Research Square

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NIPA1 Gene Mutations Cause Spastic Paraplegia Type 6 in Four Families

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Research Article

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Abstract

Objective

Spastic paraplegia type 6 (SPG6) is a rare subtype caused by Non-imprinted in Prader-Willi/Angelman syndrome region protein 1 (*NIPA1*) mutations. We reported four unrelated SPG6 families, reviewed related cases worldwide, and summarized the genotype-phenotype correlations.

Methods

We examined four probands, aged between 15 and 43 years, all presenting with progressive spasticity in the lower limbs and walking difficulty. Physical examinations consistently revealed hypertonia, hyperreflexia, and Babinski signs in lower limbs. We identified *NIPA1* mutations through whole exome sequencing, validated these findings using Sanger sequencing or MLPA, and subsequently conducted family co-segregation analysis along with a reevaluation of the phenotypes.

Results

Four SPG6 cases from different families were enrolled. One of these patients showed additional features of demyelination neuropathy. Two heterozygous mutations c.316G > A (p.Gly106Arg) in *NIPA1* and 15q11.2 BP1-BP2 microdeletion (harboring full-length *NIPA1*) were disclosed respectively.

Conclusion

Two various *NIPA1* mutations were found in four unrelated SPG6 families, leading to distinct phenotypes. While most cases presented as pure forms, one of them exhibited complicated features including demyelination neuropathy. This variability supports the notion of inter- and intra-family heterogeneity in SPG6 and indicates that the clinical boundaries of *NIPA1*-related conditions are still broadening.

1. Introduction

Hereditary spastic paraplegia (HSP) is a group of neurological degenerative diseases with high phenotypic and genotypic heterogeneity [1–3], mainly characterized by progressive spasticity of lower limbs [4–6]. Spastic paraplegia type 6 (SPG6) is a rare subtype caused by Non-imprinted in Prader-Willi/Angelman syndrome region protein 1 (*NIPA1*) gene mutations (NM_144599) [7, 8], accounting for less than 1% of autosomal dominant HSP (AD-HSP) cases in Europe [9], and 0.37% of Chinese HSP patients [10]. SPG6 usually starts in adolescence, mainly characterized by pure forms, with complicated forms accounting for 23%, including demyelination neuropathy, generalized epilepsy, cognitive decline, ataxia, amyotrophic lateral sclerosis, and schizophrenia [11, 12].

In this study, we aimed to collect clinical data from four patients with SPG6, with thorough physical and auxiliary examinations, and genetic tests, to summarize the genotype-phenotype correlations.

2. Material and Methods

2.1 Participants

We enrolled four probands diagnosed with HSP based on classic criteria of progressive lower limb spasticity and gait impairment [13], then examined them and their family members to collect clinical data.

2.2 Ethical approval

We obtained written informed consent from all patients. Formal ethical approval was given by the committee at Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine.

2.3 Mutation analysis

A standard phenol- chloroform method was applied for genomic DNA extraction from peripheral blood lymphocytes. We selected 300 healthy controls with matched geographic ancestry for comparative purposes. We then performed exome capture on the patients, for which we used Agilent SureSelect v6 reagents. Sequencing was carried out on the Illumina HiSeq X Ten platform. We aligned the resulting reads to the human reference genome (hg19, GRCh37), and after that we performed recalibration and variant calling. For initial filtering, we used population allele frequencies that came from public databases of normal human variation [1000 Genomes (1000 g; http://browser.1000genomes.org), the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org), dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/), NHLBI Exome Sequencing Project (ESP) Exome Variant Server (http://evs.gs.washington.edu/EVS), and the Genome Aggregation Database (gnomAD; http://gnomad-sg.org/)]. We removed variants with a population prevalence above 0.1%. The remaining variants were then assessed and grouped. We applied the criteria set by the American College of Medical Genetics and Genomics (ACMG) for this process [14].

2.4 Data availability statement

The corresponding author holds the original dataset from this study. It can be shared with other researchers upon reasonable request.

