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RESEARCH ARTICLE

Clinical characteristics of Taiwanese patients with Hereditary spastic paraplegia type 5

Cheng-Ta Chou[1,2], Bing-Wen Soong[3,4], Kon-Ping Lin[3,5], Yu-Shuen Tsai[6], Kang-Yang Jih[3,5], Yi-Chu Liao[3,5,7] & Yi-Chung Lee[3,5,7]

[1]Institute of Clinical Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan
[2]Department of Neurology, Neurological Institute, Taichung Veterans General Hospital, Taichung, Taiwan
[3]Department of Neurology, Neurological Institute, Taipei Veterans General Hospital, Taipei, Taiwan
[4]Department of Neurology, Taipei Neuroscience Institute, Taipei Medical University-Shuang Ho Hospital, Taipei, Taiwan
[5]Department of Neurology, National Yang-Ming University School of Medicine, Taipei, Taiwan
[6]Center for Systems and Synthetic Biology, National Yang-Ming University, Taipei, Taiwan
[7]Brain Research Center, National Yang-Ming University School of Medicine, Taipei, Taiwan

Correspondence
Yi-Chung Lee, Department of Neurology, Neurological Institute, Taipei Veterans General Hospital, No. 201, Sec 2, Shih-Pai Road, Beitou District, Taipei, Taiwan 11217. Tel: +886-2-28712121 ext 3790; Fax: +886-2-28727577; E-mail: ycli@vghtpe.gov.tw

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Abstract

Objectives: To investigate the clinical, electrophysiological, neuroimaging characteristics and genetic features of SPG5 in Taiwan. **Methods**: Mutational analysis of the coding regions of *CYP7B1* was performed by utilizing targeted resequencing analysis of the 187 unrelated Taiwanese HSP patients. The diagnosis of SPG5 was ascertained by the presence of biallelic *CYP7B1* mutations. The SPG5 patients received clinical, electrophysiological, and neuroimaging evaluations. Disease severity was assessed by using the Spastic Paraplegia Rating Scale (SPRS) and the disability score. Two microsatellite markers as well as 18 single-nucleotide polymorphism (SNP) markers flanking *CYP7B1* were genotyped to assess the founder effect of the *CYP7B1* p.R112* mutation. **Results**: Nineteen SPG5 patients from 17 families were identified. They typically presented an insidious onset progressive spastic paraparesis with proprioception involvement beginning at age 8 to 40 years. Their MRIs often showed white matter abnormalities in bilateral occipito-parietal regions, spinal cord atrophy, and mild cerebellar atrophy. Six different mutations in *CYP7B1* were recognized, including three novel ones (p.N131Ifs*4, p.A295V, and p.L439R). *CYP7B1* p.R112* was the most common mutation and present in 88.2% of the 17 SPG5 pedigrees. The patients with homozygous *CYP7B1* p.R112* mutations had a milder clinical severity. Detailed haplotype analyses demonstrated a shared haplotype in the 25 individuals carrying at least one single allele of *CYP7B1* p.R112*, suggesting a founder effect. **Interpretation**: This study delineates the distinct clinical and genetic features of SPG5 in Taiwan and provides useful information for the diagnosis and management of SPG5, especially in patients of Chinese descent.

Introduction

Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of neurodegenerative diseases characterized by slowly progressive spasticity and weakness of the lower extremities with or without other neurological system involvement. Currently, more than 80 different loci and 67 genes have been shown to be associated with HSP.[1-3] Spastic paraplegia type 5 (SPG5) is an autosomal recessive (AR) subtype of HSP and caused by mutations in the *CYP7B1* gene, which encodes the cytochrome P450 oxysterol 7a-hydroxylase (CYP7B1).[4] This enzyme participates in the acidic pathway metabolizing cholesterol to bile acid by catalyzing 7a-hydroxylation of two oxysterols: 27-hydroxycholesterol (27-OHC) and 25-hydroxycholesterol (25-OHC) to

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produce intermediate products of bile acid.[5] Mutations in *CYP7B1* were first identified in the patients with congenital cholestasis,[6,7] and later found to cause SPG5 more frequently.

SPG5 accounts for 3-7.9% of HSP patients in different populations.[8-11] The most common *CYP7B1* mutation in Caucasian populations was c.1456C> T (p.R486C), which was identified in 16-18% of the SPG5 patients.[12-14] Goizet et al. identified 13 SPG5 patients from nine families and found pure form HSP in seven families and complex from HSP in two.[12] Schöls et al. found that the concentration of serum 27-OHC was associated with the disease severity of SPG5.[13] Although SPG5 has been well characterized in West countries, the information in other populations remain sparse, especially the clinical features and genotype–phenotype correlation.

This study aims at investigating the clinical features, neuroimaging findings, and frequency and spectrum of *CYP7B1* mutations of Taiwanese patients with SPG5. We also demonstrated the genotype–phenotype correlation and the founder effect of the *CYP7B1* p.R112* mutation.

Patients and Methods

Study subjects

A consecutive series of 187 unrelated individuals with HSP were enrolled from the Neurological Services of Taipei Veterans General Hospital between 1998 and 2018. All the participants were Taiwanese of Han Chinese ethnicity. Among the 187 HSP patients, SPG5 patients were diagnosed according to the presence of biallelic *CYP7B1* mutations. Family members of the patients with SPG5 were also recruited for further studies, such as clinical evaluation and haplotype analysis to investigate the possible founder effect of *CYP7B1* p.R112* mutation. This study was approved by the institutional review board of Taipei Veterans General Hospital and the written informed consents were obtained from all the participants.

