ISSN 1022-7954, Russian Journal of Genetics, 2022, Vol. 58, No. 9, pp. 1145–1153. © Pleiades Publishing, Inc., 2022.
Russian Text © The Author(s), 2022, published in Genetika, 2022, Vol. 58, No. 9, pp. 1085–1093.

HUMAN GENETICS

Analysis of *ATL1* Gene Mutations and Clinical Features of the Disease Course in Patients with Hereditary Spastic Paraplegia

I. M. Khidiyatova[a, b, c, *], E. V. Saifullina[d], A. S. Karunas[a, b, c], A. F. Akhmetgaleyeva[d], R. F. Kutlubaeva[d], L. A. Smakova[a], S. L. Lobov[a], A. V. Polyakov[e], O. A. Shchagina[e], V. A. Kadnikova[e], O. P. Ryzhkova[e], R. V. Magzhanov[d], and E. K. Khusnutdinova[a, b, c]

[a] *Institute of Biochemistry and Genetics—Subdivision of the Ufa Federal Research Center, Russian Academy of Sciences, Ufa, 450054 Russia*
[b] *Bashkir State University, Ufa, 450076 Russia*
[c] *Saint Petersburg State University, St. Petersburg, 199034 Russia*
[d] *Bashkir State Medical University, Ufa, 450000 Russia*
[e] *Medical Genetic Research Center, Moscow, 115522 Russia*
**e-mail: imkhid@mail.ru*

Received February 1, 2022; revised March 15, 2022; accepted March 21, 2022

Abstract—Hereditary spastic paraplegia (HSP) is a group of neurodegenerative disorders with a predominant lesion of the pyramidal tract. The autosomal dominant form of SPG3A, associated with mutations in the *ATL1* gene, is one of the most common forms of HSP in European populations. Analysis of the *ATL1* gene was performed in 63 unrelated families with HSP from the Republic of Bashkortostan. Two pathogenic variants were identified: one patient had a duplication of the entire third exon, and seven patients from three unrelated families had a missense mutation c.1246C>T (p.Arg416Cys). The frequency of SPG3A among unrelated patients in Bashkortostan was 6.3%. In one of the examined families, the origin of the c.1246C>T *de novo* mutation was established. The clinical symptoms of the disease in most cases correspond to uncomplicated HSP occurring in a mild form. Intrafamilial differences in clinical manifestations of the disease, including in identical twins, were revealed. The age of manifestation in the majority of examined patients ranged from 10 to 50 years.

Keywords: hereditary spastic paraplegia, *ATL1* gene, mutations, MLPA, NGS, Republic of Bashkortostan
DOI: 10.1134/S1022795422090113

INTRODUCTION

Hereditary spastic paraplegia (HSP) is a genetically and clinically heterogeneous group of degenerative diseases of the nervous system caused by distal damage to the long axons of the corticospinal tract [1]. The main clinical manifestation of HSP is spasticity of the muscles of the lower extremities. Spastic paralysis can be combined with various additional symptoms—atrophy of the optic nerves, deafness, ataxia, polyneuropathy, epilepsy, cognitive impairment, etc. Depending on whether the main symptom is the only one or is combined with other neurological or extraneural symptoms, uncomplicated ("pure") or complicated forms of the disease are distinguished [2, 3]. The prevalence of HSP in the world varies from 1.27 to 9.6 per 100000 population [1].

Significant clinical heterogeneity of the disease is associated with a variety of pathogenetic processes that occur in neurons: defects in the formation of membrane organelles, molecular transport, disruption of myelination processes, and mitochondrial functions, which are responsible for mutations in various genes. Currently, more than 100 genes associated with HSP are known [http://www.neuromuscular.wustl.edu, October 2021]. According to modern nomenclature, gene loci and the corresponding forms of HSP are abbreviated as SPG (from the English Spastik Paraplegia Gene), with serial numbers in chronological order [1]. The disease can be inherited in both autosomal dominant and autosomal recessive and X-linked modes of inheritance. In European populations, 40–45% of cases of autosomal dominant HSP are caused by mutations in the spastin gene (*SPAST*, *SPG4*) [4], and 10% of cases are caused by mutations in the atlastin gene (*ATL1*, *SPG3A*) [5]. The protein products of these two genes are involved in many important life processes in the cell and, in particular, they jointly coordinate endoplasmic reticulum morphogenesis and microtubule dynamics, and disruption of these processes is considered one of the main proposed mechanisms of axonal degeneration in HSP [6–11].

The genetic form of SPG3A associated with mutations in the gene *ATL1* is registered in many populations of the world and is considered the third most common among autosomal dominant HSP and the most common cause of early-onset HSP [5, 12–18]. In the gene *ATL1*, localized in the chromosomal region 14q22.1, more than 80 different mutations have been registered to date, 69 of which are missense mutations; small deletions and insertions, splice site mutations, and deletions of entire exons are much less common (HGMD professional 2022.4, http://www.hgmd.cf.ac.uk). However, no clear clinical features of SPG3A have been identified so far; its significant clinical heterogeneity, including intrafamilial, is noted. The only important guide so far is the early age of disease manifestation (<10 years), but it is also not an absolute indicator, and even in the same family with the same mutation in the gene *ATL1* both early and much later onset of the disease can be observed [19]. For some types of mutations of gene *ATL1* such as deletions and insertions, it is not yet possible to establish clinical genetic correlations owing to the rarity of such cases, and information about them is only descriptive. Therefore, the accumulation of data on various genetic variants of HSP, including SPG3A, as well as on their prevalence in various populations, does not lose its relevance. Detailed information about the diversity of gene mutations and their functional role in the development of the disease is the basis for the development of pathogenetic methods of treatment, and information on the prevalence of types of genetically heterogeneous pathologies, such as HSP, and on the spectrum and frequency of mutations in the corresponding genes makes it possible to develop the most effective, optimal for individual regions, approaches to DNA diagnostics, generally improving medical genetic counseling in families of patients.

