Christine M. Freitag*, Denise Haslinger, Afsheen Yousaf, and Regina Waltes

Clinical genetic testing and counselling in autism spectrum disorder

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Abstract: Autism spectrum disorders (ASDs) are phenotypically as well as genetically heterogeneous developmental disorders with a strong heritability. Clinical and basic science research has described many replicated genetic risk factors. Many findings can well be translated into clinical human genetic practice. The current article summarizes results of genetic studies in ASD, provides a diagnostic algorithm for the clinical human genetic work-up reflecting the German health care system options and gives information with regard to the obligatory genetic counselling after a clinical genetic assessment.

Keywords: monogenic disorders, copy number variants, single nucleotide variants, heritability, human genetic diagnostic

Introduction

Autism spectrum disorders (ASDs) are behaviourally defined developmental disorders. They show a highly complex and heterogeneous underlying genetic architecture. ASD symptoms are increased in many disorders previously defined as syndromic in the clinical genetic literature. In addition, ASDs show a broad range of comorbid disorders which may indicate specific underlying genetic findings. The current article recapitulates the diagnostic criteria of ASD and summarizes the main genetic findings relevant for genetic testing and counselling after the behavioural diagnosis. Currently, no genetic test to predict ASD exists.

***Corresponding author: Christine M. Freitag,** Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Autism Research and Intervention Center of Excellence, University Hospital Frankfurt, Goethe Universität, Deutschordenstr. 50, 60528 Frankfurt am Main, Germany, e-mail: C.Freitag@em.uni-frankfurt.de
Denise Haslinger, Afsheen Yousaf, Regina Waltes, Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Autism Research and Intervention Center of Excellence, University Hospital Frankfurt, Goethe Universität, Deutschordenstr. 50, 60528 Frankfurt am Main, Germany

Diagnostic criteria of autism spectrum disorders

ASD is a chronic condition defined by behavioural symptoms in two areas: first, social communication (SC), accompanied by, second, stereotyped and repetitive behaviour and restricted as well as sensory interests, often abbreviated as restricted and repetitive behaviour (RRB) [1]. Symptoms typically occur during early development, most often at the preschool age, and show a changing pattern over development, but in most cases persist into adulthood [2]. SC symptoms include language delay, limited pragmatic language abilities, aberrant non-verbal communication and persistent problems in social interaction with peers. The severity of ASD can range from being profoundly affected to very few symptoms, a phenomenon which has been named phenotypic heterogeneity. In addition to the core symptoms in the two areas of SC and RRB, ASDs come along with a broad range of psychiatric and medical comorbidities, such as attention-deficit/hyperactivity disorder (ADHD), anxiety disorders, oppositional, irritable and aggressive behaviour [3], epilepsy, motor problems and intellectual disability [4]. The diagnosis is based on direct behavioural observation as well as reports by parents, relatives or friends on the developmental history and early occurrence of symptoms. Detailed information on the recommended diagnostic procedures can be found in the German and UK clinical guidelines on ASD [5–7].

In the literature, some disorders, such as Rett syndrome and Angelman syndrome, are often subsumed under ASD. Both syndromes are characterized by clinical and behavioural features that differ from ASD, such as motor problems and specific EEG and seizure patterns, and should therefore not be misdiagnosed as ASD. In clinical ASD populations, both syndromes are not more frequently found than by chance [8]. Rett syndrome and Angelman syndrome imply different interventions than a diagnosis of ASD, focussed on epilepsy, severe motor problems and adaptive behaviour, but not on SC.

Heritability and recurrence risk of autism spectrum disorders

Meta-analyses of twin studies estimate the heritability rate for ASD between 64 % and 91 % [9], which has been supported by recent large-scale family and molecular genetics studies [10]. Even though sporadic cases of ASD occur, ASD is often familial with a sibling recurrence risk of 10 to 20 times higher than the population prevalence of around 1 % [11]. A methodologically high-quality prospective longitudinal sibling study of the Baby Sibs Consortium reported an 18.7 % increased risk of recurrence of ASD in children with at least one older affected sibling within the family (95 % confidence interval, 13.3–25.5 %) [12].