Mutation analysis

Mutational analysis of *CYP7B1* was performed by utilizing a targeted resequencing panel covering the 76 genes associated with HSP (Table S1). The samples were sequenced on the HiSeq2500 platform (Illumina). All sequenced reads were mapped to the Human Genome version 19 (hg19/GRCh37). The BaseSpace pipeline (https://basespace.illumina.com/) and the IlluminaVariantStudio software (http://variantstudio.software.illumina.com/) were utilized to do variant calling and annotate variants, respectively. We further confirmed the *CYP7B1* variants by Sanger sequencing. The novel putative pathogenic *CYP7B1* variants were discriminated by their absence or presence with an extremely rare allele frequency in the public genome databases, including Taiwan biobank database (https://taiwanview.twbiobank.org.tw/index) containing 1517 Taiwanese healthy control genomes and the genome Aggregation Database (gnomAD, version r2.0.2; http://gnomad.broadinstitute.org).[15] *In silico* prediction of the functional effects of the variants was performed using two bioinformatic programs, Provean (http://provean.jcvi.org/index.php)[16] and Combined Annotation Dependent Depletion (CADD, GRCh38-v1.4, https://cadd.gs.washington.edu).[17] Evolutionary conservation of the mutated amino acid residue was analyzed by aligning the amino-acid sequences of CYP7B1 orthologs from several species utilizing the UniProt website (http://www.uniprot.org).

Clinical evaluation and Imaging studies

Disease severity was assessed by using the Spastic Paraplegia Rating Scale (SPRS)[18] and the disability score.[19] SPRS is a 13-item scale designed to rate functional impairment by evaluating walking ability, muscle power, spasticity, pain and urinary function. Each scale item can have a score of 0 to 4, where 4 indicates most severe impairment. The disability score grades functional impairment from 0 (no functional handicap), 1 (signs at examination), 2 (able to run, walking unlimited), 3 (unable to run, limited walking without aid), 4 (walking with one stick), 5 (walking with two sticks), 6 (requiring wheelchair) to 7 (confined to bed). Other workups included magnetic resonance imaging (MRI) of brain and spinal cord, electrophysiological studies (nerve conduction studies (NCS), somatic sensory evoked potential (SSEP) and motor evoked potential (MEP), and general blood test.

We reviewed a series of the images of the SPG5 patients, including brain MRI from 13 patients, and MRI of cervical and/or thoracic spinal cords from 14 patients. The control images came from 20 healthy individuals, which were age- and gender-matched to the 14 SPG patients (Table S2). The anterioposterior diameters of cervical and thoracic spinal cords are measured at the C2 and T4 levels,[20,21] and the measurements were carried out by two investigators (Chou C.T. and Jih K.Y.) independently in a blinded manner.

Haplotype analysis

To investigate the founder effect of the *CYP7B1*p.R112* mutation in Taiwan, haplotype analysis was performed in 15 HSP patients carrying one or two p.R112* alleles and their 11 unaffected family members by genotyping 18 single nucleotide polymorphism (SNP) markers and two

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microsatellite markers flanking the *CYP7B1* gene and covering a region of 2.4Mb in size. These genetic markers are rs10100978, rs1217095, rs6994250, rs11994609, rs2166124, rs4573320, rs59524902, D8S512, rs4367588, rs8192906, rs3779869, rs4465006, rs116843046, D8S544, rs61146973, rs7842714, rs6472155, rs6985116, rs9298109, and rs7823966. The first 12 markers are telomeric and the remaining ones are centromeric to *CYP7B1*.

Statistical analysis

We included the 19 Taiwanese patients with SPG5 in this study and the 31 Caucasian SPG5 patients reported by Schöls et al.[14] to analyze genotype–phenotype relationship. Student's t test was used to compare the clinical features between SPG5 patients carrying the homozygous p.R112* mutation and those harboring other mutations. To test the associations between SPRS and disease duration, multivariate regression analysis was performed with adjustment of sex and onset age. The diameters of spinal cord were compared between the SPG5 patients and normal controls using Student's t test. All the statistical analysis was done by SPSS Statistics version 19.

Results

Mutation analysis

Among the 187 unrelated patients, SPG5 accounts for 9.1% (17/187) of the HSP pedigrees in our cohort. Mutation analysis of *CYP7B1* in the 17 SPG5 patients revealed six different mutations, including p.R112* (c.334C> T), p.R388* (c.1162C> T), p.N131Kfs*3 (c.392_393insA), p.N131Ifs*4 (c.392_392delA), p.A295V (c.884C> T), and p.L439R (c.1316T> G) (Fig. 1). The last three mutations were never reported before. These three mutations were absent in Taiwan biobank database and absent or present with an extreme rare allele frequency ($<10^{-5}$) in GnomAD and predicted to be deleterious by Provean or CADD programs (Table 1). The *CYP7B1* p.N131Ifs*4 is a truncating frameshift mutation, which leads to a loss of approximately three fourths of the size of the protein. Both *CYP7B1* p.A295V and p.L439R mutations alter the amino acid residues which are evolutionarily conserved at least from human to reptile (Fig. 1B). Both the p.A295V and p.L439R mutations were detected *in trans* with the pathogenic p.R112* mutation in the patients. According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines, the p.N131Ifs*4 can be classified as pathogenic and the p.A295V and p.L439R can be categorized as likely-pathogenic.[22] The *CYP7B1* mutations in our cohort and those ever reported in literature were illustrated in the schematic diagram. (Fig. 2).

Thirteen of the 17 unrelated patients (76.5%) are homozygous for the *CYP7B1* p.R112* mutation and another one is homozygous for the p.R388* mutation. The other three patients are compound heterozygous for the p.[R112*];[A295V], p.[R112*];[L439R], and p.[N131Kfs*3];[N131Ifs*4] mutations, respectively.

