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Mutation analysis of four Chinese families with pure hereditary spastic paraplegia: pseudo-X-linked dominant inheritance and male lethality due to a novel *ATL1* mutation

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ABSTRACT. We studied four Chinese families with pure hereditary spastic paraplegia (HSP) to investigate the clinical features and associated genetic mutations. Linkage analysis was performed for all families to map the disease locus onto autosomal chromosomes, and related loci involved in HSP on the X chromosome were also examined. Polymerase chain reaction (PCR) sequencing was used to detect gene mutations. To confirm the influence of a splice-site mutation on mRNA, we used reverse transcription-PCR and direct sequencing. Linkage analysis and *ATL1* gene sequencing of amniocytes were performed for prenatal genetic diagnosis. One missense variant (c.1517T>A) and a splice-site mutation (c.1245+1G>A) in *SPAST*, and two missense variants (c.715C>T, c.1204T>G) in *ATL1* were identified. The c.1245+1G>A mutation caused a deletion of exon 9 in the *SPAST* gene.

Prenatal genetic diagnosis showed that fetus did not carry the *ALT1* c.1204T>G mutation. Follow-up was maintained for 5 years, and the negative result was confirmed by evidence of a healthy growing boy. We identified two novel mutations and two previously reported mutations in *SPAST* and *ATL1*, respectively. The family with the *ATL1* c.1204T>G mutation exhibited male-lethality, female infancy-onset, and pseudo-X-linked dominant transmission, which had never been previously reported for HSP. Characteristic facial features were also noticed. The boy on whom prenatal gene diagnosis was performed is healthy and without unusual facies, suggesting that the c.1204T>G mutation might be related to these features. The results extend the genetic spectrum of HSP and suggest that linkage analysis remains a powerful tool in gene discovery studies.

Key words: *SPAST*; *ATL1*; Mutation; X-linked dominant; Hereditary spastic paraplegia

INTRODUCTION

Hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of neurodegenerative diseases characterized by progressive spasticity and weakness of the lower limbs. To date, 71 different spastic gait disease loci have been identified, and 54 spastic paraplegia genes (SPGs) have already been cloned. HSP can be transmitted in an autosomal dominant (AD), autosomal recessive, X-linked, or mitochondrial manner. AD inheritance accounts for 70% of HSP. Mutations in the genes *SPAST* (SPG4) and *ATL1* (SPG3A) account for up to 50% of AD-HSP (Lo Giudice et al., 2014). Mutations in *REEP1* (SPG31) are the third most common genetic cause of AD-HSP (Züchner et al., 2006; Beetz et al., 2008). To date, five X-linked SPG loci: SPG1, SPG2, SPG16, SPG22, and SPG34, and three genes: *L1CAM*, *PLP1*, and *SLC16A2*, have been identified. Gene identities at the SPG16 and SPG34 loci are unknown.

MATERIAL AND METHODS

Patients

Four unrelated ethnic Han kindreds (marked as families 1-4) with HSP were included in this study (Figure 1). Clinical information and family histories were collected and neurological examinations were performed by a neurologist according to Harding's criteria (Harding, 1981). Families 1-3 possessed AD inheritance patterns, and family 4 appeared to segregate an X-linked dominant form of HSP, in which all six living affected individuals were females; four males had died of unknown causes within 10 days after delivery. Written informed consent was obtained from all participants prior to the study. The genetic study, conducted on the members of the four families, was approved by the local Ethics Committee. Human genomic DNA was isolated from whole blood using the DNA Isolation Kit for Mammalian Blood (Tiangen Biotech Co., Ltd., Beijing, China).

