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Diagnostics of the genetic causes of autism spectrum disorders – a clinical geneticist's view

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Summary

Explanation of the genetic basis of autism spectrum disorders has, for many decades, been a part of interest of researchers and clinicians. In recent years, thanks to modern molecular and cytogenetic techniques, a significant progress has been achieved in the diagnosis of genetic causes of autism. This applies particularly, but not exclusively, to those cases of autism that are accompanied by other clinical signs (i. e. complex phenotypes). The important clinical markers belong to different categories, and include congenital defects/anomalies, dysmorphism and macro-/microcephaly, to name the few. Thus, the choice of the diagnostic strategy depends on the clinical and pedigree information and, under Polish circumstances, the availability of specific diagnostic techniques and the amount of reimbursement under the National Health Service. Overall, the identification of the genetic causes of autism spectrum disorders is possible in about 10-30% of patients. In this paper the practical aspects of the use of different diagnostic techniques are briefly described. Some clinical examples and current recommendations for the diagnosis of patients with autism spectrum disorders are also presented. The point of view of a specialist in clinical genetics, increasingly involved, as part of the multidisciplinary care team, in the diagnostics of an autistic child has been demonstrated.

Key words: autism spectrum disorders, genetics, diagnostics

Introduction

Autism spectrum disorders comprise a broad group of developmental disorders, the diagnosis of which, according to the new DSM-V (Diagnostic and Statistical Manual of Mental Disorders-V), includes the occurrence of two core symptoms in early childhood: disorders of communication/social interaction, and stereotyped, repetitive behaviors [1]. In addition, the diagnosis is completed with an accurate assessment of intellectual functioning and language of an examined child. In contrast to the DSM-IV, the authors of DSM-V decided to merge the previously distinguished categories (including overall developmental disorders otherwise unspecified) in a large group of autism spectrum disorders (ASDs).

The population incidence of autism spectrum disorders is subject of discussion [2]. According to the DSM-IV criteria, based on studies published in this century, the incidence of autism throughout the world is 21.6/10,000 [3]. On the other hand, if we consider the much broader concept of autism spectrum disorders according to DSM-V, the rate is several times higher with 62/10,000 [3, 4]. In Poland, the lack of statistical data on the prevalence of autism spectrum disorders is apparent. SYNAPSIS Foundation study suggests that in the Mazowieckie voivodship in 2008, autism was diagnosed in about one thousand patients [5].

Autism spectrum disorders (ASDs) belong to neuropsychiatric conditions with the significant impact of genetic factors in their pathogenesis. The above is reflected by, among others, circa twentyfold increase in the risk of the disorder happening in first degree relatives (siblings, children) of the affecteds, as well as high concordance rate among monozygotic twins (probability of the disease happening in both sibs) reaching 80% [6]. So far over 100 genes have been identified, in which genetic defects (mutations) are responsible for the disease, with additional 40 chromosomal regions (i.e. *loci*), where genes causally related to autism and similar conditions of intellectual disability, epilepsy or schizophrenia reside [7]. The results of multicenter studies conducted over recent decade on large groups of patients have effectively excluded a possibility of a single mutation to be held responsible for expression of the disease in most individuals with ASDs [8]. Although the latter undoubtedly complicates the diagnostic process in ASDs, patients with a reachable diagnosis following application of modern genetic diagnostic techniques constitute a significant proportion within the whole ASDs population. In this work, practical aspects of application of various diagnostic techniques have been briefly described, some clinical examples presented together with current recommendations concerning genetic diagnostics of ASDs patients.

Genetic basis of autism

Currently, with regard to neuropsychiatric conditions, including autism spectrum disorders, there are two mutually non-exclusive working etiological hypotheses.

According to the idea of common disease-common variant (CD-CV), common DNA changes of relatively small individual impact (i.e. polymorphisms) cause clinical symptoms of the condition. In practice, this is seen as a variable (milder or more severe) disease expression in the relatives of the affected, where the closer the relation, the higher the risk of symptoms developing.