Clinical features and electrophysiological findings

Among the 17 unrelated SPG5 patients, 15 were apparently sporadic cases and two had one affected sibling each. The phenotypic and genotypic features of SPG5 patients are summarized in Table 2. The SPG5 patients in our cohort typically presented an insidious onset progressive spastic paraparesis with proprioception involvement. The mean onset age was 20.8 ± 11.5 years (range: 8–40). The mean muscle power (MRC grade) of hip flexors was 4.1 ± 0.88 (range 2–5). Exaggerated deep tendon reflex were present in nearly all patients (94.1%) at the lower limbs whereas only present in a small part of the patients (23.5%) at the upper limbs. Babinski sign was present in most of the patients (86.7%). Almost all the patients (92.9%) had position and vibration sense impairment and 64.3% of all the patients had surface sensory deficit. More than half of the patients exhibited dysmetria at the lower limbs (57.1%) and only a minor group of them had upper limb dysmetria (14.3%). None of them had other signs of cerebellar ataxia. About 43.8% of the patients reported urinary urgency or incontinence. The mean SPRS of the patients were 20.9 ± 12.0 (range 7–41). The ambulatory function was graded by disability score. One fourth of the patients (25%) could walk without assistance, that is, disability score was less than 3. The others (62.5%) required one or two sticks for walking (disability score: 3 to 5). Two patients (12.5%) were confined to bed.

Twelve of the SPG5 patients had ever received NCS and all had normal finding. Five patients underwent SSEP studies and three had absent cortical response of both median nerve and tibial nerve SSEPs. The other two patients had absent response of tibial nerve SSEPs and prolonged latencies of median nerve SSEPs. All of four patients received MEP studies showed prolonged central motor conduction time to the arms and legs.

MRI findings

The MRI findings of cerebral and spinal cord in the SPG5 patients are listed in Table 3. The main brain MRI abnormalities were white matter abnormality (WMA) in bilateral occipito-parietal regions on T2 and FLAIR images

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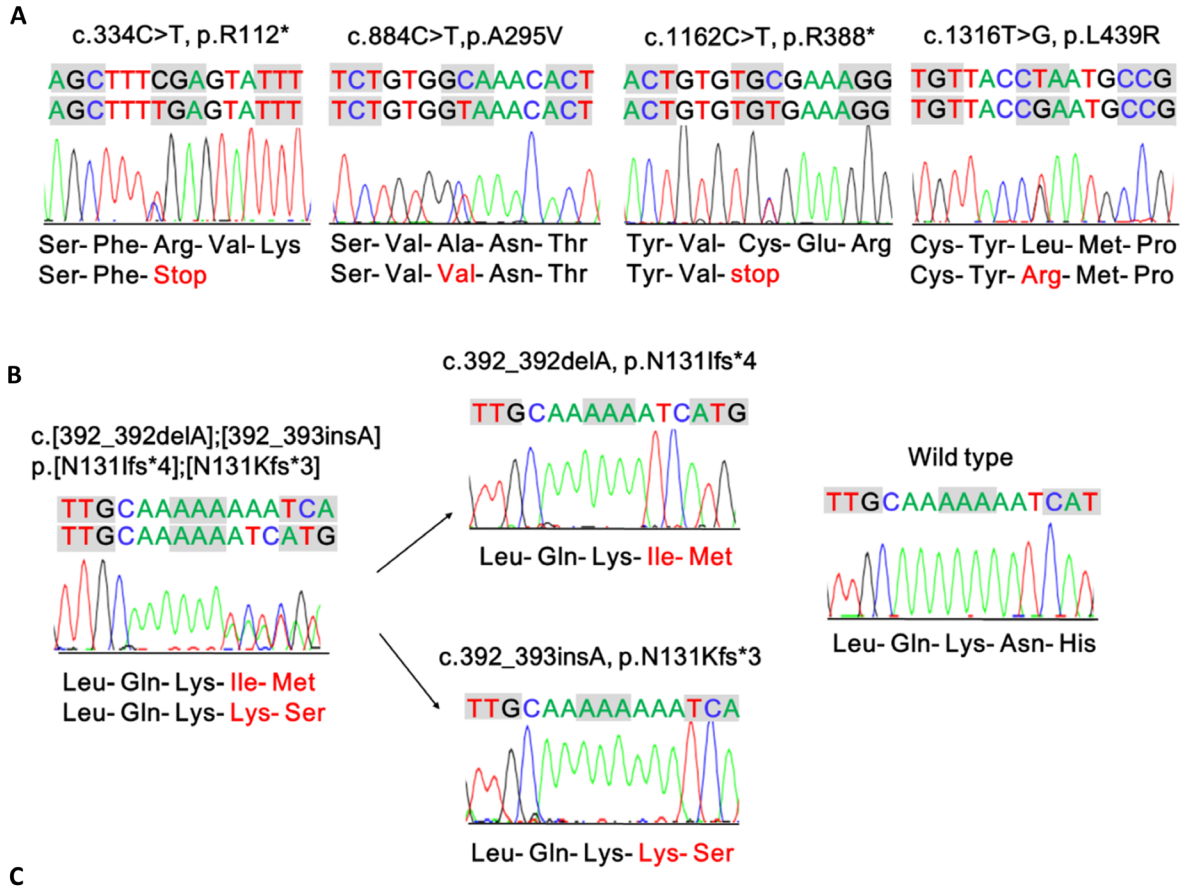

Figure 1. The *CYP7B1* mutations identified in this study. (A) Sanger sequence traces of the four missense nonsense *CYP7B1* mutations identified in our patient cohort. The mutations are labeled in red. (B) The *CYP7B1* compound heterozygous mutations, c.[392_393insA];[392_393delA], which putatively result in p.[N131Kfs*3];[N131Ifs*4], are shown by sequencing TA-subcloned PCR fragments. (C) Alignment of multiple CYP7B1 orthologues showing conservation of the Ala295 and Leu439 residues at least from human to reptile.