Different types of molecular genetic studies have been and are currently performed to elucidate the underlying genetic architecture. The earliest studies in the 1980s were conducted in the form of family-based linkage analyses and indicated several genome-wide significant loci across the chromosomes [13]. These were followed by single nucleotide polymorphism (SNP)-focussed genetic association studies, targeting common variants and estimating copy number variants from SNP chips [14]. More recently, whole exome sequencing (WES) and whole genome sequencing (WGS) studies have been performed, which reported many new single nucleotide variants (SNVs) possibly associated with ASD. For the clinical geneticist, neither the results of SNP-based genome-wide association studies (GWAS) nor recent SNV findings can currently be translated well into practice. As in other complex diseases, single SNPs only increase the risk for a disorder by a small percentage, despite their clear collective role in the genetic aetiology of especially mental disorders [15]. For specific rare SNVs, which often show a stronger genetic risk than SNPs [16], their individual role in the aetiology of a disorder is very difficult to prove, first, due to the low power of currently available sample sizes and the risk of bias induced by unknown confounding factors, such as specific additional environmental effects in some populations or families, and, second, due to the lack of functional information for many SNVs.

Still, based on the diverse genetic studies in ASD samples combined with behavioural studies in carriers of specific genetic disorders, relevant recommendations for a genetic work-up in individuals with a diagnosis of ASD can be developed. Criteria to recommend specific diagnostic methods are based on, first, the rate of genetic findings, which has been observed in previous studies in ASD, second, for specific syndromes or monogenic disorders, the increased prevalence rate of ASD in these disorders and, third, the relevance of the respective genetic findings for additional specific intervention or for genetic counselling.

Chromosomal disorders and copy number variation

Chromosomal aberrations, including sex chromosome aneuploidies, are detected by classical karyotyping techniques in 2–5 % of ASD patients [17, 18]. Recent studies have shown that males with Klinefelter syndrome (47,XXY) and Y chromosome aneuploidy (47,XYY; 47,XXYY) are characterized by increased levels of autistic features [19]. In Turner syndrome (45,X), similarly, increased rates of ASD have been observed [20].

The availability of microarray platforms enables a high-resolution and high-throughput genome scan allowing the detection of chromosomal microdeletions and duplications. To date, not only cytogenetically visible rearrangements and regions of copy number variation (CNV) with a size of >1 kb, but also smaller regions (indels) with a length of around ~40 bp, depending on arrays and platforms, are detectable [21]. With this technique even clinically relevant CNVs invisible in karyotype analysis are detectable in ASD patients [22]. Chromosomal aberrations and pathological CNV frequently occur *de novo* in ASD patients, i. e. their parents do not carry these genetic changes. Unbalanced chromosomal abnormalities are found predominantly in ASD cases with dysmorphic features, epilepsy, intellectual disability, micro/macrocephaly and other physical symptoms [23]. In particular, large *de novo* CNVs found in patients with ASD overlap with CNVs described in patients with mental retardation [14]. In patients with sporadic ASD coming from simplex families, i. e. a child without any first or second degree relative with ASD or any other developmental or mental disorder, rare *de novo* CNVs are found more frequently than in individuals from multiplex families [24]. In total, about 10–20 % of all patients with ASD carry cytogenetic alterations (e. g. chromosomal aberration, large or small (micro)deletion and -duplication, translocation or inversion) [16].

The actual pathogenicity of these genetic variants can be difficult to prove methodically since there are reports of 'double hits', i. e. the presence of several potentially pathological relevant alterations in one person [25]. Moreover, numerous CNVs, especially smaller ones, are also found in the healthy population. Nevertheless, it is generally assumed during genetic work-up that a cytogenetic finding

frequently observed in ASD is likely the cause of the carrier's disease, especially when parents are non-carriers.

