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Disease-Causing Variants in the *ATL1* Gene Are a Rare Cause of Hereditary Spastic Paraplegia among Czech Patients

Anna Uhrová Mészárosová[1]*, Dagmar Grečmalová[2], Michaela Brázdilová[3], Nina Dvořáčková[2], Zdeněk Kalina[4], Marie Čermáková[5], Dagmar Vávrová[5], Irena Smetanová[5], David Staněk[1] and Pavel Seeman[1,5]

[1]*DNA Laboratory, Department of Child Neurology, Charles University 2nd Medical School and University Hospital Motol, Prague, Czech Republic*

[2]*Department of Medical Genetics, Faculty Hospital Ostrava, Ostrava, Czech Republic*

[3]*Department of Child Neurology, Charles University 2nd Medical School and University Hospital Motol, Prague, Czech Republic*

[4]*Department of Medical Genetics, Faculty Hospital Brno, Brno, Czech Republic*

[5]*Centre for Medical Genetics and Reproductive Medicine GENNET, Prague, Czech Republic*

Summary

Variants in the *ATL1* gene have been repeatedly described as the second most frequent cause of hereditary spastic paraplegia (HSP), a motor neuron disease manifested by progressive lower limb spasticity and weakness. Variants in *ATL1* have been described mainly in patients with early onset HSP. We performed Sanger sequencing of all coding exons and adjacent intron regions of the *ALT1* gene in 111 Czech patients with pure form of HSP and additional Multiplex-Ligation Probe Analysis (MLPA) testing targeting the *ATL1* gene in 56 of them. All patients except seven were previously tested by Sanger sequencing of the *SPAST* gene with negative results. *ATL1* diagnostic testing revealed only five missense variants in the *ATL1* gene. Four of them are novel, but we suppose only two of them to be pathogenic and causal. The remaining variants are assumed to be benign. MLPA testing in 56 of sequence variant negative patients revealed no gross deletion in the *ATL1* gene. Variants in the *ATL1* gene are more frequent in patients with early onset HSP, but in general the occurrence of pathogenic variants in the *ATL1* gene is low in our cohort, less than 4.5% and less than 11.1% in patients with onset before the age of ten. Variants in the *ATL1* gene are a less frequent cause of HSP among Czech patients than has been previously reported among other populations.

Keywords: ATL1, SPG3A, hereditary spastic paraplegia, HSP

Introduction

Hereditary spastic paraplegia type 3A (SPG3A) is an autosomal dominantly inherited type of spastic paraplegia (HSP) characterized by progressive bilateral and mostly symmetric spasticity and weakness of the legs, reduced vibration sense and urinary bladder hyperactivity caused by degeneration of the corticospinal tracts and dorsal columns. More than 80% of reported individuals manifest spastic gait before the end of the first decade of life. The progression is slow, life expectancy is not limited, and wheelchair dependency or need for an assistive walking device is rather rare. The clinical findings in SPG3A tend to be more homogeneous than other forms of AD HSP (Zhao et al., 2001; Durr et al., 2004; Hedera et al., 2004). Most patients with early-onset SPG3A have a pure form of HSP; however, a few complicated HSP cases (with mental retardation, optic atrophy, scoliosis, etc.) have been reported (Haberlova et al., 2008; Yonekawa et al., 2014). Variability in phenotype and the age at onset concerning *ATL1* variants within one family have been observed (Smith et al., 2009). More than 95% of individuals diagnosed with SPG3A may have an affected parent (Hedera,

*Corresponding author: ANNA UHROVA MESZAROSOVA, DNA Laboratory, Department of Child Neurology, Charles University 2nd Medical School and University Hospital Motol, V Uvalu 84, 150 06 Prague, Czech Republic. Tel: +420 224 436 789; E-mail: anna.meszarosova@lfmotol.cuni.cz

2010). The proportion of sporadic patients is not known. Recently, a family with SPG3A with presumed autosomal recessive inheritance was reported by Khan et al. (2014). Such inheritance is extremely rare and has not been yet confirmed. SPG3A is caused by heterozygous variants in the *ATL1* gene (14q22.1), coding atlastin-1, a dynamin-related GTPase involved in the formation of tubular endoplasmatic reticulum network and axon elongation (Hu et al., 2009; Park et al., 2010). To date, more than 50 pathogenic/likely pathogenic variants (mainly missense, rarely splice and frame-shift mutation, no nonsense) and only one gross deletion have been described in the *ATL1* gene (Sulek et al., 2013). Variants in the *ATL1* gene have been described not only in spastic paraplegia but also rarely in very variable phenotypes, in patients with axonal motor neuropathy (Fusco et al., 2010; Al-Maawali et al., 2011; Leonardis et al., 2012) and hereditary sensory neuropathy (Guelly et al., 2011). According to the population databases ExAC (http://exac.broadinstitute.org/) and the Exome Variant Server (EVS) (http://evs.gs.washington.edu), a few benign missense polymorphisms also exist in this gene in the healthy population.

