ORIGINAL ARTICLE

Anticipation Can Be More Common in Hereditary Spastic Paraplegia with *SPAST* Mutations Than It Appears

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ABSTRACT: ***Background and objective:*** Hereditary spastic paraplegia (HSP) is a heterogeneous neurodegenerative disorder with lower-limb spasticity and weakness. Different patterns of inheritance have been identified in HSP. Most autosomal-dominant HSPs (AD-HSPs) are associated with mutations of the *SPAST* gene (SPG4), leading to a pure form of HSP with variable age-at-onset (AAO). Anticipation, an earlier onset of disease, as well as aggravation of symptoms in successive generations, may be correlated to SPG4. Herein, we suggested that anticipation might be a relatively common finding in SPG4 families. ***Methods:*** Whole-exome sequencing was done on DNA of 14 unrelated Iranian AD-HSP probands. Data were analyzed, and candidate variants were PCR-amplified and sequenced by the Sanger method, subsequently checked in family members to co-segregation analysis. Multiplex ligation-dependent probe amplification (MLPA) was done for seven probands. Clinical features of the probands were recorded, and the probable anticipation was checked in these families. Other previous reported SPG4 families were investigated to anticipation. ***Results:*** Our findings showed that SPG4 was the common subtype of HSP; three families carried variants in the *KIF5A*, *ATL1*, and *MFN2* genes, while five families harbored mutations in the *SPAST* gene. Clinical features of only SPG4 families indicated decreasing AAO in affected individuals of the successive generations, and this difference was significant (p-value <0.05). ***Conclusion:*** It seems *SPAST* will be the first candidate gene in families that manifests a pure form of AD-HSP and anticipation. Therefore, it may be a powerful situation of genotype–phenotype correlation. However, the underlying mechanism of anticipation in these families is not clear yet.

RÉSUMÉ : Paraplégie spastique héréditaire de type 4 et mutations du gène SPAST : l'anticipation des patients pourrait être plus fréquente qu'il n'y paraît. ***Contexte et objectif*** **:** La paraplégie spastique héréditaire (PSH) constitue une maladie neurodégénérative hétérogène se caractérisant par une spasticité et une faiblesse des membres inférieurs. À cet égard, différents profils héréditaires (*patterns of inheritance*) ont été identifiés dans le cas de la PSH. La plupart des cas autosomiques dominants de PSH (PSH-AD) sont associés à des mutations du gène *SPAST* (SPG4), ce qui conduit à une « forme pure » ou non-compliquée de PSH dont l'âge d'apparition (AA) est variable. L'anticipation des patients à un stade précoce de la maladie, de même que l'aggravation des symptômes au fil des générations, pourraient être corrélées au gène SPG4. Nous voulons donc suggérer ici que ce phénomène d'anticipation pourrait s'avérer une découverte relativement courante au sein de familles porteuses de cette mutation du gène SPG4. ***Méthodes*** **:** Un séquençage de l'exome entier (SEE) a été effectué à partir de l'ADN de 14 proposants (*probands*) iraniens atteints de PSH-AD. Les données recueillies ont été ensuite analysées. Les variants candidats ont été amplifiés par RCP et séquencés à l'aide de la méthode de Sanger pour être ensuite vérifiés chez des membres de leur famille au moyen de l'analyse de coségrégation. La méthode d'amplification multiplex de sonde dépendante d'une ligature (*multiplex ligation-dependent amplification probe)* a par ailleurs été utilisée chez sept proposants. Leurs caractéristiques ont été colligées et une forme probable d'anticipation a été vérifiée au sein de leur famille. Enfin, soulignons que d'autres familles présentant une mutation du gène SPG4 ont fait l'objet d'une analyse pour détecter une forme d'anticipation. ***Résultats*** **:** Nos résultats ont montré que le gène SPG4 était le sous-type commun de la PSH. Au total, trois familles étaient porteuses de variants pour les gènes *KIF5A*, *ATL1* et *MFN2* tandis que cinq autres familles étaient porteuses d'une mutation du gène *SPAST*. Les caractéristiques cliniques des seules familles présentant une mutation du gène SPG4 ont révélé une diminution de l'AA de la PSH chez les individus atteints au fil des générations, la différence étant ici notable (p < 0,05). ***Conclusion*** **:** Il semble donc que le gène *SPAST* soit le premier gène candidat au sein de familles qui manifestent une « forme pure » de PSH-AD et une forme d'anticipation. Il pourrait donc s'agir d'exemples indéniables de corrélation génotype-phénotype. Ceci dit, le mécanisme sous-jacent de l'anticipation au sein de ces familles n'est pas encore clair.

Keywords: Hereditary spastic paraplegia (HSP), Whole-exome sequencing (WES), MLPA, *SPAST*, SPG4, Anticipation

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Introduction

Hereditary spastic paraplegia (HSP) is a heterogeneous group of hereditary neurodegenerative disorders mainly characterized by spasticity and weakness, predominantly affecting the lower limbs.[1] Patients with additional manifestations are classified as the complicated form of HSP in contrast to the pure form of the disease. HSP has several inheritance patterns, including autosomal dominant (AD-HSP), autosomal recessive (AR-HSP), X-linked, and mitochondrial/maternal inheritance. *De novo* mutations can also happen in HSP. AD-HSP patients usually present a pure form of the disease, while AR-HSP cases often show a complicated form.[2] The most frequent mutated gene among all patients and specially AD-HSP ones is the *SPAST* gene. It usually results in a pure form of HSP and comprises approximately 60% of AD-HSPs (accounting for 17–79% of all AD-HSP cases), a third of all HSP-affected patients, and 15% of sporadic cases.[3,4] The *SPAST* gene encodes for an enzyme called spastin, an AAA protein (ATPases associated with a variety of cellular Activities).[5] Heterozygous mutations in *SPAST* cause the SPG4 type of HSP. Up to now, more than 900 mutations throughout *SPAST* have been reported in the Human Gene Mutation Database (HGMD professional 2020.4), indicating a high level of allelic heterogeneity. The most common type of *SPAST* mutations are point mutations (75–80%), and the frequency of large rearrangements such as exon deletions or duplications in *SPAST* has been estimated as 20%.[6,7] It is suggested based on type and where the mutation occurred, pathology and phenotypes might vary among SPG4 patients. So, a high level of both inter and intrafamilial clinical heterogeneity are observed in SPG4 patients[8]: scrutinized assessment of patients show differences in age at onset (AAO; ranging from early childhood to the seventh decade), disease progression pace, and clinical variability. In addition to variable expressivity in SPG4 patients, some studies indicate incomplete penetrance in these patients.[8] Thus, the role of environmental and other genetic factors should be considered for explaining this AAO and clinical heterogeneity.[8–10] Such variability could be due to (i) the genetic modifiers; for example, the presence of the c.131C>T:p. Ser44Leu variant in the patients who carry a *SPAST* mutation is associated with a lower AAO and more severe disease,[8] (ii) the type and location of *SPAST* mutations, and (iii) sex. A bimodal pattern of distribution of AAO has been reported in SPG4 patients that is related to the type of mutations; patients who carry the missense mutations in *SPAST* manifest a significantly lower AAO (often <10 years and located in the first peak) than those with truncating mutations (the interval of the second and fifth decade of life and located in the second peak).[11] There is no informative correlation between the type of mutation and severity of symptoms except for intellectual disability in some patients who carried the missense mutations[12] and psychiatric disorders among a few patients carrying a loss of function mutation in the *SPAST* gene.[13] Also, a sex-linked penetrance has been reported in SPG4 patients; a lower penetrance was observed in females.[11]