3. Results

3.1 Clinical findings

The patient (T5057, Ⅱ-2 in Fig. 1A) was a male in his twenties with progressive walking difficulty, since the age of 24 years old. Upon physical examination, he presented with hypertonia, mildly reduced strength (5-/5), hyperreflexia, bilateral ankle clonus and Babinski signs in lower limbs.

The proband (T8904, Ⅲ-6 in Fig. 1B) was a male in his thirties, with positive family history including eight affected family members. At the age of 10, he first experienced walking instability, and by age of 20, worsening of his gait was observed. Physical examination revealed hyporeflexia in four limbs, hypertonia, reduced strength (4/5), superficial hyperesthesia and reduced pinprick sensation in the lower limbs, as well as bilateral Babinski signs. Electromyography showed evidence of peripheral demyelination neuropathy, with slowed sensory conduction velocity (35.7 m/s in right sural nerve, and 31.8 m/s in left sural nerve).

The patient (T7113, II-2 in Fig. 1C) was a female in her forties , with progressive walking difficulty for more than 20 years. Gradually, she developed weakness and stiffness in both lower limbs since the age of 23. Symptoms worsened after 40, since when she had to rely on a cane, then a walker. Upon physical examination, she had hypertonia (2/4 on the modified Ashworth scale graded 0-4) and reduced strength (3/5), hyperreflexia and bilateral Babinski signs in lower limbs.

The patient (T8346, Ⅱ-1 in Fig. 1D) was an adolescent female , with bilateral Achilles tendon contracture and foot deformities with the age of onset at 1 year old. Physical examination revealed hypertonia, hyperreflexia, and Babinski signs in lower limbs.

As for all four cases, brain and spinal cord MRI showed no obvious findings. The detailed clinical features of the four patients are all summarized in Table 1.

3.2 Genetic findings

A heterozygous variant c.316G>A (p.Gly106Arg) was identified in the proband (II -2) and his mother (I-2), which was negative in the unaffected father (I-1) and elder brother (II-1) in Family 1 (Fig. 1A). The same variant was also disclosed in Family 2 (Fig. 1B), including the proband (III-6) and his father (Ⅱ-3). A heterozygous 15q11.2 BP1-BP2 microdeletion variant was identified in Family 3 (Ⅱ-1, Fig. 1C) and Family 4 (Ⅱ-1 and I-1, Fig. 1D). We applied the ACMG guidelines to support this classification [14], and both variants in the *NIPA1* gene were classified as "pathogenic" (Table 1).

4. Discussion

In this study, we enrolled four SPG6 families. The probands presented with pure or complicated forms characterized by demyelination neuropathy, due to c.316G>A (p.Gly106Arg), and 15q11.2 BP1-BP2 microdeletion. Two patients in our study carried the *NIPA1* c.316G>A (p.Gly106Arg) variant. SPG6 is relatively rare worldwide. Since 2000, a total of 33 families have been reported [7, 15], including 4 from our study. c.316G>A (p.Gly106Arg) was the most prevalent (n=19 families, 57.58%), among which 10 had complicated form (52.63%) (Fig. 2B). Three out of four families with c.316G>C (p.Gly106Arg) also had

complicated type (Fig. 2B). c.134C>G (p.Thr45Arg) was identified in 3 families, among which one had complicated form (Fig. 2B). The c.316G>A mutation is predicted to destabilize the third transmembrane domain of NIPA1 (Fig. 2B), impairing its function. The c.316G>A results in reduced NIPA1 protein expression, and among the 60 patients, 17 developed epilepsy (28.3%) while only one had demyelination neuropathy (1.7%) [7, 12, 16-27]. Across all reported *NIPA1*-related SPG6 families worldwide, only 3 families have been documented to have concurrent demyelinating neuropathy [20, 28, 29]. In our study, two patients with c.316G>A without seizure，one presented with demyelination neuropathy, with the bilateral sural nerves (right: 35.7 m/s, left: 31.8 m/s).

