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Locus and Allelic Heterogeneity in Five Families with Hereditary Spastic Paraplegia

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Abstract

Hereditary spastic paraplegias are a group of genetically heterogeneous neurological disorders characterized by progressive weakness and spasticity of lower limbs. We ascertained five families with eight individuals with hereditary spastic paraplegia. Pathogenic variants were identified by exome sequencing of index cases. The cohort consists of three families with spastic paraplegia type 47 (*AP4B1*) with a common mutation in two families, a family with spastic paraplegia type 50 *(AP4M1),* and two male siblings with X-linked spastic paraplegia 2 (*PLP1*). This work illustrates locus and allelic heterogeneity in five families with hereditary spastic paraplegia.

Keywords

Hereditary spastic paraplegia; *PLP1*; *AP4B1*; *AP4M1*; Exome sequencing

INTRODUCTION

Hereditary spastic paraplegia (HSP) are a group of neurological disorders predominantly affecting lower limbs in a progressive manner resulting in weakness and spasticity [1]. The prevalence of HSP is estimated to be around 1.8/1,00,000 general population [2]. The diagnosis of HSP is based on the presence of slowly progressive bilateral lower limb spasticity and weakness, signs of corticospinal tract involvement such as hyperreflexia and

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Author contributions

KMG and AS evaluated the patients, conceived the idea of the manuscript and planned the experiments. MH performed experiments and drafted the manuscript. SN evaluated the patients and contributed to the clinical reports. SB and KMG supervised the entire work. All authors read and approved the final manuscript.

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extensor plantar responses [3]. Complicated HSP is characterized by spastic paraplegia associated with other abnormalities like seizures, ataxia, intellectual disability, peripheral neuropathy or extrapyramidal involvement. HSPs illustrate one of the highest degree of genetic heterogeneity involving more than 70 genetic subtypes, demonstrating all patterns of Mendelian inheritance (autosomal dominant, autosomal recessive, X-linked) and mitochondrial inheritance [3, 4]. We ascertained five families with eight individuals with of HSP. Exome sequencing, Sanger validation and segregation analysis was carried out in all these families.

METHODS

We ascertained five unrelated Indian families with eight affected individuals with HSP. Subjects were evaluated at genetics clinic where patients with neurological disorders were studied under the projects titled, ‘Clinical and molecular characterization of leukodystrophies in Indian children’ and ‘Genetic Diagnosis of Heritable Neurodevelopmental Disorders in India: Investigating the Use of Whole Exome Sequencing and Genetic Counseling to Address the High Burden of Neurodevelopmental Disorders’. Patients with hereditary spastic paraplegia were selected from these research projects. Informed consent was obtained for the use of clinical and research findings for publication. The study has the approval from Institutional Ethics Committee. Exome sequencing was performed in all the families. Sanger sequencing was performed to validate the variants and to determine the segregation in the families. Exome sequencing was performed on leukocyte derived genomic DNA using Illumina’s Nextera Rapid Capture Exome Kit on the Illumina NextSeq Platform (Illumina, San Diego, California, USA) [5]. The average coverage depth was 130x, with ~95% of the bases covered at >20X, and a sensitivity of >90%. Data was stored and analyzed using a reported automated pipeline, SeqMule v1.2.5 [6]. The variant call format file was annotated by ANNOVAR v.2016Feb01 [7]. Variants were filtered to 1% allele frequency in population databases including ExAC, 1000 genome database, and an internal database of exomes of 427 individuals of Indian origin. Exonic and splice site variants were then prioritized by MIM (Mendelian Inheritance in Man) identity, phenotypic assessment, and the American College of Medical Genetics (ACMG) criteria of pathogenicity [8].

RESULTS

Clinical descriptions

Family 1—P1 was evaluated at 14 years of age. She is the first-born child to a fourth degree consanguineous marriage (Fig. S1A). She was born at term with a birth weight of 2.45 Kg (−1 SD). She achieved neck holding at five months of age, sat without support at nine months and started speaking bisyllables at three years followed by standing and walking at three years of age. On exam, she weighed 35 Kg (−2 SD) with an occipito-frontal circumference (OFC) of 47 cm (−6 SD) and a height of 138 cm (−3 SD). Facial dysmorphism included a narrow forehead, wide nasal bridge, strabismus, narrow high arched palate, irregular teeth. She had mild distal joint laxity, bilateral clinodactyly, pes planus, broad great toes, spasticity, exaggerated deep tendon reflexes, bilateral ankle

contractures. Ultrasonography of abdomen and pelvis was unremarkable. Magnetic resonance imaging of brain revealed thin splenium of corpus callosum (Fig. S1B).