In the Republic of Bashkortostan, one of the multinational regions of Russia, epidemiological and molecular genetic studies of hereditary spastic paraplegia have been carried out for several years. The overall prevalence of this group of diseases in the republic is 3.5 per 100000 population [20]. Previously, we presented information on individual cases of the genetic form of SPG4 due to mutations in the gene *SPAST*, whose contribution to the overall structure of the disease in Bashkortostan was 33.3% [21–23]. In this paper, we present the results of a study of the gene *ATL1* in patients with HSP from our region.

MATERIALS AND METHODS

The total surveyed sample of patients is represented by 130 individuals from 63 unrelated families, which is about 70% of all patients with HSP registered in the Republic of Bashkortostan. By ethnicity, the sample included 27 Tatar families, 14 Russian families, five Bashkir families, one Chuvash, Ukrainian, and Mari family each, eight mestizo families, and six families with unidentified ethnicity. In 39 families, an autosomal dominant type of inheritance of spastic paraplegia was established; in two, an autosomal recessive one was established; six patients had a sporadic nature of the disease; and for 16 patients, the type of inheritance could not be precisely established. The sample was formed on the basis of data on patients registered at the dispensary at the Republican Medical Genetic Center (RMGC) and the materials of the annual reports of the neurological service of the Republic of Bashkortostan for 2000–2018 provided by the Central District Hospital and the health care facilities of Ufa to the disposal of the Medical Information Analytical Center of the Ministry of Health of the Republic of Bashkortostan. The patients and their closest relatives were examined by the staff of the Department of Neurology with courses in neurosurgery and medical genetics of the Bashkir State Medical University. The control sample consisted of healthy residents of the Republic of Bashkortostan of different ethnicity: Russian (50 people), Tatar (50 people), Bashkir (50 people). DNA was isolated from blood by phenol-chloroform extraction [24].

Analysis of gene *ATL1* was carried out in all 63 unrelated patients, regardless of whether they had previously identified mutations in the spastin gene. Since the sample of patients was formed and studied simultaneously for several years, analysis of gene *ATL1* in different patients was carried out by different methods: in the first 57 unrelated patients, the search for mutations was carried out by the method of conformational polymorphism of single-stranded DNA (SSCP analysis), and in order to detect extended deletions or insertions, it was conducted by the method of multiplex ligase-dependent amplification (MLPA analysis). Massive parallel sequencing using a targeted panel was performed in six "new" patients with autosomal dominant HSP with an unknown genetic cause of the disease, including the analysis of the coding sequences of 63 genes responsible for various types of HSP.

All 14 exons with adjacent intron regions of the *ATL1* gene were studied by SSCP analysis. Amplification of the corresponding DNA fragments was carried out by PCR on T100 Thermal Cycler (Bio Rad, United States) and Thermal Cycler 2720 (Applied Biosystems, United States) amplifiers using oligonucleotide primers presented in [15].

MLPA analysis of gene *ATL1* was performed using the MLPA probemix P165 reagent kit, MRC-Holland. MLPA reactions were performed according to the manufacturer's instructions. Electrophoresis of PCR products was performed using an ABI 3130 genetic analyzer (Applied Biosystems). MLPA data was analyzed in the program coffalyser.net. The software calculates the relative peak heights of samples and controls (peak ratio). A heterozygous exon deletion is indicated by a relative peak height ratio between sample and control below 0.7, while a relative peak

height ratio above 1.3 indicates exon duplication or multiplication.

Targeted exome sequencing was carried out on a new generation sequencer Ion S5. For sample preparation, ultra-multiplex PCR technology coupled with subsequent sequencing (AmpliSeq™) was used. The analysis was carried out using a custom panel Spastic Paraplegia, which includes the coding sequences of the genes: *GJC2*, *AP4B1*, *AMPD2*, *IBA57*, *ALDH18A1*, *ZFYVE27*, *NT5C2*, *ENTPD1*, *MTPAP*, *CAPN1*, *BSCL2*, *KLC2*, *KIF5A*, *C12orf65*, *MARS*, *VAMP1*, *B4GALNT1*, *SPG20*, *SACS*, *ATL1*, *ZFYVE26*, *DDHD1*, *TECPR2*, *AP4S1*, *NIPA1*, *SPG11*, *SPG21*, *AP4E1*, *USP8*, *SPG7*, *FA2H*, *ARL6IP1*, *KIF1C*, *AFG3L2*, *RTN2*, *PNPLA6*, *C19orf12*, *CPT1C*, *MAG*, *HSPD1*, *KIF1A*, *REEP1*, *PGAP1*, *MARS2*, *SPAST*, *SLC33A1*, *TFG*, *WDR48*, *CYP2U1*, *ARSI*, *ZFR*, *REEP2*, *AP5Z1*, *AP4M1*, *CYP7B1*, *KIAA0196*, *ERLIN2*, *VPS37A*, *DDHD2*, *GBA2*, *L1CAM*, *PLP1*, *SLC16A2*. According to AmpliSeq™ Coverage Analysis, the mean coverage of the Spastic Paraplegia Panel is 363.1, Uniformity 92.02%. Sequencing data were processed using a standard automated algorithm offered by Thermo Fisher Scientific (Torrent Suite™) and Gene-Talk software.