15q11.2 BP1-BP2 microdeletion were identified in two patients from our study. The 15q11.2 BP1–BP2 microdeletion (encompassing *TUBGCP5*, *CYFIP1*, *NIPA1*, and *NIPA2*) (Fig. 2A) is a recognized syndrome with a prevalence of 0.57%–1.27% in patients referred for microarray analysis, representing a 2- to 4- fold increase relative to controls. Literature reviews classify its clinical manifestations into five categories: developmental (73%) and speech (67%) delay; dysmorphic ears and palatal anomalies (both 46%); learning difficulties, memory problems, and verbal IQ ≤75 (50%–60%); unspecified behavioral problems (55%); and abnormal brain imaging (43%). Less common features include seizures/epilepsy (26%), autism spectrum disorder (27%), ADHD (35%), schizophrenia/paranoid psychosis (20%), and motor delay (42%) [30]. However, hereditary spastic paraplegia is not part of this microdeletion's classically recognized phenotypic spectrum; to date, only five families worldwide (including our two families) have been reported with spastic paraplegia. The five probands and other carrier family members showed none of the cognitive or behavioral abnormalities commonly described in previous studies [31, 32]. Although *NIPA1* haploinsufficiency has not been established as a cause of the SPG phenotype, within the 15q11.2 BP1–BP2 region, *NIPA1* is the strongest candidate gene for SPG6/HSP; both prior reported cases and our patients support that deletion of this region may be associated with the HSP phenotype [32]. According to previous studies, the incomplete penetrance is common for micro deletions and duplications of 15q11.2 BP1-BP2. The calculated penetrance above the background risk was 2.18% for deletions and 1.16% for duplications of 15q11.2 BP1-BP2 [33]. Due to its low penetrance, results should be interpreted with caution when providing reproductive genetic counseling for this gene. The low penetrance may be attributed to several mechanisms: cryptic balanced insertional translocations [34]， parent-of-origin epigenetic regulation [35], breakpoint heterogeneity and oligogenic interactions [36].

Current treatment methods for SPG6 are relatively limited. However, previous studies suggested that extra supplementary Mg^{2+} in patients with 15q11.2 BP1-BP2 microdeletion might improve aberrant behavior [37]. Individual studies suggested that brain-computer interfaces may be beneficial for alleviating patients' motor dysfunction [38, 39]. In the future, gene-specific therapy for SPG6 deserves more in-depth studies.

5. Conclusion

In summary, we described four families with SPG6, two with c.316G>A and two with 15q11.2 BP1-BP2 microdeletion. In particular, one patient with c.316G>A additionally had demyelination neuropathy, while

the other three had pure forms. These findings indicate that the clinical spectrum of *NIPA1*-related disorders is still broadening.

Declarations

AcknowledgementsNot applicable.

Authors' contributions W.T. designed and organized the study, performed the experiments, designed and conducted the statistical analysis, and revised the manuscript. Q.W. performed the experiments, conducted the statistical analysis, and drafted and revised the manuscript. Y.C., X.C., and X.H. performed the experiments and contributed to data collection. L.C. conceived and supervised the study, designed the statistical analysis, reviewed the statistical results, and revised the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Shanghai Sixth People's Hospital (Approval No. 2021-219). Written informed consent was obtained from all participants prior to participation. The authors confirm that they have read the journal's position on ethical publication and affirm that this work is consistent with those guidelines.

Consent for publication No identifying images are included. Written informed consent for publication of clinical details was obtained from all participants or their parents. A copy of the consent form is available for review by the Editor of this journal.

Data Availability The 15q11.2 BP1-BP2 microdeletion has been deposited in NCBI ClinVar under accession SCV008086845 (Variation ID: 5016472). The NIPA1 c.316G>A (p.Gly106Arg) variant has been submitted to ClinVar (submission ID: SUB16539568) ; its SCV accession is currently pending and will be provided before publication. Due to ethical and privacy restrictions regarding human genetic data, the

raw whole-exome sequencing (WES) data are not publicly available. However, the de-identified variant data (VCF files) are available from the corresponding author on reasonable request.

Competing interests The authors declare no competing interests.

