www.nature.com/mp

### **FEATURE REVIEW**

# The genetics of autistic disorders and its clinical relevance: a review of the literature

CM Freitag

Department of Child and Adolescent Psychiatry, Saarland University Hospital, Homburg, Germany

Twin and family studies in autistic disorders (AD) have elucidated a high heritability of the narrow and broad phenotype of AD. In this review on the genetics of AD, we will initially delineate the phenotype of AD and discuss aspects of differential diagnosis, which are particularly relevant with regard to the genetics of autism. Cytogenetic and molecular genetic studies will be presented in detail, and the possibly involved aetiopathological pathways will be described. Implications of the different genetic findings for genetic counselling will be mentioned.

Molecular Psychiatry (2007) 12, 2-22. doi:10.1038/sj.mp.4001896; published online 10 October 2006

Keywords: review; autistic disorders; cytogenetics; molecular genetics; genetic counselling

#### Introduction

Autistic disorders (AD) are a group of disorders characterized by three core difficulties qualitative impairment in social interaction and communication, and restricted repetitive and stereotyped patterns of behaviour, interests and activities (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV);1 International Classification of Diseases-10 (ICD-10)2). The three disorders, autism, Asperger syndrome (AS) and pervasive developmental disorder-not otherwise specified (PDD-nos) differ with regard to symptom severity and early development of language, cognitive and social behaviour. Individuals with autism show impairments in all three areas and an abnormal development before age 3 years. AS is characterized by qualitative impairment in social interaction and restricted repetitive and stereotyped patterns of behaviour, interests and activities with an apparently normal language and cognitive development before age 3 years. PDD-nos is diagnosed in individuals who meet autism criteria, but show a late age of onset, or in individuals who show severe and pervasive impairment in one or two of the three core areas with or without cognitive or language delay.

Autism was first outlined in 1943 by Leo Kanner, an Austrian-US-American Professor of Child Psychiatry. He described children with mental retardation and severe social isolation not explained by the developmental level of the children.<sup>3</sup> Kanner referred to Eugen Bleuler by naming the syndrome 'infantile

autism' based on Bleulers schizophrenia criterion describing the loss of social interest in schizophrenia. At the same time, Professor Hans Asperger in Vienna, Austria, noticed similar patients with 'autistic psychopathy' and normal intellectual abilities. Hans Asperger noted that fathers of these children seemed aloof and socially isolated. Both, Kanner and Asperger, suspected a biological or even genetic origin of the disorder. However, this knowledge was lost during the 1950–1960s, until Michael Rutter and Lorna Wing resumed discussion on diagnostic concepts, differential diagnosis and aetiology of AD in the 1970s and 1980s.

In this review, we initially will delineate the phenotype of AD, discuss issues of differential diagnosis and present evidence that AD as a rule are genetically determined disorders. Cytogenetic and molecular genetic studies will be summarized, and the possibly involved aetiopathological pathways will be described. Implications of the different genetic findings for genetic counselling as well as future prospects will be pointed out.

## The phenotype of autistic and other pervasive developmental disorders

Autism, AS and PDD-nos (including atypical autism) are pervasive developmental disorders. Further pervasive developmental disorders mentioned in DSM-IV and ICD-10 are Rett syndrome and childhood disintegrative disorder.

Rett syndrome

Besides the loss of social engagement early in the course of the disorder, *Rett syndrome* is characterized by a pattern of acquired microcephaly, loss of purposeful hand skills usually in the end of the first year of life, progressive development of gait

Correspondence: Dr CM Freitag, Department of Child and Adolescent Psychiatry, Saarland University Hospital, Kirrbergerstr. 1, 66421 Homburg, Germany.

E-mail: christine.freitag@uniklinikum-saarland.de

Received 5 April 2006; revised 8 August 2006; accepted 9 August 2006; published online 10 October 2006

disturbance and stereotypic hand movements.<sup>7</sup> Females are predominately affected. Owing to these phenotypic characteristics, Rett syndrome and AD can well be differentiated clinically. In 1999, mutations in the MECP2 (methyl-CpG-binding protein 2) gene were identified, which cause the syndrome in more than 80% of the affected females. Variants of the MECP2 gene have also been assessed in AD, as affected males, a late-onset Rett syndrome variant, a preserved speech variant as well as female asymptomatic carriers have been described.8 The studies on MECP2 and AD will be mentioned below in the overview of the genetic association

#### Childhood disintegrative disorder

studies in AD.

Childhood disintegrative disorder is less distinct from AD than Rett syndrome. The central difference lies in an apparently normal development until age 2 years and a clinically significant loss of skills before age 10 years. Owing to the rarity of the disorder, 9 systematic studies regarding its aetiology are missing.

Spectrum of AD: autism, Asperger syndrome, PDD-nos and the broader autism phenotype

Regarding autism, AS and PDD-nos, these disorders are currently conceptualized by most researchers as a continuum of the same disorder with varying degrees of severity and associated intellectual functioning, possibly also including the broader autism phenotype (BAP) (Volkmar et al.,10 also see below, section on Family studies). The prevalence of autism was estimated to be 10/10000, of AS 2.5/10000 and of PDD-nos 15/10000. Recent studies have shown an increase in the prevalence of AD.9 AD are predominately genetically determined disorders. The findings of cytogenetic abnormalities and single gene disorders associated with AD indicate genetic heterogeneity and different modes of inheritance in individual families. However, for idiopathic AD, that is, cases with unknown cause, oligogenic, polygenic and multifactorial mechanisms have been proposed.

#### Cytogenetic findings and genetic syndromes in AD

There are many anecdotal reports of autism or AD with chromosomal anomalies. 11,12 In most cases, epidemiological data are missing. It has been discussed if the suspected increase in prevalence of AD might be caused primarily by cytogenetic pathologies. 13 Shortcomings of most cytogenetic studies are the lack of standardized assessment methods for AD, the inclusion of subjects with autistic features but no clear AD diagnosis and the lack of standardized assessment of cognitive and adaptive functioning.

Regarding the prevalence of cytogenetic abnormalities in AD, recent studies estimated a rate of 3-5% of cytogenetic abnormalities in AD. 13-17 Cytogenetic abnormalities have been described with regard to

most chromosomes. 11,12 Recent studies have aimed to elicit candidate genes or candidate gene regions by a detailed analysis of the boundaries of the cytogenetic abnormalities found in AD.<sup>18</sup>

With a rate of approximately 1%, the most prevalent cytogenetic abnormality is found on chromosome 15q11-13, in most cases a duplication of the maternal region or a supernumerary chromosome, that is, an inverted duplication. The AD phenotype in 15q11–13 duplication or inversion is characterized by a high incidence of epilepsies in childhood, muscular hypotonia and motor coordination problems combined with moderate to severe mental retardation and speech delay or absence of speech. Regarding additional behavioural problems, a severe hyperactivity is often noticed.19-26

Deletions of the maternal or paternal chromosome 15q11-13 regions are associated with two cytogenetic imprinting disorders, Angelman syndrome and Prader-Willi syndrome (PWS). Genomic imprinting describes the phenomenon of differences in gene expression between the allele inherited from the mother and the allele inherited from the father.

Angelman syndrome is phenotypically characterized by moderate to severe mental retardation, dyspractic gait, a happy appearance with excessive laughter, no language development, motor stereotypies (e.g., hand-flapping and mouthing of objects), characteristic electroencephalogram (EEG) findings (frontal 2-3 Hz activity; Laan and Vein<sup>27</sup>) and the development of seizures in about 80% (atypical absences, myoclonic and tonic-clonic seizures; Valente et al. 28). The characteristic EEG pattern, the happy appearance and the dyspractic gait differentiate Angelman syndrome from AD. Four major genetic mechanisms are known to cause Angelman syndrome: in 70-75% a interstitial deletion of the maternal chromosome 15q11-13; in 2-3% an uniparental disomy (UPD) of chromosome 15q11-13 with lack of the maternal copy; in 3-5% a abnormal methylation of chromosome 15q11-13; and in 20% mutations in the UBE3A gene or in the imprinting centre located on chromosome 15q11-13.29

*PWS* is phenotypically characterized by moderate mental retardation, infantile hypotonia and poor suck reflex, growth retardation, delayed sexual development and a childhood onset of pronounced hyperphagia.30 Major genetic mechanisms in PWS are as follows: in 70-80% interstitial deletions of the paternally derived chromosome 15q11-13; in 20-30% maternal UPD with lack of the paternal copy; and in 1–2% imprinting center mutation.<sup>31</sup> More autistic-like impairment in social interaction has been found in PWS subjects with UPD compared to PWS subjects with a deletion of the paternal chromosome 15q11-13.32 This emphasizes the possible relevance of maternally derived genes of the chromosome 15q11-13 region for development of AD. Several candidate genes in this region have been assessed, which will be presented

and discussed in the genetic association studies section below.

Deletions of *chromosome 2g37*, 33-39 *chromosome* 7q31<sup>40-42</sup> and *chromosome 22q11* have additionally been assessed with regard to their relevance for the development of AD. Deletions of chromosome 2q37 are often associated with dysmorphic features, hypotonia, kidney diseases and brachydactyly.33 Linkage studies have shown suggestive evidence for linkage on chromosome 2q21-q33 differing from the abovementioned cytogenetic findings. The findings of the cytogenetic studies of chromosome 7 deletions, however, overlap with the candidate region derived from genetic linkage studies (see below). With regard to the syndromes associated with a microdeletion of chromosome 22q11.2 (e.g., velocardiofacial syndrome, DiGeorge syndrome, conotruncal anomaly face syndrome), autistic features and AD have been described in these syndromes. 43 However, in a sample of 103 subjects with a strict diagnosis of autism, no single subject with a deletion of 22q11.2 has been found.44 Recently, a deletion on chromosome 22q13.3 has been suspected as cause of AD.45

In conclusion, a detailed cytogenetic evaluation has to be recommended in all subjects with AD, even more so if the subject additionally shows mental retardation, abnormal EEG patterns or seizures, muscular hypotonia, severe motor and gait problems or dysmorphic features. The finding of a chromosomal anomaly as a likely cause of AD has strong implications for genetic counselling.

#### Single gene disorders associated with AD

Several single gene disorders are associated with an increased risk of AD. The most prevalent single gene disorders in AD are tuberous sclerosis (TSC) and fragile X syndrome (FRAXA). More rare, but medically treatable single gene disorders are phenylketonuria (PK) and Smith–Lemli–Opitz syndrome (SLO). Neurofibromatosis has been suspected to be associated with AD; however, recent epidemiological studies did not show a higher than the population rate in AD, pointing towards random co-occurrence. Untreated PK as a cause of AD has become rare in countries with an established neonatal screening programme.<sup>9</sup>

TSC is an autosomal-dominant neurocutaneous disorder, characterized among others by facial angio-fibromas, ungual fibromas, cortical and cerebral tubers, calcified subependymal nodules, giant cell and retinal astrocytomas, hypomelanotic skin macules, rough atrophic skin patches, cardiac rhabdomyoma, renal lesions and infantile spasms. TSC is due to several different mutations either in the *TSC1* gene on chromosome 9q34 or in the *TSC2* gene on chromosome 16p13. Epidemiological studies have shown that the prevalence of TSC in children with autism and of autism in TSC is more than 100 times greater than expected. Children with TSC also can develop AS or PDD-nos. Risk factors for the

development of AD in TSC are a TSC2 mutation (compared to TSC1; Lewis *et al.*<sup>48</sup>), presence of temporal tubers, <sup>49,50</sup> early age of seizure onset, resistance to antiepileptic treatment and history of infantile spasms. <sup>50–53</sup>

FRAXA is one of the frequent causes of mild to moderate mental retardation in boys. The clinical picture includes macroorchidism, large ears, prominent jaw and high-pitched speech.<sup>54</sup> The incidence of the FRAXA full mutation has been estimated at one in 4000 in men and one in 8000 in women.<sup>55</sup> The molecular basis of the syndrome is an unstable expansion of a CGG repeat (>200 repeats) in the 5'UTR (untranslated region) of the FMR1 gene located on chromosome Xq27, resulting in a hypermethylation of the CGG sequence and a reduced translation of the FMR1 protein. 56,57 About 2-5% of the children and adolescents diagnosed with AD carry a full FRAXA mutation or FRAXA mosaics. 9,13,16,58 Despite this finding, no linkage or association with FMR1 gene variants<sup>59,60</sup> or the FRAXA mutation has been found in large samples diagnosed with AD by strict criteria. 61,62 As the diagnosis of FRAXA, however, has major implications for genetic counselling, it should be ruled out in all individuals with AD and mildto-severe mental retardation.

SLO is an autosomal-recessive disorder due to mutations in the gene for  $\Delta 7$ -dehydrocholesterol reductase,63,64 leading to increased serum levels of 7-dehydrocholesterol. The incidence has been estimated to be one in  $10\,000$  to one in  $60\,000.^{65}$  SLO can be improved by supplementary dietarial cholesterol. The phenotype is variable with only rare symptoms or multiple congenital anomalies comprising cleft palate, cataracts, ptosis, hypospadias, syndactyly and a distinctive craniofacial appearance. 66 The most common malformation in large-scale studies was the syndactyly of toes 2 and 3; however, only present in about 80% of affected individuals. 66,67 Two studies have shown a high rate of AD in individuals with SLO,65,68 especially in children with a start of cholesterol supplementation after age 5 years.

In conclusion, assessment of FRAXA has to be recommended in every individual with an AD with mild-to-severe mental retardation, with or without the characteristic dysmorphic features. TSC should always be excluded by a thorough skin exam with the Wood light even in absence of seizures. The diagnosis of FRAXA or TSC is particularly relevant with regard to genetic counselling. SLO at present should be suspected in individuals with AD and syndactyly of toes 2 and 3; however, more studies regarding the association of SLO and AD are needed, as SLO is a treatable disorder.

### Associated non-genetic medical or environmental conditions

Studies on associated medical conditions in autism assessed genetic and non-genetic risk factors. It is generally agreed that about 10–15% of individuals

with AD have a known medical condition that causes the disorder. 69 Most of these are the cytogenetic or single gene disorders mentioned in the previous sections. Non-genetic medical conditions are rare; however, they are especially relevant with regard to the prevention of AD. Non-genetic medical conditions are regarded as phenocopies in a genetic framework. Numerous case reports exist that reported associations of maternal thalidomide use,70 maternal valproic acid use<sup>67,71,72</sup> or maternal alcohol abuse<sup>73,74</sup> during pregnancy. The association of congenital rubella with autism has been studied in a longitudinal study on 243 children with congenital rubella,75,76 of whom 7% developed typical or atypical autism. With about 2%, another relatively frequent medical condition in AD is cerebral palsy.9

The mumps—measles—rubella (MMR) vaccine has received considerable attention as possible cause for the development of AD. Studies supporting this view, however, have not excluded children with known genetic cause nor have assessed the level of functioning of the children before the MMR vaccination.<sup>77</sup> Epidemiological and case-control studies did not show an increased risk by the vaccination.  $^{78-81}$  Therefore, the MMR vaccination currently cannot be regarded as a risk factor for the development of AD.

