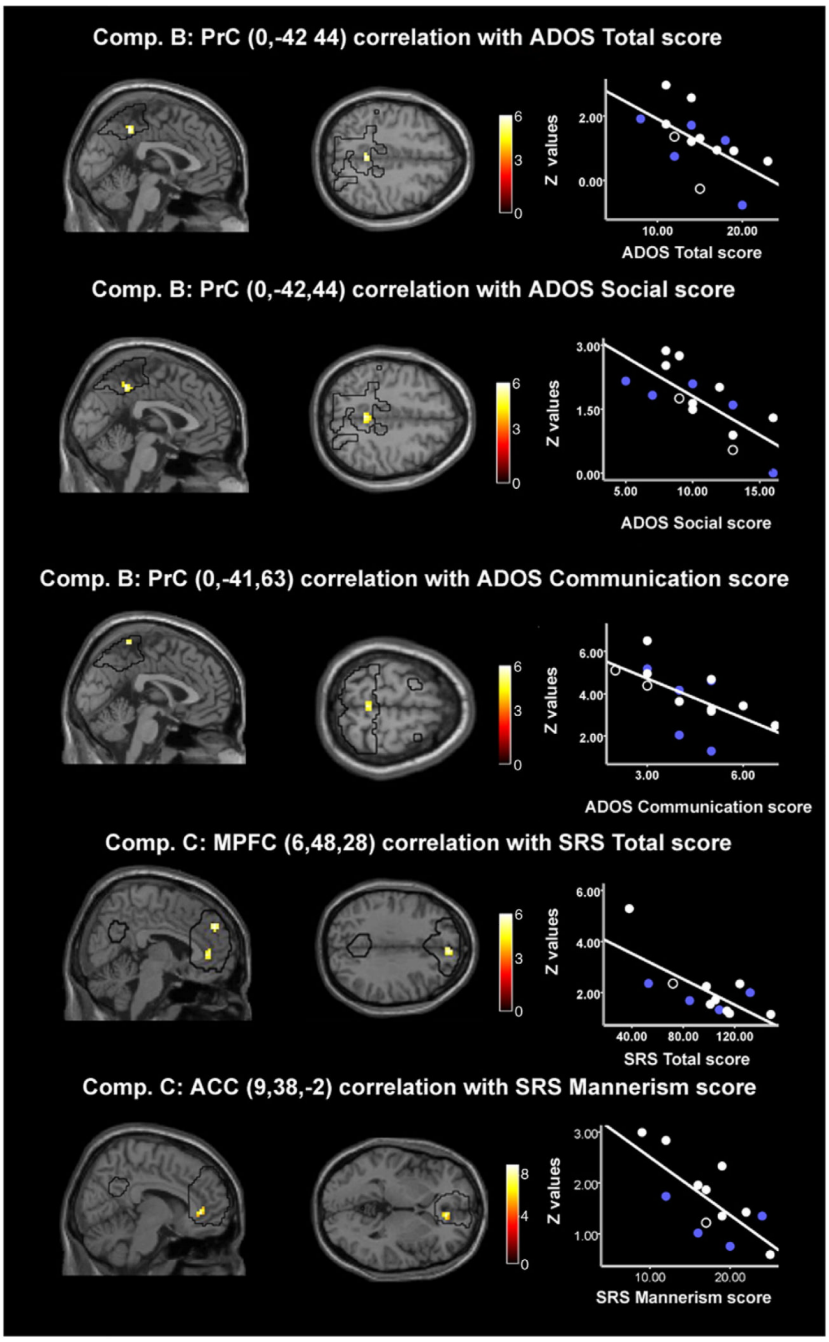


Fig. 3.

Correlations between the functional connectivity strength of the Default Mode sub-networks and ADOS and SRS scores in patients with ASD. Patients receiving treatment at the time of the scan are depicted as filled white circles, patients without drug treatment at the time of the scan are depicted as blue circles, and patients with unknown treatment status are shown as unfilled white circles. Each map is masked with the corresponding component mask generated from all participants (black outline, see Fig. 1) and threshold at $p_{FDR} < 0.05$. Note that the graph for the ACC cluster (shown in the two lower panels) correlation with SRS total scores is not displayed. Also, no significant correlation was found for component (comp.) A. ACC, anterior cingulate cortex; MPFC, medial prefrontal cortex; PrC, precuneus.

Table 1

Demographic and symptoms assessment information. (Data represents average scores ± standard deviation).

	ASD (n=15)	**HC (n=15)**	**Statistics**	***p***
Age (years) (range)	15.7±3.0 (11–20)	17.1 ±3.6 (10–23)	t(28) = –1.1	n.s.
Gender (M/F)	14/1	13/2	$\chi^2(1) = 0.4$	n.s.
Race (W/B/O)	14/0/1	10/3/2	$\chi^2(3) = 4.7$	n.s.
FSIQ (range)	113.3 ±15.0 (79–135)	117.1 ±16.9 (79–139)	t(28) = –0.7	n.s.
ADOS total (n = 15/14)	14.9 ±4.0	0.8 ±1.2	t(27) =12.7	<0.0001
ADOS communication (n = 15/14)	4.3 ±1.3	0.6 ±1.2	t(27) =7.6	<0.0001
ADOS social (n = 15/14)	10.6±3.2	0.1 ±0.4	t(27) =12.2	<0.0001
SRS Total (n = 13)	99.6 ±31.0	–		
SRS Social Awareness (n = 13)	11.5 ± 4.4	–		
SRS Social Cognition (n = 13)	16.8±6.8	–		
SRS Social Communication (n = 13)	31.5 ±11.8	–		
SRS Social Motivation (n = 13)	18.1 ±7.5	–		
SRS Social Mannerism (n=13)	17.0 ± 4.3	–		

Table 2

Brain regions identified in each of the Default Mode sub-networks (DM-SN) components. The table depicts regions' anatomic location, Talairach coordinates for peak activation voxel in each brain region, and *t* scores from random effects analyses across all participants (n=32; $p_{FWE}<0.05$) and for patients and controls (n=15 per group; $p_{FDR}<0.05$) separately.

Anatomic Location	All Participants (Fig. 1)				ASD Patients				Healthy Controls			
	Left Hemi.		Right Hemi.		Left Hemi.		Right Hemi.		Left Hemi.		Right Hemi.	
	TC	*t*	TC	*t*	TC	*t*	TC	*t*	TC	t	TC	*t*
Component A												
PCC (BA 23, 31, 30, 29)	−3,−45, 35	24.0	3, −45, 35	24.7	−6, −48, 25	25.8	6, −33, 29	17.2	−9, −42, 33	23.5	6, −45, 33	26.0
PrC (BA 7, 19, 31)	−3,−68, 34	24.7	9, −57, 30	21.4	−6, −68, 34	16.7	12, −71, 42	17.0	−6, −63, 28	25.7	12, −53, 38	20.2
IPL (BA 7, 39, 40)	−45, −62, 42	12.9	45, −62, 42	11.3	−45, −56, 44	9.9	−42, −62, 34	10.4	−50, −54, 33	10.0	−42, −62, 39	9.5
ACC (BA 24, 32)	−3, 44,−2	8.4	3, 41, 6	6.4	–3, 43, −5	8.7	3, 43, −5	5.0	−9, 35, −2	6.3	6, 44, 9	6.9
Component B												
PrC (BA 7, 19, 31)	−9, −58, 58	23.6	9, −58, 61	18.5	−21, −50, 55	19.0	9, −55, 61	12.7	−6, −58, 58	18.1	3, −52, 61	13.2
Postcentral G.	−12, −52, 63	23.1	12, −52, 66	24.0	−9, −52, 63	16.8	15, −52, 63	16.9	−12, −52, 63	14.1	12, −52, 66	16.0
Paracentral Lobule	−3, −44, 60	15.3	0, −41, 60	17.7	−3, 44, 60	10.0	3, 44, 60	11.3	−3, −41, 57	12.1	0, −38, 52	13.8
MiFG (BA 6)	−24, 3, 58	7.1	30, 6, 60	9.0	−24, 8, 47	5.7	24, 2, 47	5.2	−24, 6, 55	11.5	30, 11, 55	8.3
PCC (BA 31)	−12, −24, 37	8.5	3, −47, 41	7.4	−12, −27, 40	5.7	15, −30, 40	5.0	−15, −25, 34	8.9	6, −47, 41	6.2
Insula	−39,−12, −4	7.0			−39, −9, −5	6.3			−45, −14, 6	5.3		
SOG (BA 19)	−36, −77, 29	6.9			−39, −77, 29	5.0			−36, −83, 29	7.5		
Component C												
MFG/SFG (BA 6,8,9,10, 32)	−3, 57, 25	24.8	3, 51, 20	28.0	−6, 42, 15	35.1	3, 41, 12	25.5	−3, 59, 14	19.0	3, 51, 20	17.1
ACC (BA 24, 32)	−6, 47, 3	19.0	3, 47, −2	21.8	−9, 49, −2	13.2	6, 52, 0	15.4	−6, 49, −2	20.3	6, 47, −2	22.0
PCC/PrC (BA 23, 31)	−3, −51, 33	10.8	3, −51, 33	12.4	−3, −54, 30	7.7	3, −54, 30	8.9	9, −54, 30	8.5	6, −51, 33	9.4
IFG (BA 13, 45, 47)			36, 17, −13	8.5			48, 18, 5	7.7			30, 14, −11	6.8
MOG/SOG (BA 18, 19)			36, −87, 18	8.4			42, −84, 15	7.2			36, −86, 24	13.3
SPL (BA 7)			30, −52, 61	8.1			24, −52, 61	5.6			30, −49, 61	6.7
STG/TP (BA 38)	−45, 16, −26	7.3	45, 16, −29	7.6			53, 2, −23	7.5	−39, 16,−31	6.6	45, 13, −28	6.3
IPL (BA 38, 39, 40)	−56, −63, 28	7.5	53, −51, 33	7.3	−50, −68, 37	6.6	53, −48, 33	5.4	−56, −60, 31	4.7	56, −54, 25	7.7
Paracentral Lb. (BA 31)	−3, −15, 45	7.1	0, −12, 45	7.4	−3, −9, 47	7.2	0, −9, 45	5.2	−3, −24, 45	8.5	0, −21, 45	7.8
Precentral Gyrus	−33, −15, 56	7.0			−30, −18, 56	7.9						
Declive			33, −62, −15	6.9			18, −53,−12	7.7			33, −65, −14	4.7

Anatomic Location	All Participants (Fig. 1)				ASD Patients				Healthy Controls			
	Left Hemi.		Right Hemi.		Left Hemi.		Right Hemi.		Left Hemi.		Right Hemi.	
	TC	*t*	TC	*t*	TC	*t*	TC	*t*	TC	t	TC	*t*
Lentiform Nucleus	−15, 6, −5	6.8	18, 6, −3	6.6	−15, 6, −5	4.9			15, 6,−3	5.7	24, 12, 2	6.0

ACC, anterior cingulate cortex; BA, Brodmann's area; IFG, inferior frontal gyrus; IPL, inferior parietal lobule; MFG, medial frontal gyrus; MiFG, middle frontal gyrus; MOG, middle occipital gyrus; PCC, posterior cingulate cortex; PrC, precuneus; SFG, superior frontal gyrus; SOG, superior occipital gyrus; SPL, superior parietal lobule; STG, superior temporal gyrus; TC, Talairach coordinate; TP, temporal pole.

Neuroscience and Biobehavioral Reviews 47 (2014) 735–752

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Review

Linking neocortical, cognitive, and genetic variability in autism with alterations of brain plasticity: The Trigger-Threshold-Target model

Laurent Mottron[a,b,c,d,*], Sylvie Belleville[d,e], Guy A. Rouleau[f], Olivier Collignon[g]

[a] Centre d'excellence en Troubles envahissants du développement de l'Université de Montréal (CETEDUM), Canada
[b] Hôpital Rivière-des-Prairies, Département de Psychiatrie, Montréal, Canada
[c] Centre de recherche de l'Institut Universitaire de Psychiatrie de l'Université de Montréal, Montréal, Canada
[d] Université de Montréal, Canada
[e] Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal, Canada
[f] Montreal Neurological Institute, Université of McGill, Montréal, Canada
[g] Centre for Mind/Brain Sciences, University of Trento, Italy

ARTICLE INFO

Article history:
Received 30 October 2013
Received in revised form 2 July 2014
Accepted 12 July 2014
Available online 22 August 2014

Keywords:
Autism
Asperger
Perception
Language
Speech
Intellectual disability
Cross-modal plasticity
Synaptic plasticity
Cortical reallocation
Double-hit mechanism
Syndromic autism
Enhanced perceptual functioning
Veridical mapping

ABSTRACT

The phenotype of autism involves heterogeneous adaptive traits (strengths vs. disabilities), different domains of alterations (social vs. non-social), and various associated genetic conditions (syndromic vs. nonsyndromic autism). Three observations suggest that alterations in experience-dependent plasticity are an etiological factor in autism: (1) the main cognitive domains enhanced in autism are controlled by the most plastic cortical brain regions, the multimodal association cortices; (2) autism and sensory deprivation share several features of cortical and functional reorganization; and (3) genetic mutations and/or environmental insults involved in autism all appear to affect developmental synaptic plasticity, and mostly lead to its upregulation. We present the Trigger-Threshold-Target (TTT) model of autism to organize these findings. In this model, genetic mutations trigger brain reorganization in individuals with a low plasticity threshold, mostly within regions sensitive to cortical reallocations. These changes account for the cognitive enhancements and reduced social expertise associated with autism. Enhanced but normal plasticity may underlie non-syndromic autism, whereas syndromic autism may occur when a triggering mutation or event produces an altered plastic reaction, also resulting in intellectual disability and dysmorphism in addition to autism. Differences in the target of brain reorganization (perceptual vs. language regions) account for the main autistic subgroups. In light of this model, future research should investigate how individual and sex-related differences in synaptic/regional brain plasticity influence the occurrence of autism.

Contents

* Corresponding author at: Programme Autisme, hôpital Rivière-des-Prairies, 7070 Bvd Perras, Montréal (Qc), CANADA, H1E1A4. Tel.: +1 514 323260x2173.
E-mail address: laurent.mottron@gmail.com (L. Mottron).

http://dx.doi.org/10.1016/j.neubiorev.2014.07.012

1. Introduction: Genetically determined high plasticity is associated with both the strengths of autistic people and variability in the autistic phenotype

Phenotypic, cognitive and genetic heterogeneity of autism has complicated the understanding of its causes, but may also have a heuristic value. Autistic people display cognitive strengths which may result from differences in the plasticity of brain functions at particular regions (Mottron et al., 2013). Brain imaging studies of autism have revealed a large-scale reorganization of the autistic brain which may reflect enhanced cortical plasticity. In parallel, genetic studies of autism have recently identified many de novo mutations (Gillis and Rouleau, 2011; Ronemus et al., 2014). Most of these mutations are implicated in synaptic plasticity (Kelleher et al., 2008; Baudouin et al., 2012), which is defined as the process of microstructural construction of synapses occurring during development and the remodeling of these synapses during learning. These findings suggest that enhanced synaptic plasticity triggers a regional reorganization of brain functions that account for both the unique aspects of autism and its variability. In this paper we will review the following: (1) the main sources of inter-individual variability of the autistic phenotype, with an emphasis on the domain-general strengths of autistic people; (2) how cortical reorganization, similar to that following sensory impairment, occurs within the most "plastic" regions of the human brain in autistic people; and (3) how mutations implicated in autism alter synaptic plasticity. In addition, we propose the Trigger-Threshold-Target model which describes how genetically triggered brain reorganization may account for autism and its cognitive, phenotypic and neurogenetic variability.

2. Main sources of inter-individual variability in the autistic phenotype

2.1. Phenotypic variability

Phenotypic variability is a key feature of autism (APA, 2013). Not all signs of autism are found in every autistic person, which means that diagnosis is based on polythetic criteria as opposed to a defined set of clinical features. For instance, speech abilities in autistic people can range from none to outstanding, and intelligence from intellectual disability to genius. Various approaches have been devised to categorize this variability. The DSM-IV (APA, 2013) proposed subtyping to account for variability in language development, and differentiated autism from Asperger syndrome. The DSM-5 proposed the use of clinical specifiers (language, intelligence, neurogenetic context, severity) to describe differences in cognitive, comorbid or adaptive characteristics within the autistic spectrum. The two approaches differ in that the categorical, subtyping approach clusters different values for each source of heterogeneity, whereas the dimensional, specifier approach allows an indefinite number of possible values and combinations (Szatmari, 2011) of heterogeneity. This symptomatic variability has been seen as an obstacle to the recognition and understanding of autism. The model we propose here suggests that the phenotypic, cognitive, but also genetic heterogeneity (Girirajan and Eichler, 2010) of autism is a fundamental feature that reflects its mechanistic causes.

2.2. Cognitive variability

2.2.1. Intelligence

One source of heterogeneity in the autistic phenotype is intelligence level. The prevalence of intellectual disability, epilepsy, microcephaly, and the female to male ratio is markedly higher in syndromic autism than in nonsyndromic autism (Amiet et al., 2008). However, there are also large differences in intelligence level amongst people with nonsyndromic autism. The reported incidence of intellectual disability in nonsyndromic autism has been in constant decline in the last few decades (Prevention CfDCa, 2013), from more than two-thirds of cases to less than one-third. Part of this previously reported intellectual disability resulted from the fragmented use of language by autistic patients, which impaired their capacity to perform well in standard intelligence tests. As a result, estimates of intellectual disability were strikingly lower when nonverbal tests were used (Dawson et al., 2007). The measurement of intelligence also reveals large intra-individual variability of performance depending on the task, with some tasks performed higher than expected according to the individual's predicted IQ score.

2.2.2. Perception

Perceptual skills are the most documented cognitive strength in autistic people (Mottron et al., 2013). High perception of the surrounding environment appears to be present early in

development, with long visual fixations (Zwaigenbaum et al., 2005), early detection of audio-visual synchrony (Klin et al., 2009), interest in geometric shapes (Pierce et al., 2011) and periodic motion (Mottron et al., 2007), and superior visuo-spatial search (Kaldy et al., 2011). Perceptual strength, defined as a score superior to 1SD from the baseline IQ, is present in 30 (Howlin et al., 2009) to 50% (Caron et al., 2006) of autistic people, and is thereby a source of intra-individual heterogeneity. Perceptual strength cannot be easily classified according to perceptual levels or modalities. For instance, auditory processing is frequently altered in autism (O'Connor, 2012) and includes an enhanced ability to discriminate low level perceptual features such as pitch and loudness. Adept visual processing often involves mid-level perception, for instance the ability to detect patterns in embedded figures or visual search tasks (Mottron et al., 2012a) and more frequently involves extrastriate regions than primary regions (Schwarzkopf et al., 2014). Autistic people also have a high ability both to perceive details in compound visual stimuli (Wang et al., 2007), and to manipulate large-scale three dimensional figures (Soulieres et al., 2011). In addition, perceptual strengths in autism involve not only low or mid-level operations but also visuo-spatial reasoning (Stevenson and Gernsbacher, 2013).

2.2.3. Language

Language and speech function is another domain in which there is a striking range of strengths and deficits, both within and between individuals (Williams et al., 2008). Most autistic persons suffer from major speech onset delay (SOD), specific language impairment and/or particular deficits (deictic terms, pragmatics) early in development; however, some autistic people exhibit normal early language development. According to the DSM-IV criteria, early language impairment is associated with autism whereas individuals with Asperger Syndrome develop normal language skills. The DSM-5 no longer supports this distinction, partly due to its lack of reliability, and because features of the DSM-IV criteria of Asperger syndrome are inapplicable. Nonetheless, cognitive (Sahyoun et al., 2009; Bonnel et al., 2010; Jones et al., 2009a; Barbeau et al., 2013a) and brain imaging data (Yu et al., 2011; Sahyoun et al., 2010) of autistic spectrum (AS) people with (AS-SOD) or without (AS-NoSOD) speech onset delay and/or atypicalities, suggest that this distinction has some value.

Furthermore, there are major intra-individual variations in performance across different language functions, mostly in AS-SOD (Boucher, 2012). Despite speech onset delay, some language components are unimpaired, or even enhanced. An example of this is decoding, which is the ability to produce sounds corresponding to a graphic representation of speech (Jones et al., 2009b). This capacity has been associated with hyperlexia (Grigorenko et al., 2003), which is a precocious, transient nonlinguistic use of language. Most autistic strengths are related to pattern perception, reproduction and manipulation, for instance exceptional 3-D drawing or musical memory; however, others are not directly perceptual and are instead related to language (Klin et al., 2009) (e.g., calendar calculation, factorization, prime number detection, memory for proper names). Autistic people who possess proficient skills related to perception may show early speech alterations up to and including the absence of spontaneous speech, whereas hyperlexia or some hypermnesia imply the hyper-functioning of a component of language function (Mottron et al., 2013).

2.3. Differences in strengths according to Autism spectrum subgroups

Although autism spectrum appears as a heterogeneous condition with different patterns of enhanced or impaired perceptual and cognitive skills, patients can be divided into two main AS subgroups according to these strengths and deficits. Perceptual enhancement is largely associated with delay, deficits or abnormalities in speech (echolalia, pronoun reversal) (Caron et al., 2006). AS-SOD is characterized by strengths in reasoning (as measured with Raven matrices) and visuo-construction, combined with deficits in some, but not all, aspects of language (Dawson et al., 2007). Preserved language capacities are those that appear to involve the perceptual processing of language, for instance reading or reproducing a phonological sequence (Mottron et al., 2013). Similarly, in IQ tests, AS-SOD people perform well in visuo-spatial tasks such as the Block Design subtest, but poorly in the verbal Comprehension subtest (Stevenson and Gernsbacher, 2013). Perceptual capacity distinguishes AS-SOD from AS-NoSOD: the performance in visual inspection time tasks can correctly classify adults as AS-SOD or AS-NoSOD, because only adults with AS-SOD perform better than the comparison group (Barbeau et al., 2013a). Regarding auditory processing, AS-SOD but not AS-NoSOD is associated with an enhanced perception of low-level auditory dimensions of language such as pitch (Heaton et al., 2008a; Jarvinen-Pasley and Heaton, 2007; Eigsti and Fein, 2013).

Overall enhancement of language function, including speech, is found only in people with AS-NoSOD. These individuals develop language skills quickly with the use of polysyllabic words, exceptional mastering of syntax, and a special ability of abstract verbal reasoning as measured with the similarities subtest of the Wechsler scale. The overuse of language by AS-NoSOD people is also illustrated by occasional extreme verbosity (Adams et al., 2002), and by the "categorical", verbally defined nature of their restricted interests (Mottron et al., 2012b). These individuals do not display the visuo-spatial strengths that characterize patients with AS-SOD; they perform well in the Vocabulary and Similarities subtests of the WAIS (Nader et al., 2014) and perform poorly in the Comprehension and Coding subtests. In addition, motor clumsiness is a clinical sign associated with AS-NoSOD (Klin et al., 1995) but is rarely found in AS-SOD (Meilleur et al., 2014; Barbeau et al., 2013b). Thus, AS-NoSOD cannot simply be considered as "AS with language preserved", because these individuals display a specific pattern of enhancements in language combined with motor deficits. In contrast, perception in AS-SOD people is not only preserved, but is enhanced and is associated with speech and social alterations. This is coherent with a distinction between two AS subgroups based on their strengths, perception vs. language function, as well as their deficits, speech vs. motor function.

2.4. Variability of imaging findings in autism spectrum subgroups

In addition to differences in cognitive function, AS-SOD and AS-NoSOD markedly differ in brain reorganization. Several functional and structural Activation Likelihood Estimate (ALE) meta-analyses or systematic reviews on brain imaging in autism are now available. The analysis of functional neuroimaging data has revealed perturbations of task-related brain activity for both social and non-social tasks, and despite considerable methodological heterogeneity, main group differences can be extracted from these studies. In social tasks, these differences include greater activity in the post-central and superior temporal gyri in ASD individuals than in controls, whereas the opposite has been reported for the anterior and posterior cingulate cortex, the anterior insula and the amygdala (Di Martino et al., 2009). Also, differences between autistic individuals and nonautistic controls during task-related activity are often found in the fusiform area, with both hypo- and hyperactivation observed in the different fusiform sub-regions in autism (Di Martino et al., 2009; Samson et al., 2011a). In addition, children and adolescents within the ASD population show high activity in the post-central gyrus, with adults displaying greater superior temporal and hippocampal activity than children (Dickstein et al.,

2013). In non-social tasks, studies have consistently found that autistic individuals have greater activity in the precentral gyrus, in the fusiform gyrus and in the middle frontal cortex than nonautistic controls. In contrast, activity in the superior temporal gyrus, prefrontal cortex and cingulate cortex is frequently found to be higher in non-autistic than in autistic individuals (Di Martino et al., 2009; Samson et al., 2011a). Within ASD, activity in the insula and cingulate is stronger in children and adolescents than in adults, where as activity in the middle frontal cortex is greater in adults (Dickstein et al., 2013).

Ectopic activity in response to social and non-social information in autism is indicative of cortical reallocation. We conducted an ALE meta-analysis of 26 functional neuroimaging studies in which visual information was presented to a total of 370 controls and 357 ASD individuals. Despite similar performance levels for both groups, the activity of parietal and occipito-temporal regions associated with visual perception and expertise was higher in autistic individuals (mostly AS-SOD) than in non-autistic individuals (Samson et al., 2011a). Regarding connectivity, functional fMRI (Monk et al., 2009), EEG (Barttfeld et al., 2011; Murias et al., 2007), MEG (Kikuchi et al., 2013), and histological studies (Casanova et al., 2006; Hutsler and Zhang, 2010) have revealed limited long range connectivity between frontal and visual regions, as well as enhanced local connectivity within local cortical networks in autism. In particular, functional hyper connectivity (Khan et al., 2013) has been reported between the temporal and parietal lobe (Kikuchi et al., 2013), within the medial temporal lobe (Welchew et al., 2005), within the visual cortex (Turner et al., 2006; Noonan et al., 2009; Rudie et al., 2012; Keown et al., 2013), between the visual and frontal cortex (Leveille et al., 2010; Domínguez et al., 2013) and within the posterior cingulate cortex (Monk et al., 2009) in autism. This is indicative of highly autonomous functioning of the autistic visual cortex (see Fig. 1E).

AS-SOD individuals show reorganization of brain function during language tasks, including hyper-activation in the fusiform gyrus (Samson et al., 2011a) and atypical, strong (Leveille et al., 2010; Domínguez et al., 2013) functional connectivity between associative perceptual areas and other parts of the brain (Peters et al., 2013). This reorganization may explain why AS-SOD individuals use perception for typically nonperceptual, verbal tasks (Monk et al., 2009). However, in tasks involving the processing of non-social auditory information, AS-NoSOD individuals show greater activity in peri-auditory and language-related brain regions than AS-SOD individuals and non-autistic controls (Samson et al., 2009). This suggests that high activity and cortical reallocation in perceptual associative regions in AS-SOD individuals have an equivalent in AS-NoSOD individuals in the form of widespread allocation of auditory brain regions for language processing.

Alterations in gray and white matter have been reported in autistic persons, although there are some inconsistencies amongst studies regarding the location and direction of regional brain volume changes (Stanfield et al., 2008). Meta-analyses have established that overall brain growth is faster in the early years of life in autistic individuals than in age-matched controls (Stigler et al., 2011). Six clusters of alterations to brain structure were revealed by a recent structural meta-analysis. These structural alterations occur in the following regions (from the most to the least significantly affected): the lateral occipital lobe, the pericentral region, the medial temporal lobe, the basal ganglia, and the area proximate to the right parietal operculum. These regions contribute to the uni- and multi-modal perception of both social and non-social information (Nickl-Jockschat et al., 2012); no region is uniquely involved in the processing of social or emotional information. The analysis of combined alterations of gray and white matter (Cauda et al., 2014) has provided an additional source of information. Although autism is associated with hypertrophy of gray and white matter in the occipital regions, gray and white matter volume in the frontal and dorsal parietal brain cortices are smaller than in nonautistic controls. The most densely connected clusters of regions with volumetric variations in both directions are the left fusiform gyrus, the middle temporal gyrus, and the inferior occipital gyrus (Fig. 1C and D). A meta-analysis comparing AS-SOD and AS-NoSOD (Yu et al., 2011) has revealed structural differences between the two AS subgroups. AS-NoSOD have a reduced gray matter volume in the left occipital gyrus and enhanced volume in left fusiform than controls. AS-SOD individuals show clear structural alterations, including a lower gray matter volume than controls in the middle temporal gyrus and a larger gray matter volume in the left ventral temporal lobe.

Longitudinal studies and cross-sectional comparison of autistic children and adults indicate that the differences in overall brain volume between autistic and nonautistic individuals normalize at an adult age; however, local volumetric differences are maintained. Brain growth trajectories in autistic individuals depend on the region involved, and the growth of structures commonly affected in autism shows atypical synchronization in comparison with the rest of the cortex (Nickl-Jockschat et al., 2012). Interestingly, a longitudinal structural MRI study of ASD revealed that thickening of occipital gray matter is not present in children (Schumann et al., 2010), whereas in adults with ASD the volume of occipital regions is higher than in controls.

Overall, these studies show that: (a) brain alterations occur in regions implicated in high and low level processing of both social and non-social information and are not limited to regions implicated in “superior” or “social” functions; (b) increases of brain volume and activity consistently involve associative perceptual regions, specifically areas devoted to perceptual expertise (e.g., fusiform gyrus and lateral occipital complex) in AS-SOD and involve language regions in AS-NoSOD; (c) there is a developmental shift with overall excess in brain volume in early years being replaced by a complex pattern of regional volumetric alterations in adulthood; and (d) large cortical volume of the perceptual area appears later in life. This pattern of alterations is consistent with genetic variation, which is responsible for altered, lifelong interaction with the environment, and affects cognitive, functional, and structural properties of either the perceptual associative regions or language regions according to the subgroup of the autism spectrum.

2.5. Syndromic and non-syndromic autism

A last, major source of heterogeneity in autism is neurogenetic and differentiates nonsyndromic autism from syndromic autism. Individuals with nonsyndromic autism have no recognizable syndrome associated with autism. Persons with this condition do not present morphological characteristics such as facial dimorphisms or neural tumors. In contrast, persons with syndromic autism present phenotypic manifestations of an additional syndrome including facial dimorphisms or alterations in brain structure. Fragile X, an inherited condition associated with atypical facial morphology and intellectual disability, is an example of syndromic autism when associated with an autistic-like phenotype. Similarly, tuberous sclerosis is a neurodevelopmental genetic condition characterized by brain tumors and epilepsy, and a high prevalence of autism. Both of these conditions, when associated with autism, meet the criteria for syndromic autism, despite the fact that they occur more frequently alone than with autism. Although useful from a clinical point of view, the distinction between nonsyndromic and syndromic autism has been blurred by the discovery of morphological variations (e.g., macrocephaly curves that peak in the first year (Redcay and Courchesne, 2005) in autistic people otherwise devoid of any recognizable neurological syndrome. Moreover, causative genetic alterations are found in nonsyndromic as well

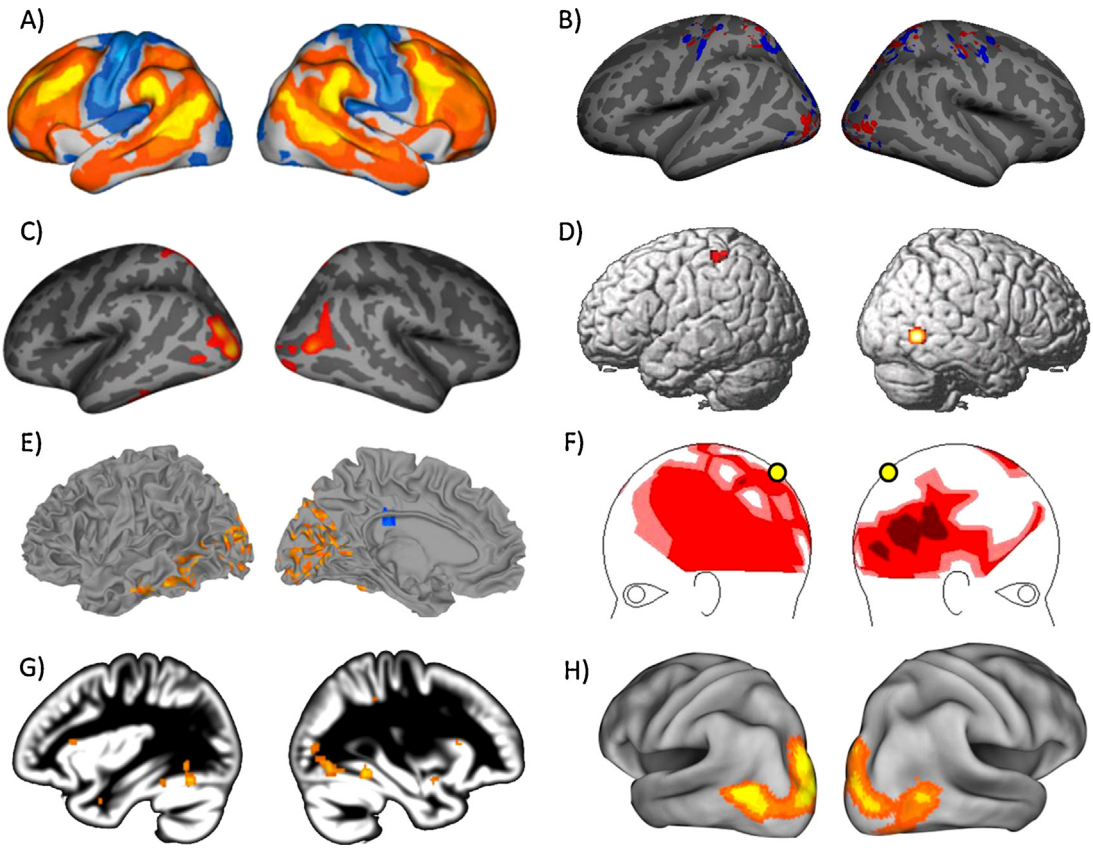


Fig. 1. Overlap between regions of: enhanced variability in non-autistic individuals (A), superior variability in autistic people (B), major gyrification (C), volumetric (D), connectivity (E, F) and functional (G) alteration in autistic people, and regions of cross modal plasticity in non-autistic, sensory-impaired people (H). (A) High inter-individual variability in resting-state functional connectivity in non-autistic individuals. Values above or below the global mean are displayed in warm and cool colors, respectively (Mueller et al., 2013); (B) The localization of the strongest peak of activity in autistic individuals (in blue) shows higher variability than in typical individuals (in red) (Poulin-Lord et al., 2014); (C) Regions showing greater cortical gyrification in autistic individuals than in non-autistic individuals. The warmer the color, the greater the significance of the group differences (Wallace et al., 2013); (D) Clusters of brain structure alterations (differences in gray matter or white matter) between autistic and non-autistic individuals (qualitative meta-analysis, whole brain FDR corrected) (Nickl-Jockschat et al., 2011); (E) Regions of enhanced resting-state local connectivity density in autistic individuals. Warm colors show the regions with greater connectivity in the autistic individuals than in non-autistic individuals, and cool colors regions of lower connectivity (Keown et al., 2013); (F) Regions where high connectivity with the right parietal region (yellow circle, MEG coherence analysis) is associated with high reading ability. The darker the color, the stronger the correlation (Kikuchi et al., 2013); (G) Regions showing more activity in autistic individuals than in non-autistic controls when processing visual information. Qualitative meta-analysis, whole brain FDR corrected (Samson et al., 2011a); (H) Regions of differences in activity between congenitally blind and sighted individuals when processing auditory information (whole brain FWE corrected; adapted from Collignon et al., 2011).

in syndromic autism. Thus, the definition of a "recognizable syndrome" (and hence the classification of syndromic autism) may be dependent on the progress of genetic knowledge.

3. Comparison between autism and cross-modal plasticity

Cognitive and imaging experiments have revealed that perceptual strengths and cortical reorganization develop during cross-modal plasticity following sensory deprivation. We will now discuss this condition in relation to autism.

3.1. Perceptual strengths in autism and cross modal plasticity

Like autistic individuals, people experiencing early and complete sensory loss (e.g., individuals born deaf or blind) have perceptual enhancements. Such enhancements involve the remaining senses (e.g., audition and smell in blind people) and are believed to be linked to the recruitment (Bavelier and Neville, 2002) of the brain areas deprived of their natural inputs to the remaining senses. This reorganization is termed cross-modal plasticity. Cross-modal plasticity in deaf or blind individuals is often considered as adaptive because it results in a more efficient interaction with the external environment. The magnitude of cross-modal recruitment may correlate with the level of performance enhancement reported in the blind (Amedi et al., 2003; Gougoux et al., 2005; Kupers et al., 2007), and with the contribution of the temporal cortex in mediating non-auditory processing in deaf adults (Bolognini et al., 2012). Cross-modal plasticity mechanisms are guided by the original computation style (Cardin et al., 2013) and/or connections (Collignon et al., 2011). Therefore, cross-modal plasticity is a particular case in which cortical recycling (Dehaene and Cohen, 2007) occurs within a sensory deprived region, with potentially beneficial effects for behaviors (Collignon et al., 2009a).

The perceptual enhancements observed in autism and sensory-deprived individuals exhibit striking similarities. For example, both blind and autistic individuals show superior pitch discrimination (Bonnel et al., 2010, 2003; Wan et al., 2010; Gougoux et al., 2004), spatial localization (Collignon et al., 2009a, 2006; Caron et al., 2004; Doucet et al., 2005), selective attention (Collignon et al., 2006, 2009b; O'Riordan et al., 2001; Plaisted et al., 1998; Collignon and De Volder, 2009; Kujala et al., 2000), tactile discrimination (Wong et al., 2011), verbal memory (Amedi et al., 2003; Mottron et al., 1996; Raz et al., 2007; Nakano et al., 2012), and an ability to quickly detect and discriminate auditory information (Hyde et al., 2011; Hertrich et al., 2013). Similarities also involve complex perceptual strategies: for instance, peripheral vision is enhanced in both autism (Mottron

et al., 2007) and early deafness (Voss et al., 2004; Chen et al., 2010; Frey et al., 2013). Parallel search, which is a widely accepted explanation for the enhanced visual search performance consistently found in autism, also occurs in deaf (Stivalet et al., 1998; Remington et al., 2012) individuals: both populations are less impaired by increasing number of visual distractors than typical individuals. People with intellectual disability plus deafness perform better in visual sorting tasks involving detailed visual information than intellectually disabled people, and autistic individuals also perform well in such tasks (Maljaars et al., 2011). Behavioral compensations in deaf individuals mostly involve inputs originating in the peripheral visual field (Neville and Lawson, 1987; Bavelier et al., 2000).

Modifications of visual strategies such as parallel search and enhanced peripheral vision that occur in deaf children strongly resemble the modification of visual attention that is beneficial to visual search in autism. These results reflect findings with congenitally blind individuals (or who became blind at a young age). Such individuals demonstrate supra-normal performances in tasks involving the localization of peripheral auditory sources, in which subtle auditory cues (i.e., spectral) have to be exploited to resolve the task efficiently (Voss et al., 2004; Röder et al., 1999; Fieger et al., 2006). Compensations involving peripheral stimuli occur preferentially in conditions where the task is difficult and where there is room for perceptual enhancement. It highlights the extreme precision of sight in deaf individuals and hearing in blind individuals (Voss et al., 2004; Röder et al., 1999; Lessard et al., 1998). Another example of similarities between AS and cross-modal plasticity is motion perception; despite some conflicting evidence, recent studies show that some aspects of visual motion perception may be enhanced in autism (Foss-Feig et al., 2013), as is the case for vision in congenitally deaf people (Hauthal et al., 2013) and for the audition of moving sounds in congenitally blind people (Lewald, 2013).

