AP4B1-associated hereditary spastic paraplegia: Expansion of Clinico-Genetic Phenotype and Geographic Range

Kamran Salayev, MD, PhD[1], Clarissa Rocca, MSc[2], Rauan Kaiyrzhanov, MD, MSc[2], Ulviyya Guliyeva, MD[3], Sughra Guliyeva, MD[3], Aytan Mursalova, MD,[4] Fatima Rahman, MD,[5] Najwa Anwar, MD[5], Faisal Zafar, MD[6], Farida Jan, MD,[7] Nuzhat Rana, MD[6], Shazia Maqbool, MD, PhD[5], SYNAPS Study Group[2], QUEEN SQUARE Genomics[2], Stephanie Efthymiou, PhD[2], Henry Houlden, MD, PhD[2*]

[1] Azerbaijan Medical University, Department of Neurology, Baku, Azerbaijan, AZ 1010.
[2] University College London, Institute of Neurology, Department of Neuromuscular Disorders, Queen Square, WC1N 3BG, London, UK.
[3] MediClub Hospital, 45, Uzeyir Hajibeyli str., Baku, Azerbaijan, AZ 1010.
[4] Baku City Gerontologic Centre, Azadliq ave., Baku, Azerbaijan
[5] Development and Behavioral Pediatrics Department, Institute of Child Health and The Children Hospital, Lahore 54000, Pakistan.
[6] Department of Paediatric Neurology, Children's Hospital and Institute of Child Health, Multan, Pakistan.
[7] Department of Paediatrics and Child Health, Aga Khan University Hospital, Karachi, Pakistan

Highlights

- Bi-allelic loss-of-function variants in genes that encode subunits of the adaptor protein complex 4 (AP-4) lead to complex hereditary spastic paraplegias.
- Disease-causing variants in the AP4B1 gene have been expanding in a number and geographic range
- Exome sequencing was performed on 7 patients from 3 unrelated Azerbaijani and Pakistani families with a clinical diagnosis of hereditary spastic paraplegia.
- We report 7 cases of *AP4B1*-associated hereditary spastic paraplegia expanding its clinico-genetic phenotype and geographic range.

Keywords: *AP4B1*, Spastic Paraplegia, epilepsy, genetics

* Corresponding author: Henry Houlden, University College London, Institute of Neurology, Department of Neuromuscular disorders. WC1N 3BG.

E-mail address: h.houlden@ucl.ac.uk

Introduction

The AP4B1 gene encodes a subunit of the heterotetrameric adaptor protein (AP) complex, a component of intracellular transport of proteins that ~~is~~are thought to have a unique role in neurons. AP4 is composed of 2 large chains, beta-4 (*AP4B1*) and epsilon-4 (*AP4E1*), a medium-chain, mu-4 (*AP4M1*), and a small chain, sigma-4 (*AP4S1*) [1-3]. A group of congenital neurological diseases characterized by spastic para- or tetraplegia, severe intellectual disability with the possible absence of speech, and microcephaly have been associated with deficiency of different subunits of adaptor protein 4 (AP-4) complex subunits [4].

Bi-allelic loss-of-function (LOF) variants in the AP4B1 gene cause autosomal recessive spastic paraplegia type 47 (SPG47). The majority of the reported families with defective *AP4B1* are from the Middle East region, but variants were also found in patients of European and South Asian descent [1, 3, 4, 5, 6, 7, 8, 9]. Most of the cases have homozygous variants and were from consanguineous families [3, 4, 5, 6]. However, patients born to non-consanguineous unions with compound heterozygous variants were reported as well [1, 7]. ~~Core~~The core clinical features in previously published patients include neonatal hypotonia that progresses to spasticity, developmental delay with prominent motor and speech delay, episodes of stereotypic laughter, and seizures including frequent febrile ~~seizures~~convulsions. The neuroimaging features include thinning of the corpus callosum~~,~~ and delayed myelination/white matter loss. Here we describe three unrelated families with seven affected individuals ~~harbouring~~harboring LOF pathogenic variants in the AP4B1 gene expanding its genetic spectrum as well as its geographic range to Transcaucasia.

