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Truncating variants in *UBAP1* associated with childhood-onset nonsyndromic hereditary spastic paraplegia

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Abstract

Hereditary spastic paraplegia (HSP) is a group of disorders with predominant symptoms of lower-extremity weakness and spasticity. Despite the delineation of numerous genetic causes of HSP, a significant portion of individuals with HSP remain molecularly undiagnosed. Through exome sequencing, we identified five unrelated families with childhood-onset nonsyndromic HSP, all presenting with progressive spastic gait, leg clonus, and toe walking starting from 7 to 8 years old. A recurrent two-base pair deletion (c.426_427delGA, p.K143Sfs*15) in the *UBAP1* gene was found in four families, and a similar variant (c.475_476delTT, p.F159*) was detected in a fifth family. The variant was confirmed to be denovo in two families and inherited from an affected parent in two other families. RNA studies performed in lymphocytes from one patient with the *de novo* c.426_427delGA variant demonstrated escape of nonsense-mediated decay of the *UBAP1* mutant transcript, suggesting the generation of a truncated protein. Both variants identified in this

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

CONFLICT OF INTERESTS

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study are predicted to result in truncated proteins losing the capacity of binding to ubiquitinated proteins, hence appearing to exhibit a dominant-negative effect on the normal function of the endosome-specific endosomal sorting complexes required for the transport-I complex.

Keywords

autosomal dominant; escape of nonsense-mediated decay; hereditary spastic paraplegia; UBAP1

1 | INTRODUCTION

Hereditary spastic paraplegia (HSP) refers to a group of inheritable disorders with axonal degeneration and predominant symptoms of lower-extremity weakness and spasticity. HSP is clinically classified as either nonsyndromic ("uncomplicated" or "pure") HSP or syndromic ("complicated") HSP based on the presence or absence of additional symptoms (Fink, 2013; Hedera, 1993; Lo Giudice, Lombardi, Santorelli, Kawarai, & Orlacchio, 2014). Uncomplicated HSP is characterized by neurologic impairment limited to progressive lower-extremity weakness and spasticity, hypertonic bladder, and lower-limb sensory disturbances, while complicated HSP includes symptoms seen in nonsyndromic HSP plus additional neurological or nonneurological findings including ataxia, seizures, intellectual disability, dementia, muscle atrophy, extrapyramidal disturbance, and peripheral neuropathy (Hedera, 1993). HSP patients may present with variable age of onset ranging from congenital to adult, and the symptoms may be nonprogressive, with slow/steady or rapid progression rate, depending on the spastic paraplegia (SPG) subtypes (Parodi, Coarelli, Stevanin, Brice, & Durr, 2018).

Currently, there are 69 subtypes of SPG with known genetic causes documented in Online Mendelian Inheritance in Man (OMIM; www.omim.org, last queried on October 3, 2019), including autosomal dominant (AD) HSP (most common type, found in 75–80% affected individuals with known genetic etiology), autosomal recessive (AR) HSP (25–30%), and rare genetic forms of X-linked and mitochondrial HSP (Hedera, 1993). Despite the numerous known genetic causes of HSP, population-based studies demonstrated 33–55% of individuals with AD HSP and 18–29% of individuals with AR HSP receive a genetic diagnosis after systemic testing, leaving a significant amount of individuals with HSP without a molecular diagnosis and HSP-associated genes likely remain to be discovered (Ruano, Melo, Silva, & Coutinho, 2014).

In this study, through exome sequencing and interinstitutional data sharing, we identified ubiquitin-associated protein 1 (*UBAP1*, MIM# 609787) as a novel disease-causing gene for AD childhood-onset nonsyndromic HSP in five unrelated families. Recently, truncating mutations in *UBAP1* were reported in multiple families with similar phenotypes of SPG (Farazi Fard et al., 2019). The finding of *de novo* or disease-segregating *UBAP1* truncating variants in additional families independently strengthens the gene-disease association and functional studies reported here support the dominant-negative disease mechanism.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

Written and oral informed consent was obtained in accordance with protocols approved by the Institutional Review Boards (IRB) of National Institutes of Health, Baylor College of Medicine, Nationwide Children's Hospital, and Research Ethics Committee of the Bellvitge University Hospital (PR076/14) for Families 1–3, and 5, respectively. Patient P1 was evaluated at Duke University as part of the Undiagnosed Diseases Network studies. Reporting of deidentified molecular data and minimum clinical information for Family 4 was approved by the IRB of Baylor College of Medicine.

