

Molecular aspects of autism spectrum disorders

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Summary

Autism, also known as autism spectrum disorders (ASD), is etiologically and clinically heterogeneous group of neurodevelopmental disabilities. ASD affects 1% of child's population. The sex difference is observed with 4:1 male to female ratio. This is descriptive diagnosis based on observation and analysis of behavior and cognitive functions. ASD does not fit the criteria of known patterns of inheritance. For the majority of patients polygenic model of inheritance with many interacting genes is the most probable. The etiology of ASD is poorly understood. It is estimated that a specific genetic etiology can be determined in up to 20% of individuals with ASD. Advances in microarray technology and next generation sequencing are revealing copy variant numbers (CNV) and single nucleotides polymorphisms (SNP) with important roles in synapse formation and function. For families where a specific etiology has been identified, the risk of recurrence in siblings generally depends on the etiologic diagnosis. For autism of unknown cause, the sibling risk varies across studies but is generally considered to range from 5 to 10 %.

Key words: autism spectrum disorders, genetic counseling

Introduction

Autism is an etiologically and clinically heterogeneous group of disorders collectively referred to as autism spectrum disorders (ASD). The first description of autism was made by Leo Kanner [1] in 1943 as „inability to interact with other people and situations” or „being alone and an “obsessive insistence on the preservation of sameness”. and Asperger [2] in Austria. Autism for many years remained mostly unnoticed outside of psychiatry. ASD is the example of very mysterious disease, because it involves core abnormalities in social cognition and language, both of which are central to what

makes as human. Understanding of autism will have impact on our knowledge about cognitive processes and development of therapeutic technics [3].

In late 80-ties monozygotic and dizygotic twins studies indicated that autism is a disorder with high heritability of around 0,85-0,92. Studies of families affected with autism showed increased risk of recurrence in siblings of proband (8,6%) [4].

Knowledge and understanding of genetic bases of autism is a challenge. ASD as a disorder does not full-fill criteria for known patterns of inheritance. In majority of cases it is not possible to establish the etiology of disorder even with the use of the newest methods of molecular biology. ASD and other psychiatric disorders belong to the polygenic disorders, where many genes are inherited in codominant fashion and function together in an additive or an synergistic manner [5].

Our knowledge about genetic mechanisms is still too incomplete to use genetic tests in making a diagnosis of ASD. It is clinical diagnosis based on observation and evaluation of behavior and cognitive functions. Prognosis is variable, the disorder has lifelong effects on the ability to socialize, to care for him/herself, and to participate meaningfully in the community. Diagnosis of ASD may impact the other family members [6,7].

After the clinical diagnosis genetic tests may be helpful in better understanding unique etiology in specific patient. "Genetic" diagnosis may be useful in contact with other families affected with the same disease, reduce the feeling of the guilty, more precise estimation of the recurrence risk in next children, possibility of more specific treatment disorders [5].

Diagnosis

Triad of the symptoms described by Wing i Gould [8] has found place in classifications DSM-IV-TR and ICD-10:

1. impairments in social interactions,
2. deficits and dysfunctions in communication (verbal and not verbal)
3. restricted repetitive and stereotyped patterns of behavior, activities and interests.

According to DSM-IV classification diagnosis of autism involves broad spectrum called autism spectrum disorders or pervasive developmental disorders ASD encompass clinically defined conditions: childhood autism, atypical autism, Asperger syndrome, disintegrative disabilities and pervasive developmental disorder-not otherwise specified. Autism represents a quantitative spectrum of impairments rather than discrete

disorders. Patients represent a clinically variable population that suffers from pathologic levels of quantitative variation in the major cognitive and behavioral domains that are disrupted. How the clinical domains relate to underlying dysfunction in specific cognitive domains is unknown [3,5,6].

Thinking about autism as „disease” is useful in the context of phenotypes, which cause that patient looks for the help of doctor. The second aspect of phenotype is severity of the disability. Genes implicated thus far in ASD seem to converge in common pathways affecting neuronal and synaptic homeostasis. Phenotype is the result of interaction between common and rare genetic variants, epigenetic factors, modifying genes and environmental factors [9].

Specific neurologic and medical symptoms occur commonly in patients with autism spectrum disorders. They involve: motor problems, anxiety, seizures, abnormal sleep patterns and psychiatric problems hyperactivity, short attention span, compulsive-obsessive disease, emotional liability, bipolar disease, schizophrenia [4].

Lack of effective preventing methods, biomarkers helpful in early diagnosis and specific treatment methods or medicine “treating” ASD. Essential is early diagnosis and correct adequate treatment and support [5,6].