Methods

This study was approved by local institutional IRB/ethical review boards, and written informed consent was obtained before genetic testing from the families involved. Clinical details were obtained through medical ~~file~~records review and clinical examination.

Genomic DNA was extracted from peripheral blood samples according to standard procedures of phenol-chloroform extraction. WES on each proband was performed as described elsewhere [10] in Macrogen, Korea. Briefly, target enrichment was performed with 2 μg genomic DNA using the SureSelectXT Human All Exon Kit version 6 (Agilent Technologies, Santa Clara, CA, USA) to generate barcoded whole-exome sequencing libraries. Libraries were sequenced on the HiSeqX platform (Illumina, San Diego, CA, USA) with 50x coverage. Quality assessment of the sequence reads was performed by generating QC statistics with FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc).

The bioinformatics filtering strategy included screening for only exonic and donor/acceptor splicing variants. In accordance with the pedigree and phenotype, priority was given to rare variants (<0.01% in public databases, including 1,000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium [ExAC v0.2]) that were fitting a recessive (homozygous or compound heterozygous) or a de novo model and/or variants in genes previously linked to spasticity, developmental delay, intellectual disability, and other neurological disorders.

Results

Clinical description

Family 1

These are two affected brothers from neurologically healthy parents of Azerbaijani origin (Figure 1A and Table 1). The parents are first cousins. The elder brother (P1) is a 5-year-old boy born full-term after uneventful pregnancy and delivery. During the first months of his life, he was hypotonic and showed delayed developmental milestones. At the age of 1 year, seizures started. Seizures partially responded to Sodium valproate, and add-on Levetiracetam proved effective. Upon examination, his head circumference was 46 cm (decreased by ≥3 SD), weight was 24 kg (1.15 SD) and height was 113 cm (–0.21 SD). He had delayed language and motor development. There was nystagmus before 2 years of age, but a recent ophthalmologic examination revealed no abnormality. He had no speech production and receptive language was limited to reaction to his name. He had spastic paraplegia with brisk tendon reflexes and was unable to walk or sit independently. There were contractures in the lower limbs and joint hyperlaxity in the upper limbs. Parents reported that the boy had spontaneous non-triggered laughter and self-injurious ~~behaviour~~behavior. He had a happy demeanor ~~and characteristic~~ and stereotypic episodes of laughter, but no "shy character." He had a thin upper vermilion and prominent cheeks (Figure ~~3~~2). MRI images showed ventriculomegaly, white matter loss, and thinning of the posterior part of the corpus callosum (Figure ~~2~~3).

The younger brother (P2) is a 2-year-old boy born full-term after uneventful pregnancy and delivery. The parents first noticed developmental problems: the child was unable to sit

independently at 9 months of age. He had no seizures. Upon examination, he had delayed language and motor development. He had a head circumference of 45.5 cm (–2 SD), weight of 11 kg (–1.38 SD), and height of 88 cm (0.32 SD). The child was able to sit independently but is unable to walk. He reacted to his name; however, he produced no words. He had hypotonia and there was neither spasticity nor contractures. However, there were already brisk tendon reflexes and extensor plantar reflexes. Generally, the boy had a happy demeanor, but neither stereotypic episodes of laughter nor "shy character." He had prominent cheeks and a thin upper vermilion (Figure 2). MRI images showed white matter loss and thinning of the posterior part of the corpus callosum (Figure 3).

