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Comprehensive Approach to Diagnosing ASD in Children Using the Example of a Clinical Case Associated with a Variation of the SOX5 Gene

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Abstract So far, more than 1,000 genes have been identified in which mutations are predictors of autism spectrum disorders. The influence of neurobiological factors has also been proven, making it possible to suspect these disorders in the early postnatal period. Despite the numerous evidence of a genetic factor in the development of autism spectrum disorders, the mechanisms of occurrence of these genetic disorders remain unclear, and therefore the description of family cases in which ASD is observed in combination with various features of the genome, which are not rarely found in conjunction with other disorders, is of particular interest psychomotor development. This study presents the results of a study conducted over five years. The results of this study were based on the complex psychological and neurophysiological status of three sibs, which have a similar developmental feature and genetic disorders inherited through the maternal line. A DNA sample from sibs and proband was searched for pathogenic and likely pathogenic variants in all coding regions of the genome. During next-generation sequencing, a variant of the nucleotide sequence **c.310C>G** in exon 3 of the **SOX5** gene (chr12:g.23999088G>C) in a heterozygous state was identified, leading to the replacement of an amino acid in position 104 of the protein chain **(p.His104Asp).**

Keywords Autism spectrum disorder, Cerebral palsy, Psychological research, Bioelectrical brain activity, Genetic disorder, Family case

1. Introduction

ASD - neuroontogenetic development disorder manifesting in early childhood. This early onset significantly reduces the ability to detect and identify specific biomarkers in time that can predict the risks of developing ASD. ASD is characterized by a wide range of clinical manifestations, some patients require lifelong care, others, on the contrary, achieve professional success and are fully socialized. Currently, there are not enough complete comprehensive methods for correcting the main symptoms of autism. For example, therapy aimed at correcting behavior supposedly increases the IQ score and reduces the severity of speech disorders, while taking medications neutralizes the symptoms of agitation and hyperactivity. Over time, studies have shown a significant role for neuroimaging and genome-wide sequencing in the structure of ASD [1].

It is important to note that the number of children with ASD is growing every year [20]. Thus, according to the US Centers for Disease Control and Prevention (CDC), the number of registered cases increased from 1 to 36 people in 2023, or 1% of the population have ASD. The increase in statistical data is unclear, but this in turn confirms the influence of combined factors. In recent decades, the growth of scientific research focused on the study of etiological factors and methods for diagnosing ASD has reached record levels. After numerous studies, RAS has now been proven to be a polygenic disease and the search for genetic markers of autism is one of the central areas of research. Currently, over 1,000 genes have been identified whose mutations may be associated with autism (AutDB database data: autism. mindspec. org/autdb). Autistic disorders have been found to be associated with genetic mutations that may be hereditary or newly emerged (de novo) [2]. The genetic causes of ASD are indicated by research data from twins, siblings and other relatives [5,10].

Hundreds of genes have now been identified, variations in which are associated with ASD [14]. Copy number variation (CNV) is a type of structural adjustment that involves changes in the number of copies of certain sections of DNA that can either be deleted or duplicated. As with other types of genetic mutations, some CNVs are inherited, while others spontaneously occur in offspring and are absent in parents (de novo). There are databases SFARI Gene, AutDB, which collect information about genes in which variations are

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associated with autism, — now has more than a thousand records [3].

In relation to the identification of the etiology and pathogenesis of autism, hereditary monogenic forms of autism are considered, which are known and sufficiently studied. It is believed that up to 10% of ASD cases are associated with known genetic syndromes, such as Fragile X syndrome, tuberous sclerosis, Rett syndrome, Angelman syndrome, etc. [4,8,12]. Combined with genetic predisposition, the action of environmental factors in the early stages of a child's development is recognized as equally important in the pathogenesis of autism [19]. The most influential factors currently considered are those whose exposure occurs in the early prenatal period of development [3,9].

Thus, autism - is a multifactorial disorder caused by genetic and environmental factors. Etiological heterogeneity, variable penetrance and broad phenotypic pleiotropy are now recognized as common characteristics of ASD genetics [5].

In this regard, detailed descriptions of family cases in which certain genome disorders accompanied by autism spectrum disorders are found are of significant interest. This article provides data from a comprehensive longitudinal psychological and neurophysiological study of three cibs with symptoms of developmental disorders, which were found to have genetic disorders inherited from their mother [6,7].

2. Materials and Research Methods

Subjects: mother, father and three children (eldest daughter M, middle daughter M, youngest son M). Complaints: developmental disorder in all children. The study was conducted 7 times over 4 years. The eldest girl was 1 year old during the first visit, during the last - 5 years 4 months; the average girl was 1.5 years old and 3 years old during the last visit; the youngest boy was 2 months old during the first visit, during the last -8 months.