In conclusion, non-genetic medical conditions are minor risk factors for AD; however, in the individual child they can be the relevant cause of the AD. They represent phenocopies of the disorder.

#### Formal genetics and patterns of inheritance

If the cause of a disorder is not known, different approaches exist to elicit if a disorder is likely to be caused by genetic or environmental risk factors or a combination of both. Twin and family studies are performed to compare concordance rates and to estimate the heritability of a disorder, that is, the variation due to additive genetic effects. Studies on twins reared apart or adoption studies are other designs to assess the influence of genetic and environmental risk factors. The latter studies have not been performed in AD due to the low prevalence of the disorders. Family studies allow one to estimate a recurrence risk for the disorder, which can be translated into a heritability estimate, and additionally may allow one to elicit a certain pattern of inheritance, if the disorder of interest seems to be a Mendelian disorder. Twin and family studies have prevailingly been performed in families with children with 'idiopathic' AD, that is, children with an abovementioned medical condition or genetic syndrome and their families have been excluded from analysis.

#### Twin studies

Four independent epidemiologically based twin studies on autism have been performed.82-85 It has been discussed that twinning in itself might be a risk factor for the development of autism.86,87 However, three large-scale epidemiological studies have refuted

this idea.88-90 In the four twin studies, pairwise concordance rates in monozygotic (MZ) twins were in the range of 36–96%, and 0–30% in same-sex dizygotic (DZ) twin pairs, resulting in heritability estimates >90%. No twin study on AS or PDD-nos has been performed to date. A re-analysis of one twin study82 with regard to the BAP, which was conceptualized for two areas, communication impairment and social dysfunction, did shown far higher rates of the BAP in discordant MZ than in discordant DZ pairs.91 Among the MZ co-twin, communication impairment and social dysfunction frequently cooccurred together, whereas restricted, stereotyped or repetitive behaviours were never seen in isolation, and were present in only one third of the individuals with BAP. This suggests that stereotyped and repetitive behaviour might be mediated by other genetic risk factors than the communication and social interaction impairments. 92,93 Other markers of genetic heterogeneity<sup>91</sup> were absence of useful speech, presence of epilepsy, severe mental retardation or head circumference, whereas the Autism Diagnostic Interview-Revised (ADI-R) total score, verbal and non-verbal IQ did show smaller within- than betweenpair variances indicating variable expression of the same genetic liability regarding these three measures.

The question of different underlying genetic liabilities in AD has been addressed by two further population-based twin studies using quantitative measurements of reciprocal social interaction and non-social behaviour. One study<sup>94,95</sup> assessed autistic traits by the Social Responsiveness Scale (SRS) in 788 pairs of twins aged 7-15 years from the Missouri Twin Study. A heritability of 0.76 in males and of 0.40 in females for social responsiveness was elucidated. Despite the differences in heritability, no evidence for the existence of sex-specific genetic influences was found. The distribution of the SRS scores gave evidence for a continuously distributed trait. In a subsample of the UK Twin Early Development Study who were followed to the age of 7 years, 10 items for social and six items for non-social autistic traits were assessed by questionnaires for parents and teachers to elicit the genetic relationship between individual differences in social and non-social behaviours characteristic of autism.<sup>96</sup> In the univariate model, genetic (0.62-0.76) and non-shared environmental effects did explain variability in social and non-social autistic traits. In the bivariate model, the genetic correlation between social and non-social behaviours, however, were below 0.40, with considerably lower values for teacher data and for female twins. This implies that social and non-social autistic traits are highly, but independently genetically determined, similar to the findings of other studies. 91,92,97

In conclusion, twin studies on AD resulted in heritability estimates >90% for the narrow phenotype of autism. They also pointed towards a common underlying genetic liability for AD and the BAP with regard to social interaction and communication. Stereotyped and repetitive behaviour,



however, might be mediated by another set of genes again underscoring genetic heterogeneity of AD. The MZ correlation <100% points to the influence of weak environmental effects on the phenotypic expression of AD.

#### Family studies

Familial aggregation of a disease can be measured by comparing the frequency of the disease in the relatives of an affected person with its prevalence in the general population. For AD, only one study<sup>5</sup> assessed the recurrence risk for siblings in case studies on autism and compared it to the population prevalence, at that time estimated at 2–5 in 10 000. This recurrence risk was 50–100 times greater than expected by chance. However, at that time, prevalence estimates for AD were very low, and no population-based studies had been performed. More recent family studies used a case—control approach to compare rates of AD and other possibly genetically determined traits in families with a child with autism and families without.

Regarding the spectrum of AD in family members, a case—control study in families with a child with autism compared to families with a child with Down's syndrome syndrome found a rate of AD in 5.8% of the siblings of children with autism, but none in the siblings of children with Down's syndrome. In addition, they described an increased rate of a combination of less severe cognitive—communication abnormalities with social impairment and/or stereotyped behaviours in 12.4% of siblings of a child with autism compared to 1.6% in siblings of a child with Down's syndrome. Mental retardation was not increased in both comparison groups indicating that cognitive abilities were independent of autistic traits.

Another study in siblings regarding the BAP found increased rates of impairment in communication abilities as assessed by the children's communication checklist in siblings of children with autism compared to typically developing children. 99 Language abilities, however, were not impaired in siblings of children with autism100 arguing against language abilities as a marker for the BAP. Two other studies, however, did find a reduced variance within autistic sib-ships regarding the onset of phrase speech, 101 and an influence of language abilities on the correlation of ICD-10 autism symptoms and the presence of the BAP in relatives, 102 arguing for a role of language abilities in the genetics of AD. A high concordance for rituals and repetitive play, for social impairments and non-verbal communication in autistic sib-pairs was found in three further studies. 101,103,104 These studies were interpreted in the same way as the twin studies suggesting the same genetic liability for social and communicative behaviour and a different genetic liability for stereotyped and repetitive behaviour and language development. 105

Assessment of the BAP in parents of children with autism has given similar results. In several studies, rates of 10–45% of social impairment,

aloofness, shyness and pragmatic language impairment were present in fathers and mothers of children with autism or AS. 106-114 This finding did not differ in parents of children with autism with and without a history of language regression. 115 Regarding obsessive-compulsive behaviours in parents of multiplex autism families, a strong correlation of the severity of restricted repetitive and stereotyped patterns of behaviour, interests and activities in the child and rates of obsessive-compulsive traits or disorders were found in parents. 116

In addition to the assessment of the BAP in relatives of children with autism, the rate of psychiatric disorders in parents of children has been assessed thoroughly (meta-analysis; Yirmiya and Shaked<sup>117</sup>). In comparison with parents of children with no known genetic risk factors parents of children with autism showed higher rates of anxiety disorders including social phobia, depression and obsessions in both mothers and fathers. The parents of low-functioning children with AD presented slightly higher rates of psychiatric disorders than the parents of high-functioning children with AD.

These findings further support the presence of sub-threshold autistic traits in parents and siblings of children with an AD, which are similar in male and female relatives. One study aimed to elicit a specific genetic model for the families with a child with idiopathic AD and resulted in an epistatic genetic model with three (range: two to ten) interacting genetic loci as the most likely genetic model for AD. Despite the male: female ratio of 4:1, no evidence for X-linked loci or a simple sex-limited additive genetic multifactorial threshold model was found in the twin and family studies, as the BAP in female and male relatives did not differ.

The phenotypic findings were adopted in the design and statistical analyses of molecular genetic studies. Findings of possibly independent risk factors for restricted repetitive and stereotyped patterns of behaviour, interests and activities, and for language abilities were incorporated into specific linkage analysis models. The asymmetric sex distribution also was assessed by specific models in linkage studies. Only a few association and linkage studies to date have tried to assess gene—gene interaction (epistasis). The increased rate of other psychiatric disorders in AD relatives has not yet been assessed by molecular genetic studies.

#### Molecular genetic studies

Similar to the twin and family studies molecular genetic studies have been performed in 'idiopathic' AD in large samples of families with at least one child with AD.

#### Linkage studies

Linkage studies aim to elicit gene loci by mapping genes in families. Linkage can be defined as the tendency for alleles close together on the same

chromosome to be transmitted together, as an intact unit, through meiosis. Linkage studies are either performed as full genome screens with a dense set of genetic markers covering all chromosomes, or locally (fine-mapping) at a certain chromosomal area of interest. Several research groups have performed full genome screens in AD. 40,119-137 Research groups, study design and main findings of linkage studies that reported positive results are summarized in Table 1 for genome-wide linkage and association studies with a qualitative AD phenotype, and in Table 2 for linkage studies with either a quantitative phenotype, a specific qualitative endophenotype, or other specific linkage models. 122,123,127,130,138-148 From Table 1, it can be seen that linkage has been found in at least two independent studies in regions 2q, 3q25-27, 3p25, 6q14–21, 7q31–36 and 17q11–21.

The locus on chromosome 7 was further supported by a regional meta-analysis <sup>149</sup> of four studies. <sup>120,125,131,132</sup> A recent heterogeneity-based genome search metaanalysis<sup>150</sup> again supported region 7q22-q32, which reached genomewide significance in studies on strictly defined autism, and revealed two loci of suggestive significance (10p12-q11.1;17p11.2-q12) in studies on AD and the BAP. Nine linkage studies 119,120,123,126,131–133,136,151 were included in this meta-analysis. Between-scan heterogeneity low for the locus on 7q, but high for the loci on 10p 12–q11.1 and 17p11.2–q12

Despite the marked sex difference in the prevalence of AD, most studies assessing the X-chromosome for linkage have resulted in negative findings. 59,152 A recent fine mapping linkage study has found suggestive evidence (criteria of Lander and Kruglyak<sup>153</sup>) for linkage at an X-chromosomal locus for the BAP. 134

Owing to the rarity of the disorder, genome scans often were first performed in a smaller set of families and again in an enlarged set of families, containing the previously assessed families as well. This, however, has not always resulted in more pronounced linkage findings at previously described loci, but on the other hand often resulted in diminished LOD (logarithm of the odds for linkage) scores. This points to the possibility of different loci containing risk genes in different populations, to false-positive or -negative findings due to differing linkage disequilibrium patterns in different populations, 154 and again towards heterogeneity of AD.

The latter has been addressed by linkage analyses in phenotypically more homogeneous samples (Table 2). Incorporating the above-mentioned findings from family studies, samples were either stratified phenotypic traits like language development, developmental milestones or developmental regression, and restricted repetitive and stereotyped behaviours, or a quantitative trait locus approach on all family members with regard to these measures was taken. From studies assessing more homogeneous samples, it can be concluded that genes influencing language development most likely will be found on chromosome 2q and 7q35, as studies in independent

samples have reported these loci. 122,138-140,142 Further unreplicated loci with possible relevance for language development have been found in the Autism Genetic Resource Exchange (AGRE) sample (Table 2). Studies on developmental milestones and developmental regression have been scarce; however, the linkage findings were in the range of significant linkage at 19p13 for more rapid achievement of developmental milestones, 130 and at chromosomes 7q and 21q for developmental regression. 43 With regard to obsessive-compulsive behaviour, significant linkage has been found on chromosome 1q.144 Orderedsubset analysis regarding the phenotype insistence on sameness has resulted in significant linkage at chromosome 15q11-13.147

Owing to the disappointing findings regarding the X-chromosome, other approaches have been chosen to elucidate the skewed sex distribution of AD. In two independent samples, two loci on 17q did show significant linkage in male only pairs. 123,148 In the International Molecular Genetic Study of Autism Consortium (IMGSAC) sample, suggestive evidence for linkage in male-only pairs was found on chromosomes 7q and 16p. Studies on maternally or paternally imprinted loci have resulted in inconclusive findings to date.

#### Association studies

Several candidate genes in regions implicated by genome scans have been examined. In Supplementary online Table 1 (selected genetic association studies), candidate gene studies are presented if the respective variant or other variants in the same gene were assessed by at least two independent studies. The diagnostic standard of the association studies differs considerably. Most, but not all studies excluded children with FRAXA. Cytogenetic assessment is not always reported.

#### Chromosome 2

Regarding the locus on chromosome 2, several studies have assessed mutations or variants in multiple candidate genes that play a role in brain development. No clear evidence for association of any of the new variants with AD was found despite relatively high LOD scores from linkage analyses in the assessed samples.38,155-157 Four independent studies compared two single-nucleotide polymorphisms (SNPs) in the gene for the mitochondrial aspartate/glutamate carrier SLC25A12 in family-based and case-control association studies. Two studies158,159 found an increased risk for autism associated with the haplotype GG (reverse strand) = CC (sense strand) consisting of SNPs rs2056202 and rs2292813. Two other studies,  $^{160,161}$ however, did not replicate this finding despite similar or greater size and power. The possible functional relevance of this haplotype has not yet become clear as it is located in an intron of the gene.

