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HUMAN GENETICS

Two Novel Mutations in Gene *SPG4* in Patients with Autosomal Dominant Spastic Paraplegia

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Abstract—Hereditary spastik paraplegias (HSP) are a group of neurodegenerative disorders with primary lesion of the pyramidal tract. The most frequent autosomal dominant form of the disease in Europeans is HSP associated with mutations in the spastin gene (*SPG4*). Analysis of the gene *SPG4* was carried out in 52 unrelated families with HSP from Bashkortostan by SSCP and following sequencing. Previously undescribed frameshift mutations c.322del29 (p.Val108SerfsX18) and c.885del10 (p.Thr295ThrfsX16) were detected in two unrelated families. Clinical studies have shown that, in both families, the disease corresponds to an uncomplicated form of hereditary spastic paraplegia, a main feature of which is the lower spastic paraparesis without any other symptoms.

Keywords: hereditary spastic paraplegia, gene *SPG4*, mutations, Republic of Bashkortostan
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INTRODUCTION

Hereditary spastic paraplegias (HSP) are a group of neurodegenerative diseases the leading pathological feature of which is the spasticity of the lower limbs which alters the gait and posture of the patient; the feet are deformed according to equinovarus type [1]. At the heart of the disease is the degeneration of the pyramidal tract of the spinal cord. Depending on whether the main symptom is single or combined with other neurological or non-neurological symptoms, there are uncomplicated or complicated forms of the disease [2]. The prevalence of HSP in the world ranges from 1.27 to 9.6 per 100000 individuals [1]; in the Republic of Bashkortostan, 3.5 per 100000 [3].

There are more than 50 genetic forms of HSP. Eleven gene loci were described for the 19 known autosomal dominant spastic paraplegias, 16 loci for 27 autosomal recessive paraplegias, and three loci for the five X-linked forms. According to the modern nomenclature, gene loci and the corresponding forms of HSP are abbreviated as *SPG* (spastik paraplegia gene) with serial numbers in chronological order [1].

The most common autosomal dominant form of the disease in the population of Europe is the HSP associated with mutations in the spastin gene (*SPG4*). Mutations in this gene are responsible for 45% of autosomal dominant HSP and 12–18% of sporadic cases. The gene encoding the spastin protein is localized in chromosomal region 2p22.3 and has 6.71 kb and 17 exons. The spastin protein is a member of the family of AAA proteins—ATPases associated with a variety of cellular activity. These proteins are involved in multiple types of cellular processes such as the cell cycle, intracellular transport, and proteolysis [4]. The main function of spastin is providing the dynamics of microtubules of the cytoskeleton. Disturbance of this process leads to the development of the SPG4 form of the disease.

SPG4 is defined as uncomplicated HSP, but lately there have been reports on its clinical diversity: patients have cognitive impairment [5], epilepsy, [6], and ataxia [7]. The age of onset of the disease varies greatly from early childhood to 60 years of age and can vary even in the same family [5]. Also, there are cases of incomplete penetrance [8] and anticipation [9–11].

To date, the *SPG4* gene has more than 500 mutations, most of which are found in exons encoding the ATPase domain (www.hgmd.org).

The aim of this work is to identify mutations in the *SPG4* gene in patients with the autosomal dominant form of HSP from the Republic of Bashkortostan and analyze the clinical features of the disease in patients with identified mutations.

[1] These authors contributed equally to this work.

