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RESEARCH ARTICLE

Clinical and genetic characterization of *NIPA1* mutations in a Taiwanese cohort with hereditary spastic paraplegia

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Abstract

Objective: *NIPA1* mutations have been implicated in hereditary spastic paraplegia (HSP) as the cause of spastic paraplegia type 6 (SPG6). The aim of this study was to investigate the clinical and genetic features of SPG6 in a Taiwanese HSP cohort. **Methods**: We screened 242 unrelated Taiwanese patients with HSP for *NIPA1* mutations. The clinical features of patients with a *NIPA1* mutation were analyzed. Minigene-based splicing assay, RT-PCR analysis on the patients' RNA, and cell-based protein expression study were utilized to assess the effects of the mutations on splicing and protein expression. **Results**: Two patients were identified to carry a different heterozygous *NIPA1* mutation. The two mutations, c.316G>A and c.316G>C, are located in the 3′ end of *NIPA1* exon 3 near the exon–intron boundary and putatively lead to the same amino acid substitution, p.G106R. The patient harboring *NIPA1* c.316G>A manifested spastic paraplegia, epilepsy and schizophrenia since age 17 years, whereas the individual carrying *NIPA1* c.316G>C had pure HSP since age 12 years. We reviewed literature and found that epilepsy was present in multiple individuals with *NIPA1* c.316G>A but none with *NIPA1* c.316G>C. Functional studies demonstrated that both mutations did not affect splicing, but only the c.316G>A mutation was associated with a significantly reduced NIPA1 protein expression. **Interpretation**: SPG6 accounted for 0.8% of HSP cases in the Taiwanese cohort. The *NIPA1* c.316G>A and c.316G>C mutations are associated with adolescent-onset complex and pure form HSP, respectively. The different effects on protein expression of the two mutations may be associated with their phenotypic discrepancy.

Introduction

Hereditary spastic paraplegias (HSPs) comprise a heterogeneous group of inherited neurodegenerative diseases characterized by slowly progressive spasticity and weakness of the lower limbs with or without involvement of other neurological systems.[1] HSPs are categorized into pure or complex form based on the clinical presentations. The typical features of pure HSPs include progressive bilateral lower extremities spasticity and weakness, hypertonic urinary bladder, and mild diminution of vibrational sensation in the lower limbs, while complex HSPs present with additional neurological or systemic abnormalities. To date, there have been more than 80 genes or genetic loci implicated in HSP.[2–4] However, only a few of them, including *SPAST*, *ATL1*, *KIF1A*, *CYP7B1*, *SPG7*, and *SPG11*, account for a significant number of patients with HSP. The contribution of mutations in other HSP disease gene, such as *NIPA1*, to HSP remains not fully clear, especially in Asian populations, because the relevant studies are still sparse.

Hereditary spastic paraplegia 6 (SPG6) is an autosomal dominant HSP caused by a mutation in the NIPA

magnesium transporter 1 gene (*NIPA1*).[5] *NIPA1* encodes magnesium transporter NIPA1 protein (NIPA1), which consists of 329 amino acids and nine transmembrane domains (Fig. 1A).[6] The NIPA1 protein is highly expressed in neuronal and epithelial cells and localized to cytoplasmic membranes.[7] The pathogenic mechanisms of *NIPA1* mutations include dysfunction of magnesium transporters,[8] neuronal cell death caused by accumulation of mutant proteins in the endoplasmic reticulum,[7] and dysregulation of axonal maintenance by inhibiting bone