#### Chromosome 6

Two studies have found evidence for association of different SNPs in the Glutamate receptor 6 (GluR6)



 Table 1
 Genomewide linkage and association studies/fine-mapping linkage studies – qualitative AD phenotype

Research group	Reference	Diagnostic criteria AD	Number of families	Linkage analysis method	Chromosomal region	Markers	Highest LOD/NPL/MLS/ Z-scores/lowest P-values
Duke	Ashley-Koch <i>et al</i> . (1999)	ADI-R ICD-10 DSM-IV	76 ASP	Non-parametric and parametric linkage analysis (fine mapping of 7q22.1–q31.2 only)	7q22.1–q31.2	D7S640	NPL = 2.0
Finland	Auranen <i>et al.</i> (2002)	CARS ASSQ ASDI DSM-IV/ICD-10	38 ASP and extended families	Non-parametric and parametric linkage analysis	3q25 <b>–</b> 27	D3S2421 D3S2427 D3S3041 D3S3037 D3S3699 D3S3730	LOD = 3.5 LOD = 3.1 LOD = 3.6 LOD = 4.3 LOD = 3.0 LOD = 3.2
CLSA	Barrett <i>et al.</i> (1999)	ADI-R ADOS-G VABS, RPM	75 ASP	Parametric linkage analysis	7q31–33 13	D7S1813 D13S800	HLOD recessive = 2.2 HLOD recessive = 3.0
AGRE <sup>a</sup>	Bartlett et al. (2005) (subset of Yonan et al. (2003))	ADI-Rª	303 ASP	Posterior probability of linkage, allowing for heterogeneity	1q23–24 17q11	Not reported Not reported	55% 15% (values > 2% indicative of linkage)
AGRE <sup>a</sup>	Buxbaum <i>et al.</i> (2001) (subset of Alarcon <i>et al.</i> (2002))		95 ASP	Non-parametric and parametric linkage analysis	2q	D2S364	HLOD dominant = 2.25 NPL = 2.45
AGRE <sup>a</sup>	Cantor <i>et al.</i> (2005)	ADI-R <sup>a</sup>	91 ASP	Non-parametric linkage analysis	3p14–12 17q11–23	D3S2406 D17S1299	MLS = 1.8 MLS = 1.9
Utah	Coon <i>et al.</i> (2005)	ADI-R ADOS-G WISC WAIS	One large family with seven affected and 24 unaffected members	Non-parametric linkage analysis (fine mapping of 3q25–27 only)	3q25–27	rs1362645 rs1402229	NPL = 3.34 NPL = 3.53
IMGSAC	IMGSAC (2001)	ADI-R ADOS-G VABS, RPM, PPVT	152 ASP	Model-free linkage analysis	2q24–q33 7q 16p	D2S2188 D7S477 D16S3102	MLS = 3.74 MLS = 3.20 MLS = 2.93
IMGSAC	IMGSAC (1998) (subset of IMGSAC (2001))	ADI-R ADOS-G VABS	99 ASP	Model-free linkage analysis	7q	D7S530- D7S684	MLS = 2.53 UK families: MLS = 3.55
IMGSAC	Lamb <i>et al.</i> (2005)	ADI-R ADOS-G VABS	219 ASP	Model-free linkage analysis	2 7 9	D2S2314-D2S2310 D7S530-D7S640 D9S171-D9S161	MLS = 2.5 MLS = 2.3 MLS = 2.1

Table 1 Continued

Research group	Reference	Diagnostic criteria AD	Number of families	Linkage analysis method	Chromosomal region	Markers	Highest LOD/NPL/MLS/ Z-scores/lowest P-values
Faroe Islands	Lauritsen et al. (2006)	ADI-R ADOS-G	12 cases and 22 controls	Genome-wide association study	3p25.3 15q21.3 18q21.1–21.1	D3S3594 D15S198 D18S536-D18S970	P-value = 0.00007 P-value = 0.005 P-value = 0.002
AGRE <sup>a</sup>	Liu <i>et al.</i> (2001) (subset of Yonan <i>et al.</i> (2003))	ADI-R <sup>a</sup>	110 ASP	Model-free linkage analysis broad and narrow phenotype	5 19 X	D5S2494 (broad) D19S714 (narrow) DXS1047 (both)	MLS = 2.55 MLS = 2.53 MLS = 2.67
CLSA AGREª	McCauley et al. (2005)	ADI-R <sup>a</sup>	158 ASP	Model-free and model-dependent methods	3p25 17q11 19p13	D3S2691 D17S1294 D19S930 D19S113	HLOD = 1.8, LOD = 2.2 HLOD = 2.9, LOD = 2.1 HLOD = 2.6, LOD = 1.9 HLOD = 2.2, LOD = 1.4
PARIS	Philippe et al. (1999)	ADI-R VABS	51 ASP	Model-free linkage analysis	6q21	D6S283	MLS = 2.23
Stanford	Risch <i>et al.</i> (1999)	ADI-R ADOS-GVABS Other IQ measures	90 ASP (stage 1) and 49 ASP (stage 2)	Model-free linkage analysis	1	D1S1675 (combined stages $1+2$ )	MLS = 2.15
CAT	Shao <i>et al.</i> (2002a)	ADI-R ADOS-G VABS	99 ASP	Non-parametric and parametric linkage analysis	3	D3S3680	Two-point MLS = 2.02 Parametric models resulted in LOD scores < 2.0
London	Vincent et al. (2005)	ADI-R ADOS-G	22 multiplex families	Non-parametric linkage analysis (fine mapping of Xq27–28 only)	Xq27–28	DXS37	HLOD = 1.1
AGRE <sup>a</sup>	Yonan et al. (2003)	ADI-R <sup>a</sup>	345 ASP (11 later diagnosed with FRAXA; Bartlett <i>et al</i> . (2005)	Model-free linkage analysis broad and narrow phenotype	5 11 17	D5S2494 (broad) D11S1392— D11S1993 (broad) D17S1800 (broad)	MLS = 2.54 MLS = 2.24 MLS = 2.83
Finland	Ylisaukko-Oja <i>et al</i> . (2004)	ICD-10 DSM-IV ASSQ	17 multiplex families Asperger syndrome only	Non-parametric and parametric linkage analysis	1q21–22 3p14–24 D13q31–33	D1S484 D3S2432 D13S793	Z-dominant = 3.6 Z-dominant = 2.5 NPL = 3.32 Z-dominant = 1.6 NPL = 2.7

_	_
4	
n	32
v	-

Research group	tesearch Reference roup	Diagnostic criteria AD	Number of families	Linkage analysis method	Chromosomal Markers region	l Markers	Highest LOD/NPL/MLS/ Z-scores/lowest P-values
AGREª Finland	Ylisaukko-Oja <i>et al.</i> (2006) (samples of Liu <i>et al.</i> (2001); Yonan <i>et al.</i> (2003); and Auranen <i>et al.</i> (2002))	ADI-R CARS ASSQ ASDI DSM-IV/ICD-10	314 ASP and extended families	Non-parametric linkage analysis 3 liability classes	1p12-q25 3p24-26 4q21-31 6q14-21 7q33-36 8q22-24 17p12-q21	D1S1677 D3S3691 D4S1591 D6S1021 D7S483 D8S1832	NPL = 2.25 NPL = 2.10 NPL = 2.53 NPL = 2.47 NPL = 2.31 NPL = 2.07 NPL = 2.30

Abbreviations: ADI-R, Autism Diagnostic Interview-Revised; ADOS-G, Autism Diagnostic Observation Schedule-Generic; AGRE, Autism Genetic Resource Exchange; ASSQ, Autism Spectrum Screening Questionnaire; CARS, Childhood autism rating scale; CAT, Collaborative Autism Team; CLSA, Collaborative Linkage Study of Autism; CPEA, Collaborative Programs of Excellence in Autism; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; FRAXA, fragile-X syndrome; ICD-10, the ICD-10 classification of mental and behavioural disorders; HLOD, heterogeneity LOD score; IMGSAC, International Molecular Genetic Study of Autism Consortium; LOD, logarithm of the odds for linkage; MLS, multipoint maximum-likelihood score; NPL, non-parametric linkage statistic; PARIS, Paris Autism Research Assessment of the ADOS-G in the AGRE sample is only mentioned in a summary publication on the sample (Gschwind et al. 268), but not in the publications cited in International Sibpair Study; RPM, Raven Progressive Matrices; VABS, Vineland Adaptive Behaviour Scales; WAIS, Wechsler Adult Intelligence Scale; WISC, Wechsler ASDI, Asperger's Syndrome Diagnostic Interview; ASP, affected sibpair design, that is, at least two affected children per family; the number of families is reported Intelligence Scale for Children; Z, non-parametric (QTL-) statistic

gene on chromosome 6 with AD. 162,163 Only one of these SNPs might have possible functional implications, and most were located in introns. However, given the importance of glutamate in brain development, learning and memory, 164 the positive linkage findings on 6q21 in two studies (Table 1) as well as post-mortem evidence of brain abnormalities of the glutamate neurotransmitter system in autism, 165 this candidate gene seems to be of relevance in the pathogenesis of AD.

#### Chromosome 7

Most candidate genes assessed in AD are at the locus on chromosome 7, as this has been the best-replicated locus from linkage studies. As linkage has been stronger in families with specific language phenotypes (Table 2), variants in the Forkhead Box P2 (FOXP2) gene, which was mutated in a severe monogenic form of speech and language impairment in one family, 166,167 have been assessed by several studies for association with AD. With the exception of nominal significance in one Chinese and one Japanese study, 168,169 none of the other studies did find an association of FOXP2 polymorphisms or mutations with AD. 170-173 Therefore, it is unlikely that this gene is of relevance in the aetiology of AD.

The Reelin (RELN) gene is another candidate gene, which might be causative for AD, as it has been shown that Reelin signalling was impaired in postmortem cortices of individuals with autism,174 and reduced plasma levels of Reelin have been found in individuals with AD and their first-degree relatives.<sup>175</sup> Reelin is a signalling protein that plays a crucial role in neuronal migration, formation of cortical layers and synaptogenesis. The most commonly assessed variant in RELN is a trinucleotide repeat polymorphism in the 5'UTR with unknown functional relevance. Three studies did find an association, 176-178 five other studies of comparable size and power did not find an association of the 5'UTR trinucleotide or other variants with AD. 179-183 The first positive finding<sup>176</sup> reported an association with the relatively rare longer alleles (>10) of the 5'UTR trinucleotide polymorphism with AD. However, in another study, 178 the most common repeat 10 was over-represented in AD. One study 177 reported an association of the more common allele of SNP rs736707, which has not yet been replicated by other studies and might not be of functional relevance, as it is located in intron 59 of RELN. Despite the biochemical evidence of a possible role of Reelin in the pathogenesis of autism, the genetic findings are still inconsistent.

Two recent studies have assessed the laminin  $\beta$ -1 (LAMB1) gene located on chromosome 7q31 for association with AD. A novel missense variant (4975C > T = I1547T) in exon 30, which was predicted to have a damaging effect on protein structure, was associated with AD in an affected sib-pair (ASP) sample, but only marginally in the singleton replication sample of the IMGSAC consortium.<sup>184</sup> Another

Table 1 Continued

Table 2 Linkage studies – quantitative and specific qualitative endophenotypes, specific linkage models

Diagnostic

criteria AD

ADI-Ra

ADI-Ra

ADI-R

data

ADI-Ra

ADI-Ra

ADOS-G

FHI: Parent

Number of

291 of 345 ASP

ADI-R language

with complete

information

phrase in

67 ASP

OSA word in

132 ASP: OSA

123 of 152 ASP

with complete

information

50 of 75 ASP

49 of 95 ASP

meeting language

criteria

criteria

228 ASP

8: 175 ASP:

16: 70 ASP

meeting language analysis

ADI-R language

families

Linkage analysis

Non-parametric

and parametric

Ordered-subset

Non-parametric and

Parametric linkage

with language delay)

Non-parametric and

parametric linkage

Non-parametric

OTL analysis

analysis

analysis

OSA chromosome and parametric

OSA chromosome Ordered-subset

(parents and children 13

parametric

OTL analysis

OTL analysis

analysis

method

Chromosomal

3q

10

15

16

17g OSA word: 7q35

10

16

17

OSA phrase:

Phrase: 10

7q

2q

1p13-q12

4q21-25

8q23-24

16p12-13

7q35

Word: 7a35-36

11

11

20

7q35

5

region

Word: 1

Phrase: 5

Markers

Not reported

D7S1813-D7S821

D13S217-D13S800

D2S335-D2S364

Not reported

Not reported

Not reported

Not reported

Not reported

D10S2327

D7S1824-D7S3058

D17S1298-

D17S1299

D3S3045-D3S1763

D17S1290-D17S1301 Z = 2.84

Highest LOD/

scores/lowest P-values

NPL/MLS/Z

Z = 2.20

Z = 3.10

Z = 2.39

Z = 2.19

Z = 2.20

Z = 2.38

Z = 2.28Z = 2.31

Z = 2.08

Z = 2.22

Z = 2.98:

Z = 2.22;

Z = 2.10; HE-LOD = 1.22

Z = 2.19:

Z = 2.29;HE-LOD = 2.21

HE-LOD = 1.14

HE-LOD = 0.96

HE-LOD = 1.21

M-HLOD = 2.2,

M-HLOD = 2.5,

dominant = 2.99

OSA MLS = 3.4

OSA MLS = 3.8

HLOD = 2.8

HLOD = 2.0

NPL = 3.32

Z = 3.63

Z = 3.13

Z = 2.45

Z = 2.61

Z = 3.09

HLOD

MLS = 2.76

MLS = 2.57

Reference

Alarcon

Alarcon

Bradford

Buxbaum

et al. (2001)

Chen et al.

(2006)

et al. (2001)

et al. (2002)

et al. (2005)

Endophenotype/linkage

1. Language measures Age at first word

Age at first phrase

Age at first word

Delayed language

Parent's language

Onset of phrase speech

development in

AD subject

development

>36 months

Non-verbal

Molecular Psychiatry

communication

Age at first phrase

model

Research

group

AGRE<sup>a</sup>

(2003))

AGRE<sup>a</sup>

(2005))

CLSA

AGRE<sup>a</sup>

(2002))

AGRE<sup>a</sup>

(2003))

(subset of

Yonan et al.

(subset of

Alarcon et al.

(subset of

Alarcon et al.

(subset of

Yonan et al.

Table 2 Continued

Endophenotype/linkage model	Research group	Reference	Diagnostic criteria AD	Number of families	Linkage analysis method	Chromosomal region	Markers	Highest LOD/ NPL/MLS/Z scores/lowest P-values
Onset of phrase speech > 36 months	CAT	Shao <i>et al.</i> (2002b)	ADI-R	45 of 82 ASP meeting language criteria	Non-parametric and parametric linkage analysis	2q	D2S116 D2S2309	HLOD recessive = 2.5, MLS = 2.9 HLOD dominant = 2.2,
								MLS = 1.6
2. Developmental milesto Motor-language, bladder and bowel control milestones	ones and develop CLSA AGRE <sup>a</sup>	mental regress McCauley et al. (2005)	ion ADI-Rª	92 of 158 ASP	Ordered-subset analysis (more rapid achievement)	19p13	D19S930	LOD = 3.4
Developmental regression	AGRE <sup>a</sup>	Molloy et al. (2005)	ADI-R <sup>a</sup>	34 of 288 ASP	Non-parametric and parametric linkage	7q	D7S483	NPL = 3.7 MLS
					analysis	21q	D21S1437	dominant = 2.0 NPL = 3.0 MLS dominant = 3.4
3. Restricted repetitive as								
Repetitive/stereotyped behaviour	AGRE <sup>a</sup> (subset of Yonan <i>et al</i> . (2003))	Alarcon et al. (2005)	ADI-Rª	291 of 345 ASP	Non-parametric and parametric QTL analysis OSA analysis	16 17	Not reported D17S1290–D17S1301	Z = 2.50 Z = 2.31
Repetitive/stereotyped behaviour	AGRE <sup>a</sup> (subset of Alarcon <i>et al.</i> (2005))	Alarcon et al. (2002)	ADI-R <sup>a</sup>	123 of 152 ASP	Non-parametric and parametric QTL analysis	7q	Not reported	Z= 2.5, HE-LOD = 0.05
Obsessive-compulsive behaviour	AGRE <sup>a</sup>	Buxbaum et al. (2004)	ADI-R <sup>a</sup> Asperger syndrome: DSM-IV criteria	62 of 115 ASP meeting obsessive- compulsive criteria	Non-parametric and parametric linkage analysis	1q24 5p14 6q14 10p14 11p13 19p13	D1S1656 D5S1473 D6S1270 D10S1412 D11S1392 D19S714	NPL = 3.1 NPL = 2.1 NPL = 2.6 NPL = 2.0 NPL = 2.1 NPL = 2.3
Savant skills	CLSA AGRE <sup>a</sup>	Ma <i>et al</i> . (2005)	ADI-R DSM-IV criteria	70 of 91 ASP	Ordered-subset analysis			No positive findings

