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SHORT COMMUNICATIONS

Major Mutation in the *SPAST* Gene in Patients with Autosomal Dominant Spastic Paraplegia from the Republic of Bashkortostan

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Abstract—Hereditary spastic paraplegia (HSP) is a group of neurodegenerative disorders with a predominant lesion of the pyramidal tract. To date, mutations responsible for the disease have been identified in more than 70 genetic loci. The main causes of HSP development are mutations in the *SPAST* gene, but major mutations are rare for this disease. Study of HSP patients from 63 unrelated families from the Bashkortostan Republic (BR) identified the c.283delG (p.Ala95Profs*66) mutation in the *SPAST* gene in families of Tatar ethnicity with a high frequency. In the general cohort of unrelated patients from the Bashkortostan Republic, its frequency was 19%, and in the cohort of Tatar patients, it was 44%. HSP was found to be inherited in an autosomal dominant manner in all families with this mutation. The clinical symptoms of the disease in most of these families corresponded to the uncomplicated phenotype, typical of the SPG4 form of HSP.

Keywords: hereditary spastic paraplegia, *SPAST* gene, NGS target exome sequencing
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Hereditary spastic paraplegia (HSP) is a genetically and clinically heterogeneous group of degenerative diseases of the nervous system caused by the distal lesion of the long axons of the corticospinal tract. Clinically, HSPs is manifested as spasticity of the muscles of the lower limbs. Depending on whether the main symptom is unique or combined with other neurological or extraneural symptoms, uncomplicated or complicated forms are distinguished [1]. The prevalence of HSP, both as a whole and as individual genetic forms, varies significantly in different populations, ranging from 0.5 to 12 per 100000 population (http://www.hspersunite.org.au); in the Bashkortostan Republic (BR), it is 3.5 per 100000 population [2].

Currently more than 70 genetic loci are known; 59 genes have been identified mutations in which cause the development of HSPs with different types of inheritance [3, 4] (http://neuromuscular.wustl.edu/spinal/fsp.html). According to the modern nomenclature, the genetic loci and the corresponding forms of HSP are designated by the SPG abbreviation (Spastic Paraplegia Gene) with sequence numbers in chronological order [5]. Epidemiological and molecular genetic studies of HSP in certain regions and ethnic groups are very relevant, making it possible to develop the most effective approaches of DNA diagnostics and medical genetic counseling in families with this pathology.

Mutations in the spastin gene (*SPAST*) are the most common cause of HSP; they are responsible for 45% of cases of autosomal dominant HSP (AD HSP) and 12–18% of sporadic cases of the disease [6]. Previously, we presented the results of the *SPAST* gene investigations in patients with HSP from the BR, partly carried out by direct sequencing methods (in some exons of the gene) and partly by SSCP analysis followed by sequencing of samples with altered electrophoretic mobility of single-stranded DNA. In the course of these studies, new, previously undescribed mutations p.322del29 (p.Val108SerfsX18), p.885del10 (p.Thr295ThrfsX16), and c.1114A>G (p.Arg372Gly) [7, 8] were identified.

Currently, the total sample of patients with HSP from the BR is represented by members of 63 unrelated families (of which 27 are Tatar; five are Bashkir; 14 are Russian; one family each is Chuvash, Ukrainian, and Mari; eight are metis families; and six families have unidentified ethnicity). In order to continue the search for genetic causes of HSP development in residents of the studied region in three unrelated families with an autosomal dominant form of the disease in probands, targeted exome sequencing was

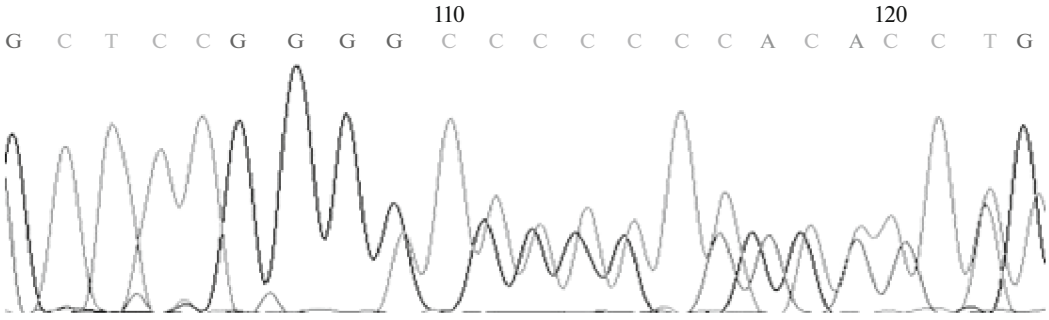

Fig. 1. Sequencing the DNA sample with the c.283delG mutation (p.Ala95Profs*66).

carried out, including the analysis of coding sequences of more than 700 genes responsible for the origin of a number of neurological diseases, including 54 HSP genes.