Anticipation is a biological event, defined by a progressive decline in AAO, and in most cases, escalation in disease severity in successive generations in a pedigree is also apparent.[14] It is typically related to the context of dynamic mutations and detected in several diseases including Huntington's disease (HD),[15] myotonic dystrophy (MD),[16] amyotrophic lateral sclerosis (ALS with repeat expansion, RE, mutations in *C9orf72*),[14] and spinocerebellar ataxia (SCA).[17] It is noteworthy that this phenomenon has been reported in other neurological and non-neurological disorders without dynamic mutations, like some types of cancers, including breast or ovarian cancer,[18,19] Lynch syndrome,[20] Crohn's disease,[21] ALS,[22,23] hypertrophic cardiomyopathies,[24] and a few subtypes of HSP such as SPG4.[25–34] However, the presence of anticipation in SPG4 cases is still doubtful, and the presence of anticipation may be due to diagnostic sensitivity of these families. So, further studies of large pedigrees with *SPAST* mutations and maybe re-analysis of previous reported SPG4 families are needed to confirm whether anticipation exists in the SPG4 families. Confirmation of anticipation in these diseases and identification of their related factors may reveal the pathophysiology of those and help the genetic counseling. The importance of analyzing genetic anticipation is that clinicians to decide from what age they should follow presymptomatic carriers.[35]

Herein we assessed this phenomenon in the identified Iranian SPG4 families and compared it to other reported families. Actually, this phenomenon might be more common than expected in these families, but due to inadequate knowledge and the small size of families, it is often overlooked or underestimated in clinical practice.

Materials and Methods

This research was performed in accordance to the Declaration of Helsinki and with the approval of the ethics board of the University of Social Welfare and Rehabilitation Sciences (USWR). All patients and their family members were informed of the nature of the research and the consent form signed.

Subjects

Fourteen unrelated Iranian families affected with AD-HSP referred to Genetics Research Center (GRC) at the USWR for genetic analysis. All affected and unaffected members of these families were recruited when available. Clinical data of unavailable affected members were also collected by in depth interviewing family members or based on their previous medical records.

Genetic Analysis

Whole-Exome Sequencing (WES)

DNA was extracted from whole blood using the salting-out protocol. DNAs of probands were whole-exome sequenced and subsequently analyzed to detect candidate disease-causing variants. Detail of analysis has been presented in the Supplementary text A.

In silico Analysis

To detect the pathogenic nature of variants in the corresponding proteins, several *in silico* bioinformatics tools were used (Supplementary text B). For the clinical interpretation of variants, the American College of Medical Genetics (ACMG) criteria were used.[36] ACMG criteria were checked in InterVar; http://wintervar.wglab.org/ and Varsome; https://varsome.com/.

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Co-segregation Analysis of Candidate Variants

Amplification of exons 5 and 13 of the *SPAST* gene that carried the candidate variants c.C782A:p. Ser261*, c.806dupA:p. Tyr269_Ser270delins*, respectively, in the probands SPG4-A-IV5, SPG4-C-II3, and c.G1496A:p. Arg499His in the probands SPG4-D-III4 and SPG4-E-III6 was done by polymerase chain reaction (PCR). The PCR products were sequenced using the Sanger method (Big Dye kit and the Prism 3130 sequencer; Applied Biosystems, Foster City, CA, USA). Sequences were checked by Sequencher 4.1 software. Sequence variants were assessed with the reference sequences available at NCBI: NC_000002.11, NM_014946.4, and NP_055761.2 and confirmed in the probands. Thereafter, to perform a co-segregation analysis of the candidate variants in affected families based on disease-status, direct sequencing of corresponding exons was performed in the parents, siblings, and other family members.

Co segregation analysis was also done for families with *KIF5A* (c.A758T:p. Lys253Met), *ATL1* (c.C715T:p. Arg239Cys), and *MFN2* (c.G380A:p. Gly127Asp) variants. Assessing of these sequence variants was done using the reference sequences available at NCBI: NC_000012.11, NM_004984.4, and NP_004975.2 for *KIF5A*, NC_000014.8, NM_015915.5, and NP_056999.2 for *ATL1*, and NC_000001.10, NM_014874.4, and NP_055689.1 for *MFN2*.

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA was performed in seven remaining families (undiagnosed families by WES), including family SPG4-B who presented anticipation. MLPA was carried out using the SALSA® MLPA® Probemix P165-C3 HSP mix-1 (MRC-Holland, Amsterdam, The Netherlands), according to the manufacturer's protocols detecting large deletions or duplications in the *SPAST* and *ATL1* genes. Data were analyzed by Coffalyser.NET software. The SALSA MLPA Probemix P165-C3 contains 47 probes with amplification products between 130 nucleotides and 481 nucleotides. It includes 16 probes for the *ATL1* gene and 20 probes for the *SPAST* gene. In addition, 11 reference probes are included that detect autosomal chromosomal locations.

Evidence for Possible Anticipation

AAO of affected parents and their affected offsprings was considered and compared to detect probable anticipation in all families, especially in five Iranian SPG4 families (families without mutation in the *SPAST* gene did not present anticipation, so their anticipation analysis was not included in this study). Clinical manifestations of 39 SPG4 patients (excluding asymptomatic individuals of SPG4-A, SPG4-C, and SPG4-E families, Table 1) from these families were recorded and used to assess anticipation (Table 2). AAO and present age of some previously reported SPG4 families were also collected and overviewed to confirm potential anticipation in these families (Supplementary Table S1).

Statistical Analysis

We used the "*t* test" to evaluate the correlation between two variables, i.e., AAO in parents and children. The correlations were mentioned as statistically significant when *p*-values were ≤0.05. Statistical calculations were done using online software (https://www.socscistatistics.com/tests/studentttest/default2.aspx).

Results

Clinical Features

Pedigrees of the five SPG4 families were shown in Figure 1A–E, and relevant information of them was presented in Table 1. Patients (total 39) showed intra- and inter familial clinical variability. The female to male ratio was 23:16. The mean AAO of all SPG4 patients was 24.46 ± 21.6 years, and the range of AAO was 2–70 years. The averages of AAO in females and males were 26.95 ± 21.1 and 20.87 ± 21.7, respectively, and the difference between them was not significant (*p*-value = 0.199878). The total average of the present age was 32.05 ± 19.2 with the range of 4–80 years (Tables 1 and 2).