Prevalence

The prevalence of ASD has been reported to be considerably higher the last years compared to numbers reported 15-20 years ago. It has raised enormous concern from parents, physicians and scientists fearing that some environmental toxins have emerged to cause an autism epidemic. Epidemiologic analyses showed that increase in number of children diagnosed with ASD was effect of increased awareness of ASD by parents and professionals and the expansion of diagnostic boundaries. The incidence of ASD appears to be as high as 1:100 children and is much more common in boys than in girls, with boys to girls ratio 4:1 [10].

A three-generation pedigree analysis is very important. ASD may occur in a sporadic patient in the otherwise healthy family, or in several children of one generation. Zhao et al. [11] hypothesized that two types of families may exist: low-risk families with sporadic autism patients being mainly caused by *de novo* mutation, with high penetrance in males and relatively poor penetrance in females, and high risk families in which ASD probands received a dominant mutation most often from females, who carry this mutation but are themselves unaffected.

The etiology of ASD is little known. The genetic cause of ASD is recognized in only 20-25% of cases. Autism is mainly caused by combinations of many common variants, copy number variants (CNV) and single nucleotide polymorphisms (SNP) with very small effect sizes [4].

The biggest advance in understanding autism pathophysiology has been the appreciation of a significant genetic contribution to the etiology of ASD. The twin studies described ASD as the most genetic of neuropsychiatric disorders, with concordance rates of 82-92 % in monozygotic twins compared with 10-20 % in dizygotic twins. In all studies, the concordance rate in monozygotic twins is not complete, indicating that epigenetic, stochastic, and/or environmental factors are present [4]. A polygenic model in which multiple genes interact either in an additive or a synergistic way appears the most plausible for the majority of ASD patients [21].

Specific forms of ASD

Autistic spectrum disorders can be divided into two groups: specific and idiopathic. Single-gene disorders associated with ASD account for ~5% of cases [12].

The most common monogenic disease, where one of the core symptoms is ASD is Fragile X syndrome (FXS), the most common familial form of mental retardation. Dynamic mutation in FXS is caused by amplification of number of repeats of triplet CGG in promoter region of *FMRI* gene causing the lack of transcription of the gene and in consequence lack of protein product of the gene FMRP. FMRP is a transporter protein binding RNA. At the level of synapse FMRP is the regulator of translation of many proteins. Lack of FMRP causes the constellation of symptoms from cognitive dysfunction to autistic behaviors. FXS affects both sexes, but clinical symptoms vary and are more severe in males. The leading symptom in males after the puberty is mental retardation, usually moderate or profound degree accompanied with behavioral problems: short attention span, emotional liability, autistic features, stereotypical. The presence of autistic features leads to the primary diagnosis of ASD, especially in older boys. Nowadays Fragile X syndrome is thought to be the most common monogenic cause of autism. 2 % of patients primary diagnosed as ASD finally have a diagnosis FXS. Recent advances in understanding neurobiology and function of FMRP are based on data from mouse model of the disease. FXS is the disease of synaptic plasticity. Known genes associated with ASD interacts with signaling pathway, which is disturbed in patients affected with FXS. If FMRP is decreased or

absent there is significant dysregulation of various pathways/proteins that that disrupt brain development pervasively leading to developmental delays, particularly autism. In future target treatment FXS may be effective in treatment of patients affected with ASD [13].

Other monogenic disorders with accompanying autism: tuberous sclerosis (*TSC1* i *TSC2*), neurofibromatosis typ I (*NFI*), Angelman syndrome (*UBE3A*), Smith-Lemli-Opitz syndrome, Rett syndrome (*MeCP2*), Joubert syndrome, *PTEN* hamartoma tumor syndrome. [4,12,14].

The risk of ASD increases also in children exposed to valproic acid and thalidomid *in utero* [4].

Genetic investigations in ASD

The latest advances in the field of autism genetics, the use of high-resolution microarrays in screening investigations of the genome caused the explosion of information about pathophysiology of ASD and allowed to detect the heterogeneous and complex genetic bases of disability and partially understanding of processes leading to different phenotypes. Identification of mutations in candidate genes such as: neuroligins, neurorexis and SHANK, encoding proteins crucial to synapse formation, maturation and stabilization showed synapsis as main player in susceptibility to ASD [14].

Genome-wide CNV and association studies allowed the identification of submicroscopic deletions and duplications called copy variant numbers (CNV), in many *loci*, observed *de novo* in 5-15% patients with ASD. Sequencing the exons allowed for detection of pathogenic *de novo* mutations in u 3,6 – 8,8 % cases with ASD.

Autism is etiologically heterogeneous group of disabilities and involves various types of copy variant numbers (CNV) and single nucleotides polymorphisms (SNP) located in almost all and characterized by incomplete penetrance. Each represent no more than 1-2% of cases individually [15].

