Received: 16 October 2023 | Revised: 13 December 2023 | Accepted: 22 December 2023

DOI: 10.1002/ajmg.a.63534

CASE REPORT

AMERICAN JOURNAL OF medical genetics PART A WILEY

Expanding the phenotype of *UPF3B*-related disorder: Case reports and literature review

Ferruccio Romano[1] | Maria K. Haanpää[2] | Pawel Pomianowski[3] | Amanda Rose Peraino[3] | John R. Pollard[4] | Maria Francesca Di Feo[1,2,3,4,5] | Monica Traverso[5,6] | Mariasavina Severino[7] | Maria Derchi[8] | Edoardo Henzen[9] | Federico Zara[5,6] | Francesca Faravelli[1] | Valeria Capra[1] | Marcello Scala[5,6]

[1]Clinical Genomics and Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy

[2]Department of Genomics and Clinical Genetics, Turku University Hospital, Turku, Finland

[3]Center for Medical Genetics and Genomics, Christiana Care Health System, Newark, Delaware, USA

[4]Epilepsy Center, Christiana Care Health System, Newark, Delaware, USA

[5]Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy

[6]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy

[7]Neuroradiology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy

[8]Cardiology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy

[9]Genomics Facility, Italian Institute of Technology (IIT), Genoa, Italy

Correspondence
Marcello Scala, University of Genoa, DINOGMI, Genoa, Italy.
Email: mscala.md@gmail.com

Valeria Capra, IRCCS Istituto Giannina Gaslini, Genoa, Italy.
Email: valeriacapra@gaslini.org

Abstract

UPF3B encodes the Regulator of nonsense transcripts 3B protein, a core-member of the nonsense-mediated mRNA decay pathway, protecting the cells from the potentially deleterious actions of transcripts with premature termination codons. Hemizygous variants in the *UPF3B* gene cause a spectrum of neuropsychiatric issues including intellectual disability, autism spectrum disorder, attention deficit hyperactivity disorder, and schizophrenia/childhood-onset schizophrenia (COS). The number of patients reported to date is very limited, often lacking an extensive phenotypical and neuroradiological description of this ultra-rare syndrome. Here we report three subjects harboring *UPF3B* variants, presenting with variable clinical pictures, including cognitive impairment, central hypotonia, and syndromic features. Patients 1 and 2 harbored novel *UPF3B* variants—the p.(Lys207*) and p.(Asp429Serfs*27) ones, respectively—while the p.(Arg225Lysfs*229) variant, identified in Patient 3, was already reported in the literature. Novel features in our patients are represented by microcephaly, midface hypoplasia, and brain malformations. Then, we reviewed pertinent literature and compared previously reported subjects to our cases, providing possible insights into genotype–phenotype correlations in this emerging condition. Overall, the detailed phenotypic description of three patients carrying *UPF3B* variants is useful not only to expand the genotypic and phenotypic spectrum of *UPF3B*-related disorders, but also to ameliorate the clinical management of affected individuals.

KEYWORDS
brain malformations, intellectual disability, microcephaly, novel clinical features, phenotype expansion, UPF3B

1 | INTRODUCTION

UPF3B (OMIM 300298) is located on the long arm of chromosome X (region Xq24). It is a core member of the nonsense-mediated mRNA decay (NMD) pathway, whose function is to rapidly degrade transcripts with premature termination codons, thereby protecting the cell against translation of truncated proteins with potentially deleterious effects (Jolly et al., 2013). This complex is considered fundamental for

a proper regulation of translation efficiency (Kunz et al., 2006). It is known that targets of NMD, like *ARHGAP24*, are involved in the regulation of neuronal growth and in neurodevelopmental issues (Nguyen et al., 2012).

The NMD machinery comprises the conserved UP-Frameshift proteins UPF1, UPF2, and UPF3. UPF3 exists as two paralogs (A and B), differentially expressed depending on cell type and developmental stage, believed to regulate NMD activity based on cellular requirements (Bufton et al., 2022). UPF3B's N-terminus comprises a conserved "RNA recognition motif-like" domain (RRM-L), interacting with a domain of UPF2. The RRM-L domain is followed by a "poorly characterized" middle domain, with two coiled-coil-like regions (CCL-1 and CCL-2), and by a C-terminal exon-junction complex binding motif. The middle domain has been implicated in translation termination. Recent evidence supports the important role of the middle domain, given its interactions with release factors, NMD substrate mRNAs, ribosomal subunits, and the MIF4GIII domain of UPF2 (Bufton et al., 2022). Though *UPF3B* is a key factor for mRNA surveillance and eukaryotic gene expression regulation, its precise mode of action in NMD still remains elusive (Bufton et al., 2022). Recent findings also indicate that *UPF3B* is a crucial regulator of brain development: Jolly et al. (2013) demonstrated on murine models that Upf3b expression is developmentally regulated, that loss of Upf3b-NMD promotes the proliferation and reduces the differentiation of neural progenitor cells, and that loss of Upf3b-NMD in primary hippocampal neurons affects neurite growth. In this study, we report three patients with *UPF3B* variants with diverse clinical features, widening the phenotypic spectrum of this ultra-rare syndrome. We also reviewed pertinent literature.

2 | METHODS

The study was conducted in accordance with the Declaration of Helsinki and approved by the local Institutional Ethics Committees. The patients were enrolled at Istituto Giannina Gaslini, Genoa, (Italy) Christiana Care, Newark, Delaware (USA), and Turku University Hospital, Turku (Finland) and clinically evaluated by pediatric geneticists and neurologists.