Patients and Methods

Patients

In total, all coding exons and adjacent intron regions of the *ATL1* gene were Sanger sequenced in 111 independent Czech patients suspected of having the pure form of HSP. No patients with clearly complicated form of spastic paraplegia were included in this study. There was no consanguinity described in patients' families. Patients were clinically sent for DNA diagnostics of HSP by different neurologists and geneticists in the Czech Republic. Examination of the *ATL1* gene was performed within the routine diagnostics of HSP patients. All but seven patients were previously tested by *SPAST* (*SPG4*) gene Sanger sequencing with negative results. Our cohort consisted of 61 sporadic patients (54.9%) and 24 familial cases with affected relatives (21.6%). In the remaining 26 cases (23.4%), we did not have enough information about the family history. Detailed patient age distribution in each group was the following: among 61 sporadic patients, 25 patients were under the age of 20 years (14 were under 10 years), among 24 familial patients 11 were under the age of 20 years (7 were under 10 years), and among 26 patients with unknown family history 10 were under the age of 20 years (6 were under 10 years). In total, 27 of our patients (24.3%; 27/111) were younger than 10 years, and together 46 patients (41.4%; 46/111) in total were younger than 20 years at the time of examination. (The age of examination did not necessarily correlate with the age of first symptoms. We did not have enough information in all cases, but in all patients the disease symptoms were present at the age of examination). Moreover, a subgroup of 56 independent patients from the whole cohort of 111 patients was additionally tested by Multiplex-Ligation Probe Analysis (MLPA) targeting the *ATL1* (and *SPAST*) gene. All these patients were *SPAST*-negative using Sanger sequencing and MLPA. From these, 29 (51.7%) were sporadic cases, 20 patients (35.7%) were familial, and the family history was unknown in the remaining 7 (12.5%) patients. Fifteen patients who were MLPA tested (26.8%) were younger than 10 years; all together 29 (51.8%) were younger than 20 years at the time of examination. All patients or their parents signed an informed consent with DNA testing for HSP.

Mutation Analysis

Genomic DNA was extracted from peripheral blood (in one case from saliva as well) according to a standard protocol. PCR products (primer sequences available upon request) were purified (FastAP/ExoI, Thermo Scientific, Waltham, MA, USA). Sanger sequencing was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). MLPA testing was performed with the P-165 Kit targeting both the *ATL1* and *SPAST* genes (MRC Holland, Amsterdam, The Netherlands). Products of Sanger sequencing and MLPA were analyzed on a Genetic Analyzer ABI 3130 (Applied Biosystems, Foster City, CA, USA). Sequencing Analysis v5.4 software (Applied Biosystems, Foster City, CA, USA) and Coffalyzer software (MRC Holland, Amsterdam, The Netherlands) were used for evaluation of the chromatograms. Human Genome Variation Society (www.hgvs.org) nomenclature and NM_181598.3 were used for assigning the mutations. Pathogenicity of detected mutations was first analyzed *in silico* using Alamut Software (Interactive Biosoftware, Rouen, France) with prediction programs Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (www.mutationtaster.org) and SIFT (http://sift.jcvi.org/) incorporated. Data about nucleotide conservation (phyloP and PhastCons), amino acid conservation and amino acid biochemical difference (Grantham distance) are automatically computed by Alamut Software (Interactive Biosoftware, Rouen, France). In three cases with revealed variants, we had DNA from other family members available and were able to perform segregation analysis in the family. To compare frequencies of revealed variants, we used frequencies available from the following public databases: Exome Variant Server (EVS) (http://evs.gs.washington.edu), ExAC (http://exac.broadinstitute.org/), dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), and 1000 Genomes (http://www.1000genomes.org).

Table 1 Patients with detected variant in the *ATL1* gene among 111 independent Czech HSP patients.

Patient number	Sex	Age at onset/Age of DNA diagnosis	Nucleotide change	Codon change	Protein change	Exon number	Novelty	Family status
1	F	52/56	**c.311A>G**	**AAT>AGT**	**p.Asn104Ser**	4	rs377736535	Sporadic
2	M	?/ 13 years	**c.505A>G**	**ATG>GTG**	**p.Met169Val**	5	Novel	Sporadic
3	M	?/3 years	**c.916A>G**	**AGT>GGT**	**p.Ser306Gly**	10	Novel	Sporadic
4	F	9 months/2 years	**c.1039A>C**	**ATG>CTG**	**p.Met347Leu**	11	Novel	Familial
5	F	6 months/1 year	**c.1064A>T**	**AAC>ATC**	**p.Asn355Ile**	12	Novel	Sporadic