Copy-Number Variants (CNV)

The use of whole-genome microarrays allowed for the detection of submicroscopic deletions and duplications in various genomic *loci* called copy-number variants (CNV – *copy numer variants*) in 10-20 % patients with idiopathic ASD [12]. CNV associated

with autism are *de novo* mutations in germline cells. The most common chromosomal aberration detected in patients with ASD was 15q11-13 duplication found in 1-3% of them. This duplication showed the parental origin effect, inherited from father who had a normal phenotype [16].

The other described CNVs were located in the regions of chromosomes deletions 1q21.1, 2p16.3, (involving *NRXN1* gene), 2q37, 7q22q13.3, 16p11.2 (600 bp) 22q21q23 i Xp22; duplications 7q11.23, 16p13.1, 17p11.2. [16-18].

In some cases such as: CNV 16p11 and 15q11-13 were transmitted by healthy parent, but caused problems in offspring. Genetic or epigenetic mechanism causing diminished penetrance of CNV in parent, carrier of mutation remains unknown [16-18].

To date, more than 3800 individuals with ASD, 1200 unaffected siblings, and 600 controls along with the genotypes of parents have been analyzed. In all studies, *de novo* copy-number variants appeared to be enriched in individuals with ASD compared with their unaffected siblings or the controls. Overall 6,6 % of individuals with sporadic ASD had at least one rare *de novo* copy-number variant, compared with 4,1 % in individuals with ASD who also had an affected first-degree relative with ASD, 1,4 % in unaffected siblings. And 1,9 % in controls. The *de novo* copy-number variants were larger and affected more genes in individuals with ASD compared with unaffected siblings and controls [15].

Mechanisms of action of rare CNV's in the pathogenesis of ASD could be:

1. through altering the necessary copy number of positional context of the key DNA sequence required for regulating the proper expression of the nearby genes.
2. affecting still undiscovered genes or non-coding RNA's residing in the CNV regions.
3. disrupting uncharacterized isoforms of the adjacent annotated genes [19].

Mutations in genes

Next generation exome sequencing have enabled the identification of many genes associated with ASD. Rare and *de novo* mutations in coding sequences were indentified in 5-10% cases with ASD. Rare alleles and single nucleotide polymorphisms (SNP) contribute to ASD predisposition with rather small effect. Mutations in genes coding proteins involved in chromatin remodelling, metabolism, mRNA translation and synapses functions seem to interact in signaling pathways important for homeostasis of neurons and synapses [20].

There are proves indicating important role of the genes coding synaptic proteins In pathophysiology of ASD. This hypothesis was supported by identification of mutations (CNV and point mutations) in multiple genes encoding proteins crucial for development maturation and stabilization of synapses. Noh et al [20] found that an usually high number of genes affected by DNA deletions/duplications are associated with the functioning of synaptic transmission. The proteins made by many of these genes are known to interact with each other and together with proteins from other duplicated/deleted genes form a large interlinked biological network. Among proteins involved in the function of synapses are: presynaptic neurexins (*NRXN1*, *NRXN2*, *NRXN3*) and their postsynaptic ligands (*NLGN1*, *NLGN3*, *NLGN4X*, *NLGN4Y*), and family of SHANK proteins enriched in the postsynaptic density (*SHANK1*, *SHANK2*, *SHANK3*). Postsynaptic neuroligins interacts with presynaptic α - and β -neurexins stimulating formation of presynaptic vesicles. Neuroligins also interact with postsynaptic SHANK proteins [14, 21-23]. Functional studies involving the creation of animal models showed that the changes of levels of these proteins changes the morphology, function and plasticity of synapses. Important is that many phenotypes may be reversed after the change of levels of protein [7].

The burden of *de novo* mutations affecting genes expressed in the brain is higher in individuals with ASD compared with controls. Two-thirds of *de novo* mutations were of parental origin, and the rate of these mutations increased with age. [20].

The accumulating number of distinct, individually rare genetic causes in ASD suggests that the genetic architecture of autism resembles that of ID, with many genetic and genomic disorders involved, each accounting for a small fraction of cases [24].

Autism represents the final common pathway for numerous genetic brain disorders. Many well-recognized intellectual disability (ID) genes can also cause ASD, with or without ID. Similarly, several genes initially identified in epilepsy patients can also result in ASD and ID. These findings indicate that these genes cause a continuum of neurodevelopmental disorders that manifest in different ways depending on other genetic, environmental or stochastic factors [24].

There are more than 100 recurrent genetic defects that can cause ASD. All the genes and chromosomal rearrangements were identified as well-known causes of ID. Several of them have also been involved in epilepsy [24].