In summary, the particular aspects of perception which are enhanced following sensory deprivation (Cohen et al., 1997; Amedi et al., 2004; Collignon et al., 2007) are also commonly enhanced in individuals with autism. Adaptive value may decide what functions are enhanced in blind and deaf people. For instance, enhanced peripheral sound or visual localization in blind and deaf individuals, respectively, enables them to rapidly detect sources of danger. A similar adaptive benefit of enhanced perceptual functions is not so obvious in autism. However, the striking similarities between domains of enhanced perception in AS and sensory deprived people suggest that common mechanisms underlie the development of these supra-normal skills. Therefore, cross-modal plasticity may be a useful "model" for autistic enhanced perceptual functioning and may provide a mechanistic explanation for some characteristics of autism. The similarity between the two groups may result from neurobiological constraints, in particular from the superior plastic potential of particular perceptual functions.

3.2. Cortical reorganization in autism and cross modal plasticity

The comparison of brain imaging results in autism and in sensory-deprived individuals provides information about the cortical reorganization that underlies cognitive changes in these two conditions. Brain areas typically devoted to the de-afferented sensory modality are diverted from their original function by another intact sensory modality in blind or deaf individuals. However, not all regions associated with the impaired modality are affected by plastic reallocation and activated by sensory input of the intact modality. The associative/multimodal perceptual areas are among the regions the most commonly affected by these reallocations, in autism, blindness (Fig. 1G) and deafness.

An ALE meta-analysis of studies of autistic individuals indicated an atypical, strong activation of associative visual areas during multiple perceptual, language, semantic and problem solving tasks (Samson et al., 2011a; Cardin et al., 2013; Koshino et al., 2008; Soulières et al., 2009; Iuculano et al., 2014). This enhanced task-related activity in associative visual regions in autism is domain-specific. When studies included in the ALE meta-analysis were stratified according to broad categories of material presented (faces, objects, letters), differences between the groups of participants varied; nonetheless, regions of enhanced activity were notably seen in the fusiform gyrus, which is a region typically dedicated to experience-dependent expertise.

Cortical reorganization in autism may be associated with a gain of function, at least for situations where an additive processing of information by visual cortex is an advantage. A study involving fMRI showed that the activity of extrastriate areas (BA18) is higher in autistic than in non-autistic individuals during a task of non-verbal reasoning. However, the activity in the lateral prefrontal cortex (BA9) and the medial posterior parietal cortex (BA7) was lower in autistic individuals and they performed the task 40% faster than non-autistic individuals. The left middle occipital gyrus and the medial precuneus were significantly more activated in autistic individuals only during the most difficult items, consistent with the implication of the visual cortex in the autism-associated strength of non-verbal reasoning. In this case, cortical reorganization involves the enhanced contribution of a region important for a particular type of task (Soulières et al., 2009).

In individuals who become deaf or blind at an early age, cross-modal cortical reorganization are widespread in the sensory deprived regions but mainly involves the recruitment of de-afferented associative areas during the stimulation of the intact modality. Recent studies on cross-modal plasticity demonstrated that the cortical reallocation of a specific brain region may maintain the cognitive role of this region, despite the change in sensory input (Collignon et al., 2009a). For instance, in blind people, auditory spatial processing relies on parts of the occipital cortex typically involved in visual spatial processing (Collignon et al., 2011, 2007; Dormal and Collignon, 2011). Thus, blind persons use the same region as sighted persons for spatial processing, but blind people use it to derive space based on auditory cues, whereas sighted people use it to derive space based on visual ones. Similarly, inner connectivity within the "Visual Word Form Area", which is inactivated in illiterate, nonautistic people, is reinforced during reading in adults leading to its activation during the acquisition of literacy (Thiebaut de Schotten et al., 2014). This region is functionally recruited in congenitally blind people during Braille reading (Reich et al., 2011). Similar reorganization patterns are associated with higher level functions. For instance, the visual cortex is strongly involved in higher order speech operations in congenitally blind people (Amedi et al., 2003; Bedny et al., 2011). Overall, the cross-modal recruitment of the occipital region in the congenitally blind follows a division of computational labor (e.g., the "what" and "where" distinction) comparable to that observed in the sighted (Dormal and Collignon, 2011; Collignon et al., 2012).

Enhanced activation of auditory perceptual regions has also been reported in autism, although few studies have investigated this modality. Higher levels of spectral/temporal complexity in speech-like stimuli are associated with greater activity in the primary auditory cortex in AS-SOD individuals than in nonautistic individuals. In contrast, AS-SOD individuals showed low levels of activity related to temporal complexity in non-primary auditory regions within the superior temporal gyrus, which is a region linked with processing temporally complex sounds (Samson et al., 2011b).

Weak hemispheric asymmetry for functions that are typically lateralized is another indicator of functional reorganization in both autistic and sensory deprived persons. In autism, weak hemispheric

asymmetry was reported for language (Lo et al., 2011; Eyler et al., 2012), face perception (Dundas et al., 2012; Scherf et al., 2010) and perceptual response in general(Dinstein et al., 2012). A similar pattern is found in sensory-deprived individuals, since some aspects of language processing are less lateralized in deaf (Emmorey et al., 2010) and blind (Hugdahl et al., 2004) individuals than in perceptually unimpaired individuals. Face (Vargha-Khadem, 1983; McCullough et al., 2005) and motion (Bavelier et al., 2001) processing are more reliant on left brain regions in deaf people, whereas these functions are preferentially implemented by right-sided brain regions in hearing individuals.

Perception of visual and auditory motion is a crucial perceptual skill for interacting with the environment. This ability relies on a set of highly specialized brain regions (Watson et al., 1993; Warren et al., 2002). Sensitivity to visual motion highly depends on early visual experience (Ellemberg et al., 2002), and is therefore a perceptual skill prone to reorganization. In deaf, visual motion relies on reorganized networks in temporal regions and in blind people the same is true for auditory motion in occipital regions (Neville and Lawson, 1987; Bosworth and Dobkins, 2002; Bedny et al., 2010; Noser and Byrne, 2007). Motion perception is also processed by modified neural networks in autism (McKay et al., 2012).

Regarding connectivity, sensory-deprived persons display alterations overlapping with those observed in autistic people. Blind people display an increase of intra-occipital and a decrease in long range resting state connectivity (Liu et al., 2011, 2007). Furthermore, task-dependent enhanced connectivity has been reported between the primary auditory and primary visual cortex (Klinge et al., 2010a; Leclerc et al., 2005; Collignon et al., 2013) in the blind, and between the parietal cortex and early visual areas in the deaf (Bavelier et al., 2001). Interestingly, absolute pitch and synesthesia, the prevalence of which is high in autism (Mottron et al., 2013), are both associated with enhanced local connectivity in the non-autistic population (Zamm et al., 2013; Loui et al., 2011), and can follow blindness (Steven and Blakemore, 2004; Pring et al., 2008) and brain damage (Bolognini et al., 2013).

In terms of the cellular mechanisms involved, both conditions are thought to be associated with disrupted pruning mechanisms. Disruption of pruning may explain high neural cell density in autism (Courchesne et al., 2011). Similarly, connections normally pruned away during development may be maintained following sensory loss due to lack of competitive perceptual input from the impaired sensory modality (Yaka et al., 1999; Berman, 1991). Studies of ocular dominance plasticity, a commonly used model to study synaptic and cortical reorganization following experience, in the fragile X mouse model revealed hyperplasticity involving an exaggerated response to visual deprivation (Dölen et al., 2007). Fragile X is a neurodevelopmental condition distinct but strongly associated with autism, for which the role of genetically-triggered enhanced plasticity is well established (see Section 4.1).

In summary, cortical reorganization in autism and in sensory-deprived individuals shares several characteristics. These include the identity of the reorganized areas, regional re-wiring of the regions affected by functional reallocation, alteration of lateralization, the reassignment of perceptual functions, and the gain of perceptual functions. Another important similarity is that the plastic modifications found in autism and sensory-deprived individuals mostly occur in specific brain regions, as will be described below.

3.3. *Regions of cortical reorganization in autism and cross-modal plasticity coincide with regions of maximal variability in humans*

Several reports suggest that the regions that are the most susceptible to reorganization in autism (the multimodal association regions) are also those that have the largest variability in terms of connectivity among non-autistic individuals (Mueller et al., 2013; Aichhorn et al., 2006) (Fig. 1A). The highest inter-individual differences in resting-state connectivity are in the multimodal association cortex and the lowest are in the unimodal sensory and motor cortices. Mueller et al. (2013) reported that regions of enhanced variability developed late during evolution, because they are the most divergent regions between monkeys and humans. They also demonstrated that cortical folding shows the highest degree of variability in these regions, and the slowest maturation. Thus, these regions appear to be highly susceptible to changes affecting ongoing learning and plasticity, and are therefore most likely subject to functional reallocation due to atypical experience (Barnes and Finnerty, 2010). These regions of maximum variability also show the largest functional activation differences between autistic individuals and non-autistic controls during the processing of visual information (Samson et al., 2011a) (Fig. 1F). In particular, the lateral occipital cortex (LOC) is a region that shows greater cortical gyrification (Wallace et al., 2013) and volumetry (Nickl-Jockschat et al., 2012) in the autistic individuals than in non-autistic individuals. This region is selectivity implicated in processing visual objects in sighted individuals (Martin, 2007) and is involved in cross-modal recruitment for auditory or tactile object processing in the congenitally blind (Amedi et al., 2007; Sathian, 2005).

For these reasons, high plasticity in autism should also be characterized by large inter-individual variability between autistic individuals in regions affected by plastic functional reallocation, with each reallocation in each individual being dependent on various environmental constraints. We scanned 23 overtly verbal autistic individuals and 22 non-autistic participants during a visuo-motor imitation task to test directly the hypothesis of high intra group variability in associative regions in autism (Poulin-Lord et al., 2014). We extracted the coordinates of the strongest activation peak in the primary and supplementary motor cortex, the visuo-motor superior parietal cortex, and the primary and associative visual areas. We then assessed the distance of each participant from their respective average group peak of activation to assess group differences in variability. The mean variability in the localization of activations in the associative visual or motor areas was higher than in the primary visual or motor areas for both groups. Importantly, we observed a greater variability in the left visuo-motor superior parietal cortex and in the left associative visual areas in the autistic group than in the control group (Fig. 1B). This indicates that the regions where autistic individuals display the maximum enhanced activity when exposed to visual information, and the regions where non-autistic individuals display the highest level of inter-subject variability are all included in the visual associative complex. Other autism studies have reported higher inter-individual variability of activation for faces than for objects, or an alteration of the typical distribution of allocation for faces vs. objects (Scherf et al., 2010; Schultz et al., 2000; Pierce et al., 2001) in the same regions.

In summary, the regions of major differences in perceptual brain activation between autistic and non-autistic individuals, as well as regions displaying the largest cross-modal plasticity in sensory-deprived individuals, overlap with regions that are the most variable and most plastic in neurotypical individuals. This overlap suggests a general mechanism for neuroplasticity, which mostly involves brain regions that are highly susceptible to reorganization. In contrast, primary sensory regions which probably require a high degree of neural constraints due to their topographic (e.g., retinotopic/tonotopic) organization may require a more hard-wired rigid organization and connectivity.

4. Genetic or prenatal risks predisposing to autism

We have presented how autistic strengths and cortical reallocations may be the result of enhanced cortical plasticity. We will now

discuss the genetic and molecular mechanisms that may explain these alterations.

4.1. Upregulation of synaptic plasticity

Due to recent advances in high throughput genomic technologies, deleterious mutations, including de novo copy number variants (CNVs) (Levy et al., 2011; Sanders et al., 2011; Marshall et al., 2008; Sebat et al., 2007), and de novo point mutations (Iossifov et al., 2012; O'Roak et al., 2012, 2011; Sanders et al., 2012; Neale et al., 2012), have been recently identified. These alterations involve a large number of genes in nonsyndromic autism and its related phenotypes, in addition to over 100 genes implicated in inherited monogenic syndromic autism (Betancur, 2011; Lim et al., 2013; Chahrour et al., 2012; Yu et al., 2013). "Animal models" (Chung et al., 2012; Shinoda et al., 2013) and neuronal cell cultures make it possible to study in vivo micro-structural modifications resulting from mutated neuroplastic genes or in utero toxic exposure. However, these are not "true" animal models of autism. Such models do not exist and may never exist. They are nonetheless experimental models of neuroplastic disruptions, which in humans, mostly result in neurodevelopmental dysmorphic syndromes with intellectual disability and a phenotype corresponding to current autism criteria in a substantial proportion of cases. They can thus contribute to understanding the mechanisms of syndromic autism, and, by extension, to autism per se. These animal models have revealed that most genes with strong effects and in utero toxic exposure implicated in autism and autistic-like phenotypes act upon a relatively small number of key biological processes affecting the structure and function of the synapses (Gillis and Rouleau, 2011).

Cascades of neuroplastic proteins control the formation of neural microcircuits between cells. This includes synaptogenesis, axonal guidance and growth, as well as synaptic plasticity, i.e., the ability of synapses to strengthen or weaken over time, in response to increased or decreased activity. Moreover, the timing of microcircuit construction follows developmental milestones, with a period of pruning starting in the second year of life, which is when most cases of autism are detected. In turn, pruning is stimulated by enriched environments (Sale et al., 2011), and coincides with learning and the formation of memory. Genes encoding many of these neuroplastic proteins have been implicated in autism, including: (1) cell adhesion molecules, e.g., neuroligin-3 (*NLGN3*) and neuroligin 4 X-linked (*NLGN4X*), mutations of which lead to high or ectopic GABAergic synapse formation (Tabuchi et al., 2007; Hoy et al., 2013); (2) postsynaptic "scaffolding" proteins, e.g., SHANK genes (i.e., *SHANK1*, *SHANK2* and *SHANK3*) which connect glutamate receptors to the actin in cytoskeleton via various intermediary elements and are a binding partner of neuroligins (Arons et al., 2012); (3) ionotropic (*AMPAR* and *NMDAR*) and metabotropic glutamate receptors (mGluR) at synapses (Chiocchetti et al., 2014); (4) transcriptional regulators of these synaptic proteins; and (5) signal transduction and tumor suppressor genes (Rinaldi et al., 2008) such as *TSC1* and *TSC2*, *NF1* and *PTEN*, involved in syndromic autism. The production of the synaptic proteins listed in (2) is controlled by genes that are in turn transcriptionally regulated by factors such as *MECP2*, a transcriptional repressor of brain-derived neurotropic factor (*BDNF*) and neuronal transcriptional regulators such as *DLX5*. Their production is also controlled by the fragile X mental retardation protein (*FMRP*) which binds mRNA transcripts in dendritic spines, exerts control over protein translation and regulates several families of synaptic proteins. Mutations of *TSC1* and *TSC2* are associated with Tuberous Sclerosis, and mutations of *NF1* and *PTEN* are associated with Neurofibromatosis and Cowden syndrome, respectively. The prevalence of autism is high in these disorders, and these mutations deregulate the production of neuroplastic proteins and modify the synaptic excitation/inhibition ratio (Bateup et al., 2013). In these cases, altered mechanisms of molecular plasticity also lead to tumors. These neuroplastic proteins regulate the production and balance of excitatory and inhibitory GABAergic synapses (Desgent and Ptito, 2012), and that of long term potentiation and depression (LTP/LTD) proteins that stabilize and remodel new circuits. Mutations in these genes cause dysregulation of activity-dependent signaling networks that control synapse development, function and plasticity (Ebert and Greenberg, 2013).

Mutations predisposing to autism appear to affect microstructural proteins and regulators. This alteration is mostly in the direction of hyper microstructural connectivity and hyper excitability (Arons et al., 2012), or more generally, the upregulation of the local plasticity (Kelleher et al., 2008; Zuko et al., 2013; Zoghbi and Bear, 2012). Disturbance of the regulation of gene expression following neural activity, or activity-dependent signaling, is among the most common functional effects of causative mutations involved in autism (see Ebert and Greenberg, 2013 for a review). Mice models have revealed that the four main mutations predisposing to autism, Nlgn3, Fmr1, Tsc2 and Shank3, produce similar physiological effects, involving the upregulation of typical synaptic plasticity mechanisms (Baudouin et al., 2012), specifically a deregulation of mGluR-LTD. In utero exposure to valproic acid (VPA) is the only environmental prenatal insult clearly associated with autism (Christensen et al., 2013). When modeled in mice, exposure to VPA also produces a hyper connectivity at the mini-columnar scale (Rinaldi et al., 2008; Silva et al., 2009), and stimulates BDNF expression (Almeida et al., 2014). The neurobiological effects of several factors predisposing to autism in humans are thus conserved in mouse models despite the questionable similarity between the phenotype of mouse models and autistic symptoms. Table 1 summarizes the main genes involved in nonsyndromic and syndromic autism, their effect on synaptic plasticity, and their "hyperplastic" consequences.

4.2. Enhanced vs. altered synaptic plasticity

One of the most complex questions raised by the involvement of upregulated synaptic plasticity in autism is how (dis) similar these processes are from their equivalent in the neurotypical population. Do these mechanisms exist in all individuals, and are they atypically *triggered* by various genetic alterations, leading to *enhanced* synaptic plasticity? Alternatively, are these mechanisms "abnormal", without an equivalent in non-autistic individuals, and thus can we describe them as *altered* synaptic plasticity? One possibility is that both exist at either end of a spectrum, with possible intermediate conditions. In this model, nonsyndromic autism is found at one end of the spectrum and syndromic autism at the other. In non-syndromic autism, the upregulated transcription of genes involved in the plasticity of network branch(es) (Kelleher et al., 2008; Gkogkas et al., 2013) may generate hyper connectivity (i.e., hyperplasticity) within certain local neuronal circuits (Tabuchi et al., 2007). Alternatively, in syndromic autism, a mutation may alter the basic synaptic mechanism involved in the construction of all synapses and neural networks (Knoth and Lippé, 2012). For instance, in "dysplastic" syndromes (e.g., Fragile X, tuberous sclerosis) that are associated with autism, neurogenetic alterations disrupt normal mechanisms of synaptic plasticity, and are associated with intellectual disability and dysmorphic features. Knockout Fmr1 mice have large dendrite spines and high spine density, enhanced long-term depression, a high rate of protein synthesis and up-regulation of the mGluR-mediated signaling pathway, indicative of hyperplasticity (Hayashi et al., 2007; Connor et al., 2011). PTEN mutations, which are associated with another neurogenetic condition characterized by macrocephaly (more severe than that commonly observed in nonsyndromic autism) and a high

Table 1
The main genes involved in nonsyndromic and syndromic autism, and their particular action on synaptic plasticity. "Syndromic" and "nonsyndromic" nature of the autistic phenotype resulting from the mutation is indicated by the name of the neurodevelopmental syndrome associated with autism.

Gene	Associated neurogenetic syndrome	Gene function	Overall mutation effects	Specific effects of mutation on synaptic plasticity
FMR1	Fragile X	Produces fragile X mental retardation protein (FMRP); Represses translation of several mRNAs.	Translational derepression of mRNAs; Up-regulation of the mGluR-mediated signaling pathway	Enhanced long-term depression (LTD); increase in the rate of cerebral protein synthesis and of excitatory activity (mGluR-dependent LTD).
TSC1/TSC2	Tuberous sclerosis	Inhibits the mTOR-raptor complex; regulator of cell growth in mitotic cells	Derepresses mTOR signaling; up-regulates the signaling pathway which promotes cell growth and proliferation	Enhanced translation in neurons; Increased excitatory activity
PTEN	Cowden syndrome	Negative regulator of PI3K-mTOR signaling	Heightened mTORC1 activity	Neuronal hypertrophy and macrocephaly; Increased excitatory activity
MCP2	Rett syndrome (mutation)	Regulates neurotrophic factors, such as brain-derived neurotrophic factor (BDNF)	Increased transcription of genes and number of excitatory hippocampal synapses	Hypoplasticity (mutation);
	MECP2 duplication Syndrome (duplication)			Hyperplasticity (duplication)
NF1	Neurofibromatose	Inhibits mTOR/PI3 K pathway	Upregulation of Ras-dependent ERK and mTOR activation	Hyperplasticity; increased availability of synaptic proteins
UBE3A	Angelman syndrome	Regulates ubiquitin-dependent protein turnover	Elevated synaptic protein levels	Increased/abnormal dendritic spine development
CACNA1C	Timothy syndrome	Regulation of inward calcium ion currents	Hyperactivation of the signal to nucleus path	Overabundance of plasticity related proteins
NLGN 3.4	None	Regulates the formation and function of excitatory and inhibitory postsynaptic transmission	Increased/ectopic GABAergic synapse formation	Gain of function when mutation does not completely inactivates the gene
NRX 1,2,3	None or Pitt–Hopkins-like syndrome	Synaptic adhesion. Interacts with NLGN to induce neurite outgrowth; initiates synapse formation	Impaired synaptic adhesion and neuron differentiation	Increase in excitatory synaptic transmission
SHANK 1,2,3	none or Phelan McDermid syndrome	Pre/postsynaptic signaling through the Neurexin–Neuroligin complex; regulates AMPA and NMDA receptor-synaptic transmission	Shank 1: reduction of dendritic spines Shank 2: increase in number of glutamate receptors and upregulation of shank 3	Shank 2: increase in the number, size, and strength of excitatory synapses; increased LTP Shank 3:alteration of glutamatergic synapses
NBEA	None	Synaptic scaffolding protein, spine formation. Regulator of membrane trafficking; formation of central synapses	Reduced number of spines	Alteration of neurotransmitter transport by large dense-core vesicles

prevalence of autism, may result in hyper connectivity in sensory areas (Xiong et al., 2012). The conditions associated with several types of syndromic autism are thus characterized by *altered* plasticity. These conditions (e.g., Fragile X or Tuberous sclerosis 1–2), are associated with dimorphism, intellectual disabilities, aberrant cell proliferation and a high prevalence of epilepsy even in the absence of autism.

5. The Trigger-Threshold-Target model of autism

A heterogeneous neurogenetic origin, a characteristic developmental course, variability in language and intelligence level, and superior perceptual or language abilities (depending on the subgroup) are key features of autism (Cowen, 2011). In parallel, there is increasing evidence for a role of genetic (Kelleher et al., 2008), micro-structural (Markram and Markram, 2010) and macrostructural cortical (Samson et al., 2011a) plasticity in autism. We propose the Trigger-Threshold-Target (TTT) model to account for this combination of features as well as for their variability. In this model, autism occurs when genetic mutation(s) ***trigger***(s) a neuroplastic reaction in individuals with a genetically-determined low ***threshold***. In this model, variability in autistic phenotype and cognitive strengths result from the unique combination of genetic triggers and thresholds, and neurofunctional ***target*** regions of this plastic reaction. Thus, the TTT model proposes that autism results from a plastic reaction targeting the most variable cortical regions; this plastic reaction may create a cascade effect yielding the particular pattern of strengths and weaknesses of each autistic individual.

5.1. Accounting for autistic strengths by cortical recycling

The "hijacking" of a region typically dedicated to a certain type of informational input by another neurological function, may result in enhanced perceptual or verbal performance in autistic individuals. For instance, there are now clear indications of cortical reallocations involving the fusiform gyrus during the processing of written material (Samson et al., 2011a), which is a strength of autistic individuals, and of enhanced temporo-occipital connectivity associated with advanced decoding ability in autism (Kikuchi et al., 2013).

This associative perceptual region appeared quite late, and its expansion was strongly selected for in human evolution (Waltereit et al., 2013). This region displays a remarkable potential for plasticity and is a striking example of a functional specialization that may be further promoted by enhanced plasticity. Similarly, autistic individuals may develop a strong perceptual "approach" to problem solving, in which case, the application of advanced perceptual processing to reasoning tasks would result in strong fluid intelligence (Dawson et al., 2007). This is confirmed by neuroimaging showing that associative visual areas are strongly activated in autistic individuals solving Raven matrices, but only for the most difficult problems (Soulières et al., 2009). The activity in BA9 and BA7 is lower in autistic individuals than in non-autistic individuals during the same task, which suggests that functional recycling of perceptual brain regions is involved in tasks normally requiring frontal and parietal regions. Reliance on perceptual regions for the completion of reasoning activities would considerably modify the approach of autistic people to these tasks such that autistic individuals complete them faster and with less verbal mediation than non-autistic individuals.

The recycling of perceptual brain regions for the performance of reasoning tasks also probably accounts for autistic strengths through the mechanism of *veridical mapping* (Mottron et al., 2013), which involves the use of typical pattern recognition to detect structural similarity among large input structures or abstract representations. *Veridical mapping* enables an individual to memorize the coupling between perceptually or structurally similar elements, either within or between perceptual modalities. This phenomenon exists in non-autistic individuals, and involves the processing of non-visual information by visual structures (Pascual-Leone and Hamilton, 2001). The retrieval of missing elements (e.g., phonological code, day-of-the-date) when provided with a fragment of the association (e.g., written code, date) is associated with many savant abilities including hyperlexia and calendar calculation, as well as absolute pitch and synesthesia. This key mechanism is similar to pattern completion, but is applied to large-scale patterns, for instance a musical phrase or a word. In some cases, such as synesthesia, these mappings are largely idiosyncratic, but in others such as hyperlexia, they are an adaptive method of processing large structures, and ultimately lead to the mastering of a socially relevant competence such as reading (Bouvet et al., 2014).

However, there may be drawback to cortical reallocations that stimulate perception. The perceptual origin of veridical mapping implies that it has domain-specificity: "restricted interests" in autistic individuals do not generalize easily to other domains of categorically similar information. Another drawback is a high dependence on access to materials, with a high level of expertise reached when autistic people have access to an input that fits their perceptual processing requirements. However, an environment lacking in material that can be processed by perceptual regions may produce deprivation (or "captivity") behaviors, and ultimately impair intellectual abilities (Lewis et al., 2007).

Last, these cortical reallocations may be involved in atypical face processing tasks. Several studies have found that the Face Fusiform Area for non-familiar faces is atypically activated in some individuals with autism (Scherf et al., 2010; Pierce et al., 2001). During facial processing, both autistic individuals and non-autistic individuals show activity in the expected Fusiform Face Area (medial fusiform gyrus); however, only autistic people display activity in the anterior portion of the fusiform gyrus, a region associated with object processing and perceptual expertise. This pattern of activity may reflect enhanced perceptual resource allocation in autism and the use of distinct perceptual strategies for the processing of social and non-social information in this population (Di Martino et al., 2009; Kana et al., 2006) (Fig. 1F). It also suggests hyper-plastic processes in perceptual associative regions, and a large contribution of experience to the development of these processes.

5.2. Accounting for AS subtypes by contrasted target/neglect components

In the TTT model, we used the term "Target" to describe the fact that a general mechanism (increase of synaptic plasticity) stimulates mainly a limited subset of functions in the autistic brain. The autistic plastic reaction has the potential to target either of the two domain-general regions, the associative perceptual cortex or language regions, because of their evolutionary, neural and developmental similarity; these regions expanded recently, are topographically variable and have a protracted period of development. The different pattern of cognitive strengths and cortical reallocations between AS-SOD and AS-NoSOD thus results from topographic and functional differences in the target of this plastic reaction.

In AS-SOD individuals, low level and mid-level perceptual strengths, combined with the strong contribution of perception to intelligence, encompass their enhanced abilities (Mottron et al., 2012a). Furthermore, performances in perceptual tasks co-vary between individuals in this subgroup, indicating that they depend on a single domain-general factor (Meilleur et al., 2014). In contrast, speech is delayed or impaired in this subgroup. However, the frequent late catch-up of delayed speech, and the preservation of some language functions in prototypical autism, suggest that the early impairment of speech does not result from a primary dysfunction of the brain mechanisms devoted to spoken language. Instead, impairment may result from the early neglect of these functions. The TTT model proposes that superior perceptual processing is an obstacle for the development of speech (Heaton et al., 2008a), because neural resources are oriented toward perceptual dimensions of language. Accordingly, the fractionation of language into perceptual and linguistic components explains why some language components are defective whereas others are over-functioning. A perceptual processing of speech would account for echolalia, the superior discrimination of pitch in speech (Heaton et al., 2008b), early decoding strengths, and the occurrence of speech delay with perceptual strengths. Conversely, perceptual processing of speech may be detrimental if speech conflicts with perception, or if speech cannot be perceptually mapped with nonlinguistic perceptual input, as is the case for joint attention including a verbal component, or in the expression of subjective states. Thus, language may be processed primarily within perceptual brain networks in individuals with AS-SOD, resulting in various impairments and strengths of verbal abilities. Alternatively, in AS-NoSOD individuals, incoming information is primarily processed by an overextension of typical language-related processes, resulting in language strengths, but not perceptual ones (Samson et al., 2009). Thus, AS-NoSOD involves *overdevelopment* of language functions, both in terms of performance and brain activity. The domain targeted by the plastic reaction would consume neuronal resources, resulting in competition between speech and motor abilities in AS-NoSOD. This explains why the early overdevelopment of speech coexists with motor clumsiness in this subgroup (Barbeau et al., 2013b).

5.3. Neglect of socially oriented behaviors in the two main ASD subgroups

We will now briefly address how the TTT model accounts for autistic social behaviors (Forgeot D'Arc and Mottron, 2012). Autistic toddlers are less overtly oriented toward social materials (Dawson et al., 1998) than non-social ones, they disengage faster from faces than typical toddlers (Chawarska et al., 2010), and they

preferentially look at audiovisual synchrony rather than biological motion (Klin et al., 2009), or at geometric figures rather than social scenes (Pierce et al., 2011). Preference for non-social over social material is therefore a diagnostic feature of autism in toddlers. However, social prioritization is not perturbed in autistic individuals (New et al., 2010) and the amygdala is activated during the processing of non-social information (Forgeot D'Arc and Mottron, 2012; Grelotti et al., 2005). Furthermore, a review of behavioral studies on face perception in adults (Weigelt et al., 2012) revealed that facial identity is processed in a similar way between autistic and non-autistic people. These observations suggest that the defective "social brain" may not be the primary cause of the cascade of alterations characterizing autism.

From a TTT perspective, the cascade of events caused by competition coming from hyperplastic functions affects social cognition to a similar extent as competition impacts different cognitive domains in non-autistic individuals. Although some "neglected" domains (motor ability and speech) differ between AS-SOD and AS-NoSOD, neglect for socially-oriented signals is shared by the two AS subgroups. In AS-SOD, perceptual cognition outcompetes social cognition for brain resources. This results in weak exposure to social information (Chawarska et al., 2010) during the development of regions dedicated to perceptual expertise in the autistic brain, which contributes further to the reallocation of brain resources. Category-specific cortical allocation appears to be built at a later age (Scherf et al., 2007) for faces than for objects, which makes it particularly sensitive to variations in early input. Similarly, the superior temporal sulcus, which is a multimodal area (Redcay, 2008) implicated in socially oriented behaviors as well as social and speech perception (Redcay, 2008; Glasel et al., 2011), is one of the cortical regions which is colonized after sensory loss (Bavelier et al., 2001; Sadato et al., 2004). It is also a region which is frequently under-activated by speech and other socially-oriented operations in autism (Redcay, 2008; Zilbovicius et al., 2006), which may result from its colonization by other functions (Paakki et al., 2010).

The example of facial processing is highly informative. The fusiform gyrus is responsible for processing faces in non-autistic individuals. It was first thought to be dysfunctional in autism (Schultz et al., 2000), because it was responsive to objects rather than faces. However, brain activity during facial processing appears to normalize with age in autism and brain regions normally involved in facial recognition are activated when familiar faces or an attention cue is used during experiments (Pierce et al., 2004; Hadjikhani et al., 2004). This indicates that faces can be processed by adult autistic people, particularly under optimal conditions. Thus, it appears that face perception in autism is initially constrained by cortical functional reallocation, and by input competition. This leads us to question the dominant view of autistic social cognition, that a weak emotional response toward socially relevant figures (as indicated by perturbed activation of the amygdala (Swartz et al., 2013; Kleinhans et al., 2014) during exposure to faces) impairs facial recognition. However, the reverse may also be true (Klin et al., 2009). Perceptually defined patterns (for AS-SOD) or verbal information (for AS-NoSOD) maybe emotionally appealing or disturbing for autistic persons because they are more salient, as is the case for blind people with auditory information (Klinge et al., 2010b).

5.4. Accounting for neurogenetic variability

Two-hit genetic models (Girirajan and Eichler, 2010; Vorstman et al., 2011; Leblond et al., 2012) and polygenic models (Murdoch and State, 2013), propose that a combination of rare genetic events and either common predisposition genes or specific environmental conditions account for the occurrence and variability of some neurodevelopmental disorders. Here, we describe a two-hit genetic model required for the occurrence of autism. A ***Trigger*** mechanism accounts for variability in causal genes, and a plasticity ***threshold*** component accounts for the fact that autistic and non-autistic outcomes are associated with similar mutations. Finally, plasticity could be further described as enhanced (associated with non-syndromic autism) or altered (associated with syndromic autism).

5.4.1. A Trigger mechanism accounts for the genetic variability of autism

It is now well established that a large series of mutations (Betancur, 2011), either de novo or transmitted, are associated with a common autistic phenotype in a subset of cases. A first generation of synthetic reviews established that these various mutations commonly affect synapses (Gillis and Rouleau, 2011; Kelleher et al., 2008; Shinoda et al., 2013; Zoghbi and Bear, 2012; Waltereit et al., 2013). A second generation of synthetic reviews established details of the underlying mechanism and revealed that enhanced plasticity is a common result of both genetic and environmental factors (e.g., VPA) associated with autism (Baudouin et al., 2012; O'Roak et al., 2012; Chung et al., 2012; Shinoda et al., 2013; Chiocchetti et al., 2014; Bateup et al., 2013; Ebert and Greenberg, 2013; Markram and Markram, 2010). In summary, we propose that de novo or inherited mutations in genes involved in synaptic plasticity *trigger* a plasticity reaction (Markram and Markram, 2010). This reaction involves a cascade of plastic mechanisms, beginning at the synapse, and ending with cortical organization, and is the final common result of an indefinite number of genetic alterations or rare, prenatal insults. Perturbation of the experience-dependent development of cortical organization and behavioral phenotypic consequences are the final results of this reaction. The events that can trigger this plastic reaction are inherently variable. Variability in the effect of the causative mutation accounts for a part of the phenotypic heterogeneity, and characteristics of each syndrome (Fig. 2).

According to the Trigger-Threshold model the link between the trigger and the subsequent plastic reaction, at least in non-syndromic autism, may be quite tenuous. Therefore, the potential inventory of genetic triggers is indefinite. Genetic events may initiate a chain of plastic modifications, they may be part of this chain, or both. Accordingly, as observed in cross modal plasticity following sensory loss, most synaptic mechanisms associated with the enhanced performance and function of neuroplastic regions are already present and ready to function in a typically developing individual. For instance, Ben-David and Shifman (2012), computed a gene co-expression network for common and rare variants described in the genetics of autism literature, and identified functionally interconnected modules involved in synaptic and neuronal plasticity that are expressed in brain areas associated with learning, memory and perception.

5.4.2. The Threshold component accounts for the moderate prevalence of autism in accompanying neurogenetic conditions

ASD mutations (or prenatal insults) predisposing to autism show tremendous phenotypic variability, with identical variants associated with a wide range of neurodevelopmental outcomes besides ASD, including schizophrenia, intellectual disability, language impairment, and epilepsy. This suggests that each of these mutations, on its own, is not sufficient to result in an autistic phenotype. The inclusion of a threshold component in the genetic mechanism of enhanced plasticity is based on the puzzling observations that: (a) neurogenetic disorders that are frequently associated with autism can occur without autism (Peters et al., 2013); (b) males are disproportionally represented in non-syndromic autism, and this cannot be explained by an excess of autism genes on the X chromosome; and (c) several common genetic variants with small effects, frequently not reproducible between studies (Girirajan and

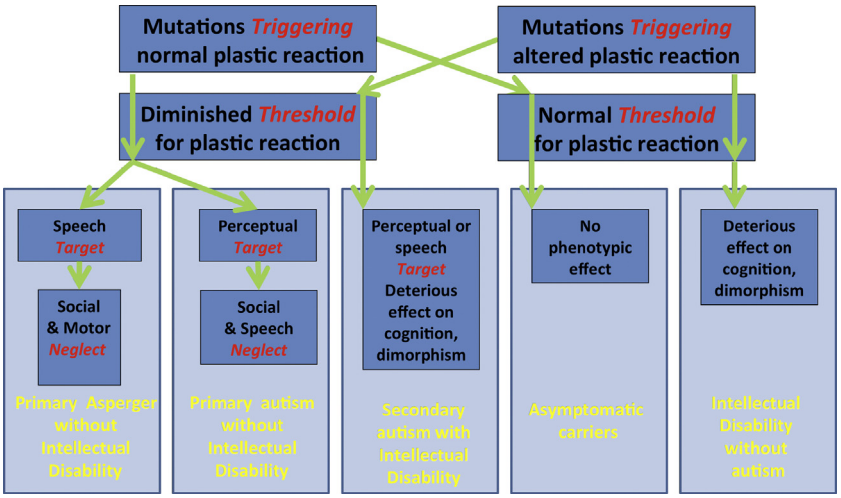


Fig. 2. The Trigger-Threshold-Target model. The causal event is a genetic alteration that affects synaptic plasticity. This mutation **triggers** a plastic reaction in individuals with a low **threshold** for plasticity, due to predisposing genes, the individual's sex and/or environmental factors. A plastic reaction **targets** a domain-general function, the identity of which determines the autism subgroup. In autism, perception is targeted and this results in enhanced visual and auditory perceptual functioning, but also in the neglect of speech. In Asperger syndrome, speech is targeted and this manifests as precocious mastering of language, concurrent with motor clumsiness. Social behaviors are **neglected** in both subgroups, as a result of the enhanced domain-specific neural investment. In nonsyndromic autism, mutations trigger a largely normal plastic reaction in individuals with a low threshold for this reaction, resulting in hyper-functioning and no intellectual disability. Individuals with a normal threshold are asymptomatic carriers of these mutations. In syndromic autism, causal mutations alter the plasticity reaction, resulting in aberrant cell proliferation, and frequent intellectual disability. In individuals with a low plastic threshold, these mutations additionally result in syndromic autism.

Eichler, 2010), slightly increase the risk for autism. It is therefore plausible that an indefinite number of factors, in addition to the causative genetic mutation, come together to lower or elevate the threshold at which the plastic reaction occurs in an individual. Among these factors, common functional polymorphisms of genes involved in plasticity may increase an individual's risk of reacting in an excessively plastic way when a triggering genetic event occurs. Males appear to be particularly sensitive to the effects of such polymorphisms. A low "threshold" would therefore account for the heterogeneity of predisposing factors, and would explain why a "trigger" mutation may or may not result in autism. It may also explain the difficulty in finding common genetic variants with significant effects, because these variants, in the absence of a trigger mechanism, cannot result in autism on their own. However, these variants may amplify the effects of de novo rare events on brain plasticity. The concept of a "threshold" may be tested by comparing plasticity mechanisms in populations possessing single gene mutations with or without autism. This strategy has proven fruitful in experiments involving neuroimaging, which demonstrated that people with Tuberous sclerosis and autism have higher local connectivity than people with Tuberous sclerosis alone (Tye and Bolton, 2013).