Sequencing was carried out on the MiSeq system (Illumina). Processing of the sequencing data was carried out using an automated algorithm, including alignment of readings to the reference human genome sequence (hg19), postprocessing of alignment, identification of variants, and filtering of variants by quality, as well as annotation of the identified variants of all known transcripts of each gene from RefSeq using several methods for predicting the pathogenicity of substitutions (SIFT, PolyPhen2-HDIV, PolyPhen2-HVAR, MutationTaster, and LRT), as well as methods for calculating the evolutionary conservatism of positions (PhyloP and PhastCons). To assess the population frequencies of the identified variants, samples of the 1000 Genomes, the ESP6500, and the Exome Aggregation Consortium projects were used.

The presumably pathogenic variant found in the *SPAST* gene was confirmed by Sanger sequencing. Further, screening was conducted for its presence/absence in other family members of the examined patients, then in 63 unrelated families with HSP from Bashkortostan, as well as in the control sample of healthy individuals (60 individuals). In all examined individuals, blood was obtained with their informed consent. The studies were approved by the Bioethics Committee of Institute of Biochemistry and Genetics, Ufa Federal Research Center, Russian Academy of Sciences.

As a result of the study in all three unrelated patients, a single nucleotide heterozygous deletion was identified in the first exon of the *SPAST* gene: c.283delG (p.Ala95Profs*66). The deletion was confirmed by Sanger sequencing (Fig. 1). It was found that this deletion can be identified using *Bsa*JI restriction enzyme, for which the restriction site is lost if there is a mutation. It can also be identified by SSCP analysis or double-stranded DNA electrophoresis in a polyacrylamide gel, having initially obtained fragments short enough for these methods (earlier, in a previous study, we did not reveal changes in the pattern of SSCP analysis of samples with this deletion at a length of the fragment of 320 bp). So, when using the *Msp*I endonuclease, the original amplification product is cut into fragments 128, 124, 35, 22, 6, and 6 bp in size, while the DNA segment with 2883delG deletion falls into the 35 bp fragment. If the deletion is heterozygous, the corresponding fragments of 35 and 34 bp are formed, which are separated by electrophoresis in 8% PAAG. This method was further used to screen for the presence of this deletion in the *SPAST* gene in the families of the patients and in the control sample. In the families of the three patients examined, the c.283delG deletion was detected only in affected relatives. In the total sample of patients with HSP (63 unrelated patients and members of their families), the deletion was detected in patients from 12 unrelated families. It was notable that all these families belong to the Tatar ethnic group. This mutation was not detected during the screening for the presence/absence of the p.283delG deletion in the control group of healthy unrelated individuals of Tatar ethnicity (60 people).

The c.283delG deletion (p.Ala95Profs*66) in the *SPAST* gene was not found in the control samples from 1000 Genomes, ESP6500, and ExAC, but was described in a British family with HSP [9] and is represented in the HGMD mutation database (www.hgmd.org).

The clinical features of all examined patients with p.Ala95Profs*66 mutation corresponded to the uncomplicated form of HSP, which is typical of most cases of the SPG4 form described in a number of studies [6, 9–11].

Spastin protein is a member of the AAA family of proteins, a special class of ATPases with multiple types of cellular activity [12]. The main function of spastin is to ensure the dynamics of the microtubules of the cytoskeleton. Violation of this process result in the development of the SPG4 form disease. As a result of the c.283delG deletion in the first exon of the *SPAST* gene, leading to a reading frame shift, a shortened protein is synthesized, in which the domains that perform the main functions of the protein are absent: MIT domain (116–194 AA), on which the ability of spastin to participate in cytokinesis and endosomal transport

depends [13, 14]; MTBD domain (270–328 AA), which plays an important role in endoplasmic reticulum (ER) morphogenesis [15–18]; ATPase domain (342–599 AA), which is responsible for the disassembly of spastic microtubules, which is one of the main functions of the protein [19].

Studies of the role of spastin in microtubule dynamics showed that mutations leading to a premature termination of protein synthesis lead to HSP owing to a lack of its amount [20], as evidenced by the absence of detectable levels of shortened spastins in cell lines [21–23]. Consequently, haploinsufficiency is the most expected mechanism for explaining the development of spastic paraplegia in the case of the synthesis of shortened protein [6, 24]. In addition, new data appeared indicating the possibility of another negative type of effect of shortened spastins on neurons: truncated spastin M1 isoform, necessary for the interaction of ER tubules and ER with microtubules, is more stable than the truncated M87 isoform and can have a toxic effect on neurons by disrupting axonal retrograde transport and because of the limited ability of neurons to eliminate damaged organelles and proteins [25].

