

**FEATURE REVIEW**

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

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**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.**

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## 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);<sup>1</sup> International Classification of Diseases-10 (ICD-10)<sup>2</sup>). 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.<sup>4</sup> 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<sup>5</sup> and Lorna Wing<sup>6</sup> 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

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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.<sup>8</sup> The studies on *MECP2* and AD will be mentioned below in the overview of the genetic association studies in AD.

#### *Childhood disintegrative disorder*

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,<sup>9</sup> 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.*,<sup>10</sup> also see below, section on Family studies). The prevalence of autism was estimated to be 10/10 000, of AS 2.5/10 000 and of PDD-nos 15/10 000. Recent studies have shown an increase in the prevalence of AD.<sup>9</sup> 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.<sup>11,12</sup> 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.<sup>13</sup> 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.<sup>13–17</sup> Cytogenetic abnormalities have been described with regard to

most chromosomes.<sup>11,12</sup> 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.<sup>19–26</sup>

*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.*<sup>28</sup>). 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.<sup>29</sup>

*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.<sup>30</sup> 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.<sup>32</sup> 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 2q37*,<sup>33–39</sup> *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.<sup>33</sup> Linkage studies have shown suggestive evidence for linkage on chromosome 2q21–q33 differing from the above-mentioned 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.<sup>43</sup> 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.<sup>44</sup> Recently, a deletion on *chromosome 22q13.3* has been suspected as cause of AD.<sup>45</sup>

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 angiofibromas, 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.<sup>46</sup> Epidemiological studies<sup>9,47</sup> 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.<sup>56,57</sup> About 2–5% of the children and adolescents diagnosed with AD carry a full FRAXA mutation or FRAXA mosaics.<sup>9,13,16,58</sup> 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.<sup>61,62</sup> As the diagnosis of FRAXA, however, has major implications for genetic counselling, it should be ruled out in all individuals with AD and mild-to-severe mental retardation.

SLO is an autosomal-recessive disorder due to mutations in the gene for  $\Delta 7$ -dehydrocholesterol reductase,<sup>63,64</sup> leading to increased serum levels of 7-dehydrocholesterol. The incidence has been estimated to be one in 10 000 to one in 60 000.<sup>65</sup> SLO can be improved by supplementary dietary 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.<sup>66</sup> 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.<sup>66,67</sup> Two studies have shown a high rate of AD in individuals with SLO,<sup>65,68</sup> 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.<sup>69</sup> 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,<sup>70</sup> 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,<sup>75,76</sup> of whom 7% developed typical or atypical autism. With about 2%, another relatively frequent medical condition in AD is cerebral palsy.<sup>9</sup>

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.<sup>78–81</sup> 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 prevalently been performed in families with children with 'idiopathic' AD, that is, children with an above-mentioned 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.<sup>82–85</sup> It has been discussed that twinning in itself might be a risk factor for the development of autism.<sup>86,87</sup> However, three large-scale epidemiological studies have refuted

this idea.<sup>88–90</sup> 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 study<sup>82</sup> with regard to the BAP, which was conceptualized for two areas, communication impairment and social dysfunction, did show far higher rates of the BAP in discordant MZ than in discordant DZ pairs.<sup>91</sup> Among the MZ co-twin, communication impairment and social dysfunction frequently co-occurred 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.<sup>92,93</sup> 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 between-pair 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.<sup>91,92,97</sup>

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<sup>98</sup> 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.<sup>99</sup> Language abilities, however, were not impaired in siblings of children with autism<sup>100</sup> 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,<sup>101</sup> and an influence of language abilities on the correlation of ICD-10 autism symptoms and the presence of the BAP in relatives,<sup>102</sup> 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.<sup>101,103,104</sup> 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.<sup>105</sup>

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.<sup>106–114</sup> This finding did not differ in parents of children with autism with and without a history of language regression.<sup>115</sup> 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.<sup>116</sup>

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 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.<sup>118</sup> Despite the male:female ratio of 4:1,<sup>9</sup> 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.<sup>40,119–137</sup> 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.<sup>122,123,127,130,138–148</sup> 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 meta-analysis<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<sup>119,120,123,126,131–133,136,151</sup> were included in this meta-analysis. Between-scan heterogeneity was low for the locus on 7q, but high for the loci on 10p12–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.<sup>59,152</sup> 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.<sup>134</sup>

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,<sup>154</sup> 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 for 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.<sup>122,138–140,142</sup> 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,<sup>130</sup> and at chromosomes 7q and 21q for developmental regression.<sup>143</sup> With regard to obsessive-compulsive behaviour, significant linkage has been found on chromosome 1q.<sup>144</sup> Ordered-subset analysis regarding the phenotype insistence on sameness has resulted in significant linkage at chromosome 15q11–13.<sup>147</sup>