All patients had spasticity of lower limbs. In 20 out of 33 patients (60.6%; clinical data for some patients were not complete), knee jerk was brisk. Babinski sign was observed in 33 out of 34 patients (97%). Impaired vibration sense in feet, urinary dysfunction, and intellectual disability were presented in 13/33 (39.4%), 3/35 (8.5%), and 2/37 (5.4%), respectively, (Figure 2). Pes cavus, epilepsy, and dysarthria were observed in 9/34 (26.5%) and 7/35 (20%), and 13/33 (39.4%) patients, respectively. Hearing impairment and scoliosis were detected in two patients (Table 1 and Figure 2).

Clinical manifestations of other AD-HSP carrying mutations in *KIF5A*, *ATL1*, and *MFN2* were shown briefly in Supplementary Table S2.

Results of WES

Assessment of the WES data confirmed the high quality of sequencing in all probands.

Filtering of WES data revealed three candidate variants in the *SPAST* gene in four families (Supplementary Table S3): variants c.C782A:p. Ser261* in SPG4-A, c.806dupA:p. Tyr269_Ser270-delins* in SPG4-C, and c.G1496A:p. Arg499His in SPG4-D and SPG4-E (Table 1). These variants were screened in the family members and co-segregated with the disease status (Figure 1). The c.G1496A:p. Arg499His variant was predicted as a deleterious/damaging variant using 9/11 *in silico* software. It had a CADD_phred score equal to 34 and was predicted as a "likely pathogenic" variant by ACMG criteria. The c.C782A:p. Ser261* variant was anticipated as "pathogenic" in the InterVar (ACMG criteria). Its CADD_phred score was 37. It was predicted as a "damaging" variant by Mutation Taster, FATHMM, DANN, fitCons and Eigen *in silico* software. C.806dupA:p. Tyr269_-Ser270delins* was also predicted as the "pathogenic" by ACMG criteria.

Filtering of WES also detected three pathogenic/likely pathogenic candidate variants in the *KIF5A*; c.A758T:p. Lys253Met, *ATL1*; c.C715T:p. Arg239Cys, and *MFN2*; c.G380A:p. Gly127Asp genes among remaining HSP families (Supplementary Table S2) that were co segregated with the disease status.

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Table 1: Clinical findings of the Iranian families harboring the mutations in the *SPAST* gene

Family ID	**SPG4-A**											**SPG4-B**							
Variant	c.782C>A:p. Ser261*											Large deletion							
Exon	5											17							
Zygosity	Heterozygous											Heterozygous							
Gender/individual ID	F/I2[&]	F/II2*	F/II3[&]	F/II4[&]	F/III4*	F/III11*	F/III12*	M/III13*	**M/IV5***	F/IV7*	F/IV10*	F/I2[#]	F/II2	M/II4	M/III10[#]	F/III15	**M/III17***	M/IV2*	M/IV3*
Clinically evaluated	-	+	−	−	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+
Present age (year)	Death in 60	75	Death in 50	Death in 60	57	55	53	43	34	40	30	Deceased	80	66	Deceased	41	35	22	12
Age at onset (year)	?	?	?	?	51	Asymp	50	40	24	20	25	~60	60	52	39	21	23	2	3
LL spasticity	Asymp	Asymp	Asymp	Asymp	+	Asymp	+	+	+	+	+	+	+	+	+	+	+	+	+
Babinski sign					+		+	+	+	+	+	+	+	+	−	+	+	+	+
Increased deep tendon reflexes					−		−	−	−	+	+	−	−	−	−	−	−	−	−
Distal LL vibratory impairment					−		−	−	−	+	+	−	−	−	−	−	+	+	−
Urinary dysfunction					−		−	−	−	−	−	−	−	+	−	−	−	−	−
Motor deficit					+		+	+	+	+	+	−	+	+	−	−	+	+	−
Intellectual disability					−		−	−	−	−	−	−	−	−	−	−	−	−	−
Pes cavus					−		−	−	−	−	−	−	−	−	−	−	−	−	−
Epilepsy					−		−	−	−	−	−	−	−	−	−	−	−	−	−
Hearing impairment					−		−	−	−	−	+	−	−	−	−	−	+	−	−
Scoliosis					−		−	−	−	−	−	−	−	−	−	−	−	−	−
Dysarthria					−		−	−	−	−	−	−	−	−	−	−	−	−	−

Family ID	**SPG4-C**								**SPG4-D**										
Variant	c.806dupA:p. Tyr269_Ser270delins*								c.1496G>A:p. Arg499His										
Exon	5								13										
Zygosity	Heterozygous								Heterozygous										
Gender/individual ID	M/I2[&]	F/I3[#]	F/II2*	**F/II3***	F/II5*	F/III2*	F/III3*	M/III4*	M/II4[&]	**F/III4***	F/III5*	F/III7*	F/III9*	M/IV1*	M/IV3*	F/IV4*	M/IV5*	F/IV6*	M/IV9*	M/IV13*
Clinically evaluated	−	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Present age (year)	Deceased	Deceased	47	46	42	23	27	19	Deceased	26	24	20	15	14	10	8	6	4	8	4
Age at onset (year)	>70	50	43	39	40	Asymp	27	Asymp	>50	8	10	10	8	5	2	2	3	3	3	3
LL spasticity	NR	+	+	+	+	Asymp	+	Asymp	NR	+	+	+	+	+	+	+	+	+	+	+
Babinski sign		NR	+	+	NR		NR			+	+	+	+	+	+	+	+	+	+	+
Increased deep tendon reflexes		NR	+	+	NR		NR			+	+	+	+	+	+	+	+	−	+	?
Distal LL vibratory impairment		NR	+	−	NR		NR			+	−	+	−	+	−	+	+	−	+	?
Urinary dysfunction		−	+	+	NR		NR			−	−	−	−	−	−	−	−	−	−	−
Motor deficit		+	+	+	+		+			+	+	+	+	+	+	+	+	+	+	+
Intellectual disability		−	−	−	−		−			+	−	−	−	−	−	−	−	−	+	−
Pes cavus		NR	−	−	NR		−			+	+	+	+	+	+	+	+	−	+	?
Epilepsy		NR	−	−	NR		−			−	−	−	−	+	−	−	−	−	−	−
Hearing impairment		−	−	−	NR		−			−	−	−	−	−	−	−	−	−	−	−
Scoliosis		−	−	−	NR		NR			+	−	−	−	+	−	−	−	−	−	−
Dysarthria		NR	−	−	NR		NR			+	+	+	+	+	+	−	−	−	+	?

