

## Genetics of autistic disorders: review and clinical implications

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# Genetics of autistic disorders: review and clinical implications

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**Abstract** Twin and family studies in autistic disorders (AD) have elucidated a high heritability of AD. In this literature review, we will present an overview on molecular genetic studies in AD and highlight the most recent findings of an increased rate of copy number variations in AD. An extensive literature search in the PubMed database was performed to obtain English published articles on genetic findings in autism. Results of linkage, (genome wide) association and cytogenetic studies are presented, and putative aetiopathological pathways are discussed. Implications of the different genetic findings for genetic counselling and genetic testing at present will be described. The article ends with a prospectus on future directions.

**Keywords** Autistic disorder · Linkage · Whole genome association · Copy number variation · Mutation

## Introduction

Autistic disorders (AD) are a group of disorders characterised by the three core problem areas: qualitative impairment in social interaction and communication, and restricted repetitive and stereotyped patterns of behaviour, interests, and activities [88]. The three disorders autism, Asperger syndrome (AS) and pervasive developmental disorder-not otherwise specified (PDD-NOS) are currently conceptualised by most researchers as a continuum of the same disorder with varying degrees of severity, associated intellectual functioning and medical conditions, possibly also including the broader autism phenotype (BAP) [80]. Recent studies estimated the prevalence of AD to around 0.5–1% [5, 17]. AD are predominantly genetically determined disorders with a heritability of around 90% [29].

Non-genetic medical conditions (phenocopies) are rare, however, they are especially relevant with regard to the prevention of AD. Retrospective case series clearly indicate maternal valproic acid use [87] during pregnancy as risk factor. The association of congenital rubella with autism has been studied in a longitudinal study on 243 children with congenital rubella of whom 7% developed typical or atypical autism [18]. With about 2%, another relatively frequent medical condition in AD is cerebral palsy [28].

The findings of cytogenetic abnormalities and single gene disorders associated with AD indicate genetic heterogeneity and different modes of inheritance in individual families. For idiopathic AD, i.e. cases with unknown genetic or environmental cause, oligogenic, polygenic, and multifactorial mechanisms have additionally been proposed. Some evidence points to a different genetic model in simplex versus multiplex AD cases [93]. Previously, hypothesis free genome wide linkage approaches were

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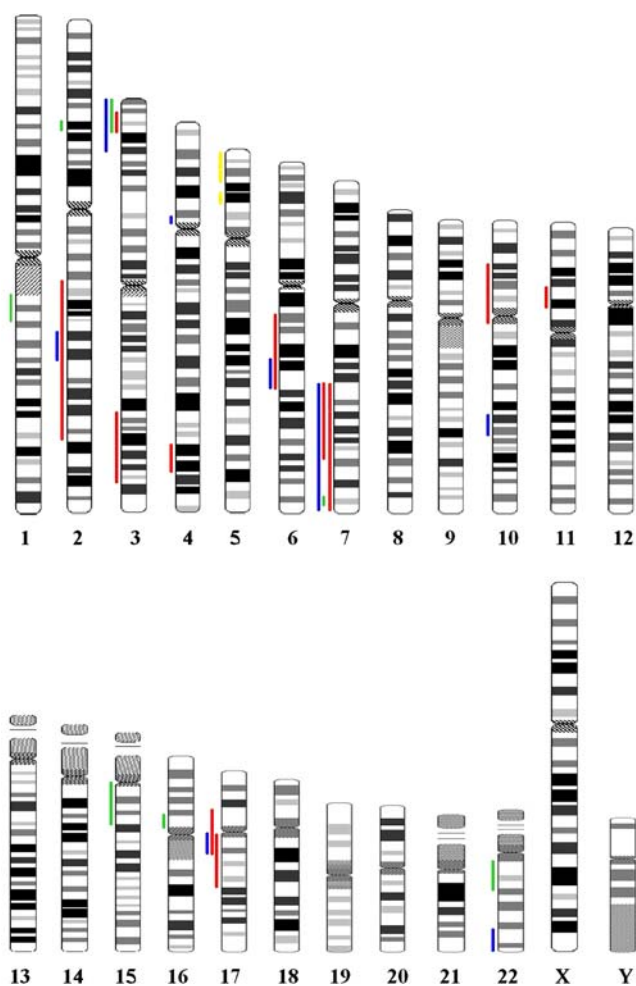
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performed to elicit relevant genetic loci. Mutation analyses and candidate gene association studies attempted to elucidate rare or common variants associated with the disorder. Owing to technical advances in CHIP based technology and cytogenetics, recent studies have focussed on hypotheses free genome wide association (GWA) and copy number variation (CNV) studies.