(Fig. 3), being present in 10 (76.9%) of the 13 patients who had received brain MRI study before. Besides, mild cerebellar atrophy also appeared in four of the seven patients with available sagittal views of brain MRI (57.1%).

In comparison with the healthy controls, the SPG5 patients had a significantly smaller anterioposterior diameter of spinal cord at C2 (6.7 ± 0.72 mm vs. 7.5 ± 0.66 mm, $P = 0.0019$) and T4 levels (5.1 ± 0.45 mm vs. 66.3 ± 0.45 mm, $P < 0.001$) (Fig. 3). The values of the C2 and T4 diameters of each individual are listed in Table S2.

Homozygous p.R112* mutations are associated with lower SPRS

The mean SPRS score was 20.9 ± 12.0 (available for 14 individuals, range 7–41) with a mean disease duration of

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Table 1. *CYP7B1* mutations identified in this study.

Mutations		Bioinformatics Prediction		Population Controls			
Nucleotide	Amino acid	Provean	CADD Score	Taiwan biobank	gnomAD	ACMG-AMP guideline	References
334C> T	p.R112*	Deleterious	24.7	0.003963 (12/3,028)[1]	0.0001469 (41/279,154)	Pathogenic	[12,24,34,36]
c.392_392delA	p.N131Ifs*4	NA	15.69	0	0	Pathogenic	This study
c.392_393insA	p.N131Kfs*3	NA	16.08	0	0.00001.994 (5/250,768)	Pathogenic	[13]
c.884C> T	p.A295V	Deleterious	24.3	0	0.000003.987 (1/250,810)	Likely pathogenic	This study
c.1162C> T	p.R388*	Deleterious	36	0	0.000007.071 (2/282,844)	Pathogenic	[4]
c.1316T> G	p.L439R	Deleterious	20.8	0	0	Likely pathogenic	This study

CADD, Combined Annotation Dependent Depletion; gnomAD, Genome Aggregation Database; ACMG-AMP, American College of Medical Genetics and Genomics and the Association for Molecular Pathology; NA, not applicable.
[1]Allele frequency (minor allele count/total allele count).

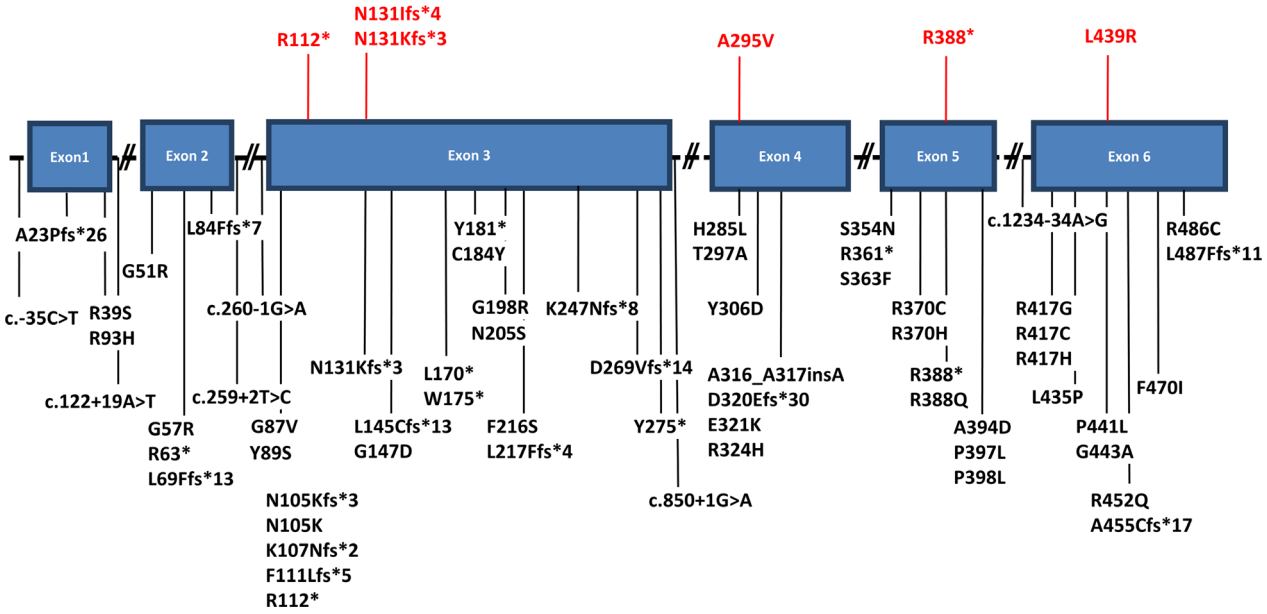

Figure 2. The *CYP7B1* mutations identified in this study (labeled in red) and in the literature (labeled in black).

20 years (range 4–44 years). When we included the data of the 31 SPG5 patients in the study by Schöls et al.[13], there was a significant correlation ($R^2 = 0.341$, $P < 0.0001$) between disease durations and SPRS scores (Fig. 4A), supporting SPRS as an appropriate scale to measure disease progression of SPG5.

To compare the clinical features between patients with homozygous p.R112* mutation and those with other mutations, we examined the onset age, disease duration, SPRS, the presence of ataxia, surface sensation, position sense, vibration sense, and urinary symptoms and included the data from the study by Schöls et al.[13]. The patients with the homozygous p.R112* mutation had a significant lower SPRS than that of patients with other *CYP7B1* mutations ($P = 0.028$) after adjusting onset age and sex. Other symptoms and parameters did not show significant difference in patients homozygous to *CYP7B1* p.R112* and those with other mutations (Table S3). The distribution of SPRS in the two groups is shown in Figure 4B.