Results

We found suspicious variants in the *ATL1* gene in 5 independent patients among the entire cohort of 111 examined patients (4.5%). All patients with variants except one were sporadic, which represents 6.6% among sporadic patients (4/61). The remaining one patient was familial, which is 4.2% among familial patients (1/24). All detected variants are missense (Table 1), four variants are novel, not yet reported and not present in public databases (ExAC, EVS, dbSNP, 1000 Genomes). Three patients with the *ATL1* variant were under 10 years (even under 5 years) of age. Concerning the age at onset, variants in the *ATL1* gene were found in total in 11.1 % (3/27) among the patients under 10 years and in 8.7% (4/46) among the patients under 20 years of age. Dividing patients younger than 10 years into sporadic and familial groups, the percentage of sporadic patients with a found variant is 14.3% (2/14), and equally the percentage of familial patients with found a variant is 14.3% (1/7). In one patient, the variant was found at his age of 13 years. In total, patients with a found variant younger than 20 years divided according the family status represent 12% (3/28) among sporadic and 9.1% (1/11) among familial patients. The last patient was genetically diagnosed at the age of 56. MLPA analysis did not reveal any gross deletion in the *ATL1* gene.

Novel Variants Detected

c.1064A>T (p.Asn355Ile) Novel missense variant c.1064A>T in exon 12 was found in a 1-year-old girl with a negative family history. The prenatal and early postnatal period were normal. The first symptoms (spasticity) manifested at the age of 6 months. Mental development milestones are normal. Neurological examination showed hyperreflexia in the lower and also in the upper limbs. Neither positive pyramidal signs (Babinski, Rossolimo) nor sphincter disturbances were present. Cranial and spinal MRI results were with normal findings. This variant is predicted to be deleterious/disease causing by all prediction programmes used. Both the nucleotide and the amino acid position are highly conserved, up to *Caenorhabditis elegans*, which supports the pathogenic character of the variant (Table 2). The same variant has been subsequently found in the patient´s father, but only in approximately 15%; therefore, we suppose mosaicism in the father, who is unaffected and asymptomatic. We sequenced DNA of the patient's father obtained from peripheral blood and from saliva with the same result (Fig. 1). The father did not manifest any neurological symptoms associated with spastic paraplegia. He was examined at the age of 32 years. He had normal gait, normal muscle strength, and normal lower limb reflexes, and no increased pyramidal signs were observed. In the past, he had manifested sporadic epileptic seizures and was then diagnosed with astrocytoma, which was successfully excised.

c.1039A>C (p.Met347Leu) This novel missense variant c.1039A>C in exon 11 was found in a 2-year-old girl who

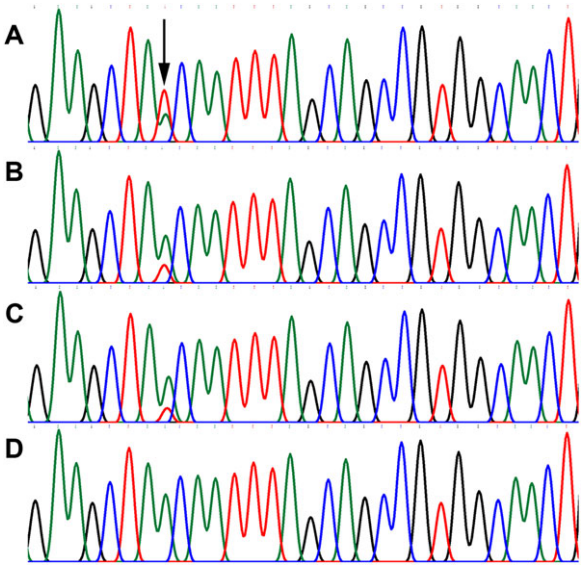

Figure 1 Novel variant c.1064A>T (p.Asn355Ile). A. Affected patient, heterozygous (blood). B. Healthy father, mosaicism (blood). C. Healthy father, mosaicism (saliva). D. Healthy mother, wild type (blood). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2 Detected variants in the *ATL1* gene: presence in protein domain, nucleotide, and amino acid conservation and *in silico* prediction.

Nucleotide change	Presence in protein domain	Nucleotide conservation		Amino acid conservation	Amino acid biochemical difference	Prediction		
		PhastCons [0–1]	phyloP [-14.1–6.4]		Grantham dist [0–215]	PolyPhen-2	Mutation Taster	SIFT
c.311A>G	Guanylate-binding protein, N-terminal	1.00	Highly [4.48]	Moderately [up to Frog]	46	Benign [0.002]	Disease causing	Tolerated
c.505A>G	Guanylate-binding protein, N-terminal	1.00	Highly [4.81]	Highly [up to *C. elegans*]	21	Possibly damaging [0.924]	Disease causing	Deleterious
c.916A>G	Guanylate-binding protein, N-terminal	1.00	Moderately [2.38]	Highly [up to Tetraodon]	56	Benign [0.124]	Disease causing	Tolerated
c.1039A>C	Guanylate-binding protein, C-terminal	1.00	Highly [4.89]	Highly [up to *C. elegans*]	15	Benign [0.257]	Disease causing	Deleterious
c.1064A>T	Guanylate-binding protein, C-terminal	1.00	Highly [4.73]	Highly [up to *C. elegans*]	149	Probably damaging [0.995]	Disease causing	Deleterious