In this review on molecular genetic findings in AD, we summarise findings of a previous review article on the genetics of AD [29] and add findings from recent studies (2007–2009). For both articles, a systematic search on molecular genetic studies in AD was performed using the database PubMed and adding information from reference lists of published articles. The following key words were used: “autistic disorder”, “autism spectrum disorder”, “autism”, “Asperger Syndrome” in combination with “genetic”, “gene”, “linkage”, “association”, “copy number variation”, “genome wide”, “cytogenetic”, “duplication”, “deletion”, “translocation”. Results of association studies were only reported if at least one replication of another or the same variant in the specific gene was published. For a graphical overview on the reported replicated findings, compare Fig. 1.

### 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. Linkage has been found in at least two independent studies in regions 2q21–33, 3q25–27, 3p25, 4q32, 6q14–21, 7q22, 7q31–36, 11p12–13, 17q11–21 [2, 24, 29, 50, 60, 74]. A meta-analysis confirmed the region 7q22–32, and reported suggestive evidence for linkage to 10p12–q11.1 and 17p11.2–q12 [75]. Due 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, did not always result in more pronounced linkage findings at previously described loci, but more often in diminished LOD scores. This might be due to different loci containing risk genes in different populations, to false positive or negative findings due to differing linkage disequilibrium patterns in different populations, towards the involvement of risk alleles of small effect, which are not detected by linkage studies, and again towards heterogeneity of AD.



**Fig. 1** Replicated findings of linkage (red bars), Genome wide association (yellow bars), copy number variation (green bars) and candidate gene (blue bars) studies as discussed in the text

### Single gene disorders and findings of association studies

Co-occurrence of AD and single gene disorders has been observed for a long time. The most prevalent single gene disorders in AD are *tuberous sclerosis* (*TSC1/TSC2*; around 1%) and *fragile X syndrome* (around 3–5%). More rare ( $\ll 1\%$ ), but medically treatable single gene disorders are *phenylketonuria* (PKU) and *Smith–Lemli–Opitz* (*SLO*) syndrome caused by mutations in 7-dehydrocholesterol reductase (*DHCR7*) [4, 29, 66]. The rate of AD in these disorders also is increased, but AD is not observed in all individuals carrying the mutation: fragile-X-syndrome ca. 25% (males), tuberous sclerosis ca. 20%; SLO ca. 50%; PKU ca. 10% [1]. Mutation screening and subsequent association studies have elucidated other rare causes of (most likely) single gene disorders in individuals with AD. Additionally, several common variants in genes located in linked regions were assessed for association with AD. In

the following overview, only replicated association findings will be presented.

#### Chromosome 2

Different common variants in the mitochondrial aspartate/glutamate carrier (*SLC25A12*) gene located on 2q24 were associated with AD in several studies [29, 67].

#### Chromosome 3

Oxytocin plays a crucial role in social cognition and behaviour [22] and the oxytocin receptor gene (*OXTR*) is located under the linkage peak on 3p24–26. Several single nucleotide polymorphism (SNP) alleles and haplotypes of *OXTR* were associated with AD [40, 46, 89].

#### Chromosome 4

One association study and a cytogenetic finding point towards the possible involvement of GABA-A receptor subunit genes encoded on chromosome 4p12 [29, 79]. In addition, a gene expression study observed down-regulation of several GABA-A-receptor subunits in the parietal cortex of individuals with autism [25]. No association studies were performed to date on genes at the 4q32 locus.

#### Chromosome 6

Three studies found evidence for association of different SNP genotypes or alleles in the Glutamate receptor 6 (*GluR6*) gene under the linkage peak on chromosome 6 [29].

#### Chromosome 7

Most candidate genes assessed in AD are located on chromosome 7q22–36, as this area is best replicated from linkage studies. Mutations or variants in the following candidate genes could not clearly be replicated as risk factors for AD [29]: *FOXP2* (a gene which was mutated in a severe monogenic form of speech and language impairment in one family) [27], *RELN* (neuronal migration, formation of cortical layers, synaptogenesis), *PTPRZ1* (highly expressed during embryogenesis), *NRCAM*, *WNT2*, and *HOXAI* (hindbrain development in mouse).