2.2 | Clinical exome sequencing and data analysis

Variants reported in this study were deposited to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Clinical trio exome sequencing for Families 1 and 3 at Baylor was performed as previously described (Wang et al., 2017; Yang et al., 2013). Briefly, genomic DNA samples were fragmented, ligated to Illumina multiplexing paired-end adaptors, amplified with indexes added, and hybridized to exome capture reagent (Roche NimbleGen). Paired-end sequencing (2 × 100 bp) was performed on the Illumina HiSeq 2500 platform with a mean sequence coverage of ~120×, with ~97% of the target bases having at least 20× coverage. The output data were converted from a BCL file to a FastQ file by Illumina Consensus Assessment of Sequence and Variation software version 1.8.3 and mapped to the human reference genome using the BWA program. Variants were called by Atlas-SNP and Atlas-indel. An in-house software program was used for variant filtering and annotation (Yang et al., 2013).

Clinical trio exome sequencing for Families 2 and 4 at GeneDx was performed as follows: using genomic DNA from the proband or proband and parents, the exonic regions and flanking splice junctions of the genome were captured using the Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA) or the IDT xGen Exome Research Panel v1.0. Massively parallel (NextGen) sequencing was done on an Illumina system with 100 bp or greater paired-end reads. Reads were aligned to human genome build GRCh37/UCSC hg19 and analyzed for sequence variants using a custom-developed analysis tool. Additional sequencing technology and a variant interpretation protocol have been previously described (Retterer et al., 2016). The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

Clinical exome sequencing on individual P5, her affected mother, her affected maternal grandmother, and her unaffected sister were performed as follows: genomic DNA was extracted from peripheral blood using standard methods. Whole-exome sequencing (WES) of index singleton cases was carried out on exon targets isolated by capture using the SureSelect XT Human All Exon V5 50 Mb kit (Agilent Technologies) with 100-bp paired-end read sequences, generated on a HiSeq2500 Platform (Illumina Inc.) at Centre Nacional d'Anàlisi Genòmica (CNAG Barcelona, Catalonia, Spain). The sequencing methodology and variant analysis protocol and interpretation followed the Genome Analysis Tool Kit (GATK)

pipeline (McKenna et al., 2010) and ACMG Guidelines (Richards et al., 2015). The research project was approved by the Clinical Research Ethics Commitee for Research Ethics Committee of the Bellvitge University Hospital (PR076/14). Informed consent was obtained from all participants in this study.

2.3 | Lymphoblastoid cell culture

Venous blood of patient P2 and her unaffected mother was collected in ACD solution A tubes. Lymphocytes from the buffy coat layer were collected and transformed with Epstein-Barr virus and cyclosporin A following standard procedures. Cell lines were maintained in RPMI 1640 medium (Thermo Fisher Scientific; #61870036) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cell cultures were maintained at 37°C in a humidified incubator supplemented with 5% CO_2.

2.4 | RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from human lymphoblast cell lines derived from P2 and her unaffected mother with the miRNeasy Mini kit (Qiagen; #217004) following the manufacturer's instructions. RNA was quantified with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Three micrograms of total RNA were reverse-transcribed to complementary DNA (cDNA) using QuantiTect Reverse Transcription kit (Qiagen; #205311). cDNA from each sample was then analyzed by PCR followed by Sanger sequencing. Semiquantitative RT-PCR cycle numbers were as follows: *UBAP1* (exon 2–4), 30 cycles; glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), 25 cycles. Quantitative RT-PCR was performed using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories) with PerfeCTa SYBR Green FastMix ROX (Quanta Biosciences; #95073). Quantitative RT-PCR results were analyzed using the comparative Ct method normalized against the housekeeping gene *GAPDH*. PCR primer sequences are available in Table S1.

3 | RESULTS

3.1 | Clinical cases

In the current multicenter study, collectively we are reporting five unrelated families of various ethnic backgrounds affected with SPG and carrying heterozygous frameshift variants in *UBAP1*. All individuals were ascertained through exome sequencing and connected through data sharing via GeneMatcher (Sobreira, Schiettecatte, Valle, & Hamosh, 2015). All affected individuals showed highly similar clinical presentations, which were predominantly SPG and progressive spastic gait with onset at age 7-8 years (Table 1). Other related clinical features included ankle/leg clonus, toe walking, and poor balance. Three out of five patients had brain magnetic resonance imaging (MRI) performed and were all normal. These three patients also showed no developmental or cognitive defects.