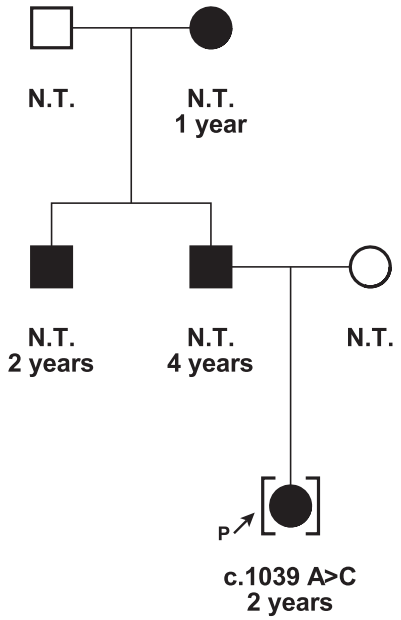

Figure 2 Family tree c.1039A>C (p.Met347Leu). Black/white shape: affected/not affected member; N.T. genetically not tested; years: age at onset.

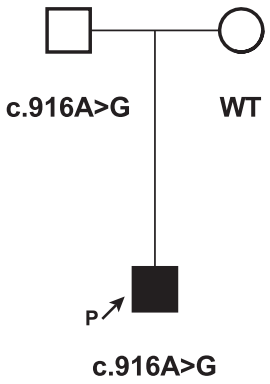

Figure 3 Family tree c.916A>G (p.Ser306Gly). Black/white shape: affected/not affected member; WT: wild type.

was adopted at the age of 9 months and lives with the adoptive-family. She has typical spastic paraplegia symptoms (spastic lower limbs, spastic gait, hyperreflexia with enlarged reflex zone on lower limbs, positive pyramidal signs). Upper limbs are without any symptoms. We have little information about the prenatal and perinatal period. Her mental development is normal, but motor milestones acquisition has been delayed. At the age of 2 years, she was able to walk only with help. She is currently 4 years old and is unable to walk without assistance. She uses crutches but prefers to crawl on all fours. *In silico* predictions show the pathogenic character of this variant, the nucleotide and the amino acid position are highly conserved. The low HumDiv score from the PolyPhen-2 prediction programme is a concern. The programme presumes this variant to be benign. We succeeded to obtain some information about the patient's biological father's family. Our patient's biological father, his brother, and their mother all have had gait problems since a young age and were diagnosed with spastic paraplegia (Fig. 2). The family follows autosomal dominant inheritance. The biological father was diagnosed at the age of 4 with the spastic paraplegia. We do not have information about the age of first symptoms. The father's brother was reported to have similar gait problems as the patient at the age of 2. He was not able to walk without help. The father's mother manifested her first symptoms at the age of 1 year. At 2 years of age, she was not able to walk without help. At 3 years, she underwent surgery of the Achilles tendons. According to available information, disease progression is very slow and has not changed since childhood. Unfortunately, we do not have information about their current state of health and the severity of their gait impairment. Unfortunately, we are not able to perform segregation analysis because DNA samples from relatives are not available.

c.916A>G (p.Ser306Gly) Missense variant c.916A>G in exon 10 was found in a 3-year-old boy who has been suspected of having a pure form of HSP. We have poor information about neurological status of this patient. He was described as a sporadic patient, with abnormal gait similar to a spastic gait and has shortened Achilles tendons. This variant is *in silico* predicted most often to be benign. The nucleotide is moderately conserved, although amino acid is conserved up to Tetraodon. We had DNA samples from his parents available, and the healthy father has the same variant c.916A>G in the *ATL1* gene (Fig. 3) in the heterozygous state; therefore, we concluded this variant to be benign and not the cause of the health problems in this patient.

c.505A>G (p.Met169Val) This missense variant in exon 5 was found in a 13-year-old boy. We do not have information about the age of first symptoms or the results of the neurological examination. The patient is described as having pure hereditary spastic paraparesis. He is a sporadic case in the family, and his family history is negative. Cranial and spinal MRIs showed normal results. This patient has been independently diagnosed with a hearing impairment since childhood, and a prevalent pathogenic variant c.35delG in *GJB2* gene in heterozygous state was previously revealed. *In silico* prediction shows the deleterious/disease-causing character of the variant in the *ATL1* gene. The conservation of nucleotide and amino acid position is also high (Table 2).

We performed Sanger sequencing of the *ATL1* gene in both healthy parents and the same variant c.505A>G was found in his unaffected mother. Subsequent neurological examination of the patient's mother showed slight hyporeflexia and reduced vibration perception in the lower limbs. No other abnormal signs indicating neuropathy problems are present' the character of her neurological examination links more to neuropathy than to central motoneuron disease. Objectively she does not manifest, and subjectively she does not complain about any neurological problems. Therefore, we assume this variant is benign and not causal for HSP.