The *RELN* (Reelin) gene codes for a signalling protein that plays a crucial role in neuronal migration, formation of cortical layers and synaptogenesis. Three studies supported the involvement of a trinucleotide repeat polymorphism in the 5'UTR region in Reelin in AD [58, 64, 69], whereas five other, at least similarly powered, studies did not [9, 21, 44, 47, 92].

Different rare and common, possibly functional variants in *LAMB1* were associated with AD in two studies [10, 29, 38].

*LAMB1* encodes the  $\beta 1$  chain of laminin, which is an important glycoprotein promoting neuronal migration and neurite outgrowth in the developing nervous system.

Different alleles of two SNPs in the Engrailed 2 (*EN2*)-gene on chromosome 7q36 were associated with AD in several independent samples [11, 29, 84, 90]. *EN2* is a homeobox transcription factor, which plays a role during the development of cerebellar and brainstem functions.

Common variants as well as rare mutations in the contactin-associated protein-like 2 (*CNTNAP2*), a member of the neurexin superfamily involved in cell-adhesion and neuronal migration [2, 3, 6] also increased the risk for AD in several independent samples. Gene-expression analyses in the developing human brain identified *CNTNAP2* as enriched in circuits important for language development.

The gene encoding the pleiotropic MET receptor tyrosine kinase plays a role in brain development and gastrointestinal repair. As some individuals with AD suffer from gastrointestinal symptoms (GIS), this gene was specifically assessed in individuals with AD and GIS. A functional promotor variant, several other SNP genotypes or alleles as well as rare mutations were associated with AD in several independent samples, predominantly in individuals with GIS [14, 16, 71]. In a post-mortem brain protein-expression analysis, AD individuals showed lower levels of the MET protein compared to controls [15].

#### Chromosome 10

*PTEN* (phosphatase and tensin homologue, located on 10q23.3) is a tumour suppressor gene that acts as a negative regulator in the PI3-kinase (PI3K) pathway. Heterozygous *PTEN* mutations were identified in a subset of individuals with autism, macrocephaly and/or developmental delay [13, 35, 78], thus rendering affected individuals *PTEN*-haploinsufficient.

#### Chromosome 15

Several variants of genes on 15q11–13 were assessed as risk factors, as cytogenetic abnormalities of this region are frequently observed in AD (see below). In candidate gene association studies to date, inconclusive findings were reported with regard to *ATP10C*, *UBE3A* and the gamma-aminobutyric acid (GABA) receptor genes located on 15q [29].

#### Chromosome 17

Due to findings of platelet hyperserotonemia in children with autism and their first-degree relatives, common variants in the Serotonin-transporter gene (*SLC6A4*) were assessed by several studies [29]. A recent meta-analysis,

however, did not report an effect of 5-HTTLPR and STin2 alleles on AD risk [37].

### Chromosome 22

Several case reports indicated 22q13.3 deletions and duplications as risk factors for AD. Therefore, *SHANK3*, the gene encoding a synaptic scaffolding protein, was assessed for mutations and common variants possibly associated with AD. Several studies observed rare mutations in individuals with AD, occurring most often de novo, but also inherited from (unaffected) parents. Similar to reports of mutations of X-chromosomal genes (see below), *SHANK3* mutations might cause a monogenic form of non-verbal or severely speech delayed AD [23, 30, 56]. The frequency of *SHANK3* mutations was estimated to 0.5–1% in AD individuals. Common variants were not detected as risk factors [73]. A recent study, however, also has reported *SHANK3* deletions in healthy individuals [31].

### X-chromosome

Despite rare positive linkage findings for loci on the X-chromosome, several variants in genes on the X-chromosome were assessed for association with AD, as the sex distribution in AD is markedly skewed (male:female = 4:1). Two neuroligin genes on Xq13 and Xp22 were screened for mutations in several studies. Neuroligins are essential components of synaptogenesis. Despite the findings of several non-conservative mutations in single families in *NLGN3* and *NLGN4* these could not be replicated in larger samples of individuals with AD [29]. Similarly, two mutations identified in the ribosomal protein gene *RPL10* on Xq28 were not replicated in a subsequent study [32, 43].