Informed consent was obtained from the parents, DNA was analyzed by comparative genome hybridization (CGH)-array, using the Human Genome CGH Microarray 4× 180 K Kit, probe design 086332 (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's instructions. The Agilent platform is an oligonucleotide-based microarray with an average resolution of about 25 kb to detect copy number variations and loss of heterozygosity (LOH) of 4 Mb. Raw data were analyzed using the Genomic Workbench 7.0.40 software (Agilent). Altered chromosomal regions and breakpoints and LOH events were detected using ADM-1 (threshold 10) with 0.5 Mb window size to reduce false positives. For aberration detection, the diploid peak centralization algorithm and the legacy centralization algorithm were applied to set the most common ploidy to zero. This is needed to ensure that the zero point reflects the most common ploidy state. Chromosome positions were determined using GRCh37/hg19 (UCSC Genome Browser).

Informed consent was obtained from the parents, trio-WES [AUTHOR: Please define (SDS, WES) in the first occurrence if necessary.] was performed in the family on genomic DNA extracted from peripheral blood. Agilent Sure Select QXT Clinical Research Exome (Agilent Technologies, Santa Clara, CA, USA) was used and Sequencing data were processed with in-house software for the GATK Best Practices pipeline for WES variant analysis execution. After filtering for allele frequency (≤0.01% in public databases, including GnomAD v2.1.1; https://gnomad.broadinstitute.org/), candidate variants were screened according to family segregation, conservation (GERP score), and predicted impact on protein function through in silico tools (including SIFT, PolyPhen-2, Mutation Taster). The variants were eventually validated through Sanger sequencing.

3 | CLINICAL CASES

3.1 | Patient 1

A boy aged 3 years and 4 months is an only child of a nonconsanguineous couple of European origin. Family history is not remarkable. During pregnancy, a tetralogy of Fallot was identified by ultrasound (US). Fetal brain magnetic resonance imaging (MRI) showed hypoplasia/dysgenesis of the corpus callosum, olfactory bulb agenesis, and a developmental venous anomaly in the left parietal lobe (Figure 1d [a–d]). The child was born at 41 weeks and 4 days by vacuum delivery. Birth weight was 3770 g, and APGAR scored 8 and 9. Other parameters at birth are not available. The child showed normal adaptation to extrauterine life. Cardiac US confirmed the prenatal diagnosis of tetralogy of Fallot. Brain MRI at birth and 2-year follow-up confirmed corpus callosum dysgenesis, anterior commissure hypoplasia, olfactory bulbs agenesis, choroidal plexus cysts, bilateral incomplete hippocampal rotation, and a left parietal developmental venous anomaly (Figure 1d[e–h]). Computed tomography highlighted choanal stenosis while the inner ear strictures were normal. CGH-array on blood's lymphocytes from the child did not identify any significant alteration.

In the following months, the child showed signs of developmental delay: evaluation with Griffith's scale at 12 months resulted with a developmental age of 5 months and 21 days. Only at the age of 1 year and 2 months did he started to take objects and feet to the mouth, but was not able to sit autonomously. His parents also reported chewing and swallowing difficulties, for which logopedic rehabilitation was started. At 1 year and 4 months axial hypotonia and limb stiffness were evident. Electroencephalography (EEG) was consistent with low voltage activity, without clear epileptic anomalies. Visual evoked potentials were altered, associated with evidence of mild blurring of optic disc. Some facial dysmorphisms can be appreciated like broad forehead, sparse eyebrows, low nasal bridge, bulbous nose tip, mandibular hypoplasia with retrognathia (Figure 1a).