Thus, taking into account all the information described above—the localization of the deletion in the first exon of the gene, which leads to a shift in the reading frame and premature termination of protein synthesis, and the presence of the mutation only in patients with HSP and not in healthy members of the family and in the control group of healthy individuals—one may reliably indicate its pathogenicity. Today more than 680 different mutations (www.hgmd.org) are described in the spastin gene, including those leading to the origin of a premature stop codon and disruption of the synthesis of a full-length protein: these are nonsense mutations [26], various deletions with a reading frame shift [6, 11, 27, 28], duplications, and insertions [26, 29]. However, basically all of the mutations described were identified in separate families with HSP, and in general, major mutations are uncommon for this disease. The c.283delG deletion found by us (p.Ala95Profs*66) was identified in 12 families of Tatar ethnicity; its frequency was 44% among Tatar families and 19% among all unrelated families with the HSP from the BR. The presence of mutations in families of Tatar ethnicity living in the BR can be attributed to the founder effect, which is typical of many known mutations that are frequent among patients with hereditary pathology living in this region. In general, the c.283delG deletion (p.Ala95-Profs*66) in the *SPAST* gene expanded the spectrum of identified mutations in patients with HSP from the Bashkortostan and, being the most frequent (major), makes a significant contribution to the development of the DNA diagnosis algorithm of HSP in this region.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The studies were approved by the Bioethics Committee of Institute of Biochemistry and Genetics, Ufa Federal Research Center, Russian Academy of Sciences. Informed consent was obtained from all individual participants involved in the study.

REFERENCES

1. Harding, A.E., Classification of the hereditary ataxias and paraplegias, *Lancet*, 1983, vol. 321, no. 8334, pp. 1151—1155.
2. Magzhanov, R.V., Saifullina, E.V., Idrisova, R.F., et al., Epidemiology of hereditary spastic paraplegias in Bashkortostan Republic, *Med. Genet.*, 2013, no. 7, pp. 12—16.
3. Novarino, G., Fenstermaker, A.G., and Zaki, M.S., Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders, *Science*, 2014, vol. 343, no. 6170, pp. 506—511. doi 10.1126/science.1247363
4. Klebe, S., Stevanin, G., and Depienne, C., Clinical and genetic heterogeneity in hereditary spastic paraplegias: from SPG1 to SPG72 and still counting, *Rev. Neurol.*, 2015, vol. 171, no. 6, pp. 505—530. doi 10.1016/j.neurol.2015.02.017
5. Fink, J.K., Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms, *Acta Neuropathol.*, 2013, vol. 126, no. 3, pp. 307—328. doi 10.1007/s00401-013-1115-8
6. Fonknechten, N., Mavel, D., Byrne, B., et al., Spectrum of SPG4 mutations in autosomal dominant spastic paraplegia, *Hum. Mol. Genet.*, 2000, vol. 9, no. 4, pp. 637—644.
7. Akhmetgaleeva, A.F., Khidiyatova, I.M., Saifullina, E.V., et al., Two novel mutations in gene *SPG4* in patients with autosomal dominant spastic paraplegia, *Russ. J.*

Genet., 2016, vol. 52, no. 6, pp. 603—607. https://doi.org/. doi 10.1134/S1022795416060028