To estimate the population frequencies of the identified variants, samples of the 1000 Genomes, ESP6500, and Genome Aggregation Database (gnomAD) projects were used. The OMIM database and HGMD® Professional Pathogen Variant Database, Version 2020.4 was used to evaluate the clinical relevance of identified variants.

To confirm the c.1246C>T mutation identified as a result of NGS sequencing in the gene *ATL1*, in the proband, sequencing of the 12th exon of the gene was conducted by the Sanger method on an ABI PRISM 3130 XL automated sequencer (Applied Biosystems). Screening for the presence/absence of this mutation in six members of the proband's family (a sick son and five healthy relatives), as well as in patients with HSP from 62 unrelated families and in population samples of healthy individuals, was carried out by RFLP analysis using endonuclease *Mwo*I.

RESULTS

As a result of SSCP analysis of 14 exons of the gene *ATL1* conducted in 57 patients with HSP from the Republic of Bashkortostan, no variants were identified that could be regarded as pathogenic or probably pathogenic.

As a result of MLPA analysis performed in the same patients, in one case, in patient G. (33.3) from a Russian family, a duplication of the third exon (dup 3 ex) of the gene *ATL1* was identified in a heterozygous state (Fig. 1).

The disease in the family of patient G. is inherited with a dominant pattern; however, for objective reasons, DNA analysis could not be performed in other sick family members. The first symptoms of HSP in the proband were weakness in the legs and change in gait. In the clinical picture, the gait is changed according to the spastic-atactic type. The strength of the muscles of the legs is reduced (proximal), and muscle tone is increased, while deep sensitivity in the legs is reduced. In addition to these symptoms, the patient has dysfunction of the pelvic organs. All symptoms correspond to the uncomplicated form of the disease. The age of onset of the disease is about 40 years.

As a result of targeted sequencing of a panel of genes in patient K. (51.0) of Tatar ethnicity with autosomal dominant HSP in the 12th exon of the gene *ATL1*, missense variant c.1246C>T (p.Arg416Cys) was identified in the heterozygous state, confirmed by Sanger sequencing (Fig. 2).

This nucleotide variant is a known pathogenic mutation; it was previously described in patients with HSP [25–27]. This mutation can be identified by RFLP analysis using endonucleases *Ngo*MIV, *Cac*8I, *Nae*I, and *Mwo*I, for which the normal restriction site disappears. By this method, using restrictase *Mwo*I (Fig. 3), we searched for the c.1246C>T mutation in other members of the proband's family, in other unrelated patients of the total surveyed sample, and in control samples of healthy individuals (150 people).

In the family of the patient K. (51.0), nucleotide substitution c.1246C>T in the gene *ATL1* was also detected in his sick son and was not found in healthy family members—brother, sister, daughter, and none of the parents. On the basis of the absence of a mutation in the parents, it was assumed that the proband had a mutation *de novo*. The conducted microsatellite analysis confirmed the consanguinity of the proband with both parents, which confirmed this assumption.

As a result of screening for the presence of the c.1246C>T mutation in other patients from the examined sample, it was detected in two more unrelated families—in patient L. (16.0) of Russian ethnicity, in his mother, and in his son, and in two sisters—twins Ch. (41.01 and 41.02) of mestizo origin (Mordva/Tatar). In control population samples of healthy individuals, this mutation was not detected.

In all patients available for study from three families with a mutation p.1246C>T, the analysis of the clinical picture of HSP was carried out.

Proband 51.0, born in 1967, from the age of 10–12 noted difficulties in walking and running fast, associated these complaints with the structure of the feet ("flat feet"). At the end of the second or beginning of the third decade of the patient's life, people around him began to notice a change in his gait: shuffling feet. Later, closer to the age of 40, the patient himself began to clearly feel stiffness in the muscles of the legs, leading to a change in gait and fatigue during long walking. An objective examination of the patient revealed an increase in muscle tone, mainly in the flexors of the