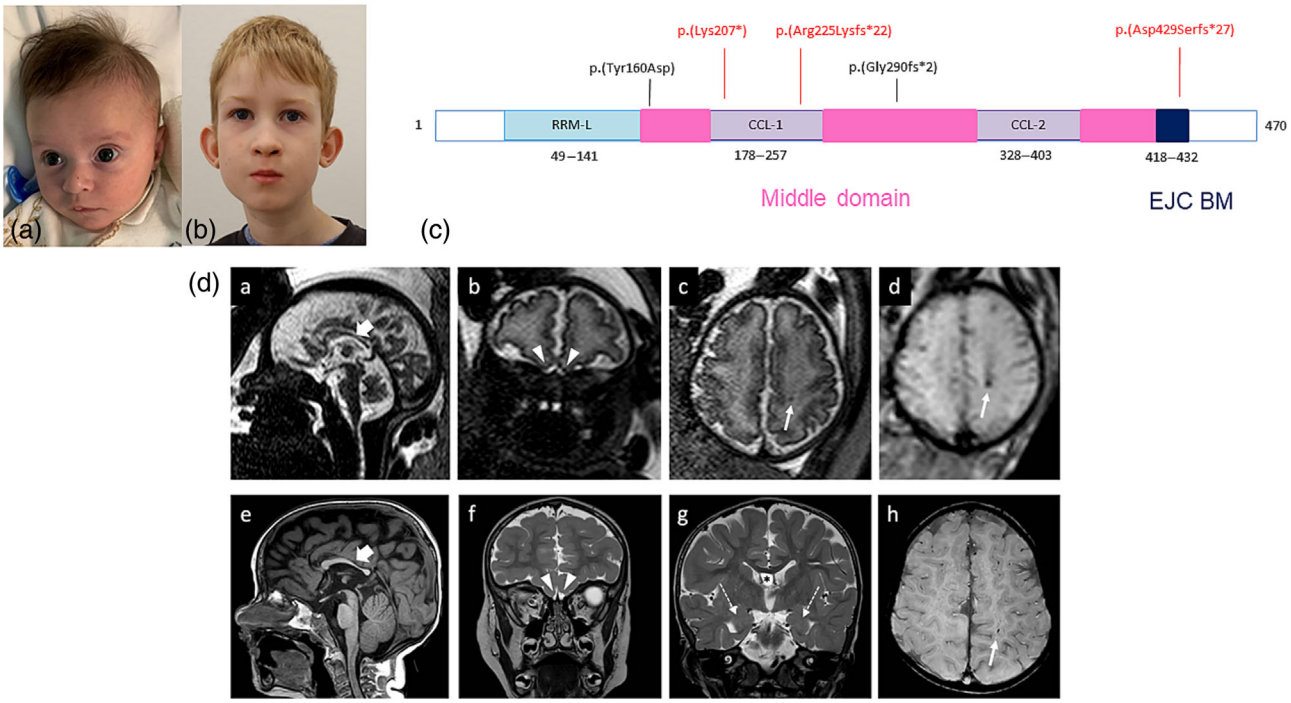

FIGURE 1 (a) Facial appearance of Patient 1: Broad forehead, sparse eyebrows, low nasal bridge, bulbous nose tip, mandibular hypoplasia, retrognathia. (b) Facial appearance of Patient 2: Triangular, hypotonic face, small mouth with tented upper lip, small chin, and protruding ears. (c) Schematic representation of protein UPF3B domain architecture; the first variants described in affected individuals are signed in black (Tarpey et al., 2007), while the three identified in our patients are highlighted in red; CCL, coiled-coil like; EJC BM, exon–junction complex binding motif; RRM-L, RNA recognition motif-like domain. Modified from Bufton et al., 2022; (d) Neuroimaging features of Patient 1. Fetal brain magnetic resonance imaging performed at 34 gestational weeks (a–d) and 2 years of age (e–h) showing callosal hypoplasia and dysgenesis (thick arrows), olfactory bulb agenesis (arrowheads), a left parietal developmental anomaly (thin arrows), and bilateral incomplete hippocampal rotation (dashed arrows). Note the persistence of cavum vergae et septi pellucidi (asterisk).

Exome sequencing (ES) was performed as previously described (Scala et al., 2022) and led to the identification of a de novo variant, c.619A > T, p.(Lys207*) in the *UPF3B* gene. This STOP variant, absent in the general population, is not reported in ClinVar. It involves a conserved amino acid and is predicted to have functional consequences on the protein. According to the American College of Medical Genetics and Genomics (ACMG) this variant is classified as likely pathogenic.

3.2 | Patient 2

Patient 2 is a 7-year-old boy of nonconsanguineous European parents. He is the youngest of the three children (older siblings are girls). Family history is unremarkable. He was born at term from an uneventful pregnancy with normal height and weight measurements. Head circumference (HC) at birth was −2.0 SDS. Other parameters at birth were not available. During the first months of life the child showed global muscular hypotonia and developmental and language delay. He started walking at the age of 20 months. Now the child produces simple sentences, with pronunciation defects. The child does not have an autism spectrum disorder (ASD) diagnosis. He however shows features of trichotillomania, and motor stereotypies, like shaking his hands on the side when excited. Global hypotonia and gross motor delay are persistent, in addition to dysarthria and feeding difficulties (but normal oral motor function). Brain MRI at the age of 2 years was normal. The child has presented with benign sleep myoclonias since early childhood, every night while falling asleep, together with bruxism. Treatment with valproate was stopped due to poor response. EEG at age 6 showed bilateral spikes, with no bursts. The boy shows some dysmorphisms like triangular, hypotonic face, midface hypoplasia, long philtrum, small open mouth with tented upper lip, small chin, and protruding ears (Figure 1b). He also has a slender build and poor muscle bulk. Cardiac evaluation (ECG, US) performed at 6 years of age was normal. The last clinical evaluation at the age of 7 years old highlighted an HC of −3 SDS, so a diagnosis of microcephaly was made. CGH-array and analysis for Fragile-X syndrome revealed no significant alterations.

Clinical exome analysis identified the hemizygous variant c.1285_1286del, p.(Asp429Serfs*27) in the *UPF3B* gene (Figure 1c). It is a small de novo deletion, occurring in exon 10, resulting in a premature truncation of the protein. It is classified as likely pathogenic. It is a novel variant, absent in the general population. Exome analysis also identified the c.2215G > A variant of uncertain significance in the *KMT2D* gene, inherited from his healthy father.

3.3 | Patient 3

Patient 3 is a now 26-year-old male, son of a nonconsanguineous couple from America. No relevant family history was reported. The child was born at 32 gestational weeks, after a pregnancy characterized by maternal hypertension. Weight at birth was 3400 g. Other parameters at birth were not available. The newborn was noticed with congenital hypotonia. A diagnosis of cerebral palsy was made. In the following months, global developmental delay was evident. At 12 months he was noticed to be floppy and could not sit autonomously. He also had significant feeding problems, including refusal to take liquids. Feeding therapy was recommended with increasing acceptance of texture food, cup drinking, and a more typical motor pattern for spoon feeding.

Currently, he is nonverbal and has a diagnosis of intellectual disability (ID). Motor stereotypies and autistic features are recognizable, including sounds and gestures mimicry, and refusal to eat solids. Seizures began approximately in the patient's early 20s characterized by shaking tremors, followed by confusion and sleepiness, with no drooling or incontinence. Frequency is around one to two events/week. One episode lasting 10–60 s was reported of focal impaired awareness seizure with stiffness, eyes rolled back, incontinence, without tongue bite, probably precipitated by concurrent sinus infection. Treatment with Levetiracetam and Lamotrigine was started with clinical response. Brain MRI at the age of 26 showed mild cerebellar atrophy.