Family 2

This case (P3) is a 31-year-old male from consanguineous parents of Pakistani origin (Figure 1A and Table 1). He is a product of full-term pregnancy. Poor head growth was reported in the last trimester. The disease presented at the age of 7 weeks with seizures along with bronchopneumonia. During the neonatal period, there was muscle hypotonia, which was followed by muscle hypertonia. He obtained independent sitting at 2 years and walking at 6 years. He started to produce his first words at 5 years. The patient had severe language and motor developmental delay. He had a head circumference of 52 cm (decreased by ≥3 SD), weight of 35 kg (decreased by ≥3 SD), and height of 150 cm (decreased by ≥3 SD). The patient had generalized tonic-clonic seizures which started at 7 weeks of age. Sodium valproate and Levetiracetam were used in order to control seizures. He had spastic tetraplegia and contractures. There were brisk tendon reflexes and extensor plantar reflexes, and bulbar palsy. He had an impairment of the sense of temperature. The patient was wheelchair-bound. The

progression of his condition ceased and stabilized when he was 18 years of age. He had a wide nasal bridge, flat nasal bridge, everted upper vermilion, and a thin upper vermilion. Generally, he had a happy demeanor, but ~~no~~neither stereotypic episodes of laughter nor shy character.

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P4 and P5 are two younger sisters of the proband. P4 is a 27-year-old female with progressive spastic paraplegia. She could sit unsupported and had prolonged ~~generizled~~generalized tonic-clonic seizures. P5 is a 22-year-old female. She wasn't able to sit unsupported. She had right~~-~~-sided hemiparesis. P5 had brief tonic seizures.

Family 3

These two cases are ~~a brother~~affected male (P6) and ~~a sister~~female (P7~~).~~) siblings. They are the third and fourth children of consanguineous parents of Pakistani origin (Figure 1A and Table 1). Parents are cousins. They have two healthy elder sisters and a younger brother.

P6 is a 9-year-old boy. Pregnancy and delivery were uneventful. He had moderate developmental delay. He acquired independent sitting at 9 months of age. He was able to walk with assistance and speak. The boy had febrile seizures. Afebrile seizures started at age of 1 year (presenting symptom). He had generalized tonic-clonic seizures controlled with Sodium valproate and Levetiracetam. Upon examination, he had spastic tetraplegia and contractures with hyperreflexia and extensor plantars.

P7 is an 8-year-old girl. Pregnancy and delivery were uneventful. She presented at age of 3 with walking difficulties. She acquired independent sitting at 9 months of age and assisted walking at 18 months. She was able to speak but not fluently. She has a mild developmental

delay. Her head circumference was 50.5 cm (–1.28 SD). Upon examination, she had spastic tetraplegia with hyperreflexia and extensor plantars and contractures. She had hypotonia and brisk reflexes. The girl had no seizures. The MRI scans were not obtained.

Genetic findings

Exome sequencing revealed three biallelic variants in the adaptin N terminal region of *AP4B1* (Table 2). In Family 1, ~~NC_000005.10~~NM_001253852.3:c.1459C>T; p.(Arg487Ter) was predicted to cause early termination of the protein (Figure 1C). The variant segregated with the disease and was found in a 9Mb large region of homozygosity. In Family 2 we observed a biallelic frameshift *AP4B1* variant c.967del; p.(Ser323ArgfsTer18) leading to a stop gained after 18 amino acids (Figure 1C). Cases ~~P4~~P6 and ~~P5~~P7 in Family 3 shared a homozygous stop gained variant in exon 8 of *AP4B1*, c.1365T>A; p.(Tyr455Ter) (Figure 1C). The variant was located in a region of homozygosity spanning 7.6Mb.

Based on the allele frequencies reported in publicly available databases (Table 2), the three reported variants are either ~~r~~ absent or extremely rare in the general population. Moreover, based on in-silico predictions, all described variants are likely to have a damaging effect on the *AP4B1* protein (Table 2).

Discussion

~~The hereditary~~Hereditary spastic paraplegias (HSP) are a group of neurodegenerative diseases that present mainly with weakness and stiffness in the ~~leg~~lower limb muscles and lead to

progressive neurological decline [11]. Bi-allelic LOF variants in genes that encode subunits of AP-4 lead to complex HSP in children, called AP-4-associated HSP [2]. SPG47 (*AP4B1*, MIM: 614066) is one of four AP4-associated HSP (others affect other proteins in AP4 family: *AP4M1*, *AP4E1*, and *AP4S1*).