The following cytogenetic findings are often found in ASD, and often come along with specific additional behavioural and somatic features, which may guide the diagnostic work-up [26]: 1q21.1-deletion, 3q29-deletion, 7q11.23-duplication (Williams–Beuren syndrome) [27], diverse 15q11.1–13.3-duplications and inversions, including imprinting disorders such as Prader–Willi syndrome [28], 16p11.2-deletion or duplication, 22q11.2-deletion and 22q13.3-deletion. The most common recurrent ASD-associated CNVs comprise a ~600 kb microdeletion or duplication at the chr16p11.2 region, identified in about 0.8 % of individuals with ASD [16]. A common phenotypic feature in patients carrying a 16p11.2-deletion is macrocephaly, whereas patients with the duplication usually show microcephaly. These and additional CNVs with their characteristic additional neuropsychiatric morbidities are shown in Table 1.

Table 1: Clinically relevant ASD-associated copy number variations and additional associated neuropsychiatric disorders [13, 29].

Chromosomal region	ASD candidate gene	Additional associated disorders
1q21.1	n. k.	ID/SCZ/BD/E
2p16.3	*NRXN1*	ID/SCZ
3q29	*DLG1/PAK2*	ID/SCZ
7q11.23	*LIMK*	ID
15q11.2	*UBE3A*	ID/SE
15q13.3	*CHRNA7*	ID/SCZ
16p11.2	*KCD13*	ID/SCZ/BD
22q11.2	n. k.	ID/SCZ
22q13.3	*SHANK3*	ID/SE/BD
Xp22.1	*PTCHD1*	ID/SE
Xp22.3	*NLGN3*	ID
Xq13.1	*NLGN4X*	ID

n. k., not known; *ID*, intellectual disability; *SCZ*, schizophrenia; *BD*, bipolar disorder; *E*, epilepsy; *SE*, seizures.

Monogenic disorders associated with ASD

Rare monogenic disorders were among the first genetic findings described as risk factors for ASD. None of these single genes account for more than 1–3 % of ASD cases, but together they are estimated to be found in up to 10 % of all ASD cases [17]. Similar to the above-mentioned cytogenetic findings, carriers of the below-mentioned monogenic disorders as a rule show additional behavioural and somatic features, which come along with the respective disorder.

The most common monogenic disorder is fragile X syndrome, occurring in ~3 % of all ASD patients [30, 31]. Other well-described single-gene disorders associated with ASD, e. g. tuberous sclerosis [32], neurofibromatosis 1 [33] and Timothy syndrome, are less frequently observed in ASD. Pathogenic *PTEN* mutations are causal for *PTEN* hamartoma tumour syndrome, which among others includes Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome and has also been found in ASD individuals [34, 35].

ASD also rarely occurs in some metabolic diseases such as phenylketonuria (*PAH* gene), Smith–Lemli–Opitz syndrome (*DHCR7* gene) [36] and adenylosuccinate lyase deficiency [37]. Impaired mitochondrial energy metabolism caused by mutations in the mitochondrial DNA has also been reported in ASD individuals [38]. There are many other single-gene disorders which may lead to increased symptoms of ASD. Most of these disorders are associated with severe mental retardation/intellectual disability and/or significant dysmorphology.

Single nucleotide variations

Current large-scale WES and WGS studies aim at describing additional mutations which may be relevant for ASD. Most studies reported a higher rate of predicted functional de novo and inherited SNVs in ASD cases compared to their unaffected siblings. In contrast, the rate of synonymous mutations was similar between affected and unaffected children. In addition, an excess of recessive mutations has been described [39–41]. Rare *de novo* mutations have been more frequently observed in offspring from older fathers. One study reported that ~75 % of *de novo* mutations originate from the father, with a 1.3-fold increase in the number of *de novo* events for every 10 years of paternal age [42]. To date, many ASD candidate genes have been identified from WES and WGS studies, but most of these studies need to be replicated and the genes have not yet been functionally described. While in most of the cases, it is likely that the combination of several SNVs or SNVs and CNVs is needed to cause ASD, some rare SNVs may also be causal and can thus be considered as monogenic forms of ASD, e. g. pathogenic point mutations of *SHANK3*, neurexins or neuroligins [29].