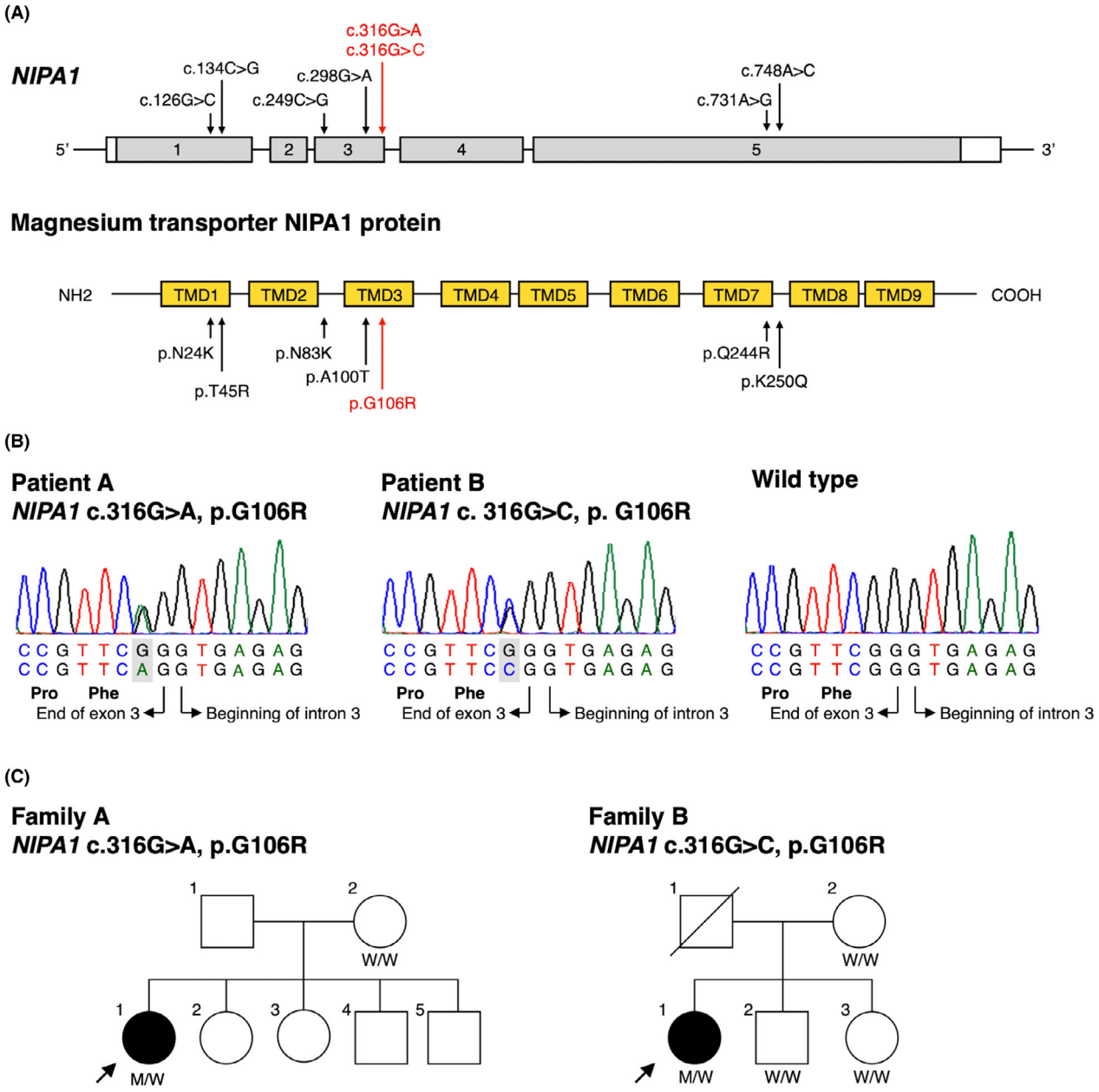

Figure 1. Genetic analysis of *NIPA1* mutations in the Taiwanese patients with hereditary spastic paraplegia (HSP). (A) Schematic illustration of structures of the *NIPA1* gene and the NIPA1 protein. The currently known pathogenic *NIPA1* mutations were labelled. The two mutations identified in this study were labelled in red. (B) Sanger sequence traces revealing the *NIPA1* c.316G>A and c.316G>C mutations, which putatively lead to the same amino acid change, p.G106R, and are located near to the exon–intron boundary. The altered nucleotides are labelled in gray. (C) The pedigrees of the two HSP patients with the *NIPA1* mutations. The probands are indicated by arrows. The "M" represents a mutant *NIPA1* allele and the "W" means a wild type allele. The squares and circles denote for males and females. The filled and open symbols represent affected and unaffected members, respectively. A slash indicates a deceased individual.

morphogenic protein (BMP) signaling pathways.[9] To date, only a few different mutations in *NIPA1* have been identified in SPG6 patients and were all missense mutations, including p.N42K (c.126C>G),[10] p.T45R (c.134C>G),[5] p.N83K (c.249C>G),[11] p.A100T (c. 298G>A),[12] p.G106R (c.316G>A or c.316G>C),[10,13–28] p.Q244R (c.731A>G),[29] and p.K250Q (c.748A>C) (Fig. 1A).[26] SPG6 typically presents as pure HSP, but may also exhibit complex HSP.[28]

To further understand the role of SPG6 in HSP in Han Chinese populations, we screened a cohort of 242 unrelated Taiwanese patients with HSP for *NIPA1* mutations. We identified two different pathogenic variants, c.316G>A and c.316G>C, which alter the same nucleotide near the exon–intron boundary region of *NIPA1* and putatively lead to the same amino acid change (p.G106R), and each was found in one single patient with apparently sporadic disease. We further characterized the clinical features of *NIPA1* mutations and demonstrated that both variants did not affect *NIPA1* splicing.