The patient shows narrow face and slightly elongated head, occipital prominence, upturned nose, short philtrum, and shortened and grayish upper central incisors. Other relevant findings include bilateral hip subluxation, flexible planovalgus feet, dysplastic hips, bilateral coxa valga deformity, and mild subluxation of the left femoral head. The patient is waiting for complete cardiac and renal evaluations.

CGH-array did not reveal any significant alteration. Trio WES analysis detected the maternally inherited c.674_677del, p.(Arg225Lysfs*22) variant in *UPF3B* (Scala et al., 2022). It is a frameshift variant predicted to result in protein truncation or nonsense mediated decay. The variant is already reported in ClinVar (Tarpey et al., 2007).

4 | DISCUSSION

Loss of function mutations in *UPF3B* are associated with "intellectual developmental disorder, X-linked syndromic 14" (OMIM 300676). Reported phenotypes are variable even within the same family and include ID, ASD, attention deficit hyperactivity disorder (ADHD), schizophrenia/childhood-onset schizophrenia (COS; Addington et al., 2011; Jolly et al., 2013; Szyszka et al., 2012; Table 1). Dysmorphisms are sometimes described in affected individuals like long, thin face, broad forehead, high nasal bridge, maxillary hypoplasia, facial asymmetry, and prominent chin (Lynch et al., 2012; Tarpey et al., 2007; Tejada et al., 2019). Also, variable degrees of joint laxity, marfanoid habitus, slender appearance, poor musculature, scoliosis, and/or pectus excavatum/carinatum can affect patients (Domingo et al., 2020; Tarpey et al., 2007; Xu et al., 2013).

Variants in this gene were first described in 2007 in four families with syndromic (suspected Lujan–Fryns syndrome), and nonsyndromic X-linked ID and ASD (Tarpey et al., 2007). Since then more patients have been studied with next generation sequencing (NGS) techniques and more genetic defects in *UPF3B* have been reported (Table 1; Chérot et al., 2018; Hu et al., 2016; Soden et al., 2014; Tzschach et al., 2015; Zhang et al., 2015). Although 21 variants have been identified in *UPF3B* according to the Human Gene Mutation Database (HGMD), the spectrum of clinical features of affected individuals remains insufficiently defined. To date, a very limited number of patients and families have been reported with full clinical/instrumental descriptions (Laumonnier et al., 2010; Lynch et al., 2012; Tejada et al., 2019; Xu et al., 2013).

Cardiac and renal issues have occasionally been reported: renal dysplasia was detected in two brothers (Lynch et al., 2012; Tarpey et al., 2007). Patent ductus arteriosus in one patient (Lynch et al., 2012), and multiple atrial septum defects in another were recorded (Lovrecic et al., 2018). Non-neurological features may be underrated in *UPF3B* individuals. However available data are still limited to infer a clear relationship between *UPF3B* variants and these clinical issues: other genetic/epigenetic or environmental factors may be contributing.

The most recent review of literature (2021) reports 18 disease-related (pathogenic/likely pathogenic) variants (Deka et al., 2021). Since then, other pathogenic variants have been signed in ClinVar (Table 1). The majority of variants are represented by *missense* and *frameshift* variants. Patients with truncating variants show variable features; on the other hand, missense variants seem associated with milder phenotypes (patients described as "not overtly dysmorphic", Bufton et al., 2022). However, only a limited number of *UPF3B* patients have been reported, and no precise genotype–phenotype correlation has been inferred. Also copy number variants (CNVs), involving the locus of *UPF3B* on chromosome X, found by microarray techniques, have been described (Deka et al., 2021). Some hypotheses have been made about phenotypic heterogeneity: while varying UPF3A protein stability appears to be one of the candidate modifying factors, other genes and proteins might also be contributing (Jolly et al., 2013; Lynch et al., 2012; Nguyen et al., 2012). To what extent the clinical expressivity of *UPF3B* mutations across neuropsychiatric conditions is mediated by the variable level of the rescue UPF3A-mediated NMD is yet to be fully investigated (Lynch et al., 2012; Tarpey et al., 2007). Additionally, the precise molecular mechanisms underlying neurodevelopmental defects still remain elusive (Bufton et al., 2022).

In our cohort, two patients harbored novel hemizygous variants in *UPF3B* and Patient 3 had an already reported one. They variably presented with neurodevelopmental impairment, and syndromic features. Patients 1 and 3 also had brain malformations. Patient 1 had ID, choanal atresia, neuroradiological findings, and tetralogy of Fallot. WES analysis led to the identification of a new variant in the *UPF3B* gene (c.619A > T). In addition, he showed developmental delay, persistent

TABLE 1 *UPF3B* variants and associated clinical phenotypes.