Family ID	**SPG4-E**						
Variant	c.1496G>A:p. Arg499His						
Exon	13						
Zygosity	Heterozygous						
Gender/individual ID	F/II2*	F/III3*	F/III4*	F/III5*	**M/III6***	F/III7*	M/III8*
Clinically evaluated	+	+	+	+	+	+	+
Present age (year)	71	44	41	36	33	30	21
Age at onset (year)	68	7	6	6	8	7	7
LL spasticity	+	+	+	+	+	+	+
Babinski sign	+	+	+	+	+	+	+
Increased deep tendon reflexes	+	+	+	+	+	+	+
Distal LL vibratory impairment	−	−	−	+	−	+	−
Urinary dysfunction	−	−	−	−	−	−	−
Motor Deficit	+	+	+	+	+	+	+
Intellectual disability	−	−	−	−	−	−	−
Pes cavus	−	−	−	−	−	−	−
Epilepsy	−	+	+	+	+	+	+
Hearing impairment	−	−	−	−	−	−	−
Scoliosis	−	−	−	−	−	−	−
Dysarthria	−	+	+	+	+	+	+

NR: not reported; F: female; M: male; LL: lower limb; -: negative; +: positive; Asymp: asymptomatic, ?: Undetermined.

All variants have been reported on the basis of NM_014946.

Probands have been shown in bold.

*Asterisks show the people in each pedigree that were genetically tested.

#AAO and clinical manifestations were determined based on their previous medical records.

&AAO and presence of spasticity were recorded based on interview with their relatives.

Table 2: Trend of alternation of age at onset in successive generations of Iranian HSP families who carried *SPAST* mutations

Family ID	Generation	# of patients	Patient ID	AAO (year)	Mean of AAO (year)	Present age (year)	Variant	Genotype
SPG4-A	3	3	III4	51	47 ± 6.08	57	c.782C>A:p.Ser261*	NM
			III12	50		53		NM
			III13	40		43		NM
	4	3	IV5	24	23 ± 2.64	34		NM
			IV7	20		40		NM
			IV10	25		30		NM
SPG4-B	1	1	I2	~60	~60	Deceased	Deletion of exon 17	Not checked
	2	2	II2	60	56 ± 5.65	80		
			II4	52		66		NM
	3	3	III10	39	27.6 ± 9.86	Deceased		Not checked
			III15	21		41		Not checked
			III17	23		35		NM
	4	2	IV2	2	2.5 ± 0.70	22		NM
			IV3	3		12		NM
SPG4-C	1	2	I2	>70	>60	Deceased	c.806dupA:p. Tyr269_Ser270delins*	Not checked
			I3	50		Deceased		Not checked
	2	3	II2	43	40.6 ± 2.08	47		NM
			II3	39		46		NM
			II5	40		42		NM
	3	1	III3	27	27	27		NM
SPG4-D	2	1	II4	>50	>50	Deceased		Not checked
	3	4	III4	8	9 ± 1.15	26	c.1496G>A:p.Arg499His	NM
			III5	10		24		NM
			III7	10		20		NM
			III9	8		15		NM
	4	7	IV1	5	3 ± 1.41	14		NM
			IV3	2		10		NM
			IV4	2		8		NM
			IV5	3		6		NM
			IV6	3		4		NM
			IV9	3		8		NM
			IV13	3		4		NM
SPG4-E	2	1	II2	68	68	71	c.1496G>A:p. Arg499His	NM
	3	6	III3	7	6.8 ± 0.53	44		NM
			III4	6		41		NM
			III5	6		36		NM
			III6	8		33		NM
			III7	7		30		NM
			III8	7		21		NM

AAO: age at onset; NM: normal-mutant.

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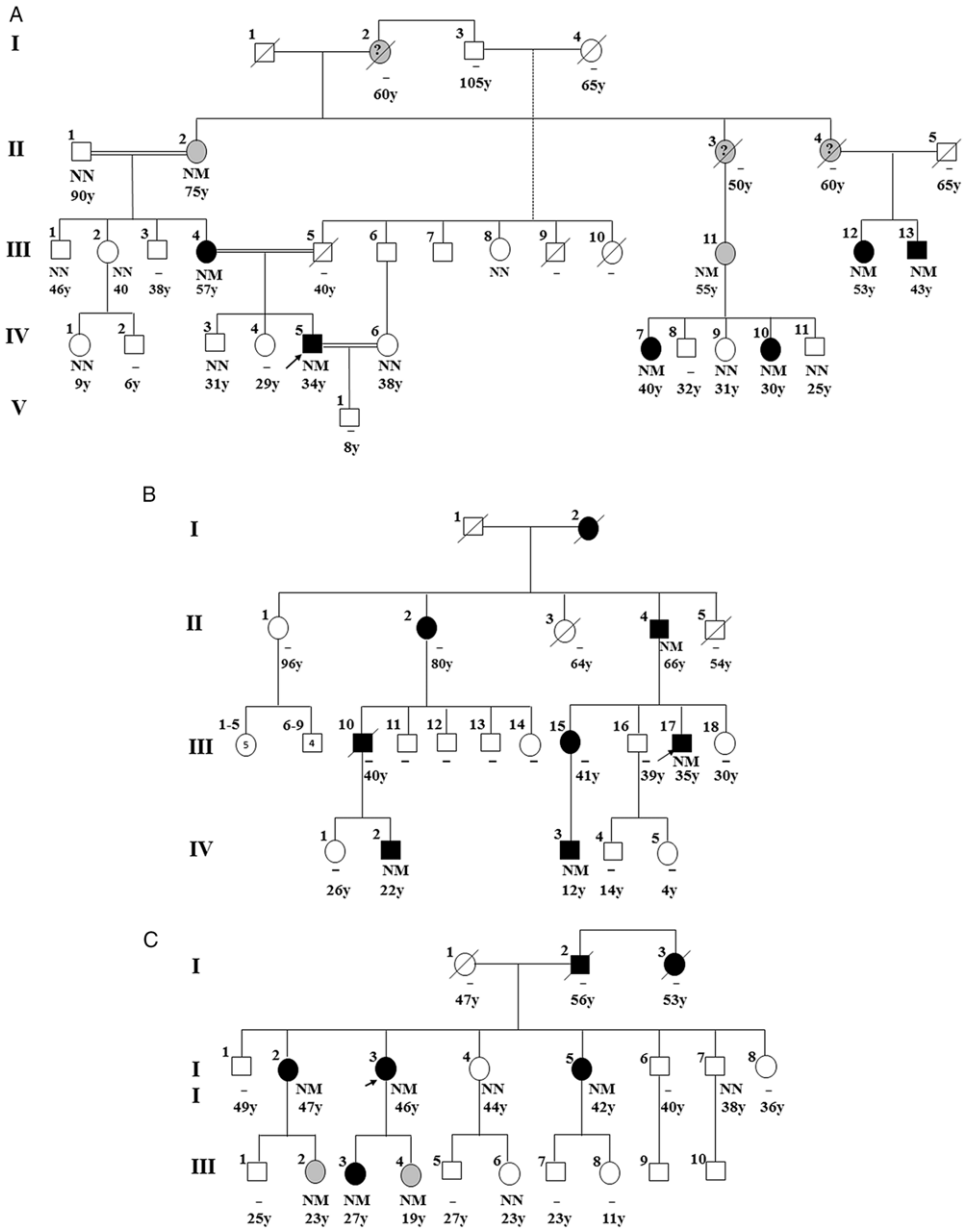