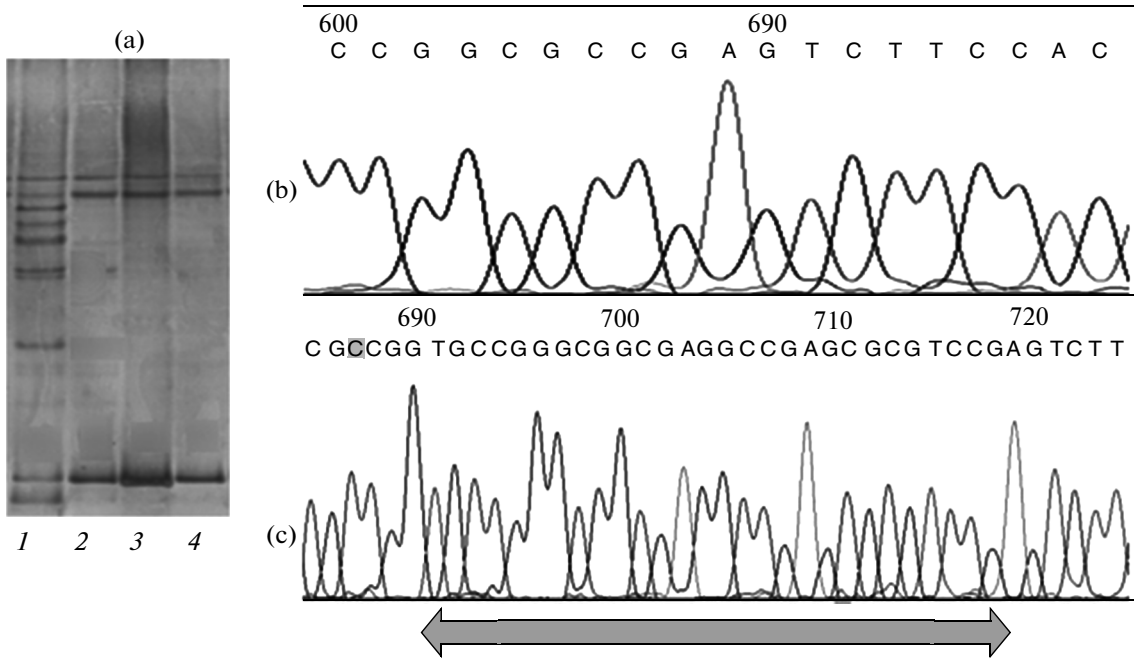

Fig. 1. Identification of mutation c.321del29 (p.Val108SerfsX18). (a) SSCP gel: (*1*) sample with mutation c.321del29 (p.Val108SerfsX18); (*2–4*) samples without changes; (b) sequencing of sample *1*; (c) sequencing of sample without changes; two-way arrow indicates a section deleted in sample *1*.

MATERIALS AND METHODS

Clinical, genealogical, and molecular genetic studies were carried out in 52 unrelated families with HSP, 18 of which were found to have a family cases of the disease and five had a sporadic cases. The control sample consisted of healthy individuals of the Republic of Bashkortostan of different ethnicity: Russian (50 ind.), Tatar (50 ind.), and Bashkir (50 ind.). All blood samples from the studied individuals was obtained with their informed consent.

Definition of the nucleotide sequence of the *SPG4* gene was carried out by analysis of DNA single-strand conformation polymorphism of (SSCP) followed by sequencing on an ABI PRISM 310 automated analyzer (Applied Biosystems). Amplification of exons was carried out using oligonucleotide primers described by Lindsey et al. [12], as well as using Genoscope Centre National de Séquençage (www.genoscope.cns.fr). Exons for which published data indicated large amounts of mutations (7, 8, 11, and 12) were analyzed by direct sequencing.

To analyze the functional significance of the identified nucleotide changes, we used the Mutationtaster (www.mutationtaster.org) and Human Splicing Finder (www.umd.be/HSF3) programs. For this purpose, control DNA samples of healthy individuals were screened for the presence of identified mutations.

RESULTS

Analysis of 17 exons and flanking intronic regions of the *SPG4* gene identified two previously undescribed mutations in patients from two unrelated families with HSP.

It was revealed a deletion of 29 nucleotides c.321del29 (p.Val108SerfsX18) (Fig. 1)in the first exon of the gene, which leads to a shift in the reading frame and the formation of a stop codon. The mutation was identified in a family of Tatar ethnicity, where HSP is inherited in a dominant mode. The age of manifestation of the disease in the proband, his uncle, and female cousin came in the fourth to fifth decade of life. Symptoms of the disease in the mother of the proband emerged later—at the end of the sixth decade of life. The clinical picture of the disease in the surveyed family members had a similar pattern and was represented by slowly progressive lower spastic paraparesis. In the group of control subjects of Tatar ethnicity (50 ind.), that mutation was not detected.

The second mutation is a deletion of ten nucleotides in the sixth exon of the gene (Fig. 2), which also leads to a shift of the reading frame and the formation of a stop codon (s.885del10 (p.Thr295ThrfsX16)); it was identified in a large family of Bashkir ethnicity in the proband and three (brother, nephew, and sister) of his relatives. In this family, HSP was detected in three generations with autosomal dominant mode of inheritance. In the proband, the disease began with a change in gait at the age of 22–23 years. Such a change in gait was seen in his brother, father, and uncle, as well