Methods

Study subjects

A consecutive series of 242 unrelated individuals with a clinical impression of HSP were recruited from the Neurology Service of Taipei Veterans General Hospital between January 1998 and January 2022. All the participants fulfilled the clinical diagnosis criteria for HSP.[30] Among the 242 patients, 95 patients (39%) had a clear family history of HSP, and 147 (61%) were apparently sporadic cases of idiopathic progressive spastic paraparesis. All the participants were Taiwanese of Han Chinese ethnicity. Blood samples were collected after written informed consents were obtained. This study was approved by the Institutional Review Board of Taipei Veterans General Hospital.

Mutational analysis

Mutational analysis of *NIPA1* was performed by utilizing a targeted resequencing panel covering 76 HSP disease genes and other 57 genes associated with diseases manifesting HSP-like phenotype (Table S1) on an Illumina HiSeq2500 platform. Alignment of the sequenced reads and the identification of sequence variants were performed with the reference Human Genome version 38 (hg38/GRCh38). The *NIPA1* variants changing the coding sequence were reconfirmed by Sanger sequencing, and the amplicon sequences were compared with reference *NIPA1* coding sequence (NM_144599.5). Multiplex ligation-dependent probe amplification (MLPA) analyses with the MLPA Probemix P211 HSP region kit (MRC-Holland, Amsterdam, the Netherlands), covering the *NIPA1* gene, were performed according to the manufacturer protocol in the 110 HSP patients whose genetic diagnosis remained uncertain after targeted resequencing. We also conducted *in silico* prediction of the functional effects of variants using two bioinformatic programs, MutationTaster (http://www.mutationtaster.org/)[31] and Combined Annotation Dependent Depletion (CADD, GRCh38-v1.6; https://cadd.gs.washington.edu).[32]

Clinical evaluations

The two probands and their family members received a complete neurological examination and history taking. Disease severity was evaluated using the Spastic Paraplegia Rating Scale (SPRS) and the SPATAX-EUROSPA disability stage.[30,33] SPRS is a validated HSP-specific severity scale, measuring 13 items designed to rate functional impairment of walking ability, muscle strength, spasticity, pain, and urinary function. Each item is scored from 0 to 4, where 0 represents full function and 4 indicates most severe impairment. SPATAX-EUROSPA disability stage 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). Mini-Mental State Examination (MMSE) was performed for neuropsychological assessment.[34]

Minigene splicing assay

A wild-type human genomic DNA fragment containing *NIPA1* exon 3 and the adjacent upstream and downstream intron sequences (>300 bps) was cloned into the pET01 exon-trap vector (MoBiTec, Göttingen, Germany) (Fig. 2A). The *NIPA1* c.316G>A and c.316G>C mutations were introduced into the construct by PCR-based site directed mutagenesis method using pfu Turbo polymerase (Agilent, Santa Clara, CA, USA), respectively. All constructs were verified by Sanger sequencing. HEK293T cells were maintained in Dulbecco's modified Eagle medium (HyClone, Cytiva, Marlborough, MA, USA) supplemented with 10% fetal bovine serum (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA) in a 5% CO_2, 37°C incubator. The Exontrap constructs were transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen). At 24 h after transfection, total RNA was extracted from cell lysates with RNeasy Mini Kit (Qiagen, Hilden, Germany). Two micrograms of RNA were reverse-transcribed with SuperScript III reverse transcriptase (Invitrogen). The plasmid-derived cDNA was amplified using the primer