UPF3B variants	Mutations/deletions (residues changed)	Neurological phenotype	Brain MRI	Syndromic features (extraneurological)	Cardiac/renal evaluation	Dysmorphisms/ physical appearance	References
Variant 1	c.478 T > G p. (Tyr160Asp)	ID, psychomotor delay, one member with high functioning autism	N.A.	No	N.A.	Long face	(Tarpey et al., 2007)
Variant 2	c.1136G > A p. (Arg379His)	ID, psychomotor delay, seizures	N.A.	Myopia, astigmatism, nystagmus, cataract	N.A.	No	(Laumonnier et al., 2010)
Variant 3	c.1103G > A p. (Arg368Gln)	ID, ASD, ADHD	Hydrocephaly	Joint laxity	N.A.	Marfanoid habitus	(Laumonnier et al., 2010)
Variant 4	c.764G > A p. (Arg255Leu)	Schizophrenia	N.A.	No	N.A.	Not reported	(Szyszka et al., 2012)
Variant 5	c.1101G > C p. (Lys367Asn)	ID, psychomotor delay	N.A.	No	N.A.	Hypertelorism, epicanthus	(Tzschach et al., 2015)
Variant 6	c.883 T > A p. (Leu295Met)	ID, psychomotor delay, infantile spasms	N.A.	No	N.A.	N.A.	(Zhang et al., 2015)
Variant 7	c.1310delC p. (Pro437fs*47)	ID, psychomotor delay	N.A.	N.A.	N.A.	N.A.	(Hu et al., 2016)
Variant 8	c.1288C > T p.(Arg430*)	ID, psychomotor delay	N.A.	No	N.A.	N.A.	(Xu et al., 2013)
Variant 9	1091_1094delAGAG p. (Glu364fs*2)	ID	N.A.	N.A.	N.A.	No	(Soden et al., 2014)
Variant 10	c.1081C > T p.(Arg361*)	ID	N.A.	strabismus	N.A.	Widow's peak, knee or elbow valgus	(Laumonnier et al., 2010)
Variant 11	c.846 + 1G > A	ID, absent speech, hypotonia	Dilated Virchow Robin spaces	Macrocephaly	N.A.	Tall stature	(Chérot et al., 2018)
Variant 12	c.867_868delAG p. (Gly290fs*2)	ID, psychotic episodes, hypotonia	N.A.	No	N.A.	Long, thin face, high nasal bridge, high arched palate. Slender build, poor musculature, pectus carinatum	(Tarpey et al., 2007)
Variant 13	c.697_698delAG p. (Arg233fs*30)	*Brother 1*: ID, ASD, ADHD, obsessive traits, dyspraxia, hand flapping; *Brother 2*: ID, hypotonia, psychomotor delay, obsessive traits	*Brother 1*: small pineal cyst *Brother 2*: prominent sub-arachnoid spaces	*Brother 2*: macrocephaly, constipation	*Brother 1*: absence of left kidney with compensatory hypertrophy of the right kidney *Brother 2*: left multicystic dysplastic kidney	*Brother 1*: long face, deep set eyes, high nasal bridge, short philtrum, prominent lips, diastema; *Brother 2*: similar to brother 1	(Lynch et al., 2012)

(Continues)

TABLE 1 (Continued)

UPF3B variants	Mutations/deletions (residues changed)	Neurological phenotype	Brain MRI	Syndromic features (extraneurological)	Cardiac/renal evaluation	Dysmorphisms/ physical appearance	References
Variant 14	c.684_685delAA p.(Glu230fs*35)	ID	N.A.	(Atopic dermatitis)	N.A.	No	(Yavarna et al., 2015)
Variant 15	c.674_677delGAAA p.(Arg225fs*20)	ID, ASD, hypotonia, one member with aggressive behavior	Dysgenesis of the corpus callosum	Mild joint laxity	N.A.	Macrocephaly, midface hypoplasia, a widow's peak, slightly upslanting palpebral fissures, bulbous distal nose, maxillary hypoplasia, Slender build, poor musculature, pectus excavatum	(Tarpey et al., 2007)
Variant 16	c.683_686delAAGA p.(Gln228fs*18)	*Brother 1*: COS, ADHD, ASD; ADHD; *Brother 2*: aphasic language delay, fine motor delay, pervasive developmental disorder	N.A.	*Brother 2*: congenital pulmonary stenosis	N.A.	No	(Addington et al., 2011)
Variant 17	c.118C > T p.(Gln40*)	ID, members with ASD, seizures, stereotypies	N.A.	Fingers/toes laxity	N.A.	High nasal bridge, short philtrum. Scoliosis, kyphosis, marfanoid habitus	(Tejada et al., 2019)
Variant 18	Xq24 loss	*Patient 1*: ID, psychomotor delay *Patient 2*: psychomotor delay	N.A.		*Patient 2*: atrial septal defect	*Patient 1*: Receding forehead, broad/ high nasal bridge, sparse eyebrows, epicanthal folds, straight eyelashes, pronounced philtrum	(Lovrecic et al., 2018)
New variant (Patient 1)	c.619A > T, p.(Lys207*)	ID, psychomotor and language delay, swallowing and chewing difficulties, hypotonia, limb stiffness	Corpus callosum hypoplasia/ dysgenesis, anterior commissure agenesis, olfactory bulbs agenesis, choroidal plexus cysts, DVA	Choanal stenosis, tetralogy of Fallot	Tetralogy of Fallot	Broad forehead, sparse eyebrows, low nasal bridge, bulbous nose tip, mandibular hypoplasia, retrognathia	This article

TABLE 1 (Continued)

UPF3B variants	Mutations/deletions (residues changed)	Neurological phenotype	Brain MRI	Syndromic features (extraneurological)	Cardiac/renal evaluation	Dysmorphisms/ physical appearance	References
New variant (Patient 2)	c.1285_1286del, p.(Asp429Serfs*27)	ID, psychomotor and language delay, hypotonia, dysarthria, feeding difficulties, stereotypies, sleep myoclonias	Normal	Microcephaly	Normal	Triangular face, midface hypoplasia, long philtrum, small mouth, tented upper lip, small chin, protruding ears. Slender build, poor musculature	This article
Variant (Patient 3)	c.674_677del p.(Arg225fs*22)	ID, developmental delay, absent speech, persistent hypotonia, feeding difficulties, autistic features, stereotypies, seizures	Mild cerebellar atrophy	Bilateral hip subluxation, flexible planovalgus feet, dysplastic hips, flat feet, bilateral coxa valga deformity, mild subluxation of the left femoral head	Normal	Narrow face, elongated head, occipital prominence, upturned nose, short philtrum, dental anomalies	This article
New variant in ClinVar 1	c.160delinsAC, p.(Val54fs)	ID	Not provided	Not provided	Not provided	Not provided	ClinVar (April 2022)
New variant in ClinVar 2	c.159_160delinsTAC p.(Val54fs)	Not provided	Not provided	Not provided	Not provided	Not provided	ClinVar (March 2023)
New variant in ClinVar 3	c.1157_1158del p.(Thr385-Phe386insTer)	Not provided	Not provided	Not provided	Not provided	Not provided	Clin Var (August 2022)

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; COS, childhood-onset schizophrenia; ID, intellectual disability; MRI, magnetic resonance imaging; N.A., not available.