Patient 1 is a 13-year-old mixed race (Mexican/Filipino) female referred due to a slowly progressive abnormal gait. Early development was normal. Symptoms started at age 7.5 years with toe walking. She was referred to neurology at age 10 years. On exam, her gait was spastic, and she had hyperreflexia and ankle clonus. Patient 1 is cognitively normal.

Previous genetic testing included a dopa-responsive dystonia panel and SPG panels, both of which returned negative. X-ray of the feet at age 10 showed severe bilateral pes planus without bone deformities. X-rays of the ankles, hips, knees, and patellae were all normal. Brain MRI was normal except for an incidental pineal cyst. MRI of the spine was unremarkable except for minimal disc bulge at C6-7. Family history was noncontributory.

Patient 2 is a 25-year-old Caucasian female. She came to medical attention at the age of 7 years due to an abnormal gait. Her foot would catch on the other foot while walking. The spastic gait had been slowly progressive until college when symptoms plateaued. Between age 13–14 years, she was trialed on dystonia treatments but was nonresponsive. Brain MRI at age 7 showed an incidental, benign pituitary adenoma. MRI of the brain and thoracic spine at age 20 was unremarkable, and MRI of the cervical spine showed minimal cervical spondylosis. Electromyography (EMG)/nerve conduction studies performed at age 19 was largely normal except for impaired activation, likely reflecting her underlying spasticity. There was no electrical evidence of large fiber neuropathy. Sural amplitudes were in the low normal range, similar to the study performed in childhood. The patient also had precocious puberty with menarche at 9 years old.

Patient 3 is a 12 years old Caucasian/American Indian male who was evaluated at 8 years of age due to spastic gait and a maternal family history of an AD pattern of spastic gait (further details are unavailable). On the neurologic exam, his tone and bulk were diffusely reduced. His gait was spastic with poor toe and heel walking, with his gait worsening with running. He also had delayed motor milestones, delayed speech/ articulation skills, as well as feeding difficulties. An HSP gene panel and dopa-responsive dystonia gene panel were both negative. Microarray identified a 0.535 Mb gain at 16p11.2 consistent with 16p11.2 microduplication syndrome (MIM# 614671), likely contributing to this patient's delayed motor and language development, a phenotype not present in other patients in this study.

Patient 5 is a 14-year-old Chilean female. She started to present with spastic paraparesis at age 8. She had brisk deep tendon reflexes in her lower extremities and bilateral ankle clonus with no Babinski sign. There were no clinical signs of ataxia or sensory deficits. She had no intellectual disability. Previous testing was all negative, including brain MRI, EMG/nerve conduction study, somatosensory evoked potentials study, and visual and brainstem evoked potentials studies. Family history is significant for similarly affected mother and maternal grandmother (Figure 2). Mother (currently 36-year-old) started a slowly progressive paraparesis at age 11 and currently has a paraparetic gait and urinary urgency (neurogenic bladder) that has been lasted for a year. Maternal grandmother (currently 69-year-old) started with gait disorder at age 10, and slowly progressed paraparesis; she currently has a paraparetic gait and urinary urgency (neurogenic bladder) that has been lasted for 5 years. Individual P5 also has one healthy sister. Consanguinity was denied.

3.2 | Identification of disease-causing variants in *UBAP1*

Exome sequencing was performed on individuals P1 and P3 at Baylor Genetics, individuals P2 and P4 at GeneDx, and P5 at Instituto de Investigación Biomédica de Bellvitge (IDIBELL). In the first four probands we identified a recurrent two base pair deletion in the *UBAP1* gene that results in a frameshift mutation (c.426_427delGA, p.K143Sfs*15,

reference NM_016525.5, Figure 1 and Table 1). This variant was found to occur *de novo* in Families 1 (Figure 1b) and 2, while in Family 3, the variant was inherited from the similarly affected mother (Figure 1c). The inheritance of the variant in Family 4 is unknown due to the unavailability of parental samples. Family 5 has three generations of affected individuals. The proband, affected mother, and affected maternal grandmother all carried another two base pair deletion (c.475_476delTT, p.F159*), while this variant was absent in the unaffected sister (Figure 2 and Table 1). Neither variant has been seen in the general population databases, including gnomAD (https://gnomad.broadinstitute.org/), 1,000 genomes (http://www.internationalgenome.org/), and the ESP database (https://evs.gs.washington.edu/EVS/). A truncated peptide with 157 amino acids in length is predicted to be generated in patients from Family 1 through Family 4 with the p.K143Sfs*15 variant. The p.F159* variant in Family 5 is predicted to result in a truncated peptide with 158 amino acids, which is almost identical in length as the first variant.