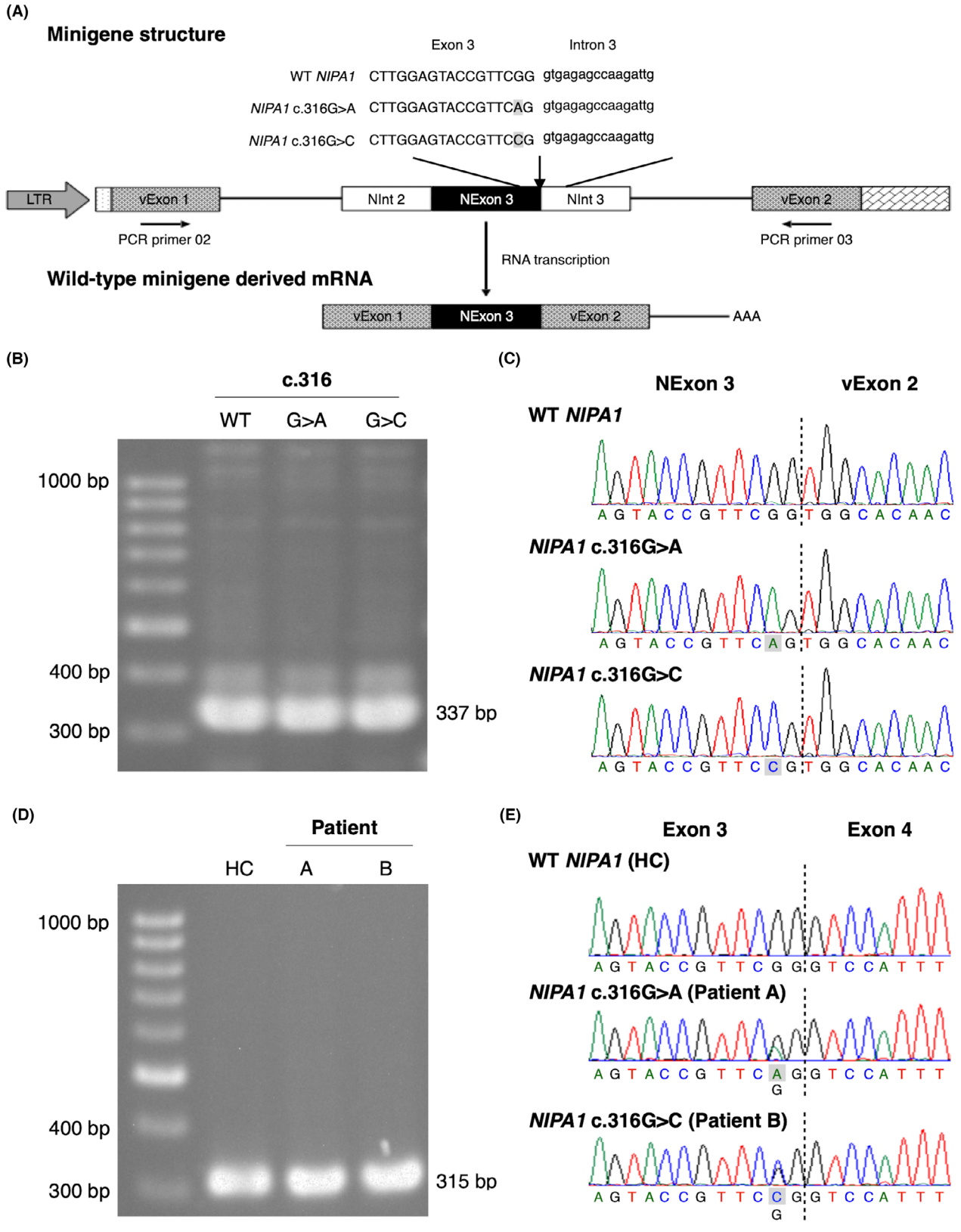

Figure 2. *In vitro* minigene splicing assay and RT-PCR analysis on the RNA from peripheral blood leukocytes of the patients. (A) The schematic structure of the minigene construct comprising the *NIPA1* genomic sequence of exon 3 (NExon 3), the adjacent upstream and downstream intron sequences (NInt 2 and NInt 3), and the backbone of pET01 exon-trap vector, including the vector exons (vExon 1 and vExon 2). The horizontal arrows depict the targeted sites of the primers used in this studies (PCR primers 02 and 03). The sequences of the wild-type (WT) and mutant minigene constructs flanking the boundary of NExon 3 and NInt 3 are shown with the mutant nucleotides labeled in gray. The mRNA derived from the WT construct contains vExon 1, NExon 3 and vExon 2. LTR: Long Terminal Repeat promoter of the Rous sarcoma virus. (B) PCR amplification of the cDNA products from WT and the two mutant minigene constructs expressed in HEK293T cells generated the same size amplicons (337 bp), suggesting that *NIPA1* mRNA from WT and the two mutant constructs are not differentially spliced. (C) The cDNA electropherograms of *NIPA1* NExon 3 and vExon 2 junction of WT and the two mutant constructs revealed the same sequence, indicating normal splicing pattern. (D) The amplicons generated by PCR amplification of the cDNA products from a healthy control (HC), patient A carrying *NIPA1* c.316G>A and patient B carrying *NIPA1* c.316G>C showed identical size (315 bp). (E) The cDNA electropherograms of *NIPA1* Exon 3 and Exon 4 junction of a healthy control and the two patients with SPG6 revealed the same splicing pattern, suggesting that both *NIPA1* c.316G>A and c.316G>C do not affect splicing.