Common variation in ASD – the role of genome-wide association studies

GWAS are mainly able to identify common variation in the form of SNPs. Although common variants have been estimated to explain the main part of ASD liability [43], due to their low individual risk effects, replication of GWAS findings has been rare. A meta-analysis of GWAS of over 16,000 ASD individuals reported one genome-wide significant signal at 10q24.32 [44]. Despite a clear role of common genetic variation in explaining heritability and phenotypic variability in ASD, they currently do not play a major role in the clinical genetic assessment of ASD.

Clinical genetic work-up in autism spectrum disorder and genetic counselling

Due to the predominantly genetic aetiology of ASD and the implications of proven genetic findings, human genetic diagnostic work-up and subsequent counselling should be offered to all parents of a child diagnosed with ASD to enable the early diagnosis of possible comorbid diseases with the aim of early treatment and intervention (such as sex hormones in Klinefelter's and growth hormones in Prader–Willi syndrome) as well as for genetic counselling [45]. In contrast, diagnostic genetic tests to predict the risk for ASD either prenatally or on the basis of early diagnostic biomarkers are not available to date. Due to the extreme genetic heterogeneity of ASD, it is unlikely that valid predictive genetic tests will be developed in the near future.

Regarding the diagnostic work-up after a behavioural diagnosis of ASD according to DSM-V, ICD-10 or – in the near future – ICD-11, the human genetic assessment contains the following steps: (1) comprehensive assessment of medical and family history; (2) physical examination including the description of dysmorphology and growth parameters; (3) if indicated, additional somatic diagnoses of possible comorbid disorders, such as epilepsy and metabolic or mitochondrial disorders; and (4) hierarchical genetic work-up (Figure 1).

The genetic laboratory assessment always needs to include a high-quality chromosomal analysis and a microarray covering at least the relevant CNVs [46]. Testing for fragile X syndrome should be done in the presence of the respective clinical features as well as in male patients with an IQ below 90. Testing for fragile X carrier status may also be done in female patients if the pedigree indicates an X-chromosomal disorder. If the physical examination or the