Source: Modified from Deka et al. (2021).

hypotonia (in common with previous cases, Laumonnier et al., 2010; Xu et al., 2013), and persistent limb stiffness. Patient 2 showed psychomotor delay, ID, facial dysmorphisms, slender build, and poor musculature. Patient 3 is an adult individual with ID, absent speech, seizures, autistic features, cerebral palsy, and mild cerebellar atrophy (the last two findings being unreported in *UPF3B* patients). Interestingly all three patients showed persistent hypotonia and chewing/feeding problems. Overall, the phenotype of our patients may contribute to expand the spectrum of the UPF3B-related disorder. Choanal stenosis and tetralogy of Fallot have never been described in association with *UPF3B* variants. Heart defects are sporadically detected in *UPF3B*-related disorder. Only very few *UPF3B* patients have undergone complete cardiac evaluation (ECG, US). Therefore, mild heart defects may be missed. Tetralogy of Fallot is a multifactorial and genetically heterogeneous condition, like most congenital heart defects (Hill et al., 2022), so inferring clear correlations with *UPF3B* variant is not possible, based on current knowledge. It is worth noticing that in Patient 1, showing choanal stenosis and heart defects, differential diagnosis with CHARGE syndrome was necessary: colobomas, inner ear anomalies, pons hypoplasia, anomalies of craniocervical junction, and growth delay were excluded. Sequence analysis of *CHD7* gene excluded the presence of disease-related variants, whereas possible minor CNVs encompassing the gene couldn't be excluded for sure by NGS. CNVs however account only for <2% of CHARGE syndrome diagnoses (van Ravenswaaij-Arts et al., 1993–2023). Other novel features in our cases included microcephaly, midface hypoplasia, long philtrum, and small mouth presented by Patient 2. The identification of microcephaly is especially interesting, since most *UPF3B* patients mostly present macrocephaly instead (Deka et al., 2021). This suggests that the differential diagnosis of *UPF3B*-related disorder needs to include other neurodevelopmental conditions with microcephaly, such as *MFSD2A*- and *EEF1A2*-related disorders (Carvill et al., 2020; Scala et al., 2020). Based on current knowledge, it is difficult to establish clear genotype–phenotypes correlations and future studies will be helpful in this regard.

As for neuroradiological anomalies Patient 1 showed corpus callosum dysgenesis, anterior commissure agenesis, olfactory bulbs agenesis, and bilateral incomplete hippocampal rotation. Patient 3 had mild cerebellar atrophy, not previously reported in affected individuals. Knowledge about neuroradiological features in *UPF3B* patients is limited, as brain MRIs are available only in a very small number of the patients described in the literature. Dysgenesis of the corpus callosum (Tarpey et al., 2007), dilatation of Virchow Robin spaces (Chérot et al., 2018), and hydrocephaly (Laumonnier et al., 2010) were respectively reported in three different works (Table 1). Lynch et al. (2012) reported two brothers with mild, not specific brain anomalies: a small pineal cyst in one, and prominent subarachnoid spaces in the other. To date, a clear correlation between *UPF3B* variants and brain anomalies is difficult to infer.

It is interesting to notice that congenital heart and brain anomalies are well-known features of other syndromes caused by genes involved in the same pathway as *UPF3B*, the NMD pathway, such as *SMG8* (Alzahrani–Kuwahara syndrome, OMIM 619268) and *SMG9* (Heart and brain malformation syndrome, OMIM 616920). *SMG8* and *SMG9* encodes for components of the SMG1–SMG8–SMG9 complex, which is activated by the UPF2–UPF3B complex (Bufton et al., 2022; Zhu et al., 2019). Even though *SMG8* and *SMG9*-related disorders are recessive, we cannot exclude possible consequences of *UPF3B* variants on these downstream target genes of the common pathway, with possible similar phenotypic features.

The pathophysiology and clinical consequences of *UPF3B* variants are still poorly understood. Further cases, along with functional studies, are needed to better understand the pathophysiology of *UPF3B*-related disorders, possible involvement of the gene in clinical phenotypes, along with clearer genotype–phenotype correlations.

5 | CONCLUSIONS

We reported three patients harboring pathogenic variants in *UPF3B* and presenting with complex neurodevelopmental phenotypes and some previously unreported features, suggesting an expansion of the phenotypic spectrum of this rare condition. Two variants were novel, while one was already described. Atypical features (especially brain and cardiac anomalies) should not be considered as exclusion criteria. We suggest that cardiac and brain evaluation should be performed in all carriers of *UPF3B* variants. Despite the growing evidence of a pathogenic role of *UPF3B* variants the phenotypic spectrum remains to be fully elucidated. An expanded cohort of patients and more in-depth studies on UPF3B protein functions will be crucial to better understand the pathomechanism behind the syndrome and to infer more precise genotype–phenotype correlations.

AUTHOR CONTRIBUTIONS

F.R. drafted the article, revised literature, and contributed to data analysis. M.H., P.P., A.R.P., J.R.P., and M.D. collected clinical and genetic data. M.F.F. contributed to literature revision and collected genetic data. M.T. and E.H. performed genetic analysis and collected genetic data. Ms.S. reviewed brain MRI scans and analyzed the neuroimaging spectrum. F.Z, F.F., and V.C. supervised the work and critically revised the article. M.S. conceived the study, supervised the work, and revised the article.