pairs targeting exon 1 and exon 2 of the pET01 vector for analyzing the splicing products (Fig. 2A). The sequences of the forward and reverse primers were 5′-GATGG ATCCGCTTCCTGCCCC-3′ and 5′-CTCCCGGGCCACCT CCAGTGCC-3′, respectively. The sizes and the sequences of the amplicons were analyzed by electrophoresis and Sanger sequencing.

Reverse transcription polymerase chain reaction (RT-PCR) analysis on the RNA from the patients' peripheral blood leukocytes

We extracted total RNA from peripheral blood leukocytes of the patients with SPG6 and one healthy control. The RNA was reverse-transcribed into double-strand cDNA with SuperScript III reverse transcriptase (Invitrogen), which was further amplified using a pair of the primers targeting the exon 1 and exon 4 of the *NIPA1* cDNA product, respectively. The sequences of the forward and reverse primers were 5′-GTCGTGTCGAGCCTGGTG-3′ and 5′-GGAGTGGATAATCAGCACGA-3′. We analyzed the sizes and the sequences of the amplicons using electrophoresis and Sanger sequencing.

Analysis of NIPA1 steady-state expression

A human *NIPA1* cDNA clone was purchased from TransOMIC (BC156247; Huntsville, AL, USA). The full-length coding region of *NIPA1* was cloned into pFLAG-CMV-5a (Sigma-Aldrich, St. Louis, MO, USA) to generate the wild-type *NIPA1* expression plasmids. The *NIPA1* mutations, c.316G>A and c.316G>C were introduced into the wild-type expression plasmids, separately, by using QuikChange Site-Directed Mutagenesis method (Stratagene; Agilent, Santa Clara, CA, USA). The plasmids expressing wild-type, each one of the two mutant *NIPA1*, or empty vector were transfected into HEK293T cells, respectively, using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Forty-eight hours post-transfection, cells were harvested and analyzed by western blotting. The steady-state NIPA1 expression levels were analyzed using the FLAG antibody (#8146, Cell Signaling Technology, Danvers, MA, USA). Actin was used as a loading control to ensure an equal amount of protein loading (MAB1501; Merck Millipore, Burlington, MA, USA).

Results

Identification of *NIPA1* mutations

Among the 242 unrelated patients with HSP, we identified two heterozygous missense mutations in *NIPA1*, and each was found in one single patient. None of the patients had large fragment deletion or duplication in *NIPA1* according to the MLPA analysis. The two mutations were *NIPA1* c.316G>A and c.316G>C, which alter the same nucleotide and putatively lead to the same amino acid substitution from glycine to arginine at the codon 106 (p.G106R) (Fig. 1B). The mutated nucleotide is located in the 3′ end of *NIPA1* exon 3 with only one nucleotide adjacent to the exon–intron boundary. Both mutations have been recognized as pathogenic mutations for SPG6 before.[10,13–28] The CADD v1.6 Phred scores of *NIPA1* c.316G>A and c.316G>C were 28 and 27.9, respectively. Both MutationTaster and CADD predicted the two variants as disease-causing mutations. The pedigrees of the two patients were shown in Figure 1C.

Clinical information of the two patients with SPG6

The genetic and clinical features of the two patients with SPG6 were summarized in Table 1. The patient A is heterozygous for *NIPA1* c.316G>A. She was born from non-consanguineous healthy parents and had no family history of neurological diseases. She started to have slowly progressive gait disturbance since age 19 years, and as the

Table 1. Clinical characteristics of the two Taiwanese SPG6 patients.