Table 2 Continued

Endophenotype/linkage model	Research group	Reference	Diagnostic criteria AD	Number of families	Linkage analysis method	Chromosomal region	Markers	Highest LOD/ NPL/MLS/Z scores/lowest P-values
Savant skills	CLSA	Nurmi <i>et al.</i> (2003)	ADI-R ADOS-G	21 of 94 ASP positive for savant skills	Parametric linkage analysis	15q11–13	D15S511	HLOD recessive = 2.6
Insistence on sameness	CAT	Shao <i>et al</i> . (2003)	ADI-R DSM-IV ADOS-G	23 of 81 ASP	Ordered-subset analysis	15q11 <b>–</b> 13	GABRB3	LOD dominant = 4.7 LOD recessive = 3.8 OSA-LOD = 3.2
4. Parent of origin analys Linkage model: paternal/ maternal contributions		Lamb <i>et al</i> . (2005)	ADI-R ADOS-G	219 ASP	Model-free linkage analysis	Maternal: 9	D9S157–D9S171	MLS = 2.0
5. Sex limited loci Male–male pairs	AGRE <sup>a</sup>	Cantor <i>et al.</i> (2005)	ADI-R <sup>a</sup>	48 male of 91 ASP	Non-parametric linkage analysis	Male–male: 17q21	D17S2180	MLS = 4.1
Male–male versus female–male and female–female pairs	IMGSAC	Lamb <i>et al.</i> (2005)	ADI-R ADOS-G VABS, RPM, PPVT	219 ASP 145 male 74 non-male	Model-free linkage analysis	Male–male: 7q 16p Non-male: 15q	D7S480–D7S530 D16S407–D16S497 D15S117–D15S125	MLS = 2.6 MLS = 2.5 MLS = 2.6
Male–male versus female–male and female–female pairs	AGRE <sup>a</sup>	Stone <i>et al.</i> (2004) (subsof Yonan <i>et al.</i> (2003))		257 ASP 148 male 109 non-male	Non-parametric linkage analysis	Male–male: 17q11 Non-male: 4q32 35	D17S1294–D17S798 2–Not reported	MLS = 4.3 MLS = 2.7

Abbreviations: ADI-R, Autism Diagnostic Interview-Revised; ADOS-G, Autism Diagnostic Observation Schedule-Generic; AGRE, Autism Genetic Resource Exchange; ASP, affected sibpair design, that is, at least two affected children per family; the number of families is reported; CAT, Collaborative Autism Team; CLSA, Collaborative Linkage Study of Autism; CPEA, Collaborative Programs of Excellence in Autism; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; FHI, Family History Interview; ICD-10, the ICD-10 classification of mental and behavioural disorders; HE-LOD, LOD score derived from parametric quantitative linkage analysis by Haseman–Elston regression; HLOD, heterogeneity LOD score; IMGSAC, International Molecular Genetic Study of Autism Consortium; LOD, logarithm of the odds for linkage; M-HLOD, multipoint heterogeneity LOD score; MLS, multipoint maximum-likelihood score; NPL, non-parametric linkage statistic; OSA, ordered-subset analysis; PARIS, Paris Autism Research International Sibpair Study; PPVT, Peabody picture vocabulary test; QTL, quantitative trait locus; RPM, Raven Progressive Matrices; VABS, Vineland Adaptive Behaviour Scales; Z, non-parametric (QTL—) statistic.

<sup>a</sup>Assessment of the ADOS-G in the AGRE sample is only mentioned in a summary publication on the sample (Gschwind *et al.*<sup>268</sup>) but not in the publications cited in Table 2.



study<sup>185</sup> similarly assessed several SNPs in the *LAMB*1 gene, and found association with the disorder for a haplotype consisting of two SNPs in intron 25. No exonic SNPs were associated with AD in this study. Besides LAMB1, the *neuronal cell adhesion molecule* (*NRCAM*) gene was assessed in both studies as well. The positive finding in the ASP, however, was again not replicated in the singleton IMGSAC sample, <sup>184</sup> and no association was found for variants in the NRCAM gene in the second study. <sup>185</sup> Taken together, LAMB1 remains an interesting candidate gene for AD, as LAMB1 encodes for the  $\beta$ 1 chain of laminin, which is an important glycoprotein promoting neuronal migration and neurite outgrowth in the developing nervous system. <sup>186,187</sup>

Variants in the protein-tyrosine phosphatase, receptor-type, zeta-1 (*PTPRZ1*) gene, which is highly expressed in the brain during embryogenesis, <sup>188</sup> have been assessed by two studies. <sup>171,184</sup> No association with AD was found.

Three studies have assessed the *WNT2* (wingless-type mouse mammary tumour virus integration site family member 2) gene. Mice lacking the protein encoded by WNT2 show reduced social interaction. The first study The reported a nominal association of a 3'UTR 783C > T SNP detected by mutation analysis in two affected siblings with AD. Subsequent studies two affected siblings with AD. Subsequent studies The could not replicate this finding. Despite an established role of WNT2 in the development of the vertebrate central nervous system, its function in human brain development has not yet been proven. The assessed variants in this gene do not seem to play an important role in the development of AD.

The engrailed 2 (EN2) gene on chromosome 7q36 has been assessed in five independent samples. 191-194 EN2 is a homeobox transcription factor that plays a role during cerebellar and brainstem development. The adult knockout mouse model shows a hypoplastic cerebellum with a decrease in number of Purkinje cells, similar to the findings in post-mortem brains of individuals with autism. 195 An association of the intronic haplotype AC of rs1861972 and rs1861973 has been replicated in two different sub-samples of the AGRE consortium and one National Institute of Mental Health (NIMH) sample. 191,192 The exonic SNP rs3735653 consistently did not show association with AD in two studies192,194 similar to other assessed exonic variants. 191 The latter study additionally assessed the effects of EN2 expression in cultures of primary neuronal precursor cells obtained from a rat cerebral cortex and found reduced neuronal differentiation in cells showing misexpression of EN2. As no association with exonic SNPs of the EN2 gene was found in this study, it was hypothesized that the intronic SNPs might potentially disrupt the binding of transcription factors for the EN2 gene. Taken together, the *EN2* gene seems to be of relevance in the pathophysiology of AD.

Owing to a reported positive association finding for the SNP rs10951154 in the *homeobox A-1* (*HOXA1*) gene on chromosome 7p, <sup>196</sup> this variant was assessed in several subsequent studies. 197-203 HOXA1 has been shown to play a role in hindbrain development in the mouse model.<sup>204</sup> The first positive finding<sup>196</sup> was not replicated despite similar or better power in most studies. The only additional study showing an association did find A as the risk allele, 198 whereas the first study discussed the G allele and the AG/GG genotypes as risk factors. In the former study, 198 an increased head circumference was associated with AG/GG, which might be of relevance, as a subgroup of individuals with AD does show macrocephalv. 205,206 However, the hypothesized disrupted development of brainstem nuclei in autism<sup>207</sup> has not been proven by brain imaging studies. 208 Therefore, it is unlikely that variants of the HOXA1 gene are of importance in the development of idiopathic AD.

#### Chromosome 15

Owing to the frequent observed cytogenetic abnormalities of chromosome 15q11-q13 in AD, several genes in this region have been assessed in idiopathic AD. The gamma-aminobutyric acid (GABA) receptor genes located on chromosome 15q11-q13 have received considerable attention, as a study has shown a decreased GABA receptor density in the hippocampus,<sup>209</sup> and a suppressed GABAergic inhibition has been suspected to be aetiologically relevant in AD.<sup>210</sup> Two studies<sup>211,212</sup> found evidence for association of a microsatellite located in intron 3 of the GABRB3 gene (GABRB3 155CA-2), whereas four other studies could not replicate this finding in samples of similar size and power.213-216 Only nominal significant associations of different haplotypes, SNPs or microsatellites located in or around the GABRB3 and the GABRG3 gene have been found in three further studies. 215,217-219 The largest study to date assessing GABA receptor subunit genes found evidence for association of a single SNP in the GABRA4 gene on chromosome 4p with AD, and for interaction effects of this variant with a SNP in the GABRB1 gene on chromosome 4p. No association for SNPs in the GABA receptor genes on chromosome 15 were found. 220 Similar inconclusive results have been obtained for variants in or close to the AT Pase, class V, type 10C (ATP10C) and the ubiquitin-protein ligase E3A (UBE3A) genes located in the maternal expression domain of chromosome 15q11–13. 212,221,222 Only one study<sup>223</sup> reported an association of D15S122/ hCV2558436 located in the intron at the 5' end of UBE3A, which remained significant after correction for multiple testing. This association, however, was not replicated in a bigger sample.222 Taken together, despite the possible role of the neurotransmitter GABA and its receptors in the aetiology of AD, the findings on genetic variants in these receptors are inconclusive to date. The complex organization of chromosome 15q11-q13 with two imprinted regions and areas of high local recombination differing between men and women<sup>217</sup> make it even more difficult to assess genes in this area with regard to their relevance for AD. The UBE3A gene seems not to be relevant for idiopathic

AD, which matches the phenotypic differences between Angelman syndrome and AD.

#### Chromosome 17

Owing to findings of platelet hyperserotonaemia in children with autism<sup>224</sup> and their first-degree relatives, 225,226 the serotonin-transporter gene (SLC6A4) on chromosome 17 was assessed by several studies. The most common assessed variants are a deletion/ insertion polymorphism in the transcriptional control region of the SLC6A4 gene with functional effects (5HTTLPR)<sup>227-229</sup> and a variable number of tandem repeat in intron 2 (STin2). Several studies have found an association of the short alleles of 5HTTLPR with AD, 217,230-233 fewer studies of the long alleles.234,235 Some studies did not replicate these findings. 214,236-243 Three studies have assessed the effects of the 5HTTLPR on whole-blood serotonin (5-HT) or platelet 5-HT parameters in AD. 236,237,244 One study<sup>244</sup> did find an increased rate of platelet-5-HT uptake in II genotypes compared to sl and ss. Another study<sup>237</sup> reported higher mean platelet 5-HT levels in haplotypes containing II of 5HTTLPR and alleles 10 or 12 of STin2 in AD. These findings are in accordance with functional effects on higher platelet serotonin uptake mediated by the long allele variants of 5HTTLPR in healthy controls.<sup>229</sup> No difference was found between genotypes for whole-blood serotonin levels in two samples of individuals with AD,236,245 which parallels the findings in healthy controls.

With the exception of one study, 246 which reported an association of an haplotype containing STin2, none of the above-mentioned studies did find an association with this variant. One study, however, reported higher obsessive-compulsive symptoms in AD individuals carrying the 12/12 genotype of STin2.<sup>239</sup> Another study similarly found a difference in obsessive-compulsive symptoms between genotypes of the two SNPs ss38318599 and ss38318601.<sup>233</sup> These findings as well as other assessed variants, 232 however, have not yet been replicated. Taken together, the above-mentioned studies as well as the reported association of the longer alleles of 5HTTLPR with less severe AD241 might point to a modulating effect of 5HTTLPR in AD. The different association findings with regard to the long and short alleles of 5HTTLPR might be caused by different sample characteristics regarding the phenotype of the disorders. It can be concluded that SLC6A4 is of relevance for the genetics of autism, either directly influencing the phenotype or modulating the severity of AD with regard to obsessive-compulsive symptoms.

#### X-chromosome

Despite rare positive linkage findings for loci on the X-chromosome, several variants in genes on the X-chromosome have been assessed for association with AD, as the sex distribution is markedly skewed. Two neuroligin (NLGN) genes on Xq13 and Xp22 have been screened for mutations in several studies. Neuroligins are essential components of synaptogenesis. Despite the findings of several non-conservative mutations in single families in the NLGN3 and NLGN4 genes, 247-250 these could not be replicated in larger samples of individuals with AD.<sup>251-253</sup> One study<sup>254</sup> detected several other variants in NLGN3 and NLGN4X; however, only nominal significance for association with AD was found. As the NLGN4X nt1253del(AG) frameshift mutation found in one study<sup>249</sup> co-segregated with unspecific mental retardation and AD in one large family, it is likely that NLGN4X mutations might be rare single gene disorders causing AD and unspecific mental retardation. Owing to the rare occurrence of the observed variants in larger samples of individuals with AD, however, it is unlikely that the NLGN3 and NLGN4X genes play an important role in idiopathic autism.

Similar findings have been obtained by several studies screening the methyl-CpG-binding protein 2 (MeCP2) gene for mutations in samples of male and female individuals with AD and mental retardation. 255-262 With the exception of two studies, 257,260 no coding mutations have been detected in AD. The latter study did not report any standardized assessment of AD; therefore, the results of this study have to be judged carefully. Generally, only a few new variants were detected in the AD samples; therefore, no association analysis has been performed to date. Variants prevailingly were found in women, 256,259 with the latter study emphasizing the differential diagnosis of the preserved speech variant of Rett syndrome with regard to AD in women. Together with the negative results of linkage studies regarding Xq28, it is unlikely that MeCP2 plays a major role in the genetics of idiopathic autism.

Owing to the elevated platelet serotonin levels in children with autism and their first relatives, variants in the monoamine-oxidase A (MAO-A) gene on the Xp11.23, which degrades serotonin, have been assessed in AD. No association of AD with different variants has been found to date.263-265

In conclusion, several interesting candidate genes and possible functional variants have been elucidated, which seem to be of relevance for the genetics of idiopathic AD. Unlike linkage studies, association studies have not made use of the findings of formal genetic studies and the detailed phenotypic assessment of the disorder. This might be due the family-based association analysis approach taken in most studies, or to the low prevalence, rendering an assessment of phenotypically defined subgroups of the disorder almost impossible. A few association studies have not reported standardized assessment of AD and have not excluded cytogenetic abnormalities, genetic syndromes or associated single gene disorders. This might have resulted in heterogeneous samples and might have lowered the power to find association. Still, the results of molecular genetic studies point to a genetic model of several genetic variants, either oligo- or polygenic, interacting with regard to the phenotypic expression of autistic traits.



Variants in the *SLC6A4* gene might modulate obsessive-compulsive behavior in AD, whereas other important genes (*GluR6*, *LAMB1*, *EN2*) might be of strong influence during neuronal and synaptic development.

#### Implications for genetic counselling

Genetic counselling for AD is challenging, as phenotype and genetic mechanisms are complex. There is a strong need to carefully assess the children and the family, and to exclude all known medical causes of the disorder. The aim of genetic counselling is to provide information to parents and children, and to estimate the recurrence risk of the disorder. Genetic counselling further is concerned with providing psychologically oriented counselling to help individuals to adapt and adjust to the impact and implications of the disorder in the family. With regard to AD, families as a rule wish to know the recurrence risk of the disorder. From the results of family studies, a sibling recurrence risk of around 5% (2-8%) can be estimated for idiopathic AD.<sup>266</sup> If a known genetic cause of the disorder is established, however, a very different recurrence risk might be present in the individual family. For dominant single gene disorders with full penetrance, like TSC, a sibling recurrence risk of 50% is present, if one of the parents carries the disease-causing variant, that is, if the variant is not a de novo mutation. In case of recessive single gene disorders, like SLO, the sibling recurrence risk is 25%. If a child suffers from FRAXA, the recurrence risk in a brother is up to 50%, and a sister will become a carrier in up to 50% or might be mildly affected. On the other hand, in the presence of cytogenetic abnormalities like a chromosome 15q11-q13 duplication or duplicated inversion, the recurrence risk is similar to the population prevalence, as most duplications and inversions arise de novo during meiosis.