Described Variant Detected

c.311A>G (p.Asn104Ser) Missense variant c.311A>G in exon 4 was found in a 56-year-old woman who first experienced HSP symptoms at the age of 52 years. She complained about gait problems and weakness of the lower limbs. Neurological examination showed hypereflexia and positive pyramidal signs. Family history is negative. We had no other family members' DNA samples available. We assume this variant to be benign because of rather benign *in silico* prediction and moderately conserved amino acid. Moreover, this variant was described with low frequency (0.01%) in the European non-Finish population only, never in homozygous status, but the prevalence of HSP in general is lower, which supports that this variant is be benign.

Discussion

Using Sanger sequencing of all coding exons and adjacent intron regions of the *ATL1* gene in the cohort of 111 Czech patients with pure spastic paraplegia, we found five independent patients with missense variants in coding regions of this gene, and four variants have not been previously described and are not present in the population databases. We found no splice-site, nonsense, or frameshift variant, which is consistent with previously reported findings. All patients except for Patient 1 manifested gait impairment beginning in childhood. Patients 3, 4, and 5 manifested gait impairment at a very early age. In all patients, where we have enough information from neurological examination, pyramidal signs and brisk lower limbs reflexes (hyperreflexia) have been observed (Patients 1, 4, and 5). Surprisingly, all but one patient (Patient 4) have been described as sporadic patients. There was no consanguinity in the family trees of patients with a variant.

Although variants in the *ATL1* gene were previously repeatedly described as a frequent cause of HSP, in approximately 10 % of all HSP patients (Fink et al., 1996; Durr et al., 2004), and the most common cause of early-onset HSP, accounting for up to 50% of all AD HSP with onset before the age of 10 years (Abel et al., 2004; Namekawa et al., 2006; Polymeris et al., 2016), our results show significantly lower frequencies among Czech HSP patients. In the whole group of patients, we found only 4.3% patients with possibly causal variants in the *ATL1* gene and in only 11.1% of patients with onset before the age of 10 years. However, we must be cautious because of the phenotypic heterogeneity of our cohort of patients. Our cohort of patients includes a broader phenotypic spectrum, not just early-onset AD patients, where the SPG3A is most frequent. For this reason, our cohort of patients was divided into groups according to the age and the family status, and we calculated the percentage of patient with found variant in the *ATL1* gene in each group separately. Nevertheless, patients with a variant found in the *ATL1* gene reach maximum 14.3% among familial patients younger than 10 years and 14.3% among sporadic patients younger than 10 years. Nevertheless, we must be cautious about the small number of examined Czech patients, but no other data are available regarding Czech HSP patients, because we are the only facility performing DNA diagnostics for HSP in the Czech Republic. Similarly, a low occurrence of *ATL1* causative variants among HSP patients has been recently described in various European and Asian populations (Kim et al., 2014; Lu et al., 2014; Elert-Dobkowska et al., 2015; Park et al., 2015).

Furthermore, we assume that the percentage of SPG3A/*ATL1*-caused HSP Czech patients is even much lower because the causality of some of detected variants in the *ATL1* gene is uncertain. We strongly assume causality only in two of the novel variants (c.1039A>C and c.1064A>T). The novel variant c.1039A>C (p.Met347Leu) segregates with the phenotype inherited by the autosomal dominant manner in the family tree, and also all other family and personal history data described (affected father and his mother, early age at onset in all affected persons, slow progression) are consistent with the typical SPG3A phenotype. The c.1064A>T variant probably emerged during the early prenatal development of our patient's unaffected father and is the cause of our patient's HSP. We do not assume that the epilepsy seizures and presence of astrocytoma in the father are related to the variant in the *ATL1* gene. The causality of these two variants (c.1039A>C and c.1064A>T) is strengthened by their presence in guanylate-binding protein domain, C-terminal. Moreover, the missense pathogenic variant in the neighbouring nucleotide within the same triplet were reported previously in both variants, p.Met347Ile in SPG3 patient (Al-Maawali et al., 2011; Leonardi et al., 2015) and p.Asn355Cys in a patient with hereditary sensory neuropathy (Guelly et al., 2011; Leonardis et al., 2012). Two of the revealed variants (c.916A>G and c.505A>G) were subsequently found in the patient's unaffected parent, which raises doubts about the pathogenicity. Moreover, the c.916A>G variant has benign