Founder effect of *CYP7B1* p.R112* in Taiwan

Haplotype analysis was performed in 25 individuals from 14 SPG5 families harboring the *CYP7B1* p.R112* mutation, including 13 patients with homozygous p.R112*

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Table 2. Clinical characteristics and *CYP7B1* mutations of the 19 patients with SPG5.

No.	Age/ Sex	*CYP7B1* Mutations	Onset age	Family history	SPRS	Disability score (0-7)[1]	Muscle Strength (hip flexors)[2]	DTR			Babi-nski sign	Sensory impairment		Dysmetria UL/LL	Urinary symptoms
								Biceps	Knee	Ankle		Surface	Position/ Vibration		
A	30/F	p.[R112*];[R112*]	7	No	10	2	5	++	+++	++++	+	+	+/+	–/–	–
B	49/F	p.[R112*];[R112*]	31	No	18	4	4	+++	+++	++++	+	+	+/+	–/+	+
C	33/M	p.[R112*];[R112*]	13	No	9	2	5	+++	+++	++++	–	+	+/+	–/+	+
D	51/M	p.[R112*];[R112*]	36	No	13	3	4	++	+++	++++	+	–	+/+	–/+	–
E	44/F	p.[R112*];[R112*]	40	No	23	3	4	+++	+++	+++	–	+	+/+	–/+	+
F	19/F	p.[R112*];[R112*]	11	No	7	2	5	++	+++	++++	+	–	–/–	–/–	–
G	42/M	p.[R112*];[R112*]	15	No	24	5	3	++	+++	++++	+	–	+/+	–/+	–
H	46/F	p.[R112*];[R112*]	36	No	13	3	4	++	+++	++++	+	–	+/+	–/–	–
I	45/M	p.[R112*];[R112*]	16	Yes	16	3	4	++	++/	++++	+	+	+/+	–/+	–
J	34/F	p.[R112*];[R112*]	15	No	11	1	5	+	+++/++++	++++	+	–	+/+	–/–	–
K	35/F	p.[R112*];[R112*]	30	No	NA	7	NA	++	+++	NA	NA	NA	NA/NA	NA/NA	NA
L	47/F	p.[R112*];[R112*]	32	No	NA	4	NA	NA	NA	NA	NA	NA	NA/NA	NA/NA	NA
M	66/M	p.[R112*];[R112*]	31	No	NA	NA	NA	NA	NA	NA	+	NA	NA/NA	–/–	NA
N	42/M	p.[R112*];[L439R]	28	No	27	4	4	–	+++	+++	+	+	+/+	–/+	+
O	52/F	p.[R112*];[A295V]	8	No	41	7	2	++	++++	++++	+	+	+/+	–/Can't	–
P–1	40/F	p.[N131lfs*4]; [N131Kfs*3]	10	Yes	40	5	3	++	+++	+++	+	+	+/+	+/+	+
P-2	39/M	p.[N131lfs*4]; [N131Kfs*3]	25	Yes	41	5	4	+++	+++	+++	+	+	+/+	+/+	+
Q-1	39/F	p.[R388*];[R388*]	8	Yes	NA	NA	NA	++	+++	NA	NA	NA	NA/NA	–/–	+
Q-2	36/M	p.[R388*];[R388*]	12	Yes	NA	NA	NA	+	+++	NA	NA	–	NA/NA	NA/NA	–

SPRS, Spastic Paraplegia Rating Scale; DTR, deep tendon reflex (+-++++); UL, upper limbs; LL, lower limbs; NA, not available; +, presence of symptoms or abnormalities; –, negative for symptoms or abnormalities.

[1]Disability score: see text.

[2]Medical Research Council (MRC) Scale 0-5.

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Table 3. MRI findings of the 15 patients with SPG5.

Patient	Brain MRI	Cord antero-posterior diameter (mm)	
		C2	T4
A	WMH	5.5	5.5
B	WMH, no CA	6.4	4.8
C	WMH	6.7	5.7
D	NA	6.7	5.6
F	Normal, no CA	8.4	5.3
G	WMH, CA	6.1	NA
H	WMH, CA	7.4	5.5
I	NA	NA	5.2
J	Normal, no CA	6.2	4.5
N	WMH	NA	4.5
O	WMH	7.1	5.2
P-1	WMH, CA	6.4	4.5
P-2	WMH, CA	NA	NA
Q-1	WMH	6.8	4.8
Q-2	Normal	6.7	5.5
mean	WMH (76.9%, 10/13) CA (57.1%, 4/7)	6.7 ± 0.72	5.1 ± 0.45
Control subjects (n = 20)		7.5 ± 0.66	6.3 ± 0.45
P-value[1]		$P = 0.0019$	$P < 0.0001$

CA, cerebellar atrophy; NA, not available; WMH, white matter T2-hyperintensity in bilateral occipito-parietal regions.
[1]Comparing the diameters of the patients with those of the controls by t-test.

mutation, two patients and nine carriers carrying a single allele of p.R112*, and one healthy individual without any *CYP7B1* mutation (Fig. 5). These SPG5 families shared a common haplotype linked to *CYP7B1* p.R112*, covering a region of 954Kb between loci rs6994250 and rs6985116 (Fig. 5), suggesting the presence of a founder effect for the p.R112* mutation.