Figure 1: *The Iranian SPG4 pedigrees: (A) SPG4-A, (B) SPG4-B, (C) SPG4-C, (D) SPG4-D, (E) SPG4-E. The present age and genotypes of the candidate variants for each family are shown when individuals were assessed. Arrows show probands. Unfilled circles and squares, normal individuals; black circles and squares indicate SPG4 patients. Gray circles indicate asymptomatic individuals with heterozygous genotype. Grey circles with "?" indicate asymptomatic individuals, who have died but, we can consider as heterozygotes due to their heterozygous affected offspring and/or sib. Abbreviations: M, mutated allele; N, normal allele.*

Results of MLPA

MLPA revealed a heterozygous deletion of exon 17 in the *SPAST* gene only in family SPG4-B (Supplementary Figure S1). We could not determine the boundaries of this deletion.

There was no deletion/duplication in the *ATL1* gene (both genes were in one probe mix).

Results of Anticipation in Iranian Families

In this study, we investigated 39 cases in five multi generation, multi affected SPG4 families for anticipation. The averages of AAO in different generations showed the progressive decline in AAO in later generations of all pedigrees (Table 2). In family SPG4-A, the mean of AAO decreased from 47 to 23 years within two generations (individuals I2, II3, and II4 died before presenting their symptoms and II2 and III11 were asymptomatic in ages 75 and 55 years, respectively; so clearly, the mean AAO in the second generation was more than 61 years), while in family SPG4-B, the mean of AAO declined from ~60 to 2.5 years during four generations. Also, in families SPG4-C and SPG4-D, the averages of AAO diminished from ~60 to 27 and ~50 to 3 years within three generations, respectively. In the last family, SPG4-E,

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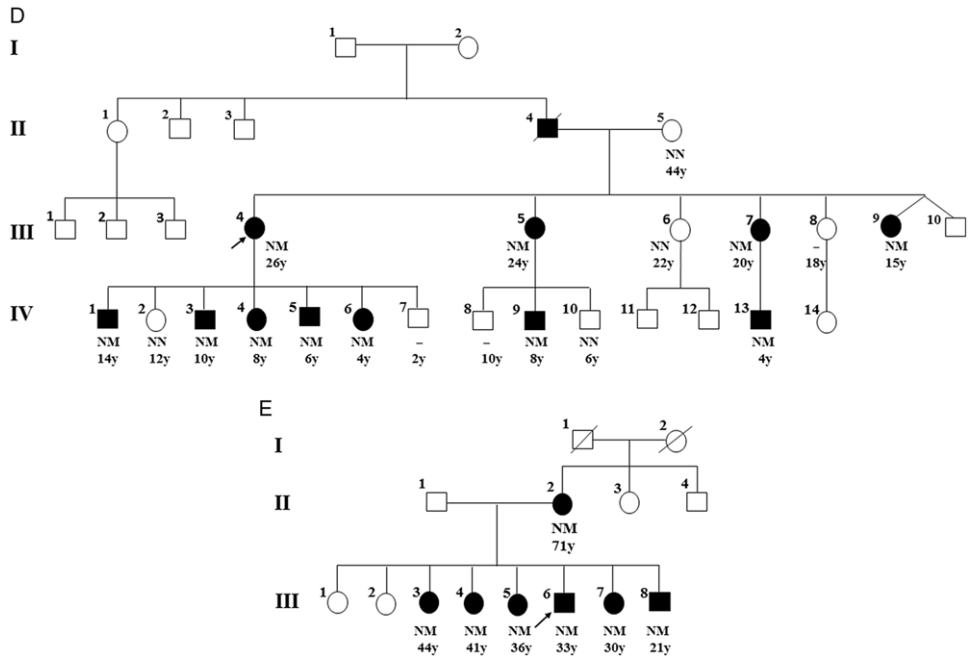

Figure 1: *(Continued)*

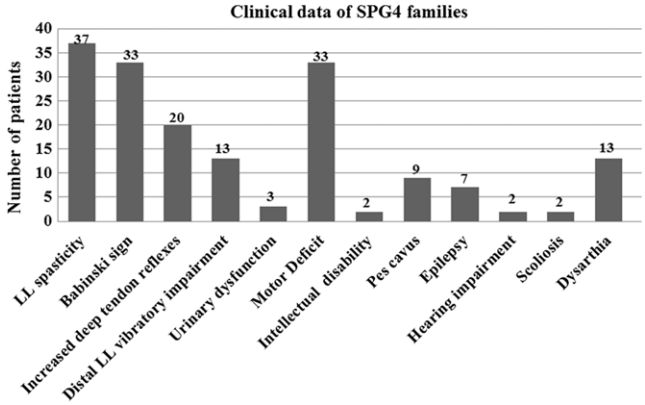

Figure 2: *Clinical data of SPG4 families. Different clinical features of the 39 SPG4 patients have been shown. Data were not available for all patients.*

the mean of AAO decreased from 68 to 6.8 years within two generations. These differences were significant between parents and their children in all five families (p-value <0.05). But the families with mutations in other genes or genetically undiagnosed families in this study did not present any significant anticipation.

Discussion

In this study, WES detected the disease-causing variants in seven out of 14 HSP families (50%). Clinical evaluation of them showed that only all SPG4 families presented a putative genetic anticipation. Among the seven remaining families, anticipation was observed only in the family SPG4-B who presented the large deletion of exon 17 of *SPAST* by MLPA as we expected. The deletion has been previously reported in several SPG4 families and suggested that the high concentration of Alu elements in the intronic and flanking regions of the *SPAST* gene likely facilitates the occurrence of intragenic Alu-mediated rearrangements especially in the final exon, exon 17.[37] Genetic anticipation was not observed in other subtypes of HSP with or without genetic diagnosis in our study (data have not been included).