In turn, the hypothesis of common disease-rare variant (CD-RV) suggests that the presence of a single rare defect (mutation) of significant impact prejudges the symptoms [9]. Mutations are further defined here as either single gene or chromosomal defects (aberrations). Both determinants of genetic variability most frequently happen *de novo*, i.e. for the first time in the affected person. Important arguments in favour of CD-RV hypothesis include: early onset of disease, frequent co-occurrence of intellectual disability and large differences in concordance rates among mono- and dizygotic twins.

Table 1. **Simplified diagnostic algorithm in ASDs taking under consideration pathogenesis of these developmental disorders.**

Likely etiologic factor	Diagnostic approach
Genetic	Specialist medical examination (including pedigree analysis, assessment of dysmorphic features and birth defects/anomalies) + genetic testing (DNA and chromosomal) +/- metabolic testing
Familial	Analysis of parental age, birth order and the presence of other relatives, including siblings, with ASDs
Prenatal	Analysis of infections, bleeding, metabolic factors, exposure to drugs and tobacco, maternal diseases (diabetes, hypothyroidism, anemia, hypertension)
Perinatal	Analysis of perinatal factors (prematurity, low birth weight, breech presentation, low Apgar scores, other complications of childbirth)
Neonatal	Assessment of hypotonia/increased muscle tone, respiratory distress, impaired movement pattern, intraventricular bleeding, jaundice, developmental defects
Other	Cigarette smoking by the mother during pregnancy, proximity of highways during pregnancy

The table does not include the "gold standard" tools utilized in the diagnosis of autism spectrum disorder (clinical observation, ADOS, ADI-R tests).

Diagnostics of causes of autism spectrum disorders

Determination of the etiology of autism spectrum disorders is a complex process that takes into account both genetic and environmental factors. Table 1 shows a simpli-

fied diagnostic algorithm including the pathogenesis of this group of diseases. Omitted in the diagram are essential tools to assist in making a diagnosis of the disease (clinical observation, ADOS and ADI-R tests). Below, the current state of knowledge with regard to recognizing only the genetic causes of autism spectrum disorders is presented.

The choice of the diagnostic strategy in establishing genetic etiology of autism in a given individual should reflect scientific hypotheses presented earlier in this paper. Thus, it depends on clinical and pedigree data, effectiveness of applied methodology as well as, under Polish circumstances, on accessibility of diagnostic techniques and National Health Service (NFZ) funding.

According to the current state of knowledge, recognizing the genetic cause of autism spectrum disorder is possible in about 10-30% of affected individuals. These are mainly the defects fulfilling CD-RV criteria, i.e. single gene mutations, chromosomal aberrations and microaberrations.

Single gene mutations

Diagnosing a single gene mutation requires DNA analysis of an affected person (molecular analysis) and most frequently relies on the precise clinical diagnosis of a specific genetic condition, e.g. fragile X syndrome, Rett syndrome, tuberous sclerosis, *PTEN*, *SHANK3*, *NLGN* or *NRXN*-related conditions, neurofibromatosis type 1 or Angelman syndrome. Some of these genes (*FMR1*, *SHANK3* or *NLGN* and *NRXN* group of genes) play an important role in interneuronal communication, *NF1* gene and a group of *TSC* gene encode tumor suppressor proteins, and the *MECP2* gene is a regulator of DNA transcription [10]. Diagnostic efficiency among single-gene disorders depends, to perhaps the greatest possible extent, on the knowledge and skills of medical specialists who care for autistic children, including clinical geneticists. Particularly useful knowledge turns out to be the natural history of diseases such as Rett syndrome and Angelman syndrome or phenotype of mutations in *PTEN* and *SHANK3*. On the other hand, in the fragile X syndrome (FRAX) additional detailed pedigree analysis is necessary, which is also indicated for other diseases. What is particularly interesting, the need to exclude fragile X syndrome is probably one of the commonest reasons for referral of patients with ASDs for genetic testing. Some useful practical clinical and diagnostic information about the FRAX is shown in Table 2.

Overall, in the relative absence of additional clues from the clinical and pedigree analysis, the diagnostic efficacy for each listed monogenic entity in patients with ASDs is about 1-5 %. The cost of the analysis ranges from 300PLN (FRAX screening) to even 7000PLN in case of neurofibromatosis type 1, however it is usually covered by NFZ. Another group of single-gene disorders are metabolic diseases, including mitochondrial disorders, where molecular analysis should be preceded by metabolic tests usually performed in specialized clinical centers.

