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SHORT COMMUNICATIONS

Estimation of Association of *CNTN6* Copy Number Variation with Idiopathic Intellectual Disability

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Abstract—Analysis of the prevalence of copy number variations of the *CNTN6* gene, recently selected as a new candidate gene for intellectual disorders, was performed. Real-time PCR did not detect any change in the number of *CNTN6* gene copies in a group of 200 patients with impaired intellectual development. However, taking into account our data from the previous aCGH analysis and published data, the overall frequency of microdeletions and microduplications of *CNTN6* was estimated as 1 : 265 (0.4%). The common phenotypic features of 40 patients with microdeletions and microduplications of *CNTN6* appeared to be the autism spectrum disorders, developmental delay, intellectual disability, seizures, cognitive impairment, cardiological defects, and behavioral problems.

Keywords: idiopathic intellectual disability, CNV, microdeletion/microduplication syndromes, *CNTN6*, real-time PCR, genotype–phenotype correlations

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The application of the modern whole-genome technologies (array comparative genomic hybridization, next-generation sequencing) to identify the genetic causes of developmental delay and intellectual disability often provides a list of copy number variations (CNVs), among which there are both polymorphic and pathogenic variants and mutations with unclear pathogenic significance (potentially pathogenic variations). The regions of chromosomal mutations usually contain several genes, making it difficult to assess the contribution of each of them to the formation of the phenotype and complicating the identification of candidate genes for the disease. In such case, in order to prove the pathogenic significance of mutations, reciprocal monogenic CNVs, represented by microdeletions and microduplications of the same chromosomal region with the involvement of a single gene, become of particular interest.

Four unique cases of reciprocal chromosomal mutations in 3p26.3, containing the single *CNTN6* gene, were for the first time described by us during the examination of patients with intellectual disability (ID) [1]. CNTN6 is a neuronal cell adhesion molecule. It is predominantly expressed in the central nervous system and is involved in the processes of development of neurons, formation of synapses, and myelination of axons [1]. Thus, CNTN6 can participate in the processes of development and functioning of the central nervous system, while mutations affecting the *CNTN6* gene may be associated with ID. The goal of this work was to find microdeletions and reciprocal microduplications of the *CNTN6* gene in the extended samples of patients with developmental delay and ID, as well as to perform a comparative analysis of the phenotypic characteristics of patients with these chromosomal mutations.

The study involved 200 children, including 46 patients aged 3 to 5 years with a delay of intellectual development (35 boys and 11 girls) and 154 children aged 5 to 18 years with a diagnosis of idiopathic intellectual disability, F79 from ICD-10 (107 boys and 47 girls). The mean age of the patients was 9 ± 3.89 years. The control group (population sample) included 97 healthy men and 103 women (altogether 200 individuals) aged from 23 to 69 years (35 ± 7.55 years). The examinations of patients and the collection of clinical material were carried out in the Genetic Clinic of the Institute of Medical Genetics, Tomsk. The informed consent from the parents of probands and individuals of the control group was obtained in all cases. The study was approved by the Committee on Biomedical Ethics of the Institute of Medical Genetics.

Quantitative real-time PCR was performed using the CFX96 Real-Time PCR system (Bio-Rad, United States) with two pairs of primers for the *CNTN6* coding region (Table 1). *HEXB* (5q13.3) was used as a reference locus.

Table 1. Structure of primers for *CNTN6* and *HEXB* genes

Region	Primer	Sequence
3p26.3	CNTN6_A F	5'-TTTGTTCCACCTGATTGTATGG-3'
	CNTN6_A R	5'-GAAGAGTTTATGAGTGGCAGCA-3'
	CNTN6_B F	5'-CTGGACAGATGACTCCAAAGAA-3'
	CNTN6_B R	5'-CCTGTCCCAGCAGTGTTGTA-3'
5q13.3	HEXB F	5'-CCGGGCACAATAGTTGAAGT-3'
	HEXB R	5'-TCCTCCAATCTTGTCCATAGC-3'

All the patients under study had a normal (disomic) number of copies of the *CNTN6* gene. No cases of microdeletion or microduplication of *CNTN6* were found in any of the studied groups. This result may be due to the fact that the mean locus-specific CNV frequency is 10^{-5}–10^{-4}; therefore, the mutation could not be found in the relatively small samples investigated in this study. However, among 79 patients with ID studied by us previously with high-resolution array comparative genomic hybridization (aCGH), the microdeletion of this gene was detected in two siblings, and the microduplication was detected in one patient [1]. So, the frequency of chromosomal mutations in the 3p26.3 region in the Russian population was 1 : 93 among patients with intellectual disability (1%).