Discussion

This study comprehensively investigates the clinical, electrophysiological, neuroimaging, and genetic features of a Taiwanese cohort of 19 patients with SPG5 from 17 families. This is the largest SPG5 cohort of Han Chinese ancestry with clear clinical and genetic information. There are several intriguing findings from this study. First, SPG5 accounts for 9.1% (17/187) of the Taiwanese HSP cohort. SPG5 is the second most common HSP subtype in Taiwan next to SPG4 (data not shown). Second, *CYP7B1* p.R112* is the most common mutation in the Taiwanese SPG5 patients and present in 88.2% of the pedigrees, including 76.4% with the homozygous p.R112* mutations. Third, the SPG5 patients typically presented with an insidious onset progressive spastic paraparesis, proprioception impairment, and lower limb dysmetria. Fourth, the electrophysiological studies revealed that the SPG5 patients had abnormal central motor and proprioception conduction with normal peripheral nerve functioning. Fifth, neuroimaging studies demonstrated that spinal cord atrophy, WHA in bilateral occipito-parietal regions and mild cerebellar atrophy. Finally, there is a founder effect for the p.R112* mutation in Taiwanese patients and the homozygous p.R112* mutation tend to have a milder clinical severity.

In the SPG5 cohort described by Schöls et al., 94% had dorsal column sensory deficits, 50% had reduced surface sensation, 47% had lower limb ataxia, and 55% had an affected urinary function.[13] Our SPG5 patients also manifested similar clinical presentations. Within our SPG5 cohort, 92.9% had proprioception deficits, 64.3% patients had abnormal surface sensation, 57.1% had ataxia at the lower limbs, and 43.8% reported urinary urgency or incontinence. The common phenotypic features found in the two cohorts of different ethnicities are proprioception deficits and lower limb ataxia, which might be helpful features for recognizing SPG5 from different HSP subtypes. The proprioception deficits in SPG5 are of central origin and peripheral nerve system is sparing. Both the study by Marelli et al. and ours showed that SPG5 patients had abnormal SSEPs but normal NCS.[14] In addition to proprioception deficits, the lower limb ataxia of SPG5 may also come from cerebellar dysfunction. Cerebellar involvement had been reported in a small number of patients previously.[11] In our study, we found that gait ataxia in our patients usually went beyond the degree of proprioception deficits and accompanied with impaired heel-knee-shin test. Brain MRIs also revealed 57.1% of the patients had mild cerebellar atrophy.

Spinal cord atrophy and WMA in bilateral occipito-parietal regions are two common MRI features in our cohort. Spinal cord atrophy in SPG5 has also been observed in previous two studies.[11,23] However, our study is the first one to investigate spinal cord diameters in SPG5 patients. The cord atrophy may reflect the damage of corticospinal and somatosensory tracts. Further studies are warranted to decide whether spinal cord diameters can be utilized as a disease progression marker for SPG5. Bilateral occipito-parietal WMA in SPG5 had also been reported in several studies.[12,24-26] The exact pathology of the cerebral WMA remains elusive. However, MR spectroscopy (MRS) revealed gliosis or demyelination in the regions of WMA in SPG5 patients.[11,24] Interestingly, several other types of HSP with disease genes involving lipid metabolism also exhibit WMA, for example, SPG26 (*B4GALNT1*),[27] SPG35 (*FA2H*),[28] SPG46 (*GBA2*),[29] SPG54 (*DDHD2*),[30] and SPG56 (*CYP2U1*).[31] Similarly,

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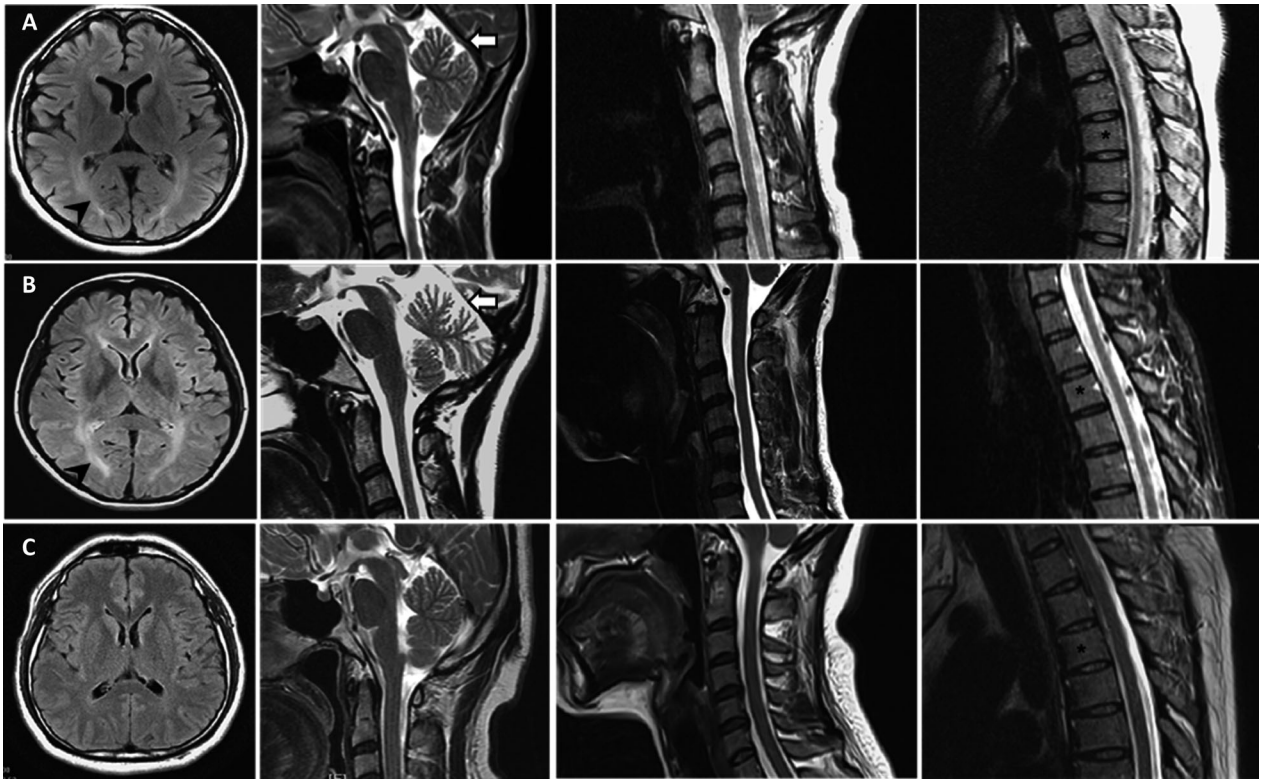

