Novel Mutations in Endoplasmic Reticulum Lipid Raft-associated Protein 2 Gene Cause Pure Hereditary Spastic Paraplegia Type 18

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Hereditary spastic paraplegia type 18 (HSP18) is a complicated form of autosomal recessive HSP characterized by progressive weakness and spasticity of the lower extremities, dysarthria, and cognitive decline.[1-3] In the year 2011, HSP18, also known as Spastic Paraplegia 18 (SPG18), was firstly identified due to a candidate gene endoplasmic reticulum lipid raft-associated protein 2 (*ERLIN2*) on chromosome 8p11.2 in one Saudis family.[1] During the past 5 years, another two families with SPG18 due to *ERLIN2* mutations have been reported presenting with complicated phenotype.[2,3] Here, we reported a patient born in a nonconsanguineous family who possessed an autosomal recessive pure form of HSP owing to novel mutations in *ERLIN2*. Patient was characterized by late-onset spasticity of lower extremities without significant speech involvement or cognitive disability.

This Chinese nonconsanguineous family [Figure 1a] was comprised of one affected individual presenting with late-onset motor involvement. The patient (II:1) was a 54-year-old female, born of full-term spontaneous vaginal delivery with unremarkable neonatal problems. She reached her early milestones for motor and speech development at an appropriate age. Initial signs of gait disturbance were first noted at the age of 39 years characterized by feet dropping when walking. Over the next 5 years, gait impairment progressed continuously, and she was unable to run. She required walking aids when going upstairs and downstairs at the age of 44 years. On evaluation, no evidence was discovered about dysarthria or cognitive decline. Assessments of mental ability were comparable for her age. There was no evidence of multiple joint contractures and no history of seizures. Neurological examination disclosed that she had weakness in the lower limbs (LLs) (3/5 on a medical research council scale graded 0–5). Muscle tone in the LLs was increased (2/4 on the modified Ashworth scale graded 0–4). It was found that marked bilateral ankle clonus and bilateral Babinski signs. Deep tendon reflexes of the lower extremities were brisk, and clonus could be elicited. The patient was able to walk alone slowly with a scissors gait. There were no abnormal movements. She had normal serum lactate and creatine kinase. Magnetic resonance imaging of the brain and spinal cord and electromyography showed no abnormalities. At the last follow-up, 18 years after onset, the rest of this family members were all normal.

Blood samples were taken from the patient and from her two unaffected descendants. Unaffected individuals ($n = 100$) of matched geographic ancestry were also included as healthy controls. The protocols were all approved by the Ruijin Hospital Ethics Committee, Shanghai Jiao Tong University

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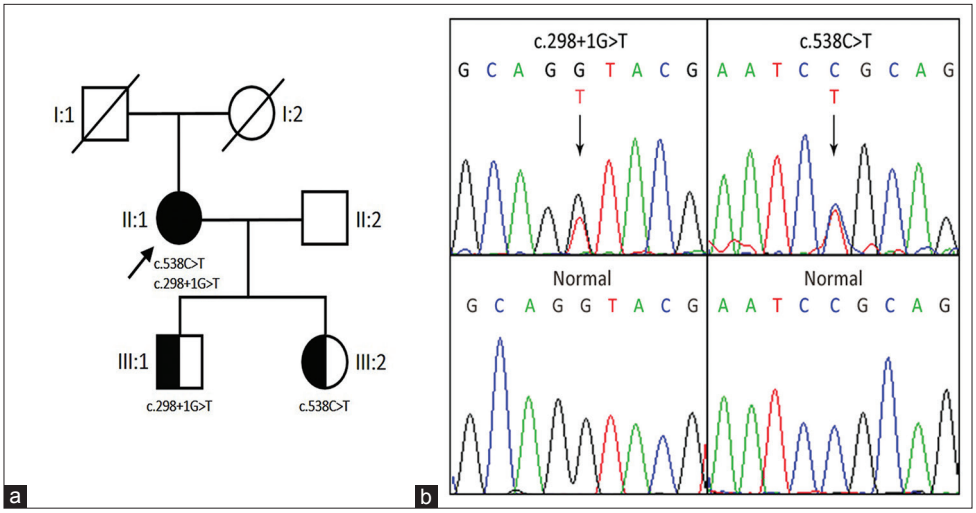

Figure 1: (a) Endoplasmic reticulum lipid raft-associated protein 2 mutations (c.538C>T and c.298+1G>T) identified in the family. (b) Sequence chromatograms from parts of endoplasmic reticulum lipid raft-associated protein 2 gene of this case. Mutations are shown in the upper, and the corresponding normal sequences are shown below. It displays one missense mutation (c.538C>T) in exon 8 and one splice site mutation (c.298+1G>T) in intron 6. Arrows illustrate the nucleotide changes which predict truncations.