Multiple hits in ASD

Each individual CNV is rare and account for fewer than 1-2 % of all ASD cases individually. In addition, most of these CNVs are not found In individuals with ASD exclusively, but also occur in unaffected controls, albeit with much lower frequency.. Any single one of them is not a fully penetrant ASD-causing variant in itself. Genetic variant is only the first hit in a two or multiple hit process that ultimately leads to disorder. The degree of mutational burden (in copy numbers or sequence) is an important determinant of the risk that a given individual carries. A multiple hit model involves relatively small number of genes in any given patient. Autism is a complex genetic disorder resulting from simultaneous genetic variations in a few, several or even multiple genes [4].

Given that *de novo* mutations account for only a small fraction of individuals with ASD (< 15%), inherited variants are expected to play a major role in genetic susceptibility to ASD. In addition inherited risk and protective alleles might contribute to the incomplete penetrance and variable expressivity observed in individuals carrying *de novo* deleterious mutations. Several studies have demonstrated the presence of more than one deleterious mutation (multiple hits) in individuals with ASD. It must involve complex and contingent functional networks and be influenced by environment al and epigenetic factors [15].

In patients affected with ASD the mutations in genes encoding a diversity of molecular mechanisms were detected: cell adhesion, synaptic vesicle release, neurotransmission, synaptic structure, RNA processing/splicing, and activity-dependent protein translation. Diversity of potential mechanisms and apparent lack of specificity of mutations for ASD begs the question as to whether ASD should be viewed as a unitary disorder. Asking whether the diverse spectrum genes implicated in ASD might converge on common pathways becomes an important question for understanding autism and developing new therapeutics [3].

Genetic counselling

There is a strong need to carefully assess the children and their families, and to exclude all known medical causes of the disorder. The aim of genetic counseling is to provide information to parents and children, and to estimate the recurrence risk of the disorder..

The American Academy of Neurology, the American Academy of Pediatrics and American College of Medical Genetics have developed guidelines for the investigation of autism spectrum disorders. Cytogenetic microarray (CMA) is recommended as a first-line test in the initial postnatal evaluation of individuals with ASD [25,26]

There are several reasons for testing, including [25]:

1. Providing medical therapy for children with treatable
2. Providing information or recurrence risks for subsequent pregnancies or for other family members
3. Defining a specific etiology, even if therapy is not available, but helping to understand pathophysiology.

When *de novo* pathogenic mutation or CNV is found in the proband the recurrence risk is typically quoted as 1%, taking into account the rare cases of gonadal mosaicism in one of the parents, or the possibility that one parent carries a chromosomal rearrangement that predisposes to the CNV [26].

One of the most difficult scenarios for genetic counseling is the situation, when proband is carrier of CNV, known to demonstrate incomplete penetrance and/or variable expressivity. Until more expansive genotype-phenotype studies are conducted, empirically derived risk figures 15-20% should be considered the minimum risk regardless of risk variants found on microarray [26].

If no apparent genetic cause is identified in the proband – as is the case presently for the majority with ASD –, the recurrence risk is extrapolated from empirical studies. Retrospective family studies published prior to the late 1990s estimated the risk for a couple with one autistic child to be 3-10 %. However, older studies are limited by small sample sizes. Because of broadening of the diagnostic criteria over time, they may have missed siblings with milder form. [12,27]. A recent large, longitudinal prospective studies of high-risk infants has shown that the recurrence risk is higher. 18,7% for the siblings of proband diagnosed with ASD. The risk did not change with the severity of ASD symptoms or with the sex of the proband. The sex of next child does impact on risk, as males were consistently at higher risk for ASD. When two siblings in one family were affected with ASD, the risk for younger sibs increased to 32,2 %, which is consistent with older estimates. (25-35%). The incidence of ASD among the offspring of individuals themselves on the spectrum is unknown but probably [28].

Population studies of 1500 000 children born in Denmark in years 1980-2004 showed that recurrence risk of ASD in siblings was 4,5-10,5%. [29].

The knowledge about genetics of autism arises the next challenge understanding the way in which ASD is a broad spectrum of phenotypes. Phenotypic heterogeneity is next serious problem in treatment of ASD. Although the magic „anti-ASD pill” may not become available soon, comprehensive genotyping of individual patients combined with the novel insights generated from the transgenic animal studies may provide us with personalized treatment options. Autism may be a phenotypically heterogeneous group of disorders caused by combination of changes in multiple possible candidate genes, being different in each patients and requiring for each individual individually-tailored treatment [20].

Future directions

There is still much to be learned about genetic causes of ASD and other neurodevelopmental disorders. Genetic testing for ASD is limited to ruling out known genetic conditions and screening the genome for rare CNVs that confer risk of unknown magnitude. Studies with the use of next-generation sequencing will allow to identify new candidate genes for ASD, *de novo* mutations in protein-coding genes that are predicted to be damaging in ASD probands, and absent in control subjects.

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