References

1. Brankovic M, Ivanovic V, Basta I, et al. Whole exome sequencing in Serbian patients with hereditary spastic paraplegia. Neurogenetics. 2024;25(3):165-77.
2. Costamagna D, Casters V, Beltrà M, et al. Autologous iPSC-Derived Human Neuromuscular Junction to Model the Pathophysiology of Hereditary Spastic Paraplegia. Cells. 2022;11(21).
3. Lallemant-Dudek P, Darios F, Durr A. Recent advances in understanding hereditary spastic paraplegias and emerging therapies. Faculty Reviews. 2021;10.
4. Albini M, Almacellas-Barbanoj A, Krawczun-Rygmaczewska A, et al. Alterations in KIDINS220/ARMS Expression Impact Sensory Processing and Social Behavior in Adult Mice. International Journal of Molecular Sciences. 2024;25(4).
5. Wang Z, Dong H, Ji X, et al. Association of an insertion mutation in PRRT2 with hereditary spastic paraplegia accompanied by polyneuropathy. Journal of Clinical Laboratory Analysis. 2021;35(6).
6. Meyyazhagan A, Kuchi Bhotla H, Pappuswamy M, et al. The Puzzle of Hereditary Spastic Paraplegia: From Epidemiology to Treatment. International Journal of Molecular Sciences. 2022;23(14).
7. Fu J, Ma M, Li G, et al. Clinical and Genetic Features of Chinese Patients With NIPA1-Related Hereditary Spastic Paraplegia Type 6. Frontiers in Genetics. 2022;13.
8. Rainier S, Chai J-H, Tokarz D, et al. NIPA1 Gene Mutations Cause Autosomal Dominant Hereditary Spastic Paraplegia (SPG6). The American Journal of Human Genetics. 2003;73(4):967-71.
9. Klebe S, Lacour A, Durr A, et al. NIPA1 (SPG6) mutations are a rare cause of autosomal dominant spastic paraplegia in Europe. Neurogenetics. 2007;8(2):155-7.
10. Cao Y, Zheng H, Zhu Z, et al. Clinical and Genetic Spectrum in a Large Cohort of Hereditary Spastic Paraplegia. Movement Disorders. 2024;39(4):651-62.
11. Boen R, Kaufmann T, van der Meer D, et al. Beyond the Global Brain Differences: Intraindividual Variability Differences in 1q21.1 Distal and 15q11.2 BP1-BP2 Deletion Carriers. Biological Psychiatry. 2024;95(2):147-60.
12. Spagnoli C, Schiavoni S, Rizzi S, et al. SPG6 (NIPA1 variant): A report of a case with early-onset complex hereditary spastic paraplegia and brief literature review. Journal of Clinical Neuroscience. 2021;94:281-5.
13. Harding AE. Classification of the Hereditary Ataxias and Paraplegias. The Lancet. 1983;321(8334):1151-5.
14. 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. Genetics in Medicine. 2015;17(5):405-24.