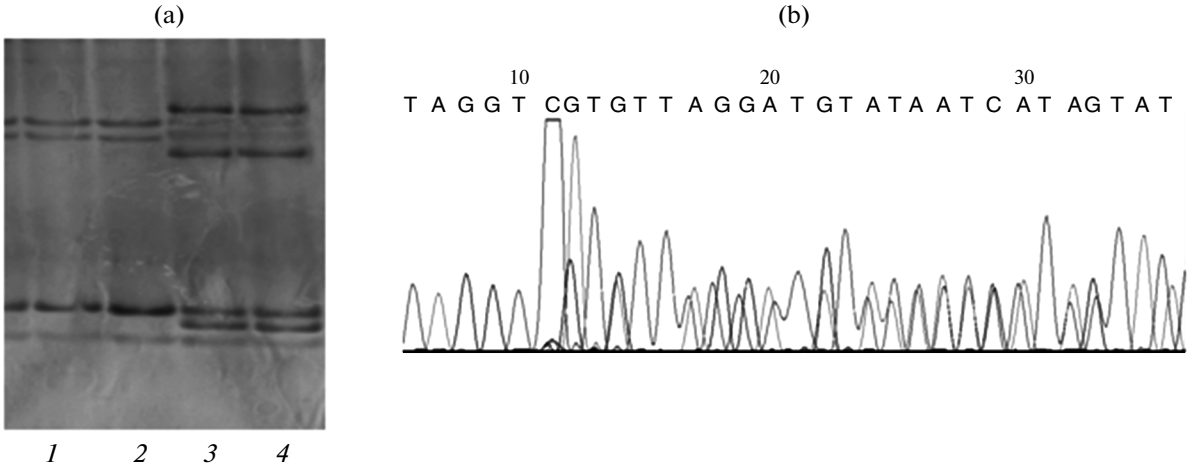

Fig. 2. Identification of mutation c.885del10 (p.Thr295ThrfsX16). (a) SSCP gel: (*1, 2*) samples without changes; (*3, 4*) samples with mutation c.885del10 (p.Thr295ThrfsX16); (b) sequencing of a sample with this mutation.

as a nephew of the proband from about the same age. The first symptoms of the disease were a feeling of tightness in the legs and a gait changes. A clinical study of the proband at the age of 47 years revealed lower central paraparesis with increased spastic hyper tone in the initiation of locomotion and the formation of the indicative pattern of walking as in skiing, as well as tendinous hyperreflexia with clonus stop and pathological reflexes of flexor and extensor types. The absence of other neurological symptoms made it possible to conclude that the family had an uncomplicated hereditary spastic paraplegia. Clinical examination of the proband's sister, who had no active complaints regarding health, revealed a slight increase in muscle tone in the distal compartments of legs and increased tendinous reflexes, especially in the legs, as well as pathological plantar and carpal reflexes. Healthy members of this family, as well as the group of control subjects of Bashkir ethnicity (50 ind.), did not show this mutation.

In addition to the revealed mutations, the examined patients with HSP was revealed to have a number of known *SPG4* polymorphisms. The 11th intron contained duplication c.1413+43_46dup (TATA); its frequency in patients was 30%; in the control group in Bashkirs, 35%; in Tatars, 42%; and in Russians, 38%. Known polymorphic variants s.1098+118 a>g (rs12617289) and c.1098+127 a>g (rs12617290), closely linked to each other, were found in patients with the **G* allele frequency of 40% in both the loci. In the fourth intron, polymorphic variant c.691-9C>T (rs202209866) was found in one patient.

DISCUSSION

Spastin protein has two isoforms: so-called M1 spastin and M87 spastin. M1 isoform consists of 616 amino acids, and M87 consists of 530 amino acids. Spastin includes several functionally distinct domains: hydrophobic (HD) domain, domain interacting with microtubules (MIT), microtubule-binding (MTBD) domain, and ATPase (AAA ATPase) domain, where the HD hydrophobic domain is present only in the M1 isoform. The MTBD and ATPase domains are required for hexamerization of spastin and severing of microtubules [4]. Coordinated interaction of atlastin 1, spastin isoform M1, and REEP1 is important for the formation of tubules of the endoplasmic reticulum (ER) and the interaction of microtubules with ER to generate the tubular endoplasmic network [4]. No major mutations in the *SPG4* gene were described; many of them are considered to be unique. The most common are missense mutations; also common are splice site mutations, deletions, insertions, and splice site deletions (www.hgmd.org). A significant proportion of all *SPG4* mutations (18%) were represented by different chromosomal aberrations such as large deletions and insertions [13]. It is noted that, in sporadic cases of HSP, the frequency of missense mutations is higher than in familial forms of the disease [14]. Population differences for the spectrum and frequency of mutations in this gene are detected. For instance, large deletions in patients with HSP reach 7% in Portugal [15], 16% in France [16], and 25% in Australia [17], whereas in patients in Estonia such mutations were not found, indicating, according to the authors, the uniqueness of this population [18].