Figure 3. Representative neuroimages of the patients with SPG5. T2-weighted images or fluid attenuation inversion recovery images of brain and spinal cord MRI from a 31-year-old female patient with p.[N131Kfs*3];[N131Ifs*4] mutation (lane A) and a 44-year-old female patient with homozygous p.R112* mutation (lane B) demonstrate white matter hyperintensity in bilateral occipito-parietal regions (arrowhead), mild cerebellar atrophy (arrow), cervical and thoracic cord atrophy (T4 is labeled with asterisks), in comparison with normal control (lane C).

cerebrotendinous xanthomatosis, caused by *CYP27A1* mutations and resulted in cholestanol accumulation, also presented with WMA in MRI.[32] These phenomena may be due to that lipid metabolism is crucial for central myelin development and maintenance. Hence, the defects of enzymes regulating lipid metabolism may lead to cerebral WMA.

The mutational spectrum of SPG5 in Taiwan is very different from those in Caucasian populations. The p.R112* mutation is very rare in Caucasian populations.[9,12-14] So far, only three families with p.R112* mutation were identified in European studies.[12,14,24] The *CYP7B1* mutations in European SPG5 patients varied widely and the most common one was p.R486C, accounting for only 18% of the patients.[8,12-13,24,33] The commonness of the p.R112* mutation in the Taiwanese SPG5 patients may come from the founder effect. One previous study reported five Taiwanese SPG5 patients, presenting spastic paraparesis, dorsal column sensory deficits with or without cerebellar ataxia. All the five patients carried one or two alleles of the *CYP7B1* p.R112* mutation and shared a common founder haplotype.[34] This and our study suggest that the allele frequency of *CYP7B1* p.R112* in Taiwan could be unexpectedly high. In Taiwan biobank database, the allele frequency of the *CYP7B1* p.R112* in Taiwan was 0.396%. Therefore, the frequency of a Taiwanese individual to carry a homozygous p.R112* mutation is approximately 1.57 per 100,000. This number is very high for a single HSP subtype. One meta-analysis study analyzed 12 HSP-related epidemiology studies and concluded that the average prevalence of AR HSP was 1.8 per 100,000.[35] In their analyses, the most common AR HSP subtypes were SPG11 and for SPG15.[35] The high allele frequency of *CYP7B1* p.R112* in Taiwan may explain why SPG5 accounts for a relatively large proportion (9.1%) of cases in Taiwan. In addition to Taiwan, *CYP7B1* p.R112* may also play an important role in SPG5 in East Asia. We checked the gnomAD database and found the allele frequency of *CYP7B1* p.R112* in East Asians was 0.171% and that in non-Finnish Europeans was 0.003%. Similar to our findings, one study from Fujian, the southeast province of China, showed that *CYP7B1* p.R112* was also common in their SPG5 patients and present in 14 of the 16 SPG5 pedigrees.[36]

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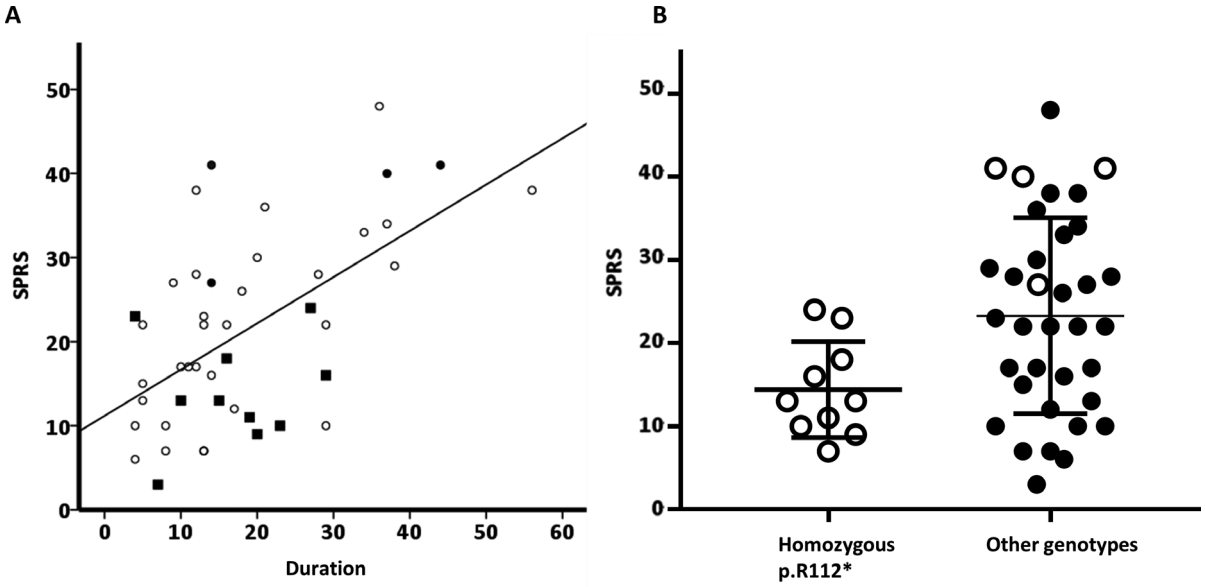