	Patient A	Patient B
NIPA1 mutation	c.316G>A (p.G106R)	c.316G>C (p.G106R)
Sex	Female	Female
Age at onset (years)	17	12
Age at examination (years)	45	34
SPATAX-EUROSPA disability stage (0–7)[a]	5	5
SPRS (0–52)	31	26
Muscle power (hip flexors)[b]	4	4
Deep tendon reflex[c]		
Biceps	+++	++
Knee	++++	+++
Ankle	++++	++++
Plantar response	Extensor	Extensor
Sensory impairment		
Pain	–	–
Proprioception	+	–
Vibration	+	+
Urinary urgency	+	–
Tremor	–	–
MMSE score	30	30
Additional manifestations	Epilepsy, schizophrenia	–

SPRS, Spastic Paraplegia Rating Scale; MMSE, mini-mental state examination; +, presence; –, absence.
[a]Disability score: 0 (no functional handicap),1 (no functional handicap but signs at examination), 2 (mild, able to run, walking unlimited), 3 (moderate, unable to run, limited walking without aid), 4 (severe, walking with one stick), 5 (walking with two sticks), 6 (unable to walk, requiring wheelchair) and 7 (confined to bed).
[b]Medical Research Council (MRC) Scale for muscle strength: 0–5.
[c]National Institute of Neurological Disorders and Stroke (NINDS) Scale for tendon reflex: 0–++++.

disease progressed, she needed two walking sticks for ambulation at age 35 years. Urinary urgency was also noticed. In addition to the motor symptoms, she was diagnosed with idiopathic generalized epilepsy at the age 17 years and schizophrenia at age 20 years, presenting with both visual and auditory hallucination. The epilepsy and psychiatric symptoms have remitted without further need of antiepileptic or antipsychotic drugs since approximately age of 30 years. Neurological examination at age 45 years revealed lower limbs spasticity and weakness, general hyperreflexia with bilateral knee and ankle clonus, and bilateral extensor plantar responses. Impaired vibration and proprioceptive sensation in the distal lower limbs were also noted. Her SPRS score was 31. Her mother, who did not carry the *NIPA1* c.316G>A mutation, presented with normal findings in the neurological examination at age 66 years. Regrettably, we cannot approach the patient's father and siblings.

The patient B was a 34-year-old lady, carrying a heterozygous *NIPA1* c.316G>C mutation, developed slowly progressive gait impairment and stiffness in the lower limbs and trunk since age 12 years. Her motor function deteriorated progressively, and she needed to use two sticks for walking since age 32 years. Neurological examination in age 34 years demonstrated lower limbs spasticity and weakness, general hyperreflexia, bilateral extensor plantar responses, and impaired vibration over distal lower limbs. Her SPRS score was 26. Her mother, elder sister, and elder brother, who did not carry the mutation, were normal in the neurological examinations at age 58, 37, and 35 years, respectively. Her father died of esophageal cancer in his fifties and was reported to have a normal gait.

NIPA1 c.316G>A and c.316G>C do not affect splicing

Although *NIPA1* c.316G>A and c.316G>C may lead to the same amino acid residue alternation, the two patients harboring either of the two mutations presented with different clinical manifestations; one carried pure HSP phenotype and the other had HSP with epilepsy and schizophrenia. In addition, we reviewed literature and found that epilepsy has been found in multiple individuals with the c.316G>A mutation but not in those with the c.316G>C mutation (Table S2).[10,13–28] Therefore, we wondered whether these two mutations might have different effects on the molecular mechanism. Since *NIPA1* c.316G>A and c.316G>C are located in the exon–intron boundary region, we investigated whether these two mutations had different impacts on splicing utilizing a minigene assay. However, PCR amplification of the cDNA products from the wild-type or mutant minigene constructs containing either of the two mutations expressed in HEK293T cells demonstrated no difference in size (Fig. 2B). Sanger sequencing analysis of the PCR products also revealed the same splicing pattern (Fig. 2C). We further analyzed the mRNA obtained from peripheral blood leukocytes of the two SPG6 patients carrying either on of the *NIPA1* c.316G>A and c.316G>C mutations by RT-PCR and Sanger sequencing. Again, there was no splicing alteration for both of the mutations (Fig. 2D and E).