Table 1: Clinical features of families with *ERLIN2* mutations

Items	Present family	Alazami *et al*.[1]	Yildırım *et al*.[2]	Wakil *et al*.[3]	Total
Ethnicity	Chinese-Han	Saudis	Turkish	Saudis	/
ERLIN2 mutation	Compound Ht c.538C>T c.298+1G>T	Hm, 20 kb deletion	Hm, c. 812_813insAC	Hm, 23 bp insertion at c.499-1G>T	/
Exon/intron	Exon 8, Intron 6	Intron 1	Exon 11	Intron 7	/
Protein alteration	p.R180C	Null mutation	p.N272Pfs*4	p.Q169Lfs*4	/
Affected individuals	1	5	13	2	21
Age at onset (years)	39	1	6 months to 2 years	1	M = 2.96
Age at the time of publication (years)	54	8, 3*	4–22†	7, 19	M = 18.33
LL spasticity	1/1	5/5	13/13	2/2	21/21
LL reflexes	1/1, +++	5/5, +++	Unable to complete	2/2, +++	/
LL amyotrophy	0/1	NR	0/13	NR	0/14
UL reflexes	1/1, ++	NR	13/13, +++	NR	/
Ankle clonus	1/1	NR	2/13	1/2	4/16
Babinski sign	1/1, BL	NR	2/13, BL	NR	3/14
Seizures	0/1	1/5	9/13	0/2	10/21
Horizontal-rotatory nystagmus	0/1	NR	2/13	NR	2/14
Ataxia	0/1	NR	NR	NR	0/1
Dysarthria	0/1	5/5	13/13	2/2	20/21
Dysphagia	0/1	NR	7/13	NR	7/14
Intellectual disability	0/1	5/5	13/13	2/2	20/21
Arthrogryposis	0/1	NR	13/13	1/2	14/16
Urinary and fecal incontinence	0/1	NR	13/13	NR	13/14
Phenotype	Pure	Complicated	Complicated	Complicated	/
EEG	NE	1/5, +	NR	NR	1/5
Cranial imaging results	1/1, NO	5/5, NO	3/13, NO 10/13, NE	2/2, NO	11/11

*Only two affected individuals from the pedigree were described; †Only seven affected individuals were evaluated again right before the time of publication, and another three individuals were dead. +: Reduced reflexes; ++: Normal reflexes; +++: Brisk reflexes; ++++: Very brisk reflexes; NR: Not reported; NE: Not examined; BL: Bilateral; NO: Normal; M: Mean; EEG: Electroencephalogram; *ERLIN2*: Endoplasmic reticulum lipid raft-associated protein 2; UL: Upper limb; LL: Lower limb; Hm: Homozygous; Ht: Heterozygous; /: Not counted.

School of Medicine. HSP panel was performed on genomic DNA from the patient. SIFT, PolyPhen2, and MutationTaster were used to predict the pathogenicity of the identified mutations. Genotyping of spinocerebellar ataxia type 3 was also performed for differentiation, and no expanded allele of *ATXN3* gene was found. The proband was identified

with compound heterozygous mutations (c.538C>T and c.298+1G>T) in exon 8 and intron 6, respectively, of the *ERLIN2* gene [Figure 1b], and either of which was inherited to her son (III:1) and daughter (III:2). Both mutations were not found in 100 healthy controls or 1000 Genome Project. c.538C>T was predicted to be probably damaging by PolyPhen2 (probability score: 0.999, sensitivity: 0.14, and specificity: 0.99), and deleterious by SIFT (score: 0.01). c.538C>T and c.298+1G>T were predicted to be disease causing by MutationTaster BioTool (probability score: 0.999 and 1.0, range: 0–1.0). According to the American College of Medical Genetics and Genomics guideline,[4] the pathogenicity of c.298+1G>T and c.538C>T was predicted to be pathogenic and likely pathogenic, respectively.

We reported a Chinese patient of SPG18 associated with compound heterozygous mutations in the *ERLIN2* gene: c.538C>T and c.298+1G>T. She presented with late-onset progressive spasticity and weakness of the LLs without speech involvement or cognitive disability. Her son and daughter each carrying one of these mutations were not clinically affected. To date, five *ERLIN2* mutations were identified among 21 patients from 4 families diagnosed as SPG18 (including this patient). Three mutations (20 kb deletion upstream of exon 2, c. 812_813insAC, 23 bp insertion at c.499-1G>T) have been reported, while two mutations (c.538C>T and c.298+1G>T) were undocumented before. We reviewed the clinical manifestation and genetic mutations of these four families from different countries [Table 1].[1-3] Initial signs appeared from 6 months to 39 years old, presenting with the LLs spasticity (21/21), seizures (10/21), horizontal-rotatory nystagmus (2/14), dysarthria (20/21), dysphagia (7/14), intellectual disability (20/21), and arthrogryposis (14/16). SPG18 patient with *ERLIN2* mutations may present pure form of HSP without speech involvement or cognitive disability.

ERLIN2 gene contains 12 exons, and the first exon encodes 5-prime untranslated regions. *ERLIN2* and its paralog *ERLIN1* encode lipid raft-associated proteins localized to the endoplasmic reticulum (ER) and nuclear envelope. They are also called Stomatin/Prohibitin/Flotillin/HflK/C (SPFH) domain family member SPFH2 and SPFH1, as their protein products belong to SPFH domain-containing proteins family. They mediate the ER-associated degradation (ERAD) pathway which is responsible for the degradation of misfolded proteins in the ER such as activated inositol 1,4,5-trisphosphate (IP_3) receptors and other substrates in mammalian cells. In our case, the compound heterozygous point mutations locate in the SPFH domain which may affect co- and post-translational modification and interaction with the inner leaflet of ER membranes. It is suggested that the mutations impaired this ERAD pathway.[5]

In summary, we identified the first Chinese family with SPG18, a pure form of HSP, due to undocumented compound heterozygous novel mutations (c.538C>T and c.298+1G>T) in the *ERLIN2* gene. This study expanded our knowledge of the phenotype of SPG18 and emphasized the importance of *ERLIN2* gene screened for the patients manifesting as a pure form of late-onset HSP.

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Conflicts of interest

There are no conflicts of interest.

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