The identified frameshift mutations c.321e129 (p.Val108SerfsX18) and c.885del10 (p.Thr295ThrfsX16) lead to the formation of a truncated protein that does not have the most important ATPase domain (amino acids 342–599) of the spastin protein. This protein is not able to perform its functions on severing of microtubules. Similar mutations leading to the synthesis of a truncated spastin protein are identified in patients with HSP often enough, accounting, according to some authors, for about half of all identified mutations in the *SPG4* gene [5, 19, 20]. The frequency of muta-

tions in different exons of the gene is different: the proportion of mutations in the first exon is small, whereas in the sixth exon the mutations occur more frequently (www.hgmd.org). The first exon turns out to have polymorphic variants which in the homozygous state or in combination with a mutation in another region of the gene can lead to HSP [12, 21]. There are deletions that start with the first exon and lead to the complete absence of the protein [16]. The sixth exon corresponds to the MTBD domain (amino acids 270–328) of the spastin protein involved in binding of microtubules prior to their severing [4]. Mutations in the MTBD domain, according to [22], account for 5% of all *SPG4* mutations, excluding extended deletions. Lacking the full MTBD domain, shorter spastin penetrates the endoplasmic reticulum membrane but is not involved in the interaction of microtubules with the ER, thus violating the ER morphogenesis [23].

The clinical effect of HSP, which is caused by mutations in the *SPG4* spastin gene, may be associated with either a decrease in protein expression (haploinsufficiency) [5, 9] or the loss of its function [24]. Studies of the role of spastin in microtubule dynamics proved that, in the case of mutations leading to premature termination of the spastin synthesis, HSP develops because of a deficiency in the amount of normally functioning protein [25], as indicated by the absence of detectable levels of the number of shortened spastins in cell lines [23]. But in the case of missense mutations, one needs to take into account the dominant-negative mechanism [25]. Missense mutations lead to the synthesis of stable mutant mRNAs and proteins that can reduce the activity of spastin encoded by the "wild" allele [23]. Thus, it is possible that different types of mutations in the *SPG4* gene can induce HSP through its pathogenic mechanism. The mutations that we identified lead to the synthesis of a truncated protein that does not contain the ATPase domain and loses its functional activity.

The surveyed families with the identified mutations were found to have a familial disease, which can be seen in three generations. Clinical symptoms correspond to the typical uncomplicated phenotype typical of the *SPG4* form [1]. In the family with mutation c.885del10 (p.Thr295ThrfsX16), the proband was diagnosed with tendinous hyperreflexia with clonus stop, indicating the SPG4 form [26]. At the moment, there are no data on association between the severity or the age of manifestation of the disease and the mutation type [5, 12, 27]. In these cases, no significant intra-family differences in the age of manifestation and the severity of the disease have been identified. But in the family with mutation c.32del29 (p.Va1108SerfsX18), in the mother of the proband, the disease manifested itself at a somewhat later age. And in the family with mutation c.885del10 (p.Thr295ThrfsX16), the sister of the proband was observed to have mild clinical symptoms. Perhaps, there is a phenomenon of sex differences in penetrance of *SPG4* mutations. On this occasion, there is evidence that women are asymptomatic carriers more often than men [10]. The previous study of the epidemiological characteristics of hereditary spastic paraplegia in the Republic of Bashkortostan discovered the ratio of diseased men and women is equal to 1.6 : 1 [3]. Similar data are presented in the work of Proukakis et al. [28], which proves that the penetrance depends on sex and that HSP is more common among men.

The polymorphic variants identified in this study are likely to be functionally neutral, and their frequency can be of interest in terms of population diversity. Thus, polymorphic variant rs202209866, which we found in one patient with HSP (1.9%), had previously been identified only in the population of South Asia with a frequency of 2% (http://www.ensemb.org/Homo_sapiens).

The frequency of major alleles of linked polymorphic variants rs12617289 and rs12617290 (alleles **A*), which in the group of patients with HSP amounted to 76%, was slightly higher compared to that in the European population (60%) (http://www.ensembl.org/Homo_sapiens).

The frequency of the allele with duplication c.1413+43_46dup (TATA), which among patients with HSP from Bashkortostan amounted to 30%, is comparable to the frequency in the control group, as well as to that in the European population (38%) [29].

FINDINGS

The two unrelated families with autosomal dominant HSP of different ethnicity from the Republic of Bashkortostan had two previously undescribed mutations in the *SPG4* spastin gene: c.321del29 (p.Val108SerfsX18) and c.885del10 (p.Thr295ThrfsX16). Both mutations relate to frameshift mutations. Clinical studies have shown that in both families the disease corresponds to an uncomplicated form of hereditary spastic paraplegia, a main feature of which is lower spastic paraparesis without any other symptoms. In the future, it will be possible to carry out presymptomatic and prenatal diagnostics in these families with autosomal dominant HSP.

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