NIPA1 c.316G>A and c.316G>C have different effects on protein expression

To investigate the influence of the two *NIPA1* mutations on protein expression, the relative abundance of the NIPA1 protein in the HEK293T cell transfection studies were determined by Western blotting analysis. As shown in Figure 3, the steady-state level of the c.316G>A NIPA1

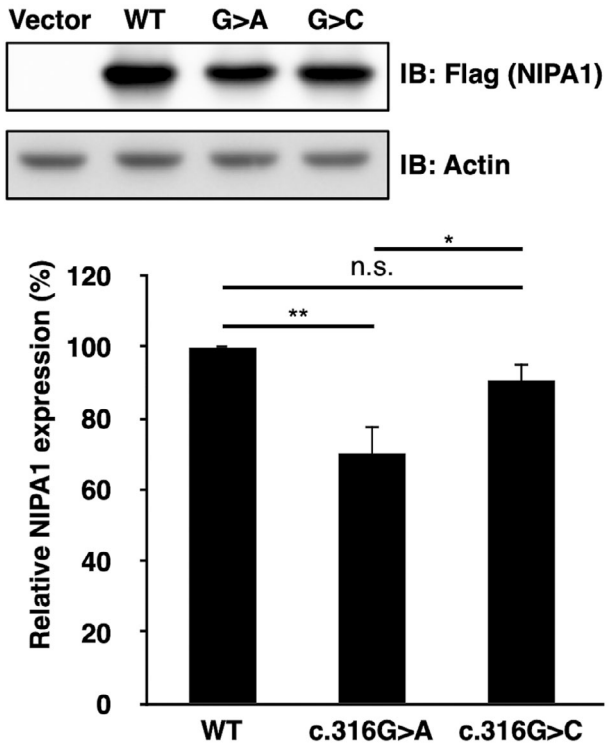

Figure 3. *In vitro* expression of the NIPA1 variants in HEK293T cells. Representative western blot analysis of steady-state expression of NIPA1 proteins in HEK293T cells transfected with NIPA1 constructs. Actin was used as a loading control. Densitometric quantification is shown below. The error bars indicate standard error of the mean (SEM) from four independent experiments. The asterisk indicates a statistically significant difference (**$P < 0.01$, *$P < 0.05$). n.s., not significant.

was significantly lower than that of the wild-type protein ($P = 0.0039$) and the c.316G>C NIPA1 ($P = 0.0324$). There was no significant difference between the wild-type protein and the c.316G>C NIPA1, although the P value was close to the boundary of significance ($P = 0.0517$).

Discussion

In this study, we identified two patients with SPG6 after screening a Taiwanese cohort of 242 unrelated HSP patients for *NIPA1* mutations. One patient had complex form HSP with epilepsy, schizophrenia, and a heterozygous c.316G>A mutation in *NIPA1*, and the other one had pure HSP and a heterozygous *NIPA1* c.316G>C mutation. Both mutations occur at the nucleotide close to the splicing site and may lead to the same amino acid alternation, p.G106R, according to the codon changes. In vitro minigene analysis and RT-PCR analysis on the RNA obtained from the patients' blood leukocytes suggest that both mutations do not affect *NIPA1* splicing. The cell-based study revealed that the c.316G>A mutation resulted in significantly reduced NIPA1 protein expression, but c.316G>C mutation did not. These findings may have the following implications.

First, although SPG6 usually manifests pure HSP with autosomal dominant inheritance, it may also present with complex HSP in individuals without family history of HSP. One study reviewed and analyzed 110 genetically confirmed SPG6 cases and found 23% of the patients had complex HSP and 10% of the patients also had generalized epilepsy.[28] The additional neurological manifestations in SPG6 could be epilepsy,[10,14,20,24,27,28] ataxia,[24,25] cognitive impairment,[14,17,21] motor neuron disease,[27] and/or peripheral neuropathy.[18,19,21,24] Interestingly, SPG6 with complex HSP is usually caused by *NIPA1* c.316G>A mutation, but may be infrequently found in patients with *NIPA1* c.316G>C, c.134C>G (p.T45R), or c.249C>G (p.N83K) mutation, too.[11,28,35] De novo *NIPA*1 mutation had also been reported in few SPG6 patients with apparently sporadic phenotype.[10,24] In this study, both SPG6 patients had no family history, and one had pure HSP and the other had HSP with epilepsy and schizophrenia. These findings suggest that SPG6 should be still considered as a potential diagnosis in patients with apparently sporadic spastic paraplegia with or without additional neurological involvement and of unknown cause.