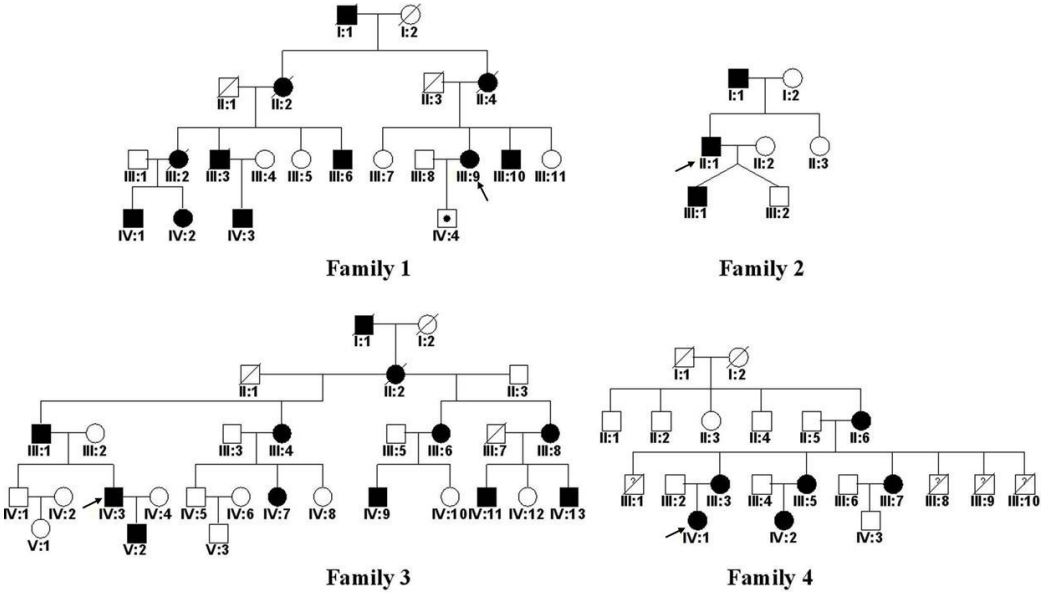

Figure 1. Pedigrees of four families with hereditary spastic paraplegia. Male (square) and female (circles) individuals with spastic paraplegia are indicated with black-filled symbols. Healthy individuals are represented with open symbols. Deceased individuals are shown with stricken-out symbols. Question marks represent possible affected individuals.

Linkage analysis

Two-point linkage analysis was performed for all 4 families using the markers 2chAAT (2chAAT position: chr2:32166435-32166465), D14S976, and D2s2232 close to SPG4, SPC3A, and SPG31, respectively, and utilizing the MLINK program of LINKAGE Package (version 5.2) (Lathrop et al., 1984) to map the disease loci related to the AD-HSP families 1-3.

For family 4, in consideration of the suspected X-linked dominant transmission, 5 loci (SPG1, SPG2, SPG16, SPG22, and SPG34) on the X chromosome previously found to be involved in HSP were selected, and two-point linkage analysis was performed using the markers DXS8087, XchCA (XchCA position: chrX: 102911057-102911103), XchAT (XchAT position: chrX: 69925512-69925549), DXS8066, and DXS8057, which are close to SPG1, SPG2, SPG16, SPG22, and SPG34, respectively, utilizing the MLINK program of LINKAGE Package (version 5.2) (Lathrop et al., 1984) to map the disease loci.

Mutation analysis

For these 4 families, two affected and one unaffected individual for each family were selected for mutation analysis. All exons and intron-exon boundaries of *SPAST* and *ATL1* were amplified by polymerase chain reaction (PCR) as previously described (Hazan et al., 1999; Zhao et al., 2001) (http://genome.ucsc.edu/). The amplified fragments were then subjected to direct sequencing. Both novel variations were further confirmed by digestion with restriction enzyme in all affected individuals, normal family members, and 100 health controls. The digested products were separated and analyzed by 8% polyacrylamide gel electrophoresis and

silver staining. The *SPAST* c.1517T>A variation was digested by restriction enzyme *Hinf*I. All affected individuals show three fragments of 298, 96 and 202 bp. The *ATL1* c.1204T>G variation was digested by restriction enzyme *Alu*I with mismatch primer method. All affected individuals show two fragments of 135 and 117, the 17-bp product cannot be visualized on the gel.

mRNA analysis

Total mRNA was extracted from blood leukocytes (RNAliquid Speeding whole blood (liquid) total RNA extraction kit; Aidlab Biotech, Beijing, China) and skin sample from skin biopsies (EASYspin Plus tissue/cell RNA Rapid extraction kit; Aidlab Biotech) of individual II:1 of family 2, and converted to cDNA using the Prime Script™ RT reagent Kit (TaKaRa Biotechnology (Dalian) Co., Ltd., Osaka, Japan). Primers were designed for PCR amplification of the cDNA segments that encompassed the splice-site variation. The sequences of the forward and reverse primers were 5'-GGCTGTTACTCTTTGGTC-3' and 5'-CAACGTATGTACGCTTCT-3', respectively. The PCR conditions for the cDNA segment amplification were as follows: denaturing at 95°C for 5 min; 35 cycles of denaturing at 95°C for 30 s, annealing at 46°C for 30 s, and extension at 72°C for 30 s; and a final step for 5 min at 72°C, using the 96-well Thermal Cycler (Applied Biosysterms). The amplified fragments were then subjected to direct sequencing.