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.<sup>123,148</sup> 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.<sup>38,155–157</sup> 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 studies<sup>158,159</sup> 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,<sup>160,161</sup> 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	3q25–27	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 <i>et al.</i> (2005) (subset of Yonan <i>et al.</i> (2003))	ADI-R <sup>a</sup>	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))	ADI-R ADOS-G	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 Markers region	Highest LOD/NPL/MLS/Z-scores/lowest P-values
Faroe Islands	Lauritsen <i>et al.</i> (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 <i>P</i> -value = 0.00007 <i>P</i> -value = 0.005 <i>P</i> -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 AGRE <sup>a</sup>	McCauley <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (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



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
AGRE <sup>a</sup> 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 D17S1294	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; 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; 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 International Sibpair Study; RPM, Raven Progressive Matrices; VABS, Vineland Adaptive Behaviour Scales; WAIS, Wechsler Adult Intelligence Scale; WISC, Wechsler Intelligence Scale for Children; 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 1.

gene on chromosome 6 with AD.<sup>162,163</sup> 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,<sup>164</sup> 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,<sup>165</sup> 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,<sup>166,167</sup> have been assessed by several studies for association with AD. With the exception of nominal significance in one Chinese and one Japanese study,<sup>168,169</sup> none of the other studies did find an association of *FOXP2* polymorphisms or mutations with AD.<sup>170-173</sup> 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 post-mortem cortices of individuals with autism,<sup>174</sup> 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,<sup>176-178</sup> five other studies of comparable size and power did not find an association of the 5'UTR trinucleotide or other variants with AD.<sup>179-183</sup> 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,<sup>178</sup> the most common repeat<sup>10</sup> was over-represented in AD. One study<sup>177</sup> 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 β-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 2** Linkage studies – quantitative and specific qualitative endophenotypes, specific linkage models

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
<i>1. Language measures</i>								
Age at first word	AGRE <sup>a</sup> (subset of Yonan <i>et al.</i> (2003))	Alarcon <i>et al.</i> (2005)	ADI-R <sup>a</sup>	291 of 345 ASP with complete ADI-R language information OSA word in 132 ASP; OSA phrase in 67 ASP	Non-parametric and parametric QTL analysis Ordered-subset analysis	Word: 1	Not reported D3S3045–D3S1763	Z = 2.20
Age at first phrase						3q		Z = 3.10
						5		Z = 2.39
						10		Z = 2.19
						15		Z = 2.20
						16		Z = 2.38
						17q		Z = 2.84
						OSA word: 7q35		MLS = 2.57
						Phrase: 5		Z = 2.28
						10		Z = 2.31
	16	Z = 2.08						
	17	Z = 2.22						
	OSA phrase: 7q35	MLS = 2.76						
Age at first word	AGRE <sup>a</sup> (subset of Alarcon <i>et al.</i> (2005))	Alarcon <i>et al.</i> (2002)	ADI-R <sup>a</sup>	123 of 152 ASP with complete ADI-R language information	Non-parametric and parametric QTL analysis	Word: 7q35–36	D7S1824–D7S3058	Z = 2.98;
Age at first phrase						11		HE-LOD = 1.14
						Phrase: 10		Z = 2.22;
						11		HE-LOD = 0.96
						20	D10S2327	Z = 2.10;
								HE-LOD = 1.22
								Z = 2.19;
								HE-LOD = 1.21
								Z = 2.29;
								HE-LOD = 2.21
Delayed language development in AD subject	CLSA	Bradford <i>et al.</i> (2001)	ADI-R ADOS-G FHI: Parent data	50 of 75 ASP meeting language criteria	Parametric linkage analysis (parents and children with language delay)	7q	D7S1813–D7S821	M-HLOD = 2.2,
Parent's language development						13		D13S217–D13S800
Onset of phrase speech > 36 months	AGRE <sup>a</sup> (subset of Alarcon <i>et al.</i> (2002))	Buxbaum <i>et al.</i> (2001)	ADI-R <sup>a</sup>	49 of 95 ASP meeting language criteria	Non-parametric and parametric linkage analysis	2q	D2S335–D2S364	HLOD dominant = 2.99 NPL = 3.32
Non-verbal communication	AGRE <sup>a</sup> (subset of Yonan <i>et al.</i> (2003))	Chen <i>et al.</i> (2006)	ADI-R <sup>a</sup>	228 ASP OSA chromosome 8: 175 ASP; OSA chromosome 16: 70 ASP	Non-parametric and parametric QTL analysis Ordered-subset analysis	1p13–q12 4q21–25 7q35 8q23–24 16p12–13	Not reported Not reported Not reported Not reported	Z = 3.63 Z = 3.13 Z = 2.45 Z = 2.61 OSA MLS = 3.4 Z = 3.09 OSA MLS = 3.8