Second, *NIPA1* mutation appears to be an uncommon cause of HSP in Taiwan. Our study identified *NIPA1* mutations in 2 of the 242 unrelated HSP patients, suggesting that SPG6 may account for approximately 0.8% (2/242) of HSP patients in Taiwan. However, the percentage of individuals with SPG6 to overall HSP patients varies across different populations. Previous studies demonstrated that the prevalence of *NIPA1* mutations in HSP patients ranged from 0% in Japanese people,[36] 0.9%–1.9% in European populations,[17,20,29] 5.6% in Korean individuals,[25] and 3.6%–8.6% in Chinese cohorts.[10,24] In addition to ethnic and geographic factors, the wide variation of the prevalence of SPG6 in different populations may result from disease rarity. It is always challenging to precisely estimate the prevalence of a rare disease like SPG6, as single individuals may have a large impact on the percentage in a cohort. On the other hand, comparing to mutations in most of other HSP disease genes, *NIPA1* mutations might be not so rare. In our Taiwanese HSP cohort, *NIPA1* mutations are only obviously rarer than mutations in *SPAST*, *CYP7B1*, or *ATL1*,[37,38] but are comparable to or more common than mutations in other HSP disease genes. Therefore, *NIPA1* mutations are a relatively uncommon but still appreciable cause for HSP.

Third, the two SPG6 cases in the study suggest that *NIPA1* c.316G>A seems to be associated with epilepsy, but the c.316G>C variant does not. We further reviewed literature and found that epilepsy was present in 17 of

the 60 SPG6 cases with *NIPA1* c.316G>A and none of the 20 individuals with SPG6 cause by the c.316G>C mutation (Table S2).[10,13–28] Therefore, epilepsy is much more frequently associated with *NIPA1* c.316G>A than c.316G>C (P = 0.0046, Fisher's exact test). This phenotypic discrepancy is surprising because both the c.316G>A and c.316G>C putatively lead to the same p.G106R amino acid alteration according to their codon changes (GGG>AGG, GGG>CGG). Furthermore, the nucleotide altered by both mutations is located very close to the boundary between exon 3 and intron 3 of *NIPA1*. Hence, the two *NIPA1* mutations might have different influences on *NIPA1* splicing. However, the *in vitro* minigene-based splicing assays and the RT-PCR analysis on the RNA from the patients' blood leukocytes revealed that both *NIPA1* c.316G>A and c.316G>C did not affect splicing. Interestingly, the cell-based study showed that the c.316G>A mutation but not c.316G>C mutation resulted in a significantly reduced NIPA1 protein expression, which may be responsible for the phenotypic discrepancy. However, it remains elusive how *NIPA1* c.316G>A and c.316G>C mutations could exert a different effect on *NIPA1* expression. Despite synonymous codons encode the same amino acids, the different mRNA structures and codon usage bias may still influence the initiation and elongation, and further the efficacy and accuracy of translation.[39–41] Further study is mandatory to elucidate the underlying mechanism responsible for the difference between the phenotypes associated the c.316G>A and c.316G>C mutations.

Epilepsy is not common in patients with HSP and may serve as a diagnostic clue for a few HSP subtypes. It has been reported in patients with several different autosomal dominant HSPs, including SPG3A (*ATL1*), SPG6 (*NIPA1*), SPG18 (*ERLIN2*), and SPG31 (*REEP1*). Among autosomal recessive HSPs, epilepsy was found with SPG11 (*SPG11*), SPG15 (*ZFYVE26*), SPG35 (*FA2H*), SPG44 (*GJC2*), SPG47 (*AP4B1*), SPG49 (*TECPR2*), SPG50 (*AP4M1*), SPG51 (*AP4E1*) and SPG77 (*FARS2*). Patients with the X-linked inherited SPG2 (*PLP1*) may also have epilepsy.[42] In addition, it is worthy to note that some other monogenic diseases may also present with spastic paraplegia and epilepsy, such as Friedreich's ataxia (*FXN*), adrenoleukodystrophy (*ABCD1*), and GLUT-1 deficiency syndrome (*SLC2A1*). Epilepsy may provide an extra diagnostic clue in patients presenting spastic paraplegia, but others clinical features or genetic characteristics should always be taken into consideration.

In conclusion, SPG6 accounted for 0.8% (2 out of 242) of HSP cases in the Taiwanese cohort. The *NIPA1* c.316G>A and c.316G>C mutations are associated with adolescent-onset complex and pure form HSP, respectively. Although neighbouring to the exon–intron boundary, both *NIPA1* mutations do not affect splicing. The c.316G>A but not c.316G>C is associated with a significantly reduced NIPA1 protein expression, which may explain for the phenotypic discrepancy.

Acknowledgments

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Conflict of Interest

The authors disclose 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 for hereditary spastic paraparesis.
Table S2. Previously published SPG6 patients carrying *NIPA1* c.316G>A or c.316G>C variants.