15. Boysen S, Elumalai V, ElSheikh RH, et al. Epilepsy in hereditary spastic paraplegia associated with NIPA1 gene. Journal of Clinical Neuroscience. 2022;100:212-3.
16. Fang SY, Chou YT, Hsu KC, et al. Clinical and genetic characterization of NIPA1 mutations in a Taiwanese cohort with hereditary spastic paraplegia. Annals of Clinical and Translational Neurology. 2023;10(3):353-62.
17. Tanti M, Cairns D, Mirza N, et al. Is NIPA1-associated hereditary spastic paraplegia always 'pure'? Further evidence of motor neurone disease and epilepsy as rare manifestations. neurogenetics. 2020;21(4):305-8.
18. Zhao M, Chen Y-J, Wang M-W, et al. Genetic and Clinical Profile of Chinese Patients with Autosomal Dominant Spastic Paraplegia. Molecular Diagnosis & Therapy. 2019;23(6):781-9.
19. Kim A, Kumar KR, Davis RL, et al. Increased Diagnostic Yield of Spastic Paraplegia with or Without Cerebellar Ataxia Through Whole-Genome Sequencing. The Cerebellum. 2019;18(4):781-90.
20. Lu C, Li L-X, Dong H-L, et al. Targeted next-generation sequencing improves diagnosis of hereditary spastic paraplegia in Chinese patients. Journal of Molecular Medicine. 2018;96(7):701-12.
21. Hedera P. Recurrent de novo c.316G>A mutation in NIPA1 hotspot. Journal of the Neurological Sciences. 2013;335(1-2):231-2.
22. Arkadir D, Noreau A, Goldman JS, et al. Pure hereditary spastic paraplegia due to a de novo mutation in the NIPA1 gene. European Journal of Neurology. 2013;21(1).
23. Martinez-Lage M, Molina-Porcel L, Falcone D, et al. TDP-43 pathology in a case of hereditary spastic paraplegia with a NIPA1/SPG6 mutation. Acta Neuropathologica. 2012;124(2):285-91.
24. Svenstrup K, Møller RS, Christensen J, et al. NIPA1mutation in complex hereditary spastic paraplegia with epilepsy. European Journal of Neurology. 2011;18(9):1197-9.
25. Munhoz RP, Kawarai T, Teive HA, et al. Clinical and genetic study of a Brazilian family with spastic paraplegia (SPG6 locus). Movement Disorders. 2006;21(2):279-81.
26. Reed JA, Wilkinson PA, Patel H, et al. A novel NIPA1 mutation associated with a pure form of autosomal dominant hereditary spastic paraplegia. Neurogenetics. 2005;6(2):79-84.
27. Chen S, Song C, Guo H, et al. Distinct novel mutations affecting the same base in the NIPA1 gene cause autosomal dominant hereditary spastic paraplegia in two Chinese families. Human Mutation. 2005;25(2):135-41.
28. Liu SG, Zhao JJ, Zhuang MY, et al. Clinical and genetic study of SPG6 mutation in a Chinese family with hereditary spastic paraplegia. J Neurol Sci. 2008;266(1-2):109-14.
29. Du J, Hu Y-c, Tang B-s, et al. Expansion of the phenotypic spectrum of SPG6 caused by mutation in NIPA1. Clinical Neurology and Neurosurgery. 2011;113(6):480-2.
30. Cox D, Butler M. The 15q11.2 BP1–BP2 Microdeletion Syndrome: A Review. International Journal of Molecular Sciences. 2015;16(2):4068-82.

31. Sha Q, Xia Y, Shen X, et al. 15q11.2 BP1-BP2 microdeletion presenting as progressive spastic paraplegia and brain images of small vessel disease. Neurosciences. 2022;27(3):191-6.
32. Dobkowska EE. Familial 15q11.2 Micro deletions are not Fully Penetrant in Two Cases with Hereditary Spastic Paraplegia and Dysmorphic Features. Journal of Genetic Syndromes & Gene Therapy. 2014;05(05).
33. Maya I, Perlman S, Shohat M, et al. Should We Report 15q11.2 BP1-BP2 Deletions and Duplications in the Prenatal Setting? Journal of clinical medicine. 2020;9(8).
34. Koochek M, Harvard C, Hildebrand MJ, et al. 15q duplication associated with autism in a multiplex family with a familial cryptic translocation t(14;15)(q11.2;q13.3) detected using array-CGH. Clinical genetics. 2006;69(2):124-34.
35. Davis KW, Serrano M, Loddo S, et al. Parent-of-Origin Effects in 15q11.2 BP1-BP2 Microdeletion (Burnside-Butler) Syndrome. International Journal of Molecular Sciences. 2019;20(6).
36. Butler MG. Clinical and genetic aspects of the 15q11.2 BP1–BP2 microdeletion disorder. Journal of Intellectual Disability Research. 2017;61(6):568-79.
37. Butler MG. Magnesium Supplement and the 15q11.2 BP1–BP2 Microdeletion (Burnside–Butler) Syndrome: A Potential Treatment? International Journal of Molecular Sciences. 2019;20(12).
38. Chai X, Cao T, He Q, et al. Brain–computer interface digital prescription for neurological disorders. CNS Neuroscience & Therapeutics. 2024;30(2).
39. Lun X, Liu J, Zhang Y, et al. A Motor Imagery Signals Classification Method via the Difference of EEG Signals Between Left and Right Hemispheric Electrodes. Frontiers in Neuroscience. 2022;16.