The limited clinical validity of genetic testing for autism and the related ethical concerns have recently been delineated by McMahon *et al.*<sup>267</sup> It seems of particular relevance to keep in mind the complex genetics and uncertainty principle as well as the right of the individual and the family not to participate in genetic testing.

#### **Future directions**

The presented association studies have shown the difficulties in finding disease-causing genetic variants based on a small number of microsatellites, SNPs or haplotypes. High-density SNP association studies might become feasible in the near future, which might enable researchers to assess linkage patterns and haplotype structure at a genome-wide level in different populations and choose the relevant tagging SNPs for adequate haplotype association studies. In addition to more sophisticated association technology, functional analyses of new variants in coding regions should be brought forward. Gene—gene inter-

actions and epigenetic mechanisms additionally seem to be of relevance in  $\rm AD.^{187}$ 

#### **Conclusions**

Despite the high heritability estimates for AD, only a few genes increasing the risk for idiopathic AD have been elucidated. As the disorder shows a high phenotypic variability and additional genetic heterogeneity, it is of crucial importance to, first, clearly define the phenotype, especially with regard to the broader spectrum of AD and to the differential diagnosis of other pervasive developmental disorders like Rett syndrome, and, second, to perform a detailed cytogenetic analysis in every individual with AD and additional testing for FRAXA in individuals with AD and mental retardation in clinical and research settings. With regard to molecular genetic studies on AD, promising new technologies have been developed, and larger samples with higher power might eventually lead to more stable results.

#### References

- 1 American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Association: Washington, DC, 1994.
- 2 World Health Organisation. The ICD-10 Classification of Mental and Behavioural Disorders. Clinical Descriptions and Diagnostic Guidelines. World Health Organisation: Geneva, 1992.
- 3 Kanner L. Autistic disturbance of affective contact. *Nervous Child* 1943; **2**: 217–250.
- 4 Asperger H. Die 'Autistischen Psychopathen' im Kindesalter. Arch Psychiat Nerven 1944; 117: 73–136.
- 5 Rutter M. Concepts of autism: a review of research. *J Child Psychol Psychiatry* 1968; 9: 1–25.
- 6 Wing L. Asperger's syndrome: a clinical account. *Psychol Med* 1981; 11: 115–129.
- 7 Percy AK, Lane JB. Rett syndrome: clinical and molecular update. Curr Opin Pediatr 2004; 16: 670–677.
- 8 Erlandson A, Hagberg B. MECP2 abnormality phenotypes: clinicopathologic area with broad variability. *J Child Neurol* 2005; **20**: 727–732.
- 9 Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev Disord* 2003; **33**: 365–382.
- 10 Volkmar FR, Lord C, Bailey A, Schultz RT, Klin A. Autism and pervasive developmental disorders. J Child Psychol Psychiatry 2004; 45: 135–170.
- 11 Gillberg C. Chromosomal disorders and autism. J Autism Dev Disord 1998; 28: 415–425.
- 12 Lauritsen M, Mors O, Mortensen PB, Ewald H. Infantile autism and associated autosomal chromosome abnormalities: a register-based study and a literature survey. *J Child Psychol Psychiatry* 1999; **40**: 335–345.
- 13 Reddy KS. Cytogenetic abnormalities and fragile-X syndrome in autism spectrum disorder. *BMC Med Genet* 2005; **6**: 3–19.
- 14 Chakrabarti S, Fombonne E. Pervasive developmental disorders in preschool children. JAMA 2001; 285: 3093–3099.
- 15 Ritvo ER, Mason-Brothers A, Freeman BJ, Pingree C, Jenson WR, McMahon WM et al. The UCLA University of Utah epidemiologic survey of autism: the etiologic role of rare diseases. Am J Psychiatry 1990; 147: 1614–1621.
- 16 Wassink TH, Piven J, Patil SR. Chromosomal abnormalities in a clinic sample of individuals with autistic disorder. *Psychiatr Genet* 2001; 11: 57–63.

- 17 Wassink TH, Piven J, Vieland VJ, Huang J, Swiderski RE, Pietila J et al. Evidence supporting WNT2 as an autism susceptibility gene. Am J Med Genet 2001; 105: 406–413.
- 18 Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol Psychiatry* 2006; 11, 1–18, 28.
- 19 Bolton PF, Dennis NR, Browne CE, Thomas NS, Veltman MW, Thompson RJ et al. The phenotypic manifestations of interstitial duplications of proximal 15q with special reference to the autistic spectrum disorders. Am J Med Genet 2001; 105: 675–685.
- 20 Borgatti R, Piccinelli P, Passoni D, Dalpra L, Miozzo M, Micheli R et al. Relationship between clinical and genetic features in 'inverted duplicated chromosome 15' patients. Pediatr Neurol 2001; 24: 111–116.
- 21 Gurrieri F, Battaglia A, Torrisi L, Tancredi R, Cavallaro C, Sangiorgi E et al. Pervasive developmental disorder and epilepsy due to maternally derived duplication of 15q11–q13. Neurology 1999; 52: 1694–1697.
- 22 Repetto GM, White LM, Bader PJ, Johnson D, Knoll JH. Interstitial duplications of chromosome region 15q11q13: clinical and molecular characterization. Am J Med Genet 1998; 79: 82–89.
- 23 Schroer RJ, Phelan MC, Michaelis RC, Crawford EC, Skinner SA, Cuccaro M et al. Autism and maternally derived aberrations of chromosome 15q. Am J Med Genet 1998; 76: 327–336.
- 24 Sutcliffe JS, Nurmi EL, Lombroso PJ. Genetics of childhood disorders: XLVII. Autism, part 6: duplication and inherited susceptibility of chromosome 15q11-q13 genes in autism. J Am Acad Child Adolesc Psychiatry 2003; 42: 253-256.
- 25 Thomas JA, Johnson J, Peterson Kraai TL, Wilson R, Tartaglia N, LeRoux J et al. Genetic and clinical characterization of patients with an interstitial duplication 15q11–q13, emphasizing behavioral phenotype and response to treatment. Am J Med Genet A 2003; 119: 111–120.
- 26 Wolpert CM, Menold MM, Bass MP, Qumsiyeh MB, Donnelly SL, Ravan SA et al. Three probands with autistic disorder and isodicentric chromosome 15. Am J Med Genet 2000; 96: 365–372.
- 27 Laan LA, Vein AA. Angelman syndrome: is there a characteristic EEG? Brain Dev 2005; 27: 80–87.
- 28 Valente KD, Koiffmann CP, Fridman C, Varella M, Kok F, Andrade JQ et al. Epilepsy in patients with Angelman syndrome caused by deletion of the chromosome 15q11-13. Arch Neurol 2006; 63: 122–128.
- 29 Clayton-Smith J, Laan L. Angelman syndrome: a review of the clinical and genetic aspects. *J Med Genet* 2003; **40**: 87–95.
- 30 State MW, Dykens EM. Genetics of childhood disorders: XV. Prader-Willi syndrome: genes, brain, and behavior. J Am Acad Child Adolesc Psychiatry 2000; 39: 797–800.
- 31 Vogels A, Fryns JP. The Prader-Willi syndrome and the Angelman syndrome. Genet Counsel 2002; 13: 385–396.
- 32 Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S et al. Prader–Willi syndrome: intellectual abilities and behavioural features by genetic subtype. *J Child Psychol Psychiatry* 2005; **46**: 1089–1096.
- 33 Casas KA, Mononen TK, Mikail CN, Hassed SJ, Li S, Mulvihill JJ et al. Chromosome 2q terminal deletion: report of 6 new patients and review of phenotype-breakpoint correlations in 66 individuals. Am J Med Genet A 2004; 130: 331–339.
- 34 Gallagher L, Becker K, Kearney G, Dunlop A, Stallings R, Green A et al. Brief report: a case of autism associated with del(2)(q32.1q32.2) or (q32.2q32.3). J Autism Dev Disord 2003; 33: 105–108.
- 35 Ghaziuddin M, Burmeister M. Deletion of chromosome 2q37 and autism: a distinct subtype? *J Autism Dev Disord* 1999; **29**: 259–263.
- 36 Lukusa T, Vermeesch JR, Holvoet M, Fryns JP, Devriendt K. Deletion 2q37.3 and autism: molecular cytogenetic mapping of the candidate region for autistic disorder. *Genet Counsel* 2004; 15: 293–301.
- 37 Smith M, Escamilla JR, Filipek P, Bocian ME, Modahl C, Flodman P et al. Molecular genetic delineation of 2q37.3 deletion in autism and osteodystrophy: report of a case and of new

- markers for deletion screening by PCR. Cytogenet Cell Genet 2001; 94: 15–22.
- 38 Wassink TH, Piven J, Vieland VJ, Jenkins L, Frantz R, Bartlett CW et al. Evaluation of the chromosome 2q37.3 gene CENTG2 as an autism susceptibility gene. Am J Med Genet B 2005; 136: 36–44
- 39 Wolff DJ, Clifton K, Karr C, Charles J. Pilot assessment of the subtelomeric regions of children with autism: detection of a 2q deletion. Genet Med 2002: 4: 10-14.
- 40 Ashley-Koch A, Wolpert CM, Menold MM, Zaeem L, Basu S, Donnelly SL et al. Genetic studies of autistic disorder and chromosome 7. Genomics 1999; 61: 227–236.
- 41 Vincent JB, Herbrick JA, Gurling HM, Bolton PF, Roberts W, Scherer SW. Identification of a novel gene on chromosome 7q31 that is interrupted by a translocation breakpoint in an autistic individual. *Am J Hum Genet* 2000; **67**: 510–514.
- 42 Warburton P, Baird G, Chen W, Morris K, Jacobs BW, Hodgson S et al. Support for linkage of autism and specific language impairment to 7q3 from two chromosome rearrangements involving band 7q31. Am J Med Genet 2000; 96: 228–234.
- 43 Fine SE, Weissman A, Gerdes M, Pinto-Martin J, Zackai EH, McDonald-McGinn DM et al. Autism spectrum disorders and symptoms in children with molecularly confirmed 22q11.2 deletion syndrome. J Autism Dev Disord 2005; 35: 461–470.
- 44 Ogilvie CM, Moore J, Daker M, Palferman S, Docherty Z. Chromosome 22q11 deletions are not found in autistic patients identified using strict diagnostic criteria. IMGSAC. International Molecular Genetics Study of Autism Consortium. Am J Med Genet 2000; 96: 15–17.
- 45 Manning MA, Cassidy SB, Clericuzio C, Cherry AM, Schwartz S, Hudgins L et al. Terminal 22q deletion syndrome: a newly recognized cause of speech and language disability in the autism spectrum. Pediatrics 2004; 114: 451–457.
- 46 Roach ES, Sparagana SP. Diagnosis of tuberous sclerosis complex. J Child Neurol 2004; 19: 643–649.
- 47 Harrison JE, Bolton PF. Annotation: tuberous sclerosis. *J Child Psychol Psychiatry* 1997; **38**: 603–614.
- 48 Lewis JC, Thomas HV, Murphy KC, Sampson JR. Genotype and psychological phenotype in tuberous sclerosis. *J Med Genet* 2004; 41: 203–207.
- 49 Bolton PF, Griffiths PD. Association of tuberous sclerosis of temporal lobes with autism and atypical autism. *Lancet* 1997; 349: 392–395.
- 50 Bolton PF, Park RJ, Higgins JN, Griffiths PD, Pickles A. Neuro-epileptic determinants of autism spectrum disorders in tuberous sclerosis complex. *Brain* 2002; 125: 1247–1255.
- 51 Gutierrez GC, Smalley SL, Tanguay PE. Autism in tuberous sclerosis complex. J Autism Dev Disord 1998; 28: 97–103.
- 52 Hunt A, Dennis J. Psychiatric disorder among children with tuberous sclerosis. *Dev Med Child Neurol* 1987; 29: 190–198.
- 53 Jambaque I, Chiron C, Dumas C, Mumford J, Dulac O. Mental and behavioural outcome of infantile epilepsy treated by vigabatrin in tuberous sclerosis patients. *Epilepsy Res* 2000; 38: 151–160.
- 54 Cianchetti C, Sannio-Fancello G, Fratta AL, Manconi F, Orano A, Pischedda MP et al. Neuropsychological, psychiatric, and physical manifestations in 149 members from 18 fragile X families. Am J Med Genet 1991; 40: 234–243.
- 55 Lombroso PJ. Genetics of childhood disorders: XLVIII. Learning and memory. Part 1: Fragile X syndrome update. J Am Acad Child Adolesc Psychiatry 2003; 42: 372–375.
- 56 Oostra BA, Chiurazzi P. The fragile X gene and its function. *Clin Genet* 2001; **60**: 399–408.
- 57 Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D *et al.* DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum Mol Genet* 1992; 1: 397–400.
- 58 Bailey AJ. The biology of autism. Psychol Med 1993; 23: 7–11.
- 59 Hallmayer J, Pintado E, Lotspeich L, Spiker D, McMahon W, Petersen PB et al. Molecular analysis and test of linkage between the FMR-1 gene and infantile autism in multiplex families. Am J Hum Genet 1994; 55: 951–959.
- 60 Vincent JB, Thevarkunnel S, Kolozsvari D, Paterson AD, Roberts W, Scherer SW. Association and transmission analysis of the FMR1 IVS10+14C-T variant in autism. Am J Med Genet B 2004; 125: 54–56.