The mother was 22 years old during the first examination, the father - was 29 years old. Thus, the mother gave birth to her first child at 21, her last - at 24 years old.

Pregnancy and childbirth proceeded in all cases without complications. Delayed psycho-speech development was observed in all children, and in the older child - of the girl - it was most noticeable and previously caused concern among parents. Based on a consultation with a psychiatrist, the children were diagnosed as follows:

F, eldest child, female, - Mixed specific psychological development disorders. With attention deficit hyperactivity disorder; ASD.

M, middle child, girl, - delayed psycho-speech development with ASD elements;

M., the youngest of the children, a boy, delayed psychomotor development.

Psychological research: methods included in the diagnostic examination protocol for suspected autism spectrum disorders were used [2].

1. Vineland Adaptive Behavior Scale VABS; allows you to identify the overall level of development of adaptive skills, assessing them in four areas of life: communication, everyday life skills, socialization, motor skills. The average (normative) level of development corresponds to 100+15 points.
2. Intelligence test KABC-II (Kaufman Assessment Battery for Children); allows you to assess the level of development of nonverbal cognitive abilities in the form of a general index of nonverbal intelligence, the average standard value of which corresponds to 100 points (+15 points).
3. The Social Communication Questionnaire (SCQ) is a parent-based screening technique that helps identify symptoms associated with autism spectrum disorders. Form «During life» is filled out taking into account the entire development history of the child and its use gives results (above 15 points for all cases, above 12 points, if there are accompanying factors such as hereditary burden), which may be grounds for referral for a more detailed examination [8,9].

Result of analysis of full exome sequencing data.

After consultation, parents were asked to do a genetic study on the eldest child, whose clinical picture was pronounced and the debut of manifestations was noted in the early stages of development.

Genetic research

A genetic examination of full-exome – sequencing identified a heterozygous variant of unknown clinical significance c.310C>G (p.His104Asp) (potentially affecting splicing) in the **SOX5** gene. (Table 1).

Table 1. Results of genetic examination of children and parents During full exome sequencing, the following nucleotide variants were identified in the proband

Pathogenic variants:

Gen	Position (GRCh37/hg19)	Genotype	Exon	Option for DNA (AA)	Allele frequency *	Reference sequence	Reading depth **
Not found							

Options of unknown clinical significance:

Gen	Position (GRCh37/hg19)	Genotype	Exon	CDNA variant (AC)	Allele frequency *	Reference sequence	Reading depth **
SOX5	chr12:g.23999088G>C	heterozygote	3	c.310C>G (p.His104Asp)	н/д	NM_006940.6	52x

Asymptomatic heterozygous carrier:

Gen	Position (GRCh37/hg19)	Genotype	Э Exon	CDNA variant (AC)	Allele frequency *	Reference sequence	Reading depth **
NBN	chr8:g.90983441del5	heterozygote	6	c.657_661del (p.Lys219fs)	0.000225	NM_002485.5	91x
TMEM38B	chr9:g.108484867G>A	heterozygote	4	c.507G>A (p.Trp169Ter)	0.00000434	NM_018112.3	77x

* Allele frequencies are given from samples of conventionally healthy volunteers of the Genome Aggregation Database project (gnomAD v4, more than 807,000 people). n/a=no data (not described)
** Number of independent readings of a region of the genome containing a variant of the nucleotide sequence.

Interpretation of research results

A proband DNA sample was searched for pathogenic and likely pathogenic variants in all coding regions of the genome.

During next-generation sequencing, a variant of the nucleotide sequence **c.310C>G** in exon 3 of the **SOX5** gene (chr12:g.23999088G>C) in a heterozygous state was identified, leading to the replacement of an amino acid in position 104 of the protein chain (**p.His104Asp**). The **SOX5** gene encodes a transcription factor protein that is involved in the process of neurogenesis and chondrogenesis. Pathogenic and likely pathogenic variants in the heterozygous state in the **SOX5** gene suggest an autosomal dominant inheritance pattern. The identified replacement for **c.310C>G** has not previously been described in the scientific and medical literature in sick or healthy individuals, and is absent in control samples of gnomAD. The replacement of histidine with aspartic acid **p.His104Asp** (amino acids of different physicochemical properties) occurred in the evolutionarily conserved position of the protein. Pathogenicity prediction algorithms evaluate this variant with discrepancies (PROVEAN, SIFT – disrupting the function of Polyphen2 _HDIV, Polyphen2_HVAR; - benign) [9,10]. Splice prediction algorithms (SpliceAI) estimate **c.310C>G** substitution as likely to disrupt splicing. Based on the totality of information, the identified replacement of **c.310C>G (p.His104Asp)** in a heterozygous state in the SOX5 gene should be considered as a variant of unknown clinical significance that may be relevant to the patient's phenotype if additional supporting data is obtained.