Table

Table 1. Summary of clinical and genetic characteristics of 4 patients with spastic paraplegia type 6.

Patient		Case 1 (T5057)	Case 2 (T8904)	Case 3 (T7113)	Case 4 (T8346)
Gender		M	M	F	F
AAO (years)		26	10	21	1
Duration (years)		3	20	22	14
Family History		+	+	-	-
Initial Symptoms		Difficulty in walking	Difficulty in walking	Difficulty in walking	Difficulty in walking
Other Symptoms		-	-	-	Foot deformities
LL	Hypertonia	+	+	+	+
	Hyperreflexia	+	-	+	+
Babinski signs		+	+	+	+
Ankle clonus		+	-	-	+
SPRS		8	12	30	12
Additional features		-	Demyelination neuropathy reduced pinprick sensation	-	-
NIPA1 mutation		c.316G>A (p. Gly106Arg)	c.316G>A (p. Gly106Arg)	15q11.2 BP1-BP2 microdeletion	15q11.2 BP1-BP2 microdeletion
ACMG		P	P	P	P

AAO, age at onset; +, positive; -, none; LL, lower limbs; SPRS, Spastic Paraplegia Rating Scale; NIPA1, nonimprinted in Prader-Willi/Angelman loci 1 (NM_144599),ACMG, American College of Medical Genetics and Genomics (Richards et al., 2015); M, male; F, female; P, pathogenic.