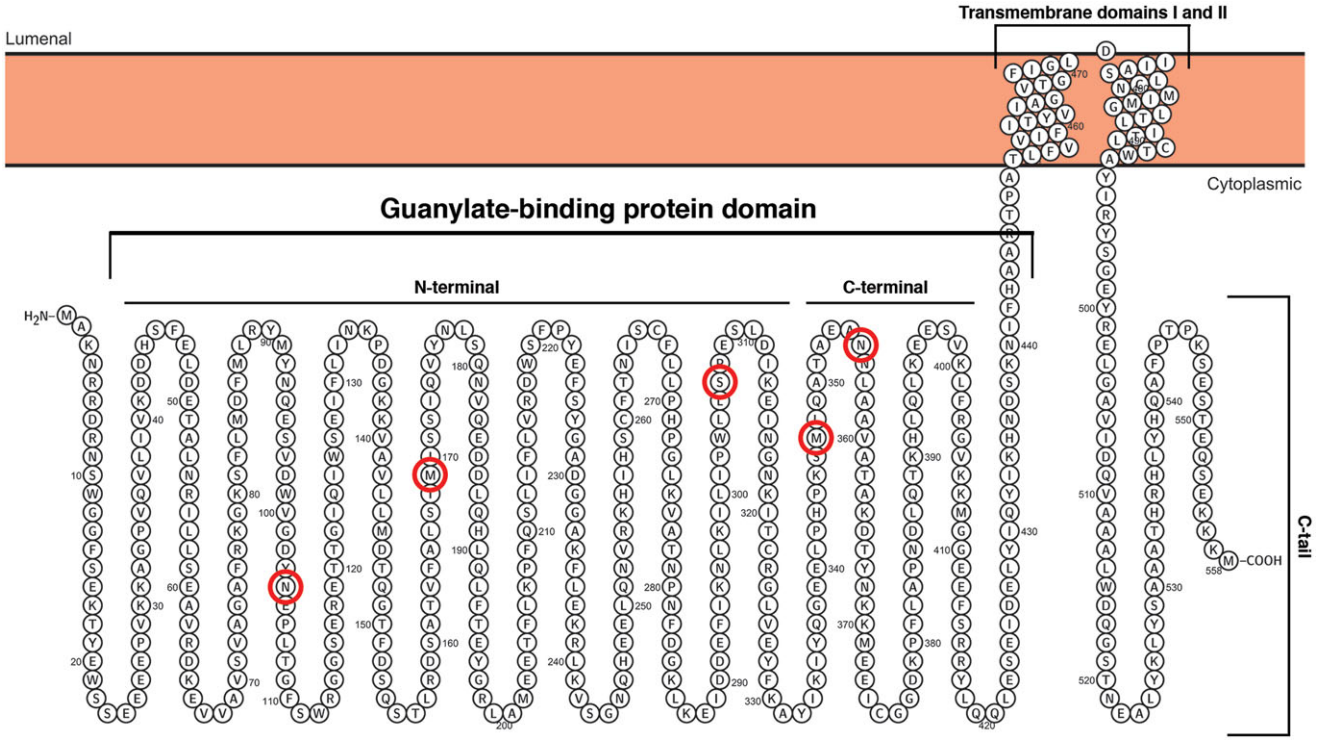

Figure 4 Protein atlastin-1 coded by *ATL1* gene has two cytoplasmic (animo acids 1–449; and 493–558) and two transmebrane domains (animo acids 450–470; and 472–492). All variants revealed in our cohort (highlighted with the red circle) are localised in the Guanylate-binding protein domain
(amino acids 43–438; N-terminal 43–312, C-terminal 317–438). Described variants in *ATL1* gene are localised in all domains of the protein. Protein structure is visualised using http://wlab.ethz.ch/protter/. [Colour figure can be viewed at wileyonlinelibrary.com]

in silico predictions. The c.505A>G variant was found in the patient's mother with unclear neurological findings. Although few variants in the *ATL1* gene have been described with axonal neuropathies (Fusco et al., 2010; Al-Maawali et al., 2011; Leonardis et al., 2012), variability in phenotype and penetrance in the son and his mother greatly differ. On the other hand, we must beware that both these variants (c.505A>G and c.916A>G) are in a Guanylate-binding protein (N-terminal) protein domain (Fig. 4). In this domain, both pathogenic variants for SPG3A as well as variants with uncertain significance were described. Moreover, reduced penetrance and differences in age at onset in some *ATL1* variants have been repeatedly described in the SPG3A phenotype/*ATL1* gene, which should be taken into account (Durr et al., 2004; D'Amico et al., 2004). Varga et al. (2013) described two families with variants in *ATL1* manifesting reduced phenotype penetrance and even sex-dependent penetrance. A similar situation could exist in our patients, particularly in the c.505A>G variant with the healthy mother carrying the same variant.

MLPA testing of 56 independent patients selected form the main cohort did not reveal any gross deletion in the *ATL1* gene among our patients. This result is consistent with previously published data. Only one large deletion was described in exon 4 of the *ATL1* gene (Sulek et al., 2013). The character of variants found in our patients (all of missense type) is also consistent with previously described data. The majority of previously described mutations are of missense/nonsense character. Only two variants affecting splicing and few truncating variants in *ATL1* have been described to date.