*MeCP2*-mutations are risk factors for Rett-Syndrome, which by some authors is regarded as an AD, but clearly differs phenotypically from AD by deterioration of motor abilities and very low IQ. In addition, in males with mental retardation and progressive spasticity, duplications of the *MECP2* region were repeatedly observed [48]. In AD samples in contrast, no association of *MeCP2* variants was found [29].

### Y-chromosome

There are only a few studies, which have investigated variants of Y chromosomal genes in autism. A recent study reported nominal association with a SNP variant in *NLGN4Y* [63] in contrast to previous findings [42, 91]. Thus, an influence of genes on the Y chromosome has not been established to date.

## Genome wide association studies

The above reported candidate gene association studies are based on specific hypotheses. Results in one sample often were not replicated in subsequent samples, possibly indicating false positive reports. Other causes might be heterogeneity across populations, bias due to technical artefacts, population stratification or environmental modifiers. False negative reports, especially in light of genes of small effect, hamper further understanding of genetic mechanisms in autism. Similar to linkage studies, genome wide association (GWA) studies are considered hypothesis-free studies. Performing a GWA study in a sufficiently large sample and replicating the most significant SNPs in an independent sample with sufficient power is currently considered as a design avoiding the report of false positive findings [70]. However, GWA studies to date did not replicate any of the previous linkage or association findings, most likely due to the same reasons as the non-replication of hypotheses based association studies. In addition, the “common disease–common variant” genetic model underlying GWA studies might not hold true for the majority of AS cases [51]. GWA studies done on currently available samples and with the currently available SNP assays cannot detect risk alleles with low minor allele frequency or rare variants which might be relevant in the aetiology of AD [53]. To date, three GWA studies were performed in AD [49, 83, 85]. Common genetic variants on 5p14.1 and 5p15 were replicated in two independent samples, respectively, each carrying a small increased risk (OR 1.2) or protective effect (OR 0.6) [83, 85].

At 5p14.1 segments with a high degree of evolutionary conservation can be found, suggesting potential regulatory function. Genes *CDH9* and *CDH10* are near the two replicated SNPs [83]. These genes encode cadherins, which are a group of proteins that are involved in calcium-dependent cell–cell junctions in the nervous system, thereby shaping the physical structure and functional connectivity of the brain. The one replicated SNP at 5p15 is located near *TAS2RI*, a gene encoding a bitter taste receptor, and the semaphorin 5A gene (*SEMA5A*) implicated in axonal guidance. *SEMA5A* was downregulated in lymphoblastoid cell lines as well as occipital lobe cortex in AD [85].

## Chromosomal abnormalities

For a long time suspected causes of heterogeneity in AD are chromosomal abnormalities which can be observed by standard karyotyping. Numerous case reports of chromosomal abnormalities on almost each single chromosome in AD do exist [82], however, epidemiological data are



missing to date. Shortcomings of most cytogenetic studies are the lack of standardised assessment methods for AD, the inclusion of subjects with autistic features but no clear AD diagnosis, and the lack of standardised assessment of cognitive and adaptive functioning. Recent studies estimated a rate of 3–5% of cytogenetic abnormalities in AD [59]. Some studies aimed to elicit candidate genes or candidate gene regions by a detailed analysis of the boundaries of the cytogenetic abnormalities found in AD [82].

With a rate of approximately 1%, the most prevalent cytogenetic abnormalities are observed on *chromosome 15q11-13* [20, 29], in most cases maternally, but also paternally inherited duplications. Further relatively frequent findings are deletions of *chromosome 2q37*, *chromosome 7q31* and deletions or duplications of *chromosome 22q13*. In addition, *Klinefelter Syndrome (XXY)* as well as duplications of the Williams–Beuren–Syndrome region *7q11.23* and deletions of *22q11 (Velo-cardio-facial Syndrome)* are associated with increased autistic traits [8, 77, 81].