ACKNOWLEDGMENTS

One of the authors of this publication is a member of the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability ERN-ITHACA. [EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516]. The authors aknowledge Yeraldin Chiquinquira Castillo De Spelorzi and all the staff of Genomics Facility, Istituto Italiano di Tecnologia (IIT), Genova, Italy for their precious contribution to genetic analyses. Open access funding provided by BIBLIOSAN.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (M.S.) upon request.

ORCID

Ferruccio Romano https://orcid.org/0009-0005-3933-0597

REFERENCES

Addington, A. M., Gauthier, J., Piton, A., Hamdan, F. F., Raymond, A., Gogtay, N., Miller, R., Tossell, J., Bakalar, J., Inoff-Germain, G., Gochman, P., Long, R., Rapoport, J. L., & Rouleau, G. A. (2011). A novel frameshift mutation in UPF3B identified in brothers affected with childhood onset schizophrenia and autism spectrum disorders. *Mol Psychiatry*, *16*(3), 238–239. https://doi.org/10.1038/mp.2010.59. Epub 2010 May 18. Erratum in: Mol Psychiatry. 2012 Apr;17(4):468. Germain, G [corrected to Inoff-Germain, G].

Bufton, J. C., Powers, K. T., Szeto, J. A., Toelzer, C., Berger, I., & Schaffitzel, C. (2022). Structures of nonsense-mediated mRNA decay factors UPF3B and UPF3A in complex with UPF2 reveal molecular basis for competitive binding and for neurodevelopmental disorder-causing mutation. *Nucleic Acids Research*, *50*(10), 5934–5947. https://doi.org/10.1093/nar/gkac421

Carvill, G. L., Helbig, K. L., Myers, C. T., Scala, M., Huether, R., Lewis, S., Kruer, T. N., Guida, B. S., Bakhtiari, S., Sebe, J., Tang, S., Stickney, H., Oktay, S. U., Bhandiwad, A. A., Ramsey, K., Narayanan, V., Feyma, T., Rohena, L. O., Accogli, A., … Kruer, M. C. (2020). Damaging de novo missense variants in EEF1A2 lead to a developmental and degenerative epileptic-dyskinetic encephalopathy. *Human Mutation*, *41*(7), 1263–1279. https://doi.org/10.1002/humu.24015. Epub 2020 Apr 6.

Chérot, E., Keren, B., Dubourg, C., Carré, W., Fradin, M., Lavillaureix, A., & Mignot, C. (2018). Using medical exome sequencing to identify the causes of neurodevelopmental disorders: Experience of 2 clinical units and 216 patients. *Clinical Genetics*, *93*(3), 567–576.

Deka, B., Chandra, P., & Singh, K. K. (2021). Functional roles of human up-frameshift suppressor 3 (UPF3) proteins: From nonsense-mediated mRNA decay to neurodevelopmental disorders. *Biochimie*, *180*, 10–22. https://doi.org/10.1016/j.biochi.2020.10.011. Epub 2020 Oct 24.

Domingo, D, Nawaz, U, Corbett, M, Espinoza, JL, Tatton-Brown, K, Coman, D, Wilkinson, MF, Gecz, J, Jolly, LA. (2020). A synonymous UPF3B variant causing a speech disorder implicates NMD as a regulator of neurodevelopmental disorder gene networks. *Hum Mol Genet*, *29*(15), 2568–2578. doi:10.1093/hmg/ddaa151

Hill, M. C., Kadow, Z. A., Long, H., Morikawa, Y., Martin, T. J., Birks, E. J., Campbell, K. S., Nerbonne, J., Lavine, K., Wadhwa, L., Wang, J., Turaga, D., Adachi, I., & Martin, J. F. (2022). Integrated multi-omic characterization of congenital heart disease. *Nature*, *608*(7921), 181–191. https://doi.org/10.1038/s41586-022-04989-3. Epub 2022 Jun 22.

Hu, H., Haas, S. A., Chelly, J., Van Esch, H., Raynaud, M., de Brouwer, A. P., & Kalscheuer, V. M. (2016). X-exome sequencing of 405 unresolved families identifies seven novel intellectual disability genes. *Molecular Psychiatry*, *21*(1), 133–148.

Jolly, L. A., Homan, C. C., Jacob, R., Barry, S., & Gecz, J. (2013). The UPF3B gene, implicated in intellectual disability, autism, ADHD and childhood onset schizophrenia regulates neural progenitor cell behaviour and neuronal outgrowth. *Human Molecular Genetics*, *22*(23), 4673–4687. https://doi.org/10.1093/hmg/ddt315. Epub 2013 Jul 2.

Kunz, J. B., Neu-Yilik, G., Hentze, M. W., Kulozik, A. E., & Gehring, N. H. (2006). Functions of hUpf3a and hUpf3b in nonsense-mediated mRNA decay and translation. *RNA*, *12*(6), 1015–1022. https://doi.org/10.1261/rna.12506. Epub 2006 Apr 6.