- 61 Klauck SM, Munstermann E, Bieber-Martig B, Ruhl D, Lisch S, Schmotzer G et al. Molecular genetic analysis of the FMR-1 gene in a large collection of autistic patients. Hum Genet 1997; 100: 224–229.
- 62 Gurling HM, Bolton PF, Vincent J, Melmer G, Rutter M. Molecular and cytogenetic investigations of the fragile X region including the Frax A and Frax E CGG trinucleotide repeat sequences in families multiplex for autism and related phenotypes. Hum Hered 1997; 47: 254–262.
- 63 Irons M, Elias ER, Salen G, Tint GS, Batta AK. Defective cholesterol biosynthesis in Smith–Lemli–Opitz syndrome. *Lancet* 1993; **341**: 1414.
- 64 Tint GS, Irons M, Elias ER, Batta AK, Frieden R, Chen TS et al. Defective cholesterol biosynthesis associated with the Smith– Lemli–Opitz syndrome. N Engl J Med 1994; 330: 107–113.
- 65 Tierney E, Nwokoro NA, Porter FD, Freund LS, Ghuman JK, Kelley RI. Behavior phenotype in the RSH/Smith-Lemli-Opitz syndrome. Am J Med Genet 2001; 98: 191–200.
- 66 Ryan AK, Bartlett K, Clayton P, Eaton S, Mills L, Donnai D *et al.* Smith-Lemli-Opitz syndrome: a variable clinical and biochemical phenotype. *J Med Genet* 1998; **35**: 558–565.
- 67 Cunniff C, Kratz LE, Moser A, Natowicz MR, Kelley RI. Clinical and biochemical spectrum of patients with RSH/Smith– Lemli–Opitz syndrome and abnormal cholesterol metabolism. Am J Med Genet 1997; 68: 263–269.
- 68 Sikora DM, Pettit-Kekel K, Penfield J, Merkens LS, Steiner RD. The near universal presence of autism spectrum disorders in children with Smith-Lemli-Opitz syndrome. Am J Med Genet A 2006; 140: 1511–1518.
- 69 Folstein SE, Rosen-Sheidley B. Genetics of autism: complex aetiology for a heterogeneous disorder. Nat Rev Genet 2001; 2: 943-955.
- 70 Stromland K, Nordin V, Miller M, Akerstrom B, Gillberg C. Autism in thalidomide embryopathy: a population study. *Dev Med Child Neurol* 1994; 36: 351–356.
- 71 Moore SJ, Turnpenny P, Quinn A, Glover S, Lloyd DJ, Montgomery T et al. A clinical study of 57 children with fetal anticonvulsant syndromes. J Med Genet 2000; 37: 489–497.
- 72 Williams G, King J, Cunningham M, Stephan M, Kerr B, Hersh JH. Fetal valproate syndrome and autism: additional evidence of an association. Dev Med Child Neurol 2001; 43: 202–206.
- 73 Aronson M, Hagberg B, Gillberg C. Attention deficits and autistic spectrum problems in children exposed to alcohol during gestation: a follow-up study. *Dev Med Child Neurol* 1997; **39**: 583–587.
- 74 Nanson JL. Autism in fetal alcohol syndrome: a report of six cases. *Alcohol Clin Exp Res* 1992; **16**: 558–565.
- 75 Chess S, Fernandez P, Korn S. Behavioral consequences of congenital rubella. *J Pediatr* 1978; **93**: 699–703.
- 76 Chess S. Follow-up report on autism in congenital rubella. *J Autism Child Schizophr* 1977; 7: 69–81.
- 77 Wakefield AJ, Murch SH, Anthony A, Linnell J, Casson DM, Malik M et al. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Lancet 1998: 351: 637-641.
- 78 Chen W, Landau S, Sham P, Fombonne E. No evidence for links between autism, MMR and measles virus. *Psychol Med* 2004; 34: 543–553.
- 79 Honda H, Shimizu Y, Rutter M. No effect of MMR withdrawal on the incidence of autism: a total population study. *J Child Psychol Psychiatry* 2005; 46: 572–579.
- 80 Smeeth L, Cook C, Fombonne E, Heavey L, Rodrigues LC, Smith PG et al. MMR vaccination and pervasive developmental disorders: a case-control study. Lancet 2004; 364: 963-969.
- 81 Taylor B, Miller E, Farrington CP, Petropoulos MC, Favot-Mayaud I, Li J et al. Autism and measles, mumps, and rubella vaccine: no epidemiological evidence for a causal association. Lancet 1999; 353: 2026–2029.
- 82 Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E *et al.* Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med* 1995; **25**: 63–77.
- 83 Folstein S, Rutter M. İnfantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry* 1977; **18**: 297–321.

- 84 Ritvo ER, Spence MA, Freeman BJ, Mason-Brothers A, Mo A, Marazita ML. Evidence for autosomal recessive inheritance in 46 families with multiple incidences of autism. *Am J Psychiatry* 1985; 142: 187–192.
- 85 Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G et al. A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. J Child Psychol Psychiatry 1989; 30: 405–416.
- 86 Betancur C, Leboyer M, Gillberg C. Increased rate of twins among affected sibling pairs with autism. Am J Hum Genet 2002; 70: 1381–1383.
- 87 Greenberg DA, Hodge SE, Sowinski J, Nicoll D. Excess of twins among affected sibling pairs with autism: implications for the etiology of autism. *Am J Hum Genet* 2001; **69**: 1062–1067.
- 88 Croen LA, Grether JK, Selvin S. Descriptive epidemiology of autism in a California population: who is at risk? J Autism Dev Disord 2002; 32: 217–224.
- 89 Hallmayer J, Glasson EJ, Bower C, Petterson B, Croen L, Grether J et al. On the twin risk in autism. Am J Hum Genet 2002; 71: 941–946.
- 90 Hultman CM, Sparen P, Cnattingius S. Perinatal risk factors for infantile autism. *Epidemiology* 2002; **13**: 417–423.
- 91 Le Couteur A, Bailey A, Goode S, Pickles A, Robertson S, Gottesman I *et al.* A broader phenotype of autism: the clinical spectrum in twins. *J Child Psychol Psychiatry* 1996; **37**: 785–801.
- 92 Freitag C, IMGSAC. Phenotypic characteristics of siblings with autism and/or pervasive developmental disorder: evidence for heterogeneity. *Am J Med Genet* 2002; **114**: 723.
- 93 Walker DR, Thompson A, Zwaigenbaum L, Goldberg J, Bryson SE, Mahoney WJ et al. Specifying PDD-NOS: a comparison of PDD-NOS, Asperger syndrome, and autism. J Am Acad Child Adolesc Psychiatry 2004; 43: 172–180.
- 94 Constantino JN, Todd RD. Genetic structure of reciprocal social behavior. *Am J Psychiatry* 2000; **157**: 2043–2045.
- 95 Constantino JN, Hudziak JJ, Todd RD. Deficits in reciprocal social behavior in male twins: evidence for a genetically independent domain of psychopathology. J Am Acad Child Adolesc Psychiatry 2003; 42: 458–467.
- 96 Ronald A, Happe F, Plomin R. The genetic relationship between individual differences in social and nonsocial behaviours characteristic of autism. *Dev Sci* 2005; **8**: 444–458.
- 97 Kolevzon A, Smith CJ, Schmeidler J, Buxbaum JD, Silverman JM. Familial symptom domains in monozygotic siblings with autism. *Am J Med Genet B Neuropsychiatr Genet* 2004; **129**: 76–81.
- 98 Bolton P, Macdonald H, Pickles A, Rios P, Goode S, Crowson M et al. A case–control family history study of autism. *J Child Psychol Psychiatry* 1994; **35**: 877–900.
- 99 Bishop DV, Maybery M, Wong D, Maley A, Hallmayer J. Characteristics of the broader phenotype in autism: a study of siblings using the children's communication checklist-2. Am J Med Genet B 2006; 141: 117–122.
- 100 Pilowsky T, Yirmiya N, Shalev RS, Gross-Tsur V. Language abilities of siblings of children with autism. J Child Psychol Psychiatry 2003; 44: 914–925.
- 101 Silverman JM, Smith CJ, Schmeidler J, Hollander E, Lawlor BA, Fitzgerald M et al. Symptom domains in autism and related conditions: evidence for familiality. Am J Med Genet 2002; 114: 64-73.
- 102 Pickles A, Starr E, Kazak S, Bolton P, Papanikolaou K, Bailey A et al. Variable expression of the autism broader phenotype: findings from extended pedigrees. J Child Psychol Psychiatry 2000; 41: 491–502.
- 103 MacLean JE, Szatmari P, Jones MB, Bryson SE, Mahoney WJ, Bartolucci G et al. Familial factors influence level of functioning in pervasive developmental disorder. J Am Acad Child Adolesc Psychiatry 1999; 38: 746–753.
- 104 Spiker D, Lotspeich L, Kraemer HC, Hallmayer J, McMahon W, Petersen PB et al. Genetics of autism: characteristics of affected and unaffected children from 37 multiplex families. Am J Med Genet 1994; 54: 27–35.
- 105 Folstein SE, Santangelo SL, Gilman SE, Piven J, Landa R, Lainhart J et al. Predictors of cognitive test patterns in autism families. J Child Psychol Psychiatry 1999; 40: 1117–1128.

npg

- 106 Bailey A, Palferman S, Heavey L, Le Couteur A. Autism: the phenotype in relatives. *J Autism Dev Disord* 1998; **28**: 369–392.
- 107 Bishop DV, Maybery M, Maley A, Wong D, Hill W, Hallmayer J. Using self-report to identify the broad phenotype in parents of children with autistic spectrum disorders: a study using the Autism-Spectrum Quotient. J Child Psychol Psychiatry 2004; 45: 1431–1436.
- 108 Ghaziuddin M. A family history study of Asperger syndrome. *J Autism Dev Disord* 2005; **35**: 177–182.
- 109 Landa R, Folstein SE, Isaacs C. Spontaneous narrative-discourse performance of parents of autistic individuals. *J Speech Hear Res* 1991; **34**: 1339–1345.
- 110 Landa R, Piven J, Wzorek MM, Gayle JO, Chase GA, Folstein SE. Social language use in parents of autistic individuals. *Psychol Med* 1992; 22: 245–254.
- 111 Piven J, Wzorek M, Landa R, Lainhart J, Bolton P, Chase GA et al. Personality characteristics of the parents of autistic individuals. Psychol Med 1994; 24: 783–795.
- 112 Piven J, Palmer P. Cognitive deficits in parents from multiple-incidence autism families. J Child Psychol Psychiatry 1997; 38: 1011-1021.
- 113 Szatmari P, MacLean JE, Jones MB, Bryson SE, Zwaigenbaum L, Bartolucci G et al. The familial aggregation of the lesser variant in biological and nonbiological relatives of PDD probands: a family history study. J Child Psychol Psychiatry 2000; 41: 579–586.
- 114 Wolff S, Narayan S, Moyes B. Personality characteristics of parents of autistic children: a controlled study. *J Child Psychol Psychiatry* 1988; **29**: 143–153.
- 115 Lainhart JE, Ozonoff S, Coon H, Krasny L, Dinh E, Nice J et al. Autism, regression, and the broader autism phenotype. Am J Med Genet 2002; 113: 231–237.
- 116 Hollander E, King A, Delaney K, Smith CJ, Silverman JM. Obsessive-compulsive behaviors in parents of multiplex autism families. *Psychiatry Res* 2003; 117: 11–16.
- 117 Yirmiya N, Shaked M. Psychiatric disorders in parents of children with autism: a meta-analysis. J Child Psychol Psychiatry 2005: 46: 69–83.
- 118 Pickles A, Bolton P, Macdonald H, Bailey A, Le Couteur A, Sim CH et al. Latent-class analysis of recurrence risks for complex phenotypes with selection and measurement error: a twin and family history study of autism. Am J Hum Genet 1995; 57: 717–726.
- 119 Auranen M, Vanhala R, Varilo T, Ayers K, Kempas E, Ylisaukko-Oja T et al. A genomewide screen for autism-spectrum disorders: evidence for a major susceptibility locus on chromosome 3q25–27. Am J Hum Genet 2002; 71: 777–790.
- 120 Barrett S, Beck JC, Bernier R, Bisson E, Braun TA, Casavant TL et al. An autosomal genomic screen for autism. Collaborative linkage study of autism. Am J Med Genet 1999; 88: 609–615.
- 121 Bartlett CW, Goedken R, Vieland VJ. Effects of updating linkage evidence across subsets of data: reanalysis of the autism genetic resource exchange data set. Am J Hum Genet 2005; 76: 688–695.
- 122 Buxbaum JD, Silverman JM, Smith CJ, Kilifarski M, Reichert J, Hollander E et al. Evidence for a susceptibility gene for autism on chromosome 2 and for genetic heterogeneity. Am J Hum Genet 2001; 68: 1514–1520.
- 123 Cantor RM, Kono N, Duvall JA, Alvarez-Retuerto A, Stone JL, Alarcon M et al. Replication of autism linkage: fine-mapping peak at 17q21. Am J Hum Genet 2005; 76: 1050–1056.
- 124 Coon H, Matsunami N, Stevens J, Miller J, Pingree C, Camp NJ *et al.* Evidence for linkage on chromosome 3q25–27 in a large autism extended pedigree. *Hum Hered* 2005; **60**: 220–226.
- 125 IMGSAC. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. International Molecular Genetic Study of Autism Consortium. Hum Mol Genet 1998; 7: 571-578.
- 126 IMGSAC. A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. Am J Hum Genet 2001; 69: 570-581.
- 127 Lamb JA, Barnby G, Bonora E, Sykes N, Bacchelli E, Blasi F et al. Analysis of IMGSAC autism susceptibility loci: evidence for sex limited and parent of origin specific effects. J Med Genet 2005; 42: 132–137.