Table 2. **Practical remarks about fragile X syndrome (FRAX) (based on [6, 7] and www.cdc.gov/ncbddd/autism)**

Disease prevalence = about 1-3% of ASDs patients
About 60% of FRAX patients fulfill ASDs criteria according to DSM-IV
Recommendations of Center for Disease Control regarding indications for FRAX testing (dynamic mutation of *FMR1* gene): family history suggesting FRAX and/or intellectual disability in male relatives, accompanying intellectual disability or whenever intellectual disability in ASDs patients cannot be excluded
Family history of intellectual disability, large head circumference (macrocephaly) and characteristic dysmorphism are the most significant diagnostic markers of FRAX

Chromosomal aberrations and microaberrations

Searching for chromosomal aberration (cytogenetic analysis/karyotype) necessitates microscopic analysis of fixed patient's chromosomes searching for loss (deletion) or doubling (duplication) of large chromosomal segments, including key ASDs fragments, e.g. 15q11-13 or 16p11.2. Karyotype in an unselected group of patients with ASDs leads to the identification of the disease in approximately 1-5% of patients. This proportion is likely to be significantly greater when the autistic traits coexist with malformations/body anomalies, growth retardation, mental retardation and dysmorphic features (symptoms suggestive of chromosomal aberrations). The cost of about 500PLN is reimbursed by the state.

Chromosomal aberrations of considerably (up to 1000 times) smaller segments, so called microdeletions or microduplications, cannot be envisaged using standard cytogenetic methods. Therefore, they require the use of special techniques, including e.g. multiplex ligation-dependent probe amplification (MLPA) and array comparative genomic hybridisation (aCGH), where patient's DNA is applied on a special glass plate with probes against various chromosomal regions and compared with control DNA. Whereas MLPA method allows investigation of only some DNA fragments (so called MLPA autism kits), the sensitivity of aCGH is unlimited and depends merely on the number of used probes. In practice, arrays of mean resolution of about 100kbp (kbp=a thousand base pairs) are used. Array CGH reveals the diagnosis in at least 6% of patients with ASDs (unpublished data from Baylor College of Medicine, Houston, Texas for September, 2009), wherein, as in routine cytogenetic diagnosis, the likelihood is significantly increased in cases of clinical suspicion of chromosomal aberrations, up to 60%. Research conducted at the Institute of Mother and Child allowed the identification of chromosomal microaberrations responsible for the clinical symptoms in 12 of 145 patients (8%). The presence of abnormalities did not correlate with the severity of the disease [13]. Other data suggest the diagnostic efficacy of aCGH in ASDs of up

to 30% [14]. The application of aCGH usually associated with the cost of roughly about 1600-2200PLN, which is not covered by NFZ. Thus, in many clinical centers the only alternative left is relatively cheap and technically simple MLPA method. Apart from already mentioned techniques, diagnostics of autism (both of frequent and rare variants) in the future will likely rely on so called exome sequencing (analysis of all coding regions of the known genes, at present not available in Poland in ASDs patients diagnostics [15].

Autistic child in clinical practice

Clinical diagnostics process of an autistic child may be complicated by the fact of various phenotypes overlapping in a single patient, including autistic features, intellectual disability or epilepsy [16]. On the other hand, it allows for selection of those affected, in whom the chance of establishing genetic cause of the disorder using available diagnostic armamentarium is the highest.

Clinical features important from the genetic point of view

Phenotypic variables of diagnostic importance (markers) increasing the likelihood of arriving at diagnosis can be divided into disordered physical development, neurological problems, aspects of natural history of the disease and some data from family history [17]. The most important symptoms belonging to the first category are dysmorphism and micro-/macrocephaly, as well as congenital defects, including CNS anomalies. The combination of dysmorphic features found in children with ASDs is specific to a particular genetic syndrome (e.g. long, triangular face, and long earlobes in fragile X syndrome). Although any one dysmorphic feature does not stand out as characteristic of the entire population of ASDs, the examiner should focus on the face region and distal limb segments.