After the study result, a search was carried out for the **c.310C>G (p.His104Asp)** variant in the **SOX5** gene using the Sanger sequencing method in parents and siblings of the proband (Table 2).

3. Research Results

Table 2. DNA analysis of patients

Sibs probanda	Option c.310C>G in the **SOX5** gene
Mother	Found in heterozygous state
Average girl M.	Found in heterozygous state
Younger son M.	Not found
Father	Not found

DNA analysis of patients was carried out on a SeqStudio sequencer using direct automatic sequencing of PCR products.

Variant annotations were performed on reference sequences NM_006940.6 (RefSeq database). The standard HGVS nomenclature (https://mutalyzer.nl/ version 2.0.25) was used to name the variants [11,12].

The search for the c.310C>G variant (p.His104Asp) in the **SOX5** gene using the Sanger sequencing method in family members – in the father and younger brother was not identified; in the mother of the proband and younger sister, the desired variant was identified in a heterozygous state.

Psychological research:

Children were examined 7 times over 4 years. Table 2 presents the summarized results of the psychological examination at the time of the first and last meeting with the family.

Subject F. (the eldest child in the family, a girl) was examined three times using the Vineland Adaptive Behavior Scale, as well as the SCQ social but communication questionnaire. At the first meeting, parents were concerned about the expressed difficulties of communication, maladaptive behavior, and significant delay in psycho-speech development. At the age of 4.5, the girl did not attend preschool educational institutions and studied at home with her parents. At the age of 6, the girl was diagnosed with ASD and prescribed antipsychotic therapy. At all ages, the level of adaptation was assessed as low with a moderate deficit of 4 years 6 months (50 points) and a severe deficit of 6 years 9 months (38 points). The decrease in the last examination was associated with an episode of developmental regression that occurred at the age of 6 years (this fact was reported by the mother), with a subsequent improvement by the age of 7 years associated with the administration of drug maintenance therapy in the form of rispolept. Selected areas of adaptive behavior were investigated and the following patterns were identified. At all ages, the strongest side of subject B. is the field of motor skills; the least developed areas of socialization and communication [13,14]. Interestingly, at the age of 5 years 11 months there was a decrease in raw scores (1st examination — 25 points, 2nd examination — 15 points, 3rd — 27 points) and the overall standard score on the «Communication» scale, which indicated a deterioration in the development of communication skills in this age period,

followed by improvement at the age of 6 years 9 months. The socio-communicative questionnaire (form«During life») was filled out by parents for age F. 5 years 4 months. The score is — 18, which exceeds the threshold and indicates a high risk of autism spectrum disorder.

Subject M. (the middle child in the family, a girl) was examined three times using the Vineland Adaptive Behavior Scale, as well as the SCQ Social Communication Questionnaire. On the first visit, the parents were concerned about their son's delayed psycho-speech development, which, however, was less pronounced than that of his sister. The child did not attend the preschool educational institution; during the last visit, the boy was worried, it was difficult to establish contact with him, he refused to complete tasks.

At all ages, the level of adaptation was assessed as low with a moderate deficit (at 2 years 8 months and 4 years 11 months) and with a mild deficit (at 4 years 1 month). This pattern is associated with a slowdown in the development of motor and everyday life skills in V. after 4 years. The evaluation of selected areas of adaptive behavior revealed the following patterns. At all ages, the strongest side of subject V. is the sphere of everyday and motor skills, the least developed sphere of communication and socialization. However, the line of development of socialization and communication skills, unlike skills in the motor and household spheres, had a continuous, positively progressive character [15,16].

ScQ Score Sum (Form «Lifetime») — 14. Given the unfavorable family history of the presence of developmental characteristics in the other two sibs, this value can be considered sufficient to be identified as a risk group for the development of autistic disorders.