5.4.3. Enhanced and altered plasticity accounts for the distinction between syndromic and non-syndromic autism

In non-syndromic autism, a normal or quasi normal plastic reaction is believed to occur. In this situation, the triggering mutations would have no biological effect on the neural network and would instead initiate a cascade of plastic reactions, meaning that the stimulus needed to change synaptic connectivity in targeted regions would be decreased. In this case, most alterations would be functional reallocations resulting in the hyper functioning of targeted functions and subsequent neglect of non-targeted functions. Variations in the target of the plastic reaction and in raising conditions would account for inter-individual differences in symptoms (e.g., with or without SOD) and in the nature of the cognitive enhancements. Conversely, in syndromic autism, the triggering mutations would introduce an aberrant plasticity process where synaptic connectivity would occur in an abnormal way with no equivalent in non-autistic individuals. Thus, the causal genetic event has *altered* the plastic reaction, and the same alteration would characterize the associated condition, regardless of whether it is accompanied by autism. As is the case for non-syndromic autism, a mutation which produces a neurodevelopmental disorder would result in autism only when it occurs in individuals possessing a low threshold for a plastic reaction (Fig. 2). In individuals with a normal plasticity threshold, the mutation would only perturb the construction of neural networks, resulting in dimorphism, intellectual disability and/or epilepsy.

6. Concluding remarks

6.1. Summary of the TTT model

We suggest that a plastic reaction triggered by a series of mutations in genes encoding proteins involved in the construction of synapses accounts for enhanced perceptual or speech processing associated with autism. This alteration of synaptic plasticity affects the balance between the social vs. the perceptual or linguistic properties of materials that are preferentially processed. The structural and functional alterations targeting perceptual associative regions account for the superior performance of autistic individuals, and for the influence of perception in autistic phenotype. Competition with other cortical allocations results in the neglect of non-targeted functions, leading to autistic "negative" social behaviors. Sensory driven activity present at early stages of development influences existing organization dictated by genes, and can fundamentally alter the organization of the cortex, its connectivity and its function, resulting in enhanced perceptual functioning in autism, and poor mastering of social interactions, speech, or motor coordination. The large number of mutations with the potential to trigger a plastic reaction may explain the variety of neurological conditions associated with autism. Conversely, the specific nature of the plastic reaction found in autism and the resulting behavioral phenotype may result from the genetic source of its trigger.

6.2. Plasticity, interventions and pharmacological treatment

A plasticity model is also compatible with quantitative and qualitative variations in the post-natal environment, which is a prominent additional source of phenotypic variability (Dawson et al., 2008; Schneider et al., 2006). If autism results from a plasticity reaction, with potentially adaptive and non-adaptive consequences, at least a part of these mechanisms should be modulated by exposure to (or availability of) material associated with a high performance in autistic individuals. Plasticity processes can be modified by external interventions, and use of alternative brain regions to support impaired processes forms the basis of numerous intervention strategies (Belleville et al., 2011).

With this in mind, most early intervention programs adopt a "restorative" approach by stimulating the neglected function; for instance, social interest and competence are targetted by modeling socio-communicative markers of social reciprocity, joint attention or speech, and by limiting functions that are spontaneously enhanced in autism (like non-social perception) (Dawson et al., 2010). However, focus on only impaired functions may monopolize resources in favor of non-immediately processable material, and is unlikely to reverse the reallocation process (Dawson et al., 2008; Lyness et al., 2013). We therefore suggest that early intervention in autism should be based on lessons learned from sensory loss or memory impaired patients. For example, the congenitally deaf children with late access to sign language develop lower cross-modal plasticity (Pénicaud et al., 2012), and have generally poorer language development than children with early access to sign language (Lyness et al., 2013). A recent retrospective study compared deaf children with early cochlear implantation coming from deaf family (and thus native signers) with early-implanted deaf children coming from hearing family (and thus with limited, if any, access to sign language) at various times following implantation. Implanted deaf native signers outperformed implanted deaf non-signers on measures of speech perception, speech production and language development (Lyness et al., 2013; Hassanzadeh, 2012). These initial results then suggest that early exposure to a sign language paired with early cochlear implantation may be beneficial for an optimal spoken language development, rather than interfering with it. Interventions in blind persons promote tactile stimulation and the learning of Braille reading for the development of literacy. In another field, episodic memory training in memory-impaired patients most often relies on the teaching of non-conventional alternative encoding strategies (for instance, using visual imagery to encode verbal material) that relies on intact brain regions (Belleville et al., 2011, 2006; Belleville, 2008; Belleville and Bherer, 2012). In sum, rethinking early intervention within a TTT framework leads us to reconsider (a) the reversibility of the neglected functions; (b) the efficiency of harnessing targeted vs. neglected functions for the restoration of social functions; and (c) the possible adverse effects on the construction of cognitive architecture as a result of competition between various types of input and material.

Aberrant mechanisms of synaptic plasticity may also be treated by new pharmacological approaches (Pignatelli et al., 2013; Delorme et al., 2013; Walsh et al., 2008) in autism. One frequent suggestion is to *reduce* plasticity to diminish autistic symptoms. However, this should be done with caution, because it is difficult to distinguish the effects of the detrimental trigger from the adaptive effects of the plastic reaction. In cases where plasticity is altered, leading to an associated neurogenetic syndrome and intellectual disability, "repairing" the alteration is conceivable. However, if plasticity is a partially adaptive reaction to a genetic event, blocking these mechanisms may deprive the autistic person of unique strengths, and may bring back the initial, more detrimental deficit (Auerbach et al., 2011).

6.3. Limitation of the model and future research priorities

Several limitations of the TTT model need to be considered. The threshold component describes individual differences in plasticity. Alternative theories of factors favoring autism suggest that a continuous distribution of autistic traits exists in the population, which may be considered as favoring conditions, as minimal expression of the variants that cause autism, or as unrelated phenotypic overlap (Barbeau et al., 2009). Differences amongst individuals or between the sexes in the processing of social information may favor the development of an autistic or autistic-like phenotype, which may occur independently from the genetic mechanism involved in prototypical autism.

With the exception of one preliminary study that reported high LTP/LTD (Oberman et al., 2010) activity in AS individuals, most genetic and micro-structural data reported here come from experiments in animals, looking at genes involved in "altered" synaptic plasticity of syndromic autism. The next step needed is to validate our model in studies involving autistic individuals and cell cultures derived from them. In particular, it will be important to validate our principal hypothesis that mutations involved in non-syndromic autism can be understood within the context of normal plasticity. We also assume that autism associated with a neurogenetic syndrome, and non-syndromic autism, are more similar than dissimilar. Alternatively, neurogenetic syndromes may be considered as producing "phenocopies" of autism, with a low degree of similarity with non-syndromic autism. However, the convergence between synaptic processes involved in different types of syndromic autism argues against this idea, and supports instead the "Trigger" component of our model.

Enhanced micro-structural plasticity in perceptual associative brain regions has not yet been directly linked to over-performance in autism (see Hoy et al., 2013; Desgent and Ptito, 2012 for a review and for an animal example). This gap in our understanding is partially filled by sensory loss, which suggests that normal brain microstructure has the potential, under an environmental trigger, to over-develop perceptual function with measurable regional effects. However, a more direct link between synaptic and regional plasticity has to be empirically validated by combining genetic investigation, cell cultures and fMRI studies in the same group of individuals. In addition, the TTT model does not account for the mechanism of choice among the two domain-general targets, and why the two domains of enhancement are not frequently found together. Moreover, enhanced cortical allocation in the AS-NoSOD subgroup is based only on preliminary data, despite the fact that speech can be considered as a target of plastic reaction. Finally, the threshold component is the most difficult part of the genetic model to define and validate. Investigation of the sex-component of regional plasticity, in both the normal and sensory-impaired population may constitute a way to answer this question.

Acknowledgements

A special thanks to Michelle Dawson, Lan Xiong, Fabienne Samson, Tyler Cowen and Jacques Michaud, and to anonymous reviewers, for discussion, comments and informed suggestions. The manuscript also benefited from the support of other current and past members of the Montréal group, Christiane Belleville, Armando Bertone, Jessica Bertrand-Rivest, Anna Bonnel, Lucie Bouvet, Elise Brochu-Barbeau, Chantal Caron, Marie-Josée Caron, Baudouin Forgeot d'Arc, Claudine Jacques, Patricia Jelenic, Annie Lahaie, Véronique Langlois, Edith Ménard, Marie-Pierre Poulin-Lord, and Isabelle Soulières. This work was supported by the Marcel and Rolande Gosselin Research Chair on the cognitive neuroscience of autism, and a grant from the Canadian Institute for Health

Research (CIHR), *Atypical low-level perception in autism: Brain mechanisms and behavioral relevance*, MOP-84243. This is part of another CIHR grant, *Perception–Language relationships in autism*. Both grants were awarded to LM.

References

Adams, C., Green, J., Gilchrist, A., Cox, A., 2002. Conversational behaviour of children with Asperger syndrome and conduct disorder. J. Child Psychol. Psychiatry 43 (5), 679–690.

Aichhorn, M., Perner, J., Kronbichler, M., Staffen, W., Ladurner, G., 2006. Do visual perspective tasks need theory of mind? Neuroimage 30 (3), 1059–1068.

Almeida, L.E., Roby, C.D., Krueger, B.K., 2014. Increased BDNF expression in fetal brain in the valproic acid model of autism. Mol. Cell. Neurosci. 59C, 57–62.

Amedi, A., Raz, N., Pianka, P., Malach, R., Zohary, E., 2003. Early 'visual' cortex activation correlates with superior verbal memory performance in the blind. Nat. Neurosci. 6 (7), 758–766.

Amedi, A., Floel, A., Knecht, S., Zohary, E., Cohen, L.G., 2004. Transcranial magnetic stimulation of the occipital pole interferes with verbal processing in blind subjects. Nat. Neurosci. 7 (11), 1266–1270.

Amedi, A., Stern, W.M., Camprodon, J.A., Bermpohl, F., Merabet, L., Rotman, S., et al., 2007. Shape conveyed by visual-to-auditory sensory substitution activates the lateral occipital complex. Nat. Neurosci. 10 (6), 687–689.

Amiet, C., Gourfinkel-An, I., Bouzamondo, A., Tordjman, S., Baulac, M., Lechat, P., et al., 2008. Epilepsy in autism is associated with intellectual disability and gender: evidence from a meta-analysis. Biol. Psychiatry 64 (7), 577–582.

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders DSM-5. American Psychiatric Association.

Arons, M.H., Thynne, C.J., Grabrucker, A.M., Li, D., Schoen, M., Cheyne, J.E., et al., 2012. Autism-associated mutations in ProSAP2/Shank3 impair synaptic transmission and neurexin–neuroligin-mediated transsynaptic signaling. J. Neurosci. 32 (43), 14966–14978.

Auerbach, B.D., Osterweil, E.K., Bear, M.F., 2011. Mutations causing syndromic autism define an axis of synaptic pathophysiology. Nature 480 (7375), 63–68.

Barbeau, E.B., Mendrek, A., Mottron, L., 2009. Are autistic traits autistic? Br. J. Psychol. 100 (Pt 1), 23–28.

Barbeau, E.B., Soulières, I., Dawson, M., Zeffiro, T.A., Mottron, L., 2013a. The level and nature of autistic intelligence III: inspection time. J. Abnorm. Psychol. 122 (1), 295–301.

Barbeau, E.B., Meilleur, A.-A., Zeffiro, T., Mottron, L., 2013b. Comparing Visuomotor skills in autism spectrum individuals with and without speech delay. Autism Res. (under revision).

Barnes, S.J., Finnerty, G.T., 2010. Sensory experience and cortical rewiring. Neuroscientist 16 (2), 186–198.

Barttfeld, P., Wicker, B., Cukier, S., Navarta, S., Lew, S., Sigman, M., 2011. A big-world network in ASD: dynamical connectivity analysis reflects a deficit in long-range connections and an excess of short-range connections. Neuropsychologia 49 (2), 254–263.

Bateup, H.S., Johnson, C.A., Denefrio, C.L., Saulnier, J.L., Kornacker, K., Sabatini, B.L., 2013. Excitatory/inhibitory synaptic imbalance leads to hippocampal hyperexcitability in mouse models of tuberous sclerosis. Neuron 78 (3), 510–522.

Baudouin, S.J., Gaudias, J., Gerharz, S., Hatstatt, L., Zhou, K., Punnakkal, P., et al., 2012. Shared synaptic pathophysiology in syndromic and nonsyndromic rodent models of autism. Science 338 (6103), 128–132.

Bavelier, D., Neville, H.J., 2002. Cross-modal plasticity: where and how? Nat. Rev. Neurosci. 3 (6), 443–452.

Bavelier, D., Tomann, A., Hutton, C., Mitchell, T., Corina, D., Liu, G., et al., 2000. Visual attention to the periphery is enhanced in congenitally deaf individuals. J. Neurosci. 20 (17), RC93.

Bavelier, D., Brozinsky, C., Tomann, A., Mitchell, T., Neville, H., Liu, G., 2001. Impact of early deafness and early exposure to sign language on the cerebral organization for motion processing. J. Neurosci. 21 (22), 8931–8942.

Bedny, M., Konkle, T., Pelphrey, K., Saxe, R., Pascual-Leone, A., 2010. Sensitive period for a multimodal response in human visual motion area MT/MST. Curr. Biol. 20 (21), 1900–1906.

Bedny, M., Pascual-Leone, A., Dodell-Feder, D., Fedorenko, E., Saxe, R., 2011. Language processing in the occipital cortex of congenitally blind adults. Proc. Natl. Acad. Sci. U.S.A. 108 (11), 4429–4434.

Belleville, S., 2008. Cognitive training for persons with mild cognitive impairment. Int. Psychogeriatr. 20 (1), 57–66.

Belleville, S., Bherer, L., 2012. Biomarkers of cognitive training effects in aging. Curr. Transl. Geriatr. Exp. Gerontol. Rep. 1 (2), 104–110.

Belleville, S., Gilbert, B., Fontaine, F., Gagnon, L., Ménard, E., Gauthier, S., 2006. Improvement of episodic memory in persons with mild cognitive impairment and healthy older adults: evidence from a cognitive intervention program. Dement. Geriatr. Cogn. Disord. 22 (5–6), 486–499.

Belleville, S., Clément, F., Mellah, S., Gilbert, B., Fontaine, F., Gauthier, S., 2011. Training-related brain plasticity in subjects at risk of developing Alzheimer's disease. Brain 134 (Pt 6), 1623–1634.

Ben-David, E., Shifman, S., 2012. Networks of neuronal genes affected by common and rare variants in autism spectrum disorders. PLoS Genet. 8 (3), e1002556.

Berman, N.E., 1991. Alterations of visual cortical connections in cats following early removal of retinal input. Brain Res. Dev. Brain Res. 63 (1–2), 163–180.

Betancur, C., 2011. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. Brain Res. 1380, 42–77.

Bolognini, N., Cecchetto, C., Geraci, C., Maravita, A., Pascual-Leone, A., Papagno, C., 2012. Hearing shapes our perception of time: temporal discrimination of tactile stimuli in deaf people. J. Cogn. Neurosci. 24 (2), 276–286.

Bolognini, N., Convento, S., Rossetti, A., Merabet, L.B., 2013. Multisensory processing after a brain damage: clues on post-injury crossmodal plasticity from neuropsychology. Neurosci. Biobehav. Rev. 37 (3), 269–278.

Bonnel, A., Mottron, L., Peretz, I., Trudel, M., Gallun, E., Bonnel, A.-M., 2003. Enhanced pitch sensitivity in individuals with autism: a signal detection analysis. J. Cogn. Neurosci. 15 (2), 226–235.

Bonnel, A., McAdams, S., Smith, B., Berthiaume, C., Bertone, A., Ciocca, V., et al., 2010. Enhanced pure-tone pitch discrimination among persons with autism but not Asperger syndrome. Neuropsychologia 48 (9), 2465–2475.

Bosworth, R.G., Dobkins, K.R., 2002. Visual field asymmetries for motion processing in deaf and hearing signers. Brain Cogn. 49 (1), 170–181.

Boucher, J., 2012. Research review: structural language in autistic spectrum disorder – characteristics and causes. J. Child Psychol. Psychiatry 53 (3), 219–233.

Bouvet, L., Donnadieu, S., Valdois, S., Caron, C., Dawson, M., Mottron, L., 2014. Veridical mapping in savant abilities, absolute pitch, and synesthesia: an autism case study. Front. Psychol. 5, 106.

Cardin, V., Orfanidou, E., Rönnberg, J., Capek, C.M., Rudner, M., Woll, B., 2013. Dissociating cognitive and sensory neural plasticity in human superior temporal cortex. Nat. Commun. 4, 1473.

Caron, M.J., Mottron, L., Rainville, C., Chouinard, S., 2004. Do high functioning persons with autism present superior spatial abilities? Neuropsychologia 42 (4), 467–481.

Caron, M.J., Mottron, L., Berthiaume, C., Dawson, M., 2006. Cognitive mechanisms, specificity and neural underpinnings of visuospatial peaks in autism. Brain 129 (Pt 7), 1789–1802.

Casanova, M.F., van Kooten, I.A., Switala, A.E., van Engeland, H., Heinsen, H., Steinbusch, H.W., et al., 2006. Minicolumnar abnormalities in autism. Acta Neuropathol. 112 (3), 287–303.

Cauda, F., Costa, T., Palermo, S., D'Agata, F., Diano, M., Bianco, F., et al., 2014. Concordance of white matter and gray matter abnormalities in autism spectrum disorders: a voxel-based meta-analysis study. Hum. Brain Mapp. 35 (5), 2073–2098.

Chahrour, M.H., Yu, T.W., Lim, E.T., Ataman, B., Coulter, M.E., Hill, R.S., et al., 2012. Whole-exome sequencing and homozygosity analysis implicate depolarization-regulated neuronal genes in autism. PLoS Genet. 8 (4), e1002635.

Chawarska, K., Volkmar, F., Klin, A., 2010. Limited attentional bias for faces in toddlers with autism spectrum disorders. Arch. Gen. Psychiatry 67 (2), 178–185.

Chen, Q., He, G., Chen, K., Jin, Z., Mo, L., 2010. Altered spatial distribution of visual attention in near and far space after early deafness. Neuropsychologia 48 (9), 2693–2698.

Chiocchetti, A.G., Bour, H.S., Freitag, C.M., 2014. Glutamatergic candidate genes in autism spectrum disorder: an overview. J. Neural Transm. [Epub ahead of print].

Christensen, J., Grønborg, T.K., Sørensen, M.J., Schendel, D., Parner, E.T., Pedersen, L.H., et al., 2013. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA 309 (16), 1696–1703.

Chung, L., Bey, A.L., Jiang, Y.H., 2012. Synaptic plasticity in mouse models of autism spectrum disorders. Kor. J. Physiol. Pharmacol. 16 (6), 369–378.

Cohen, L.G., Celnik, P., Pascual-Leone, A., Corwell, B., Falz, L., Dambrosia, J., et al., 1997. Functional relevance of cross-modal plasticity in blind humans. Nature 389 (6647), 180–183.

Collignon, O., De Volder, A.G., 2009. Further evidence that congenitally blind participants react faster to auditory and tactile spatial targets. Can. J. Exp. Psychol. 63 (4), 287–293.

Collignon, O., Renier, L., Bruyer, R., Tranduy, D., Veraart, C., 2006. Improved selective and divided spatial attention in early blind subjects. Brain Res. 1075 (1), 175–182.

Collignon, O., Lassonde, M., Lepore, F., Bastien, D., Veraart, C., 2007. Functional cerebral reorganization for auditory spatial processing and auditory substitution of vision in early blind subjects. Cereb. Cortex 17 (2), 457–465.

Collignon, O., Voss, P., Lassonde, M., Lepore, F., 2009a. Cross-modal plasticity for the spatial processing of sounds in visually deprived subjects. Exp. Brain Res. 192 (3), 343–358.

Collignon, O., Charbonneau, G., Lassonde, M., Lepore, F., 2009b. Early visual deprivation alters multisensory processing in peripersonal space. Neuropsychologia 47 (14), 3236–3243.

Collignon, O., Vandewalle, G., Voss, P., Albouy, G., Charbonneau, G., Lassonde, M., et al., 2011. Functional specialization for auditory-spatial processing in the occipital cortex of congenitally blind humans. Proc. Natl. Acad. Sci. U.S.A. 108 (11), 4435–4440.

Collignon, O., Dormal, G., Lepore, F., 2012. Building the brain in the dark: functional and specific crossmodal reorganization in the occipital cortex of blind individuals. In: Steeves, J.K.E., Harris, L.R. (Eds.), Plasticity in Sensory Systems. Cambridge University Press, Cambridge, UK, p. 24.

Collignon, O., Dormal, G., Albouy, G., Vandewalle, G., Voss, P., Phillips, C., et al., 2013. Impact of blindness onset on the functional organization and the connectivity of the occipital cortex. Brain 136 (Pt 9), 2769–2783.

Connor, S.A., Hoeffer, C.A., Klann, E., Nguyen, P.V., 2011. Fragile X mental retardation protein regulates heterosynaptic plasticity in the hippocampus. Learn. Mem. 18 (4), 207–220.
Courchesne, E., Mouton, P.R., Calhoun, M.E., Semendeferi, K., Ahrens-Barbeau, C., Hallet, M.J., et al., 2011. Neuron number and size in prefrontal cortex of children with autism. JAMA 306 (18), 2001–2010.
Cowen, T., 2011. An Economic and Rational Choice Approach to the Autism Spectrum and Human Neurodiversity. GMU Working Paper in Economics [Internet], December 22, 2011, pp. 11–58. Available from: http://ssrn.com/abstract=1975809
Dawson, G., Meltzoff, A.N., Osterling, J., Rinaldi, J., Brown, E., 1998. Children with autism fail to orient to naturally occurring social stimuli. J. Autism Dev. Disord. 28 (6), 479–485.
Dawson, M., Soulieres, I., Gernsbacher, M.A., Mottron, L., 2007. The level and nature of autistic intelligence. Psychol. Sci. 18 (8), 657–662.
Dawson, M., Mottron, L., Gernsbacher, M.A., 2008. Learning in autism. In learning and memory: a comprehensive reference. In: Roediger, J.B.H.L. (Ed.), Cognitive Psychology. Elsevier, Oxford, UK, pp. 759–772.
Dawson, G., Rogers, S., Munson, J., Smith, M., Winter, J., Greenson, J., et al., 2010. Randomized, controlled trial of an intervention for toddlers with autism: the Early Start Denver Model. Pediatrics 125 (1), e17–e23.
Dehaene, S., Cohen, L., 2007. Cultural recycling of cortical maps. Neuron 56 (2), 384–398.
Delorme, R., Ey, E., Toro, R., Leboyer, M., Gillberg, C., Bourgeron, T., 2013. Progress toward treatments for synaptic defects in autism. Nat. Med. 19 (6), 685–694.
Desgent, S., Ptito, M., 2012. Cortical GABAergic interneurons in cross-modal plasticity following early blindness. Neural Plast. 2012, 590725.
Di Martino, A., Ross, K., Uddin, L.Q., Sklar, A.B., Castellanos, F.X., Milham, M.P., 2009. Functional brain correlates of social and nonsocial processes in autism spectrum disorders: an activation likelihood estimation meta-analysis. Biol. Psychiatry 65 (1), 63–74.
Dickstein, D.P., Pescosolido, M.F., Reidy, B.L., Galvan, T., Kim, K.L., Seymour, K.E., et al., 2013. Developmental meta-analysis of the functional neural correlates of autism spectrum disorders. J. Am. Acad. Child Adolesc. Psychiatry 52 (3), 279.e16–289.e16.
Dinstein, I., Heeger, D.J., Lorenzi, L., Minshew, N.J., Malach, R., Behrmann, M., 2012. Unreliable evoked responses in autism. Neuron 75 (6), 981–991.
Dölen, G., Osterweil, E., Rao, B.S., Smith, G.B., Auerbach, B.D., Chattarji, S., et al., 2007. Correction of fragile X syndrome in mice. Neuron 56 (6), 955–962.
Domínguez, L.G., Velázquez, J.L., Galán, R.F., 2013. A model of functional brain connectivity and background noise as a biomarker for cognitive phenotypes: application to autism. PLOS ONE 8 (4), e61493.
Dormal, G., Collignon, O., 2011. Functional selectivity in sensory-deprived cortices. J. Neurophysiol. 105 (6), 2627–2630.
Doucet, M.E., Guillemot, J.P., Lassonde, M., Gagné, J.P., Leclerc, C., Lepore, F., 2005. Blind subjects process auditory spectral cues more efficiently than sighted individuals. Exp. Brain Res. 160 (2), 194–202.
Dundas, E.M., Best, C.A., Minshew, N.J., Strauss, M.S., 2012. A lack of left visual field bias when individuals with autism process faces. J. Autism Dev. Disord. 42 (6), 1104–1111.
Ebert, D.H., Greenberg, M.E., 2013. Activity-dependent neuronal signalling and autism spectrum disorder. Nature 493 (7432), 327–337.
Eigsti, I.M., Fein, D.A., 2013. More is less: pitch discrimination and language delays in children with optimal outcomes from autism. Autism. Res.
Ellemberg, D., Lewis, T.L., Maurer, D., Brar, S., Brent, H.P., 2002. Better perception of global motion after monocular than after binocular deprivation. Vis. Res. 42 (2), 169–179.
Emmorey, K., Xu, J., Gannon, P., Goldin-Meadow, S., Braun, A., 2010. CNS activation and regional connectivity during pantomime observation: no engagement of the mirror neuron system for deaf signers. Neuroimage 49 (1), 994–1005.
Eyler, L.T., Pierce, K., Courchesne, E., 2012. A failure of left temporal cortex to specialize for language is an early emerging and fundamental property of autism. Brain 135 (Pt 3), 949–960.
Fieger, A., Röder, B., Teder-Sälejärvi, W., Hillyard, S.A., Neville, H.J., 2006. Auditory spatial tuning in late-onset blindness in humans. J. Cogn. Neurosci. 18 (2), 149–157.
Forgeot D'Arc, B., Mottron, L., 2012. Social cognition in autism. In: Beauchamp, V.A.a.M. (Ed.), Developmental Social Neuroscience and Childhood Brain Insult: Implications for Theory and Practice. Guilford Press, New-York, USA, pp. 299–324.
Foss-Feig, J.H., Tadin, D., Schauder, K.B., Cascio, C.J., 2013. A substantial and unexpected enhancement of motion perception in autism. J. Neurosci. 33 (19), 8243–8249.
Frey, H.P., Molholm, S., Lalor, E.C., Russo, N.N., Foxe, J.J., 2013. Atypical cortical representation of peripheral visual space in children with an autism spectrum disorder. Eur. J. Neurosci. 38 (1), 2125–2138.
Gillis, R.F., Rouleau, G.A., 2011. The ongoing dissection of the genetic architecture of autistic spectrum disorder. Mol. Autism 2 (1), 12.
Girirajan, S., Eichler, E.E., 2010. Phenotypic variability and genetic susceptibility to genomic disorders. Hum. Mol. Genet. 19 (R2), R176–R187.
Gkogkas, C.G., Khoutorsky, A., Ran, I., Rampakakis, E., Nevarko, T., Weatherill, D.B., et al., 2013. Autism-related deficits via dysregulated eIF4E-dependent translational control. Nature 493 (7432), 371–377.
Glasel, H., Leroy, F., Dubois, J., Hertz-Pannier, L., Mangin, J.F., Dehaene-Lambertz, G., 2011. A robust cerebral asymmetry in the infant brain: the rightward superior temporal sulcus. Neuroimage 58 (3), 716–723.
Gougoux, F., Lepore, F., Lassonde, M., Voss, P., Zatorre, R.J., Belin, P., 2004. Neuropsychology: pitch discrimination in the early blind. Nature 430 (6997), 309.
Gougoux, F., Zatorre, R.J., Lassonde, M., Voss, P., Lepore, F., 2005. A functional neuroimaging study of sound localization: visual cortex activity predicts performance in early-blind individuals. PLoS Biol. 3 (2), e27.
Grelotti, D.J., Klin, A.J., Gauthier, I., Skudlarski, P., Cohen, D.J., Gore, J.C., et al., 2005. fMRI activation of the fusiform gyrus and amygdala to cartoon characters but not to faces in a boy with autism. Neuropsychologia 43 (3), 373–385.
Grigorenko, E.L., Klin, A., Volkmar, F., 2003. Annotation: hyperlexia: disability or superability? J. Child Psychol. Psychiatry 44 (8), 1079–1091.
Hadjikhani, N., Joseph, R.M., Snyder, J., Chabris, C.F., Clark, J., Steele, S., et al., 2004. Activation of the fusiform gyrus when individuals with autism spectrum disorder view faces. Neuroimage 22 (3), 1141–1150.
Hassanzadeh, S., 2012. Outcomes of cochlear implantation in deaf children of deaf parents: comparative study. J. Laryngol. Otol. 126 (10), 989–994.
Hauthal, N., Sandmann, P., Debener, S., Thorne, J.D., 2013. Visual movement perception in deaf and hearing individuals. Adv. Cogn. Psychol. 9 (2), 53–61.
Hayashi, M.L., Rao, B.S., Seo, J.S., Choi, H.S., Dolan, B.M., Choi, S.Y., et al., 2007. Inhibition of p21-activated kinase rescues symptoms of fragile X syndrome in mice. Proc. Natl. Acad. Sci. U.S.A. 104 (27), 11489–11494.
Heaton, P., Hudry, K., Ludlow, A., Hill, E., 2008a. Superior discrimination of speech pitch and its relationship to verbal ability in autism spectrum disorders. Cogn. Neuropsychol. 25 (6), 771–782.
Heaton, P., Davis, R.E., Happe, F.G., 2008b. Research note: exceptional absolute pitch perception for spoken words in an able adult with autism. Neuropsychologia 46 (7), 2095–2098.
Hertrich, I., Dietrich, S., Ackermann, H., 2013. Tracking the speech signal-time-locked MEG signals during perception of ultra-fast and moderately fast speech in blind and in sighted listeners. Brain Lang. 124 (1), 9–21.
Howlin, P., Goode, S., Hutton, J., Rutter, M., 2009. Savant skills in autism: psychometric approaches and parental reports. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 364 (1522), 1359–1367.
Hoy, J.L., Haeger, P.A., Constable, J.R., Arias, R.J., McCallum, R., Kyweriga, M., et al., 2013. Neuroligin1 drives synaptic and behavioral maturation through intracellular interactions. J. Neurosci. 33 (22), 9364–9384.
Hugdahl, K., Ek, M., Takio, F., Rintee, T., Tuomainen, J., Haarala, C., et al., 2004. Blind individuals show enhanced perceptual and attentional sensitivity for identification of speech sounds. Brain Res. Cogn. Brain Res. 19 (1), 28–32.
Hutsler, J.J., Zhang, H., 2010. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain Res. 1309, 83–94.
Hyde, K.L., Foster, N.E., Simard-Meilleur, A.A., Mottron, L., 2011. Enhanced perception of pitch direction in young adults with autism spectrum disorder. In: 10th International Meeting for Autism Research, San Diego, CA, May 12–14.
Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., et al., 2012. De novo gene disruptions in children on the autistic spectrum. Neuron 74 (2), 285–299.
Iuculano, T., Rosenberg-Lee, M., Supekar, K., Lynch, C.J., Khouzam, A., Phillips, J., et al., 2014. Brain organization underlying superior mathematical abilities in children with autism. Biol. Psychiatry 75 (3), 223–230, http://dx.doi.org/10.1016/j.biopsych.2013.06.018.
Jarvinen-Pasley, A., Heaton, P., 2007. Evidence for reduced domain-specificity in auditory processing in autism. Dev. Sci. 10 (6), 786–793.
Jones, C.R., Happé, F., Baird, G., Simonoff, E., Marsden, A.J., Tregay, J., et al., 2009a. Auditory discrimination and auditory sensory behaviours in autism spectrum disorders. Neuropsychologia 47 (13), 2850–2858.
Jones, C.R., Happe, F., Golden, H., Marsden, A.J., Tregay, J., Simonoff, E., et al., 2009b. Reading and arithmetic in adolescents with autism spectrum disorders: peaks and dips in attainment. Neuropsychology 23 (6), 718–728.
Kaldy, Z., Kraper, C., Carter, A.S., Blaser, E., 2011. Toddlers with Autism Spectrum Disorder are more successful at visual search than typically developing toddlers. Dev. Sci. 14 (5), 980–988.
Kana, R.K., Keller, T.A., Cherkassky, V.L., Minshew, N.J., Just, M.A., 2006. Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. Brain 129 (Pt 9), 2484–2493.
Kelleher 3rd, R.J., Bear, M.F., 2008. The autistic neuron: troubled translation? Cell 135 (3), 401–406.
Keown, C.L., Shih, P., Nair, A., Peterson, N., Mulvey, M.E., Müller, R.A., 2013. Local functional overconnectivity in posterior brain regions is associated with symptom severity in autism spectrum disorders. Cell Rep. 5 (3), 567–572.
Khan, S., Gramfort, A., Shetty, N.R., Kitzbichler, M.G., Ganesan, S., Moran, J.M., et al., 2013. Local and long-range functional connectivity is reduced in concert in autism spectrum disorders. Proc. Natl. Acad. Sci. U.S.A. 110 (8), 3107–3112.
Kikuchi, M., Yoshimura, Y., Shitamichi, K., Ueno, S., Hirosawa, T., Munesue, T., et al., 2013. A custom magnetoencephalography device reveals brain connectivity and high reading/decoding ability in children with autism. Sci. Rep. 3, 1139.
Kleinhans, N.M., Richards, T., Weaver, K., Johnson, L.C., Greenson, J., Dawson, G., et al., 2014. Association between amygdala response to emotional faces and social anxiety in autism spectrum disorders. Neuropsychologia 48 (12), 3665–3670.
Klin, A., Volkmar, F.R., Sparrow, S.S., Cicchetti, D.V., Rourke, B.P., 1995. Validity and neuropsychological characterization of Asperger syndrome: convergence with nonverbal learning disabilities syndrome. J. Child Psychol. Psychiatry 36 (7), 1127–1140.