Seizures are present in about 25% of autistic population, whereas abnormal EEG concerns 50% of the affected. No less important is the disease onset (the earlier, the higher the likelihood of presence of a rare *de novo* mutation) and accompanying developmental regression. Family history information should include the presence of ASDs phenotype as well as other neuropsychiatric conditions (especially intellectual disability, epilepsy and schizophrenia but also bipolar affective disorder or alcoholism).

Autism and Autism+

Knowledge and appropriate interpretation of the above mentioned diagnostic markers allow for a preliminary assignment of the patient to a category of autism without other accompanying features (*essential autism* or autism) and autism with other clinical symptoms (*autism complex* or autism+). An especially high chance of identifica-

tion of the genetic defect, up to 25%, concerns autism+ patients. These are still more likely (but the proportion is significantly less than in autism) boys. In autism+ group there is a lower average IQ and frequency of seizures, abnormal EEG, dysmorphia, macro-/microcephaly and central nervous system defects are all increased, whereas the prognosis for the disease course is worse. The risk of the disease recurring in siblings of individuals diagnosed with autism is increased in relation to population risk and amounts to 3-10%, with the average risk being lower when the patient is female (4%) compared to males (7%) [18]. In the autism+ group it is suggested that the disease is usually the result of a *de novo* mutation (insignificant family history), but the final risk depends on the specific diagnosis [19]. Comparative characteristics of other clinical features of autism and autism+ is shown in Table 3.

Table 3. **Comparison of alternative types of autism (modified after [13])**

	Autism complex, more severe, with genetic etiology (autism+)	Milder sole autism, of unknown etiology
Mean IQ	lower	higher
Etiology	point mutations or aberrations, *de novo* or inherited	unknown mutations, unknown inheritance
Clinical heterogeneity	significant	possibly less
Environmental contribution	less important	more important
Paternal age effect	relevant	less relevant
Sex ratio (male/female)	2-4/1	4-8/1
Dysmorphism	frequent	usually absent
Head circumference	microcephaly or extreme macrocephaly	mild microcephaly possible
Developmental regression	rare	more common

Clinical recommendations

A broad knowledge of the genetic causes of autism spectrum disorders is being increasingly translated into practical changes in diagnostic and therapeutic procedures. This is reflected in the specific recommendations intended for physicians of different specialties. In addition to the already mentioned guidelines on the diagnosis of fragile X syndrome, the noteworthy diagnostic recommendations are those of American College of Medical Genetics in 2013, shown in Table 4 [21,22]. According to them, in all patients with the diagnosis of ASDs aCGH should be performed, whereas in the

absence of such technical opportunities, one should consider karyotype and MLPA with probes for common regions correlated with autism (e.g., 15q11-q13, 16p11.2, 22q13), so-called MLPA autism. Other recommendations relate to specific phenotypes of monogenic syndromes, including metabolic diseases. In the latter group proposed diagnostic panel includes blood count, serum metabolic profile (analyzed with mass spectrometry MS/MS), plasma aminogram and test for the presence of glycosaminoglycans in the urine [22].

Table 4. **Clinical guidance on the use of genetic testing in patients with ASDs [21,22]**

Genetic test or gene	Clinical indications in asds population
Array CGH (aCGH)	All children, especially with dysmorphism, intellectual disability, developmental delay, growth retardation, congenital malformations or anomalies
Karyotype/MLPA autism	All children whenever aCGH cannot be used
FMR1 (FRAX)	Intellectual disability (ID) (or if it cannot be ruled out) +/- large head AND/OR family history of ID in male relatives)
MECP2	Rett syndrome phenotype in girls (stereotypic hand movements, fine motor regression, gait disturbance), especially Rett preserved speech variant, AND symptoms suggestive of *MECP2* duplication in boys (hypotonic facies, frequent respiratory infections, drooling)
PTEN	Head circumference above the 98th percentile for age OR Cowden syndrome phenotype (skin lesions, Lhermitte-Duclos disease, cancer)
Metabolic testing including mitochondrial disease	Electrolyte disturbances, anemia with MCV ↑, cyclic vomiting, skin lesions, regression during infection or fever, gastrointestinal dysfunction, hypotonia, dystonia, metabolic acidosis, multisystem disease (especially involvement of liver, heart and kidneys), apathy, symptoms of neurodegeneration other than loss of speech typical for ASDs, growth retardation, microcephaly, seizures/epilepsy