Conclusion

We examined all coding exons and exon/intron boundaries of the *ATL1* gene using Sanger sequencing in 111 independent Czech patients with pure spastic paraplegia where all but 7 are concluded as *SPAST* negative, and further we did gross deletion analysis in the same gene using MLPA in 56 of these patients. Approximately half of the examined patients were younger than 20 years. We found only five suspicious missense variants among our 111 patients, four of them are novel, but we assume only two of them (1.8%) are causal for HSP. We have not found any gross deletion in the *ATL1* gene in a subgroup of 56 patients. We can conclude that the occurrence

of *ATL1* disease-causing variants among Czech pure spastic paraplegia patients is low. It is less than 5% in the whole cohort and probably less than 11% in the group of patients younger than 10 years due to the ambiguous causality of some variants. Separate Sanger sequencing of the *ATL1* gene does not seem to be cost-effective even in patients with early onset of the disease. It is more useful to include the *ATL1* gene into a gene panel for next-generation targeted sequencing.

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Conflict of Interest

All authors declare that they do not have any conflict of interest.

References

Abel, A., Fonknechten, N., Hofer, A., Durr, A., Cruaud, C., Voit, T., Weissenbach, J., Brice, A., Klimpe, S., Auburger, G. & Hazan, J. (2004) Early onset autosomal dominant spastic paraplegia caused by novel mutations in SPG3A. *Neurogenetics* **5**, 239–243.

Al-Maawali, A., Rolfs, A., Klingenhaeger, M. & Yoon, G. (2011) Hereditary spastic paraplegia associated with axonal neuropathy: a novel mutation of SPG3A in a large family. *J Clin Neuromuscul Dis* **12**, 143–146.

D'amico, A., Tessa, A., Sabino, A., Bertini, E., Santorelli, F. M. & Servidei, S. (2004) Incomplete penetrance in an SPG3A-linked family with a new mutation in the atlastin gene. *Neurology* **62**, 2138–2139.

Durr, A., Camuzat, A., Colin, E., Tallaksen, C., Hannequin, D., Coutinho, P., Fontaine, B., Rossi, A., Gil, R., Rousselle, C., Ruberg, M., Stevanin, G. & Brice, A. (2004) Atlastin1 mutations are frequent in young-onset autosomal dominant spastic paraplegia. *Arch Neurol* **61**, 1867–1872.

Elert-Dobkowska, E., Stepniak, I., Krysa, W., Rajkiewicz, M., Rakowicz, M., Sobanska, A., Rudzinska, M., Wasielewska, A., Pilch, J., Kubalska, J., Lipczynska-Lojkowska, W., Kulczycki, J., Kurdziel, K., Sikorska, A., Beetz, C., Zaremba, J. & Sulek, A. (2015) Molecular spectrum of the SPAST, ATL1 and REEP1 gene mutations associated with the most common hereditary spastic paraplegias in a group of Polish patients. *J Neurol Sci* **359**, 35–39.

Fink, J. K., Heiman-Patterson, T., Bird, T., Cambi, F., Dube, M. P., Figlewicz, D. A., Haines, J. L., Hentati, A., Pericak-Vance, M. A., Raskind, W., Rouleau, G. A. & Siddique, T. (1996) Hereditary spastic paraplegia: advances in genetic research. Hereditary Spastic Paraplegia Working group. *Neurology* **46**, 1507–1514.

Fusco, C., Frattini, D., Farnetti, E., Nicoli, D., Casali, B., Fiorentino, F., Nuccitelli, A. & Giustina, E. D. (2010) Hereditary spastic paraplegia and axonal motor neuropathy caused by a novel SPG3A de novo mutation. *Brain Dev* **32**, 592–594.

Guelly, C., Zhu, P. P., Leonardis, L., Papic, L., Zidar, J., Schabhuttl, M., Strohmaier, H., Weis, J., Strom, T. M., Baets, J., Willems, J., De Jonghe, P., Reilly, M. M., Frohlich, E., Hatz, M., Trajanoski, S., Pieber, T. R., Janecke, A. R., Blackstone, C. & Auer-Grumbach, M. (2011) Targeted high-throughput sequencing identifies mutations in atlastin-1 as a cause of hereditary sensory neuropathy type I. *Am J Hum Genet* **88**, 99–105.

Haberlova, J., Claeys, K. G., Zamecnik, J., De Jonghe, P. & Seeman, P. (2008) Extending the clinical spectrum of SPG3A mutations to a very severe and very early complicated phenotype. *J Neurol* **255**, 927–928.

Hedera, P. (2010) Spastic paraplegia 3A. In: Pagon, R. A., Adam, M. P., Ardinger, H. H., Wallace, S. E., Amemiya, A., Bean, L. J. H., Bird, T. D., Ledbetter, N., Mefford, H. C., Smith, R.J.H., Stephens, K. *GeneReviews*, Sep 21.

Hedera, P., Fenichel, G. M., Blair, M. & Haines, J. L. (2004) Novel mutation in the SPG3A gene in an African American family with an early onset of hereditary spastic paraplegia. *Arch Neurol* **61**, 1600–1603.