Due to autistic traits in several chromosomal aneuploidies, a cytogenetic evaluation has to be recommended in all subjects with AD. Some studies reported higher rates of deletions, duplications or translocations in AD individuals with mental retardation, abnormal EEG patterns or seizures, muscular hypotonia, severe motor and gait problems, or dysmorphic features [41], however, chromosomal anomalies are also observed in individuals without dysmorphic features [82]. The finding of a chromosomal anomaly as a likely cause of AD has strong implications for genetic counselling and in some cases, like Klinefelter or Velo-cardio-facial Syndrome, also for the treatment of other medical conditions associated with the respective syndrome.

### Submicroscopic copy number variations

Due to technological advances, it is now possible to also assess small cytogenetic abnormalities not detected by standard karyotyping [34]. Until recently, it was thought that only 0.1% base pair changes accounted for major genetic variation between individuals. With the advance of micro-array-based comparative genomic hybridization (array-CGH), this view has been challenged by studies that showed that the genomes of unrelated healthy individuals vary significantly with respect to the number of copies an individual has of each DNA segment [39, 62]. A copy number variation (CNV) is currently defined as a DNA segment longer than 1 kb, with a variable copy number compared to a reference genome [26]. A CNV can be a deletion, insertion, duplication or a complex multi-site variant. It can be inherited or may arise de novo on a paternally or maternally inherited chromosome. To be

pathogenic, a CNV must affect a gene in a molecular pathway important in the development or maintenance of the human body. CNVs might change the transcription rate of a gene product by increased or decreased transcription, in case of deletions, they may unmask recessive mutations, or they might change the coding sequence of a gene.

Recent publications show that some CNVs are observed more frequently in AD patients compared to control subjects [12, 31, 45, 52, 55, 61, 86]. Similar to the cytogenetic findings obtained by standard karyotyping, most CNVs represent rare, unique events rather than representing recurrent deletions or duplications. Replicated CNVs from genome wide studies, which were observed more frequently in AD compared to control individuals, are located on the following chromosomes: 1q21, 2p16.3 (*NRXN1*), 3p25-26 (*CNTN4*), 7q36.2 (*DPP6*), 15q11-13 (*UBE3A*, *OR4M2*, *OR4N4*); 16p11.2 (*MAPK3*, *MAZ*, *DOC2A*, *SEZ6L2*, *HIRIP3*, *IL6*); 22q11.2. Some of these CNV were observed also more frequently in individuals with mental retardation or schizophrenia than in controls. ASD specific CNVs were not exclusively observed in AD individuals with specific dysmorphic features or mental retardation but were also present in high-functioning patients with autism with only minor dysmorphology [76].

### Summary on genetic mechanisms and possible functional pathways involved in AD

Molecular genetic studies in AD have come a long way from the early linkage studies, which aimed at describing a few loci and subsequently finding one or a few genes of major effect relevant for all cases of AD. It has now become clear, that AD are heterogeneous disorders, caused by several rare—most likely—monogenetic disorders (as fragile X syndrome, mutations in *TSC1/TSC2*, *LAMB1*, *CNTNAP2*, *PTEN*, *DHCR7*, *SHANK3*, *NLGN3/4*, or *RPL10*). In addition, “contiguous gene syndromes” are likely causes of AD, as the overall rate of CNVs and large chromosomal deletions, duplications, and translocations is increased in individuals with AD compared to controls.

Common variants, on the other hand, may shape the phenotype or eventually may lead to the disorder by interacting with rare mutations or CNVs. A mechanism like this has been shown for *PTEN* haploinsufficient individuals. The serotonin-transporter gene *SLC6A4* has been discussed as both an AD susceptibility gene and a second-site modifier in AD [7, 36]. A study in *PTEN* haploinsufficient mice [57] demonstrated that the phenotypes of these mice were modified in an additive fashion by *SLC6A4* haploinsufficiency. In addition, the role of *PTEN* in the maintenance of genomic stability [65, 72] makes it likely that *PTEN* haploinsufficiency may increase the probability of a

secondary modifying event, such as a copy number variation in a chromosomal region relevant to AD. Common variants also might increase the risk for autistic traits in the general population as well as for less severe autistic disorders as Asperger Syndrome or PDD-NOS.