Clinical examples

Suggested below in Table 5 on specific examples from author's own experience is the clinical diagnostic flow illustrating the complexity of the process from the point of the clinical geneticist. Of note is the fact of examining in the Genetic Counseling Unit two patients, both with the same diagnosis of pervasive developmental disorders, not otherwise specified. The presence of additional problems in the form of congenital malformations and dysmorphic features in one of them allows the examiner to qualify this child in a group with autism+, reaching the diagnosis of genetic syndrome, which further allows for provision of accurate and reliable genetic counseling and helps

formulate specific recommendations for care. In many cases though, especially in the youngest patients, with no genetic causation established, the most appropriate next step is further diagnostic outpatient observation following the preliminary assignment of the patient to one of the mentioned diagnostic groups.

Table 5. **Steps in the diagnostics of two 2-year-old boys sent to Genetic Clinic with the same diagnosis ICD10 F84.9, but significantly different clinical and pedigree data, final diagnosis and management (examples 1 and 2).**

Diagnostic step	Clinical and pedigree data	Example 1	Example 2
1	Pedigree	irrelevant	irrelevant
2	Psychomotor development	delayed	delayed
3	Physical development	normal	normal
4	Physical defects/anomalies	none	hypodysplastic kidneys, bilateral inguinal hernia
5	Dysmorphism	none	present
6	Macro-/microcephaly	none	none
7	Seizure/epilepsy	none	none
	Diagnosis and management		
8	Autism type	autism (*essential*)	autism+ (*complex*)
9	Genetic test	karyotype/aCGH	karyotype/aCGH
10	Diagnosis	none	syndromic (Pallister-Killian syndrome)
11	Management	further outpatient observation	recommendations for care, genetic counselling, further outpatient observation

Indications for clinical geneticist's consultation

The role of clinical genetics specialist is to determine the genetic causes of autism spectrum disorder after detailed clinical assessment and the use of available diagnostic techniques, the formulation of practical recommendations for the therapeutic management and prevention of symptoms and complications of the disease, as well as provision of a reliable genetic counseling regarding the risk of recurrence of autism in the

family. Suggested indications for consultation with a specialist in clinical genetics should include cases of ASDs accompanied by:

a. dysmorphism;
b. intellectual disability/psychomotor development delay or whenever intellectual disability cannot be excluded;
c. congenital defect/malformation or anomalies;
d. large head> 3 SD or microcephaly>-3SD;
e. family history of ASDs or neuropsychiatric disorders in first degree relatives (siblings, parents);
f. any abnormal result of genetic testing;
g. developmental regression.

Cited indications for specialist consultations reflect the results of clinical trials suggest that the more complex and the more severe the clinical picture of autism spectrum disorder the more likely it is to identify pathogenic changes (mutation or chromosomal aberration) (see Table 3).

Conclusions

Modern techniques of molecular and cytogenetic analysis have revolutionized the diagnostics of the causes of autism spectrum disorders. This applies particularly, but not exclusively, to those cases of autism that are accompanied by other clinical signs (i.e. complex phenotypes). The role of the specialist in clinical genetics is the clinical assessment of patients for the presence of the above phenotypic traits, selection of an appropriate genetic test and interpretation of its result. It is worth remembering that, not unusually, only the diagnostic reassessment of the developing child allows to formulate a specific diagnosis. Establishing clinical diagnosis and its molecular/cytogenetic verification end the diagnostic process and allow for the development of optimal therapeutic treatment and provision of reliable advice on the risk of the recurrence of genetic condition in the family. This publication is one of the first such works in Polish describing the practical aspects of the diagnostics of the genetic causes of autism. In an era of personalized medicine, the parents of autistic children obtain access to modern genetic diagnostic techniques. Yet, it is the awareness and knowledge of these parents and medical specialists, including clinical geneticists, that allow for a proper management of the diagnostic process in order to maximize the chances of identifying the cause of the disease.

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