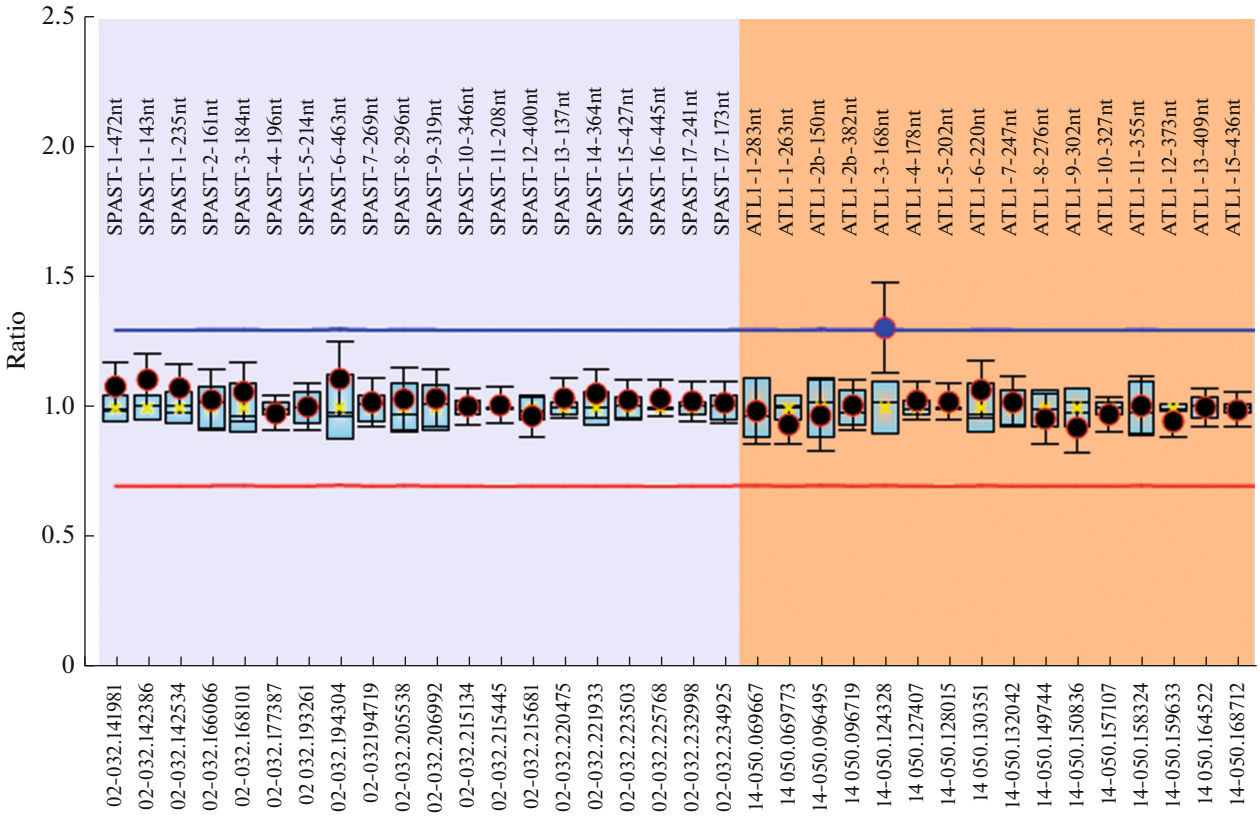

Fig. 1. Results of MLPA analysis after processing in Cofalyser.net: identification of duplication of the third exon of the gene *ATL1* (probe ATL1-3-168nt).

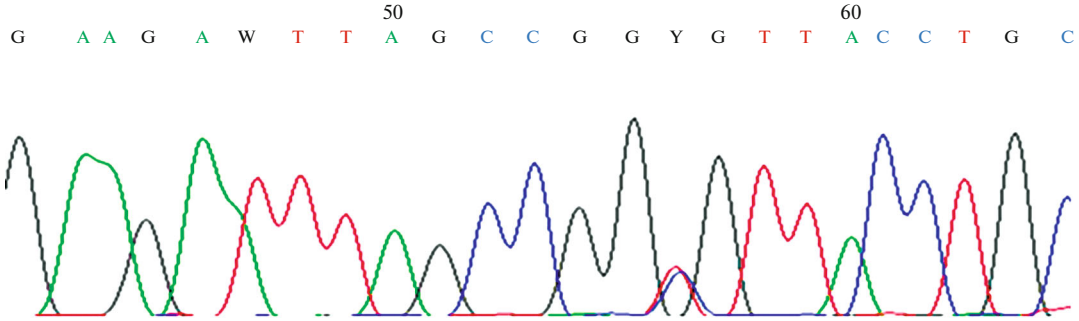

Fig. 2. Sequencing of the 12th exon of the gene *ATL1* in a patient with HSP: nucleotide substitution c.1246C>T in the heterozygous state.

legs and feet according to the spastic type up to three points on the Ashworth scale, and a decrease in muscle strength up to four points in the proximal sections of legs. "Hollow" feet. Tendon reflexes in the legs are increased; clonuses of both feet and pathological foot signs of flexion and extensor types are noted. He walks independently, his gait is changed according to the "skier" type. The son of the proband, born in 1990, clinically examined at the age of 30, has a similar change in gait and an increase in muscle tone in the legs up to one point, as well as tendon reflexes from the legs, clonuses of both feet, and pathological foot signs.

In the family of L. under our supervision were the proband (16.0), his mother, and his son. The proband noticed the first signs of the disease at the age of 20, when he began to notice a change in gait due to a feeling of stiffness in the legs. When examining the patient at the age of 30 years, the clinical symptoms corresponded to uncomplicated spastic paraplegia: lower spastic paraparesis, hyperreflexia of tendon reflexes,

positive foot and hand pathological reflexes, a slight decrease in vibration sensitivity in the legs, dysfunction of the pelvic organs by the type of urinary retention. The mother of the proband had no active complaints at the time of examination (at the age of 56 years), her gait was not changed, but tendon reflexes from the legs were increased and extensor foot pathological reflexes were noted. The son of the proband was examined at the age of 16, he had no active health complaints, his gait was not changed, the muscle tone in the legs was satisfactory, but there was tendon hyperreflexia from the legs. Thus, the disease in the family is represented by an uncomplicated form with variable expressivity.