AP-4 belongs to a family of adaptor proteins consisting of the five heterotetramers AP-1 to AP-5, which have key functions in vesicle trafficking [12]. AP-4 is composed of four subunits that form ~~an~~a heterotetrameric complex [13]. AP-4 mediates protein trafficking from the trans-Golgi network endosomes [14, 15]. Loss of one AP-4 subunit results in defective AP-4 complex formation and leads to impaired endosomal trafficking. Deleterious variants in genes encoding different subunits of the AP-4 complex have been associated with autosomal-recessive intellectual disability with spastic paraplegia, though it is still largely unclear how AP-4 impairment leads to the clinical presentation of spastic paraplegia.

Several pathogenic variants in the AP4B1 gene have been described in individuals with spastic diplegia [1, 3, 4, 5, 6]. Based on the similarity in clinical features in patients with variants affecting different AP-4 subunits, delineation of features of the recognizable AP-4 deficiency syndrome is important [6]. *AP4B1*-positive individuals present with a similar phenotype. Ebrahimi-Fakhari et al. compared ~~their~~ eight patients with bi-allelic variant in *AP4B1* to eleven patients with the same gene defects described ~~by others~~previously. They proposed a list of core clinical and imaging features ~~of~~for *AP4B*1-associated SPG47 [1]. All of the affected individuals had delayed motor development and neonatal or infantile hypotonia. Most of them also had delayed speech development (94%), progression to spastic diplegia (89%), loss of independent walking (88%), episodes of stereotypic laughter (77%), microcephaly (69%), short stature (57%), and epilepsy (47%). Imaging studies showed thin corpus callosum (73%), delayed

myelination or white matter loss (67%), and ventriculomegaly (40%). Our cases showed all of these symptoms and features except for short stature, which was present only in P3 (Table 1). Case P3 is the oldest reported patient with *AP4B1*-associated SPG47. Cases reported by Abdollahpour et al. were ~~social~~sociable and ~~had a~~ generally had a happy ~~demeanour~~demeanor, whereas in previous reports "shy character" was described in two families [6]. None of our cases had "shy character," and stereotypical laughter was reported in one case (P1). In addition, P1 has frequent self-injurious ~~behaviour~~behavior. MR scans of two of our cases showed typical *AP4B1* changes in the posterior part of the corpus callosum and delayed myelination (Figure ~~2~~3).

Five of our cases had epileptic seizures (Table 1). Cases P1, P3, and P6 experienced seizures before age of 1 year. In case ~~P4~~P6, seizures manifested as febrile convulsions. In all three cases, a combination of Sodium valproate and Levetiracetam was used. In case P1, adding Levetiracetam to Sodium valproate led to seizure control. In the case described by Ruan et al. (2020) Levetiracetam was effective after the failure of Topiramate to control febrile seizures [16].

In a previous report [2], 13 out of 19 patients with *AP4B1*-associated SPG47 were females, which led the author to the suggestion of a possible sex imbalance. However, adding our cases to the total cohort of reported cases rectifies the skewed sex ratio previously noted.

There are in total twenty-seven reported pathogenic variants in *AP4B1* (Table 3). Three variants were reported by different authors: homozygous variant c.304C > T, p.(~~Arg102*)~~Arg102Ter) [1, 17], c.664delC, p.(~~Leu222Cysfs*31~~Leu222CysfsTer31) [1, 3, 5], and c.1160_1161delCA (~~p.~~.(Thr387fs) [6, 8]. The variants described to date, including the three truncating variants in

this study, are found in the adaptin N terminal region of *AP4B1* (Figure 1C). According to gnomAD probability of LOF intolerance score (pLI), *AP4B1* is intolerant to LOF (pLI=0.83).

In summary, here we describe seven additional affected individuals from three consanguineous families presenting with hypotonia, developmental delay, and seizures. All our patients were from consanguineous marriages and therefore homozygous for their respective *AP4B1* variants. P3 case in this publication is the oldest reported *AP4B1*-associated hereditary spastic paraplegia patient. This patient had a previously unreported symptom of poor sense of temperature. We also reported the first case from Transcaucasia expanding the geographic range of *AP4B1*-associated hereditary spastic paraplegia.