From results of current genetic findings in AD, it is likely that mutations or common variants in genes coding for gene products involved in (1) cell–cell interaction and synaptic function, including development of dendritic spines, (2) neuronal migration and growth, or (3) excitatory and inhibitory neurotransmission are causes of AD. The pathway influencing cell–cell interaction and synaptic function includes *NRXNs*, *NLNGs*, *CNTN3/4*, *CNTNAP2*, and *SHANK3*. In addition, the FMR protein, which is missing in fragile X syndrome, modulates dendritic spine formation and synaptic plasticity by inhibiting mGluR1/5 mediated dendritic protein synthesis [33]. Neuronal migration and growth are influenced by gene products of *LAMB1*, *EN2* or the MET receptor tyrosine kinase gene. The mTor/PI3-kinase (PI3K) pathway involves *PTEN*, *TSC1/2*, and several other genes, which were observed in CNVs in individuals with AD [19]. It strongly influences (neuronal) cell growth. Gene products influencing the regulation of excitatory and inhibitory neurotransmission are GABA and glutamate receptors. In addition, dysbalance of excitatory and inhibitory neurotransmission was also observed in fragile X syndrome. Clearly, this list of possibly involved pathways is not exhaustive, and other mechanisms or pathways may emerge as results of further studies will be published.

### 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 their 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 not caused by any of the currently known mechanisms [68]. 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 *TSC1/2*-mutations, a sibling recurrence risk of 50% is present, if

one of the parents carries the disease-causing variant, i.e. if the variant is not a de-novo mutation. In case of recessive single gene disorders, like Smith–Lemli–Opitz syndrome, the sibling recurrence risk is 25%. If a child suffers from fragile X syndrome, 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 15q11-13 duplication or duplicated inversion, the recurrence risk can be similar to the population prevalence, as most duplications and inversions arise de-novo during meiosis. The same is true for CNVs. However, they also can be inherited, which will result in an entirely different recurrence risk.

The limited clinical validity of genetic testing for autism and the related ethical concerns have been delineated in detail [54]. 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. Despite contrasting information to patients, no genetic test does exist to date, by which an AD can be diagnosed. It remains a diagnosis based on behavioural observation.

### Future directions

Due to technical advances, sequencing of the genome will be available and feasible in the near future. Similar to results of genome-wide CNV and association studies, the detection of new mutations, rare and common variants in cases as well as controls will make it difficult to prove the relevance of the findings for AD in general as well as for the affected individual specifically. Researchers are faced with genetic heterogeneity, reduced penetrance, unmasking of recessive alleles, “second hit” mechanisms, pleiotropy, epigenetic mechanisms, and possible environmental mediators. It therefore is of crucial relevance to include on the one hand more differentiated phenotypic measures, on the other hand functional aspects on the cellular and molecular level as well as biological network analysis approaches to focus on the most relevant findings. This approach will need large and thoroughly diagnosed samples with AD. In parallel, knowledge about population wide variation at the genomic level in comparison with phenotypic expression of AD symptoms will add information towards involved functional pathways. The final goal anticipated is the development of therapeutic targets for drug treatments. The development of a molecular genetic test kit to assist in AD diagnosis might also be achieved. At the current stage of research, however, a genetic test does not seem feasible nor ethical due to the heterogeneity of the disorder, the lack of population-based

studies on the genetics of AD and the lack of sensitivity and specificity analyses in comparison to other psychiatric or neurological disorders in childhood.

## Conclusions

Despite the high-heritability estimates for AD, no major gene has been observed to be relevant for the majority of AD diagnoses. In contrast, rare mutations of larger effect and a few common variants of small effect in several different genes, which are also involved in different cellular pathways, seem to be causal for many cases of AD. From a statistical point of view therefore, large AD and control samples are necessary, to prove the causality of specific rare mutations (including CNVs) or variants of small effect as risk factors for AD. As the disorder shows a high-phenotypic variability and additional genetic heterogeneity, it is of crucial importance to (1) define clear phenotypes especially with regard to the broader spectrum of AD and to the differential diagnosis of other pervasive developmental disorders like Rett syndrome, and (2) to perform a detailed cytogenetic analysis in every individual with AD and additional testing for fragile X syndrome in individuals with AD and low intelligence/mental retardation in clinical and research settings. It also might be feasible in the near future, to employ kits assessing the most prevalent cytogenetic findings in individuals with AD. In addition, samples with mental retardation or other psychiatric disorders, like schizophrenia, should be compared to elicit the most specific risk factors for AD. New technologies, such as more sophisticated chip based arrays as well as large scale sequencing will lead to new results which need to be integrated into meaningful biological models.

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