3.3 | Truncated transcript identified in patientderived lymphocytes

Given the identification of frameshift variants in multiple families with HSP, significantly underrepresented loss-of-function variants in the general population (gnomAD database, probability of loss-of-function intolerance = 0.91, observed/expected = 0.12), and the embryonic lethal phenotype in *UBAP1* homozygous knockout mice (International Mouse Phenotyping Consortium, http://www.mousephenotype.org/), loss-of-function was initially suspected as the disease-causing mechanism for the *UBAP1* variants in our cohort. However, the finding of a recurrent variant in Families 1–4 and the almost identical predicted protein product in Family 5 led to the hypothesis that truncated *UBAP1* proteins may confer deleterious effects through a mechanism other than haploinsufficiency. Upon careful inspection, a short palindromic sequence was predicted at the breakpoint of the recurrent two base pair deletion variant found in Families 1–4 (Figure S1), which could be a potential structural characteristic for the recurrence of the frameshift deletion in unrelated families (Ripley, 1982).

To test whether the *UBAP1* transcript with the frameshift variant escapes nonsense-mediated decay (NMD) and potentially leads to a truncated protein, we obtained lymphocytes from individual P2 and her unaffected mother. Sanger sequencing of the *UBAP1* cDNA demonstrated the presence of stable transcripts from both the wildtype allele and the mutant allele in patient P2, whereas messenger RNA (mRNA) from her unaffected mother only showed the wildtype allele (Figure 3b). Additionally, semiquantitative (Figure 3a) and quantitative (Figure 3c) PCR analysis revealed equal levels of mRNA expression in P2 and her unaffected mother at various exons of *UBAP1*, further indicating that the mRNA generated from the mutant allele does not undergo NMD.

4 | DISCUSSION

We report frameshift *UBAP1* variants in five individuals with childhood-onset AD HSP. While preparing this manuscript, an article describing truncating variants in *UBPA1* causing HSP was published (Farazi Fard et al., 2019), with eight different truncating mutations from 10 families, including a recurrent *de novo* c.426_427delGA variant in two families, which is

identical to the variant in Families 1–4 reported here (Table 1). Therefore, with our patients, there are now six HSP families with the c.426_427delGA variant identified, providing additional evidence for a mutational hotspot in *UBAP1*. Taken together, these two reports include four patients with a *de novo* change and one patient with the variant inherited from a similarly affected mother. A predicted palindromic sequence at the 2 base pair deletion breakpoint potentially justified it to be a mutation hotspot (Figure S1). Variant c.475_476delTT in Family 5 in the current study is a novel variant not reported previously.

Phenotypic comparison of patients currently and previously reported (Table 1) demonstrated highly uniform clinical presentations of childhood-onset progressive SPG. Intelligence and development were generally normal. Patient P3 had feeding difficulties and delayed speech and articulation skills, likely attributed to the 0.535 Mb copy-number gain at 16p11.2, consistent with 16p11.2 microduplication syndrome (MIM# 614671). Patient P4 had abnormal behavioral issues including aggressiveness and attention deficit hyperactivity disorder. Since no other genetic testing results were available for Patient P4, it is difficult to ascertain if his behavioral difficulties are related to the *UBAP1* variant.