P2 is a 13-years-old male sibling of P1. He had similar complaints and facial appearance as his elder sister. He weighed 1.75 Kg (−3 SD) at birth. Developmental milestones were delayed. He achieved neck holding at three months of age, crawled at 3 years, sat without support at four years, and spoke bisyllables at 13 years. He is non-ambulatory. Examination revealed an OFC of 46 cm (−5 SD), height 117 cm (−5 SD) and weight 17 Kg (−5 SD). He also had post-axial polydactyly on left hand and both feet, broad great toes, contractures at knee and ankle joints, spasticity, and exaggerated deep tendon reflexes. Ultrasonography of abdomen and pelvis were unremarkable. Magnetic resonance imaging of brain revealed thin splenium of corpus callosum and periventricular white matter abnormalities (Fig. S1C, S1D).

Family 2—P3 and P4 are male siblings. Their parents were consanguineously married (third degree) (Fig. S2A). P3 was evaluated at 4 years of age. He had an uneventful birth history and neonatal period. His weight at birth was 2.6 Kg (+1 SD). He had global developmental delay. He walked at 4 years of age and has not attained speech. There were no seizures in him. Parents complained of frequent falls and drooling. His weight was 11 Kg (−2 SD), OFC 44 cm (−5 SD) and height 95 cm (−1 SD) at 4 years. He was not dysmorphic. He has an unsteady gait and walked on toe tips. Bilateral contractures of tendoachilles was noted. High resolution karyotype revealed a karyotype of 46,XYqh+. Magnetic resonance imaging of brain showed hypoplastic corpus callosum (Fig. S2B).

P4 is a one-year-old boy with unremarkable birth history. He weighed 2.8 Kg (+1 SD) at birth. He attained head control at 4 months, sits with support at present. He had an episode of febrile seizure. His anthropometry showed an OFC of 44 cm (−3 SD), height of 75 cm (−1 SD), weight, 9.5 Kg (−1 SD). He had coarse facies, broad forehead, hypertelorism, short nose, anteverted nares, long philtrum. Other findings on exam were hypotonia, bilateral undescended testes. Creatinine kinase (CK) levels were normal. GCMS, sub-telomeric MLPA were unremarkable. Magnetic resonance imaging of brain revealed hypoplastic corpus callosum.

Family 3—P5 was second born to a non-consanguineous marriage and evaluated at 2 years (Fig. S3A). Birth history and neonatal period was normal. He weighed 3 Kg (+2 SD) at birth. Neck holding and partial roll over was achieved before 6 months of age. He does not sit. He had 3–4 episodes of generalized tonic-clonic seizures daily starting from age 6 months and was on antiepileptic drugs. At age 2 years, his OFC was 40.5 cm (−6 SD), length was 76 cm (−3 SD), weight 8.5 Kg (−3 SD). Deep tendon reflexes were diminished, and tone was variable. His hematology and urine routine examinations revealed normal results. Hypoplasia of corpus callosum and periventricular white matter abnormalities were observed on magnetic resonance imaging of brain (Fig. S3B, S3C).

Family 4—P6 was 6 years when she was evaluated. She was the first born to a consanguineous marriage (Fig. S4A). She was noted to have developmental delay and seizures (precipitated by fever). At age 6 years she spoke disyllables and stood with support.

She was not toilet trained. She was noted to have microcephaly (OFC 42 cm, −3 SD), mild facial dysmorphism comprising sagging cheeks and downturned corners of the mouth. Hypotonia, joint laxity in upper limbs, spasticity of lower limbs and contractures at knees were observed. Magnetic resonance imaging of brain showed mild prominence and asymmetry of the lateral ventricles, mild hypoplasia of posterior part of the body of corpus callosum (Fig. S4B and S4C).

Family 5—P7 and P8 are male siblings aged 19 and 17 years respectively, born to non-consanguineous family (Fig. S5A). Both had global developmental delay. P8 achieved roll over at 1 year and walked with support at 2.5 years. Both were noted to have progressive difficulty in walking and feeding difficulty. Both of them had normal growth parameters. P8 had brisk deep tendon reflexes, spasticity, contractures, extensor plantar response and distal joint laxity. Magnetic resonance imaging of brain was unremarkable. P7 had a similar but milder phenotype as compared to P8.

Molecular analysis

Molecular findings in the affected individuals from all the six families are summarized in Table 1. Family 1, family 2 and family 3 (Fig. S1E, S2C, S3D) were diagnosed with spastic paraplegia type 47 (#MIM 614066). Family 2 and 3 had the same genotype, c.304C>T, p.(Arg102Ter). P6 from family 4 was noted to have a canonical splice-site variant, c.1026–1G>T in *AP4M1* (Fig. S4D) causing spastic paraplegia type 50 (#MIM 615905). A known pathogenic variant, c.2T>G, p.Met1Arg in *PLP1* was identified in family 5 with X-linked spastic paraplegia 2 (#MIM 312920) [9] (Fig. S6B). We report six novel and three known variants. All the variants were not observed in homozygous state in population databases like 1000 Genomes Project, ExAC database and in-house data of 437 exomes and occur at highly conserved amino acids sequence. One was a canonical splice site variant (*AP4M1*) and another frameshift deletion (*AP4B1*). Other novel variants were missense changes. *In-silico* analysis tools were consistent in predicting that they may damage the protein function. Clinical findings observed in the patients were in concordance with respective phenotypes caused due to these variations. Biallelic segregation and validation of the variants were done by Sanger sequencing. The missense variants therefore fell into the category, “likely pathogenic” according to ACMG criteria.