- 128 Lauritsen MB, Als TD, Dahl HA, Flint TJ, Wang AG, Vang M et al. A genome-wide search for alleles and haplotypes associated with autism and related pervasive developmental disorders on the Faroe Islands. *Mol Psychiatry* 2006; 11: 37–46.
- 129 Liu J, Nyholt DR, Magnussen P, Parano E, Pavone P, Geschwind D et al. A genomewide screen for autism susceptibility loci. Am J Hum Genet 2001; 69: 327–340.
- 130 McCauley JL, Li C, Jiang L, Olson LM, Crockett G, Gainer K et al. Genome-wide and Ordered-Subset linkage analyses provide support for autism loci on 17q and 19p with evidence of phenotypic and interlocus genetic correlates. BMC Med Genet 2005; 6: 1.
- 131 Philippe A, Martinez M, Guilloud-Bataille M, Gillberg C, Rastam M, Sponheim E et al. Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sibpair Study. Hum Mol Genet 1999; 8: 805–812.
- 132 Risch N, Spiker D, Lotspeich L, Nouri N, Hinds D, Hallmayer J et al. A genomic screen of autism: evidence for a multilocus etiology. Am J Hum Genet 1999; 65: 493–507.
- 133 Shao Y, Wolpert CM, Raiford KL, Menold MM, Donnelly SL, Ravan SA et al. Genomic screen and follow-up analysis for autistic disorder. Am J Med Genet 2002; 114: 99–105.
- 134 Vincent JB, Melmer G, Bolton PF, Hodgkinson S, Holmes D, Curtis D *et al.* Genetic linkage analysis of the X chromosome in autism, with emphasis on the fragile X region. *Psychiatr Genet* 2005; **15**: 83–90.
- 135 Yonan AL, Alarcon M, Cheng R, Magnusson PK, Spence SJ, Palmer AA et al. A genomewide screen of 345 families for autism-susceptibility loci. Am J Hum Genet 2003; 73: 886–897.
- 136 Ylisaukko-Oja T, Nieminen-von Wendt T, Kempas E, Sarenius S, Varilo T, von Wendt L et al. Genome-wide scan for loci of Asperger syndrome. Mol Psychiatry 2004; 9: 161–168.
- 137 Ylisaukko-Oja T, Alarcon M, Cantor RM, Auranen M, Vanhala R, Kempas E et al. Search for autism loci by combined analysis of Autism Genetic Resource Exchange and Finnish families. Ann Neurol 2006; 59: 145–155.
- 138 Alarcon M, Cantor RM, Liu J, Gilliam TC, Geschwind DH. Evidence for a language quantitative trait locus on chromosome 7q in multiplex autism families. Am J Hum Genet 2002; 70: 60–71
- 139 Alarcon M, Yonan AL, Gilliam TC, Cantor RM, Geschwind DH. Quantitative genome scan and Ordered-Subsets Analysis of autism endophenotypes support language QTLs. *Mol Psychiatry* 2005; 10: 747–757.
- 140 Bradford Y, Haines J, Hutcheson H, Gardiner M, Braun T, Sheffield V et al. Incorporating language phenotypes strengthens evidence of linkage to autism. Am J Med Genet 2001; 105: 539–547.
- 141 Chen GK, Kono N, Geschwind DH, Cantor RM. Quantitative trait locus analysis of nonverbal communication in autism spectrum disorder. Mol Psychiatry 2006; 11: 214–220.
- 142 Shao Y, Raiford KL, Wolpert CM, Cope HA, Ravan SA, Ashley-Koch AA et al. Phenotypic homogeneity provides increased support for linkage on chromosome 2 in autistic disorder. Am J Hum Genet 2002; 70: 1058–1061.
- 143 Molloy CA, Keddache M, Martin LJ. Evidence for linkage on 21q and 7q in a subset of autism characterized by developmental regression. *Mol Psychiatry* 2005; 10: 741–746.
- 144 Buxbaum JD, Silverman J, Keddache M, Smith CJ, Hollander E, Ramoz N et al. Linkage analysis for autism in a subset families with obsessive-compulsive behaviors: evidence for an autism susceptibility gene on chromosome 1 and further support for susceptibility genes on chromosome 6 and 19. Mol Psychiatry 2004; 9: 144–150.
- 145 Ma DQ, Jaworski J, Menold MM, Donnelly S, Abramson RK, Wright HH et al. Ordered-subset analysis of savant skills in autism for 15q11–q13. Am J Med Genet B 2005; 135: 38–41.
- 146 Nurmi EL, Dowd M, Tadevosyan-Leyfer O, Haines JL, Folstein SE, Sutcliffe JS. Exploratory subsetting of autism families based on savant skills improves evidence of genetic linkage to 15q11-q13. J Am Acad Child Adolesc Psychiatry 2003; 42: 856-863.
- 147 Shao Y, Cuccaro ML, Hauser ER, Raiford KL, Menold MM, Wolpert CM et al. Fine mapping of autistic disorder to

- npg
- chromosome 15q11–q13 by use of phenotypic subtypes. Am J Hum Genet 2003; **72**: 539–548.
- 148 Stone JL, Merriman B, Cantor RM, Yonan AL, Gilliam TC, Geschwind DH *et al.* Evidence for sex-specific risk alleles in autism spectrum disorder. *Am J Hum Genet* 2004; **75**: 1117–1123.
- 149 Badner JA, Gershon ES. Regional meta-analysis of published data supports linkage of autism with markers on chromosome 7. Mol Psychiatry 2002; 7: 56–66.
- 150 Trikalinos TA, Karvouni A, Zintzaras E, Ylisaukko-Oja T, Peltonen L, Jarvela I et al. A heterogeneity-based genome search meta-analysis for autism-spectrum disorders. Mol Psychiatry 2006; 11: 29–36.
- 151 Yonan AL, Alarcon M, Cheng R, Magnusson PK, Spence SJ, Palmer AA et al. A genomewide screen of 345 families for autism-susceptibility loci. Am J Hum Genet 2003; 73: 886–897.
- 152 Hallmayer J, Spiker D, Lotspeich L, McMahon WM, Petersen PB, Nicholas P et al. Male-to-male transmission in extended pedigrees with multiple cases of autism. Am J Med Genet 1996; 67: 13–18.
- 153 Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995; 11: 241–247.
- 154 Nothnagel M, Rohde K. The effect of single-nucleotide polymorphism marker selection on patterns of haplotype blocks and haplotype frequency estimates. *Am J Hum Genet* 2005; 77: 988–998.
- 155 Bacchelli E, Blasi F, Biondolillo M, Lamb JA, Bonora E, Barnby G et al. Screening of nine candidate genes for autism on chromosome 2q reveals rare nonsynonymous variants in the cAMP–GEFII gene. Mol Psychiatry 2003; 8: 916–924.
- 156 Hamilton SP, Woo JM, Carlson EJ, Ghanem N, Ekker M, Rubenstein JL. Analysis of four DLX homeobox genes in autistic probands. *BMC Genet* 2005; **6**: 52.
- 157 Rabionet R, Jaworski JM, Ashley-Koch AE, Martin ER, Sutcliffe JS, Haines JL et al. Analysis of the autism chromosome 2 linkage region: GAD1 and other candidate genes. Neurosci Lett 2004; 372: 209–214.
- 158 Segurado R, Conroy J, Meally E, Fitzgerald M, Gill M, Gallagher L. Confirmation of association between autism and the mitochondrial aspartate/glutamate carrier SLC25A12 gene on chromosome 2q31. Am J Psychiatry 2005; 162: 2182–2184.
- 159 Ramoz N, Reichert JG, Smith CJ, Silverman JM, Bespalova IN, Davis KL et al. Linkage and association of the mitochondrial aspartate/glutamate carrier SLC25A12 gene with autism. Am J Psychiatry 2004; 161: 662–669.
- 160 Blasi F, Bacchelli E, Carone S, Toma C, Monaco AP, Bailey AJ et al. SLC25A12 and CMYA3 gene variants are not associated with autism in the IMGSAC multiplex family sample. Eur J Hum Genet 2006; 14: 123–126.
- 161 Rabionet R, McCauley JL, Jaworski JM, Ashley-Koch AE, Martin ER, Sutcliffe JS et al. Lack of association between autism and SLC25A12. Am J Psychiatry 2006; 163: 929–931.
- 162 Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B *et al.* Linkage and association of the glutamate receptor 6 gene with autism. *Mol Psychiatry* 2002; 7: 302–310.
- 163 Shuang M, Liu J, Jia MX, Yang JZ, Wu SP, Gong XH et al. Family-based association study between autism and glutamate receptor 6 gene in Chinese Han trios. Am J Med Genet B 2004; 131: 48–50.
- 164 Watkins JC, Jane DE. The glutamate story. Br J Pharmacol 2006; 147(Suppl 1): S100–S108.
- 165 Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology 2001; 57: 1618–1628.
- 166 Fisher SE, Vargha-Khadem F, Watkins KE, Monaco AP, Pembrey ME. Localisation of a gene implicated in a severe speech and language disorder. Nat Genet 1998; 18: 168–170.
- 167 Lai CS, Fisher SE, Hurst JA, Levy ER, Hodgson S, Fox M et al. The SPCH1 region on human 7q31: genomic characterization of the critical interval and localization of translocations associated with speech and language disorder. Am J Hum Genet 2000; 67: 357–368.
- 168 Gong X, Jia M, Ruan Y, Shuang M, Liu J, Wu S *et al.* Association between the FOXP2 gene and autistic disorder in Chinese population. *Am J Med Genet B* 2004; **127**: 113–116.

- 169 Li H, Yamagata T, Mori M, Momoi MY. Absence of causative mutations and presence of autism-related allele in FOXP2 in Japanese autistic patients. *Brain Dev* 2005; **27**: 207–210.
- 170 Gauthier J, Joober R, Mottron L, Laurent S, Fuchs M, De K *et al.* Mutation screening of FOXP2 in individuals diagnosed with autistic disorder. *Am J Med Genet A* 2003; **118**: 172–175.
- 171 Marui T, Koishi S, Funatogawa I, Yamamoto K, Matsumoto H, Hashimoto O et al. No association of FOXP2 and PTPRZ1 on 7q31 with autism from the Japanese population. Neurosci Res 2005; 53: 91–94.
- 172 Newbury DF, Bonora E, Lamb JA, Fisher SE, Lai CS, Baird G *et al.* FOXP2 is not a major susceptibility gene for autism or specific language impairment. *Am J Hum Genet* 2002; **70**: 1318–1327.
- 173 Wassink TH, Piven J, Vieland VJ, Pietila J, Goedken RJ, Folstein SE et al. Evaluation of FOXP2 as an autism susceptibility gene. Am J Med Genet 2002; 114: 566–569.
- 174 Fatemi SH, Snow AV, Stary JM, Araghi-Niknam M, Reutiman TJ, Lee S *et al.* Reelin signaling is impaired in autism. *Biol Psychiatry* 2005; **57**: 777–787.
- 175 Fatemi SH, Stary JM, Egan EA. Reduced blood levels of reelin as a vulnerability factor in pathophysiology of autistic disorder. *Cell Mol Neurobiol* 2002; 22: 139–152.
- 176 Persico AM, D'Agruma L, Maiorano N, Totaro A, Militerni R, Bravaccio C et al. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. Mol Psychiatry 2001; 6: 150–159.
- 177 Serajee FJ, Zhong H, Mahbubul Huq AH. Association of Reelin gene polymorphisms with autism. *Genomics* 2006; **87**: 75–83.
- 178 Skaar DA, Shao Y, Haines JL, Stenger JE, Jaworski J, Martin ER et al. Analysis of the RELN gene as a genetic risk factor for autism. Mol Psychiatry 2005; 10: 563–571.
- 179 Bonora E, Beyer KS, Lamb JA, Parr JR, Klauck SM, Benner A et al. Analysis of reelin as a candidate gene for autism. Mol Psychiatry 2003; 8: 885–892.
- 180 Devlin B, Bennett P, Dawson G, Figlewicz DA, Grigorenko EL, McMahon W et al. Alleles of a reelin CGG repeat do not convey liability to autism in a sample from the CPEA network. Am J Med Genet B 2004; 126: 46–50.
- 181 Krebs MO, Betancur C, Leroy S, Bourdel MC, Gillberg C, Leboyer M. Absence of association between a polymorphic GGC repeat in the 5' untranslated region of the reelin gene and autism. Mol Psychiatry 2002; 7: 801–804.
- 182 Li J, Nguyen L, Gleason C, Lotspeich L, Spiker D, Risch N et al. Lack of evidence for an association between WNT2 and RELN polymorphisms and autism. Am J Med Genet B 2004; 126: 51–57.
- 183 Zhang H, Liu X, Zhang C, Mundo E, Macciardi F, Grayson DR et al. Reelin gene alleles and susceptibility to autism spectrum disorders. Mol Psychiatry 2002; 7: 1012–1017.
- 184 Bonora E, Lamb JA, Barnby G, Sykes N, Moberly T, Beyer KS et al. Mutation screening and association analysis of six candidate genes for autism on chromosome 7q. Eur J Hum Genet 2005; 13: 198–207.
- 185 Hutcheson HB, Olson LM, Bradford Y, Folstein SE, Santangelo SL, Sutcliffe JS et al. Examination of NRCAM, LRRN3, KIAA0716, and LAMB1 as autism candidate genes. BMC Med Genet 2004; 5: 12.
- 186 Powell SK, Rao J, Roque E, Nomizu M, Kuratomi Y, Yamada Y et al. Neural cell response to multiple novel sites on laminin-1. J Neurosci Res 2000; 61: 302–312.
- 187 Persico AM, Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. *Trends Neurosci* 2006; 29: 349–358.
- 188 Levy JB, Canoll PD, Silvennoinen O, Barnea G, Morse B, Honegger AM *et al.* The cloning of a receptor-type protein tyrosine phosphatase expressed in the central nervous system. *J Biol Chem* 1993; **268**: 10573–10581.
- 189 Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, Herrup K et al. Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. Cell 1997; 90: 895–905.
- 190 McCoy PA, Shao Y, Wolpert CM, Donnelly SL, Ashley-Koch A, Abel HL *et al.* No association between the WNT2 gene and autistic disorder. *Am J Med Genet* 2002; **114**: 106–109.
- 191 Benayed R, Gharani N, Rossman I, Mancuso V, Lazar G, Kamdar S  $et\ al.$  Support for the homeobox transcription factor gene

- ENGRAILED 2 as an autism spectrum disorder susceptibility locus. Am J Hum Genet 2005; 77: 851–868.
- 192 Gharani N, Benayed R, Mancuso V, Brzustowicz LM, Millonig JH. Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. *Mol Psychiatry* 2004; 9: 474–484.
- 193 Petit E, Herault J, Martineau J, Perrot A, Barthelemy C, Hameury L et al. Association study with two markers of a human homeogene in infantile autism. J Med Genet 1995; 32: 269–274.
- 194 Zhong H, Serajee FJ, Nabi R, Huq AH. No association between the EN2 gene and autistic disorder. J Med Genet 2003; 40: e4.
- 195 Bauman ML, Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. *Int J Dev Neurosci* 2005; 23: 183–187.
- 196 Ingram JL, Stodgell CJ, Hyman SL, Figlewicz DA, Weitkamp LR, Rodier PM. Discovery of allelic variants of HOXA1 and HOXB1: genetic susceptibility to autism spectrum disorders. *Teratology* 2000; 62: 393–405.
- 197 Collins JS, Schroer RJ, Bird J, Michaelis RC. The HOXA1 A218G polymorphism and autism: lack of association in white and black patients from the South Carolina Autism Project. J Autism Dev Disord 2003; 33: 343–348.
- 198 Conciatori M, Stodgell CJ, Hyman SL, O'Bara M, Militerni R, Bravaccio C *et al.* Association between the HOXA1 A218G polymorphism and increased head circumference in patients with autism. *Biol Psychiatry* 2004; **55**: 413–419.
- 199 Devlin B, Bennett P, Cook Jr EH, Dawson G, Gonen D, Grigorenko EL et al. No evidence for linkage of liability to autism to HOXA1 in a sample from the CPEA network. Am J Med Genet 2002; 114: 667–672.
- 200 Gallagher L, Hawi Z, Kearney G, Fitzgerald M, Gill M. No association between allelic variants of HOXA1/HOXB1 and autism. *Am J Med Genet B* 2004; **124**: 64–67.
- 201 Li J, Tabor HK, Nguyen L, Gleason C, Lotspeich LJ, Spiker D et al. Lack of association between HoxA1 and HoxB1 gene variants and autism in 110 multiplex families. Am J Med Genet 2002; 114: 24–30.
- 202 Romano V, Cali F, Mirisola M, Gambino G, D' Anna R, Di Rosa P et al. Lack of association of HOXA1 and HOXB1 mutations and autism in Sicilian (Italian) patients. Mol Psychiatry 2003; 8: 716–717.
- 203 Talebizadeh Z, Bittel DC, Miles JH, Takahashi N, Wang CH, Kibiryeva N et al. No association between HOXA1 and HOXB1 genes and autism spectrum disorders (ASD). J Med Genet 2002; 39: e70.
- 204 Barrow JR, Stadler HS, Capecchi MR. Roles of Hoxa1 and Hoxa2 in patterning the early hindbrain of the mouse. *Development* 2000; **127**: 933–944.
- 205 Lainhart JE, Piven J, Wzorek M, Landa R, Santangelo SL, Coon H et al. Macrocephaly in children and adults with autism. J Am Acad Child Adolesc Psychiatry 1997; 36: 282–290.
- 206 Fombonne E, Roge B, Claverie J, Courty S, Fremolle J. Microcephaly and macrocephaly in autism. J Autism Dev Disord 1999; 29: 113–119.
- 207 Rodier PM, Ingram JL, Tisdale B, Nelson S, Romano J. Embryological origin for autism: developmental anomalies of the cranial nerve motor nuclei. J Comp Neurol 1996; 370: 247-261.
- 208 Lainhart JE. Advances in autism neuroimaging research for the clinician and geneticist. Am J Med Genet C 2006; 142: 33–39.
- 209 Blatt GJ, Fitzgerald CM, Guptill JT, Booker AB, Kemper TL, Bauman ML. Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. J Autism Dev Disord 2001; 31: 537–543.
- 210 Hussman JP. Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. J Autism Dev Disord 2001; 31: 247–248.
- 211 Buxbaum JD, Silverman JM, Smith CJ, Greenberg DA, Kilifarski M, Reichert J et al. Association between a GABRB3 polymorphism and autism. Mol Psychiatry 2002; 7: 311–316.
- 212 Cook Jr EH, Courchesne RY, Cox NJ, Lord C, Gonen D, Guter SJ et al. Linkage-disequilibrium mapping of autistic disorder, with 15q11–13 markers. Am J Hum Genet 1998; 62: 1077–1083.