It is noteworthy that, in August 2015, foreign researchers published the results of a retrospective search for copy number variations of *CNTN6* among 3724 patients with multiple congenital malformations, heart defects, short stature, developmental delay, intellectual disabilities, autism spectrum disorders, and seizures [2]. Fourteen CNVs affecting *CNTN6* were identified in children aged 1 year to 15 years. Seven of the patients had either complete or intragenic deletion of *CNTN6*. Five patients had intragenic duplication of *CNTN6*. Another two patients had duplication of co-localized *CHL1/CNTN6* and *CHL1/CNTN6/CNTN4* genes, respectively. The researchers suggest that the frequency of *CNTN6* copy number variation was 4 : 1000 (0.4%); however, in our opinion, two patients with the adjacent gene microduplications affecting the neighboring genes which are considered pathogenic for the syndrome of deletion of the short arm of chromosome 3 (3p- syndrome; OMIM 613792) should be excluded from this group [3]. Thus, the frequency of isolated variations in the number of *CNTN6* copies detected by Hu et al. [2] was 1 : 310 (0.3%). They note that the incomplete penetrance and variable expressivity of CNV may impede the counting of the frequency of chromosomal mutations in the 3p26.3 region, as some patients may have poorly marked clinical signs of the disease or normal phenotype [2]. Detection of CNVs affecting *CNTN6* in clinically healthy individuals does not exclude the possibility that these mutations may be a risk factor for neurological disorders. That is why the control group in this study consists of adults.

Taking into account the new data provided by Hu et al. and also a number of other studies in which patients with *CNTN6* microdeletions and microduplications were mentioned [4–7], the cases present in the DECIPHER [8], and our earlier results, we conducted a comparative analysis of phenotypic characteristics of these patients (Table 2). The group for the study consisted of 40 children aged from 1 year to 15 years with developmental delay or ID. Among the 40 probands, regardless of the type of chromosomal mutation, most frequently, the autism spectrum disorders and delayed physical and psychomotor development were reported. Slightly less frequently, ID and cognitive impairment were identified. A more detailed clinical description was available only for 16 patients [1, 2]. Among them, in about half of the cases, marked seizures and cardiological defects were reported. Some children had behavioral disorders, abnormalities of the hair, skull, musculoskeletal system, and fingers, and some facial dysmorphic features. Additionally, in the group of patients with microduplication, the cases with attention deficit hyperactivity disorder and reflux were noted, and among patients with microdeletion, thyroid hypoplasia and kidney disease. Thus, these reciprocal microstructural aberrations can be attributed to the chromosomal mutations with partially overlapping and unique phenotypes, and the change in the *CNTN6* gene dose can affect the normal development of not only nerve tissue but also other organs and systems.

Using the high-resolution genomic technologies makes it possible in the case of the known chromosomal syndromes, diagnosed by the standard cytogenetic method and associated with extended chromosomal regions, to identify smaller and even monogenic clinically relevant variants. It is possible that some of them, when clinical and genetic data are accumulated and compared, can form separate syndromes. To date, the microdeletion and/or microduplication of *CNTN6* are not associated with distinct syndromes; however, these CNVs fall within the region of known 3p- syndrome (OMIM 613792). The presence of such clinical signs as microcephaly and growth and psychomotor retardation, both in patients with the 3p deletion syn-

Table 2. Phenotypic features of patients with *CNTN6* microdeletion and microduplication

Clinical signs	*CNTN6* microduplication	*CNTN6* microdeletion
Common	Autism spectrum disorders (17/40), delayed physical and psychomotor development (15/40), intellectual disability (8/40), cognitive impairment (7/40), seizures (7/16), cardiological defects (6/16), behavioral disorders (5/16), abnormal hair structure and growth (5/16), abnormalities of fingers and toes (5/16), wide nasal bridge (4/16), high-arched palate (4/16), microcephaly (4/16), short stature (4/16), abnormal EEG (4/16), epicanthus (4/16), antimongoloid slant (3/16), abnormalities of teeth and gums (3/16), ear abnormalities (3/16), joint hypermobility (3/16), frontal bossing (3/16), hydrocephalus (3/16), abnormal skull shape (3/16), scoliosis (3/16), strabismus (2/16), hypothyroidism (2/16), dysarthria (2/16), speech development delay (2/16), short philtrum (2/16), macrocephaly (2/16), spasticity (2/16)	
Unique	Attention deficit hyperactivity disorder (3/16), reflux (2/16), cryptorchidism (1/16), micrognathia (1/16)	Thyroid hypoplasia (2/16), kidney disease (2/16)

The table contains clinical data from the articles [1, 2, 4–7] and DECIPHER [8]. The frequencies of clinical signs are shown in parentheses.

drome and in patients with an isolated change in the number of copies of *CNTN6*, allows one to suggest that the contactin 6 protein plays some role in their formation. In addition, in the region of 3p- syndrome, the clinically significant microdeletions and microduplications of another neighboring gene *CHL1* were detected [9].

To date 40 cases of isolated microdeletions and microduplications of *CNTN6* among patients with neuropsychiatric and neurological disorders are presented in the literature and DECIPHER [1, 2, 4–8]. However, the sizes of samples in which these patients were detected are shown only in some studies [1, 2, 4, 5]. With this in mind, the total frequency of *CNTN6* microdeletions and microduplications among patients with idiopathic ID can be estimated as 1 : 265 (0.4%, 19 out of 5049 individuals). This assessment considers the variations in the 3p26.3 region as widespread among the patients with idiopathic intellectual disabilities. For example, the frequencies of the most common 22q11.2 and 16p11.2 microdeletion syndromes are 1 : 167 and 1 : 241, respectively, in patients with ID [10]. Taking into account the functional features, spatial and temporal expression of the gene, and the presence of its copy number changes in patients with intellectual disabilities, it is obvious that the change in the dose of the CNTN6 protein may be important for the pathogenesis of neurocognitive disorders.

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