Figures

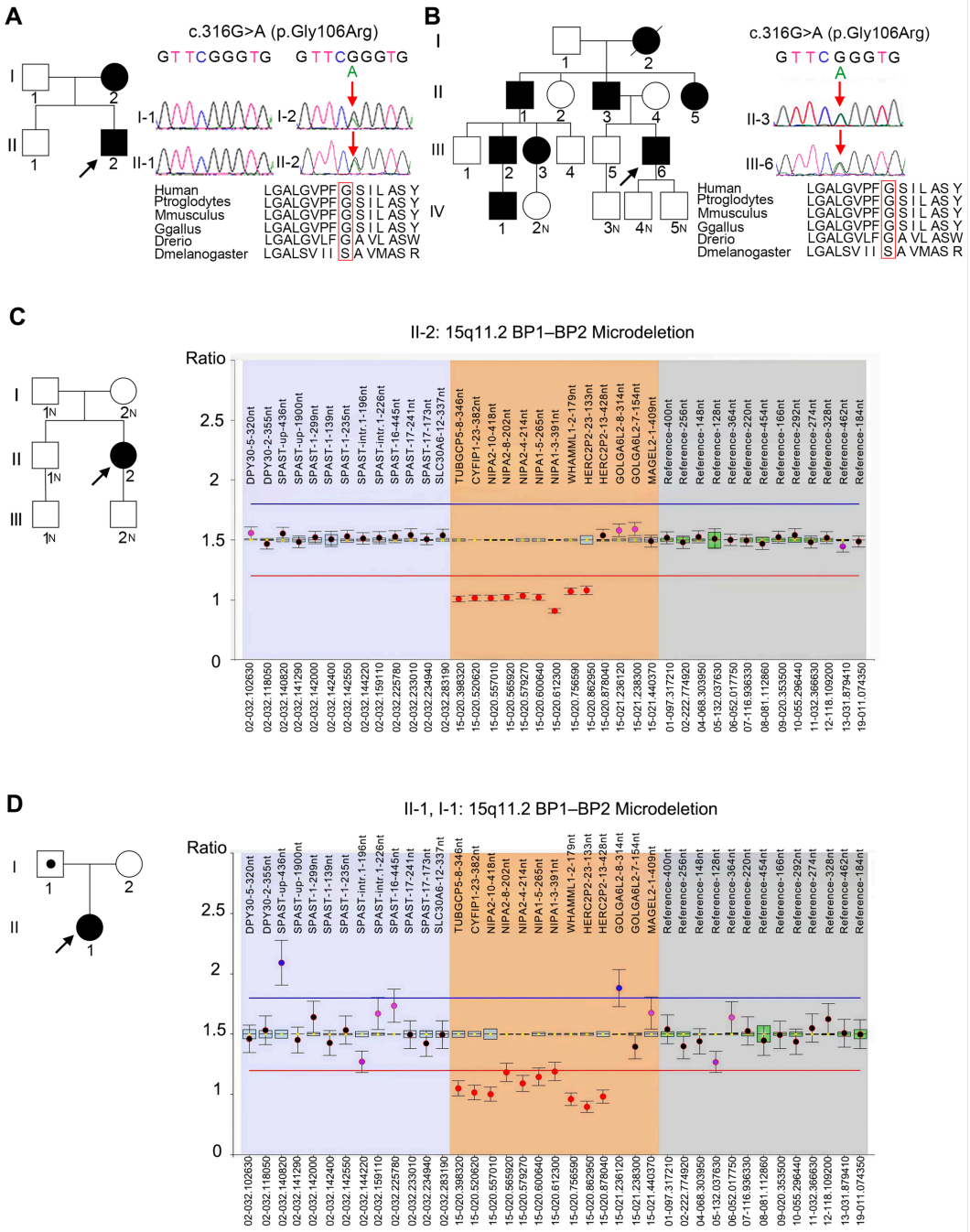


Figure 1

Analysis of Pedigrees and *NIPA1* Mutation Conservation Across Species in SPG6.

The patient's family pedigree and the Sequence chromatogram of the *NIPA1*gene(NM_144599) (red arrows indicate mutation sites). In the pedigree, squares represent males, circles represent females. black-filled symbols indicate affected individuals, white symbols indicate unaffected individuals, and the

point in the white symbol indicates a heterozygous carrier. N, not tested. All other individuals shown underwent genetic testing.

A: Pedigree of Family 1. sequence chromatogram of *NIPA1* gene displays one hemizygous mutation c.316G>A (p.Gly106Arg) in the proband (II:2)which was identified in his mother (I:2) but negative in his father (I:2) and brother (II:1). The mutations located in the highly conserved region of proteins are shown in the bottom half. Red square frame: mutant amino acid.

B: Pedigree of Family 2. sequence chromatogram of *NIPA1* gene displays one hemizygous mutation c.316G>A (p.Gly106Arg) in the proband (III:6)which was identified in his Father (II:3) but negative in his mother (II:4)and brother (III:5).

C: Pedigree of Family 3. MLPA results indicate missing genes (*TUBGCP5*, *CYFIP1*, *NIPA1, NIPA2*) involved in hemizygous 15q11.2 BP1-BP2 microdeletion in the proband (I:2).

D: Pedigree of Family 4. MLPA results indicate missing genes (*TUBGCP5*, *CYFIP1*, *NIPA1*, *NIPA2*) involved in hemizygous 15q11.2 BP1-BP2 microdeletion in the proband (II:1) and his father (I:1), but negative in his mother (I:2).