In the identical twin sisters Ch. (41.01 and 41.02), there was a difference both in the age of disease manifestation and in the spectrum of clinical symptoms. Unfortunately, it was not possible to examine the parents or other siblings and the sisters' children for objective reasons. In the first sister, a change in gait appeared at the age of 43–44 years; the clinical picture at the age of 46 corresponded to an uncomplicated form of the disease; in the second sister, complaints of gait changes were noted from the age of 49–50 years; clinical symptoms at the age of 53 included, in addition to lower spastic paraparesis, a slight decrease in vibration sensitivity in the legs and a moderate decrease in cognitive functions. Electrophysiological parameters (speed of propagation of excitation and amplitude of M-responses along the peripheral nerves of the arms and legs) were within normal limits; neuroimaging parameters of the spinal cord were normal; and according to MRI of the brain, there were signs of microangiopathy and initial manifestations of the atrophic process. To clarify the relationship between cognitive decline and the underlying disease, further examination of both the patient herself and her identical twin sister is required.

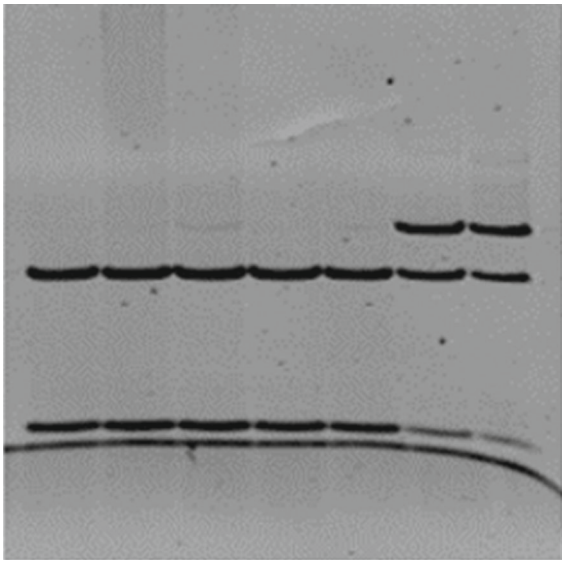

Fig. 3. Sample of identification of the mutation c.1246C>T (p.Arg416Cys) in the gene *ATL1* by RFLP analysis using endonuclease *Mwo*I (7% PAAG): (*1–5*) DNA samples without mutation; (*6*, *7*) DNA samples with a mutation in the heterozygous state.

DISCUSSION

In our sample of patients with HSP from the Republic of Bashkortostan, in four out of 63 unrelated families, two different changes in the nucleotide sequence were identified in the gene *ATL1*: in one family, a previously undescribed duplication of the third exon of the gene; in three families, a known missense mutation c.1246C>T (p.Arg416Cys). In all these families, an autosomal dominant type of inheritance of HSP was established. A patient with a duplication of the third exon had an uncomplicated form of the disease, accompanied by dysfunction of the pelvic organs, which manifested itself at the age of 40 years. In seven examined patients from three families with the p.Arg416Cys mutation, the age of disease manifestation varied from 10 to 50 years; in most of them the clinical picture corresponded to an uncomplicated form of the disease; in one case, it was combined with dysfunction of the pelvic organs; in one patient, the main clinical symptoms of HSP were complicated by moderate cognitive impairment. The severity of the disease in most of our HSP patients was mild, and almost asymptomatic cases have been noted (2/7—28.6%).

As already mentioned, with SPG3A, the most common mutations in the gene *ATL1* are missense variants, while deletions or insertions are much less common, and in a single case, an exon deletion is described: Sulek et al. [28] screened 93 patients for the presence of extended deletions/duplications in genes *SPAST* and *ATL1* using MLPA analysis, revealing 11 different deletions and one duplication in the spastin gene and a deletion of the fourth exon in the atlastin gene. About the complete deletion of one allele of the gene *ATL1* in a heterozygous state, combined with a deletion of an exon of gene *SPAST*, Beetz et al. [29] previously reported, at the same time assuming that the disease in the patient examined by them was caused by a mutation of the gene *SPAST* rather than a deletion of the whole gene *ATL1*. The transmembrane protein atlastin-1 encoded by the gene *ATL1* belongs to the dynamin subfamily of large GTPases and has an N-terminal GTP-binding domain and two closely spaced hydrophobic segments at the C-terminus, which form a transmembrane domain [8, 9]. The third exon of the gene *ATL1* corresponds to the GTPase domain of the protein. This region contains catalytically active glutamic acid at the 117th position, which is involved in the hydrolysis of GTP [30]. To date, autosomal dominant cases of the disease associated

with pathogenic variants in the third exon of the gene are unknown, and only two mutations, p.R118Q and p.R217*, have been described, identified in the homozygous state in families with an autosomal recessive form of HSP [31, 32]. Both of these mutations are located in a conserved nucleotide-binding site GTPase domain and are expected to result in loss of gene function in a manner that does not interfere with the wild-type allele. All patients from families with these mutations had an early age of manifestation—the first year of life, an uncomplicated form of HSP, with the development of pelvic organ dysfunction, in particular, urinary incontinence, in the third decade of life [31, 32]. Most of the clinical signs described in sick members of these families coincide with those found in our patient with a duplication of the third exon, except for her much later age of HSP manifestation—about 40 years. On the basis of information about the functional significance of this region of the gene, it can be assumed that the extended duplication of the third exon of gene *ATL1*, which we identified in the heterozygous state in a patient with HSP, can have a significant negative effect on the GTPase function of atlastin, when the compensation of the function due to the wild-type allele is incomplete. This effect of mutation can be associated with different mechanisms. If the duplication of an exon is an insertion of a nucleotide sequence by the number of nucleotides not multiple of three, then a shift in the reading frame is obvious, disrupting the entire coding sequence of the gene, usually leading to the formation of a premature stop codon and, as a result, to nonsense-mediated protein destruction. If the number of nucleotides of the insert is a multiple of three, then the presumably negative effect can be associated with a significant size of its coding part and with a high probability that this part will contain other amino acids, since the beginning of the exon does not always coincide with the triplet. However, these conclusions are conjectural and require further research, since the mutation was detected by MLPA analysis, which does not allow us to establish the exact boundaries of the insertion.