Figure 4. (A) Linear regression of SPRS scores by disease durations in our cohort (n = 14, solid squares: homozygous *CYP7B1* p.R112* mutation, solid circles: other genotypes) and data from Schöls et al. (n = 31, hollow circles). There was a moderate correlation between the SPRS and disease duration with an estimated disease progression rate (SPRS points per year with the disease) of 0.55 points/year (R^2 = 0.341, P < 0.001). (B) The scatter plot of SRPS in the SPG5 patients with a homozygous *CYP7B1* p.R112* mutation and those with other genotypes (including data from Schöls et al13). The SPRS were significantly lower in patients with homozygous p.R112* mutation than those with other genotypes despite the two groups having similar disease durations.

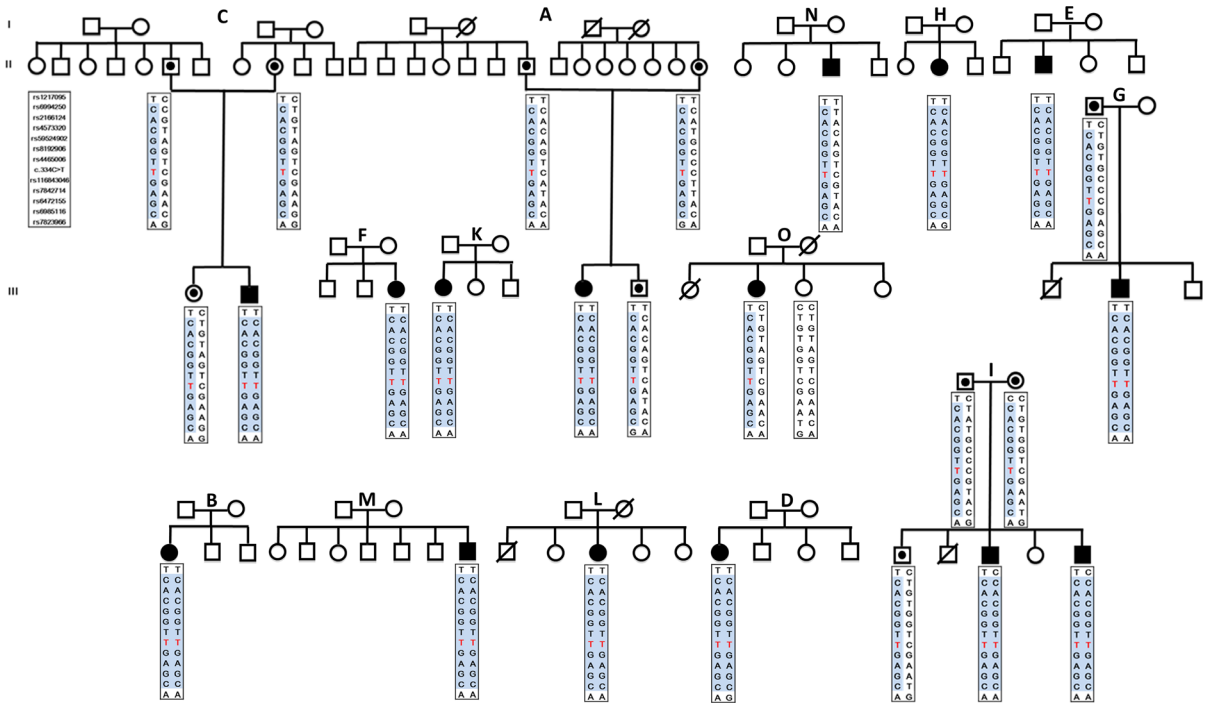

Figure 5. Haplotype analysis of 12 single nucleotide polymorphism (SNP) markers flanking the *CYP7B1* gene in 13 pedigrees harboring the *CYP7B1* p.R112* (c.334C> T) mutation. The squares and circles denote males and females, respectively. The filled symbols represent patients and open symbols designate unaffected members. Dotted symbols indicate asymptomatic carriers. A slash indicates deceased individuals. The mutated nucleotide is labeled in red and the linked haplotype is labeled with blue.

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We demonstrated a common haplotype linked to p.R112* mutation in 14 Taiwanese SPG5 families, covering a region of 954Kb between loci rs6994250 and rs6985116. These findings support that individuals carrying p.R112* mutation might be descendants from a common ancestor. Given the geographical proximity between Taiwan and Fujian, individuals with *CYP7B1* p.R112* in both regions may share the same founder haplotype. We compared the data of haplotype analyses from the study by Dong et al.[36] and our study. The carriers harboring *CYP7B1* p.R112* in both Taiwan and Fujian shared a shorter common haplotype, covering a region of approximately 200Kb between loci rs8192906 and rs7842714. Therefore, the *CYP7B1* p.R112* mutation in Taiwan or Fujian may arise from a single founder mutation. A milder clinical severity associated with the homozygous p.R112* mutation may make this mutation more easily inherited from parents to offspring than other *CYP7B1* mutations.

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Conflict of Interest

All authors read and approved the final manuscript. They declared no conflicts of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The gene list of the targeted resequencing panel.
Table S2. The spinal cord diameters measured by the 2 investigators.
Table S3. Comparison of clinical profiles between the patients with *CYP7B1* homozygous p. R112* mutation and those with other genotypes (including data from Schöls et al.).

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