Prenatal genetic diagnosis

Individual III:7 of family 4 was tested at 20 weeks of pregnancy. A 20-mL sample of amniotic fluid was withdrawn from the amniotic sac under the guidance of ultrasound. The amniocytes were separated by centrifugation. The DNA was isolated from the amniocytes using a DNA Extraction Kit (Spin-column) (Norgen BioTek, Thorold, ON, Canada). The DNA was directly analyzed by two-point linkage inferential analysis using the marker D14s976, and sequencing of the *ATL1* gene was performed for the prenatal genetic diagnosis of the fetus.

RESULTS

The clinical characteristics of the affected members in the four families are shown in Table 1. This study included 26 subjects (female:male ratio of 1:1; age range: 5-64 years). The ages of onset exhibited a wide range from 0 to 47 years. All symptomatic patients manifested as uncomplicated phenotypes and presented motor dysfunction due to weakness or severe spasticity of the lower limbs. Considerable intrafamilial variation in terms of age of onset, rate of progression, and severity of disease were observed in families 1 and 2. In families 3 and 4, all patients suffered from onset in infancy. In family 4, all affected individuals were females. They showed very similar phenotypes, and had to walk on double crutches. In addition, their facial features were distinct, with a prominent mouth lip and a long nasal bridge. Subject II:6 had an abnormal childbearing history: four male newborns all died of unknown causes within 2-10 days after normal delivery.

For families 1 and 2, we obtained positive LOD scores of 1.47 and 1.53, respectively, at $\theta = 0$ with marker 2chAAT in the genomic region close to the *SPAST* gene. *SPAST* mutations were discovered in both kindreds. In family 1, we identified the missense mutation c.1517T>A in exon 13 of the *SPAST* gene, which resulted in a valine to glutamine substitution at amino

Table 1. Clinical characteristics and identified mutations of hereditary spastic paraplegia patients.

Family	Case	Gender	Age at exam (years)	Age at onset (years)	Spastic gait	Difficulty in walking	LL spasticity	UL spasticity	Scoliosis	Facial figures	Pes cavus	Babinski reflex	Clonus	Walking assistance	Brain MRI	Detected mutation
1 (c.1517T>A)	III:6	M	53	41	-	-	+	-	-	-	-	+	-	-	NA	*SPAST*
	III:9	F	55	38	+	+	+	-	-	-	-	+	+	+	NA	
	III:10	M	50	47	-	-	+	-	-	-	-	+	-	-	NA	
	IV:1	M	55	44	-	-	+	-	-	-	-	+	+	-	Normal	
	IV:2	F	48	3	+	+	+	-	+	-	-	+	-	+	Normal	
	IV:3	M	40	10	-	-	+	-	-	-	-	+	-	-	NA	
	IV:4	F	20	-	-	-	-	-	-	-	-	-	-	-	NA	
2 (c.1245+1G>A)	I:1	M	64	16	+	+	+	-	-	-	-	+	+	+	NA	*SPAST*
	II:1	M	34	20	-	-	+	-	-	-	-	+	-	-	NA	
	III:1	M	12	4	-	-	+	-	-	-	-	+	-	-	Normal	
3 (c.715C>T)	III:1	M	56	4	+	-	+	+	-	-	-	+	-	-	NA	*ATL1*
	III:4	F	54	3	-	-	+	-	-	-	-	+	-	-	NA	
	III:6	F	52	6	+	-	+	-	-	-	-	+	-	-	NA	
	III:8	F	49	1	-	-	+	-	-	-	-	+	-	-	NA	
	IV:3	M	30	2	-	-	+	-	-	-	-	+	-	-	NA	
	IV:7	F	26	5	-	-	+	-	-	-	-	-	-	-	NA	
	IV:9	M	21	4	-	-	+	-	-	-	-	-	-	-	NA	
	IV:11	M	23	5	-	-	+	-	-	-	-	+	-	-	Normal	
	IV:13	M	18	4	-	-	+	-	-	-	-	+	-	-	NA	
	V:2	M	6	1	-	-	+	-	-	-	-	-	-	-	Normal	
4 (c.1204T>G)	II:6	F	55	0	+	+	+	-	-	+	+	+	+	+	NA	*ATL1*
	III:3	F	35	0	+	+	+	-	-	+	+	+	+	+	NA	
	III:5	F	33	0	+	+	+	-	-	+	+	+	+	+	NA	
	III:7	F	31	0	+	+	+	-	-	+	+	+	+	+	NA	
	IV:1	F	12	0	+	+	+	-	-	+	+	+	+	+	Normal	
	IV:2	F	5	0	+	+	+	-	-	+	+	+	+	+	NA	

LL = lower limbs; UL = upper limbs; MRI = magnetic resonance imaging; M = male; F = female; NA = not available; (+) = present; (-) = absent.

acid residue 506 (p.Val506Glu). The mutation was not detected in 100 unrelated ethnic Han Chinese individuals. Individual IV:11 of family 1, who had a normal phenotype, also carried the c.1517T>A mutation found in the symptomatic familial members. In family 2, the splice mutation c.1245+1G>A was identified, which has previously been reported (Svenson et al., 2001). This mutation caused the deletion of exon 9 of the *SPAST* gene as confirmed by RT-PCR and direct sequencing.