UBAP1 encodes ubiquitin-associated protein 1. It is a component of the endosome-specific endosomal sorting complexes required for transport-I (ESCRT-I) complex that functions to sort ubiquitylated cargo such as epidermal growth factor receptor to the multivesicular body (MVB) and for endosomal ubiquitin homeostasis (Stefani et al., reaction; *UBAP1*, ubiquitin-associated protein 1 2011). There are two functionally critical domains in *UBAP1*—the UMA (*UBAP1*-MVB12-associated) domain for ESCRT-I binding near the N-terminus (de Souza & Aravind, 2010; Wunderley, Brownhill, Stefani, Tabernero, & Woodman, 2014), and the solenoid of overlapping ubiquitin-associated (SOUBA) domains for ubiquitin interaction near the C-terminus (Agromayor et al., 2012; Figure 1a). The two predicted truncated proteins reported in this study and all proteins reported by Farazi Fard et al. have an intact UMA domain while lacking the SOUBA domains. One possible model would be the mutant proteins are being recruited by the ESCRT-I complex, however, have lost the ability to interact with ubiquitinated proteins. As a result, the truncated *UBAP1* may exert a dominant-negative effect on the normal function of the ESCRT-I protein complex. Supporting this model, in Farazi Fard et al., it was demonstrated that truncated *UBAP1* protein was able to colocalize and bind to its binding partner VSP28 but was unable to bind to ubiquitinated proteins, corroborating the dominant-negative disease mechanism. Interestingly, one of the selective assembly partners of *UBAP1* for the ESCRT-I protein complex, VPS37A, is known to be associated with AR spastic paraplegia 53 (SPG53; MIM# 614898; Zivony-Elboum et al., 2012), lending further credence to the potential pathogenicity of deleterious variants in *UBAP1* in causing HSP.

HSP is known to be clinically and genetically heterogeneous. Proteins implicated in HSP are part of specific molecular pathways or functional modules when mutated, leading to degeneration or abnormal development of long spinal cord axons. One of the known disturbed pathways or functional modules involves endosome membrane trafficking, vesicle formation and selective uptake of proteins into vesicles (Blackstone, 2018; Fink, 2013; Lo Giudice et al., 2014). Several of the known HSP-causing genes encode proteins function in this pathway, including *AP4B1* associated AR SPG47 (MIM# 614066) (Abou Jamra et al.,

2011), *AP5Z1* associated AR SPG48 (MIM# 613647; Slabicki et al., 2010), *AP4M1* associated AR SPG50 (MIM# 612936; Verkerk et al., 2009), *AP4E1* associated AD SPG51 (MIM# 613744; Abou Jamra et al., 2011), *AP4S1* associated AR SPG52 (MIM# 614067; Abou Jamra et al., 2011), and the above mentioned *VPS37A* associated AR SPG53. In addition, proteins functioning in the ubiquitination machinery have also been associated with HSP (Bilguvar et al., 2013; Blackstone, 2018; Lo Giudice et al., 2014). Therefore, it is conceivable that deleterious variants in *UBAP1*, which disturb its regular functions in endosomal trafficking and interaction with ubiquitinated proteins, could potentially cause HSP.