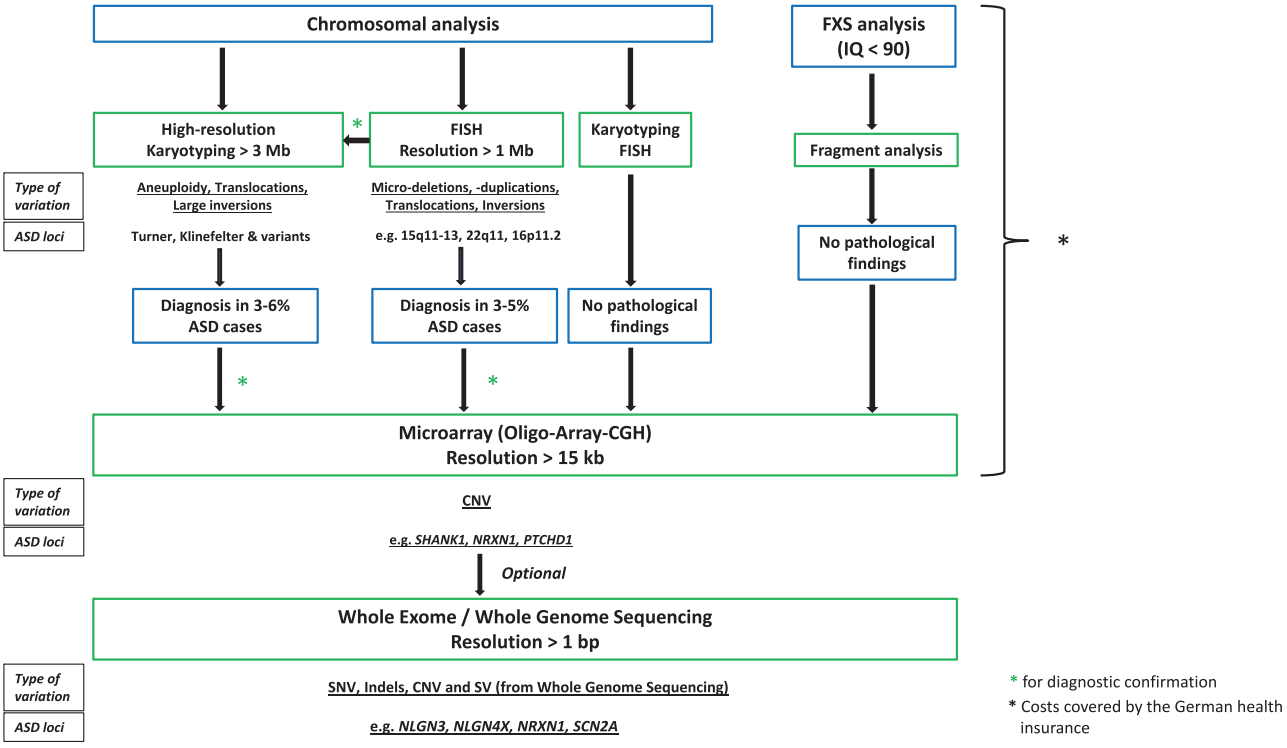

Figure 1: Genetic testing of individuals diagnosed with ASD.

comorbidity pattern is indicative of a specific genetic disorder, this disorder should be directly tested, most often by targeted sequencing of the involved genes. In the case of a positive result, the parents should also be tested to assess whether a particular finding has been passed on from parents to the child or whether it is a *de novo* event.

Regarding the interpretation of genetic findings in ASD, the following databases may be useful.

The database AutismKB (db.cbi.pku.edu.cn/autismkb_v2/; last update 08/26/2018) allows to distinguish between syndromic (currently 99 genes) and non-syndromic (currently 1280 genes) ASD risk genes. Furthermore, it contains lists including the respective references for (1) CNV regions, (2) linkage regions and (3) SNPs and variable number tandem repeats previously described in ASD.

Another well-established database for ASD risk genes is SFARI GENE (https://gene-archive.sfari.org/; last update 06/20/2019). With a gene scoring into category S for syndromic genes (89 genes), followed by categories 1–6 with decreasing evidence (category 1 [25 genes], 2 [66 genes], 3 [202 genes], 4 [463 genes], 5 [177 genes] and 6 [25 genes]), SFARI GENE has the advantage of a first classification and allows a fast assessment of each gene's evidence and function.

Based on the results of the clinical and laboratory human genetic work-up, genetic counselling needs to be offered to all parents and patients, if possible. If no likely underlying genetic disorder has been found in an individual with ASD, the recurrence risk is approximately 20 % [12]. If a *de novo* event has been described, the recurrence risk equals the prevalence of ASD in the population (around 1 %), multiplied by the increased risk coming along with advanced paternal and maternal age [47]. If in the case of fragile X syndrome a recessive or dominant mutation is found, the genetic counselling follows in a standard way regarding autosomal or sex chromosome-specific inheritance patterns.

In conclusion, genetic research in ASD has strongly advanced the field, and the results of basic science regarding chromosomal disorders, CNV and specific monogenic disorders can well be translated into the clinic. Results of WES and WGS studies need to be replicated and the functional relevance of SNVs additionally needs to be clarified before these results can be fully translated into a standard genetic work-up offered to affected individuals and their families.

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Professor Christine M. Freitag
Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Autism Research and Intervention Center of Excellence, University Hospital Frankfurt, Goethe Universität, Deutschordenstr. 50, 60528 Frankfurt am Main, Germany
C.Freitag@em.uni-frankfurt.de

Denise Haslinger
Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Autism Research and Intervention Center of Excellence, University Hospital Frankfurt, Goethe Universität, Deutschordenstr. 50, 60528 Frankfurt am Main, Germany

Afsheen Yousaf
Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Autism Research and Intervention Center of Excellence, University Hospital Frankfurt, Goethe Universität, Deutschordenstr. 50, 60528 Frankfurt am Main, Germany

Regina Waltes
Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Autism Research and Intervention Center of Excellence, University Hospital Frankfurt, Goethe Universität, Deutschordenstr. 50, 60528 Frankfurt am Main, Germany