Klin, A., Lin, D.J., Gorrindo, P., Ramsay, G., Jones, W., 2009. Two-year-olds with autism orient to non-social contingencies rather than biological motion. Nature 459 (7244), 257–261.
Klinge, C., Eippert, F., Röder, B., Büchel, C., 2010a. Corticocortical connections mediate primary visual cortex responses to auditory stimulation in the blind. J. Neurosci. 30 (38), 12798–12805.
Klinge, C., Röder, B., Büchel, C., 2010b. Increased amygdala activation to emotional auditory stimuli in the blind. Brain 133 (Pt 6), 1729–1736.
Knoth, I.S., Lippé, S., 2012. Event-related potential alterations in fragile X syndrome. Front. Hum. Neurosci. 6, 264.
Koshino, H., Kana, R.K., Keller, T.A., Cherkassky, V.L., Minshew, N.J., Just, M.A., 2008. fMRI investigation of working memory for faces in autism: visual coding and underconnectivity with frontal areas. Cereb. Cortex 18 (2), 289–300.
Kujala, T., Alho, K., Näätänen, R., 2000. Cross-modal reorganization of human cortical functions. Trends Neurosci. 23 (3), 115–120.
Kupers, R., Pappens, M., de Noordhout, A.M., Schoenen, J., Ptito, M., Fumal, A., 2007. rTMS of the occipital cortex abolishes Braille reading and repetition priming in blind subjects. Neurology 68 (9), 691–693.
Leblond, C.S., Heinrich, J., Delorme, R., Proepper, C., Betancur, C., Huguet, G., et al., 2012. Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders. PLoS Genet. 8 (2), e1002521.
Leclerc, C., Segalowitz, S.J., Desjardins, J., Lassonde, M., Lepore, F., 2005. EEG coherence in early-blind humans during sound localization. Neurosci. Lett. 376 (3), 154–159.
Lessard, N., Paré, M., Lepore, F., Lassonde, M., 1998. Early-blind human subjects localize sound sources better than sighted subjects. Nature 395 (6699), 278–280.
Leveille, C., Barbeau, E.B., Bolduc, C., Limoges, E., Berthiaume, C., Chevrier, E., et al., 2010. Enhanced connectivity between visual cortex and other regions of the brain in autism: a REM sleep EEG coherence study. Autism Res. 3 (5), 280–285.
Levy, D., Ronemus, M., Yamrom, B., Lee, Y.H., Leotta, A., Kendall, J., et al., 2011. Rare de novo and transmitted copy-number variation in autistic spectrum disorders. Neuron 70 (5), 886–897.
Lewald, J., 2013. Exceptional ability of blind humans to hear sound motion: implications for the emergence of auditory space. Neuropsychologia 51 (1), 181–186.
Lewis, M.H., Tanimura, Y., Lee, L.W., Bodfish, J.W., 2007. Animal models of restricted repetitive behavior in autism. Behav. Brain Res. 176 (1), 66–74.
Lim, E.T., Raychaudhuri, S., Sanders, S.J., Stevens, C., Sabo, A., MacArthur, D.G., et al., 2013. Rare complete knockouts in humans: population distribution and significant role in autism spectrum disorders. Neuron 77 (2), 235–242.
Liu, Y., Yu, C., Liang, M., Li, J., Tian, L., Zhou, Y., et al., 2007. Whole brain functional connectivity in the early blind. Brain 130 (Pt 8), 2085–2096.
Liu, C., Liu, Y., Li, W., Wang, D., Jiang, T., Zhang, Y., et al., 2011. Increased regional homogeneity of blood oxygen level-dependent signals in occipital cortex of early blind individuals. Neuroreport 22 (4), 190–194.
Lo, Y.C., Soong, W.T., Gau, S.S., Wu, Y.Y., Lai, M.C., Yeh, F.C., et al., 2011. The loss of asymmetry and reduced interhemispheric connectivity in adolescents with autism: a study using diffusion spectrum imaging tractography. Psychiatry Res. 192 (1), 60–66.
Loui, P., Li, H.C., Hohmann, A., Schlaug, G., 2011. Enhanced cortical connectivity in absolute pitch musicians: a model for local hyperconnectivity. J. Cogn. Neurosci. 23 (4), 1015–1026.
Lyness, C.R., Woll, B., Campbell, R., Cardin, V., 2013. How does visual language affect crossmodal plasticity and cochlear implant success? Neurosci. Biobehav. Rev. 37 (10 Pt 2), 2621–2630.
Maljaars, J.P., Noens, I.L., Scholte, E.M., Verpoorten, R.A., van Berckelaer-Onnes, I.A., 2011. Visual local and global processing in low-functioning deaf individuals with and without autism spectrum disorder. J. Intellect. Disabil. Res. 55 (1), 95–105.
Markram, K., Markram, H., 2010. The intense world theory – a unifying theory of the neurobiology of autism. Front. Hum. Neurosci. 4, 224.
Marshall, C.R., Noor, A., Vincent, J.B., Lionel, A.C., Feuk, L., Skaug, J., et al., 2008. Structural variation of chromosomes in autism spectrum disorder. Am. J. Hum. Genet. 82 (2), 477–488.
Martin, A., 2007. The representation of object concepts in the brain. Annu. Rev. Psychol. 58, 25–45.
McCullough, S., Emmorey, K., Sereno, M., 2005. Neural organization for recognition of grammatical and emotional facial expressions in deaf ASL signers and hearing nonsigners. Brain Res. Cogn. Brain Res. 22 (2), 193–203.
McKay, L.S., Simmons, D.R., McAleer, P., Marjoram, D., Piggot, J., Pollick, F.E., 2012. Do distinct atypical cortical networks process biological motion information in adults with Autism Spectrum Disorders? Neuroimage 59 (2), 1524–1533.
Meilleur, S.A., Bertone, A., Berthiaume, C., Mottron, L., 2014. Autism-specific covariation of perceptual performances: "g" or "p" factor? PLoS One (in press).
Monk, C.S., Peltier, S.J., Wiggins, J.L., Weng, S.J., Carrasco, M., Risi, S., et al., 2009. Abnormalities of intrinsic functional connectivity in autism spectrum disorders. Neuroimage 47 (2), 764–772.
Mottron, L., Belleville, S., Stip, E., 1996. Proper name hypermnesia in an autistic subject. Brain Lang. 53 (3), 326–350.
Mottron, L., Mineau, S., Martel, G., Bernier, C.S., Berthiaume, C., Dawson, M., et al., 2007. Lateral glances toward moving stimuli among young children with autism: early regulation of locally oriented perception? Dev. Psychopathol. 19 (1), 23–36.
Mottron, L., Soulières, I., Dawson, M., et al., 2012a. Perception. In: Volkmar, F. (Ed.), Encyclopedia of Autism. Springer New York, New York, NY.
Mottron, L., Soulières, I., Dawson, M., et al., 2012b. Circumscribed Interest. In: Volkmar, F. (Ed.), Encyclopedia of Autism. Springer New York, New York, NY.
Mottron, L., Bouvet, L., Bonnel, A., Samson, F., Burack, J.A., Dawson, M., et al., 2013. Veridical mapping in the development of exceptional autistic abilities. Neurosci. Biobehav. Rev. 37 (2), 209–228.
Mueller, S., Wang, D., Fox, M.D., Yeo, B.T., Sepulcre, J., Sabuncu, M.R., et al., 2013. Individual variability in functional connectivity architecture of the human brain. Neuron 77 (3), 586–595.
Murdoch, J.D., State, M.W., 2013. Recent developments in the genetics of autism spectrum disorders. Curr. Opin. Genet. Dev. 23 (3), 310–315.
Murias, M., Webb, S.J., Greenson, J., Dawson, G., 2007. Resting state cortical connectivity reflected in EEG coherence in individuals with autism. Biol. Psychiatry 62 (3), 270–273.
Nader, A.M., Jelenic, P., Soulières, I., 2014. Cognitive profile in autistic versus Asperger children: a comparison of WISC-III and WISC-IV profiles. J. Child Psychol. Psychiatry (submitted for publication).
Nakano, T., Kato, N., Kitazawa, S., 2012. Superior haptic-to-visual shape matching in autism spectrum disorders. Neuropsychologia 50 (5), 696–703.
Neale, B.M., Kou, Y., Liu, L., Ma'ayan, A., Samocha, K.E., Sabo, A., et al., 2012. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485 (7397), 242–245.
Neville, H.J., Lawson, D., 1987. Attention to central and peripheral visual space in a movement detection task: an event-related potential and behavioral study. II. Congenitally deaf adults. Brain Res. 405 (2), 268–283.
New, J.J., Schultz, R.T., Wolf, J., Niehaus, J.L., Klin, A., German, T.C., et al., 2010. The scope of social attention deficits in autism: prioritized orienting to people and animals in static natural scenes. Neuropsychologia 48 (1), 51–59.
Nickl-Jockschat, T., Habel, U., Maria Michel, T., Manning, J., Laird, A.R., Fox, P.T., et al., 2012. Brain structure anomalies in autism spectrum disorder-a meta-analysis of VBM studies using anatomic likelihood estimation. Hum. Brain Mapp. 33 (6), 1470–1489, http://dx.doi.org/10.1002/hbm.21299.
Noonan, S.K., Haist, F., Müller, R.A., 2009. Aberrant functional connectivity in autism: evidence from low-frequency BOLD signal fluctuations. Brain Res. 1262, 48–63.
Noser, R., Byrne, R.W., 2007. Mental maps in chacma baboons (*Papio ursinus*): using inter-group encounters as a natural experiment. Anim. Cogn. 10 (3), 331–340.
Oberman, L., Ifert-Miller, F., Najib, U., Bashir, S., Woollacott, I., Gonzalez-Heydrich, J., et al., 2010. Transcranial magnetic stimulation provides means to assess cortical plasticity and excitability in humans with fragile x syndrome and autism spectrum disorder. Front. Synaptic Neurosci. 2, 26.
O'Connor, K., 2012. Auditory processing in autism spectrum disorder: a review. Neurosci. Biobehav. Rev. 36 (2), 836–854.
O'Riordan, M.A., Plaisted, K.C., Driver, J., Baron-Cohen, S., 2001. Superior visual search in autism. J. Exp. Psychol. Hum. Percept. Perform. 27 (3), 719–730.
O'Roak, B.J., Deriziotis, P., Lee, C., Vives, L., Schwartz, J.J., Girirajan, S., et al., 2011. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. Nat. Genet. 43 (6), 585–589.
O'Roak, B.J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B.P., et al., 2012. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485 (7397), 246–250.
Paakki, J.J., Rahko, J., Long, X., Moilanen, I., Tervonen, O., Nikkinen, J., et al., 2010. Alterations in regional homogeneity of resting-state brain activity in autism spectrum disorders. Brain Res. 1321, 169–179.
Pascual-Leone, A., Hamilton, R., 2001. The metamodal organization of the brain. Prog. Brain Res. 134, 427–445.
Pénicaud, S., Klein, D., Zatorre, R.J., Chen, J.K., Witcher, P., Hyde, K., et al., 2012. Structural brain changes linked to delayed first language acquisition in congenitally deaf individuals. Neuroimage 66C, 42–49.
Peters, J.M., Taquet, M., Vega, C., Jeste, S.S., Sanchez Fernandez, I., Tan, J., et al., 2013. Brain functional networks in syndromic and non-syndromic autism: a graph theoretical study of EEG connectivity. BMC Med. 11 (1), 54.
Pierce, K., Muller, R.A., Ambrose, J., Allen, G., Courchesne, E., 2001. Face processing occurs outside the fusiform 'face area' in autism: evidence from functional MRI. Brain 124 (Pt 10), 2059–2073.
Pierce, K., Haist, F., Sedaghat, F., Courchesne, E., 2004. The brain response to personally familiar faces in autism: findings of fusiform activity and beyond. Brain 127 (Pt 12), 2703–2716.
Pierce, K., Conant, D., Hazin, R., Stoner, R., Desmond, J., 2011. Preference for geometric patterns early in life as a risk factor for autism. Arch. Gen. Psychiatry 68 (1), 101–109.
Pignatelli, M., Feligioni, M., Piccinin, S., Molinaro, G., Nicoletti, F., Nisticò, R., 2013. Synaptic plasticity as a therapeutic target in the treatment of autism-related single-gene disorders. Curr. Pharm. Des. 19 (36), 6480–6490.
Plaisted, K., O'Riordan, M., Baron-Cohen, S., 1998. Enhanced visual search for a conjunctive target in autism: a research note. J. Child Psychol. Psychiatry 39 (5), 777–783.
Poulin-Lord, M.-P., Barbeau, E.B., Soulières, I., Monchi, O., Doyon, J., Benali, H., Mottron, L., 2014. Increased topographical variability of task-related activation in perceptive and motor associative regions in adult autistics. Neuroimage: Clin. 4, 443–453.
Prevention CfDCa, 2013. Data and Statistics Autism Spectrum Disorders (ASDs), Available from: http://www.cdc.gov/ncbddd/autism/data.html
Pring, L., Woolf, K., Tadic, V., 2008. Melody and pitch processing in five musical savants with congenital blindness. Perception 37 (2), 290–307.
Raz, N., Striem, E., Pundak, G., Orlov, T., Zohary, E., 2007. Superior serial memory in the blind: a case of cognitive compensatory adjustment. Curr. Biol. 17 (13), 1129–1133.

Redcay, E., 2008. The superior temporal sulcus performs a common function for social and speech perception: implications for the emergence of autism. Neurosci. Biobehav. Rev. 32 (1), 123–142.
Redcay, E., Courchesne, E., 2005. When is the brain enlarged in autism? A meta-analysis of all brain size reports. Biol. Psychiatry 58 (1), 1–9.
Reich, L., Szwed, M., Cohen, L., Amedi, A., 2011. A ventral visual stream reading center independent of visual experience. Curr. Biol. 21 (5), 363–368.
Remington, A.M., Swettenham, J.G., Lavie, N., 2012. Lightening the load: perceptual load impairs visual detection in typical adults but not in autism. J. Abnorm. Psychol. 121 (2), 544–551.
Rinaldi, T., Perrodin, C., Markram, H., 2008. Hyper-connectivity and hyper-plasticity in the medial prefrontal cortex in the valproic acid animal model of autism. Front. Neural Circuits 2, 4.
Röder, B., Teder-Sälejärvi, W., Sterr, A., Rösler, F., Hillyard, S.A., Neville, H.J., 1999. Improved auditory spatial tuning in blind humans. Nature 400 (6740), 162–166.
Ronemus, M., Iossifov, I., Levy, D., Wigler, M., 2014. The role of de novo mutations in the genetics of autism spectrum disorders. Nat. Rev. Genet. 15 (2), 133–141.
Rudie, J.D., Brown, J.A., Beck-Pancer, D., Hernandez, L.M., Dennis, E.L., Thompson, P.M., et al., 2012. Altered functional and structural brain network organization in autism. Neuroimage Clin. 16 (2), 79–94, http://dx.doi.org/10.1016/j.nicl.2012.11.006. eCollection 2012.
Sadato, N., Yamada, H., Okada, T., Yoshida, M., Hasegawa, T., Matsuki, K., et al., 2004. Age-dependent plasticity in the superior temporal sulcus in deaf humans: a functional MRI study. BMC Neurosci. 5, 56.
Sahyoun, C.P., Soulieres, I., Belliveau, J.W., Mottron, L., Mody, M., 2009. Cognitive differences in pictorial reasoning between high-functioning autism and Asperger's syndrome. J. Autism Dev. Disord. 39 (7), 1014–1023.
Sahyoun, C.P., Belliveau, J.W., Soulieres, I., Schwartz, S., Mody, M., 2010. Neuroimaging of the functional and structural networks underlying visuospatial vs. linguistic reasoning in high-functioning autism. Neuropsychologia 48 (1), 86–95.
Sale, A., De Pasquale, R., Bonaccorsi, J., Pietra, G., Olivieri, D., Berardi, N., et al., 2011. Visual perceptual learning induces long-term potentiation in the visual cortex. Neuroscience 172, 219–225.
Samson, F., Zeffiro, T.A., Mendrek, A., Hyde, K.L., Mottron, L. (Eds.), 2009. International Meeting for Autism Research (IMFAR). Chicago, IL, USA, 2009-05-07.
Samson, F., Mottron, L., Soulieres, I., Zeffiro, T.A., 2011a. Enhanced visual functioning in autism: an ALE meta-analysis. Hum. Brain Mapp.
Samson, F., Hyde, K.L., Bertone, A., Soulières, I., Mendrek, A., Ahad, P., et al., 2011b. Atypical processing of auditory temporal complexity in autistics. Neuropsychologia 49 (3), 546–555.
Sanders, S.J., Ercan-Sencicek, A.G., Hus, V., Luo, R., Murtha, M.T., Moreno-De-Luca, D., et al., 2011. Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70 (5), 863–885.
Sanders, S.J., Murtha, M.T., Gupta, A.R., Murdoch, J.D., Raubeson, M.J., Willsey, A.J., et al., 2012. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485 (7397), 237–241.
Sathian, K., 2005. Visual cortical activity during tactile perception in the sighted and the visually deprived. Dev. Psychobiol. 46 (3), 279–286.
Scherf, K.S., Behrmann, M., Humphreys, K., Luna, B., 2007. Visual category-selectivity for faces, places and objects emerges along different developmental trajectories. Dev. Sci. 10 (4), F15–F30.
Scherf, K.S., Luna, B., Minshew, N., Behrmann, M., 2010. Location, location, location: alterations in the functional topography of face- but not object- or place-related cortex in adolescents with autism. Front. Hum. Neurosci. 4, 26.
Schneider, T., Turczak, J., Przewłocki, R., 2006. Environmental enrichment reverses behavioral alterations in rats prenatally exposed to valproic acid: issues for a therapeutic approach in autism. Neuropsychopharmacology 31 (1), 36–46.
Schultz, R.T., Gauthier, I., Klin, A., Fulbright, R.K., Anderson, A.W., Volkmar, F., et al., 2000. Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. Arch. Gen. Psychiatry 57 (4), 331–340.
Schumann, C.M., Bloss, C.S., Barnes, C.C., Wideman, G.M., Carper, R.A., Akshoomoff, N., et al., 2010. Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. J. Neurosci. 30 (12), 4419–4427.
Schwarzkopf, D.S., Anderson, E.J., de Haas, B., White, S.J., Rees, G., 2014. Larger extrastriate population receptive fields in autism spectrum disorders. J. Neurosci. 34 (7), 2713–2724.
Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., et al., 2007. Strong association of de novo copy number mutations with autism. Science 316 (5823), 445–449.
Shinoda, Y., Sadakata, T., Furuichi, T., 2013. Animal models of autism spectrum disorder (ASD): a synaptic-level approach to autistic-like behavior in mice. Exp. Anim. 62 (2), 71–78.
Silva, G.T., Le Bé, J.V., Riachi, I., Rinaldi, T., Markram, K., Markram, H., 2009. Enhanced long-term microcircuit plasticity in the valproic acid animal model of autism. Front. Synaptic Neurosci. 1, 1.
Soulieres, I., Zeffiro, T.A., Girard, M.L., Mottron, L., 2011. Enhanced mental image mapping in autism. Neuropsychologia 49 (5), 848–857.
Soulières, I., Dawson, M., Samson, F., Barbeau, E.B., Sahyoun, C.P., Strangman, G.E., et al., 2009. Enhanced visual processing contributes to matrix reasoning in autism. Hum. Brain Mapp. 30 (12), 4082–4107.
Stanfield, A.C., McIntosh, A.M., Spencer, M.D., Philip, R., Gaur, S., Lawrie, S.M., 2008. Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. Eur. Psychiatry 23 (4), 289–299.
Steven, M.S., Blakemore, C., 2004. Visual synaesthesia in the blind. Perception 33 (7), 855–868.
Stevenson, J.L., Gernsbacher, M.A., 2013. Abstract spatial reasoning as an autistic strength. PLOS ONE 8 (3), e59329.
Stigler, K.A., McDonald, B.C., Anand, A., Saykin, A.J., McDougle, C.J., 2011. Structural and functional magnetic resonance imaging of autism spectrum disorders. Brain Res. 1380, 146–161.
Stivalet, P., Moreno, Y., Richard, J., Barraud, P.A., Raphel, C., 1998. Differences in visual search tasks between congenitally deaf and normally hearing adults. Brain Res. Cogn. Brain Res. 6 (3), 227–232.
Swartz, J.R., Wiggins, J.L., Carrasco, M., Lord, C., Monk, C.S., 2013. Amygdala habituation and prefrontal functional connectivity in youth with autism spectrum disorders. J. Am. Acad. Child Adolesc. Psychiatry 52 (1), 84–93.
Szatmari, P., 2011. New recommendations on autism spectrum disorder. BMJ 342, d2456.
Tabuchi, K., Blundell, J., Etherton, M.R., Hammer, R.E., Liu, X., Powell, C.M., et al., 2007. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 318 (5847), 71–76.
Thiebaut de Schotten, M., Cohen, L., Amemiya, E., Braga, L.W., Dehaene, S., 2014. Learning to read improves the structure of the arcuate fasciculus. Cereb. Cortex 24 (4), 989–995.
Turner, K.C., Frost, L., Linsenbardt, D., McIlroy, J.R., Müller, R.A., 2006. Atypically diffuse functional connectivity between caudate nuclei and cerebral cortex in autism. Behav. Brain Funct. 2, 34.
Tye, C., Bolton, P., 2013. Neural connectivity abnormalities in autism: insights from the Tuberous Sclerosis model. BMC Med. 11, 55.
Vargha-Khadem, F., 1983. Visual field asymmetries in cogenitally deaf and hearing children. Br. J. Dev. Psychol. 74, 13.
Vorstman, J.A., van Daalen, E., Jalali, G.R., Schmidt, E.R., Pasterkamp, R.J., de Jonge, M., et al., 2011. A double hit implicates DIAPH3 as an autism risk gene. Mol. Psychiatry 16 (4), 442–451.
Voss, P., Lassonde, M., Gougoux, F., Fortin, M., Guillemot, J.P., Lepore, F., 2004. Early- and late-onset blind individuals show supra-normal auditory abilities in far-space. Curr. Biol. 14 (19), 1734–1738.
Wallace, G.L., Robustelli, B., Dankner, N., Kenworthy, L., Giedd, J.N., Martin, A., 2013. Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders. Brain 136 (Pt 6), 1956–1967.
Walsh, C.A., Morrow, E.M., Rubenstein, J.L., 2008. Autism and brain development. Cell 135 (3), 396–400.
Waltereit, R., Banaschewski, T., Meyer-Lindenberg, A., Poustka, L., 2013. Interaction of neurodevelopmental pathways and synaptic plasticity in mental retardation, autism spectrum disorder and schizophrenia: implications for psychiatry. World J. Biol. Psychiatry [Epub ahead of print].
Wan, C.Y., Wood, A.G., Reutens, D.C., Wilson, S.J., 2010. Early but not late-blindness leads to enhanced auditory perception. Neuropsychologia 48 (1), 344–348.
Wang, L., Mottron, L., Peng, D., Berthiaume, C., Dawson, M., 2007. Local bias and local-to-global interference without global deficit: a robust finding in autism under various conditions of attention, exposure time, and visual angle. Cogn. Neuropsychol. 24 (5), 550–574.
Warren, J.D., Zielinski, B.A., Green, G.G., Rauschecker, J.P., Griffiths, T.D., 2002. Perception of sound-source motion by the human brain. Neuron 34 (1), 139–148.
Watson, J.D., Myers, R., Frackowiak, R.S., Hajnal, J.V., Woods, R.P., Mazziotta, J.C., et al., 1993. Area V5 of the human brain: evidence from a combined study using positron emission tomography and magnetic resonance imaging. Cereb Cortex. 3 (2), 79–94.
Weigelt, S., Koldewyn, K., Kanwisher, N., 2012. Face identity recognition in autism spectrum disorders: a review of behavioral studies. Neurosci. Biobehav. Rev. 36 (3), 1060–1084.
Welchew, D.E., Ashwin, C., Berkouk, K., Salvador, R., Suckling, J., Baron-Cohen, S., et al., 2005. Functional disconnectivity of the medial temporal lobe in Asperger's syndrome. Biol. Psychiatry 57 (9), 991–998.
Williams, D., Botting, N., Boucher, J., 2008. Language in autism and specific language impairment: where are the links? Psychol. Bull. 134 (6), 944–963.
Wong, M., Gnanakumaran, V., Goldreich, D., 2011. Tactile spatial acuity enhancement in blindness: evidence for experience-dependent mechanisms. J. Neurosci. 31 (19), 7028–7037.
Xiong, Q., Oviedo, H.V., Trotman, L.C., Zador, A.M., 2012. PTEN regulation of local and long-range connections in mouse auditory cortex. J. Neurosci. 32 (5), 1643–1652.
Yaka, R., Yinon, U., Wollberg, Z., 1999. Auditory activation of cortical visual areas in cats after early visual deprivation. Eur. J. Neurosci. 11 (4), 1301–1312.
Yu, K.K., Cheung, C., Chua, S.E., McAlonan, G.M., 2011. Can Asperger syndrome be distinguished from autism? An anatomic likelihood meta-analysis of MRI studies. J. Psychiatry Neurosci. 36 (6), 412–421.
Yu, T.W., Chahrour, M.H., Coulter, M.E., Jiralerspong, S., Okamura-Ikeda, K., Ataman, B., et al., 2013. Using whole-exome sequencing to identify inherited causes of autism. Neuron 77 (2), 259–273.
Zamm, A., Schlaug, G., Eagleman, D.M., Loui, P., 2013. Pathways to seeing music: enhanced structural connectivity in colored-music synesthesia. Neuroimage 74, 359–366.

Zilbovicius, M., Meresse, I., Chabane, N., Brunelle, F., Samson, Y., Boddaert, N., 2006. Autism, the superior temporal sulcus and social perception. Trends Neurosci. 29 (7), 359–366.

Zoghbi, H.Y., Bear, M.F., 2012. Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. Cold Spring Harb. Perspect. Biol. 4 (3.).

Zuko, A., Kleijer, K.T., Oguro-Ando, A., Kas, M.J., van Daalen, E., van der Zwaag, B., et al., 2013. Contactins in the neurobiology of autism. Eur. J. Pharmacol. 719 (1–3), 63–74.

Zwaigenbaum, L., Bryson, S., Rogers, T., Roberts, W., Brian, J., Szatmari, P., 2005. Behavioral manifestations of autism in the first year of life. Int. J. Dev. Neurosci. 23 (2–3), 143–152.

Article

The Worldwide Prevalence of ADHD: A Systematic Review and Metaregression Analysis

Guilherme Polanczyk, M.D.

Maurício Silva de Lima, M.D., Ph.D.

Bernardo Lessa Horta, M.D., Ph.D.

Joseph Biederman, M.D.

Luis Augusto Rohde, M.D., Ph.D.

Objective: The worldwide prevalence estimates of attention deficit hyperactivity disorder (ADHD)/hyperkinetic disorder (HD) are highly heterogeneous. Presently, the reasons for this discrepancy remain poorly understood. The purpose of this study was to determine the possible causes of the varied worldwide estimates of the disorder and to compute its worldwide-pooled prevalence.

Method: The authors searched MEDLINE and PsycINFO databases from January 1978 to December 2005 and reviewed textbooks and reference lists of the studies selected. Authors of relevant articles from North America, South America, Europe, Africa, Asia, Oceania, and the Middle East and ADHD/HD experts were contacted. Surveys were included if they reported point prevalence of ADHD/HD for subjects 18 years of age or younger from the general population or schools according to DSM or ICD criteria.

Results: The literature search generated 9,105 records, and 303 full-text articles were reviewed. One hundred and two studies comprising 171,756 subjects from all world regions were included. The ADHD/HD worldwide-pooled prevalence was 5.29%. This estimate was associated with significant variability. In the multivariate metaregression model, diagnostic criteria, source of information, requirement of impairment for diagnosis, and geographic origin of the studies were significantly associated with ADHD/HD prevalence rates. Geographic location was associated with significant variability only between estimates from North America and both Africa and the Middle East. No significant differences were found between Europe and North America.

Conclusions: Our findings suggest that geographic location plays a limited role in the reasons for the large variability of ADHD/HD prevalence estimates worldwide. Instead, this variability seems to be explained primarily by the methodological characteristics of studies.

(Am J Psychiatry 2007; 164:942–948)

Attention deficit hyperactivity disorder (ADHD) is characterized by pervasive and impairing symptoms of inattention, hyperactivity, and impulsivity according to DSM-IV (1). The World Health Organization (WHO) (2) uses a different name—hyperkinetic disorder (HD)—but lists similar operational criteria for the disorder. Regardless of the name used, ADHD/HD is one of the most thoroughly researched disorders in medicine (3). It has been associated with a broad range of negative outcomes for affected subjects (4, 5) and with a serious financial burden to families and society (6), which characterizes it as a major public health problem (7).

An understanding of the epidemiological aspects of ADHD/HD may provide insight into its distribution and etiology as well as information for planning the allocation of funds for mental health services (8). In past decades, investigators from all regions of the world have made substantial efforts to define the prevalence of the disorder. Several literature reviews have reported highly variable rates worldwide, ranging from as low as 1% to as high as nearly 20% among school-age children (8, 9).

Although the reasons for variability across studies remain poorly understood, it has been hypothesized that geographical and demographic factors are associated with it (10). Several investigators have suggested that prevalence rates in Europe were significantly lower than rates found in North America (8, 11, 12). This hypothesis fueled the concern that ADHD/HD may be a product of cultural factors and promoted an enduring debate in the medical literature (8, 11, 12). Other experts have argued that the variability of ADHD/HD prevalence estimates may be best explained by the use of different case definitions and that no variability of the actual prevalence across geographical sites should be found when case definitions are the same (5, 8, 13). A similar position has been adopted by the American Academy of Child and Adolescent Psychiatry, which states that conflicting results of ADHD/HD prevalence estimates can be explained by methodological dif-

This article is discussed in an editorial by Drs. Moffitt and Melchior on p. 856.

ferences across studies, such as method of ascertainment, diagnostic systems and associated criteria (e.g., situational versus pervasive, degree of impairment), assessment methods, informants, and the population studied (4). Unfortunately, no empirical evidence to date supports these assumptions, which led the American Medical Association (3), the National Institute of Mental Health (6), and the Centers for Disease Control and Prevention (7) to call for further research on this issue.

Therefore, the main purpose of this study was to conduct an extensive review of the literature on the prevalence of ADHD/HD to 1) calculate a worldwide-pooled prevalence estimate and 2) determine factors implicated in the variability of estimates by examining the assumptions of different estimates based on geographic location of studies. Since some studies suggest that ADHD/HD prevalence is higher in North America, cross-national comparisons were conducted to investigate differences between North American studies and those conducted elsewhere. The main hypothesis was that geographic location would not significantly explain the variability of worldwide ADHD/HD prevalence estimates after adjustment for methodological differences across studies.

Method

Data Sources

The following four search strategies were used for this systematic review of the literature: 1) computer search of databases, 2) review of specialized textbooks, 3) review of reference lists of all articles retrieved, and 4) contact with authors and experts on ADHD/HD epidemiology. This search strategy is in accordance with expert recommendations on the subject (14).

Computer search. We searched MEDLINE and PsycINFO from Jan. 1978 to Dec. 2005 for articles in English, German, French, Spanish, and Portuguese. Search words/terms were as follows: child*, adolesc*, epidemiology, prevalence, rate, mental disorder*, psychiatric disorder*, ADHD, ADD, attention-deficit, attention-deficit/hyperactivity disorder, hyperactiv*, overactiv*, inattent*, hyperkinetic disorder, and minimal brain dysfunction. An asterisk after a term means that all terms that begin with that root were included in the search.

Step 1. Abstracts were reviewed by the first author (Dr. Polanczyk) and selected for further review if they met one of the following three criteria: 1) significant reviews of literature that covered ADHD/HD prevalence, 2) major guidelines on ADHD/HD, or 3) original investigation of prevalence based on nonreferred samples (schools or community) of subjects 18 years of age or younger diagnosed according to criteria established by any DSM (III, III-R, or IV) (1) or ICD (9 or 10) versions (2). If a criterion was not met because not enough information was provided, the abstract was set aside for further evaluation.

Step 2. Abstracts were reviewed independently by two authors (Drs. Polanczyk and Rohde) and were selected based on their consensus according to the same criteria used in Step 1. If consensus was not reached, the abstract was set aside for further evaluation.

Step 3. Full-text articles of abstracts selected in Step 2 were retrieved and reviewed by one author (Dr. Polanczyk). Inclusion was based on consensus between two investigators (Drs. Polanczyk and Rohde). Disagreements were discussed with a third author (Dr. de Lima). Studies were included if they met the following criteria: 1) original surveys on ADHD/HD prevalence (point prevalence), 2) diagnoses based on any DSM (III, III-R, or IV) or ICD (9 or 10) versions, 3) probabilistic sample from the general population (e.g., households, birth registers) or from schools, and 4) subjects 18 years of age or younger. Twin studies and data from the first evaluation of longitudinal studies were included. The exclusion criteria were 1) studies that did not report confidence intervals (CI) or standard errors (SE) and that did not report data that allowed the calculation of these parameters and whose authors did not provide such data upon request and 2) studies that adopted a retrospective diagnostic approach based on subjects' records. Authors were contacted via e-mail if data were missing.

TABLE 1. Methodological Characteristics and Geographic Location of the Studies (N=102)

Variable	No. of Studies
Geographic location	
North America	32
Europe	32
Asia	15
South America	9
Oceania	6
Middle East	4
Africa	4
Origin of sample	
Community	42
School	60
Diagnostic criteria	
ICD-10	13
DSM-IV	44
DSM-III-R	36
DSM-III	9
Impairment criterion[a]	
Yes	44
No	48
Number of stages of evaluation	
One	61
Two	33
Two, only screens positive at first stage	8
Source of information[a]	
Best-estimate procedure	23
"And rule"	6
Parents	33
"Or rule"	9
Teachers	15
Subjects	7

[a] For some studies, data were not available for extraction.

Review of specialized textbooks. The ADHD/HD sections of five major textbooks on child and adolescent psychiatry and on general psychiatry were reviewed to identify references to original surveys that might meet Step 1 criteria.

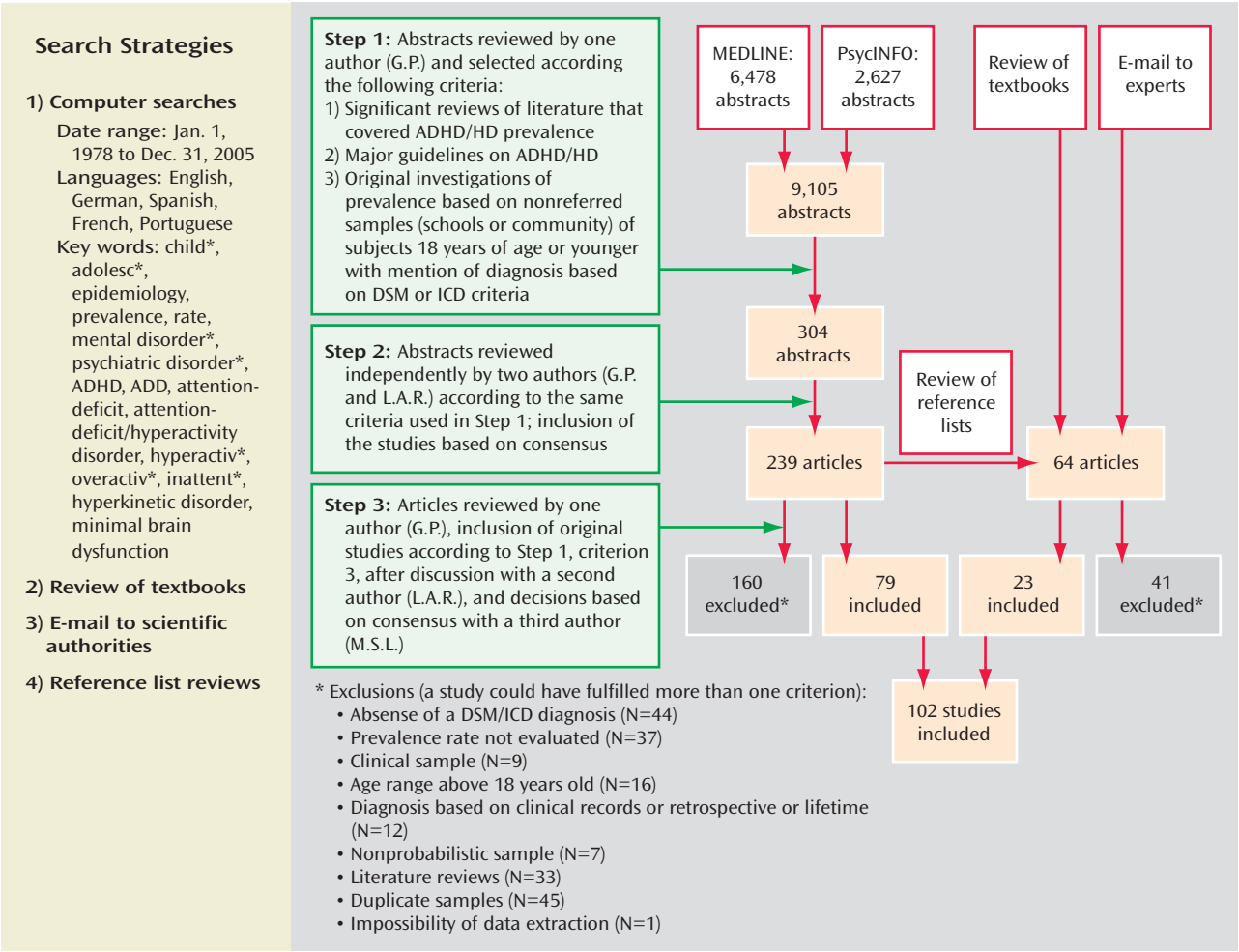
Contact with experts. Forty-five scientific authorities in the field of ADHD/HD from North America, South America, Europe, Africa, Asia, Oceania, and the Middle East were contacted via e-mail and asked whether they were aware of unpublished or ongoing studies, and 28 experts returned responses.

Review of reference lists. The reference lists of all articles selected in Step 3 were reviewed, and the full texts of potentially interesting studies were examined.

Data Extraction

A protocol for data extraction was defined and evaluated by two authors (Drs. Polanczyk and Rohde) (available from authors upon request). Data were extracted from full-text articles by one author (Dr. Polanczyk) and reviewed by a second author (Dr. Rohde). Disagreements were discussed with a third author (Dr. de Lima).

FIGURE 1. Flow Diagram of Study Selection



To determine pooled estimates, each study contributed with only one estimate. Whenever a study generated multiple estimates according to different diagnostic or methodological definitions, one estimate was selected according to a previously defined protocol. This protocol ensured that data on each subject were extracted only once.

The computer program Epi Info version 6.04d (15) was used to calculate CIs if they were not reported in the study.

Data Analyses

The first step of our previously defined strategy of data analysis was to determine an overall pooled-prevalence estimate of ADHD/HD based on all studies. A test of heterogeneity (Q test) was used to determine whether the differences in prevalence estimates across studies were greater than expected by chance. Significant heterogeneity was detected for the pooled estimate. At this point, sensitivity analysis was also performed, and findings showed that no study had skewed the overall result.

Since significant heterogeneity was found across studies, the second step was to conduct univariate analyses to test the individual association of each variable (methodological variables + geographic location of the studies) with the overall pooled-prevalence estimate of ADHD/HD using metaregression analyses (16). This analytical strategy evaluated which variables affected the results. Methodological variables were method of ascertainment of the sample (community or school), sample size, diagnostic criteria (DSM-III, -III-R, or -IV; ICD-9 or -10), source of information (best-estimate procedure, parents, "and rule," "or rule," teachers, or subjects), requirement of impairment for the diagnosis (yes or no), number of stages, and response rate (1-attrition rate).

We also tested the association between geographic location and variability of the overall pooled-prevalence estimate of ADHD/HD. Studies were divided into seven groups according to their geographic location: Africa, Asia, Europe, the Middle East, North America, Oceania, and South America. Three studies were fully conducted in Central America (Costa Rica), and one was a multisite study conducted in the United States and Costa Rica. These studies were grouped with those from North America.

In the third step, a random-effects regression model was used to evaluate sources of variability in the overall pooled-prevalence estimate of ADHD/HD. All covariates associated with ADHD/HD prevalence rates for a flexible $p \leq 0.2$ (17) in univariate analyses were included in the final multivariate metaregression model. For these analyses, a significance level of 5% was established.

Age and gender were not included in the final multivariate metaregression model because less than 50% of the studies reported findings stratified by these variables, but individual estimates were computed according to these strata (age was stratified into the following ranges: 6–11 and 12–18). At this stage, we also determined pooled estimates for each geographic region. All analyses were performed using Stata 9.0 (Stata Corp, College Station, Tex.).

TABLE 2. Association Between Methodological Covariates and Geographic Area With ADHD/HD Prevalence Estimates

Variable	Univariate Model p	Metaregression (Multivariate Model)[a] p
Origin of sample	<0.001	
Community		index
School		0.38
Source of information	<0.001	
Best-estimate procedure		index
"And rule"		0.04
Parents		0.03
"Or rule"		0.003
Teachers		<0.001
Subjects		0.46
Impairment criterion	<0.001	
Yes		index
No		0.001
Diagnostic criteria	<0.001	
DSM-IV		index
DSM-III-R		0.02
DSM-III		0.69
ICD-10		0.005
Number of stages of evaluation	<0.001	
One		index
Two		0.25
Two, only screens positive at first stage[b]		0.31
Response rate	0.25	—
Sample size	<0.001	0.81
Geographic area	0.009	
North America		index
Europe		0.40
Oceania		0.45
South America		0.83
Asia		0.85
Africa		0.03
Middle East		0.01

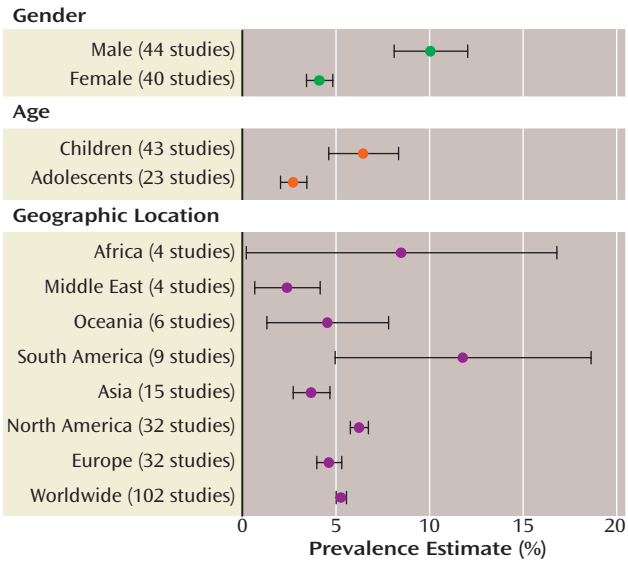
[a] Between-study variance assessed by moment-based estimate (tau 2=7.815).
[b] Studies using two-stage sampling where only screening positives were assessed in the second stage (for details see the data supplement of the online version of this article).

Results

We screened 9,105 abstracts published in the last 27 years. A total of 303 full-text articles were reviewed, and 102 studies comprising 171,756 subjects were included in this systematic review (Figure 1). The list of reviewed references is available at http://www.ufrgs.br/psiq/prodah-e.html. Studies from all continents were retrieved. Of the 102 studies included, 32 were conducted in North America and 32 in Europe. The most frequent diagnostic system was DSM-IV. Characteristics of studies included in the analyses are described in Table 1.

Overall, the pooled prevalence of ADHD/HD was 5.29% (95% CI=5.01–5.56). This analysis revealed significant heterogeneity across studies (p<0.001). In univariate metaregression analyses (Table 2), all methodological variables evaluated were significantly associated with ADHD/HD prevalence rates, except response rate (p=0.25). The geographic location of studies was also significantly associated with the variability of ADHD/HD prevalence rates and was included in the final metaregression model.