In summary, we identified two truncating variants in *UBAP1* in similarly affected patients with nonsyndromic HSP from five families, including one recurrent frameshift change in four unrelated families. mRNA studies demonstrated an escape of NMD for the recurrent frameshift variant, predicting the generation of a truncated protein. Our current study supports the recent publication on truncating variants in *UBPA1* causing HSP (Farazi Fard et al., 2019), further delineating *UBAP1* as an HSP-causing gene. Overall, *UBAP1*-HSP is characterized as a “pure” HSP that is typical of childhood-onset and should be considered in the differential diagnosis when evaluating children who present with progressive spastic gait, and with no associated symptoms affecting the nervous system except the corticospinal tract.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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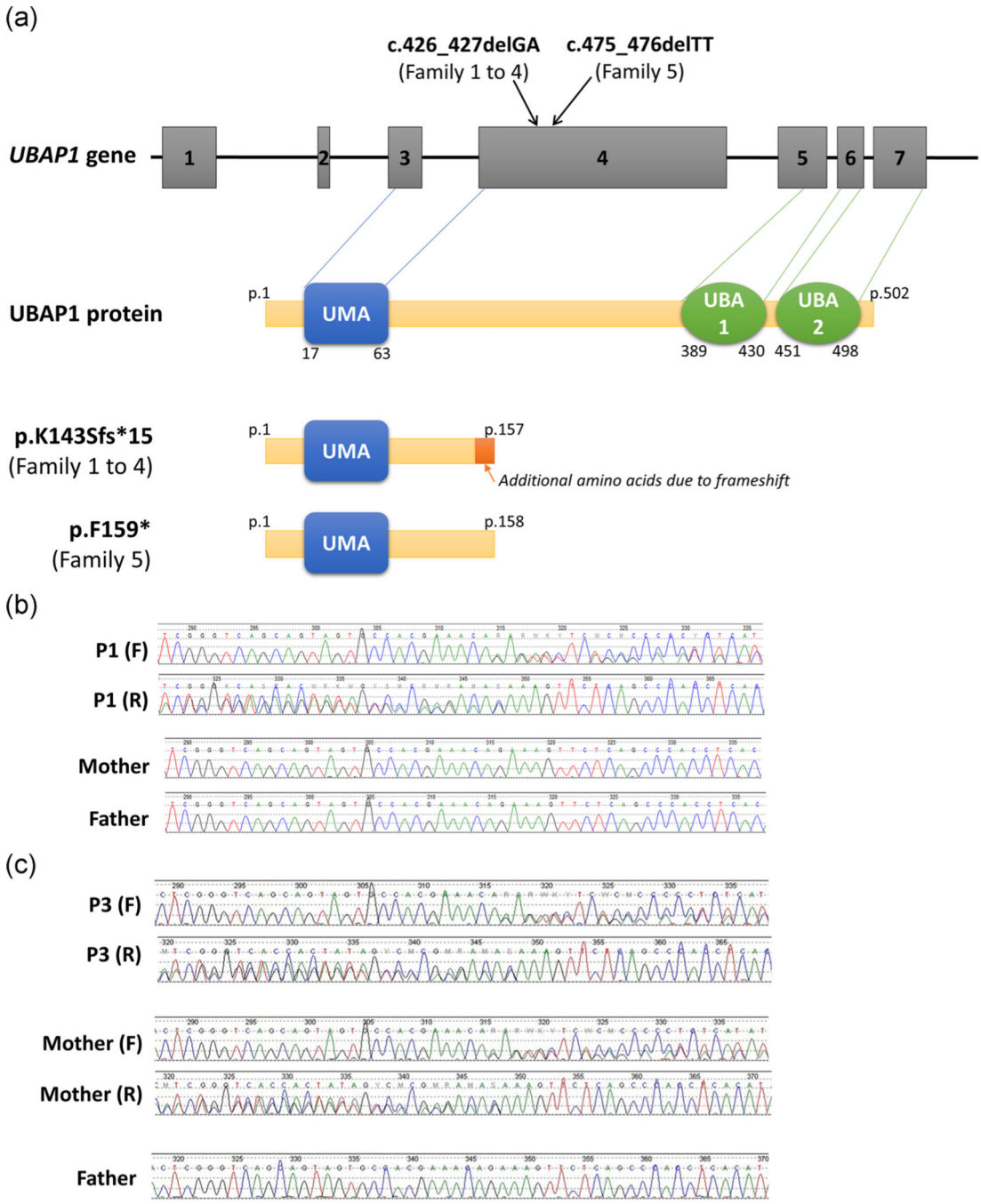

FIGURE 1.

(a) Gene and protein structure of *UBAP1* and the locations of the two truncating variants identified in this study based on transcript NM_016525.5. Gray boxes represent exons of the gene. Major functional domains UMA and UBA1/2 are depicted according to the UniProt database (www.uniprot.org, protein ID: Q9NZ09). (b) Sanger sequencing confirmed the apparently *de novo* variant c.426_427delGA in *UBAP1* in individual P1. (c) Sanger sequencing confirmed the variant c.426_427delGA in *UBAP1* and his affected mother, while

his father is negative for the change. F, forward primer; R, reverse primer; *UBAP1*, ubiquitin-associated protein 1