For family 3, we obtained a positive LOD score of 1.78 at $\theta = 0$ with marker D14S976, which is close to the *ATL1* gene. The missense mutation c.715C>T in exon 7 of *ATL1* was detected, which has previously been reported (Zhao et al., 2001; Alvarez et al., 2010; McCorquodale et al., 2011). This mutation caused an amino acid change from arginine to cysteine at amino acid residue 239 (p.Arg239Cys).

For family 4, negative LOD scores were detected with all 5 markers on the X chromosome and with marker 2chAAT near the *SPAST* gene. We obtained a maximal LOD score of 0.97 at D14S976 near the *ATL1* locus. Sequencing of the *ATL1* gene in two affected individuals identified the missense mutation c.1204T>G in exon 10, which caused an amino acid change from serine to alanine at amino acid residue 346 (p.Ser346Ala); this mutation was not present in the selected normal family member, nor was it detected in 100 unrelated ethnic Han Chinese.

Two-point linkage with marker D14S976 revealed that individual IV:3 of family 4 did not have the same haplotype as the other patients. *ATL1* sequencing showed that this subject did not carry the c.1204T>G mutation. After 5 years of follow-up, individual IV:3 had grown into a healthy boy without the characteristic facial features of the affected individuals of family 4.

DISCUSSION

Spastin is a member of the "ATPases associated with diverse cellular activities" (AAA) family, which have roles in microtubule dynamics and membrane trafficking. Spastin has two main structural domains: the microtubule interacting and transport domain at the N-terminus, and the AAA catalytic domain in C-terminus. Over 370 mutations have been described in spastin to date including missense, nonsense, and splice-site point mutations as well as insertions and deletions, and almost all seem to affect, either directly or indirectly, the AAA cassette-encoding region of the gene. In the present study, we found a novel missense mutation and a splice mutation in *SPAST*. Both the mutations affected the conserved AAA cassette, and were predicted to result in haploinsufficiency (a loss-of-function mechanism). In family 1, individual IV:11 was found to carry the c.1517T>A mutation, but he showed no abnormal features on neurological examination at the age of 20 years. We could not determine whether incomplete dominance existed in this family with AD-HSP, as the penetrance might be age-dependent or incomplete, or the subject might manifest only subtle symptoms (Meijer et al., 2002; Alvarez et al., 2010). Subtle symptoms might progress slowly and go unnoticed for years. Consequently, long-term follow-up is needed to confirm these conditions because individual IV:11 was still young.

In families 3 and 4, we identified two missense mutations in *ATL1*: c.715C>T and c.1204T>G. The c.715C>T variant has been described previously (Zhao et al., 2001; Alvarez et al., 2010; McCorquodale et al., 2011). Most patients with *ATL1* mutations present the pure form of HSP (Kwon et al., 2010), with onset before 10 years (Namekawa et al., 2006). Patients with very early onset develop a severe spastic gait, contrary to those with late onset. Complicated SPG3A and the late-onset form are very rare (Sauter et al., 2004; Ivanova et al.,

2007). AD inheritance is common, although autosomal recessive inheritance and sex-related penetrance have also been reported (Varga et al., 2013; Khan et al., 2014). In this study, family 4, which segregates the c.1204T>G *ATL1* mutation, presents infancy-onset HSP, and the lower limb spasticity of female patients in this family is too severe to permit walking without double crutches. These features are consistent with SPG3A, but the inheritance pattern in this family shows pseudo-X-linked dominant transmission and, strikingly, male-lethality, neither of which have never previously been reported for HSP. We also noted characteristic facial features in this family. So far, approximately 43 mutations of *ATL1* have been identified, which are predominantly of the missense type. However, only one mutation, c.1006C>T, has involved exon 10 (Ivanova et al., 2007). The family that carried c.1006C>T is very small, and only had two male patients, who presented pure HSP and onset at 1 year old. Both patients walk with assistance, corresponding with our family. The c.1204T>G substitution altered residue 346 of atlastin-1, which does not localize to a known protein domain, and is not highly conserved. The function of this coding region is still unknown. A previous study suggested that such mutations exert pathogenic effects either by introducing aberrant secondary structure that disrupts GTPase activity, by disturbing multimerization, or by altering the interactions of atlastin with other proteins (Zhao et al., 2001). These unique characters could not been verified caused by mutation of c.1204T>G in *ATL1*, but the boy who had been subjected to prenatal genetic diagnosis and was shown not to carry this mutation was healthy and without the characteristic facial features of the affected individuals. It was indirectly proved that the *ATL1* c.1204T>G mutation caused the unique characters in family 4.