FIGURE 2. ADHD/HD Pooled Prevalence According to Demographic Characteristics and Geographic Location



In the final multivariate metaregression model (Table 2), the methodological variables that remained significantly associated with the prevalence rates were the requirement of impairment for the diagnosis, diagnostic criteria, and source of information. As expected, studies without a definition of impairment had significantly higher ADHD/HD prevalence rates than those with a definition of impairment (p<0.001). Studies based on DSM-III-R or ICD-10 criteria, respectively, had significantly lower ADHD/HD prevalence rates than those using DSM-IV criteria (p=0.02 and p=0.005, respectively). Studies that relied on information provided by parents, teachers, and "or rule," respectively, were associated with significantly higher ADHD/HD prevalence rates than those relying on a best-estimate procedure (p=0.02, p<0.001, and p=0.003, respectively), whereas those relying on information provided using the "and rule" criterion were associated with significantly lower ADHD/HD prevalence estimates (p=0.04) (Table 2).

Geographic location was associated with significant variability between estimates from North America and both Africa (p=0.03) and the Middle East (p=0.01). Estimates from these areas were significantly lower than estimates from North America. No significant differences were found in prevalence rates between North America and Europe (p=0.40), South America (p=0.83), Asia (p=0.85), or Oceania (p=0.45) (Table 2).

The multivariate metaregression model using Europe for the comparison yielded similar findings. Impairment, diagnostic criteria, and source of information remained significantly associated with prevalence rates. Significant differences were found only between Europe and both Africa (p=0.05) and the Middle East (p=0.03).

To increase power by decreasing degrees of freedom, an additional model was run using only methodological vari-

ables initially. Methodological variables associated with the prevalence rate for a p≤0.20 in univariate analyses were initially included and progressively deleted from the model using a backward procedure. Then, the geographic location of the studies was entered. In this multivariate model, geographic location was not significantly associated with prevalence rates after adjustment for methodological variables (data available from authors upon request).

Finally, since most studies were conducted in Europe and North America (N=64), the same data analysis strategy was applied using only these two regions. In the final multivariate metaregression model, the same methodological variables remained significantly associated with prevalence rates: impairment, diagnostic criteria, and source of information. Again, geographical location (Europe versus North America) was not significantly associated with ADHD/HD prevalence rates (p=0.61).

ADHD/HD prevalence rates were stratified by age in 43 studies and by gender in 44 studies. In these studies, both age and gender were significantly associated with prevalence rates (p<0.001). The pooled prevalence of ADHD/HD stratified by gender and age can be found in Figure 2. The pooled prevalence in the seven geographic areas assessed is also shown in Figure 2. These analyses revealed significant heterogeneity across studies (p<0.001).

Discussion

We have conducted a comprehensive systematic review of studies addressing prevalence rates of ADHD/HD worldwide and a metaregression analysis to understand the reasons of estimate variability. Our findings show that 1) the ADHD/HD worldwide-pooled prevalence is 5.29% (95% CI=5.01–5.56); 2) the large variability of ADHD/HD prevalence rates worldwide resulted mainly from methodological differences across studies; and 3) adjusting for methodological differences, prevalence rate variability was only detected between studies conducted in North America and those conducted in Africa and the Middle East. Moreover, no significant differences in ADHD/HD prevalence rates between North America and Europe were detected in the overall metaregression analysis and in the analysis restricted to studies conducted on these two continents. To our knowledge, this is the broadest systematic review of this subject to date.

Our search strategy identified original studies of ADHD/HD prevalence conducted on all continents. Despite the higher number of studies conducted in North America and Europe, our literature search showed that ADHD/HD is a well-studied disorder and that its prevalence rate has been estimated in several different cultures.

Although the ADHD/HD worldwide-pooled prevalence is consistent with results reported in most previous reviews (9, 18, 19), our results should be interpreted with caution because of the large variability found in all analyses. Our metaregression analysis reports, for the first time, on the critical role that methodological variables (i.e., impairment criterion, diagnostic criteria, and source of information) play in the large variability of ADHD/HD prevalence estimates in different geographic locations. This finding empirically supports assumptions previously discussed in the literature (8, 9). For instance, applying the same methodological procedures and diagnostic criterion, very similar rates of ADHD/HD were found in Russia (20) and Britain (21) (1.3% and 1.4%, respectively). However, when the diagnosis of ADHD/HD was made in the same geographic location but according to a different methodological criterion (i.e., with or without the requirement of functional impairment) estimates ranged from 3.7% to 8.9% (22).

ADHD/HD terminology has undergone significant changes over the past decades. The current ICD-10 and DSM-IV criteria provide very similar lists of symptoms but recommend different ways of establishing a diagnosis. The ICD-10 requires a minimum number of symptoms in all three dimensions (inattention, overactivity, and impulsivity). The DSM-IV defines only two dimensions (with hyperactivity and impulsivity symptoms included in the same dimension), and a diagnosis can be made if there is a minimum number of symptoms in only one dimension. The ICD-10 requires that all criteria be met in at least two different situational contexts, whereas DSM-IV requires the presence of some impairment in more than one setting. The ICD-10 includes mood, anxiety, and developmental disorders as exclusion diagnoses. In DSM-IV, these diagnoses may be classified as comorbid conditions. Therefore, ADHD prevalence rates based on DSM-IV are expected to be higher than those based on ICD-10, which was demonstrated by individual studies (23) and corroborated by our analyses.

Our finding of lower ADHD/HD prevalence rates in Africa and the Middle East than in North America should be carefully interpreted. These geographic areas contributed with only a few studies to the overall analysis, and therefore we cannot rule out the possibility of lower accuracy of the adjustment for methodological aspects of these studies. Nevertheless, data concerning the lack of significant differences in ADHD/HD prevalence rates between North America and Europe in all sets of metaregression analyses are extremely relevant. This finding argues against the view that ADHD/HD is a culturally-based construct peculiar to the North American culture (12). It is important to note, however, that we do not suggest that culture has no influence on the prevalence of this disorder in a given population. Environmental and cultural aspects should play a role in the etiology of ADHD/HD, since the estimates of heritability in the disorder are approximately 80% (24, 25).

Our findings should be understood in the context of some limitations. First, characteristics of the subjects (age and gender) were not included in the final metaregression model because less than 50% of the studies reported re-

sults stratified by age or gender or provided data that allowed us to calculate these distributions. Second, other characteristics of the countries, besides geographic location, could not be assessed. However, the geographic location of a country is conceptually relevant for the purpose of this study and is often cited as a factor that may explain the variability of ADHD/HD prevalence rates. Finally, we did not provide estimates of ADHD/HD prevalence among adults, an emerging new area of research (26) that should be addressed in future studies.

Despite these limitations, we found a large variability in ADHD/HD prevalence rates worldwide. Results suggest that geographic location may play a limited role in the explanation of this variability. The many methodological differences in the 102 studies assessed may be the most important source of variability in the pooled estimate of ADHD/HD prevalence. These results indicate that international efforts from institutions such as WHO should be directed to the standardization of epidemiological studies so that comparable epidemiological data from all regions of the world are made available for the study of relevant mental disorders such as ADHD/HD.

Received Aug. 9, 2006; revisions received Nov. 9, 2006 and Jan. 8, 2007; accepted Jan. 8, 2007. From the ADHD Outpatient Program, Child and Adolescent Psychiatric Division, Hospital de Clinicas de Porto Alegre, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Pediatric Psychopharmacology Unit, Massachusetts General Hospital, Harvard Medical School, Boston; Federal University of Pelotas and Catholic University of Pelotas, Pelotas, Brazil; and the Post-Graduate Program in Epidemiology, Federal University of Pelotas, Pelotas, Brazil. Address correspondence and reprint requests to Dr. Rohde, Child and Adolescent Psychiatric Division, Hospital de Clinicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Porto Alegre, RS, Brazil 90035-003; lrohde@terra.com.br or Dr. Polanczyk at gvp.ez@terra.com.br (e-mail).

The ADHD outpatient program receives research support from the following pharmaceutical companies: Bristol-Myers Squibb, Eli Lilly, Janssen-Cilag, and Novartis. Dr. Rohde is on the speakers' bureau and is a consultant for Bristol-Myers Squibb, Eli Lilly, Janssen-Cilag, and Novartis; he also serves on the advisory board for Eli Lilly. Dr. de Lima is Medical Director at Eli Lilly, Brazil. Dr. Biederman receives research support from Shire Laboratories, Inc., Eli Lilly, Wyeth-Ayerst, Pfizer, Cephalon, Novartis, and Janssen; he also serves on the speakers' bureaus of Eli Lilly, Pfizer, Novartis, Wyeth-Ayerst, Shire Laboratories, Inc., McNeil, and Cephalon and is on the advisory board of Eli Lilly, CellTech, Shire Laboratories, Inc., Novartis, Noven, McNeil, Janssen, Johnson & Johnson, Pfizer, and Cephalon. Drs. Polanczyk and Horta report no competing interests.

Supported in part by research grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) (grants 471761/03-6 and 300226/2002-0), Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil, and Eli Lilly. There was no involvement of any funding source in the study design, data collection, analysis, interpretation of data, and writing of this article or in the decision to submit the article for publication.

The authors thank the following individuals: Glorisa Canino, Valsamma Eapen, Steve Faraone, Martine Flament, Susan Shur-Fen Gau, Robert Goodman, Peter Jensen, Florence Levy, Anneke Meyer, Cecilia Montiel-Nava, Helmut Remschmidt, Russell Schachar, Joseph Sergeant, James Swanson, and Erik Taylor for their opinions on methodological issues of studies; Steven P. Cuffe, Cristiane Duarte, Bacy Fleitlich-Bilik, Sam Goldstein, Richard Hackett, David Hay, Rolf Loeber, Magda Stouthamer-Loeber, Pall Magnusson, Savita Malhotra, Christa Winkler Metzke, Emma Van der Meulen, Irma Moilanen, Rosalind Neuman, Lawrence Scahill, Martin Schmidt, John Seeley, Jakob Smári, Kenneth P. Tercyak, Márcio Vasconcelos, Kenneth Whiting, and Alessandro Zuddas for providing data about their studies; and Sandra Costa Fuchs, Jair Mari, Carisi Anne Polanczyk, and Luis Eduardo Rohde for their helpful comments.

References

1. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Diseases (DSM-IV), 4th ed. Washington, DC, American Psychiatric Publishing, 1994
2. World Health Organization: The ICD-10 Classification of Mental and Behavioral Disorders: Diagnostic Criteria for Research. Geneva, Switzerland, World Health Organization, 1993
3. Goldman LS, Genel M, Bezman RJ, Slanetz PJ: Diagnosis and treatment of attention-deficit/hyperactivity disorder in children and adolescents: Council on Scientific Affairs, American Medical Association. JAMA 1998; 279:1100–1107
4. Dulcan M: Practice parameters for the assessment and treatment of children, adolescents, and adults with attention-deficit/hyperactivity disorder: American Academy of Child and Adolescent Psychiatry. J Am Acad Child Adolesc Psychiatry 1997; 36(suppl 10):85S–121S
5. Swanson JM, Sergeant JA, Taylor E, Sonuga-Barke EJ, Jensen PS, Cantwell DP: Attention-deficit hyperactivity disorder and hyperkinetic disorder. Lancet 1998; 351:429–433
6. National Institutes of Health: National Institutes of Health Consensus Development Conference Statement: diagnosis and treatment of attention-deficit/hyperactivity disorder (ADHD). J Am Acad Child Adolesc Psychiatry 2000; 39:182–193
7. Lesesne C, Abramowitz A, Perou R, Brann E: Attention Deficit/Hyperactivity Disorder: A Public Health Research Agenda. http://www.cdc.gov/ncbddd/adhd/dadphra.htm, 2000 (Accessed Aug. 2006)
8. Bird HR: The diagnostic classification, epidemiology and cross-cultural validity of ADHD, in Attention Deficit Hyperactivity Disorder: State of the Science: Best Practices. Edited by Jensen PCJ. Kingston, NJ, Civic Research Institute, 2002
9. Faraone SV, Sergeant J, Gillberg C, Biederman J: The worldwide prevalence of ADHD: is it an American condition? World Psychiatry 2003; 2:104–113
10. Rappley MD: Attention deficit-hyperactivity disorder. N Engl J Med 2005; 352:165–173
11. Anderson JC: Is childhood hyperactivity the product of Western culture? Lancet 1996; 348:73–74
12. Timimi S, Taylor E: ADHD is best understood as a cultural construct. Br J Psychiatry 2004; 184:8–9
13. Rohde LA, Szobot C, Polanczyk G, Schmitz M, Martins S, Tramontina S: Attention-deficit/hyperactivity disorder in a diverse culture: do research and clinical findings support the notion of a cultural construct for the disorder? Biol Psychiatry 2005; 57: 1436–1441
14. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB: Meta-analysis of observational studies in epidemiology: a proposal for reporting: Meta-Analysis of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283:2008–2012
15. Center for Disease Control and Prevention, World Health Organization: Epi Info, Version 6.04d, 2001
16. Thompson SG, Higgins JP: How should meta-regression analyses be undertaken and interpreted? Stat Med 2002; 21:1559–1573
17. Maldonado G, Greenland S: Simulation study of confounder-selection strategies. Am J Epidemiol 1993; 138:923–936
18. Scahill L, Schwab-Stone M: Epidemiology of ADHD in school-age children. Child Adolesc Psychiatr Clin N Am 2000; 9:541–555, vii
19. Szatmari P: The epidemiology of attention-deficit hyperactivity disorders. Child Adolesc Psychiatr Clin N Am 1992; 1:361–371

20. Goodman R, Slobodskaya H, Knyazev G: Russian child mental health: a cross-sectional study of prevalence and risk factors. Eur Child Adolesc Psychiatry 2005; 14:28–33
21. Ford T, Goodman R, Meltzer H: The British Child and Adolescent Mental Health Survey 1999: the prevalence of DSM-IV disorders. J Am Acad Child Adolesc Psychiatry 2003; 42:1203–1211
22. Canino G, Shrout PE, Rubio-Stipec M, Bird HR, Bravo M, Ramirez R, Chavez L, Alegria M, Bauermeister JJ, Hohmann A, Ribera J, Garcia P, Martinez-Taboas A: The DSM-IV rates of child and adolescent disorders in Puerto Rico: prevalence, correlates, service use, and the effects of impairment. Arch Gen Psychiatry 2004; 61:85–93
23. Goodman R, Ford T, Richards H, Gatward R, Meltzer H: The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. J Child Psychol Psychiatry 2000; 41:645–655
24. Biederman J, Faraone SV: Attention-deficit hyperactivity disorder. Lancet 2005; 366:237–248
25. Sergeant J: Are we ready for endophenotypes in attention deficit hyperactivity disorder? Rev Bras Psiquiatr 2005; 27:262–263
26. Wilens TE, Faraone SV, Biederman J: Attention-deficit/hyperactivity disorder in adults. JAMA 2004; 292:619–623

informa
healthcare

The genetics and neurobiology of ESSENCE: The third Birgit Olsson lecture

THOMAS BOURGERON

Bourgeron T The genetics and neurobiology of ESSENCE: The third Birgit Olsson lecture Nord J Psychiatry 2015;Early Online:1–9.

ESSENCE refers to early symptomatic syndromes eliciting neurodevelopmental clinical examinations. It includes a broad range of early onset neurodevelopmental disorders affecting more than 10% of children before 5 years of age. ESSENCE includes among others attention deficit hyperactivity disorder (ADHD), intellectual disability (ID) and autism spectrum disorders (ASD). Some degree of disability is the rule rather than the exception. The causes are heterogeneous ranging from extreme social deprivation, pre- and perinatal risk factors, genetic and metabolic diseases, immune and infectious disorders, nutritional factors, physical trauma, and postnatal toxic and environmental factors (and combinations/interactions of some or several of these). Treatments often involve a combination of psychoeducational interventions, home- and school-based programmes, and medication. Here, I will first briefly review our main knowledge on the biological pathways associated with early onset neurodevelopmental disorders and will provide useful links to be informed of the progress in the field. Five main pathways are associated with ASD and ID: chromatin remodelling, cytoskeleton dynamics, mRNA translation, metabolism and synapse formation/function. I will then detail three propositions coming from institutions, researchers and/or communities of patients and families to foster research: 1) to use more dimensional and quantitative data than diagnostic categories; 2) to increase data sharing and research on genetic and brain diversity in human populations; 3) to involve patients and relatives as participants for research. Finally, I will provide examples of very stimulating initiatives towards a more inclusive world for individuals with ESSENCE.
• *Autism, Genes, Synapses.*

Thomas Bourgeron, Human Genetics and Cognitive Functions Unit, 25 rue du docteur Roux, 75015, Paris, France. E-mail: thomasb@pasteur.fr; Accepted 14 April 2015.

Children with neurodevelopmental disorders can experience problems with language and speech, motor skills, behaviour, memory, learning, or other neurological functions. These difficulties are also frequently associated with co-morbidities such as sensory-motor, sleep, and gastrointestinal problems (1). To better tackle this heterogeneity Christopher Gillberg coined the acronym ESSENCE, referring to "early symptomatic syndromes eliciting neurodevelopmental clinical examinations". It is a term to refer to the reality of children (and their parents) presenting in clinical settings with impairing child symptoms before age 3–5 years in the fields of 1) general development, 2) communication and language, 3) social interrelatedness, 4) motor coordination, 5) attention, 6) activity, 7) behaviour, 8) mood, and/or 9) sleep. Symptoms of neurodevelopmental disorders often evolve and may improve as a child grows older, but many disabilities are permanent. Diagnosis and treatment of these disorders can be difficult; treatment often involves a combination of professional therapy, pharmaceuticals, and home- and school-based programmes. With progress in genetics and neurobiology, the causes of early onset neurodevelopmental disorders (or ESSENCE) are better understood. Here, I will summarize the current knowledge on the genetic causes. Then I will summarize propositions that were suggested to improve research in this field.

Definition and prevalence

Early onset neurodevelopmental disorders affect more than 10% of children (Table 1) often with consequences throughout their lives and with significant effects on their families (1–3). This grouping is diverse in terms of severity and pathophysiology: fetal alcohol syndrome (FAS), attention deficit hyperactivity disorder (ADHD), intellectual disability (ID), tic disorder, developmental coordination

DOI: 10.3109/08039488.2015.1042519

Table 1. Prevalence and biological pathways associated with ESSENCE.

Neurodevelopmental disorders	Prevalence%	Proteins or biological pathways
Learning disabilities	2–4	Chromatin remodelling Metabolism Actin skeleton organization Channels Synaptogenesis Neurotransmission
Dyslexia	5–15	Neuronal migration?
Attention deficit hyperactivity disorder	1.7–9	Synapses? Cortical maturation?
Autism spectrum disorders	0.6–1.2	Chromatin remodelling Metabolism Actin skeleton organization Channels Synapses
Epilepsy	0.45–1	Synapses Channels
Fetal alcohol syndrome	0.1–5	–

disorder, dyslexia, specific language disorders and autism spectrum disorders (ASD). Neuromuscular disorders such as Becker or Duchenne muscular dystrophies could also be included in neurodevelopmental disorders since they also affect cognition in a subset of patients, but such disorders are often considered as a separate cluster because of their predominant symptoms. Boys seem to be at elevated risk compared with girls for most neurodevelopmental disorders, suggesting gender-specific risk and protective factors.

The amount of funding and research dedicated to a disorder is often correlated to its prevalence and its severity (4). Thus, it is noteworthy that the amount of research on intellectual disability is below the predicted level (4). Causes of ESSENCE range from severe social deprivation, genetic risk factors, metabolic diseases, immune disorders, infectious diseases, nutritional factors, physical trauma, and toxic and environmental factors. Among these factors, we have recently gained better knowledge concerning genetic risk factors, which is, in turn, motivating new neurobiological research.

The genetics of ESSENCE

The growing list of genes that contribute to early onset developmental disorders includes hundreds of genes. However, the complexity is multiplied by the observation that each patient can carry a specific combination of alleles of large and small effect that occur *de novo* or inherited.

De novo mutations in ESSENCE

De novo mutations include single base mutations, amplification of trinucleotide repeats, copy-number variations (CNVs), large chromosomal rearrangements and chromosomal aneuploidy (5). Chromosomal aneuploidy (an abnormal number of chromosomes) is observed in syndromic forms of neurodevelopmental disorders such as Down, Klinefelter or Turner syndromes. Large chromosomal rearrangements and CNVs can be recurrent in some regions of the genome such as on chromosome 22q11 (velocardiofacial syndrome), 15q (Angelman and Prader-Willi syndromes), or 17p (Smith-Magenis syndrome). However, in most cases, CNVs are unique to each patient, affecting from one to hundreds of genes. A trinucleotide repeat expansion of CGG repeats is observed in fragile X syndrome. This expansion upstream of the *FMR1* gene impedes its expression resulting in increased translation at the synapse. An additional example: single nucleotide mutations can affect X-linked genes such as *MECP2* to cause Rett syndrome or autosomal genes such as *CDH8* or *SHANK3* to cause ASD.

Highly penetrant *de novo* mutations probably account for a significant fraction (15–50%) of severe early onset developmental disorders (6, 7–13). This has been clearly demonstrated for ID (14) and ASD (11, 12, 15). The risk factors that increase the occurrence of *de novo* mutations, amplifications, deletions or duplications are better understood (16). For example, regions of the human genome flanked by large segmental duplications (such as on chromosome 15, 16p,) are more prone to be deleted/duplicated through illegitimate recombination. Increased paternal age was also shown to be a factor in *de novo* single base pair change. For ASD and ID, *de novo* chromosomal rearrangements and CNVs are more frequently observed in patients compared with controls. In contrast, patients and controls usually carry the same number of *de novo* single base mutations (on average 60–70 *de novo* mutations in each genome of 3 billion base pairs and one in each exome of 60 million base pairs). However, in patients, there is a significant increase, compared with controls, of damaging mutations (e.g. loss of function) in evolutionarily constrained genes expressed in the brain (Fig. 1) (11–13, 17).

The vast majority of mutations reported in patients with ASD were identified using DNA isolated from their blood (or from saliva in some projects). As a consequence, *de novo* somatic mutations occurring in specific brain cell lineage are missed (18, 19). Only studies using deep genomic sequencing and post-mortem brain tissues of the patients will be able to inform us as to whether somatic mutations in the brain are increased in early onset neurodevelopmental disorders.

Inherited monogenic and polygenic forms of ESSENCE

Among patients with early onset developmental disorders, inherited monogenic forms might account for a relatively significant fraction (>10%) (20). In ASD it was estimated that 3–6% of patients are "homozygous knock-out" carriers of two loss of function mutations in the same

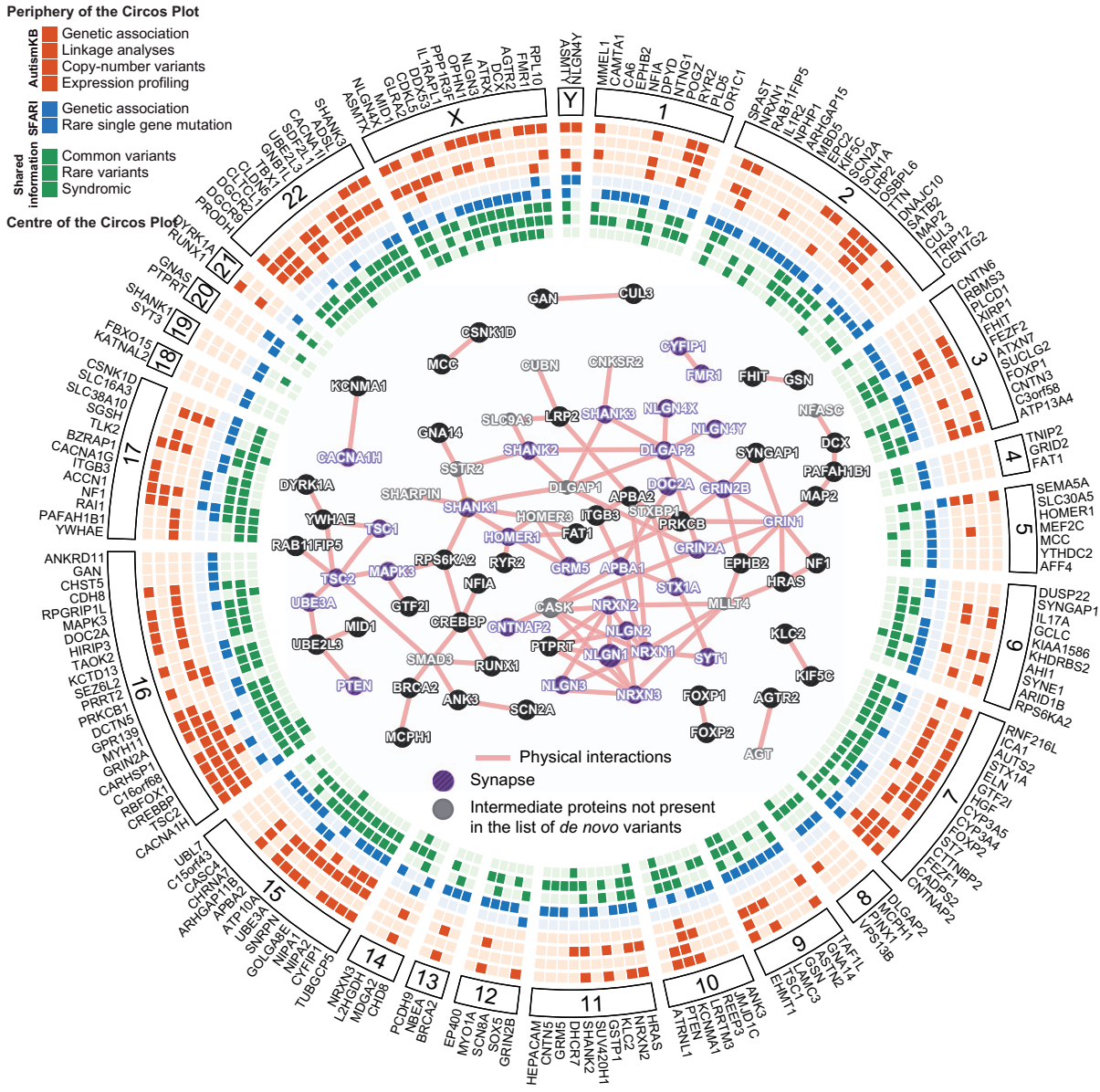


Fig. 1. Circos plot of *de novo* mutations in ASD. All coding-sequence variants and copy-number variants present in AutismKB and SFARI Gene are shown. A GeneMANIA network analysis (centre) highlights proteins with synaptic function (36% of the proteins have at least one interaction with another protein, 61% are expressed in the brain, and 14% are known to be involved in synaptic function). From Huguet et al. 2013 (36).

gene (21, 22). In countries with higher consanguinity, the impact of recessive mutations is likely to be higher (23).

Multiple hits in different regions of the genome might also contribute to susceptibility to early onset neurodevelopmental disorders. Several studies have demonstrated the presence of more than one deleterious mutation in such patients (24–26). In a large-scale study of 2312 children known to carry a CNV associated with ID and congenital abnormalities, 10% carried a second large CNV in addition to the primary genetic lesion (25). Children who carried two large CNVs of unknown clinical significance were eight times more likely than controls to have developmental delay than controls. Among affected children, inherited CNVs tended to co-occur with a second-site large CNV. No parental bias was observed for the primary *de novo* or inherited site, but for the second-site, 72% of the second-site CNVs were inherited from the mother (25).

Other studies have supported a multiple-hits model in patients carrying a similar "first hit". In 42 carriers of a 16p11.2 microdeletion, 10 carried an additional large copy-number variant, a significantly higher proportion when compared with controls conditional on a large first hit (10 of 42 cases, 21 of 471 controls; $P = 0.000057$, odds ratio = 6.6) (24). The clinical features of individuals

with two mutations were distinct from and/or more severe than those of individuals carrying only the co-occurring mutation. Another study showed that three patients with ASD carrying a *de novo* SHANK2 deletion were also carriers of a second CNV at the 15q11 locus (26). Two were carrying CNVs including *CHRNA7* and *ARHGAP11B*; the third was carrying a mutation that removed *CYFIP1*, *NIPA1*, *NIPA2*, and *TUBGCP5*. After this initial publication, another child with neurodevelopmental disorder carrying a *SHANK2* translocation and a *CHRNA7* duplication was reported (27).

Beside *de novo* and inherited rare mutations, one of the current challenges for geneticists is to identify the myriad of frequent alleles across the genome, which in an additive manner increase the risk of developing a disorder. Common variants could contribute to 17–60% and 25–30% of the heritability of ASD and ADHD, respectively (28–30). The same methodology was also used to estimate the contribution of genotyped single nucleotide polymorphisms (SNPs) in the heritability of the IQ (>40%) (31, 32) and on the human brain anatomy (50%) estimates that common variants might contribute to such quantitative phenotypes (Toro et al. Molecular Psychiatry, in press. Given that these common variants have individually only a weak additive effect (33), genome-wide association studies (GWAS) to date have been significantly underpowered and identified very few if any replicated common sequence variants that contribute to risk of early onset neurodevelopmental disorders (34). Based on these results, even if this genetic information is difficult to translate into clinical diagnosis, the identification of low risk alleles represents an important goal for understanding the genetic architecture of early onset neurodevelopmental disorders (35). Moreover, even weak alleles shown with confidence to influence disease risk, point to genes and pathways involved in pathogenesis.

Database of genes associated with ESSENCE

Several genetic databases provide clinical and functional annotation of genes associated with early onset neurodevelopmental disorders. The Online Mendelian Inheritance in Man (OMIM) database catalogues more than 5000 human genetic diseases (http://www.omim.org/). Decipher (http://decipher.sanger.ac.uk/) and the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home) are interactive Web-based databases which incorporate a suite of tools designed to aid the interpretation of genomic variants. Two databases of genes associated with ASD are updated regularly: AutismKB (http://autismkb.cbi.pku.edu.cn) and SFARI Gene (https://gene.sfari.org) (36). A total of 197 genes are included in both databases, and 481 are additionally included in either one or the other (255 in AutismKB and 226 in SFARI Gene). The main difference between the two databases concerns the selection of the genes. AutismKB usually selects genes from linkage analyses, copy-number variant studies, and GWAS, whereas SFARI Gene usually selects genes from copy-number variant studies, sequencing analyses of large cohorts, and case reports.

Biological pathways involved in ESSENCE

In the last 10 years, tremendous progress has been made in our comprehension of early onset developmental disorders. Animal models (37–47) as well as induced pluripotent stem cells (48, 49) have both contributed to better understanding of pathophysiology and to suggest new treatments. Understanding the symptoms and course for each individual, and the biology ranging from genetic and environmental risk factors to the neural circuits involved remains a substantial challenge for geneticists and neurobiologists (50–52).

Several pathway analyses have been performed using either genetic or transcriptome data to gain insight into the biological functions associated with ASD. Pinto et al. (53) recently analysed 2446 ASD-affected families and confirmed an excess of genic deletions and duplications in affected versus control groups (1.41-fold, $p = 0.00001$) and an increase in affected subjects carrying exonic pathogenic CNVs overlapping known loci associated with dominant or X-linked ASD and intellectual disability (odds ratio = 12.62, $p = 02.7 \times 10^{-15}$, ~3% of ASD subjects). Consistent with hypothesized sex-specific modulators of risk, female patients with ASD were more likely to have highly penetrant CNVs ($p = 0.017$) and were also overrepresented among subjects with mutations in genes that encode fragile X syndrome protein targets ($p = 0.02$) suggesting that severe genetic lesions were required to overcome the lower liability to ASD in girls. Genes affected by *de novo* CNVs and/or loss-of-function single-nucleotide variants converged on networks related to neuronal signalling and development, synaptic function, and chromatin regulation. Voineagu and colleagues (54) analysed genes that are differentially expressed between two brain regions (frontal and temporal lobes) in patients with ASD and controls. Interestingly, the typical regional differences between the gene expression profiles of the frontal and temporal lobes were attenuated in patients. A first network module was related to interneurons and to genes involved in synaptic function, and was down-regulated in brains from patients compared with those from controls; a second module was enriched for genes related to immunity and microglial activation, and was up-regulated in brains from patients with ASD compared with those of controls.

To date, five main pathways have been identified as candidates for early onset neurodevelopmental disorders (Fig. 2): chromatin remodelling, cytoskeleton dynamics, mRNA translation, metabolism and synapse formation/function. This list is, however, far from exhaustive.

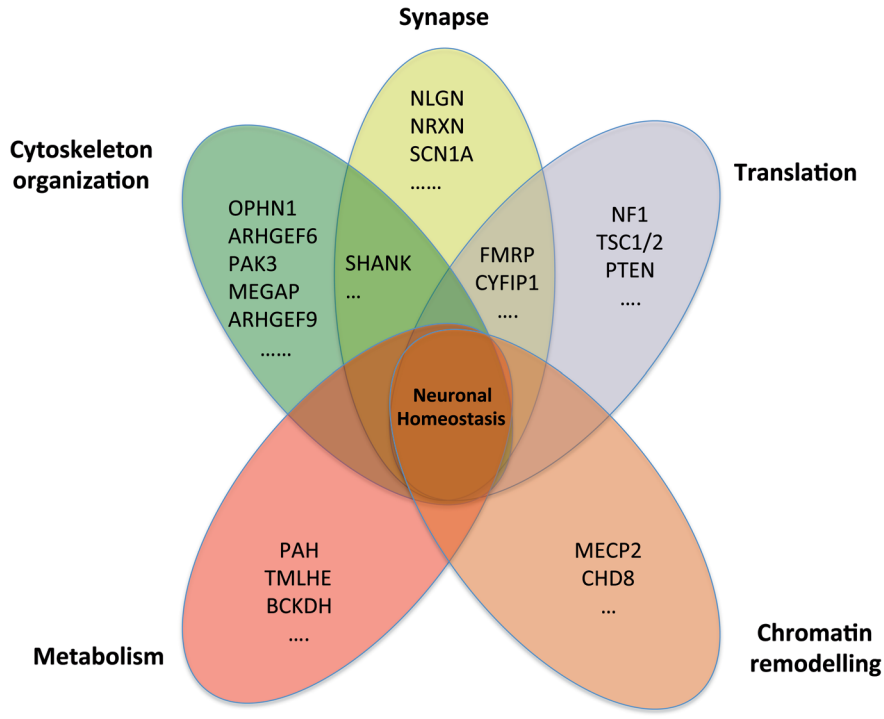


Fig. 2. Five pathways are associated with early onset neurodevelopmental disorders. For each pathway, examples of mutated genes are indicated.

The first pathway concerns chromatin remodelling and was suggested by reports of mutations in genes such as *MECP2* or *CDH8* in Rett syndrome and ASD, respectively (11, 12, 55, 56). A second pathway is related to metabolism and includes mutations in genes such as *PAH* in phenylketonuria, *BCKDH* in disorders of branched-chain amino acids, *TMLHE* in carnitine deficiency or *AGAT* and *GAMT* in creatine deficiency syndromes. Interestingly, patients with mild forms of inborn errors of metabolism may present with predominantly autistic symptoms (22). Identifying such mutations is of clinical importance since treatments may already be available (57). A third pathway is related to aberrant translation of mRNA encoding synaptic proteins, (58) and includes mutations affecting several proteins that normally inhibit translation through the PI3K-mTOR signalling pathway (TSC1, TSC2, NF1, and PTEN) as well as mutations affecting proteins directly involved in inhibiting mRNA translation at the synapse (FMRP, CYFIP1, and EIF4E) (58, 59). A fourth pathway concerns the actin cytoskeleton organization and includes mutations affecting OPHN1, ARHGEF6, PAK3, MEGAP, ARHGEF9 and the regulation of the RhoGTPase, the Ras, the Rab, the Arf and the JNK pathways (60). While mutations affecting these pathways were mostly identified in patients with ID, they might account for a fraction of patients with ASD (53). Finally, a fifth pathway is involved in synapse formation and excitation/inhibition balance (17, 61). Several genes associated with ASD, such as *NLGN3/4X*, *NRXN*, and *SHANK1-3*, appear to be involved in the formation of excitatory and inhibitory synapses (62–64). In addition, genes associated with epilepsy, such as *SCN1A*, which encodes a voltage-gated sodium channel, were also found mutated in patients with ASD (7).

While different, these pathways most likely affect neuronal homeostasis at the end point (17, 65). Some suggest potential drug targets; indeed some early clinical trials are ongoing to determine whether targeting some such proteins could improve the symptoms of patients (see (66, 67) for reviews).

Three propositions to improve research in the field of ESSENCE

While tremendous progress has been made in the understanding of the causes of early onset neurodevelopmental disorders, several issues detailed below represent potential breaks for research in this field. Three propositions are listed below.

Proposition 1: fewer categories, more dimensions

The recent advances in genomics have demonstrated that an identical genetic variant may increase the risk for a wide range of diagnoses formerly thought of as distinct (29, 68, 69). These findings are contributing to an ongoing re-conceptualization of the current psychiatric nosology. The use of epidemiological samples, studies grouping individuals based first on genetic findings, and efforts at combining existing categorical schema with dimensional phenotypes

and biomarkers, all promise to provide important new insights into the aetiology and classification of these disorders. DSM-5 now makes it easier to recognize overlap between different diagnostic categories, but in the main the existing narrow and rigid categories tend to disconnect researchers from the real phenotypes. Recently, several initiatives such as the ESSENCE from Christopher Gillberg were undertaken to improve phenotype characterization using more dimensional approaches. The Research Domain Criteria (RDoC) project has been launched by the US National Institute of Mental Health (NIMH), calling for the development, for research purposes, of new ways of classifying psychopathology based on dimensions of observable behaviour and neurobiological and genetic measures (http://www.nimh.nih.gov/research-priorities/rdoc/nimh-research-domain-criteria-rdoc.shtml). This effort is attempting to define basic dimensions of functioning related to known neural circuitry to be studied across multiple units of analysis, from genes to neural circuits to behaviours, cutting across disorders as traditionally defined.

In summary, it is most likely that progress in the comprehension of the risk factors for neurodevelopmental disorders will come from dimensional and quantitative data that goes well beyond current psychiatric classification. One first step would be to gather the information that is currently separated by DSM-5 diagnostic categories and dispersed in different laboratories that may fail to communicate. To achieve this, there is a need for more data sharing (see below).

Proposition 2: more research on genetic and brain diversity in human populations and more data sharing

Based on current case-control design, there is a tendency for researchers to know better the genotypes and phenotypes of the patients than those of the controls. Indeed, in the vast majority of genetic studies, controls are often not investigated at the phenotypic level, and in phenotypic studies, controls are very limited in their number and their cultural and socioeconomic status diversity (70). As a consequence, early onset developmental disorders are therefore considered as binary traits "affected" versus "non-affected" without taking into account the genetic and phenotypic diversity of both "affected" and "non-affected" individuals. The same is true for studies using transgenic mice; most of our knowledge is based on the effect of the mutations in C57BL6 mice. However, we know that mutations might produce a different phenotype in a different strain. The crucial role of the genetic background was very nicely illustrated in a recent paper showing the phenotypic consequence of the scalloped mutation in different strains of *Drosophila melanogaster* (71).

No progress could have been made in the genetics of neurodevelopmental disorders if thousands of genomes had not been sequenced to ascertain their genetic diversity. The same is true for human brains. The first initiatives of the Allen Brain Institute (http://www.brain-map.org) or the Sestan Laboratory (http://medicine.yale.edu/lab/sestan/index.aspx) are impressive in their description of human gene expression at very high resolution. However, if we want to better ascertain the links that exist between the variability of genomes and human brains, thousands of brains will need to be studied at the gene expression level as well as the functional level, even if this proves costly and difficult.