Anticipation usually resulted from the dynamic mutations/repeat expansions.[15–17] Furthermore, it has also been reported occasionally in several diseases with static mutations. For instance, a few missense and splice-site mutations in the *SOD1* gene, which causes ALS, already have been associated with anticipation.[22,23,38] Or, anticipation has been considered with several mutations in the *SPAST* gene. Before discovering *SPAST* as a SPG4-causing gene, anticipation has been mentioned in a few families linked to *SPAST* locus,[39–43] but there was insufficient clinical evidence to support anticipation in SPG4 families. Bruyn et al. reported anticipation for the first time in a large Dutch family with clinically probable Strumpell's disease with autosomal dominant inheritance. They observed that the AAO in offsprings declined in the successive generations from 45.8 to 9.6 years and suggested possible anticipation[39] (Supplementary Table S1). After identifying the *SPAST* gene, anticipation has been noted in the small number of families affected by SPG4 (Supplementary Table S1).[25–34,39–43] All these families were diagnosed with static mutations in the *SPAST* gene. Neither nature of the mutation nor position of the alteration changed among generations. However, high variability in AAO in the SPG4 patients in different generations of the families suggests that additional genetic or environmental modifiers also contribute to their phenotypes.[28] Reddy et al. found a deletion mutation in *SPAST*, and they concluded it might be dynamic and variability in the size of the deletion could account for anticipation.[34]

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Nevertheless, this hypothesis does not seem to be correct because this mutation did not alter transmission from one generation to another, and therefore, it cannot be dynamic. Also, anticipation was seen in some other SPG4 families with missense mutations. So, it seems that other molecular mechanisms or environmental factors may play roles in the development of anticipation in SPG4 families.[34] Considering the same living environment for different generations, it looks unlikely that environmental factors play an important role in anticipation in these families.[11] Potentially, unknown intracellular factors like telomere position effect (TPE) or miscellaneous modifiers like sex and hormones or additional genetic modifiers are involved in such cases.[8] It is also suggested that some genes such as *SPAST* contain a few microsatellites in introns that are often highly mutable, and their copy numbers vary in different generations. These copy number variations may affect gene expression and thus AAO of disease.[34] If this hypothesis is correct, the lack of anticipation in some families could be justified. However, the correctness of this hypothesis needs to be proven.

Although many SPG4 families have been reported worldwide, in many of them, anticipation was not reported, which may be due to (1) the lack of anticipation may be due to the above hypothesis, (2) the lack of attention (Supplementary Table S4),[44–47] (3) the cohort studies to detect genotype–phenotype correlation have not investigated anticipation, [7,13] (4) the small number of affected individuals in these families and the lack of access to several generations of patients[48] or (5) the lack of access to clinical data of the earlier generations. Regarding the last one, the determination of AAO of the older affected individuals from the first affected generations in each family can be associated with recollection/ascertainment bias.[49] On the other hand, the presence of disease may not be recognized at all when there is very late onset and possibility of misdiagnosis with primary lateral sclerosis (PLS).[50] These limitations can affect the prediction of anticipation. Thus, its confirmation needs to focus on a large number of SPG4 families or maybe apply of objective rating scales such as Spastic Paraplegia Rating Scale (SPRS)[51] and Expanded Disability Status Scale (EDSS)[52] or even designing of a novel rating scales to better compare phenotypes between generations. All in all, the existence of anticipation in SPG4 has remained controversial; however, it seems that anticipation can be relatively more common in SPG4 than it appears (Supplementary Table S4).

The literature search also showed anticipation might rarely be observed in some other subtypes of AD-HSP, including a few SPG3A (with mutations in *ATL1*)[53] and SPG31 (with mutations in *REEP1*)[54] families. Although anticipation may be observed in other subtypes of HSP, it seems *SPAST* will be the first candidate gene in families who present autosomal dominant inheritance, the pure form of disease as well as anticipation. This seems to be a powerful genotype-phenotype correlation.

Altogether, further research about *SPAST* mutations in the affected families should be validated experimentally for possible anticipation and finding its causes. Confirmation of anticipation can be valuable for genetic counseling in presymptomatic carriers and eventually their follow-up. Furthermore, providing more information about the underlying mechanisms has resulted in a less obscure understanding of the pathophysiology of SPG4.[8,11]

Clinical manifestations of SPG4 families in this study revealed intra- and interfamilial variability with differences in AAO and disease severity: intellectual disability was observed only in two affected individuals of the family SPG4-D but not in other affected individuals of the family or the patients of the SPG4-E family with the same mutation. These results confirm intra and inter-familial heterogeneity among SPG4 families (Table 1). Despite these heterogeneities, interestingly, the AAO between affected individuals in a given generation especially between siblings was less variable than between different generations (Table 2). Parodi et al. reported such correlation and suggested that other genetic factors may significantly impact the AAO.[8]

Our results also showed another genotype–phenotype correlation: missense mutations seem to be associated with a more severe form of the disease. Intellectual disability, dysarthria, pes cavus, and epilepsy were observed only in two families with a missense mutation in the AAA ATPase cassette, and none of the SPG4 patients with truncating mutations developed these symptoms. Although these manifestations are not common in SPG4 patients, those have been previously reported in other SPG4 families.[55,56] Similar results were reported by Parodi et al. in 2018. They reported an increasing severity in patients harboring a missense mutation and mentioned that intellectual disability was significantly more frequent among SPG4 patients with missense mutations.[8] It is suggested that these interfamilial phenotypic variabilities among SPG4 patients might be due to type and location of mutations. Missense mutations that are often located in AAA-ATPase domain (such as p. Arg499His) result in weaken the microtubule severing activity of the protein or sometimes lack of affinity to ATP, suggesting a loss-of-function mechanism.[57,58] Whereas missense mutations in other domains of spastin may alter the endosomal tubule fusion function.[59,60] or may increase the lifespan of the mutant protein, leading to toxic accumulation and interaction with the wild-type SPAST proteins, highlighting a negative dominant mechanism.[61] Nonsense mutations (such as p. Ser261*), resulting in truncated protein, can trigger nonsense-mediated decay machinery to cleanse the cell from such abnormalities, but given the rapid pileup, slight increase in stability, and the less susceptibility to degradation, neurotoxicity of truncated SPAST subunits results in defective outgrowth of neurites.[57,62,63] This phenomenon can be a gain-of-function that occurs in even very low doses of truncated spastin protein, which plays an essential role in axonal transportation. It has been suggested that this accumulation is progressive in nature, which is in concert with that progressive nature of SPG4, as well.[63]

Briefly, although we observed a presumptive anticipation among SPG4 families, several researchers have suggested the earlier AAO might be linked to a potential ascertainment bias.[13,34] Due to the lack of knowledge and attention of these families regarding this disease, diagnostic sensitivity might have been enhanced in subsequent generations. Also, the lack of access to details of clinical data and assessment of the precise AAO, especially in the earlier generations, and the limited number of studied families can affect the prediction of anticipation. However, considering the observation of anticipation in a large number of previous reported SPG4 patients (Supplementary Table S1), as well as a significant difference in the AAO of the disease (>50 years), cannot be accidental.

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Conflicts of Interest

All authors claim absence of financial interests and absence of conflicts of interest. All authors read and approved the final version of the manuscript.

Statement of Authorship

1) Research project: A. Conception, B. Organization, C. Execution;
2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique;
3) Manuscript: A. Writing of the first draft, B. Review and Critique.