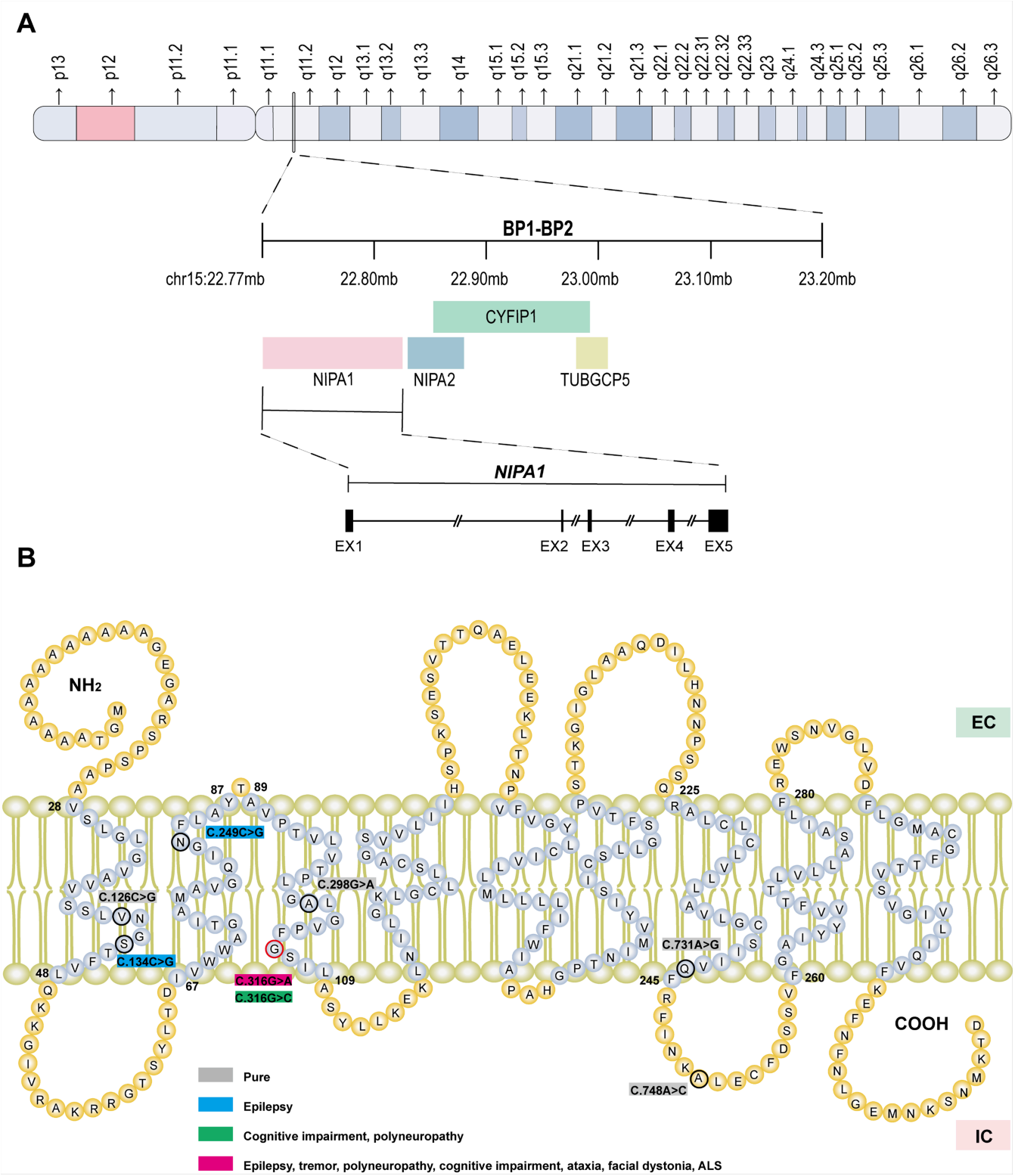


Figure 2

Diagram of Chromosome 15, genes in the BP1-BP2 region and NIPA1 protein structure.

A: Gene distribution of 15q11.2 BP1-BP2 region and gene structure of *NIPA1* (NM_144599). BP, breakpoint; EX, exon.

B: Schematic of NIPA1 Structure and Summary of SPG6 Genotype-Phenotype Correlations. Yellow balls represent amino acids in topological domains, and blue balls represent amino acids in transmembrane domains. Amino acid mutations causing different genotypes markedwith black (reported before) or red circles (reported in this study). Genotype-phenotype correlations of SPG6 were highlighted with different colors: grey= pure form; blue= epilepsy; green= cognitive impairment, polyneuropathy; magenta= epilepsy, tremor, polyneuropathy, cognitive impairment, ataxia, facial dystonia, amyotrophic lateral sclerosis (ALS). EC, extracellular; IC, intracellular; NH2, amino terminal; COOH, carboxyl terminal.