Integrating diversity into our experimental design will require an increase in the sample size of our study populations. Indeed, risk factors for early onset neurodevelopmental disorders are either rare with large effect or frequent but with a small effect (72). In both situations, robust genotype–phenotype relationships are difficult to ascertain in small samples. One opportunity to increase sample size is to foster data sharing. Many constraints reduce efficient data sharing (73). Hence, there is need 1) to agree on an ethical informed consent for research subjects that will allow data sharing; 2) to agree on standardized measures, 3) to change the reward system regarding publications, 4) to set up systems to make data sharing easy and secure.

There is an emerging community of researchers involved in data sharing. Specifically in neuroscience, initiatives such as the Neuroscience Information Framework (NIF) or the International Neuroinformatics Coordinating Facility (INCF) were launched recently. NIF (http://www.neuinfo.org) is a dynamic inventory of Web-based neuroscience resources: data, materials, and tools accessible via any computer connected to the Internet. This should advance neuroscience research by enabling discovery and access to public research data and tools worldwide through an open source, networked environment. INCF (http://www.incf.org/) develops collaborative neuroinformatics infrastructure and promotes the sharing of data and computing resources to the international research community. Neuroinformatics integrates information across all levels and scales of neuroscience to help understand the brain and treat disease. In addition to increasing sample size of the studies, these initiatives for more data sharing in the scientific community should also lead to a reduction of the important publication bias in the field of early onset neurodevelopmental disorders (74).

Proposition 3: patients and relatives as participants for research

Many aspects of the quality of life of patients and their relatives are not adequately taken into account by researchers. For example, in ASD, co-morbidities such as gastrointestinal and sensory problems are under-explored. The movement "no research about me, without me" is calling for patients and their relatives to be more involved in research designs. For example, the UK National health Service (NHS) initiative INVOLVE (http://www.invo.org.

uk) is a national advisory group that supports greater public involvement in NHS, public health and social care research. There is also the James Lind Alliance (http://www.lindalliance.org), the Patient-Centered Outcomes Research Institute (PCORI) (http://www.pcori.org) and PatientsLikeMe (www.patientslikeme.org) initiatives for patients who want to monitor their own health and chronic illness. Using such frameworks, patients can propose and conduct their own studies among memberships – with some successes to report already. For example, a trial of lithium for amyotrophic lateral sclerosis (ALS) was completed faster than randomized control trials (RCTs) (75). In this study, PatientsLikeMe reached exactly the same conclusion as previous RCTs suggesting that data reported by patients over the Internet may be useful for accelerating clinical discovery and evaluating the effectiveness of drugs already in use. Another example is the initiative for cancer research at Sage Bionetworks (http://sagebase.org). Sage develops tools so that medical patients can keep their own data rather than storing it in particular medical institutions. The aim is to offer predictive, personalized, preventive, participatory (P4) cancer medicine (76). These types of initiatives require "the creation of new types of strategic partnerships – between patients, large clinical centers, consortia of clinical centers and patient-advocate groups. For some clinical trials it will be necessary to recruit very large numbers of patients – and one powerful approach to this challenge is the crowd-sourced recruitment of patients by bringing large clinical centers together with patient-advocacy groups. p. 184" (76)

Perspectives: towards a more inclusive world

For patients, the burden of neurodevelopmental disorders makes daily activities difficult and lowers the odds of living an independent life. Progress on the causes of neurodevelopmental disorders hopefully will provide knowledge-based treatments to improve quality of life for those affected. Nevertheless, in addition to improved medical care, innovative initiatives towards a more inclusive world point towards other important advances. For example, Aspiritech, a non-profit organization based in Highland Park, Illinois, USA, places people who have autism (mainly Asperger's syndrome) in jobs testing software (http://www.aspiritech.org). The Danish company Specialisterne has helped more than 170 individuals with autism obtain jobs since 2004. Its parent company, the Specialist People Foundation, aims to connect one million autistic people with meaningful work (http://www.specialistpeople.com). Laurent Mottron, a psychologist working in Montreal, has offered jobs for patients with ASD in his group and this new perspective has had a positive impact on his research on autism. As he said "The hallmark of an enlightened society is its inclusion of non-dominant behaviours and phenotypes, such as homosexuality, ethnic differences and disabilities. Governments have spent time and money to accommodate people with visual and hearing impairments, helping them to navigate public places and find employment, for instance – we should take the same steps for autistics" (77). As suggested by Waterhouse and Gillberg, it might be better to abandon the belief that there is a single defining ASD brain dysfunction (78). Instead, we should understand the diversity of ASD (or autismS). Considering autism not as a single entity, but as a continuum of human diversity and tackling this heterogeneity using information coming from different fields of research (including direct information from the affected individuals and their families (79)) should allow a better diagnostic, care and integration of individuals with autism (77).

Acknowledgements—I want to thank Steve Hyman and Roberto Toro for their helpful reading of the manuscript. This work was funded by the Institut Pasteur, the Bettencourt-Schueller Foundation, Centre National de la Recherche Scientifique, University Paris Diderot, Agence Nationale de la Recherche (ANR-13-SAMA-0006; SynDivAutism), the Conny-Maeva Charitable Foundation, the Cognacq Jay Foundation, the Orange Foundation, and the Fondation FondaMental.

Disclosure of interest: The author declares that there are no conflicts of interest. The author alone is responsible for the content and writing of the paper.

References

1. Gillberg C. The ESSENCE in child psychiatry: early symptomatic syndromes eliciting neurodevelopmental clinical examinations. Res Dev Disabil 2010;31:1543–51.
2. Developmental Disabilities Monitoring Network Surveillance Year Principal I. Prevalence of autism spectrum disorder among children aged 8 years – autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ 2014;63(Suppl2):S1–21.
3. Perou R, Bitsko RH, Blumberg SJ, Pastor P, Ghandour RM, Gfroerer JC, et al. Mental health surveillance among children – United States, 2005–2011. MMWR Surveill Summ 2013;62(Suppl2):S1–35.
4. Bishop DV. Which neurodevelopmental disorders get researched and why? PLoS One 2010;5:e15112.
5. Hoischen A, Krumm N, Eichler EE. Prioritization of neurodevelopmental disease genes by discovery of new mutations. Nat Neurosci 2014;17:764–72.
6. Krumm N, O'Roak BJ, Shendure J, Eichler EE. A de novo convergence of autism genetics and molecular neuroscience. Trends Neurosci 2014;37:95–105.
7. O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. Nat Genet 2011;43:585–9.
8. Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, et al. Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 2011;70:863–85.
9. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, et al. De novo gene disruptions in children on the autistic spectrum. Neuron 2012;74:285–99.
10. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, et al. Rate of de novo mutations and the importance of father's age to disease risk. Nature 2012;488(7412):471–5.

11. Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, et al. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 2012;485(7397):242–5.
12. O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 2012;485(7397):246–50.
13. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 2012;485(7397):237–41.
14. Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, Willemsen MH, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature 2014;511(7509):344–7.
15. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 2012;485(7397):237–41.
16. Campbell CD, Eichler EE. Properties and rates of germline mutations in humans. Trends Genet 2013;29:575–84.
17. Toro R, Konyukh M, Delorme R, Leblond C, Chaste P, Fauchereau F, et al. Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. Trends Genet 2010;26:363–72.
18. Frank SA. Somatic mosaicism and disease. Curr Biol 2014;24:R577–81.
19. Poduri A, Evrony GD, Cai X, Walsh CA. Somatic mutation, genomic variation, and neurological disease. Science 2013;341:1237758.
20. Zhu X, Need AC, Petrovski S, Goldstein DB. One gene, many neuropsychiatric disorders: lessons from Mendelian diseases. Nat Neurosci 2014;17:773–81.
21. Lim ET, Raychaudhuri S, Sanders SJ, Stevens C, Sabo A, MacArthur DG, et al. Rare complete knockouts in humans: population distribution and significant role in autism spectrum disorders. Neuron 2013;77:235–42.
22. Yu TW, Chahrour MH, Coulter ME, Jiralerspong S, Okamura-Ikeda K, Ataman B, et al. Using whole-exome sequencing to identify inherited causes of autism. Neuron 2013;77:259–73.
23. Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS, et al. Identifying autism loci and genes by tracing recent shared ancestry. Science 2008;321:218–23.
24. Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, Itsara A, et al. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. Nat Genet 2010;42:203–9.
25. Girirajan S, Rosenfeld JA, Coe BP, Parikh S, Friedman N, Goldstein A, et al. Phenotypic heterogeneity of genomic disorders and rare copy-number variants. N Engl J Med 2012;367:1321–31.
26. Leblond CS, Heinrich J, Delorme R, Proepper C, Betancur C, Huguet G, et al. Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders. PLoS Genet 2012;8:e1002521.
27. Chilian B, Abdollahpour H, Bierhals T, Haltrich I, Fekete G, Nagel I, et al. Dysfunction of SHANK2 and CHRNA7 in a patient with intellectual disability and language impairment supports genetic epistasis of the two loci. Clin Genet 2013;84:560–5.
28. Klei L, Sanders SJ, Murtha MT, Hus V, Lowe JK, Willsey AJ, et al. Common genetic variants, acting additively, are a major source of risk for autism. Mol Autism 2012;3:9.
29. Cross-Disorder Group of the Psychiatric Genomics Consortium,Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet 2013;45:984–94.
30. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, et al. Most genetic risk for autism resides with common variation. Nat Genet 2014;46:881–5.
31. Deary IJ, Yang J, Davies G, Harris SE, Tenesa A, Liewald D, et al. Genetic contributions to stability and change in intelligence from childhood to old age. Nature 2012;482(7384):212–15.
32. Davies G, Tenesa A, Payton A, Yang J, Harris SE, Liewald D, et al. Genome-wide association studies establish that human intelligence is highly heritable and polygenic. Mol Psychiatry 2011;16:996–1005.
33. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. Nat Genet 2010;42:565–9.
34. Anney R, Klei L, Pinto D, Almeida J, Bacchelli E, Baird G, et al. Individual common variants exert weak effects on the risk for autism spectrum disorderspi. Hum Mol Genet 2012;21:4781–92.
35. Gratten J, Wray NR, Keller MC, Visscher PM. Large-scale genomics unveils the genetic architecture of psychiatric disorders. Nat Neurosci 2014;17:782–90.
36. Huguet G, Ey E, Bourgeron T. The genetic landscapes of autism spectrum disorders. Annu Rev Genomics Hum Genet 2013;14:191–213.
37. Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, et al. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. Nature 2011;472(7344):437–42.
38. Bozdagi O, Sakurai T, Papapetrou D, Wang X, Dickstein DL, Takahashi N, et al. Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. Mol Autism 2010;1:15.
39. Won H, Lee H-R, Gee HY, Mah W, Kim J-I, Lee J, et al. Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. Nature 2012;486(7402):261–5.
40. Schmeisser MJ, Ey E, Wegener S, Bockmann J, Stempel AV, Kuebler A, et al. Autistic-like behaviours and hyperactivity in mice lacking ProSAP1/Shank2. Nature 2012;486(7402):256–60.
41. Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, et al. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 2007;318:71–6.
42. Jamain S, Radyushkin K, Hammerschmidt K, Granon S, Boretius S, Varoqueaux F, et al. Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. Proc Natl Acad Sci USA. 2008;105:1710–15.
43. Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K, et al. Neuroligins determine synapse maturation and function. Neuron 2006;51:741–54.
44. Baudouin SJ, Gaudias J, Gerharz S, Hatstatt L, Zhou K, Punnakkal P, et al. Shared synaptic pathophysiology in syndromic and nonsyndromic rodent models of autism. Science 2012;338:128–32.
45. Han K, Holder JL Jr, Schaaf CP, Lu H, Chen H, Kang H, et al. SHANK3 overexpression causes manic-like behaviour with unique pharmacogenetic properties. Nature 2013;503(7474):72–7.
46. Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. Nat Rev Neurosci 2010;11:490–502.
47. Ey E, Leblond CS, Bourgeron T. Behavioral profiles of mouse models for autism spectrum disorders. Autism Res 2011;4:5–16.
48. Shcheglovitov A, Shcheglovitova O, Yazawa M, Portmann T, Shu R, Sebastiano V, et al. SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients. Nature 2013;503(7475):267–71.
49. Boissart C, Poulet A, Georges P, Darville H, Julita E, Delorme R, et al. Differentiation from human pluripotent stem cells of cortical neurons of the superficial layers amenable to psychiatric disease modeling and high-throughput drug screening. Transl Psychiatry 2013;3:e294.
50. Willsey AJ, Sanders SJ, Li M, Dong S, Tebbenkamp AT, Muhle RA, et al. Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. Cell 2013;155:997–1007.
51. Parikshak NN, Luo R, Zhang A, Won H, Lowe JK, Chandran V, et al. Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. Cell 2013;155:1008–21.
52. Gokhale A, Larimore J, Werner E, So L, Moreno-De-Luca A, Lese-Martin C, et al. Quantitative proteomic and genetic analyses of the schizophrenia susceptibility factor dysbindin identify novel roles of the biogenesis of lysosome-related organelles complex 1. J Neurosci 2012;32:3697–711.
53. Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, et al. Functional impact of global rare copy number variation in autism spectrum disorders. Nature 2010;466(7304):368–72.
54. Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 2011;474(7351):380–4.
55. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 1999;23:185–8.

56. O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science 2012;338: 1619–22.
57. Novarino G, El-Fishawy P, Kayserili H, Meguid NA, Scott EM, Schroth J, et al. Mutations in BCKD-kinase lead to a potentially treatable form of autism with epilepsy. Science 2012;338:394–7.
58. Kelleher RJ, III, Bear MF. The autistic neuron: troubled translation? Cell 2008;135:401–6.
59. Costa-Mattioli M, Monteggia LM. mTOR complexes in neurodevelopmental and neuropsychiatric disorders. Nat Neurosci 2013;16:1537–43.
60. Ba W, van der Raadt J, Nadif Kasri N. Rho GTPase signaling at the synapse: implications for intellectual disability. Exp Cell Res 2013;319:2368–74.
61. Bourgeron T. A synaptic trek to autism. Curr Opin Neurobiol 2009;19:231–4.
62. Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, et al. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat Genet 2003;34:27–9.
63. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 2007;39:25–7.
64. Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat Genet 2007;39:319–28.
65. Ramocki MB, Zoghbi HY. Failure of neuronal homeostasis results in common neuropsychiatric phenotypes. Nature 2008;455(7215): 912–18.
66. Spooren W, Lindemann L, Ghosh A, Santarelli L. Synapse dysfunction in autism: a molecular medicine approach to drug discovery in neurodevelopmental disorders. Trends Pharmacol Sci 2012;33:669–84.
67. Delorme R, Ey E, Toro R, Leboyer M, Gillberg C, Bourgeron T. Progress toward treatments for synaptic defects in autism. Nat Med 2013;19:685–94.
68. Moreno-De-Luca A, Myers SM, Challman TD, Moreno-De-Luca D, Evans DW, Ledbetter DH. Developmental brain dysfunction: revival and expansion of old concepts based on new genetic evidence. Lancet Neurol 2013;12:406–14.
69. Kim YS, State MW. Recent challenges to the psychiatric diagnostic nosology: a focus on the genetics and genomics of neurodevelopmental disorders. Int J Epidemiol 2014;43:465–75.
70. Manly JJ. Critical issues in cultural neuropsychology: profit from diversity. Neuropsychol Rev 2008;18:179–83.
71. Chari S, Dworkin I. The conditional nature of genetic interactions: the consequences of wild-type backgrounds on mutational interactions in a genome-wide modifier screen. PLoS Genet 2013;9:e1003661.
72. McCarroll SA, Feng G, Hyman SE. Genome-scale neurogenetics: methodology and meaning. Nat Neurosci 2014;17:756–63.
73. Poline JB, Breeze JL, Ghosh S, Gorgolewski K, Halchenko YO, Hanke M, et al. Data sharing in neuroimaging research. Front Neuroinform 2012;6:9.
74. Joober R, Schmitz N, Annable L, Boksa P. Publication bias: what are the challenges and can they be overcome? J Psychiatry Neurosci 2012;37:149–52.
75. Wicks P, Vaughan TE, Massagli MP, Heywood J. Accelerated clinical discovery using self-reported patient data collected online and a patient-matching algorithm. Nat Biotechnol 2011;29:411–14.
76. Hood L, Friend SH. Predictive, personalized, preventive, participatory (P4) cancer medicine. Nat Rev Clin Oncol 2011;8:184–7.
77. Mottron L. Changing perceptions: The power of autism. Nature 2011;479(7371):33–5.
78. Waterhouse L, Gillberg C. Why autism must be taken apart. J Autism Dev Disord 2014;44:1788–92.
79. Kohane IS, Eran A. Can we measure autism? Sci Transl Med 2013;5:209ed18.

Thomas Bourgeron, Human Genetics and Cognitive Functions Unit, Institut Pasteur, Paris, France, CNRS UMR3571 Genes, Synapses and Cognition, Institut Pasteur, Paris, France, Human Genetics and Cognitive Functions, University Paris Diderot, Sorbonne Paris Cité, Paris, France, Fondation FondaMental, Créteil, France, Gillberg Neuropsychiatry Centre, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Research in Developmental Disabilities 31 (2010) 1543–1551

Contents lists available at ScienceDirect

Research in Developmental Disabilities

The ESSENCE in child psychiatry: Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations☆

Christopher Gillberg

Institute of Neuroscience and Physiology, Child and Adolescent Psychiatry, University of Göteborg, Sweden

ARTICLE INFO

Article history:
Received 7 May 2010
Received in revised form 25 May 2010
Accepted 4 June 2010

Keywords:
Autism
ADHD
Developmental disorders
Learning disability
ESSENCE

ABSTRACT

Co-existence of disorders – including attention-deficit/hyperactivity disorder, oppositional defiant disorder, tic disorder, developmental coordination disorder, and autism spectrum disorder – and sharing of symptoms across disorders (sometimes referred to as comorbidity) is the rule rather than the exception in child psychiatry and developmental medicine. The acronym ESSENCE refers to Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations. It is a term I have coined to refer to the reality of children (and their parents) presenting in clinical settings with impairing child symptoms before age 3 (−5) years in the fields of (a) general development, (b) communication and language, (c) social inter-relatedness, (d) motor coordination, (e) attention, (f) activity, (g) behaviour, (h) mood, and/or (i) sleep. Children with major difficulties in one or more (usually several) of these fields, will be referred to and seen by health visitors, nurses, social workers, education specialists, pediatricians, GPs, speech and language therapists, child neurologists, child psychiatrists, psychologists, neurophysiologists, dentists, clinical geneticists, occupational therapists and physiotherapists, but, usually they will be seen only by one of these specialists, when they would have needed the input of two or more of the experts referred to. Major problems in at least one ESSENCE domain before age 5 years often signals major problems in the same or overlapping domains years later. There is no time to wait; something needs to be done, and that something is unlikely to be just in the area of speech and language, just in the area of autism or just in special education.

1. Introduction

While in the past, child psychiatry had little interest in operationalised diagnosis, recent trends have made categorical diagnosis an integral part of everyday clinical and research practice (Sonuga-Barke, 2009). So focused are we now on the dichotomous distinction between disorder and not disorder that clinics become more and more specialised and cater to the needs of children with "autism only", "attention-deficit/hyperactivity disorder/ADHD only" or "Tourette syndrome only". This has led to a situation in which the diffuseness of disorder has come to be underestimated.

At the same time, rather belatedly, there is a growing realisation that co-existence of disorders and sharing of symptoms across disorders (so called comorbidity, a misnomer if ever there was one) is the rule rather than the exception (e.g. Kadesjö & Gillberg, 2001). I pointed this out more than a quarter of a century ago (Gillberg, 1983), but, in clinical practice, this insight has not led to new approaches to addressing the needs of children and families with "complex needs". Instead, diversification has boomed.

☆ Presented as The Blake Marsh Lecture at the RCPsych meeting in Liverpool, June 2009.

0891-4222/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ridd.2010.06.002

There are legislational, scientific, and clinical attempts to separate out children with autism spectrum disorders (ASD) from those who do not have ASD, all aiming to provide better societal guidelines, more focused attempts at finding the causes, and autism-specific services. Children with ADHD are targeted in similar ways, even though legislation has yet to catch up with them. The same holds for children with language impairments (often erroneously referred to as "specific" language impairment (SLI); erroneous because the impairments are almost never specific), visual impairments and hearing deficits (children who may, or may not, have additional impairments as regards general cognition, motor performance, ASD or ADHD).

There is good evidence that ASD and ADHD can be separate and recognisable "disorders", but, equally, there is mounting evidence that they often overlap, constitute amalgams of problems, and that in some families they separate together and probably represent different aspects of the same underlying disorder (Reiersen, Constantino, Volk, & Todd, 2007).

With growing insight that early onset childhood problems, such as those reflected in children who are diagnosed in early childhood as suffering from ASD or ADHD, have long-term, indeed probably often, lifetime consequences (Billstedt, Gillberg, & Gillberg, 2005; Cederlund, Hagberg, & Gillberg, 2010; Rasmussen & Gillberg, 2000), the incentive to screen and diagnose these conditions has become a main priority for clinicians and administrators hoping to alter the often negative course inherent in cases who have had little or no intervention (or indeed an exclusionary attitude on the part of those "responsible") during the course of growing up. The question to be addressed is: would making discrete diagnosis (of, say, ASD or ADHD) before age 5 years contribute to a better understanding, better intervention, and more positive outcome in children who present with problems that potentially could be indices of these disorders.

2. What is ESSENCE?

The acronym ESSENCE refers to Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations. It is a term I have coined to refer to the reality of children (and their parents) presenting in clinical settings with impairing child symptoms before age 3 (−5) years in the fields of (a) general development, (b) communication and language, (c) social interrelatedness, (d) motor coordination, (e) attention, (f) activity, (g) behaviour, (h) mood, and/or (i) sleep. Children with major difficulties in one or more (usually several) of these fields, will be referred to and seen by health visitors, nurses, social workers, education specialists, pediatricians, GPs, speech and language therapists, child neurologists, child psychiatrists, psychologists, neurophysiologists, dentists, clinical geneticists, occupational therapists and physiotherapists, but, in the vast majority of cases they will be seen only by one of these specialists, when, in fact, they would have needed the input of two or more (occasionally even all) of the experts referred to.

The syndromes encompassed under the ESSENCE umbrella acronym are listed in Table 1.

Most of these syndromes are conceptualised as more or less discrete disorders in the DSM-IV-TR, and in the ICD-10. Here, they are listed, not because I believe they exist "in their own right" (even though occasionally they do show up as isolated conditions in individuals), but because they currently drive development in the whole field of child health, and all of them have links to one or more of the other conditions on the list.

2.1. An example from the field of SLI

In a recent population study, Miniscalco identified 25 children with "specific language impairment" (SLI) at age 2.5 years (Miniscalco, Nygren, Hagberg, Kadesjö, & Gillberg, 2006). They had been screened by child health nurses and had both screened positive (on one or more of the items (i) fewer than twenty-five communicative words, (ii) comprehension difficulties, (iii) articulation difficulties) *and* been deemed to have some degree of speech and language impairment after formal testing made by a pediatric speech and language therapist (SLT). They were contrasted with 80 children from the general population without SLI and followed as regards speech and language development for a period of 5 years (seen by an SLT at ages 4, 6 and 7.5 years). When they were 7.5 years they were, in addition, examined by a neuropsychiatric team, who remained blind to the original assessments. At this age, more than 70% of the children with SLT had ASD, ADHD,

Table 1
Syndromes encompassed under the ESSENCE acronym.

Syndrome	Prevalence	Key reference
ASD/PDD	1%	Gillberg and Wing (1999)
ADHD	5%	Swanson, Wigal, and Lakes (2009)
ODD	4%	Pliszka (2000)
SLI	6%	Miniscalco et al. (2006)
LD	1.5%	Gillberg and Söderström (2003)
NVLD	?	Rourke (1988)
Tic disorders/Tourette syndrome	1%	Comings (1990)
Bipolar disorder	?	Biederman et al. (2003)
Behavioural phenotype syndromes	.7%	O'Brien (2000)
Rare epilepsy syndromes	.01%	Aicardi, Bax, and Gillberg (2009)
Reactive attachment disorder	?	Minnis et al. (2009)
Total taking overlap into account	7–10%	Gillberg (1995)

mental retardation, or borderline learning disability (or combinations of these). None of them had been suspected of having any of these problems at the original diagnosis of SLI. By age 4 and 6 years, only a small fraction had been recognised to suffer from ASD or ADHD, and an even smaller proportion had received appropriate interventions for such problems.

What can we conclude on the basis of these and similar findings from previous studies? Children with SLI at 2.5 years are a large group—several per cent are affected according to UK and Swedish studies (Law et al., 2006; Miniscalco, Westerlund, & Lohmander, 2005). When a child is recognised as having SLI in a child health setting he/she is usually referred for a hearing test and assessment and possibly speech and language therapy to a pediatric SLT. The results of the study referred to indicate that this might not be appropriate. It would probably be reasonable to characterise the problem signalled by the SLI as belonging to the ESSENCE group and refer the child for multidisciplinary evaluation by a community pediatrician, a psychologist *and* an SLT.

2.2. An example from the field of ASD

Two decades ago, our group demonstrated that autism diagnoses made before age 3 years were relatively stable over time, 75% still meeting criteria for ASD at follow-up years later (Gillberg et al., 1990). However, in 25% this was not the case, but all the children in this latter group met criteria for another developmental disorder, such as non-autism learning disability or ADHD. Other groups (e.g. Chawarska, Klin, Paul, Macari, & Volkmar, 2009) have found similar results. In a new study of more than 300 preschool children with a clinical diagnosis of ASD, the vast majority met research DSM-IV criteria for autistic disorder, Asperger's disorder, or pervasive developmental disorder not otherwise specified at follow-up after 2 years. However, about one in 10 was not diagnosed with ASD, but had other developmental disorder diagnoses, such as non-autism learning disability or ADHD (Fernell et al., 2010). Rates of speech and language problems, ADHD, DCD, gastrointestinal problems, epilepsy, and learning disability in the ASD group varied from about 10% to 60%, but this had not been revealed in connection with the original clinical diagnosis of ASD. The findings provide good support for the notion that these were children suffering from ESSENCE, and, depending on the inclination, interest, and training of the professional first seeing the child because of ESSENCE problems, the child may first have been diagnosed with SLI, ADHD, learning disability or ASD, and any number of the comorbid problems might have been missed.

2.3. The early symptoms of ESSENCE

The "typical" symptoms of ESSENCE are listed in Table 2. These symptoms should not be seen as "specific" for ESSENCE. Rather, they should be considered markers for the (very likely) presence of a neurodevelopmental disorder that (very likely) will continue to cause symptoms long after their clinical surfacing in the first few years of life.

3. Scope of the problem

The estimated prevalence rates of the syndromes subsumed under the ESSENCE acronym are listed in Table 1. Most of the disorders listed have been epidemiologically surveyed during the early or middle school ages, and only a few have been the subject of prevalence studies in the preschool years. Even though all of the syndromes are present (and usually symptomatic) from the preschool years, many cases will not have come to the attention of clinicians before school age. Thus, the sum prevalence of about 10% of the general population of children suffering from these syndromes, may not reflect how many children come to clinical attention during the preschool period. On the basis of preschool studies of ASD, ODD and ADHD (Fernell et al., 2010; Kadesjö, Hägglöf, Kadesjö, & Gillberg, 2003), a reasonable estimate would be that about 5–7% of children under age 6 years would meet "criteria" for ESSENCE (i.e. have clinical symptoms of a syndrome and have presented at a clinic with a view to diagnosis and intervention). Boys would be extremely overrepresented in this group, even though they probably would not outnumber girls by more than 2–3:1 had parents, teachers and clinicians been more aware that girls with ASD, ADHD, ODD, and SLI, while meeting full criteria for these disorders, might have a slightly/clearly different pattern

Table 2
Symptoms (causing major impairment) signalling ESSENCE in the first 4 years of life.

Symptom	Reference
Motor abnormality	Gillberg (1995)
General developmental delay	Gillberg (1995)
Speech and language delay	Law et al. (2006)
Social interaction/communication problems	Wing (2005)
Behaviour problems	Richman, Stevenson, and Graham (1975)
Hyperactivity/impulsivity	Spencer, Biederman, and Mick (2007)
Hypoactivity	Lundervold, Posserud, Sørensen, Ullebø, and Gillberg (submitted for publication)
Inattention/does not listen	Bishop et al. (1999)
Sleep problems	Stores (2006)
Feeding difficulties	McDougall, Drewett, Hungin, and Wright (2009)

of comorbidity (Kopp, Berg Kelly, & Gillberg, 2009; Mahone & Wodka, 2008; Pinkhardt et al., 2009). Girls, as a group, tend to be less violent, less motorically active, more socially adept, and better at using language skills for communication. All of these factors contribute to masking the early symptomatic presentation of disorders such as ASD, and ADHD. With better awareness about the presence of such disorders in preschool girls, more and more female cases are likely to come to attention over the next several years.

4. ASD

ASD is no longer considered a rare condition (Baird et al., 2006), rather, its prevalence during the school years is believed to be slightly higher than 1% of the general population of children. Boys are clearly much more often affected than girls, at least if we are referring to the clinically impairing variant of the autism phenotype. Skuse (2009) has argued that the autism phenotype might be equally common in males and females, and that other factors are responsible for the large discrepancy in male:female ratios seen in clinical and epidemiological populations. However, others (including Baron-Cohen, 2005) have proposed that the autistic phenotype is an expression of the "extreme male brain", which would make the male preponderance in ASD a very real thing and not due to gender roles, comorbidity or other factors making boys more likely to be diagnosed with the condition.

ASD is a group of multifactorially determined conditions, and there are almost as many different causes as there are cases (Gillberg & Coleman, 2000). The prefrontal, temporal, brainstem and cerebellar regions of the central nervous system are usually affected. These areas constitute a functional network, "the default network", which appears to be critically differently functioning in ASD (Buckner & Vincent, 2007; Iacoboni, 2006; Monk et al., 2009). ASD with some degree of cognitive impairment is probably associated with life-long disability in the vast majority of cases (e.g. Billstedt et al., 2005), but it is unclear to what extent higher functioning individuals with ASD (including the group with Asperger syndrome) continue to show pervasive impairments in adult life (e.g. Cederlund et al., 2010), even though there are indications that persistence of some problems throughout life are more common than not. There is now good evidence that early intensive training programmes have lasting beneficial effects on a number of aspects of the disorder.

ASD is almost never an isolated phenomenon. Co-existing problems and disorders are the rule. These include learning disability (including non-verbal learning disability), epilepsy, motor control problems, ADHD, depression, and anxiety, gastrointestinal problems, and sleep disorders. These problems and disorders are sometimes the reason for referral to a specialist for evaluation. For instance, it is not uncommon for an extremely hyperactive child to be referred for evaluation of ADHD, but the full appraisal, once considered, will reveal that the child's main diagnosis is ASD, and it may, or may not, be motivated to diagnose co-existing ADHD.

5. ADHD (and oppositional-defiant disorder)

ADHD (with or without ODD) is a very common condition, affecting at least 5% of school age children (Faraone, Sergeant, Gillberg, & Biederman, 2003). In about 60% of the cases it is associated with ODD, which is usually symptomatic already around 3 years of age (Kadesjö et al., 2003). Again, boys are affected much more often than girls, and, particularly in the preschool period it is unusual for a girl to be recognised as having the condition (unless it is in the context of having another diagnosis, such as ASD or learning disability). It appears that at least half of individuals diagnosed in childhood with ADHD continue to have impairing ADHD in adult life, and that the majority have some remaining problems, even if they do not meet full criteria for "clinical" ADHD (Dopheide & Pliszka, 2009; Rasmussen & Gillberg, 2000). There is evidence that several aspects of the disorder can be positively affected by short- and long-term interventions combining a psychoeducational and pharmacological approach (Ghuman, Arnold, & Anthony, 2008; Vaughan, Fegert, & Kratochvil, 2009). There are indications, that at least when it comes to certain associated conditions (such as ASD), "comorbidity" needs to influence intervention choice in important ways in order to achieve the best possible outcome (Ollendick, Jarrett, Grills-Taquechel, Hovey, & Wolff, 2008). Preschool ODD, perhaps the most common of all the associated problems in the field of ADHD, indicates a much increased risk that the child may go on to develop conduct disorder, which, in turn, is a strong predictor of later antisocial personality disorder. Recognising and intervening for ODD in ADHD would probably ameliorate prognosis in a number of cases. Similarly, recognising and intervening for DCD in ADHD, has the potential of improving outcome even further. DCD in ADHD is also a strong predictor/marker for associated ASD (Kadesjö & Gillberg, 1999).

ADHD is largely genetic (Curatolo, Paloscia, D'Agati, Moavero, & Pasini, 2009), but a very similar phenotype can develop after various types of brain damage/environmentally caused brain dysfunction (Strang-Karlsson et al., 2008). The brain develops differently in children with ADHD than in typically developing children, with loss of the prefrontal component of normal asymmetrical brain development (Shaw et al., 2009). There is also growing evidence that the brain's dopamine-dependent reward system is dysfunctional in ADHD (Volkow et al., 2009). Interestingly, there is now good evidence that ASD and ADHD are clearly related in some families, and that CNS connectivity genes involved in ASD may also be relevant for the development of ADHD symptoms (Kopp et al., 2009; Mulligan et al., 2009; Sharp, McQuillin, & Gurling, 2009).

ADHD, like ASD, is usually not a discrete disorder. Instead, even in the community, not just in clinics dealing with severely impaired individuals, "co-morbidity" is the rule (Kadesjö & Gillberg, 2001). ODD, DCD, depression, anxiety, ASD, substance use disorder, and conduct disorder are all relatively or very common co-existing disorders.

6. Learning disability, non-verbal learning disability, and dyslexia

Learning problems, including learning disability, borderline intellectual functioning, non-verbal learning disability, and precursors of dyslexia (including phonological awareness problems) are common in the preschool period, and affect several per cent of both boys and girls. More often than not, such learning problems co-exist with other neurodevelopmental/neuropsychiatric disorders, such as ADHD, ASD and ODD. There is currently a clinical diagnostic substitution trend, at least in the UK, Scandinavia, and the US (Bishop, Whitehouse, Watt, & Line, 2008; Coo et al., 2008; Fernell et al., 2010; Howlin, 2008), leading to fewer children being diagnosed with learning disability and more being labelled as suffering from ASD. The problem with this trend is that the very real learning problems suffered by many individuals with ASD and ADHD may go undiagnosed for long periods of time. In the past, the opposite was often true. Non-verbal learning disability is common in Asperger syndrome (Cederlund & Gillberg, 2004; Klin, Volkmar, Sparrow, Cicchetti, & Rourke, 1995), but often not recognised, much less diagnosed. This is unhelpful for those who are clearly impaired by "both conditions". Many individuals with Asperger syndrome – and their parents and teachers – benefit greatly from a better understanding of the particular neuropsychological profile (with its characteristic peaks and troughs) associated with non-verbal learning disability. The reverse is also true, and Asperger syndrome is often missed by neuropsychologists who specialise in non-verbal learning disability. Phonological awareness problems, a common precursor of dyslexia, are frequent in ADHD (with or without associated autistic symptoms), but are often missed, once the "overshadowing" diagnosis of ADHD/ASD has been established (Asberg, Kopp, Berg-Kelly, & Gillberg, 2009). Many of these clinical problems, stemming from the overfocus on one or other of all the preschool neurodevelopmental disorders, could be avoided if clinicians were more aware of the implications of ESSENCE, and had several different diagnoses (and associated/comorbid diagnoses) in mind whenever examining a child presenting with impairing symptoms of ESSENCE.

7. Developmental coordination disorder

DCD has recently become the subject of more intense systematic study, after having been virtually neglected as an important clinical problem and focus of research. It is quite common, affecting about 5% of all school age children (Gillberg & Kadesjö, 2003), the majority of whom should be recognisable before age 5 years. However, currently, it is rare for a child to be given this diagnosis before school age. There is now a need for all child psychiatrists to be trained in the field of motor coordination assessment, and for pediatricians and other "non-psychiatry" physicians to keep abreast of developments in the field of ASD and ADHD, the two psychiatric disorders that appear to be most commonly associated with DCD. A Swedish population-study has suggested that there might be a specific connection between ADHD and ASD, and that it is mediated by DCD (Kadesjö & Gillberg, 1999): children with ADHD who also have DCD (about half the group of all with ADHD) have a very high risk of having impairing autistic symptomatology, whereas those without DCD have a low risk, and a much higher risk for ODD and conduct problems.

8. Tics and Tourette syndrome

Tics are extremely common in middle childhood and probably affect at least 15% of all children at some time. Severe, chronic motor and vocal tics (the combination that is referred to as Tourette syndrome) are much less common, probably affecting about 1% of all school age children (Kadesjö & Gillberg, 2000). Tics fluctuate in intensity and over time, which means that even some severely affected individuals may not actually show any tics during consultation. Tics are rarely diagnosed in the preschool years, but various forerunners of Tourettés disorder (such as impulsivity and a variety of obsessive compulsive phenomena) are usually present long before the typical, sometimes striking, even dramatic, tics occur or surface at early school age. Tourettés disorder is considered to be a strongly genetic disorder (but more heterogeneous than previously believed) (Keen-Kim & Freimer, 2006; State, Pauls, & Leckman, 2001).

One of the clinically most important aspects of Tourette syndrome (and other severe motor or vocal tic disorders) is its strong association with ADHD and OCD (Debes, Hjalgrim, & Skov, 2009). Almost all severely handicapped children with Tourette syndrome are affected by either ADHD or OCD or both, and are usually more impaired by these "comorbid" conditions than by the tic disorder itself. These associated problems, particularly ADHD (and perhaps particularly extremes of impulsive-hyperactive behaviours), are often apparent already during the preschool years, and they, rather than the tics, are what will lead to referral for clinical neurodevelopmental/neuropsychiatric examination.

9. Bipolar disorder

Pediatric bipolar disorder is still a somewhat controversial diagnosis (Biederman et al., 2003). However, it is becoming increasingly recognised that bipolar disorder can present with symptoms already in the preschool years. Children with "ADHD" and/or depression who have a family history of bipolar disorder may actually be presenting with prodromal signs and symptoms of a bipolar disorder (Chang, 2008). Extremes of irritability, mood swings, and even classic manic symptoms may onset in the first several years of life and signal the possibility of an underlying bipolar disorder. ADHD and ASD can both occur in conjunction with bipolar disorder (and can probably overshadow the affective disorder). Longitudinal systematic study of large groups of children with ESSENCE will help clarify the prevalence and importance of pediatric bipolar disorder.