- 213 Curran S, Roberts S, Thomas S, Veltman M, Browne J, Medda E et al. An association analysis of microsatellite markers across the Prader–Willi/Angelman critical region on chromosome 15 (q11–13) and autism spectrum disorder. Am J Med Genet B 2005; 137: 25–28.
- 214 Maestrini E, Lai C, Marlow A, Matthews N, Wallace S, Bailey A et al. Serotonin transporter (5-HTT) and gamma-aminobutyric acid receptor subunit beta3 (GABRB3) gene polymorphisms are not associated with autism in the IMGSA families. The International Molecular Genetic Study of Autism Consortium. Am J Med Genet 1999; 88: 492–496.
- 215 Martin ER, Menold MM, Wolpert CM, Bass MP, Donnelly SL, Ravan SA et al. Analysis of linkage disequilibrium in gammaaminobutyric acid receptor subunit genes in autistic disorder. Am J Med Genet 2000; 96: 43–48.
- 216 Salmon B, Hallmayer J, Rogers T, Kalaydjieva L, Petersen PB, Nicholas P *et al.* Absence of linkage and linkage disequilibrium to chromosome 15q11–q13 markers in 139 multiplex families with autism. *Am J Med Genet* 1999; **88**: 551–556.
- 217 McCauley JL, Olson LM, Delahanty R, Amin T, Nurmi EL, Organ EL et al. A linkage disequilibrium map of the 1-Mb 15q12 GABA(A) receptor subunit cluster and association to autism. Am J Med Genet B 2004; 131: 51–59.
- 218 Menold MM, Shao Y, Wolpert CM, Donnelly SL, Raiford KL, Martin ER et al. Association analysis of chromosome 15 gabaa receptor subunit genes in autistic disorder. J Neurogenet 2001; 15: 245–259.
- 219 Ashley-Koch AE, Mei H, Jaworski J, Ma DQ, Ritchie MD, Menold MM et al. An analysis paradigm for investigating multi-locus effects in complex disease: examination of three GABA receptor subunit genes on 15q11–q13 as risk factors for autistic disorder. Ann Hum Genet 2006; 70: 281–292.
- 220 Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H et al. Identification of significant association and gene–gene interaction of GABA receptor subunit genes in autism. Am J Hum Genet 2005; 77: 377–388.
- 221 Kim SJ, Herzing LB, Veenstra-VanderWeele J, Lord C, Courchesne R, Leventhal BL et al. Mutation screening and transmission disequilibrium study of ATP10C in autism. Am J Med Genet 2002: 114: 137–143.
- 222 Nurmi EL, Amin T, Olson LM, Jacobs MM, McCauley JL, Lam AY et al. Dense linkage disequilibrium mapping in the 15q11–q13 maternal expression domain yields evidence for association in autism. Mol Psychiatry 2003; 8: 624–634, 570.
- 223 Nurmi EL, Bradford Y, Chen Y, Hall J, Arnone B, Gardiner MB et al. Linkage disequilibrium at the Angelman syndrome gene UBE3A in autism families. Genomics 2001; 77: 105–113.
- 224 Anderson GM, Freedman DX, Cohen DJ, Volkmar FR, Hoder EL, McPhedran P et al. Whole blood serotonin in autistic and normal subjects. J Child Psychol Psychiatry 1987; 28: 885–900.
- 225 Abramson RK, Wright HH, Carpenter R, Brennan W, Lumpuy O, Cole E et al. Elevated blood serotonin in autistic probands and their first-degree relatives. J Autism Dev Disord 1989; 19: 397–407.
- 226 Leboyer M, Philippe A, Bouvard M, Guilloud-Bataille M, Bondoux D, Tabuteau F et al. Whole blood serotonin and plasma beta-endorphin in autistic probands and their first-degree relatives. Biol Psychiatry 1999; 45: 158–163.
- 227 Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL et al. Organization of the human serotonin transporter gene. J Neural Transm Gen Sect 1994; 95: 157–162.
- 228 Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996; 66: 2621–2624.
- 229 Greenberg BD, Tolliver TJ, Huang SJ, Li Q, Bengel D, Murphy DL. Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. Am J Med Genet 1999: 88: 83–87.
- 230 Cook Jr EH, Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A et al. Evidence of linkage between the serotonin transporter and autistic disorder. Mol Psychiatry 1997; 2: 247–250.
- 231 Devlin B, Cook Jr EH, Coon H, Dawson G, Grigorenko EL, McMahon W *et al.* Autism and the serotonin transporter: the long and short of it. *Mol Psychiatry* 2005; **10**: 1110–1116.



- 232 Kim SJ, Cox N, Courchesne R, Lord C, Corsello C, Akshoomoff N et al. Transmission disequilibrium mapping at the serotonin transporter gene (SLC6A4) region in autistic disorder. Mol Psychiatry 2002; 7: 278–288.
- 233 Sutcliffe JS, Delahanty RJ, Prasad HC, McCauley JL, Han Q, Jiang L et al. Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. Am J Hum Genet 2005; 77: 265–279.
- 234 Klauck SM, Poustka F, Benner A, Lesch KP, Poustka A. Serotonin transporter (5-HTT) gene variants associated with autism? *Hum Mol Genet* 1997; **6**: 2233–2238.
- 235 Yirmiya N, Pilowsky T, Nemanov L, Arbelle S, Feinsilver T, Fried I et al. Evidence for an association with the serotonin transporter promoter region polymorphism and autism. Am J Med Genet 2001; 105: 381–386.
- 236 Betancur C, Corbex M, Spielewoy C, Philippe A, Laplanche JL, Launay JM et al. Serotonin transporter gene polymorphisms and hyperserotonemia in autistic disorder. Mol Psychiatry 2002; 7: 67-71.
- 237 Coutinho AM, Oliveira G, Morgadinho T, Fesel C, Macedo TR, Bento C et al. Variants of the serotonin transporter gene (SLC6A4) significantly contribute to hyperserotonemia in autism. Mol Psychiatry 2004; 9: 264–271.
- 238 Koishi S, Yamamoto K, Matsumoto H, Koishi S, Enseki Y, Oya A et al. Serotonin transporter gene promoter polymorphism and autism: a family-based genetic association study in Japanese population. Brain Dev 2006; 28: 257–260.
- 239 Mulder EJ, Anderson GM, Kema IP, Brugman AM, Ketelaars CE, de Bildt A *et al.* Serotonin transporter intron 2 polymorphism associated with rigid-compulsive behaviors in Dutch individuals with pervasive developmental disorder. *Am J Med Genet B* 2005; **133**: 93–96.
- 240 Persico AM, Militerni R, Bravaccio C, Schneider C, Melmed R, Conciatori M *et al.* Lack of association between serotonin transporter gene promoter variants and autistic disorder in two ethnically distinct samples. *Am J Med Genet* 2000; **96**: 123–127.
- 241 Tordjman S, Gutknecht L, Carlier M, Spitz E, Antoine C, Slama F et al. Role of the serotonin transporter gene in the behavioral expression of autism. Mol Psychiatry 2001; 6: 434–439.
- 242 Wu S, Guo Y, Jia M, Ruan Y, Shuang M, Liu J et al. Lack of evidence for association between the serotonin transporter gene (SLC6A4) polymorphisms and autism in the Chinese trios. Neurosci Lett 2005; 381: 1–5.
- 243 Zhong N, Ye L, Ju W, Brown WT, Tsiouris J, Cohen I. 5-HTTLPR variants not associated with autistic spectrum disorders. *Neuro-genetics* 1999; 2: 129–131.
- 244 Anderson GM, Gutknecht L, Cohen DJ, Brailly-Tabard S, Cohen JH, Ferrari P et al. Serotonin transporter promoter variants in autism: functional effects and relationship to platelet hyperserotonemia. Mol Psychiatry 2002; 7: 831–836.
- 245 Persico AM, Pascucci T, Puglisi-Allegra S, Militerni R, Bravaccio C, Schneider C et al. Serotonin transporter gene promoter variants do not explain the hyperserotonemia in autistic children. Mol Psychiatry 2002; 7: 795–800.
- 246 Conroy J, Meally E, Kearney G, Fitzgerald M, Gill M, Gallagher L. Serotonin transporter gene and autism: a haplotype analysis in an Irish autistic population. *Mol Psychiatry* 2004; 9: 587–593.
- 247 Blasi F, Bacchelli E, Pesaresi G, Carone S, Bailey AJ, Maestrini E. Absence of coding mutations in the X-linked genes neuroligin 3 and neuroligin 4 in individuals with autism from the IMGSAC collection. Am J Med Genet B Neuropsychiatr Genet 2006; 141: 220–221.
- 248 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: 27–29.

- 249 Laumonnier F, Bonnet-Brilhault F, Gomot M, Blanc R, David A, Moizard MP et al. X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family. Am J Hum Genet 2004; 74: 552–557.
- 250 Yan J, Oliveira G, Coutinho A, Yang C, Feng J, Katz C et al. Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric patients. Mol Psychiatry 2005; 10: 329–332.
- 251 Gauthier J, Bonnel A, St Onge J, Karemera L, Laurent S, Mottron L et al. NLGN3/NLGN4 gene mutations are not responsible for autism in the Quebec population. Am J Med Genet B 2005; 132: 74–75.
- 252 Talebizadeh Z, Bittel DC, Veatch OJ, Butler MG, Takahashi TN, Miles JH. Do known mutations in neuroligin genes (NLGN3 and NLGN4) cause autism? *I Autism Dev Disord* 2004: 34: 735–736.
- 253 Vincent JB, Kolozsvari D, Roberts WS, Bolton PF, Gurling HM, Scherer SW. Mutation screening of X-chromosomal neuroligin genes: no mutations in 196 autism probands. Am J Med Genet B 2004: 129: 82–84.
- 254 Ylisaukko-Oja T, Rehnstrom K, Auranen M, Vanhala R, Alen R, Kempas E et al. Analysis of four neuroligin genes as candidates for autism. Eur J Hum Genet 2005; 13: 1285–1292.
- 255 Beyer KS, Blasi F, Bacchelli E, Klauck SM, Maestrini E, Poustka A. Mutation analysis of the coding sequence of the MECP2 gene in infantile autism. *Hum Genet* 2002; 111: 305–309.
- 256 Carney RM, Wolpert CM, Ravan SA, Shahbazian M, Ashley-Koch A, Cuccaro ML et al. Identification of MeCP2 mutations in a series of females with autistic disorder. *Pediatr Neurol* 2003; 28: 205–211.
- 257 Lam CW, Yeung WL, Ko CH, Poon PM, Tong SF, Chan KY et al. Spectrum of mutations in the MECP2 gene in patients with infantile autism and Rett syndrome. J Med Genet 2000; 37: E41.
- 258 Li H, Yamagata T, Mori M, Yasuhara A, Momoi MY. Mutation analysis of methyl-CpG binding protein family genes in autistic patients. *Brain Dev* 2005; 27: 321–325.
- 259 Lobo-Menendez F, Sossey-Alaoui K, Bell JM, Copeland-Yates SA, Plank SM, Sanford SO et al. Absence of MeCP2 mutations in patients from the South Carolina autism project. Am J Med Genet B 2003; 117: 97–101.
- 260 Shibayama A, Cook Jr EH, Feng J, Glanzmann C, Yan J, Craddock N et al. MECP2 structural and 3'-UTR variants in schizophrenia, autism and other psychiatric diseases: a possible association with autism. Am J Med Genet B 2004; 128: 50–53.
- 261 van Karnebeek CD, van GI, Nijhof GJ, Abeling NG, Vreken P, Redeker EJ *et al.* An aetiological study of 25 mentally retarded adults with autism. *J Med Genet* 2002; **39**: 205–213.
- 262 Zappella M, Meloni I, Longo I, Canitano R, Hayek G, Rosaia L et al. Study of MECP2 gene in Rett syndrome variants and autistic girls. Am J Med Genet B 2003; 119: 102–107.
- 263 Cohen IL, Liu X, Schutz C, White BN, Jenkins EC, Brown WT et al. Association of autism severity with a monoamine oxidase A functional polymorphism. Clin Genet 2003; 64: 190–197.
- 264 Philippe A, Guilloud-Bataille M, Martinez M, Gillberg C, Rastam M, Sponheim E et al. Analysis of ten candidate genes in autism by association and linkage. Am J Med Genet 2002; 114: 125–128.
- 265 Yirmiya N, Pilowsky T, Tidhar S, Nemanov L, Altmark L, Ebstein RP. Family-based and population study of a functional promoter-region monoamine oxidase A polymorphism in autism: possible association with IQ. Am J Med Genet 2002; 114: 284–287.
- 266 Simonoff E. Genetic counseling in autism and pervasive developmental disorders. *J Autism Dev Disord* 1998; **28**: 447–456.
- 267 McMahon WM, Baty BJ, Botkin J. Genetic counseling and ethical issues for autism. Am J Med Genet C 2006; 142: 52–57.
- 268 Gschwind DH, Sowinski J, Lord C, Iversen P, Shestack J, Jones P et al. The autism genetic resource exchange: a resource for the study of autism and related neuropsychiatric conditions. Am J Hum Genet 2001; 69: 463–466.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)