In conclusion, we analyzed four Chinese families with pure HSP. One of these represents the first reported family with pure HSP characterized by pseudo-X-linked dominant transmission, infancy-onset, male lethality, and unusual facies. We identified two novel mutations and two repeating mutations in *SPAST* and *ATL1*, respectively. These results extend the genetic spectrum of HSP, and contribute to the molecular genetic understanding of HSP and its clinical diagnosis. Our findings suggest that linkage analysis remains a powerful tool in gene discovery studies.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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REFERENCES

Alvarez V, Sánchez-Ferrero E, Beetz C, Díaz M, et al. (2010). Mutational spectrum of the SPG4 (*SPAST*) and SPG3A (*ATL1*) genes in Spanish patients with hereditary spastic paraplegia. *BMC Neurol*. 10: 89.

Beetz C, Schüle R, Deconinck T, Tran-Viet KN, et al. (2008). *REEP1* mutation spectrum and genotype/phenotype correlation in hereditary spastic paraplegia type 31. *Brain* 131: 1078-1086.

Harding AE (1981). Hereditary "pure" spastic paraplegia: a clinical and genetic study of 22 families. *J. Neurol. Neurosurg. Psychiatry* 44: 871-883.

Hazan J, Fonknechten N, Mavel D, Paternotte C, et al. (1999). Spastin, a novel AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nat. Genet*. 23: 296-303.

Ivanova N, Claeys KG, Deconinck T, Litvinenko I, et al. (2007). Hereditary spastic paraplegia 3a associated with axonal neuropathy. *Arch. Neurol*. 64: 706-713.

Khan TN, Klar J, Tariq M, Anjum Baig S, et al. (2014). Evidence for autosomal recessive inheritance in *SPG3A* caused by homozygosity for a novel *ATL1* missense mutation. *Eur. J. Hum. Genet.* 22: 1180-1184.

Kwon MJ, Lee ST, Kim JW, Sung DH, et al. (2010). Clinical and genetic analysis of a Korean family with hereditary spastic paraplegia type 3. *Ann. Clin. Lab. Sci*. 40: 375-379.

Lathrop GM, Lalouel JM, Julier C and Ott J (1984). Strategies for multilocus linkage analysis in humans. *Proc. Natl. Acad. Sci. U. S. A.* 81: 3443-3446.

Lo Giudice T, Lombardi F, Santorelli FM, Kawarai T, et al. (2014). Hereditary spastic paraplegia: Clinical-genetic characteristics and evolving molecular mechanisms. *Exp. Neurol*. 261: 518-539.

McCorquodale DS 3rd, Ozomaro U, Huang J, Montenegro G, et al. (2011). Mutation screening of *spastin*, *atlastin*, and *REEP1* in hereditary spastic paraplegia. *Clin. Genet*. 79: 523-530.

Meijer IA, Hand CK, Cossette P, Figlewicz DA, et al. (2002). Spectrum of *SPG4* mutations in a large collection of North American families with hereditary spastic paraplegia. *Arch. Neurol*. 59: 281-286.

Namekawa M, Ribai P, Nelson I, Forlani S, et al. (2006). *SPG3A* is the most frequent cause of hereditary spastic paraplegia with onset before age 10 years. *Neurology* 66: 112-114.

Sauter SM, Engel W, Neumann LM, Kunze J, et al. (2004). 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.* 23: 98.

Svenson IK, Ashley-Koch AE, Gaskell PC, Riney TJ, et al. (2001). Identification and expression analysis of spastin gene mutations in hereditary spastic paraplegia. *Am. J. Hum. Genet*. 68: 1077-1085.

Varga RE, Schüle R, Fadel H, Valenzuela I, et al. (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.

Zhao X, Alvarado D, Rainier S, Lemons R, et al. (2001). Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. *Nat. Genet.* 29: 326-331.

Züchner S, Wang G, Tran-Viet KN, Nance MA, et al. (2006). Mutations in the novel mitochondrial protein REEP1 cause hereditary spastic paraplegia type 31. *Am. J. Hum. Genet.* 79: 365-369.