Table 3. Results of psychological examination

Subject, age (at the time of the first and last examination)	**Art. — points general level of adaptation (at the time of the first and last examination)**	**SCQ values (form «During life»)**	**Nonverbal intelligence assessment (at the time of the first and last examination)**
Subject F.			
4 y. 6 m.	50 tbsp. — points low level with moderate deficiency	18 points High risk	The test is not available due to severe behavioral problems
6 y. 9 m.	38 tbsp. — points are low with a deep deficit		
Subject M.			
2 y. 8 m.	40 tbsp. — points low level with moderate deficiency	14 points Threshold value	The test is not available due to severe behavioral problems
4 y. 11 m.	51 tbsp. point — is low with a moderate deficit		

Subject M. (younger child in the family, boy). Considering the age of the child, it is not possible to conduct psychological tests used in older children. The child was examined 3 times. In which the psychomotor and emotional development of the child was assessed. Up to 5 months. The child did not experience developmental delay. At the last appointment at the age of 8 months, signs of developmental delay began to appear: the shape of the head is rachitic, the child does not hold his head confidently, does not turn over, does not try to sit, support when verticalizing on «chickens», in the position on the stomach, support on the handles is weak. He doesn't stretch his hands to objects, holds the nested objects for a short time, tries to present them to his mouth, the tendon reflexes are animated, muscle strength is 4b. Psycho-emocyanal development: sterotypic movements in the form of constant clenching and unclenching of the fingers, accompanied by general anxiety. It should be noted that in a child there is no walking in the presence of eye contact. He smiles at the sight of bright objects and parents, but there is no emotionally enriched walking in the form of syllables. According to the mother, the child is very calm, for a long time he can be in the hands of strangers - a comfortable« child. An examination using the neuroimaging method NSH showed hypoxic changes in the brain. No organic disturbances were identified [17,18].

Parents: during a joint examination by a psychoneurologist and psychologist, during the study of the geniological history of parents, he showed the direct role of this gene mutation in the maternal family. The mother of the proband also showed a delay in psycho-speech development, speech after 5 years, when she began attending preschool educational institutions. The mother has a meager vocabulary, answers questions with a dagger, and has a hard time perceiving new skills. Notes difficulties in communication and has a hard time adapting to a new environment. He does not communicate with neighbors and partially masters household skills. In caring for children, he needs help from strangers. The mother has 2 sisters who also experienced a delay in psycho-speech development. But at present they have socialized, although there are difficulties in communication. No communication or social disorders were identified in the maternal grandmother of the proband. Whereas in the maternal grandfather of the proband, a psychological examination showed violations of the sphere of socialization and communication. The father of the proband did not have any violations in this area.

4. Discussion

In the described family with hereditary maternal burden, three children were born with certain functioning disorders. The children were born to young parents who did not have bad habits.

Based on the results of a full-exome sequencing study, a heterozygous variant of an unknown clinical value of c.310C>G (p.His104Asp) (potentially affects splicing) in the **SOX5** gene was identified.

The search for the c.310C>G variant (p.His104Asp) in the **SOX5** gene using the Sanger sequencing method in family

members – in the father and younger brother was not identified; in the mother of the proband and younger sister, the desired variant was identified in a heterozygous state [19,20].

Based on the clinical picture, family history and data and molecular genetic study, we found that copy number variations (CNVs) in the SOX5 gene identified in these ASD patients may also contribute to the development of other neurodevelopmental disorders. Characterized by delayed speech and psychomotor development, decreased intelligence, autistic behavioral traits, etc. Genetic analysis found various genome disorders (CNVs) in children, two of which were inherited from their mother. In 2 cases out of three, a significant increase in the altered area of the chromosome inherited from the material can be associated with disruption of the functioning of certain genes of this chromosome, which manifested itself in autism spectrum disorder diagnosed by psychiatrists and confirmed methods of psychological examination. The older and younger daughters showed the same mutations, and since the clinical picture of the children is significantly similar, it can be assumed that these cases are associated with the identified mutation. Moreover, the identified gene is part of the database of genes whose mutations may be associated with autism (data from the AutDB database: autism.mindspec. org/autdb). In the average child, autistic disorders were significantly milder than in the eldest daughter.

In conclusion, I would like to note that this is the first description of a family case in which three children had some kind of developmental disorder, and in two cases these disorders were pronounced and associated with ASD and gross delay in psycho-speech development. All family members underwent a genetic examination, the results of which showed that the mother had severe genomic instability. An extremely important observation has been made based on genetic analysis: all children inherited these mutations with a significantly increased number of repetitions. The older and middle child has a very high probability that the cause of autism spectrum disorders is a mutation in the SOX5 gene. The youngest child was not identified and is probably not related to his mental state.

Thus, a fairly complete comprehensive genetic, psychological and neurophysiological study of a family case made it possible to understand the structure of the identified disorders and suggest their possible causes. The disease is associated with heterozygous mutations in the **SOX5** gene. This gene encodes a protein that is involved in the processes of regulation of transcriptional activity. Variations appear with different phenotypic expressions, suggesting that these changes are only one of a variety of factors contributing to the development of autistic traits. In the future, further investigation of their association with neurodevelopment and autism is needed to better understand the pathogenicity of CNVs.

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