Acknowledgment:

We thank all patients and relatives for consent to be part of the study. Families were collected as part of the SYNaPS Study Group collaboration funded by The Wellcome Trust and strategic award (Synaptopathies) funding (WT093205 MA and WT104033AIA) and research was conducted as part of the Queen Square Genomics group at University College London, supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. SE and HH were supported by an MRC strategic award to establish an International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) MR/S005021/1'. We are also grateful to Queen Square genomics at the Institute of Neurology University College London, supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre, for the bioinformatics support.

Data availability

The pathogenic variants identified in this work have been submitted to ClinVar with accession numbers:

NM_001253852.3(AP4B1):c.1459C>T (p.Arg487Ter) - VCV001334912

NM_001253852.3(AP4B1):c.1365T>A (p.Tyr455Ter) - VCV001335806

NM_001253852.3(AP4B1):c.967del (p.Ser323fs) - VCV001335882

Author Roles:

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Manuscript Preparation: A. Writing of the first draft, B. Review and Critique.

K.S.: 1A, 1B, 1C, 2A

CR: 1B, 1C, 2A

R.K.: 1A, 1B, 1C, 2A

U.G.: 1C, 2B

S.G: 1C, 2B

A.M.: 1C, 2B

FR: 1B, 1C

NA: 1B, 1C

FZ: 1B, 1C

FJ: 1B, 1C

NZ: 1B, 1C

SM: 1B, 1C

SE: 1A, 1B, 1C, 2A, 2B

H.H.: 1A, 1B, 2B

Disclosures:

Funding Sources and Conflict of Interest: This research was funded in part, by the Wellcome Trust [WT093205MA, WT104033AIA and the Synaptopathies Strategic Award, 165908]. This study was funded by the Medical research council (MRC) (MR/S01165X/1, MR/S005021/1, G0601943). The authors declare that there are no conflicts of interest relevant to this work.

Financial Disclosures for the previous 12 months: The authors declare that there are no additional disclosures to report.

Ethical Compliance Statement:

Written informed consent for genetic testing and photo/video materials were obtained from the parents. The study was conducted in accordance with the Declaration of Helsinki and approved by the relevant institutional review boards.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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Figure legends

Figures are not for colour print

Figure 1. Pedigrees of the three families described, the position of variants in a schematic diagram of gene and protein, and evolutionary conservation. (A) Pedigrees of the three families described. The variant is indicated by the + sign. Square = male; circle = female; black filled symbol = affected individual; white symbols = unaffected individuals; diagonal line = deceased individual. Double lines indicate consanguinity. (B) Schematic representation of the AP4B1 gene showing previously described variants in black and novel variants in red. (C) Schematic diagram indicating *AP4B1* protein. The blue shape represents

the adaptin N terminal domain, where all the variants described to date are found. The yellow shape indicates the Beta2-adaptin appendage, C-terminal sub-domain.

~~**Figure 2.**~~

Figure 2. Facial features of patient P1 (Family 1, IV.1) and patient P2 (Family 1, IV.2). Shared facial characteristics individuals include prominent cheeks, a thin upper vermilion border, and a flat nasal bridge.

Figure 3. MRI scans of patient P1 (Family 1, IV.1) and patient P2 (Family 1, IV.2). The left scan is sagittal T1 weighted images while the right scan is axial T2 weighted images. The main features of *AP4B1*-associated SPG47: a) a thin corpus callosum, particularly affecting the posterior aspect~~;~~ (arrow); 2) delayed or decreased myelination; and 3) ventriculomegaly.

~~**Figure 3.** Facial features of patient P1 (Family 1, IV.1) and patient P2 (Family 1, IV.2). Shared facial characteristics individuals include prominent cheeks, a thin upper vermilion border, and a flat nasal bridge.~~

Table 1. Clinical features of presented cases.

Table 2. Summary of *AP4B1* variants identified in the present cohort.

Table 3. *AP4B1* variants spectrum.

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