DISCUSSION

Currently, HSP is a syndromic designation for a clinically and genetically heterogeneous group of inherited neurodevelopmental disorders in which the main symptoms and signs are weakness and spasticity of the lower limbs primarily due to retrograde dysfunction of the descending fibers of the corticospinal tract [3] With increasing number of genetic etiologies, it poses a significant challenge for diagnosis of these conditions in the clinic.

We observed three families with five affected individuals aged 1–14 years with spastic paraplegia 47. All of them had classical symptoms of SPG47 [3, 10, 11]. Family 1 (P1 and P2), family 2 (P3 and P4) and family 3 (P5) presented with global developmental delay, progressive difficulty in walking and speech delay in the probands. Additionally, facial dysmorphism was noted in P1 and P2 of family 1. P5 also had generalized tonic-clonic

seizures at the time of presentation. Age related progression was striking as the younger patients P4 and P5 displayed generalized hypotonia with diminished reflexes and the older patients P1, P2 and P3 manifested contractures and spasticity. Hypoplasia of corpus callosum was the most common brain imaging finding in all of them with periventricular white matter abnormalities in P2 and P5. As reported in the literature earlier, we too observed loss-of-function variants (frameshift and nonsense) in our patients [10–13]. Family 2 and family 3 shared the same nonsense variant. We identified another novel frameshift variant, c.1181_1182del, p.(Gln394ArgfsTer23) and a reported nonsense variant, c.304C>T p.(Arg102Ter) in *AP4B1*[14].

Autosomal recessive HSP due to pathogenic variants in *AP4M1* (Spastic paraplegia 50) is a rare neurodevelopmental disorder reported for only a few patients. Seven pathogenic *AP4M1* mutations in seven families have been reported to date [15]. Copy number variants including the *AP4M1* have also been implicated in developmental anomalies [16]. The spectrum of clinical findings observed in the patients in the literature are of infantile hypotonia, intellectual disability, developmental delay, early-onset spastic paraplegia, variable white matter and cerebellar involvement on brain MRI [17–21]. A genotype-phenotype correlation has been proposed earlier as early onset and severe phenotype was often noted in individuals with truncating variants in comparison to missense variants in individuals with milder phenotype [15]. Proband in family 4 (P6) possessed a canonical splicing variant and manifested classical signs of AP-4 complex deficiency like seizures (precipitated by fever), distinctive facial appearance, hypotonia, developmental delay, early-onset spastic paraplegia and signs of cerebellar volume loss.

Pathogenic variants in the *PLP1* have been identified in families with X-linked recessive spastic paraplegia type 2 and Pelizaeus-Merzbacher disease. Multiple affected males from a large French-Canadian family is reported with early with developmental delay and progressive spastic paraplegia with the same variant observed in our probands, P7 and P8. Our probands had global developmental delay, progressive difficulty in walking, spasticity of lower limbs and contractures. Magnetic resonance imaging of brain had normal brain myelination and development in contrast to periventricular and deep white matter changes in the reported patient with the same variant. Vision and hearing was not evaluated in P7 and P8. WES revealed the previously reported variant, c.2T>G, p.Met1Arg in *PLP1* in the siblings [9].

In conclusion, this report adds novel genotypes for rare subtypes of hereditary spastic paraplegias and further emphasizes the phenotypic and genetic heterogeneity of these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Results of genomic testing in the cohort with hereditary spastic paraplegia

Family	Patient	Gene	Variants	Transcript ID	CADD score	GERP score	Known/Novel variant	GnomAD frequency	Zygosity	Phenotype	OMIM number
1	1,2	*AP4B1*	c.1181_1182del, p.(Gln394ArgfsTer23)	NM_001253852.1	NA	NA	Novel	0	Homozygous	spastic paraplegia type 47	614066
2	3,4	*AP4B1*	c.304C>T, p.(Arg102Ter)	NM_001253852.1	36	3.36	Known	0.00003249	Homozygous	Spastic paraplegia type 47	614066
3	5	*AP4B1*	c.304C>T, p.(Arg102Ter)	NM_001253852.1	36	3.36	Known	0.00003249	Homozygous	Spastic paraplegia type 47	614066
4	6	*AP4M1*	c.1026–1G>T	NM_004722.3	25.5	4.81	Novel	0	Homozygous	Spastic paraplegia type 50	615905
5	7,8	*PLP1*	c.2T>G p.Met1Arg	NM_000533.3	13.24	5.44	Known	0	Hemizygous	Spastic paraplegia 2, X-linked	312920