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
<i>2. Developmental milestones and developmental regression</i>								
Motor-language, bladder and bowel control milestones	CLSA AGRE <sup>a</sup>	McCauley <i>et al.</i> (2005)	ADI-R <sup>a</sup>	92 of 158 ASP	Ordered-subset analysis (more rapid achievement)	19p13	D19S930	LOD = 3.4
Developmental regression	AGRE <sup>a</sup>	Molloy <i>et al.</i> (2005)	ADI-R <sup>a</sup>	34 of 288 ASP	Non-parametric and parametric linkage analysis	7q  21q	D7S483  D21S1437	NPL = 3.7 MLS dominant = 2.0 NPL = 3.0 MLS dominant = 3.4
<i>3. Restricted repetitive and stereotyped patterns of behaviour, interests and activities</i>								
Repetitive/stereotyped behaviour	AGRE <sup>a</sup> (subset of Yonan <i>et al.</i> (2003))	Alarcon <i>et al.</i> (2005)	ADI-R <sup>a</sup>	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 <i>et al.</i> (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 <i>et al.</i> (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

<i>Endophenotype/linkage model</i>	<i>Research group</i>	<i>Reference</i>	<i>Diagnostic criteria AD</i>	<i>Number of families</i>	<i>Linkage analysis method</i>	<i>Chromosomal region</i>	<i>Markers</i>	<i>Highest LOD/NPL/MLS/Z scores/lowest P-values</i>
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	15q11–13	GABRB3	LOD dominant = 4.7 LOD recessive = 3.8 OSA-LOD = 3.2
<i>4. Parent of origin analysis</i>								
Linkage model: paternal/ maternal contributions	IMGSAC	Lamb <i>et al.</i> (2005)	ADI-R ADOS-G	219 ASP	Model-free linkage analysis	Maternal: 9	D9S157–D9S171	MLS = 2.0
<i>5. Sex limited loci</i>								
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) (subset of Yonan <i>et al.</i> (2003))	ADI-R <sup>a</sup>	257 ASP 148 male 109 non-male	Non-parametric linkage analysis	Male–male: 17q11 Non-male: 4q32–Not reported 35	D17S1294–D17S798	MLS = 4.3 MLS = 2.7

Abbreviations: ADI-R, Autism Diagnostic Interview-Revised; ADOS-G, Autism Diagnostic Observation Schedule-Generative; 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 *LAMB1* 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.<sup>189</sup> The first study<sup>17</sup> reported a nominal association of a 3'UTR 783C>T SNP detected by mutation analysis in two affected siblings with AD. Subsequent studies<sup>182,190</sup> 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.<sup>191–194</sup> *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.<sup>195</sup> 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.<sup>191,192</sup> The exonic SNP rs3735653 consistently did not show association with AD in two studies<sup>192,194</sup> similar to other assessed exonic variants.<sup>191</sup> 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.<sup>197–203</sup> *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,<sup>198</sup> whereas the first study discussed the G allele and the AG/GG genotypes as risk factors. In the former study,<sup>198</sup> an increased head circumference was associated with AG/GG, which might be of relevance, as a subgroup of individuals with AD does show macrocephaly.<sup>205,206</sup> However, the hypothesized disrupted development of brainstem nuclei in autism<sup>207</sup> has not been proven by brain imaging studies.<sup>208</sup> 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.<sup>213–216</sup> 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.<sup>215,217–219</sup> 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.<sup>220</sup> Similar inconclusive results have been obtained for variants in or close to the *ATPase, class V, type 10C (ATP10C)* and the *ubiquitin-protein ligase E3A (UBE3A)* genes located in the maternal expression domain of chromosome 15q11–13.<sup>212,221,222</sup> 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.<sup>222</sup> 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 hyperserotonemia in children with autism<sup>224</sup> and their first-degree relatives,<sup>225,226</sup> 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,<sup>217,230–233</sup> fewer studies of the long alleles.<sup>234,235</sup> Some studies did not replicate these findings.<sup>214,236–243</sup> Three studies have assessed the effects of the 5HTTLPR on whole-blood serotonin (5-HT) or platelet 5-HT parameters in AD.<sup>236,237,244</sup> 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,<sup>236,245</sup> which parallels the findings in healthy controls.

With the exception of one study,<sup>246</sup> 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,<sup>232</sup> 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 AD<sup>241</sup> 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 synapto-

genesis. Despite the findings of several non-conservative mutations in single families in the *NLGN3* and *NLGN4* genes,<sup>247–250</sup> 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.<sup>255–262</sup> With the exception of two studies,<sup>257,260</sup> 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 prevalently were found in women,<sup>256,259</sup> 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.<sup>263–265</sup>

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 AD.<sup>187</sup>

### 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.

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