10. Behavioural phenotype syndromes

As many as 0.7–0.8% of all preschool children may be affected by one (or more) of the "rare disorders", also referred to as behavioural phenotype syndromes (Gillberg, 2009, chap. 23–25). Examples of such disorders are the fragile X syndrome, 22q11deletion syndrome, tuberous sclerosis and Smith-Lemli-Opitz syndrome. Each of these disorders is really "rare" (occurring, usually in fewer than 1 in 2000 children), but given that there are hundreds of them, taken as a group they are actually quite common. The majority of these syndromes have a large subgroup – usually the majority – with some degree of cognitive impairment, although it is important to point out that there are quite a number of affected individuals who do not have learning disability, and some have high IQ (e.g. most individuals with Marfan syndrome and about half the group with 22q11deletion syndrome). Large subgroups of individuals within each category of the rare disorders in addition have ASD or ADHD or both, and other individuals may have other neuropsychiatric/neurodevelopmental problems that are symptomatic from a very young age (Hagerman et al., 2009; Niklasson, Rasmussen, Óskardòttir, & Gillberg, 2009; Sikora, Pettit-Kekel, Penfield, Merkens, & Steiner, 2006). Indeed it is very common for such problems to be the original reason for referral. In our centre we see quite a number of cases each year, in which the behavioural phenotype syndrome (and the genetic abnormality usually underlying it) has been missed.

11. Rare epilepsy syndromes

Landau-Kleffner syndrome or "verbal auditory agnosia with seizures" is a relatively rare syndrome which often presents in the preschool years and which is sometimes "misdiagnosed" as ASD, ADHD or both. Children with Landau Kleffner syndrome very often meet criteria for one or both of these types of conditions, but it is essential that the underlying epileptic syndrome not remain undiagnosed. Pulsed steroids, and, in certain cases, surgical treatments may be indicated (Cross & Neville, 2009). The overlap with the syndrome referred to as Continuous Spike Wave activity during Slow-wave sleep (CSWS) is considerable, and it is probably more a matter of the child's age than of any intrinsic difference between Landau-Kleffner syndrome (preschool children) and CSWS (older children) which of the named conditions gets a label in the individual case.

Infantile spasms (Saemundsen, Ludvigsson, & Rafnsson, 2008) and Dravet syndrome with SCN1A mutations (Arzimanoglou, 2009) carry high risks of intellectual disability, ASD and ADHD. It is important that such additional diagnoses are not overlooked in the follow-up of preschool children with these rare epilepsy syndromes, given that clinical experience suggest beneficial effects of ASD and ADHD interventions even in the presence of the severe underlying seizure disorder. Other rare epilepsy syndromes with onset in the preschool period is usually of such devastating character that making additional diagnoses of neuropsychiatric disorders such as ASD and ADHD is often not discussed, nor indeed relevant. However, just occasionally, epilepsies of the Lennox-Gastaut type (and other, even rarer conditions) can be sufficiently well controlled and ASD or ADHD type problems so pronounced that the issue of ESSENCE might be raised. In such instances it would not be appropriate to conclude that given the nature and severity of the epilepsy syndrome an additional diagnosis of ASD, ADHD, or another ESSENCE behaviour disorder would makes little difference. There is sufficient anecdotal support for the notion that even in cases considered "hopeless", interventions targeting ASD and/or ADHD may drastically improve quality of life for affected families.

12. Reactive attachment disorder

There is emerging evidence that reactive attachment disorder as defined under the DSM-IV-TR exists as a relatively distinct problem (Minnis et al., 2009). It can be recognised in the preschool years (Zeanah, Keyes, & Settles, 2003), and separated from – although symptomatically overlapping with – ADHD during the early school years (Minnis & Follan, in press). It also is associated with severe pragmatic language problems that are not explained by the occasional co-occurrence with ASD (Sadiq et al., submitted for publication). It is of considerable interest that a large subgroup of children meeting symptomatic criteria for reactive attachment disorder have not been severely abused or deprived in early childhood (Minnis, Marwick, Arthur, & McLaughlin, 2006). A brief screen for the disorder is available for school age children (Minnis et al., 2007), but there is a need for development of more refined screening and diagnostic tools for preschoolers. The disorder should be considered in all children who have suffered severe maltreatment or deprivation in the early years, and, perhaps also in all children with any kind of impairing ESSENCE symptom who show the possibly discriminating feature of cuddliness with strangers (Minnis & Follan, in press).

13. Overlap, co-existence and "comorbidity"

The word comorbidity is inadequate when it comes to describing and delineating the reality and meaning of the co-occurrence of phenomena, problems, symptoms, syndromes and disorders and diseases in the clinical and research field of ESSENCE. Most clinicians and researchers attach different meanings to the word comorbidity (Caron & Rutter, 1991). Using the word in a literal sense, one would assume that a person diagnosed with "comorbid" ASD and ADHD would have two different morbid ("disease") conditions. These morbid conditions could have different etiologies, the same etiology, or have no known etiology ("idiopathic"). In actual fact, when we talk about comorbidity, what we are usually referring to is

"co-existence", "association", "overlap", "additional problems" or suchlike. When the word comorbidity has been used here (usually within quotes), it has been "in that sense".

The syndromes subsumed under the ESSENCE label constitute collections of symptoms – sometimes, but certainly not always, operationalised under rigidly structured algorithms – that, at the current state of our knowledge, appear to delineate clinically meaningful conditions. However, as our knowledge base increases, so the algorithm barriers for making the specific diagnoses will need to be reviewed, and, quite often, changed. This has happened over the past 30 years in ASD and ADHD. The DSM-V is going to introduce another, probably major, change in how these categories are conceptualised, operationalised, algorithmised, and diagnosed. There is growing realisation that (a) most so called syndromes, including ASD, are, at least to some extent, partly arbitrary end- or cut-off-points on normal distribution curves, and depending on where you draw the line you may be referring to autistic disorder or Asperger syndrome; (b) most syndromes comprise a mixture of symptom collections from end- or cut-off points of different normal distribution curves, so that, at intersections, some individuals affected will meet criteria for ASD, others for ASD with ADHD, and others still for ADHD "only"; (c) most syndromes can be "mimicked by" (or may have actually be modelled around) more circumscribed brain disorders (genetic or environmental) or diffuse or unspecific/specific brain injury/dysfunction (temporary or chronic) caused by a variety of factors including the effects of myelin disorder after extreme prematurity, periventricular bleeding after perinatal asphyxia, thalidomide – or extremes of alcohol – exposure in fetal life, and exposure to products included in diets currently considered to be "normal", or, at least, not harmful. Against this background, it should come as no surprise that the introduction of the term ESSENCE, as suggested by the definition of the acronym, is nothing but an attempt to acknowledge this state of affairs, and the fact that there is a need to implement this approach to thinking about the problems in the whole wide field of child health and development services.

14. The implications of ESSENCE

What then are the implications of introducing a term such as ESSENCE? Let me list them, in no specific order, but with the most important conclusion summarised at the end:

(1) ESSENCE is a new acronym but not a new way of thinking about early onset childhood problems that continue to affect children's development long after the preschool period;
(2) ESSENCE is introduced so as to detract from the current trend towards compartmentalising syndromes in child and adolescent psychiatry and developmental medicine to the extent that "things" such as ASD and ADHD are considered "boxes" that are exclusive and separable from each other;
(3) ESSENCE is a term that draws attention to the fact that there is no easy way out in terms of diagnosis in preschool children who present with ESSENCE symptoms. All children presenting with an ESSENCE problem need to be considered from the point of view of multiproblem and multidisciplinary assessment;
(4) Children with ESSENCE need to get a holistic approach – on first presenting to services – to diagnosis and intervention. If the child suffers from ASD, it is likely that he/she also suffers from LD, ADHD, etc., and if the child suffers from ADHD, it is likely that he/she also suffers from ODD and DCD. The approach to diagnosis is likely to be unhelpful if it is exclusively directed to the diagnosis of one of these "disorders";
(5) The overlap of problems encountered in the field of ESSENCE indicates that we are not dealing with discrete disorders or syndromes, but with brain dysfunctions/neurodevelopmental problems that reflect circuitry breakdown, network dysfunctions and decreased/aberrant/increased connectivity, or, indeed, in quite a number of cases, "normal" brain function variants, and, that, therefore, it would be inappropriate to diagnose one problem and not consider the implication of the other(s). Currently, there is a trend towards compartmentalisation, services and clinics being developed specifically for ASD or ADHD or Tourette syndrome. This does not appear to be a helpful approach. In the future, as we learn more about the extent of normality, and as we teach a growing generation of children that we are all different, there may not be a need for lumping diagnoses (such as ESSENCE), but for specific diagnosis of genetic and environmental contributors to the problems encountered in each individual case;
(6) Taken together, all of the above would appear to combine to suggest the obvious solution. There is a need for Child ESSENCE Centres (rather than Community Pediatrics, GP centres, CAMHS, SLT-services, Special Education Units, Child Neurology, ASD, ADHD, Tourette or Affective disorder centres) to be organised for all preschool children, catering to the diagnostic, intervention planning, and follow-up requirements that are clearly warranted for all preschool children presenting with a major ESSENCE symptom. There is abundant evidence that major problems in at least one ESSENCE domain before age 5 years signals major problems in the same or overlapping domains several years later. There is no time to "sit down and wait"; something needs to be done, and that something is unlikely to be "just" in the area of speech and language. "just" in the areas of ASD or ADHD or "just" in special education.

14.1. Lumping, splitting, splitting, lumping, or both?

Progress in medical research usually leads to refinement of diagnostic criteria and increasingly sophisticated methods of subgrouping according to etiology, with important consequences for intervention. Superficially, this much-accepted view of cutting-edge medicine could be seen as support for a "splitter" approach to medical progress. The introduction of the

ESSENCE label could, by some, be taken to mean a step backwards in development in child psychiatry, given its implicit support for a "lumper" view. However, lumping of ESSENCE is only meaningful and, I would suggest, very helpful, if clinicians and researchers start by approaching the area of early child developmental problems by accepting that splitting in a state-of-the-art way (making refined and individualised diagnosis and intervention plans) will only be possible if there is anything to start splitting from (i.e. from a "lumped" group of cases). Also, if splitting occurs already in the mind of the original beholder/referrer (i.e. delayed language is seen as the "property" of the SLT, social interaction problems is seen as the "property" of the "autism centre", and delayed overall development with behaviour problem is seen as the "business of the learning disability psychiatrist") this would lead to inadvertent delay in recognising that the child with ESSENCE very likely will have more than one problem (i.e. ASD with ADHD, ASD with ADHD and epilepsy, ADHD with DCD and reactive attachment disorder, etc.). In summary, the introduction of the ESSENCE-mode of thinking about problems to do with deviations from normal child development, should not be taken as support for lumping rather than splitting, but for the order in which those two aspects of diagnosis is approached.

References

Aicardi, J., Bax, M., & Gillberg, C. (2009). *Diseases of the nervous system in childhood*. Cambridge: MacKeith Press.

Arzimanoglou, A. (2009). Dravet syndrome: From electroclinical characteristics to molecular biology. *Epilepsia,* Suppl. 8: 3–9.

Asberg, J., Kopp, S., Berg-Kelly, K., & Gillberg, C. (2009). Reading comprehension, word decoding and spelling in girls with autism spectrum disorders or AD/HD: Performance and predictors. *International Journal of Language and Communication Disorders, 2*, 1–16.

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., & Charman, T. (2006). Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: The Special Needs and Autism Project (SNAP). *Lancet, 368*, 210–215.

Baron-Cohen, S. (2005). Testing the extreme male brain (EMB) theory of autism: Let the data speak for themselves. *Cognitive Neuropsychiatry, 10*, 77–81.

Biederman, J., Mick, E., Faraone, S. V., Spencer, T., Wilens, T. E., & Wozniak, J. (2003). Current concepts in the validity, diagnosis and treatment of paediatric bipolar disorder. *International Journal of Neuropsychopharmacology, 6*, 293–300.

Billstedt, E., Gillberg, I. C., & Gillberg, C. (2005). Autism after adolescence: Population-based 13–22-year follow-up study of 120 individuals with autism diagnosed in childhood. *Journal of Autism and Developmental Disorders, 35*, 351–360.

Bishop, D. V., Bishop, S. J., Bright, P., James, C., Delaney, T., & Tallal, P. (1999). Different origin of auditory and phonological processing problems in children with language impairment: Evidence from a twin study. *Journal of Speech, Language, and Hearing Research, 42*, 155–168.

Bishop, D. V., Whitehouse, A. J., Watt, H. J., & Line, E. A. (2008). Autism and diagnostic substitution: Evidence from a study of adults with a history of developmental language disorder. *Developmental Medicine and Child Neurology, 50*, 341–345.

Buckner, R. L., & Vincent, J. L. (2007). Unrest at rest: Default activity and spontaneous network correlations. *Neuroimage, 37*, 1091–1096.

Caron, C., & Rutter, M. (1991). Comorbidity in child psychopathology: Concepts, issues and research strategies. *Journal of Child Psychology and Psychiatry, 32*, 1063–1080.

Cederlund, M, & Gillberg, C. (2004). One hundred males with Asperger syndrome. A clinical study of background and associated factors. *Developmental Medicine and Child Neurology, 46*, 652–660.

Cederlund, M., Hagberg, B., & Gillberg, C. (2010). Asperger syndrome in adolescent and young adult males. Interview, self- and parent assessment of social, emotional and cognitive problems. *Research in Developmental Disabilities, 31*, 287–298.

Chang, K. D. (2008). The bipolar spectrum in children and adolescents: Developmental issues. *Journal of Clinical Psychiatry, 69*, e9.

Chawarska, K., Klin, A., Paul, R., Macari, S., & Volkmar, F. (2009). A prospective study of toddlers with ASD: Short-term diagnostic and cognitive outcomes. *Journal of Child Psychology and Psychiatry, 50*, 1235–1245.

Comings, D. E. (1990). *Tourette syndrome and human behavior*. Hope Press.

Coo, H., Ouellette-Kuntz, H., Lloyd, J. E., Kasmara, L., Holden, J. J., & Lewis, M. E. (2008). Trends in autism prevalence: Diagnostic substitution revisited. *Journal of Autism and Developmental Disorders, 8*, 1036–1046.

Cross, J. H., & Neville, B. G. (2009). The surgical treatment of Landau-Kleffner syndrome. *Epilepsia,* Suppl. 7: 63–67.

Curatolo, P., Paloscia, C., D'Agati, E., Moavero, R., & Pasini, A. (2009). The neurobiology of attention deficit/hyperactivity disorder. *European Journal of Paediatric Neurology, 13*, 299–304.

Debes, N. M., Hjalgrim, H., & Skov, L. (2009). The presence of comorbidity in Tourette syndrome increases the need for pharmacological treatment. *Journal of Child Neurology, 24*, 1504–1512.

Dopheide, J. A., & Pliszka, S. R. (2009). Attention-deficit-hyperactivity disorder: An update. *Pharmacotherapy, 29*, 656–679.

Faraone, S. V., Sergeant, J., Gillberg, C., & Biederman, J. (2003). The worldwide prevalence of ADHD: Is it an American condition? *World Psychiatry, 2*, 104–113.

Fernell, E., Hedvall, A., Norrelgen, F., Erikssson, M., Höglund-Carlsson, L., Barnevik-Olsson, M., Svensson, L., et al. (2010). Developmental profiles in preschool children with autism spectrum disorders referred for intervention. *Research om Developmental Disabilities, 31*, 790–799.

Ghuman, J. K., Arnold, L. E., & Anthony, B. J. (2008). Psychopharmacological and other treatments in preschool children with attention-deficit/hyperactivity disorder: Current evidence and practice. *Journal of Child Adolescent and Psychopharmacology, 18*, 413–447.

Gillberg, C. (1983). Perceptual, motor and attentional deficits in Swedish primary school children. Some child psychiatric aspects. *Journal of Child Psychology and Psychiatry, 24*, 377–403.

Gillberg, C. (1995). *Clinical child neuropsychiatry*. Cambridge: Cambridge University Press.

Gillberg, C. (2009). Developmental and neuropsychiatric disorders of childhood. In J. Aicardi (Ed.), *Diseases of the nervous system in childhood* (3rd ed., pp. 889–932). London: Mac Keith Press.

Gillberg, C., & Coleman, M. (2000). *The biology of the autistic syndromes* (3rd ed.). Cambridge: Cambridge University Press.

Gillberg, C., Ehlers, S., Schaumann, H., Jakobsson, G., Dahlgren, S. O., Lindblom, R., et al. (1990). Autism under age 3 years: A clinical study of 28 cases referred for autistic symptoms in infancy. *Journal of Child Psychology and Psychiatry, 31*, 921–934.

Gillberg, C., & Kadesjö, B. (2003). Why bother about clumsiness? The implications of having developmental coordination disorder (DCD). *Neural Plasticity, 10*, 59–68.

Gillberg, C., & Söderström, H. (2003). Learning disability. *Lancet, 362*, 11–21.

Gillberg, C., & Wing, L. (1999). Autism: Not an extremely rare disorder. *Acta Psychiatrica Scandinavica, 99*, 399–406.

Hagerman, R. J., Berry-Kravis, E., Kaufmann, W. E., Ono, M. Y., Tartaglia, N., Lachiewicz, A., Kronk, R., Delahunty, C., Hessl, D., Visootsak, J., Picker, J., Gane, L., & Tranfaglia, M. (2009). Advances in the treatment of fragile X syndrome. *Pediatrics, 123*, 378–390 (review).

Howlin, P. (2008). Autism and diagnostic substitution. *Developmental Medicine and Child Neurology, 50*, 325.

Iacoboni, M. (2006). Failure to deactivate in autism: The co-constitution of self and other. *Trends in Cognitive Sciences, 10*, 431–433.

Kadesjö, B., & Gillberg, C. (1999). Developmental coordination disorder in Swedish 7-year-old children. *Journal of the American Academy of Child and Adolescent Psychiatry, 38*, 820–828.

Kadesjö, B., & Gillberg, C. (2000). Tourette's disorder: Epidemiology and comorbidity in primary school children. *Journal of American Academy and Child Adolescent Psychiatry, 39*, 1466.

Kadesjö, B., & Gillberg, C. (2001). The comorbidity of ADHD in the general population of Swedish school age children. *Journal of Child Psychology and Psychiatry, 42*, 487–492.
Kadesjö, C., Hägglöf, B., Kadesjö, B., & Gillberg, C. (2003). Attention-deficit-hyperactivity disorder with and without oppositional defiant disorder in 3-7-year-old children. *Developmental Medicine and Child Neurology, 45*, 693–699.
Keen-Kim, D., & Freimer, N. B. (2006). Genetics and epidemiology of Tourette syndrome. *Journal of Child Neurology, 21*, 665–671.
Klin, A., Volkmar, F. R., Sparrow, S. S., Cicchetti, D. V., & Rourke, B. P. (1995). Validity and neuropsychological characterization of Asperger syndrome: Convergence with nonverbal learning disabilities syndrome. *Journal of Child Psychology and Psychiatry, 36*, 1127–1140.
Kopp, S., Berg Kelly, K., & Gillberg, C. (2009, on-line). Girls with social and/or attention deficit: A descriptive study of 100 clinic attenders. *Journal of Attention Disorders*.
Law, J., Dockrell, J. E., Castelnuovo, E., Williams, K., Seef, B., & Normand, C. (2006). Early Years Centres for pre-school children with primary language difficulties: What do they cost and are they cost-effective? *International Journal of Communication Disorders, 41*, 67–81.
Lundervold, A. J., Posserud, M. B., Sørensen, L., Ullebø, A. K., & Gillberg, C. (submitted for publication). Processing speed in hypoactive 7–9 years old children with internalizing or/and externalizing disorders.
Mahone, E. M., & Wodka, E. L. (2008). The neurobiological profile of girls with ADHD. *Developmental Disability Research Review, 14*, 276–284.
McDougall, P., Drewett, R. F., Hungin, A. P., & Wright, C. M. (2009). The detection of early weight faltering at the 6–8-week check and its association with family factors, feeding and behavioural development. *Archives of Disease in Childhood, 94*, 549–552.
Miniscalco, C., Nygren, G., Hagberg, B., Kadesjö, B., & Gillberg, C. (2006). Neuropsychiatric and neurodevelopmental outcome at school age 6 and 7 years of children who screened positive for language problems at 2.5 years. A community-based study. *Developmental Medicine and Child Neurology, 48*, 361–366.
Miniscalco, C., Westerlund, M., & Lohmander, A. (2005). Language skills at age 6 years in Swedish children screened for language delay at 2(1/2) years of age. *Acta Paediatrica, 94*, 1798–1806.
Minnis, H., & Follan, M. (in press). Forty-four Juvenile Thieves revisited: From Bowlby to reactive attachment disorder. *Child, Care Health and Development*.
Minnis, H., Green, J., ÓConnor, T. G., Liew, A., Glaser, D., Taylor, E., Follan, M., Young, D., Barnes, J., Gillberg, C., Pelosi, A., Arthur, J., Burston, A., Connolly, B., & Sadiq, F. A. (2009). An exploratory study of the association between reactive attachment disorder and the attachment narratives in early school-age children. *Journal of Child Psychology and Psychiatry, 50*, 931–942.
Minnis, H., Marwick, H., Arthur, J., & McLaughlin, A. (2006). Reactive attachment disorder—A theoretical model beyond attachment. *European Child and Adolescent Psychiatry, 15*, 336–342.
Minnis, H., Reekie, J., Young, D., O'Connor, T., Ronald, A., Gray, A., & Plomin, R. (2007). Genetic, environmental and gender influences on attachment disorder behaviours. *British Journal of Psychiatry, 190*, 490–495.
Monk, C. S., Peltier, S. J., Wiggins, J. L., Weng, S. J., Carrasco, M., Risi, S., & Lord, C. (2009). Abnormalities of intrinsic functional connectivity in autism spectrum disorders. *Neuroimage, 47*, 764–772.
Mulligan, A., Anney, R. J., O'Regan, M., Chen, W., Butler, L., Fitzgerald, M., et al. (2009). Autism symptoms in attention-deficit/hyperactivity disorder: A familial trait which correlates with conduct, oppositional defiant, language and motor disorders. *Journal of Autism and Developmental Disorders, 39*, 197–209 (Erratum in: Journal of Autism and Developmental Disorders, 39, 210–211).
Niklasson, L., Rasmussen, P., Óskardòttir, S., & Gillberg, C. (2009). Autism, ADHD, mental retardation and behavior problems in 100 individuals with 22q11 deletion syndrome. *Research in Developmental Disabilities, 30*, 763–773.
O'Brien, G. (2000). Behavioural phenotypes. *Journal of the Royal Society of Medicine, 93*, 618–620.
Ollendick, T. H., Jarrett, M. A., Grills-Taquechel, A. E., Hovey, L. D., & Wolff, J. C. (2008). Comorbidity as a predictor and moderator of treatment outcome in youth with anxiety, affective, attention deficit/hyperactivity disorder, and oppositional/conduct disorders. *Clinical Psychology Review, 28*, 1447–1471.
Pinkhardt, E. H., Kassubek, J., Brummer, D., Koelch, M., Ludolph, A. C., Fegert, J. M., & Ludolph, A. G. (2009). Intensified testing for attention-deficit hyperactivity disorder (ADHD) in girls should reduce depression and smoking in adult females and the prevalence of ADHD in the longterm. *Medical Hypotheses, 72*, 409–412.
Pliszka, S. R. (2000). Patterns of comorbidity with attention-deficit/hyperactivity disorder. *Child and Adolescent Psychiatry Clinics of North America, 9*, 525–540.
Rasmussen, P., & Gillberg, C. (2000). Natural outcome of ADHD with Developmental Coordination Disorder at age 22 years: A controlled, longitudinal, community-based study. *Journal of American Academy of Child and Adolescent Psychiatry, 39*, 1424–1431.
Reiersen, A. M., Constantino, J. N., Volk, H. E., & Todd, R. D. (2007). Autistic traits in a population-based ADHD twin sample. *Journal of Child Psychology and Psychiatry, 48*, 464–472.
Richman, N., Stevenson, J. E., & Graham, P. J. (1975). Prevalence of behaviour problems in 3-year-old children: An epidemiological study in a London borough. *Journal of Child Psychology and Psychiatry, 16*, 277–287.
Rourke, B. P. (1988). Socioemotional disturbances of learning disabled children. *Journal of Consulting and Clinical Psychology, 56*, 801–810 (Review).
Sadiq, A., Slator, L., Law, J., Gillberg, C., Skuse, D., & Minnis, H. (submitted for publication). A comparison of the pragmatic skills of children with reactive Attachment Disorder and High functioning Autism.
Saemundsen, E., Ludvigsson, P., & Rafnsson, V. (2008). Risk of autism spectrum disorders after infantile spasms: A population-based study nested in a cohort with seizures in the first year of life. *Epilepsia, 49*, 1865–1870.
Sharp, S. I., McQuillin, A., & Gurling, H. M. (2009). Genetics of attention-deficit hyperactivity disorder (ADHD). *Neuropharmacology, 57*, 590–600.
Shaw, P., Lalonde, F., Lepage, C., Rabin, C., Eckstrand, K., Sharp, W., Greenstein, D., Evans, A., Giedd, J. N., & Rapoport, J. (2009). Development of cortical asymmetry in typically developing children and its disruption in attention-deficit/hyperactivity disorder. *Archives of General Psychiatry, 66*, 888–896.
Sikora, D. M., Pettit-Kekel, K., Penfield, J., Merkens, L. S., & Steiner, R. D. (2006). The near universal presence of autism spectrum disorders in children with Smith-Lemli-Opitz syndrome. *American Journal of Medicine Genetics A, 40*, 1511–1518.
Skuse, D. H. (2009). Is autism really a coherent syndrome in boys, or girls? *British Journal of Psychology, 100*, 33–37.
Sonuga-Barke, E. (2009). Gained in translation: How can we facilitate science-driven innovations in child mental health therapeutics? *Journal of Child Psychology and Psychiatry, 50*, 655–656.
Spencer, T. J., Biederman, J., & Mick, E. (2007). Attention-deficit/hyperactivity disorder: Diagnosis, lifespan. Comorbidities, and neurobiology. *Journal of Pediatric Psychology, 32*, 631–642.
State, M. W., Pauls, D. L., & Leckman, J. F. (2001). Tourette's syndrome and related disorders. *Child and Adolescent Psychiatric Clinics of North American, 10*, 317–323.
Stores. (2006). Sleep disorders. In C. Gillberg, R. Harrington, & H. C. Steinhausen (Eds.), *Clinician's deskbook of child and adolescent psychiatry* (pp. 304–338). Cambridge: Cambridge University Press.
Strang-Karlsson, S., Räikkönen, K., Pesonen, A. K., Kajantie, E., Paavonen, E. J., Lahti, J., Hovi, P., Heinonen, K., Järvenpää, A. L., Eriksson, J. G., & Andersson, S. (2008). Very low birth weight and behavioral symptoms of attention deficit hyperactivity disorder in young adulthood: The Helsinki study of very-low-birth-weight adults. *American Journal of Psychiatry, 65*, 1345–1353.
Swanson, J. M., Wigal, T., & Lakes, K. (2009). DSM-Vand the future diagnosis of attention-deficit/hyperactivity disorder. *Current Psychiatry Reports, 11*, 399–406.
Vaughan, B., Fegert, J., & Kratochvil, C. J. (2009). Update on atomoxetine in the treatment of attention-deficit/hyperactivity disorder. *Expert Opinion on Pharmacotherapy, 10*, 669–676.
Volkow, N. D., Wang, G. J., Kollins, S. H., Wigal, T. L., Newcorn, J. H., Telang, F., et al. (2009). Evaluating dopamine reward pathway in ADHD: Clinical implications. *Journal of the American Medical Association, 302*, 1084–1091 (Erratum in: Journal of the American Medical Association, 302, 1420).
Wing, L. (2005). Reflections on opening Pandora's box. *Journal of Autism and Developmental Disorders, 35*, 197–203.
Zeanah, C. H., Keyes, A., & Settles, L. (2003). Attachment relationship experiences and childhood psychopathology. *Annals of the New York Academy of Sciences, 1008*, 22–30.

Attention-Deficit/Hyperactivity Disorder: Diagnosis, Lifespan, Comorbidities, and Neurobiology

Thomas J. Spencer, MD, Joseph Biederman, MD, and Eric Mick, ScD
Department of Psychiatry, Massachusetts General Hospital, Boston, Mass.

In this report, we provide an evidence-based overview of attention-deficit/hyperactivity disorder (ADHD), including diagnosis, prevalence, developmental expression of symptoms, persistence, the heterogeneity of functional outcome, impairment in afflicted adults, psychiatric comorbidity, pathophysiology, genetics, psychosocial and biologic risk factors, and neurobiology. Attention-deficit/hyperactivity disorder is an early-onset, highly prevalent neurobehavioral disorder, with genetic, environmental, and biologic etiologies, that persists into adolescence and adulthood in a sizable majority of afflicted children of both sexes. It is characterized by behavioral symptoms of inattention, hyperactivity, and impulsivity across the life cycle and is associated with considerable morbidity and disability. Comorbidity is a distinct clinical feature of both childhood and adult ADHD. Although its etiology remains unclear, emerging evidence documents its strong neurobiologic and genetic underpinnings. Despite the high diagnostic reliability and the robust evidence of the validity of ADHD, there are many underlying issues that remain to be resolved. These include establishing developmentally appropriate diagnostic criteria at older ages, further elaborating the impact of gender on symptom expression, and examining risk and protective factors in relationship to prevention or amelioration of ADHD as well as related functional impairments.

Key words attention-deficit/hyperactivity disorder; comorbidity; diagnosis; neurobiology.

Attention-deficit/hyperactivity disorder (ADHD) is the most common emotional, cognitive, and behavioral disorder treated in youth.[1,2] Epidemiological studies indicate that ADHD is a prevalent disorder affecting from 4% to 7% of children worldwide, including the United States, New Zealand/Australia, Germany, and Brazil.[3] Although previously thought to remit largely in adolescence, a growing literature supports the persistence of the disorder and/or associated impairment into adulthood in a majority of cases. It is a major clinical and public health problem because of its associated morbidity and disability in children, adolescents, and adults.[2] Data from cross-sectional, retrospective, and follow-up studies indicate that youth with ADHD are at risk for developing other psychiatric difficulties in childhood, adolescence, and adulthood, including delinquency as well as mood, anxiety, and substance use disorders.[4]

Early reports

The first coherent description of ADHD was by George Still[5–7] in the Coombs lectures of 1902. He described an ''abnormal defect in moral control in children.'' Moral control was defined as ''the control of action in conformity with the idea of the good of all ... (that) can only exist when there is a cognitive relationship to the environment.'' Thus, moral control required a ''consciousness'' that informed the capacity of ''inhibitory volition.''[5–7] Other early observations on the relationship between behavioral disorders and cerebral trauma or encephalitis supported theories of a biologic etiology. For example, Strecker and Ebaugh[8] and Ebaugh and Franklin[9] noted behavior disorders, including hyperkinesis, explosive behavior, fatigability, and attention deficit, after acute epidemic encephalitis and cerebral trauma in children.

All correspondence concerning this article should be addressed to Thomas Spencer, MD, Yawkey Center for Outpatient Care, 32 Fruit St, Boston, MA 02114. E-mail: tspencer@partners.org.

Journal of Pediatric Psychology 32(6) pp. 631–642, 2007
doi:10.1093/jpepsy/jsm005
Advance Access publication June 7, 2007
Journal of Pediatric Psychology vol. 32 no. 6 ADHD Special Issue, reprinted by permission from *Ambulatory Pediatrics*, Vol. 7, Number 2 (Supplement), Jan./Feb. 2007,

Diagnostic Criteria

In the 1930s, hyperkinesis, impulsivity, learning disability, and short attention span were described as ''minimal brain damage''—and later as ''minimal brain dysfunction''—due to similarities to patients with frank central nervous system (CNS) injuries. In the 1950s, this label was modified to ''hyperactive child syndrome'' and then ''hyperkinetic reaction of childhood'' in Diagnostic and Statistical Manual of Mental Disorders (DSM)-II in 1968. Each of these labels and sets of criterion were focused exclusively on children and placed the greatest emphasis on motoric hyperactivity and overt impulsivity as hallmarks of the disorder.

The DSM-III represented a paradigm shift as it began to emphasize inattention as a significant component of the disorder. The DSM-III definition also recognized developmental variability presentation of the disorder at different ages. DSM-III introduced a residual type of ADHD if the remaining symptoms continued to cause significant levels of impairment. DSM-IV now defines 3 subtypes of ADHD: predominantly inattentive, predominantly hyperactive-impulsive, and a combined subtype. Criteria for each DSM-IV subtype require 6 or greater of 9 symptoms in each respective category. There are 4 additional criteria that include age of onset by 7, ADHD-specific adaptive impairments, pervasiveness, and separation from other existing conditions. The combined subtype is the most commonly represented subgroup accounting for from 50% to 75% of all ADHD individuals, followed by the inattentive subtype (20%–30%), and the hyperactive-impulsive subtype (less than 15%).[10–13]

Factor analyses have revealed that ADHD is comprised of 2 separate dimensions of symptoms (hyperactive/impulsive and inattentive).[14] Accordingly, DSM-IV moved away from the unitary model of DSM-III-R and returned to a model with separate dimensions. In addition, the 2 dimensions are associated with a different developmental course, comorbid disorders, sex ratios, and forms of functional impairment. In a multivariate analysis that included ADHD, oppositional defiant disorder (ODD), conduct disorder (CD), anxiety and depressive symptoms, hyperactive/impulsive symptoms were strongly related to Children's Global Assessment Scores and inattentive symptoms were related to academic impairment. Further analysis revealed that both combined and inattentive types of ADHD were associated with significant social impairment. Cluster analyses revealed the 3 subtypes now codified in DSM-IV.[14]

Children, adolescents, and adults with the inattentive subtype of ADHD are more likely to be female and have fewer other emotional or behavioral problems compared with the other subtypes. Youth with prominent inattentive problems as part of their ADHD (combined or inattentive subtype) have greater academic impairment compared with those with predominate hyperactivity/impulsivity. The combined-type ADHD individuals have more co-occurring psychiatric and substance abuse disorders and are the most impaired overall.

Diagnostic Considerations

The diagnosis of ADHD is made by careful clinical history.[15] A child with ADHD is characterized by a considerable degree of inattentiveness, distractibility, impulsivity, and often hyperactivity that is inappropriate for the developmental stage of the child. Although ADHD is often first observed in early childhood, many overactive toddlers will not develop ADHD.[16] Other common symptoms include low frustration tolerance, shifting activities frequently, difficulty organizing, and daydreaming. These symptoms are usually pervasive; however, they may not all occur in all settings. Children with predominately inattentive symptoms may have more difficulties in school and in completing homework, and somewhat fewer difficulties with peers or family. Conversely, children with excessive hyperactive or impulsive symptoms may do relatively well in school but have difficulties at home or in situations with less guidance and structure.

Adults must have childhood-onset, persistent, and current symptoms of ADHD to be diagnosed with the disorder. Adults with ADHD often present with marked inattention, distractibility, organization difficulties, and poor efficiency, which culminate in life histories of academic and occupational failure.[4,13]

Rating scales are extremely helpful in documenting the individual profile of ADHD symptoms as well as assessing the response to treatments. It is important to emphasize that they should not be used for diagnosis without careful clinical confirmation and elicitation of the other criteria necessary for diagnosis. Although neuropsychological testing is not relied upon to diagnose ADHD, it may serve to identify particular weaknesses within ADHD[17] or specific learning disabilities co-occurring with ADHD (for review see Barkley[15]).

A thorough review of rating scales for ADHD was recently published.[18] Rating scales are available for all age groups and can be useful in assessing and monitoring home, academic, and occupational performance.

In general, ADHD rating scales have evolved over the last few decades in a manner consistent with the evolution of understanding and the general shift from hierarchal to nonhierarchal models of diagnosis in DSM. For example, there has been a growing appreciation of the relative independence of ADHD and aggression dimensions.[19] Factor analyses, including the DSM field trials, have consistently found that these are separate dimensions.[14]

Increasingly, there has been a remarkable congruence of opinion in this area, with a number of the most widely used scales consisting of Likert ratings of the existing DSM-IV criteria.[20] There are 2 types of scales in wide use, the so-called ''narrow'' scales that are specific for ADHD and ''broad'' scales that measure additional dimensions, including comorbidity.[21] The broad scales are useful for separating straightforward and complex cases, and the narrow scales are most useful for honing in on exclusively ADHD dimensions—for diagnosis and to monitor specific responses to treatment.

In looking to the future, there are proposals to expand the set of diagnostic symptoms to include executive functions (such as time management and multitasking), especially in older individuals.[22] In fact, the symptom ''often has difficulties organizing'' is the most complex DSM item and appeared for the first time in DSM-IV. Field trials will have to clarify whether the executive function items identify a somewhat different population or are developmentally analogous to current criteria.

Prevalence of ADHD

Prevalence estimates of childhood ADHD in the USA are estimated to be 5% to 8%.[23] Estimates vary predictably depending on methodology. Definitions that require both symptom dimensions (hyperactivity/impulsivity and inattention) are more restrictive than those that require only one of these dimensions. Thus, estimates based on pre-DSM-III definitions or the International Classification of Diseases (ICD) codes of hyperkinetic disorder produce lower estimates. In addition, the surveys that estimate based on symptoms alone and do not include impairment yield higher estimates.[12] As recently reviewed by Faraone et al,[24] other factors that affect apparent prevalence estimates include pervasiveness criteria, informants (teacher, parent, and child), use of rating scales versus clinical interviews, and ascertainment issues. Community samples have higher rates than school samples.[25]

Gender and age of the sample also affect estimates of prevalence. Girls more commonly have the inattentive type and also less commonly have accompanying ODD/CD and disruptive disorders, factors leading to lower rates of diagnosis. The original descriptions were derived from a child-focused perspective and do not reflect what are thought to be more salient aspects of adult ADHD: the executive function disorders of poor organization, poor time management, and memory disturbance associated with academic and occupational failure. The lack of appropriate description of adult symptoms may reduce the true prevalence of ADHD in adulthood.

While there is a popular conception that ADHD is a cultural phenomenon, much of the cross-cultural disagreement has been due to criterion variance. In a scholarly review, Faraone et al[24] reviewed 20 US studies and 30 non-US studies. The results revealed that the prevalence in non-US studies was at least as high as that in US studies, especially when using DSM-IV criteria. In addition, studies of ADHD outside of the US have reported remarkable concordance with those of the US in external correlates of diagnosis, such as the pattern of adaptive impairments and neuropsychologic deficits; degree of familiality; and estimation of the magnitude of genetic influence; association of specific candidate genes; structural, functional, and molecular imaging findings; and response to specific pharmacological treatment.[26]

Despite the existence of studies from 5 continents, there is a paucity of studies in developing countries. Based on the higher prevalence of psychosocial risk factors in these countries, there may be a higher prevalence of ADHD and other disorders. Epidemiological studies in developing countries are needed to determine the nature of the condition in these countries.

Impact of Normal Development on ADHD Symptom Expression

We addressed the relative rate of decline of the core symptoms of ADHD from childhood into early adulthood to offer a developmental perspective on symptom decline.[27] As seen in Figure 1 we found a differential rate of symptomatic decline for inattention and hyperactivity/impulsivity. Although symptoms of inattention declined at a very modest rate, those of hyperactivity and impulsivity remitted much more abruptly. Hart et al[28] also documented a similar pattern of ADHD-subtype specific persistence: the mean number of hyperactive/impulsive symptoms declined with age, whereas the mean number of inattentive symptoms remained stable from age 8 to 15 years.