Seyyed-Saleh Hashemi: 1C, 2B, and 3B
Reza Hajati: 1C, 2B, and 3B
Atefeh Davarzani: 1C, 2B, and 3B
Mohammad Rohani: 1A, 3A, and 3B
Mohammad Masoud Rahimi Bidgoli: 1C, 2B, 3B
Fardad DanaeeFard: 1C, 2B, and 3B
Farzad Fatehi: 1A, 3B
Ariana Kariminejad: 1A, 3B
Hossein Najmabadi: 1A, 3B
Shahriar Nafissi: 1A, 3B
Afagh Alavi: 1A, 1B, 2A, 2B, 3A, and 3B

Supplementary Material

To view supplementary material for this article, please visit https://doi.org/10.1017/cjn.2021.188

References

1. Harding AE. Classification of the hereditary ataxias and paraplegias. Lancet. 1983;1:1151–5.
2. Klebe S, Stevanin G, Depienne C. Clinical and genetic heterogeneity in hereditary spastic paraplegias: from SPG1 to SPG72 and still counting. Rev Neurol. 2015;171:505–30.
3. Erfanian Omidvar M, Torkamandi S, Rezaei S, et al. Genotype-phenotype associations in hereditary spastic paraplegia: a systematic review and meta-analysis on 13,570 patients. J Neurol. 2021;268:2065–82.
4. Shribman S, Reid E, Crosby AH, Houlden H, Warner TT. Hereditary spastic paraplegia: from diagnosis to emerging therapeutic approaches. Lancet Neurol. 2019;18:1136–46.
5. Errico A, Ballabio A, Rugarli EI. Spastin, the protein mutated in autosomal dominant hereditary spastic paraplegia, is involved in microtubule dynamics. Hum Mol Genet. 2002;11:153–63.
6. Beetz C, Nygren A, Schickel J, et al. High frequency of partial SPAST deletions in autosomal dominant hereditary spastic paraplegia. Neurology. 2006;67:1926–30.
7. Kadnikova V, Rudenskaya G, Stepanova A, Sermyagina I, Ryzhkova O. Mutational spectrum of Spast (Spg4) and Atl1 (Spg3a) genes in Russian patients with hereditary spastic paraplegia. Sci Rep. 2019;9:1–8.
8. Parodi L, Fenu S, Barbier M, et al. Spastic paraplegia due to SPAST mutations is modified by the underlying mutation and sex. Brain. 2018;141:3331–42.
9. de Souza PVS, de Rezende Pinto WBV, de Rezende Batistella GN, Bortholin T, Oliveira ASB. Hereditary spastic paraplegia: clinical and genetic hallmarks. Cerebellum. 2017;16:525–51.
10. Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol. 2013;126:307–28.
11. Solowska JM, Baas PW. Hereditary spastic paraplegia SPG4: what is known and not known about the disease. Brain. 2015;138:2471–84.
12. Loureiro JL, Brandao E, Ruano L, et al. Autosomal dominant spastic paraplegias: a review of 89 families resulting from a portuguese survey. JAMA Neurol. 2013;70:481–7.
13. Chelban V, Tucci A, Lynch DS, et al. Truncating mutations in SPAST patients are associated with a high rate of psychiatric comorbidities in hereditary spastic paraplegia. J Neurol Neurosurg Psychiatry. 2017;88:681–7.
14. Paulson H. Repeat expansion diseases. Handbook Clin Neurol. 2018;147:105–23.
15. Ranen NG, Stine OC, Abbott MH, et al. Anticipation and instability of IT-15 (CAG) n repeats in parent-offspring pairs with Huntington disease. Am J Hum Genet. 1995;57:593.
16. Redman JB, Fenwick RG, Fu Y-H, Pizzuti A, Caskey CT. Relationship between parental trinucleotide GCT repeat length and severity of myotonic dystrophy in offspring. JAMA. 1993;269:1960–5.
17. Imbert G, Saudou F, Yvert G, et al. Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. Nat Genet. 1996;14:285–91.
18. El Tannouri R, Albuisson E, Jonveaux P, Luporsi E. Is there a genetic anticipation in breast and/or ovarian cancer families with the germline c. 3481_3491del11 mutation? Fam Cancer. 2018;17:5–14.
19. McInnis MG. Anticipation: an old idea in new genes. Am J Hum Genet. 1996;59:973.
20. Bozzao C, Lastella P, Stella A. Anticipation in lynch syndrome: where we are where we go. Curr Genomics. 2011;12:451–65.
21. Bayless TM, Picco MF, LaBuda MC. Genetic anticipation in Crohn's disease. Am J Gastroenterol. 1998;93:2322–5.
22. Alavi A, Nafissi S, Rohani M, et al. Genetic analysis and SOD1 mutation screening in Iranian amyotrophic lateral sclerosis patients. Neurobiol Aging. 2013;34:1516. e1–, e8.
23. Iwai K, Yamamoto M, Yoshihara T, Sobue G. Anticipation in familial amyotrophic lateral sclerosis with SOD1-G93S mutation. J Neurol Neurosurg Psychiatry. 2002;72:819–20.
24. Guo X, Fan C, Wang Y, et al. Genetic anticipation in a special form of hypertrophic cardiomyopathy with sudden cardiac death in a family with 74 members across 5 generations. Medicine. 2017;96:e6249.
25. Bönsch D, Schwindt A, Navratil P, et al. Motor system abnormalities in hereditary spastic paraparesis type 4 (SPG4) depend on the type of mutation in the spastin gene. J Neurol Neurosurg Psychiatry. 2003;74:1109–12.
26. Kawarai T, Montecchiani C, Miyamoto R, et al. Spastic paraplegia type 4: a novel SPAST splice site donor mutation and expansion of the phenotype variability. J Neurol Sci. 2017;380:92–7.
27. Lan M-Y, Fu S-C, Chang Y-Y, et al. Clinical and genetic analysis of four Taiwanese families with autosomal dominant hereditary spastic paraplegia. J Formosan Med Assoc. 2012;111:380–5.
28. Morita M, Ho M, Hosler BA, McKenna-Yasek D, Brown RHJr. A novel mutation in the spastin gene in a family with spastic paraplegia. Neurosci Lett. 2002;325:57–61.
29. Namekawa M, Takiyama Y, Sakoe K, et al. A Japanese SPG4 family with a novel missense mutation of the SPG4 gene: intrafamilial variability in age at onset and clinical severity. Acta Neurol Scand. 2002;106:387–91.
30. Orlacchio A, Gaudiello F, Totaro A, et al. A new SPG4 mutation in a variant form of spastic paraplegia with congenital arachnoid cysts. Neurology. 2004;62:1875–8.