Nucleotide substitution c.1246C>T (p.Arg416Cys) in the gene *ATL1* was previously described by Orlacchio et al. [25], Magariello et al. [26], and Luo et al. [27] as a pathogenic mutation causing HSP of the SPG3A form with an autosomal dominant type of inheritance. The clinical picture of HSP in patients presented in these works is somewhat different. For example, Orlacchio et al. [25] identified this mutation in ten patients with HSP AD from the same South African family: all of them had a late age of disease manifestation (from 39 to 51 years), and all of them had mental retardation (IQ from 32 to 67).

Magariello et al. [26] identified the same mutation in a patient with HSP, in whom the disease was first diagnosed at the age of 40; however, the patient herself noted its first signs in the form of difficulties in running, cramps in the lower extremities, and lumbar pain from an earlier age, after puberty. The disease in this patient corresponded to uncomplicated HSP; in particular, no cognitive impairment was detected in her. According to Luo et al. [27], in one Chinese family with HSP caused by the mutation p.Arg416Cys, most of the sick family members developed the disease in an uncomplicated form before the age of 10 years, but in some of them at a later age. Summarizing the above information, we can conclude that HSP caused by the c.1246C>T mutation in the 12th exon of the gene *ATL1* can manifest itself both at an early age (before 10 years) and at a much later age (often after 40 years). In most cases, HSP corresponds to the uncomplicated type, in which sometimes there may be a decrease in vibration sensitivity in the legs or signs of pelvic dysfunction in the form of difficulty urinating or urinary incontinence; less commonly, in carriers of this mutation, spastic paraplegia may be complicated by cognitive impairment. According to clinical manifestations, both interfamilial and intrafamilial variability can be observed, even in the case of identical twins, which may indicate the role of epigenetic factors in the development and course of the disease. In one of our families, the origin of the c.1246C>T mutation was established *de novo*, which confirms the existing assumption about the presence of a "hot spot" in the 12th exon of the gene. Also, along with the 12th exon, exons 4, 7, and 8 of the gene *ATL1* are considered "hot," and the majority (87.32%) of known mutations in the gene occur in these exons [19].

Zhao and Liu [19] conducted a generalized analysis of genophenotypic correlation based on data on patients with SPG3A from 142 families presented in 51 publications. The authors showed that, in 84.79% of patients with mutations in the gene *ATL1*, an early form of SPG3A is observed, with age of onset <10 years, and 15.21% have a later (>10 years) form of the disease. This circumstance, which is also confirmed by our data, emphasizes the need for analysis of gene *ATL1* not only in the early forms of HSP. In addition, it was shown that both interfamilial and intrafamilial heterogeneity can be observed in terms of the age of onset of the disease, even with the same mutation (in particular, with p.A161P and p.R495W mutations [33, 34]). Comparison of clinical data in the same study did not reveal any genotype–phenotype correlation for various mutations in the gene *ATL1* [19]. Of the 440 patients for whom information was available in the study of these authors, 378 patients (85.90%) had a "pure" form of HSP and 62 (14.10%) had a complicated form of the disease. Complicating symptoms in patients with SPG3A spastic paraplegia included seizures, optic nerve atrophy, sensory impairment, mental retardation, ataxia, distal atrophy, and peripheral axonal neuropathy. At the same time, the authors found that distal atrophy is the most common symptom in patients with complicated forms of SPG3A. Reduced vibrational sensitivity in the legs and dysfunction of the pelvic organs, which are included in

the clinical picture of the uncomplicated form of the disease and were detected in the L. family examined by us, are much less common with SPG3A than with other forms of AD HSP [19].

The severity of the disease in most patients with SPG3A is noted as mild, but the degree of spasticity increases with the duration of the disease. The penetrance of the disease is 80–90%, but in a number of family cases in heterozygous carriers of a mutation in the gene *ATL1* in old age, the neurological status remains normal, which testifies against age-dependent penetrance [15]. The lowest percentage of penetrance (30%) was described in a family with a mutation in the adjacent, 415th, codon of the gene *ATL1*: c.1243C>T (p.Arg415Trp) [35].

In general, for mutations in the gene *ATL1*, it is still difficult to draw clear genophenotypic correlations, but the accumulation of data on specific pathogenic variants can contribute to the understanding of such relationships. In particular, the data we obtained indicate a later age of HSP manifestation and its relatively mild course with mutations in exons 3 and 12 identified in our patients and confirm the information about the incomplete penetrance of the c.1246C>T mutation and the existence of a hot spot in the 12th exon. Our population study also demonstrates the heterogeneity of the distribution of individual genetic forms of HSP in different regions. In the total sample of 63 probands with HSP, representing approximately 70% of all unrelated families of patients registered in the Republic of Bashkortostan, the contribution of the SPG3 genetic form to the structure of the disease was 6.3%, and the frequency of the c.1246C>T (p.Arg416Cys) mutation was 4.7%. These data complement the overall picture of the spectrum and frequency of pathogenic variants leading to the development of HSP in patients in the region under study and contribute to an increase in the effectiveness of genetic counseling in the families of patients.