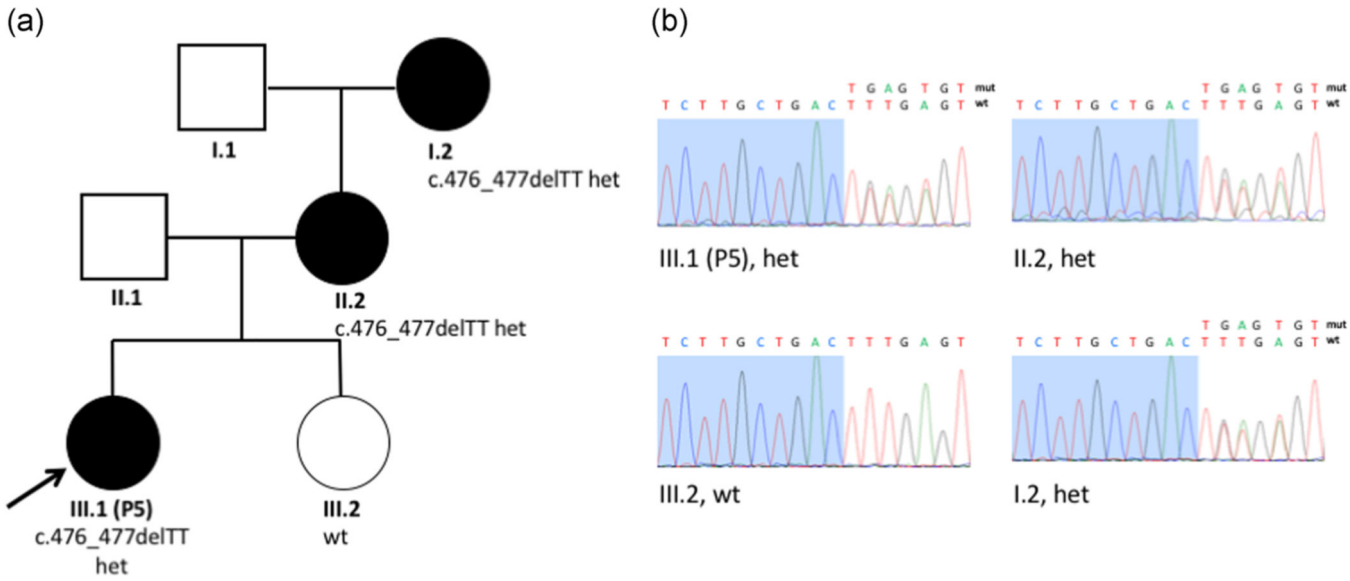

FIGURE 2.

(a) Pedigree of family 5 with three generations of affected individuals with HSP. Proband P5 is indicated by an arrow. Proband (P5 or III.1), mother (II.2), and maternal grandmother (I.2) are all affected and carry the variant c.476_477delTT, while unaffected sister (III.2) does not carry the variant. (b) Sanger sequencing results of the above mentioned four individuals. Het, heterozygous; HSP, hereditary spastic paraplegia; wt, wide-type

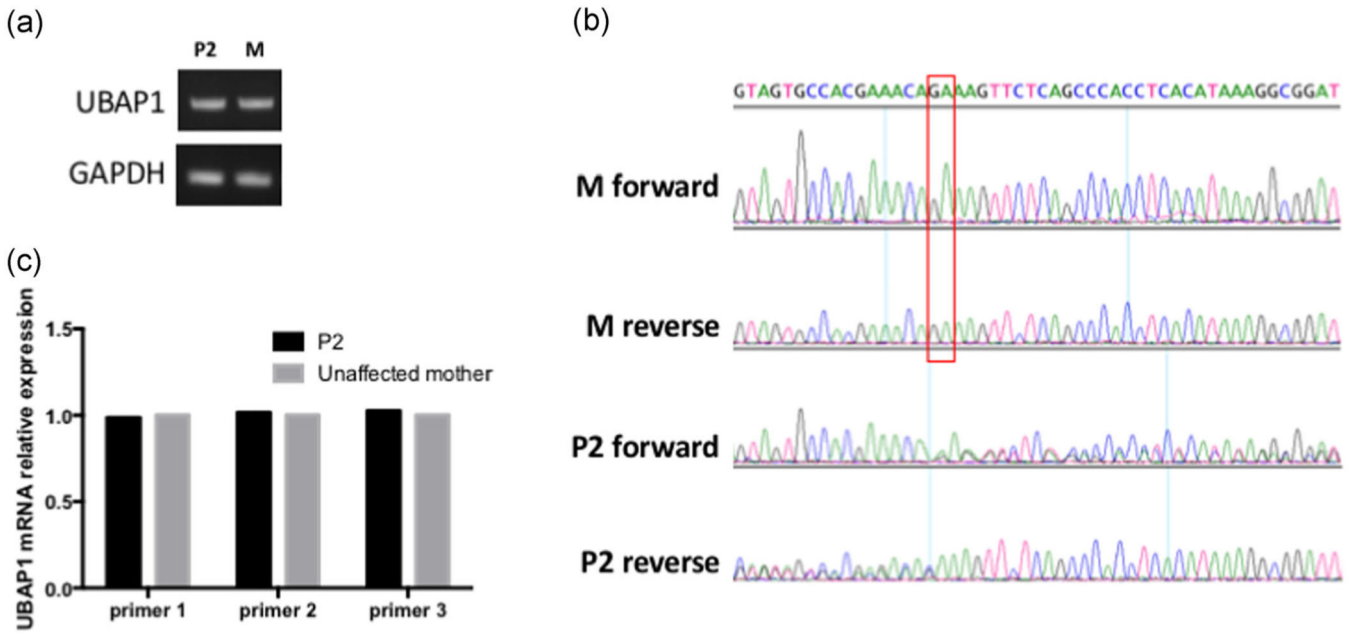

FIGURE 3.