31. Wang K, Zhao G. Exon 8-17 deletions of SPAST in a Chinese family with hereditary spastic paraplegia: a case report and literature review. J Neurol Sci. 2015;357:282–4.
32. Wei Q-Q, Chen Y, Zheng Z-Z, et al. Spastin mutation screening in Chinese patients with pure hereditary spastic paraplegia. Parkinsonism Relat Disord. 2014;20:845–9.
33. Yu W, Jin H, Deng J, Nan D, Huang Y. A novel SPAST gene mutation identified in a Chinese family with hereditary spastic paraplegia. BMC Med Genet. 2020;21:1–7.
34. Reddy PL, Seltzer WK, Grewal RP. Possible anticipation in hereditary spastic paraplegia type 4 (SPG4). Can J Neurol Sci. 2007; 34:208–10.
35. Van Mossevelde S, van der Zee J, Gijselinck, I, etal. Clinical evidence of disease anticipation in families segregating a C9orf72 repeat expansion. JAMA Neurol. 2017;74:445–52.
36. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–23.
37. Boone PM, Liu P, Zhang F, et al. Alu-specific microhomology-mediated deletion of the final exon of SPAST in three unrelated subjects with hereditary spastic paraplegia. Genet Med. 2011; 13:582–92.
38. Kim W, Kim J-S, Lee K-S, Gwoun Y-J, Kim J-M, Lee K-H. Anticipation and phenotypic heterogeneity in korean familial amyotrophic lateral sclerosis with superoxide dismutase 1 gene mutation. J Clin Neurol. 2007;3:38–44.
39. Bruyn R, Van Deutekom J, Frants R, Padberg G. Hereditary spastic paraparesis: clinical and genetic data from a large Dutch family. Clin Neurol Neurosurg. 1993;95:125–9.
40. Matsuura T, Sasaki H, Wakisaka A, Hamada T, Moriwaka F, Tashiro K. Autosomal dominant spastic paraplegia linked to chromosome 2p: clinical and genetic studies of a large Japanese pedigree. J Neurol Sci. 1997;151:65–70.
41. Raskind WH, Pericak-Vance MA, Lennon F, Wolff J, Lipe HP, Bird TD. Familial spastic paraparesis: evaluation of locus heterogeneity, anticipation, and haplotype mapping of the SPG4 locus on the short arm of chromosome 2. Am J Med Genet. 1997;74:26–36.
42. Scott WK, Gaskell PC, Lennon F, et al. Locus heterogeneity, anticipation and reduction of the chromosome 2p minimal candidate region in autosomal dominant familial spastic paraplegia. Neurogenetics. 1997;1:95–102.
43. Thurmon T, He C, Haskell C, Thorpe P, Thurmon S, Rosen D. Genetic anticipation in a large family with pure autosomal dominant hereditary spastic paraplegia. Am J Med Genet. 1999;83:392–6.
44. Mitne-Neto M, Kok F, Beetz C, et al. A multi-exonic SPG4 duplication underlies sex-dependent penetrance of hereditary spastic paraplegia in a large Brazilian pedigree. Eur J Hum Genet. 2007;15:1276–9.
45. Zhao G, Liu X, Jiang PJNS. Identification of a novel SPG4 tandem base substitution in a Chinese hereditary spastic paraplegia family. Neurol Sci. 2017;38:903–5.
46. Ki C-S, Lee WY, Han DH, et al. A novel missense mutation (I344K) in the SPG4 gene in a Korean family with autosomal-dominant hereditary spastic paraplegia. J Hum Genet. 2002;47:473–7.
47. Nielsen JE, Johnsen B, Koefoed P, et al. Hereditary spastic paraplegia with cerebellar ataxia: a complex phenotype associated with a new SPG4 gene mutation. Eur J Neurol. 2004;11: 817–24.
48. Basri R, Yabe I, Soma H, et al. Four mutations of the spastin gene in Japanese families with spastic paraplegia. J Hum Genet. 2006; 51:711–5.
49. Minikel EV, Zerr I, Collins SJ, et al. Ascertainment bias causes false signal of anticipation in genetic prion disease. Am J Hum Genet. 2014;95:371–82.
50. Almomen M, Martens K, Quadir A, et al. High diagnostic yield and novel variants in very late-onset spasticity. J Neurogenet. 2019;33:27–32.
51. Schüle R, Holland-Letz T, Klimpe S, et al. The spastic paraplegia rating scale (SPRS): a reliable and valid measure of disease severity. Neurology. 2006;67:430–4.
52. Guthrie G, Pfeffer G, Bailie M, et al. The neurological and ophthalmological manifestations of SPG4-related hereditary spastic paraplegia. J Neurol. 2013;260:906–9.
53. Ming L. SPG3A-hereditary spastin paraplegia with genetic anticipation and incomplete penetrance. Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chin J Med Genet. 2007;24:15–8.
54. Kamada M, Kawarai T, Miyamoto R, et al. Spastic paraplegia type 31: a novel REEP1 splice site donor variant and expansion of the phenotype variability. Parkinsonism Relat Disord. 2018; 46:79–83.
55. de Bot ST, van den Elzen RT, Mensenkamp A, et al. Hereditary spastic paraplegia due to SPAST mutations in 151 Dutch patients: new clinical aspects and 27 novel mutations. J Neurol Neurosurg Psychiatry. 2010;81:1073–8.
56. Lu C, Li L-X, Dong H-L, et al. Targeted next-generation sequencing improves diagnosis of hereditary spastic paraplegia in Chinese patients. J Mol Med. 2018;96:701–12.
57. Bürger J, Fonknechten N, Hoeltzenbein M, et al. Hereditary spastic paraplegia caused by mutations in the SPG4 gene. Eur J Hum Genet. 2000;8:771–6.
58. Evans KJ, Gomes ER, Reisenweber SM, Gundersen GG, Lauring BP. Linking axonal degeneration to microtubule remodeling by Spastin-mediated microtubule severing. J Cell Biol. 2005;168:599–606.
59. Allison R, Edgar JR, Reid E. Spastin MIT domain disease-associated mutations disrupt lysosomal function. Front Neurosci. 2019;13:1179.
60. Boutry M, Morais S, Stevanin G. Update on the genetics of spastic paraplegias. Curr Neurol Neurosci Rep. 2019;19:1–19.
61. Al Mutairi F, Alfadhel M, Nashabat M, et al. Phenotypic and molecular spectrum of Aicardi-Goutières syndrome: a study of 24 patients. Pediatr Neurol. 2018;78:35–40.
62. Qiang L, Piermarini E, Muralidharan H, et al. Hereditary spastic paraplegia: gain-of-function mechanisms revealed by new transgenic mouse. Hum Mol Genet. 2019;28:1136–52.
63. Solowska JM, Rao AN, Bass PW. Truncating mutations of SPAST associated with hereditary spastic paraplegia indicate greater accumulation and toxicity of the M1 isoform of spastin. Mol Biol Cell. 2017;28:1728–37.

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