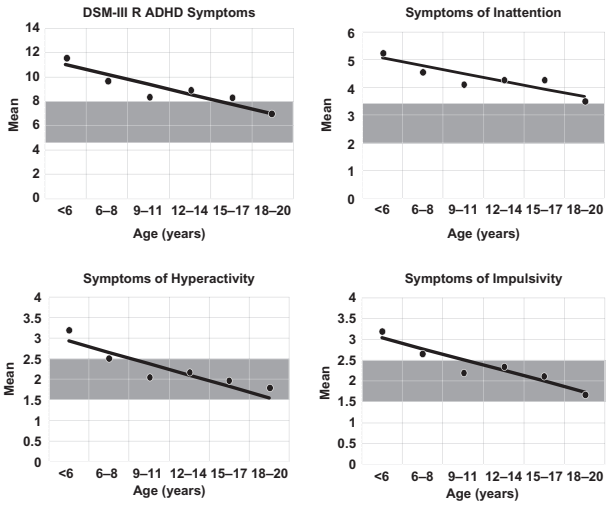


Figure 1. Age-dependent decline of symptoms of attention-deficit/hyperactivity disorder. Adapted from Biederman et al.[27]

Follow-up Studies of ADHD

Samples ascertained before the publication of DSM-III relied on earlier definitions that highlighted hyperactivity as a hallmark of ADHD. Since it is hyperactivity that wanes earliest, it may be that older samples were enriched with subjects more likely to remit from ADHD than individuals identified today. There is evidence for this hypothesis in the data. A combined estimate of the persistence of ADHD is shown in the Table I. The persistence rate was lowest in studies ascertained according to DSM-II attention-deficit disorder and highest in those studies ascertained according to DSM-III-R ADHD.

Heterogeneous Outcome in Persistent ADHD

The adolescent and young adult with ADHD is at risk for school failure, emotional difficulties, poor peer relationships, and trouble with the law.[29,30] Factors identifiable in younger youth that predict the persistence of ADHD into adulthood include familiality with ADHD and psychiatric comorbidity—particularly aggression or delinquency problems.[28,29,31,32]

Although the literature provides compelling evidence that the diagnosis of ADHD in childhood predicts persistent ADHD and poor outcome in adolescence, these findings also suggest that all children with ADHD do not share such a compromised outcome. The discussion thus far has not addressed a related clinical question: can the functioning of children with ADHD normalize in the context of persistent ADHD? We analyzed data from a 4-year-longitudinal study of referred children and adolescents with ADHD to assess normalization of functioning and its predictors among boys with persistent ADHD.[33]

Using indices of emotional, educational, and social adjustment, we found that 20% of children with persistent ADHD functioned poorly at follow-up in all 3 domains, 20% did well in all 3 domains, and 60% had intermediate outcomes.[33] These findings suggested that the syndromic persistence of ADHD is not associated with a uniform functional outcome but leads instead to a wide range of emotional, educational, and social adjustment outcomes that can be partially predicted by exposure to maternal psychopathology, larger family size, psychiatric comorbidity, and impulsive symptoms.[33]

Related Impairment in Untreated Adults With Persistent ADHD

If adult ADHD is a clinically significant disorder, then ADHD adults should show functional impairments in multiple domains. Several studies suggest this to be true. In an early study, Borland and Heckman[34] compared ADHD adults with their non-ADHD siblings. The ADHD adults had lower socioeconomic status, more work difficulties, and more frequent job changes. Morrison[35,36] compared ADHD adults with psychiatric controls matched for age and sex. The ADHD adults had fewer years of education and lower rates of professional employment. Similarly, others have shown that among patients with substance use disorders, ADHD predicts social maladjustment, immaturity, fewer social assets, lower occupational achievement, and high rates of separation and divorce.[37,38]

Murphy and Barkley[39] compared 172 ADHD adults with 30 non-ADHD adults. The ADHD adults reported more psychological maladjustment, more speeding violations, and more frequent changes in employment. Compared with the non-ADHD adults, more ADHD adults had had their drivers license suspended, had performed poorly at work, and had quit or been fired from their job. Moreover, the ADHD adults were more likely to have had multiple marriages.

Given that academic underachievement is a well-known correlate of ADHD in childhood,[40] ADHD adults ought to have histories reflecting school problems. Our work[41,42] demonstrated that compared with control adults, ADHD adults had significantly higher rates of repeated grades, tutoring, placement in special classes, and reading disability. Similarly, Murphy and Barkley[39] showed that ADHD adults had histories marked by poorer educational performance and more frequent school disciplinary actions against them.

Table I. Follow-Up Studies of ADHD

Study	Baseline Mean Age	Follow-Up Mean Age	Follow-Up Diagnostic Criteria		Persistence N%
		DSM-II Diagnosis at Baseline			
Mendelson ([82])[a]	9.9	13.4	DSM-II[a]	42	50
Borland ([34])[a]	7.5	30.4	DSM-II[a]	10	50*
Mannuzza ([83])	7.9	17.4	DSM-III	12	33
Gittelman ([29])	9.3	18.3	DSM-III	31	31
Mannuzza ([84])	7.3	18.5	DSM-III	21	22
Mannuzza ([85])	9.3	25.5	DSM-III, IIIR	7	8
Mannuzza ([81])	7.3	24.1	DSM-IIIR	3	4
Lambert ([77])	7.7	14.3	DSM-III	25	43
Lambert ([86])	9.3	18.3	DSM-III	47	80*
Feldman ([87])[a]	10.0	15.5	DSM-II[a]	35	43
August ([19])[a]	10.7	14.2	DSM-III	19	86*
Weiss ([88])[a]	6–12	25.1	DSM-III	42	66*
Yan ([89])[a]	10.0	25.5	DSM-IIIR[d]	140	70*
Combined estimate; Rate (95%CI)					**40 (36–45)**
		DSM-III Diagnosis at Baseline			
Cantwell ([90])	5.5	9.7	DSM-III	28	80
Offord ([91])	4–12	8–16	DSM-III	16	34
Claude ([92])	7.3	19.7	DSM-IIIR	26	50
Rasmussen ([93])[c]	7	22	DSM-IV	28	56*
Rasmussen ([93])[c]	7	22	DSM-IV	24	48
Combined estimate; Rate (95%CI)					**52 (39–67)**
		DSM-IIIR Diagnosis at Baseline			
Barkley ([79])[b]	4–12	14.9	DSM-IIIR	88	72
Barkley ([79])[b]	4–12	14.9	DSM-IIIR	102	83*
Barkley ([80])[b]	4–12	21.1	DSM-IV	78	58
Barkley ([80])[b]	4–12	21.1	DSM-IV	89	66*
Hart [28]	9.4	10.4	DSM-IIIR	89	77
Hart ([28])	9.4	11.4	DSM-IIIR	90	85
Hart ([28])	9.4	12.4	DSM-IIIR	92	84
Biederman ([78])	10.5	14.5	DSM-IIIR	109	85*
Biederman ([78])	10.5	14.5	DSM-IIIR	78	61
Combined estimate; Rate (95%CI)					**74 (69–79)**

*Residual ADHD diagnosis.

[a]Diagnostic system not stated but completed in DSM-II era.

[b]Diagnoses shown to be equivalent to DSM-IIIR.

[c]Diagnoses shown to be equivalent to DSM-III.

[d]Diagnostic system not stated but completed in DSM-IIIR era; adapted from Faraone S, Biederman J, Mick E: A Re-evaluation of the age dependent decline of attention deficit hyperactivity disorder. American Journal of Psychiatry 2003.

Psychiatric Comorbidity

Oppositional Defiant Disorder and Conduct Disorder

There are important nosologic distinctions between attention and hyperactivity per se and that of associated symptoms common to the disruptive behavioral disorder category. Oppositional defiant disorder is characterized by a pattern of negativistic, hostile, and defiant behavior.[16] Attention-deficit/hyperactivity disorder and ODD/CD have been found to co-occur in 30% to 50% of cases in both epidemiologic and clinical samples.[43] In contrast, CD is a more severe disorder of habitual rule breaking defined by a pattern of aggression, destruction, lying, stealing, or truancy.

A recent follow-up study of children with ADHD confirmed that the overlap of CD and ODD was asymmetric.[44] While CD was almost always comorbid with and was preceded by ODD, ODD at baseline was a weak predictor of new onsets of CD at follow-up into midadolescence 4 years later. In addition, while CD was a strong predictor of substance abuse at follow-up, ODD without CD was not.

Mood Disorders

Major depression in a child may be apparent from a sad or irritable mood or a persistent loss of interest or pleasure in the child's favorite activities. Other signs and symptoms include physiologic disturbances, such as in changes in appetite and weight, abnormal sleep patterns, psychomotor abnormalities, fatigue, and diminished ability to think, as well as feelings of worthlessness or guilt and suicidal preoccupation. Associated features of depression in children include school difficulties, school refusal, withdrawal, somatic complaints, negativism, aggression, and antisocial behavior. Conduct disorder and substance abuse commonly co-occur with depression in older children and adolescents.

Classical mania in adults is characterized by euphoria, elation, grandiosity, and increased energy. However, in many adults and most children, mania is more commonly manifested by extreme irritability or explosive mood with associated poor psychosocial functioning that is often devastating to the patient and family. In milder conditions, additional symptoms include unmodulated high energy such as a decreased sleep, overtalkativeness, racing thoughts, or increased goal-directed activity (social, work, school, and sexual) or an associated manifestation of markedly poor judgment, such as thrill seeking or reckless activities. It is often difficult to differentiate juvenile mania from ADHD, CD, depression, and psychotic disorders because of overlapping developmental features. In adolescent-onset mania, one may obtain a clearer picture of childhood onset disorders such as ADHD, whose symptoms may precede the first manic episode by many years.

In several prospective studies, our group at Massachusetts General Hospital examined rates of depression in children with ADHD.[45] In a 4-year follow-up, lifetime rates of comorbid depression in children with ADHD increased from 29% at baseline to 45% at average age 15. A baseline diagnosis of major depression predicted lower psychosocial functioning, a higher rate of hospitalization, and impairments in interpersonal and family functioning. Similarly, mania was detected in 11% of children at baseline (mean age 11) and increased to 23% at 4-year follow-up. Children with ADHD with comorbid mania at either baseline or follow-up assessment had other correlates expected in mania, including additional psychopathology, psychiatric hospitalization, severely impaired psychosocial functioning, and a greater family history of mood disorders.

Childhood Anxiety Disorders

Anxiety symptoms are generally expressed in 4 domains: cognitive, affective, physical, and behavioral.[46] Cognitive elements may range from rumination and vigilant apprehension to catastrophic thinking, such as the anticipation of great embarrassment or threat to life. Behavioral features may include agitation, tantrums, attention seeking, overdependence, and rituals. Many of these symptoms may be misinterpreted because of overlap with ADHD. Childhood anxiety disorders are often not suspected in an overactive child, just as ADHD is often not assessed in inhibited children. When present, both contribute to social, behavioral, and academic dysfunction. In addition, anxiety may be associated with intense intrapsychic suffering. Thus, having both ADHD and anxiety disorders may substantially worsen the outcome of children with both disorders. In the Massachusetts General Hospital (MGH) follow-up study, children with ADHD with comorbid anxiety disorder had increased psychiatric treatment, more impaired psychosocial functioning, and a greater family history of anxiety disorders.[47]

Cognitive Performance and Learning Disabilities

Children with ADHD perform more poorly than controls on standard measures of intelligence and achievement.[48] In addition, children with ADHD perform more poorly in school than do controls, as evidenced by more grade repetitions, poorer grades in academic subjects, more placement in special classes, and more tutoring.[49–51] The reported degree of overlap ranges from as low[52,53] as 10% to as high as 92%.[54] The prevalence varies by definition, the more restrictive definition has a rate of 20% to 25%.

ADHD Plus Tics

Children with ADHD have higher rates of tic disorders,[55] which may contribute additional dysfunction due to distractions and social impairments directly attributable to the movements or vocalizations themselves. A number of studies have noted that anti-ADHD treatment is highly effective for ADHD behaviors, aggression, and social skill deficits in children with Tourette's Syndrome (TS) or chronic tics.

Substance Use Disorders

Combined data from retrospective accounts of adults and prospective observations of youth indicates that juveniles with ADHD are at increased risk for cigarette smoking and substance abuse during adolescence. Recent work

suggests that ADHD youth disproportionately become involved with cigarettes, alcohol, and then drugs.[56,57] Individuals with ADHD, independent of comorbidity, tend to maintain their addiction longer compared with their non-ADHD peers.[58]

Pathophysiology and Genetics

Genetics and ADHD

Because ADHD is believed to be highly genetic, studies of twins have been used to establish its heritability, or the degree to which this disorder is influenced by genetic factors. Based on numerous studies of twins, which varied considerably in methodology and definitions of ADHD, the mean heritability for ADHD was shown to be 77% (Figure 2).

Molecular Genetics Studies

Two approaches are used to evaluate the genetic etiology of ADHD: 1) the genome scan, which examines all chromosomal locations without a priori guessing as to which genes underlie ADHD and 2) the candidate gene approach, which examines 1 or more genes based on theory and empirical evidence. A genome-wide linkage scan in 204 nuclear families (853 individuals and 270 affected sibling pairs) suggests that regions 16p13 and 17p11 likely harbor risk genes for ADHD.[59]

Seven candidate genes show statistically significant evidence of association with ADHD on the basis of the pooled odds ratio (1.18–1.46) across studies: DRD4, DRD5, DAT, DBH, 5-HTT, HTR1B, and SNAP-25.[60]

Other Etiologic Factors

Biologic Adversity

Several biologic factors have been proposed as contributors to ADHD, including food additives/diet, lead contamination, cigarette and alcohol exposure, maternal smoking during pregnancy, and low birth weight. Although the Feingold Diet for ADHD was popularized by the media and accepted by many parents, systematic studies showed that this diet was ineffective and that food additives do not cause this disorder.[61] Several investigators have shown that lead contamination can cause symptoms of ADHD. However, lead does not account for the majority of ADHD cases, and many children with high lead exposure do not develop ADHD. An emerging literature documents that maternal smoking and alcohol exposure during pregnancy, low birth weight, and psychosocial adversity are additional independent risk factors for ADHD.[62,63]

Pregnancy and delivery complications (i.e., toxemia, eclampsia, poor maternal health, maternal age, fetal postmaturity, duration of labor, fetal distress, low birth weight, and antepartum hemorrhage) appear to have a predisposition for ADHD.[64] Several studies documented that maternal smoking during pregnancy is an independent risk factor for ADHD.[63,65,66]

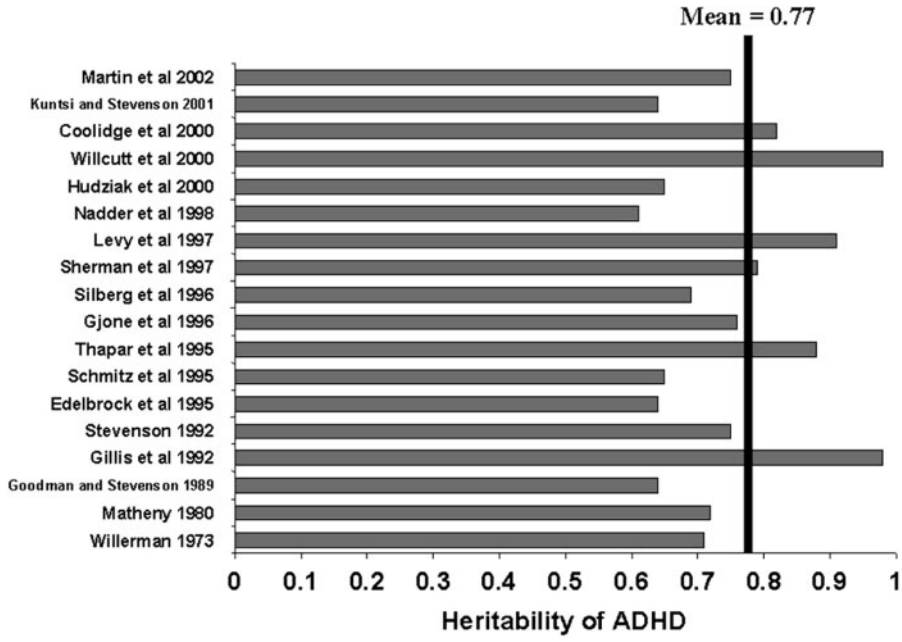


Figure 2. Heritability of attention-deficit/hyperactivity disorder. Adapted from Faraone et al.[60]

Psychosocial Adversity

Compelling work by Rutter and colleagues[67] in the classic Isle of Wight studies revealed that the aggregate of adversity factors (i.e., severe marital discord, low social class, large family size, paternal criminality, maternal mental disorder, and foster care placement), rather than the presence of any single factor, led to psychopathology. Findings of more recent studies support the previous work of Rutter et al[67] and stress the importance of adverse family-environment variables as risk factors for ADHD.[63,68] In one of these, chronic family conflict, decreased family cohesion, and exposure to parental psychopathology (particularly maternal) were more common in ADHD families compared with control families.[63] However, some of the findings are likely secondary to ADHD in the child and/or parent, rather than primary.

It remains unclear whether exposure to violence in childhood is a risk factor for ADHD. There are theoretic reasons to examine this potential association. Exposure to violence may act through psychosocial adversity but may also lead to permanent brain changes, based on the impact of prolonged exposure of the developing brain to steroid hormones.[69]

It is important to note that, although many studies provide powerful evidence for the importance of psychosocial adversity in ADHD, such factors tend to emerge as universal predictors of children's adaptive functioning and emotional health, rather than specific predictors of ADHD. As such, they can be conceptualized as nonspecific triggers of an underlying predisposition or as modifiers of the course of illness.

Neurobiology of ADHD

The neurobiology of ADHD is not completely understood, although imbalances in dopaminergic and noradrenergic systems have been implicated in the core symptoms that characterize this disorder.[70,71] As reviewed by Seidman et al,[72] many brain regions are candidates for impaired functioning in ADHD. Prefrontal hypotheses in ADHD have primarily involved the dorsolateral prefrontal cortex, associated with organizational, planning, working memory, and attentional dysfunctions, and orbital lesions associated with social disinhibition and impulse control disorders.

While morphometric imaging studies cannot be used to make diagnoses, they are ideal for testing hypotheses about the locus of brain dysfunction in ADHD and provide direct assessments of brain structure and function. Structural imaging studies using computerized tomography or magnetic resonance imaging found evidence of structural brain abnormalities among ADHD patients, with the most common findings being smaller volumes in frontal cortex, cerebellum, and subcortical structures. One of the most important neuroimaging studies of ADHD is that of Castellanos et al.[73] They found smaller total cerebral brain volumes from childhood through adolescence. This work suggested that genetic or early environmental influences on brain development in ADHD are fixed, nonprogressive, and unrelated to stimulant treatment. Limitations included the combining of both longitudinal and cross-sectional assessments. As reviewed by Bush et al,[74] numerous functional magnetic resonance imaging studies have reported dorsal anterior cingulate cortex hypofunction in ADHD on tasks of inhibitory control.

Brain imaging studies fit well with the concept that dysfunction in frontosubcortical pathways occurs in ADHD. Three subcortical structures implicated by the imaging studies (i.e., caudate, putamen, and globus pallidus) are part of the neural circuitry underlying motor control, executive functions, inhibition of behavior, and the modulation of reward pathways. These frontal-striatal-pallidal-thalamic circuits provide feedback to the cortex for the regulation of behavior.[75]

The frontosubcortical systems pathways associated with ADHD are rich in catecholamines, which are involved in the mechanism of action of stimulant medications used to treat this disorder. A plausible model for the effects of medications in ADHD suggests that, through dopaminergic and/or noradrenergic pathways, these agents increase the inhibitory influences of frontal cortical activity on subcortical structures.[70]

Imaging studies also implicate the cerebellum and corpus callosum in the pathophysiology of ADHD. The cerebellum contributes significantly to cognitive functioning, presumably through cerebellar-cortical pathways involving the pons and thalamus. The corpus callosum connects homotypic regions of the 2 cerebral hemispheres. Size variations in the callosum and volume differences in number of cortical neurons may degrade communication between these 2 hemispheres, which may account for some of the cognitive and behavioral symptoms of ADHD.[73,76]

Received January 7, 2006; accepted July 22, 2006

References

1. Jensen, P., Kettle, L., Roper, M., et al. (1999). Are stimulants overprescribed? Treatment of ADHD in four U. S. communities, *J Am Acad Child Adolesc Psychiatry, 38*, 797–804.

2. Goldman, L., Genel, M., Bezman, R., & Slanetz, P. (1998). Diagnosis and treatment of attention-deficit/hyperactivity disorder in children and adolescents. *JAMA, 279*, 1100–1107.
3. Szatmari, P. (1992). The epidemiology of attention-deficit hyperactivity disorders. In G. Weiss (Ed.), *Attention-Deficit Hyperactivity Disorder*. Vol 1 (pp. 361–371). Philadelphia, Pa: Saunders.
4. Biederman, J., Newcorn, J., & Sprich, S. (1991). Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. *Am J Psychiatry, 148*, 564–577.
5. Still, G. (1902). The Goulstonian lectures on some abnormal psychical conditions in children, Lecture I, *Lancet, 1*, 1008–1012.
6. Still, G. (1902). The Goulstonian lectures on some abnormal psychical conditions in children, Lecture II, *Lancet, 1*, 1077–1082.
7. Still, G. (1902). The Goulstonian lectures on some abnormal psychical conditions in children, Lecture III, *Lancet, 1*, 1163–1168.
8. Strecker, E., & Ebaugh, F. (1924). Neuropsychiatric sequelae of cerebral trauma in children. *Arch Neurol Psychiatry, 12*, 443–453.
9. Ebaugh, F., & Franklin, G. (1923). Neuropsychiatric sequelae of acute epidemic encephalitis in children. *Am J Dis Child, 25*, 89–97.
10. Morgan, A., Hynd, G., Riccio, C., & Hall, J. (1996). Validity of DSM-IV ADHD predominantly inattentive and combined types: relationship to previous DSM diagnoses/subtype differences. *J Am Acad Child Adolesc Psychiatry, 35*, 325–333.
11. Paternite, C., Loney, J., & Roberts, M. (1995). External validation of oppositional disorder and attention deficit disorder with hyperactivity. *J Abnorm Child Psychol, 23*, 453–471.
12. Wolraich, M., Hannah, J., Pinnock, T., et al. (1996). Comparison of diagnostic criteria for attention-deficit hyperactivity disorder in a county-wide sample. *J Am Acad Child Adolesc Psychiatry, 35*, 319–324.
13. Millstein, R. B., Wilens, T. E., Biederman, J., & Spencer, T. J. (1997). Presenting ADHD symptoms and subtypes in clinically referred adults with ADHD. *J Atten Disord, 2*, 159–166.
14. Lahey, B., Applegate, B., McBurnett, K., et al. (1994). DSM-IV field trials for attention deficit hyperactivity disorder in children and adolescents. *Am J Psychiatry, 151*, 1673–1685.
15. Barkley, R. (1998). *Attention-Deficit/Hyperactivity Disorder: A Handbook for Diagnosis and Treament*. 2nd ed. (pp. 1–628). New York: Guilford Press.
16. American Psychiatric Association. (1994). Diagnostic and Statistical Manual of Mental Disorders. 4th ed. (pp. 886). Washington, DC: American Psychiatric Association.
17. Seidman, L. J., Biederman, J., Faraone, S. V., et al. (1997). Toward defining a neuropsychology of ADHD: performance of children and adolescents from a large clinically referred sample. *J Consult Clin Psychol, 65*, 150–160.
18. Collett, B. R., Ohan, J. L., & Myers, K. M. (2003). Ten-year review of rating scales, V: Scales assessing attention-deficit/hyperactivity disorder, *J Am Acad Child Adolesc Psychiatry, 42*, 1015–1037.
19. August, G. J., Stewart, M. A., & Holmes, C. S. (1983). A four-year follow-up of hyperactive boys with and without conduct disorder. *Br J Psychiatry, 143*, 192–198.
20. DuPaul, G. (1991). Parent and teacher ratings of ADHD symptoms: psychometric properties in a community-based sample. *J Clin Child Psychol, 20*, 245–253.
21. Achenbach, T. (1995). Diagnosis, assessment, and comorbidity in psychosocial treatment research. *J Abnorm Child Psychol, 23*, 45–65.
22. Barkley, R. A., Edwards, G., Laneri, M., et al. (2001). Executive functioning, temporal discounting, and sense of time in adolescents with attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD). *J Abnorm Child Psychol, 29*, 541–555.
23. Dulcan, M. (1997). Practice parameters for the assessment and treatment of children, adolescents, and adults with attention-deficit/hyperactivity disorder. American Academy of Child and Adolescent Psychiatry. *J Am Acad Child Adolesc Psychiatry, 36*, 10(Suppl), 85S–121S.
24. Faraone, S. V., Sergeant, J., Gillberg, C., & Biederman, J. (2003). The worldwide prevalence of ADHD: is it an American condition? *World Psychiatry, 2*, 104–112.
25. Brown, R. T., Freeman, W. S., Perrin, J. M., et al. (2001). Prevalence and assessment of attention-deficit/hyperactivity disorder in primary care settings. *Pediatrics, 107*, E43.
26. Spencer, T. J., Biederman, J., Wilens, T. E., & Faraone, S. V. (2002). Overview and neurobiology

of attention-deficit/hyperactivity disorder. *J Clin Psychiatry. 63*(Suppl 12), 3–9.
27. Biederman, J., Mick, E., & Faraone, S. V. (2000). Age-dependent decline of symptoms of attention deficit hyperactivity disorder: impact of remission definition and symptom type. *Am J Psychiatry, 157*, 816–818.
28. Hart, E., Lahey, B., Loeber, R., et al. (1995). Developmental change in attention-deficit hyperactivity disorder in boys: a four-year longitudinal study. *J Abnorm Child Psychol, 23*, 729–749.
29. Gittelman, R., Mannuzza, S., Shenker, R., & Bonagura, N. (1985). Hyperactive boys almost grown up: I, Psychiatric status, *Arch Gen Psychiatry, 42*, 937–947.
30. Hechtman, L., & Weiss, G. (1986). Controlled prospective fifteen year follow-up of hyperactives as adults: non-medical drug and alcohol use and anti-social behaviour. *Can J Psychiatry, 31*, 557–567.
31. Loney, J., Kramer, J., & Milich, R. S. (1981). The hyperactive child grows up: predictors of symptoms, delinquency and achievement at follow-up. In K. D. Gadow, & J. Loney (Eds.), *Psychosocial Aspects of Drug Treatment for Hyperactivity* (pp. 381–416). Boulder, CO: Westview Press.
32. Taylor, E., Sandberg, S., Thorley, G., & Giles, S. (1991). The epidemiology of childhood hyperactivity (pp. 158). New York: Oxford University Press.
33. Biederman, J., Mick, E., & Faraone, S. (1998). Normalized functioning in youths with persistent ADHD. *J Pediatr, 133*, 544–551.
34. Borland, B. L., & Heckman, H. K. (1976). Hyperactive boys and their brothers: a 25-year follow-up study. *Arch Gen Psychiatry, 33*, 669–675.
35. Morrison, J. R. (1980). Adult psychiatric disorders in parents of hyperactive children. *Am J Psychiatry, 137*, 825–827.
36. Morrison, J. R. (1980). Childhood hyperactivity in an adult psychiatric population: social factors. *J Clin Psychiatry, 41*, 40–43.
37. Wilens, T. E., Biederman, J., & Mick, E. (1998). Does ADHD affect the course of substance abuse? Findings from a sample of adults with and without ADHD, *Am J Addict,* 7, 156–163.
38. Tarter, R. E. (1982). Psychosocial history, minimal brain dysfunction and differential drinking patterns of male alcoholics. *J Clin Psychol, 38*, 867–873.
39. Murphy, K., & Barkley, R. A. (1996). Attention deficit hyperactivity disorder adults: comorbidities and adaptive impairments. *Compr Psychiatry, 37*, 393–401.
40. Hinshaw, S. P. (1992). Externalizing behavior problems and academic underachievement in childhood and adolescence: causal relationships and underlying mechanisms. *Psychol Bull, 111*, 127–155.
41. Biederman, J., Faraone, S. V., Spencer, T., et al. (1993). Patterns of psychiatric comorbidity, cognition, and psychosocial functioning in adults with attention deficit hyperactivity disorder. *Am J Psychiatry, 150*, 1792–1798.
42. Biederman, J., Faraone, S. V., Spencer, T., et al. (1994). Gender differences in a sample of adults with attention deficit hyperactivity disorder. *Psychiatry Res, 53*, 13–29.
43. Biederman, J., Newcorn, J., & Sprich, S. (1991). Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. *Am J Psychiatry, 148*, 564–577.
44. Biederman, J., Faraone, S. V., Milberger, S., et al. (1996). Is childhood oppositional defiant disorder a precursor to adolescent conduct disorder? Findings from a four-year follow-up study of children with ADHD, *J Am Acad Child Adolesc Psychiatry, 35*, 1193–1204.
45. Biederman, J., Faraone, S. V., Keenan, K., et al. (1992). Further evidence for family-genetic risk factors in attention deficit hyperactivity disorder, Patterns of comorbidity in probands and relatives in psychiatrically and pediatrically referred samples, *Arch Gen Psychiatry*, 49, 728–738.
46. Pollack, M. H., Otto, M. W., Sabatino, S., et al. (1996). Relationship of childhood anxiety to adult panic disorder: correlates and influence on course. *Am J Psychiatry, 153*, 376–381.
47. Biederman, J., Faraone, S. V., Mick, E., et al. (1996). Attention deficit hyperactivity disorder and juvenile mania: an overlooked comorbidity? *J Am Acad Child Adolesc Psychiatry, 35*, 997–1008.
48. Campbell, S. B., & Werry, J. S. (1986). Attention deficit disorder (hyperactivity). In H. C. Quay, & J. S. Werry (Eds.), *Psychopathologic Disorders of Childhood* (pp. 1–35). New York: Wiley & Sons.
49. Lahey, B. B., Schaughency, E. A., Strauss, C. C., & Frame, C. L. (1984). Are attention deficit disorders with and without hyperactivity similar or dissimilar disorders? *J Am Acad Child Adolesc Psychiatry, 23*, 302–309.

50. Edelbrock, C., Costello, A. J., & Kessler, M. D. (1984). Empirical corroboration of attention deficit disorder. *J Am Acad Child Adolesc Psychiatry, 23*, 285–290.
51. Semrud-Clikeman, M. S., Biederman, J., Sprich, S., et al. (1992). Comorbidity between ADHD and learning disability: a review and report in a clinically referred sample. *J Am Acad Child Adolesc Psychiatry, 31*, 439–448.
52. August, G. J., & Holmes, C. S. (1984). Behavior and academic achievement in hyperactive subgroups and learning-disabled boys. *Am J Dis Child, 138*, 1025–1029.
53. Halperin, J. M., Gittelman, R., Klein, D. F., & Rudel, R. G. (1984). Reading-disabled hyperactive children: a distinct subgroup of attention deficit disorder with hyperactivity. *J Abnorm Child Psychol, 12*, 1–14.
54. Silver, L. B. (1981). The relationship between learning disabilities, hyperactivity, distractibility, and behavioral problems. *J Am Acad Child Psychiatry, 20*, 385–397.
55. Spencer, T., Biederman, J., Coffey, B., et al. (1999). The 4-year course of tic disorders in boys with attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry, 56*, 842–847.
56. Biederman, J., Wilens, T., Mick, E., et al. (1998). Does attention-deficit hyperactivity disorder impact the developmental course of drug and alcohol abuse and dependence? *Biol Psychiatry, 44*, 269–273.
57. Milberger, S., Biederman, J., Faraone, S., et al. (1997). ADHD is associated with early initiation of cigarette smoking in children and adolescents. *J Am Acad Child Adolesc Psychiatry, 36*, 37–43.
58. Wilens, T., Biederman, J., & Mick, E. (1998). Does ADHD Affect the course of substance abuse? Findings from a sample of adults with and without ADHD, *Am J Addict, 7*, 156–163.
59. Ogdie, M. N., Macphie, I. L., Minassian, S. L., et al. (2003). A genomewide scan for attention-deficit/hyperactivity disorder in an extended sample: suggestive linkage on 17p11. *Am J Hum Genet, 72*, 1268–1279.
60. Faraone, S. V., Perlis, R. H., Doyle, A. E., et al. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry, 57*, 1313–1323.
61. Conners, C. K. (1980). *Food Additives and Hyperactive Children*. New York: Plenum.
62. Mick, E., Biederman, J., Prince, J., et al. (2002). Impact of low birth weight on attention-deficit/hyperactivity disorder. *J Dev Behav Pediatr, 23*, 16–22.
63. Biederman, J., Milberger, S., Faraone, S. V., et al. (1995). Family-environment risk factors for attention deficit hyperactivity disorder: a test of Rutter's indicators of adversity. *Arch Gen Psychiatry, 52*, 464–470.
64. Sprich-Buckminster, S., Biederman, J., Milberger, S., et al. (1993). Are perinatal complications relevant to the manifestation of ADD? Issues of comorbidity and familiality, *J Am Acad Child Adolesc Psychiatry, 32*, 1032–1037.
65. Mick, E., Biederman, J., Faraone, S. V., et al. (2002). Case-control study of attention-deficit/hyperactivity disorder and maternal smoking, alcohol use, and drug use during pregnancy. *J Am Acad Child Adolesc Psychiatry, 41*, 378–385.
66. Milberger, S., Biederman, J., Faraone, S., et al. (1996). Is maternal smoking during pregnancy a risk factor for attention deficit hyperactivity disorder in children? *Am J Psychiatry, 153*, 1138–1142.
67. Rutter, M., Cox, A., Tupling, C., et al. (1975). Attainment and adjustment in two geographical areas: Vol 1, The prevalence of psychiatric disorders, *Br J Psychiatry, 126*, 493–509.
68. Biederman, J., Milberger, S. V., Faraone, S., et al. (1995). Impact of adversity on functioning and comorbidity in children with attention-deficit hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry, 34*, 1495–1503.
69. Yehuda, R. (2000). Biology of posttraumatic stress disorder. *J Clin Psychiatry, 61*, 14–21.
70. Zametkin, A. J., & Rapoport, J. L. (1987). Noradrenergic hypothesis of attention deficit disorder with hyperactivity: a critical review. In H. Y. Meltzer (Ed.), *Psychopharmacology: The Third Generation of Progress* (pp. 837–842). New York: Raven Press.
71. Pliszka, S. R. (1998). Comorbidity of attention-deficit/hyperactivity disorder with psychiatric disorder: an overview. *J Clin Psychiatry, 59*, 50–58.
72. Seidman, L. J., Valera, E. M., & Makris, N. (2005). Structural brain imaging of attention-deficit/hyperactivity disorder. *Biol Psychiatry, 57*, 1263–1272.
73. Castellanos, F. X., Lee, P. P., Sharp, W., et al. (2002). Developmental trajectories of brain volume abnormalities in children and adolescents with

attention-deficit/hyperactivity disorder. *JAMA, 288*, 1740–1748.
74. Bush, G., Valera, E. M., & Seidman, L. J. (2005). Functional neuroimaging of attention-deficit/hyperactivity disorder: a review and suggested future directions. *Biol Psychiatry, 57*, 1273–1284.
75. Alexander, G. E., DeLong, M. R., & Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci, 9*, 357–381.
76. Berquin, P. C., Giedd, J. N., Jacobsen, L. K., et al. (1998). Cerebellum in attention-deficit hyperactivity disorder: a morphometric MRI study. *Neurology, 50*, 1087–1093.
77. Lambert, N., Hartsough, C., Sassone, D., & Sandoval, J. (1987). Persistence of hyperactivity symptoms from childhood to adolescence and associated outcomes. *Am J Orthopsychiatry. 57*(1), 22–32.
78. Biederman, J., Faraone, S. V., Milberger, S., Curtis, S., Chen, L., Marrs, A., Ouellette, C., Moore, P., & Spencer, T. (1996). Predictors of persistence and remission of ADHD: results from a four-year prospective follow-up study of ADHD children. *J Am Acad Child Adolescent Psychiatry. 35*(3), 343–351.
79. Barkley, R. A., Fischer, M., Edelbrock, C. S., & Smallish, L. (1990). The adolescent outcome of hyperactive children diagnosed by research criteria: I. An 8-year prospective follow-up study. *J Am Acad Child Adolescent Psychiatry, 29*(4), 546–557.
80. Barkley, R. A., Fischer, M., Smallish, L., & Fletcher, K. (2002). The persistence of attention-deficit/hyperactivity disorder into young adulthood as a function of reporting source and definition of disorder. *J Abnorm Psychol, 111*(2), 279–289.
81. Mannuzza, S., Klein, R., Bessler, A., Malloy, P., & LaPadula, M. (1998). Adult psychiatric status of hyperactive boys grown up. *Am J Psychiatry, 155*(4), 493–498.
82. Mendelson, W., Johnson, N., & Stewart, M. (1971). Hyperactive children as teenagers: A follow-up study. *J Nervous and Mental Diseases, 153*(4), 273–279.
83. Mannuzza, S., & Gittelman, R. (1984). The adolescent outcome of hyperactive girls. *Psychiatry Research, 13*, 19–29.
84. Mannuzza, S., Gittelman Klein, R., Bonagura, N., Malloy, P., Giampino, T. L., & Addalli, K. A. (1991). Hyperactive boys almost grown up: V. Replication of psychiatric status. *Arch Gen Psychiatry*, *48*(1), 77–83.
85. Mannuzza, S., Klein, R. G., Bessler, A., Malloy, P., & LaPadula, M. (1993). Adult outcome of hyperactive boys: Educational achievement, occupational rank and psychiatric status. *Arch Gen Psychiatry, 50*, 565–576.
86. Lambert, N. M. (1988). Adolescent outcomes for hyperactive children: Perspectives on general and specific patterns of childhood risk for adolescent educational, social and mental health problems. *Am Psychologist*, *43*(10), 786–799.
87. Feldman, S., Denhoff, E., & Denhoff, J. (1979). *The attention disorders and related syndromes: Outcome in adolescent and young adult life, in Minimal Brain Dysfunction: A Developmental Approach.* In E. Denhoff, & L. Stern (Eds.) (pp. 133–148). New York: Masson Publishing Inc.
88. Weiss, G., Hechtman, L., Milroy, T., & Perlman, T. (1985). Psychiatric status of hyperactives as adults: a controlled prospective 15-year follow-up of 63 hyperactive children. *J Am Acad Child Adolescent Psychiatry, 24*(2), 211–220.
89. Yan, W. (1996). An investigation of adult outcome of hyperactive children in Shanghai. *Chinese Medical Journal, 109*(11), 877–880.
90. Cantwell, D. P., & Baker, L. (1989). Stability and natural history of DSM-III childhood diagnoses. *J Am Acad Child Adolescent Psychiatry, 28*(5), 691–700.
91. Offord, D. R., Boyle, M. H., Racine, Y. A., Fleming, J. E., Cadman, D. T., Blum, H. M., Byrne, C., Links, P. S., Lipman, E. L., & Macmillan, H. L. (1992). Outcome, prognosis and risk in a longitudinal follow-up study. *J Am Acad Child Adolescent Psychiatry, 31*(5), 916–923.
92. Claude, D., & Firestone, P. (1995). The development of ADHD boys: a 12-year follow-up. *Canadian Journal of Behavioural Science, 27*(2), 226–249.
93. Rasmussen, P., & Gillberg, C. (2000). Natural outcome of ADHD with developmental coordination disorder at age 22 years: a controlled, longitudinal, community-based study. *J Am Acad Child and Adolesc Psychiatry, 39*(11), 1424–1431.