(a) Semiquantitative RT-PCR products encompassing the variant, c.426_427delGA, from the lymphoblastoid cell lines of patient P2 and her unaffected mother. (b) Sanger sequencing analysis of the RT-PCR products shown in (b). The heterozygous 2-nucleotide deletion is marked within the red box. (c) *UBAP1* mRNA quantification by quantitative RT-PCR from the lymphoblastoid cell lines of the patient P2 and her unaffected mother. The relative expression of *UBAP1* mRNA in the unaffected mother is set as 1.0. Data are normalized to *GAPDH* mRNA levels. *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; M, mother; mRNA, messenger RNA; RT-PCR, reverse-transcription polymerase chain reaction; UBAP1, ubiquitin-associated protein 1

TABLE 1

Detailed clinical information of affected individuals with truncating variants in *UBAP1*

Patient ID	**PI**	**P2**	**P3**	**P4**	**P5**	**Farazi Fard et al. (2019) (30 individuals from 10 families)**
Gender	F	F	M	M	F	
Ethnicity	Filipino/Mexican	German, Irish, Greek/ Greek	Germany/Irish, American Indian	African American	Hispanic (Chilean)	
Age of onset (years)	7.5	7	8	7	8	Median: 8 years lst-3rd quartile: 4–9 years Range: 1–26 years
Major phenotype	Progressive spastic gait, hyperreflexia, ankle clonus, toe walking, severe bilateral pes planus	Spastic paraplegia, progressive spastic gait	Spastic paraplegia, progressive spastic gait, toe and heel walking, bilateral clonus	Spasticity, abnormal gait, clonus of legs, hypotonia of hands and fingers, poor balance	Progressive spastic gait, brisk deep tendon reflexes with ankle clonus. No Babinski sign	Progressive lower-1 ¡mb spastic paraparesis, accompanied by brisk lower-1 ¡mb tendon reflexes
Development and cognition	Normal	Normal	Delayed speech and articulation skills, feeding difficulties	Aggressive behavior, ADHD	Normal	8/10 families with no neurologic involvement
Cerebellar involvement	None	None	None	Unknown	None	2/10 families with features included saccadic pursuit, gaze-evoked nystagmus, dysmetric saccades, and limb ataxia
Other findings	Subcentimeter pineal cyst noted incidentally by brain MRI, feet/ankle/ knee pain	Benign pituitary adenoma noted incidentally by brain MRI, precocious puberty, lower back pain	N/A	N/A	Head circumference −1.5 SD at 11 yo	Urinary urgency, mild sensory deficits, cerebellar involvement
Family history	None	None	Spastic gait in mother and several maternal relatives	None	Mother and maternal grandmother affected. One healthy sister	Isolated or familial
UBAP1 variant[a]	c.426_427delGA (p.K143Sfs*15)	c.426_427delGA (p.K143Sfs*15)	c.426_427delGA (p.K143Sfs*15)	c.426_427delGA (p.K143Sfs*15)	c.475_476delTT (p.F159*)	All producing truncated proteins at N terminal, except one at C terminal
UBAP1 variant inheritance	*De novo*	*De novo*	Inherited from similarly affected mother	Unknown	Inherited from similarly affected mother	Autosomal dominant, either *de novo* or segregated with disease
Previous genetic testing	Negative SNP array and HSP panel (59 genes)	Negative SNP array and mitochondria genome testing	Negative HSP panel; SNP array identified 16pll.2 microduplication (MIM# 614671)	Unknown	Not performed	

Abbreviations: ADHD, attention deficit hyperactivity disorder; HSP, hereditary spastic paraplegia; MRI, magnetic resonance imaging; SNP, single-nucleotide polymorphism; UBAP1, ubiquitin-associated protein 1.

[a]UBAP1 reference: NM_016525.5.