FUNDING

This work was carried out within the framework of the State Order of the Ministry of Education and Science of the Russian Federation (no. AAAA-A16-116020350032-1), with partial financial support from St. Petersburg State University (project no. 93025749), a grant of the Russian Foundation for Basic Research (no. 17-44-020951), and a Megagrant of the Government of the Russian Federation (agreement no. 075-15-2021-595).

ACKNOWLEDGMENTS

For the study, the equipment of the Center for Collective Use Biomika (Department of Biochemical Research Methods and Nanobiotechnology of the Republican Center for Collective Use Agidel) and UNU KODINK was used. The DNA samples for the study were taken from the "Collection of Human Biological Materials" of the Institute of Biochemistry and Genetics, Ufa Federal Research Center of the Russian Academy of Sciences, supported by the Program of Bioresource Collections of FASO of Russia (agreement no. 007-030164/2).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in a study involving people comply with the ethical standards of the institutional and/or national committee for research ethics and the 1964 Helsinki Declaration and its subsequent changes or comparable ethical standards. The studies were approved by the bioethics committee of the Institute of Biochemistry and Genetics, Ufa Federal Research Center of the Russian Academy of Sciences.

Informed consent was obtained from each of the participants included in the study.

REFERENCES

1. Fink, J.K., Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms, *Acta Neuropathol.*, 2013, vol. 126, no. 3, pp. 307—328. https://doi.org/10.1007/s00401-013-1115-8
2. Hazan, J., Fonknechten, N., Mavel, D., et al., Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia, *Nat. Genet.*, 1999, vol. 23, no. 3, pp. 296—303. https://doi.org/10.1038/15472
3. Finsterer, J., Löscher, W., Quasthoff, S., et al., Hereditary spastic paraplegias with autosomal dominant, recessive, X-linked, or maternal trait of inheritance, *J. Neurol. Sci.*, 2012, vol. 318, no. 1, pp. 1—18. https://doi.org/10.1016/j.jns.2012.03.025
4. 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. https://doi.org/10.1093/hmg/9.4.637
5. Namekawa, M., Nelson, I., Ribai, P., et al., A founder effect and mutational hot spots may contribute to the most frequent mutations in the SPG3A gene, *Neurogenetics*, 2006, vol. 7, no. 2, pp. 131—132. https://doi.org/10.1007/s10048-006-0028-2
6. Zhu, P.P., Soderblom, C., Tao-Cheng, J.H., et al., SPG3A protein atlastin-1 is enriched in growth cones and promotes axon elongation during neuronal development, *Hum. Mol. Genet.*, 2006, vol. 15, no. 8, pp. 1343—1353. https://doi.org/10.1093/hmg/ddl054
7. Namekawa, M., Muriel, M.P., Janer, A., et al., Mutations in the SPG3A gene encoding the GTPase atlastin interfere with vesicle trafficking in the ER/Golgi interface and Golgi morphogenesis, *Mol. Cell. Neurosci.*,