Hu, J., Shibata, Y., Zhu, P. P., Voss, C., Rismanchi, N., Prinz, W. A., Rapoport, T. A. & Blackstone, C. (2009) A class of dynamin-like GTPases involved in the generation of the tubular ER network. *Cell* **138**, 549–561.

Khan, T. N., Klar, J., Tariq, M., Anjum Baig, S., Malik, N. A., Yousaf, R., Baig, S. M. & Dahl, N. (2014) Evidence for autosomal recessive inheritance in SPG3A caused by homozygosity for a novel ATL1 missense mutation. *Eur J Hum Genet* **22**, 1180–1184.

Kim, T. H., Lee, J. H., Park, Y. E., Shin, J. H., Nam, T. S., Kim, H. S., Jang, H. J., Semenov, A., Kim, S. J. & Kim, D. S. (2014) Mutation analysis of SPAST, ATL1, and REEP1 in Korean patients with hereditary spastic paraplegia. *J Clin Neurol* **10**, 257–261.

Leonardi, L., Marcotulli, C., Santorelli, F. M., Tessa, A. & Casali, C. (2015) De novo mutations in SPG3A: a challenge in differential diagnosis and genetic counselling. *Neurol Sci* **36**, 1063–1064.

Leonardis, L., Auer-Grumbach, M., Papic, L. & Zidar, J. (2012) The N355K atlastin 1 mutation is associated with hereditary sensory neuropathy and pyramidal tract features. *Eur J Neurol* **19**, 992–998.

Lu, X., Cen, Z., Xie, F., Ouyang, Z., Zhang, B., Zhao, G. & Luo, W. (2014) Genetic analysis of SPG4 and SPG3A genes in a cohort of Chinese patients with hereditary spastic paraplegia. *J Neurol Sci* **347**, 368–371.

Namekawa, M., Ribai, P., Nelson, I., Forlani, S., Fellmann, F., Goizet, C., Depienne, C., Stevanin, G., Ruberg, M., Durr, A. & Brice, A. (2006) SPG3A is the most frequent cause of hereditary spastic paraplegia with onset before age 10 years. *Neurology* **66**, 112–114.

Park, H., Kang, S. H., Park, S., Kim, S. Y., Seo, S. H., Lee, S. J., Lee, J. A., Cho, S. I., Sung, J. J., Lee, K. W., Kim, J. Y., Park, S. S. & Seong, M. W. (2015) Mutational spectrum of the SPAST and ATL1 genes in Korean patients with hereditary spastic paraplegia. *J Neurol Sci* **357**, 167–172.

Park, S. H., Zhu, P. P., Parker, R. L. & Blackstone, C. (2010) Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network. *J Clin Invest* **120**, 1097–1110.

Polymeris, A. A., Tessa, A., Anagnostopoulou, K., Rubegni, A., Galatolo, D., Dinopoulos, A., Gika, A. D., Youroukos, S., Skouteli, E., Santorelli, F. M. & Pons, R. (2016) A series of Greek

children with pure hereditary spastic paraplegia: clinical features and genetic findings. *J Neurol* **263**, 1604–1611.

Smith, B. N., Bevan, S., Vance, C., Renwick, P., Wilkinson, P., Proukakis, C., Squitieri, F., Berardelli, A., Warner, T. T., Reid, E. & Shaw, C. E. (2009) Four novel SPG3A/atlastin mutations identified in autosomal dominant hereditary spastic paraplegia kindreds with intra-familial variability in age of onset and complex phenotype. *Clin Genet* **75**, 485–489.

Sulek, A., Elert, E., Rajkiewicz, M., Zdzienicka, E., Stepniak, I., Krysa, W. & Zaremba, J. (2013) 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* **34**, 239–242.

Varga, R. E., Schule, R., Fadel, H., Valenzuela, I., Speziani, F., Gonzalez, M., Rudenskaia, G., Nurnberg, G., Thiele, H., Altmuller, J., Alvarez, V., Gamez, J., Garbern, J. Y., Nurnberg, P., Zuchner, S. & Beetz, C. (2013) Do not trust the pedigree: reduced and sex-dependent penetrance at a novel mutation hotspot in ATL1 blurs autosomal dominant inheritance of spastic paraplegia. *Hum Mutat* **34**, 860–863.

Yonekawa, T., Oya, Y., Higuchi, Y., Hashiguchi, A., Takashima, H., Sugai, K. & Sasaki, M. (2014) Extremely severe complicated spastic paraplegia 3A with neonatal onset. *Pediatr Neurol* **51**, 726–729.

Zhao, X., Alvarado, D., Rainier, S., Lemons, R., Hedera, P., Weber, C. H., Tukel, T., Apak, M., Heiman-Patterson, T., Ming, L., Bui, M. & Fink, J. K. (2001) Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. *Nat Genet* **29**, 326–331.

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