8. Akhmetgaleeva, A.F., Khidiyatova, I.M., Saifullina, E.V., et al., Clinical case of sporadic spastic paraplegia with a new mutation in the *SPAST* gene, *Med. Genet.*, 2016, vol. 15, no. 7, pp. 11—13.
9. Lindsey, J.C., Lusher, M.E., McDermott, C.J., et al., Mutation analysis of the spastin gene (SPG4) in patients with hereditary spastic paraparesis, *J. Med. Genet.*, 2000, vol. 37, no. 10, pp. 759—765.
10. Hentati, A., Deng, H.X., Zhai, H., et al., Novel mutations in spastin gene and absence of correlation with age at onset of symptoms, *Neurology*, 2000, vol. 55, no. 9, pp. 1388—1390.
11. Basri, R.1., Yabe, I., Soma, H., et al., Four mutations of the spastin gene in Japanese families with spastic paraplegia, *J. Hum. Genet.*, 2006, vol. 51, no. 8, pp. 711—715. doi 10.1007/s10038-006-0412-7
12. Lumb, J.H., Connell, J.W., Allison, R., and Reid, E., The AAA ATPase spastin links microtubule severing to membrane modelling, *Biochim. Biophys. Acta*, 2012, vol. 1823, no. 1, pp. 192—197. doi 10.1016/j.bbamcr.2011.08.010
13. Guizetti, J., Schermelleh, L., Mäntler, J., et al., Cortical constriction during abscission involves helices of ESCRT-III-dependent filaments, *Science*, 2011, vol. 331, no. 6024, pp. 1616—1620. doi 10.1126/science.1201847
14. Allison, R.1., Lumb, J.H., Fassier, C., et al., An ESCRT—spastin interaction promotes fission of recycling tubules from the endosome, *J. Cell Biol.*, 2013, vol. 202, no. 3, pp. 527—543. doi 10.1083/jcb.201211045
15. White, S.R. and Lauring, B., AAA+ ATPases: achieving diversity of function with conserved machinery, *Traffic*, 2007, vol. 8, no. 12, pp. 1657—1667. doi 10.1111/j.1600-0854.2007.00642.x
16. Park, S.H., Zhu, P.P., Parker, R.L., and Blackstone, C., Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network, *J. Clin. Invest.*, 2010, vol. 120, no. 4, pp. 1097—1110. doi 10.1172/JCI40979
17. Blackstone, C., O'Kane, C.J., and Reid, E., Hereditary spastic paraplegias: membrane traffic and the motor pathway, *Nat. Rev. Neurosci.*, 2011, vol. 12, no. 1, pp. 31—42. doi 10.1038/nrn2946
18. Blackstone, C., Cellular pathways of hereditary spastic paraplegia, *Annu. Rev. Neurosci.*, 2012, vol. 35, pp. 25—47. doi 10.1146/annurev-neuro-062111-150400
19. Evans, K.J., Gomes, E.R., Reisenweber, S.M., et al., Linking axonal degeneration to microtubule remodeling by spastin-mediated microtubule severing, *J. Cell. Biol.*, 2005, vol. 168, no. 4, pp. 599—606.
20. Errico, A., Ballabio, A., and Rugarli, E.I., Spastin, the protein, mutated in autosomal dominant hereditary spastic paraplegia, is involved in microtubule dynamics, *Hum. Mol. Genet.*, 2002, vol. 11, no. 2, pp. 153—163.
21. Solowska, J.M. and Baas, P.W., Hereditary spastic paraplegia SPG4: what is known and not known about the disease, *Brain*, 2015, pp. 2471—2484.
22. Rebbapragada, I. and Lykke-Andersen, J., Execution of nonsense-mediated mRNA decay: what defines a substrate?, *Curr. Opin. Cell Biol.*, 2009, vol. 21, no. 3, pp. 394—402. doi 10.1016/j.ceb.2009.02.007
23. Lykke-Andersen, S. and Jensen, T.H., Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes, *Nat. Rev. Mol. Cell Biol.*, 2015, vol. 16, no. 11, pp. 665—677. doi 10.1038/nrm4063
24. Burger J., Fonknechten N., Hoeltzenbein M. et al. Hereditary spastic paraplegia caused by mutations in the SPG4 gene, *Eur. J. Hum. Genet.* 2000, vol. 8, no. 10, pp. 771—776. doi 10.1038/sj.ejhg.5200528
25. Solowska, J.M., Rao, A.N., and Baas, P.W., Truncating mutations of SPAST associated with hereditary spastic paraplegia indicate greater accumulation and toxicity of the M1 isoform of spastin, *Mol. Biol. Cell.*, 2017, vol. 28, no. 13, pp. 1728—1737. doi 10.1091/mbc.E17-01-0047
26. de Bot, S.T., Elzen, R.T., and Mensenkamp, A.R., Hereditary spastic paraplegia due to SPAST mutations in 151 Dutch patients: new clinical aspects and 27 novel mutations, *J. Neurol. Neurosurg. Psychiatry*, 2010, vol. 81, no. 10, pp. 1073—1078. doi 10.1136/jnnp.2009.201103
27. Sauter, S., Miterski, B., Klimpe, S., et al., Mutation analysis of the spastin gene (SPG4) in patients in Germany with autosomal dominant hereditary spastic paraplegia, *Hum. Mutat.*, 2002, vol. 20, no. 2, pp. 127—132. doi 10.1002/humu.10105
28. Magariello, A., Muglia, M., Patitucci, A., et al., Novel spastin (SPG4) mutations in Italian patients with hereditary spastic paraplegia, *Neuromusc. Disord.*, 2006, vol. 16, no. 6, pp. 387—390. doi 10.1016/j.nmd.2006.03.009
29. Crippa, F., Panzeri, C., Martinuzzi, A., et al., Eight novel mutations in SPG4 in a large sample of patients with hereditary spastic paraplegia, *Arch. Neurol.*, 2006, vol. 5, pp. 750—755. doi 10.1001/archneur.63.5.750

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