2007, vol. 35, no. 1, pp. 1—13.
https://doi.org/10.1016/j.mcn.2007.01.012

8. Rismanchi, N., Soderblom, C., Stadler, J., et al., Atlastin GTPases are required for Golgi apparatus and ER morphogenesis, *Hum. Mol. Genet.*, 2008, vol. 17, no. 11, pp. 1591—1604.
https://doi.org/10.1093/hmg/ddn046
9. Hu, J., Shibata, Y., Zhu, P.P., et al., A class of dynamin-like GTPases involved in the generation of the tubular ER network, *Cell*, 2009, vol. 138, no. 3, pp. 549—561.
https://doi.org/10.1016/j.cell.2009.05.025
10. Orso, G., Pendin, D., Liu, S., et al., Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin, *Nature*, 2009, vol. 460, no. 7258, pp. 978—983.
https://doi.org/10.1038/nature08280
11. 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.
https://doi.org/10.1172/JCI40979
12. Zhao, X., Alvarado, D., Rainier, S., et al., Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia, *Nat. Genet.*, 2001, vol. 29, no. 3, pp. 326—331.
https://doi.org/10.1038/ng758
13. Tessa, A., Casali, C., Damiano, M., et al., SPG3A an additional family carrying a new atlastin mutation, *Neurology*, 2002, vol. 59, no. 12, pp. 2002—2005
https://doi.org/10.1212/01.wnl.0000036902.21438.98
14. Abel, A., Fonknechten, N., Hofer, A., et al., Early onset autosomal dominant spastic paraplegia caused by novel mutations in SPG3A, *Neurogenetics*, 2004, vol. 5, no. 4, pp. 239—243.
https://doi.org/10.1007/s10048-004-0191-2
15. Dürr, A., Camuzat, A., Colin, E., et al., Atlastin1 mutations are frequent in young-onset autosomal dominant spastic paraplegia, *Arch. Neurol.*, 2004, vol. 61, pp. 1867—1872.
https://doi.org/10.1001/archneur.61.12.1867
16. Hedera, P., Fenichel, G.M., Blair, M., and Haines, J.L., Novel mutation in the SPG3A gene in an African American family with an early onset of hereditary spastic paraplegia, *Arch. Neurol.*, 2004, vol. 61, no. 10, pp. 1600—1603.
https://doi.org/10.1001/archneur.61.10.1600
17. Sauter, S.M., Engel, W., Neumann, L.M., et al., Novel mutations in the atlastin gene (SPG3A) in families with autosomal dominant hereditary spastic paraplegia and evidence for late onset forms of HSP linked to the SPG3A locus, *Hum. Mutat.*, 2004, vol. 23, no. 1, p. 98.
https://doi.org/10.1002/humu.9205
18. Erfanian Omidvar, M., Torkamandi, S., Rezaei, S., et al., Genotype—phenotype associations in hereditary spastic paraplegia: a systematic review and meta-analysis on 13570 patients, *J. Neurol.*, 2021, vol. 268, pp. 2065—2082.
https://doi.org/10.1007/s00415-019-09633-1
19. Zhao, G.-H. and Liu, X.-M., Clinical features and genotype—phenotype correlation analysis in patients with ATL1 mutations: a literature reanalysis, *Transl. Neurodegen.*, 2017, vol. 6, no. 1, p. 9.
https://doi.org/10.1186/s40035-017-0079-3
20. 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.
21. Akhmetgaleeva, A.F., Khidiyatova, I.M., Saifullina, E.V., et al., Two novel mutations in gene *SPG4* in patients with autosomal dominant spastic paraplegia from the Republic of Bashkortostan, *Russ. J. Genet.*, 2016, vol. 52, no. 6, pp. 603—607.
https://doi.org/10.1134/S1022795416060028
22. 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.
23. Khidiyatova, I.M., Akhmetgaleyeva, A.F., Saifullina, E.V., et al., Major mutation in the *SPAST* gene in patients with autosomal dominant spastic paraplegia from the Republic of Bashkortostan, *Russ. J. Genet.*, 2019, vol. 55, no. 2, pp. 259—262.
https://doi.org/10.1134/S1022795419020091
24. Mathew, C.G.P., The isolation of high molecular weight eukaryotic DNA, *Nucleic Acids Res.*, 1984, pp. 31—34.
25. Orlacchio, A., Montieri, P., Babalini, C., et al., Late-onset hereditary spastic paraplegia with thin corpus callosum caused by a new SPG3A mutation, *J. Neurol.*, 2011, vol. 258, no. 7, pp. 1361—1363.
https://doi.org/10.1007/s00415-011-5934-z
26. Magariello, A., Tortorella, C., Citrigno, L., et al., The p.Arg416Cys mutation in SPG3a gene associated with a pure form of spastic paraplegia, *Muscle Nerve*, 2012, vol. 45, no. 6, pp. 919—920.
https://doi.org/10.1002/mus.23360
27. Luo, Y., Chen, C., Zhan, Z., et al., Mutation and clinical characteristics of autosomal-dominant hereditary spastic paraplegias in China, *Neurodegen. Dis.*, 2014, vol. 14, no. 4, pp. 176—183.
https://doi.org/10.1159/000365513
28. Sulek, A., Elert, E., Rajkiewic, M., et al., Screening for the hereditary spastic paraplaegias SPG4 and SPG3A with the multiplex ligation-dependent probe amplification technique in a large population of affected individuals, *Neurol. Sci.*, 2012, vol. 34, no. 2, pp. 239—242.
https://doi.org/10.1007/s10072-011-0899-3
29. Beetz, C., Nygren, A.O.H., Deufel, T., and Reid, E., An SPG3A whole gene deletion neither co-segregates with disease nor modifies phenotype in a hereditary spastic paraplegia family with a pathogenic SPG4 deletion, *Neurogenetics*, 2007, vol. 8, pp. 317—318.
https://doi.org/10.1007/s10048-007-0099-8
30. Bian, X., Klemm, R.W., Liu, T.Y., et al., Structures of the atlastin GTPase provide insight into homotypic fusion of endoplasmic reticulum membranes, *Proc. Natl.*

Acad. Sci. U.S.A., 2011, vol. 108, no. 10, pp. 3976—3981.
https://doi.org/10.1073/pnas.1101643108

31. Khan, T.N., Klar, J., Tariq, M., et al., Evidence for autosomal recessive inheritance in SPG3A caused by homozygosity for a novel ATL1 missense mutation, *Eur. J. Hum. Genet.*, 2014, vol. 22, pp. 1180—1184.
https://doi.org/10.1038/ejhg.2014.5

32. Willkomm, L., Heredia, R., Hoffmann, K., et al., Homozygous mutation in atlastin GTPase 1 causes recessive hereditary spastic paraplegia, *J. Hum. Genet.*, 2016, vol. 61, no. 6, pp. 571—573.
https://doi.org/10.1038/jhg.2016.6

33. Scarano, V., Mancini, P., Criscuolo, C., et al., The R495W mutation in SPG3A causes spastic paraplegia associated with axonal neuropathy, *J. Neurol.*, 2005, vol. 252, pp. 901—903.
https://doi.org/10.1007/s00415-005-0768-1

34. Ivanova, N., Claeys, K.G., Deconinck, T., et al., Hereditary spastic paraplegia 3A associated with axonal neuropathy, *Arch. Neurol.*, 2007, vol. 64, pp. 706—713.
https://doi.org/10.1001/archneur.64.5.706

35. D'Amico, A., Tessa, A., Sabino, A., et al., Incomplete penetrance in an SPG3A-linked family with a new mutation in the atlastin gene, *Neurology*, 2004, vol. 62, pp. 2138—2139.
https://doi.org/10.1212/01.wnl.0000127698.88895.85