Laumonnier, F., Shoubridge, C., Antar, C., Nguyen, L. S., Van Esch, H., Kleefstra, T., Briault, S., Fryns, J. P., Hamel, B., Chelly, J., Ropers, H. H., Ronce, N., Blesson, S., Moraine, C., Gécz, J., & Raynaud, M. (2010). Mutations of the UPF3B gene, which encodes a protein widely expressed in neurons, are associated with nonspecific mental retardation with or without autism. *Molecular Psychiatry*, *15*, 767–776. https://doi.org/10.1038/mp.2009.14

Lovrecic, L., Rajar, P., Volk, M., Bertok, S., Gnidovec Strazisar, B., Osredkar, D., Jekovec Vrhovšek, M., & Peterlin, B. (2018). Diagnostic efficacy and new variants in isolated and complex autism spectrum disorder using molecular karyotyping. *Journal of Applied Genetics*, *59*, 179–185. https://doi.org/10.1007/s13353-018-0440-y

Lynch, S. A., Nguyen, L. S., Ng, L. Y., Waldron, M., McDonald, D., & Gecz, J. (2012). Broadening the phenotype associated with mutations in UPF3B: Two further cases with renal dysplasia and variable developmental delay. *European Journal of Medical Genetics*, *55*(8-9), 476–479. https://doi.org/10.1016/j.ejmg.2012.03.010. Epub 2012 Apr 25.

Nguyen, L. S., Jolly, L., Shoubridge, C., Chan, W. K., Huang, L., Laumonnier, F., Raynaud, M., Hackett, A., Field, M., Rodriguez, J., Srivastava, A. K., Lee, Y., Long, R., Addington, A. M., Rapoport, J. L., Suren, S., Hahn, C. N., Gamble, J., Wilkinson, M. F., … Gecz, J. (2012). Transcriptome profiling of UPF3B/NMD-deficient lymphoblastoid cells from patients with various forms of intellectual disability. *Mol Psychiatry*, *17*(11), 1103–1115. https://doi.org/10.1038/mp.2011.163. Epub 2011 Dec 20.

Scala, M., Chua, G. L., Chin, C. F., Alsaif, H. S., Borovikov, A., Riazuddin, S., Riazuddin, S., Chiara Manzini, M., Severino, M., Kuk, A., Fan, H., Jamshidi, Y., Toosi, M. B., Doosti, M., Karimiani, E. G., Salpietro, V., Dadali, E., Baydakova, G., Konovalov, F., … Silver, D. L. (2020). Biallelic MFSD2A variants associated with congenital microcephaly, developmental delay, and recognizable neuroimaging features. *European Journal of Human Genetics*, *28*(11), 1509–1519. https://doi.org/10.1038/s41431-020-0669-x. Epub 2020 Jun 22.

Scala, M., Nishikawa, M., Ito, H., Tabata, H., Khan, T., Accogli, A., Davids, L., Ruiz, A., Chiurazzi, P., Cericola, G., Schulte, B., Monaghan, K. G., Begtrup, A., Torella, A., Pinelli, M., Denommé-Pichon, A. S., Vitobello, A., Racine, C., Mancardi, M. M., … Nagata, K. I. (2022). Variant-specific changes in RAC3 function disrupt corticogenesis in neurodevelopmental phenotypes. *Brain*, *145*(9), 3308–3327. https://doi.org/10.1093/brain/awac106

Soden, S. E., Saunders, C. J., Willig, L. K., Farrow, E. G., Smith, L. D., Petrikin, J. E., LePichon, J. B., Miller, N. A., Thiffault, I., Dinwiddie, D. L., Twist, G., Noll, A., Heese, B. A., Zellmer, L., Atherton, A. M., Abdelmoity, A. T., Safina, N., Nyp, S. S., Zuccarelli, B., … Kingsmore, S. F. (2014). Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Science Translational Medicine*, *6*(265), 265ra168.

Szyszka, P., Sharp, S. I., Dedman, A., Gurling, H. M., & McQuillin, A. (2012). A nonconservative amino acid change in the UPF3B gene in a patient with schizophrenia. *Psychiatric Genetics*, *22*(3), 150–151.

Tarpey, P. S., Raymond, F. L., Nguyen, L. S., Rodriguez, J., Hackett, A., Vandeleur, L., Smith, R., Shoubridge, C., Edkins, S., Stevens, C., O'Meara, S., Tofts, C., Barthorpe, S., Buck, G., Cole, J., Halliday, K., Hills, K., Jones, D., Mironenko, T., … Gécz, J. (2007). Mutations in UPF3B, a member of the nonsense-mediated mRNA decay complex, cause syndromic and nonsyndromic mental retardation. *Nature Genetics*, *39*(9), 1127–1133. https://doi.org/10.1038/ng2100. Epub 2007 Aug 19.

Tejada, M. I., Villate, O., Ibarluzea, N., de la Hoz, A. B., Martínez-Bouzas, C., Beristain, E., Martínez, F., Friez, M. J., Sobrino, B., & Barros, F. (2019). Molecular and clinical characterization of a novel nonsense variant in exon 1 of the *UPF3B* gene found in a large Spanish Basque family (MRX82). *Frontiers in Genetics*, *31*(10), 1074. https://doi.org/10.3389/fgene.2019.01074

Tzschach, A., Grasshoff, U., Beck-Woedl, S., Dufke, C., Bauer, C., Kehrer, M., Evers, C., Moog, U., Oehl-Jaschkowitz, B., Di Donato, N., Maiwald, R., Jung, C., Kuechler, A., Schulz, S., Meinecke, P., Spranger, S., Kohlhase, J., Seidel, J., Reif, S., … Bauer, P. (2015). Next-generation sequencing in X-linked intellectual disability. *European Journal of Human Genetics*, *23*(11), 1513–1518.

van Ravenswaaij-Arts, C. M., Hefner, M., Blake, K., & Martin, D. M. (1993–2023). CHD7 disorder. 2006 Oct 2 [updated 2022 Sep 29]. In M. P. Adam, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. W.

Gripp, & A. Amemiya (Eds.), *GeneReviews® [Internet]*. University of Washington, Seattle.

Xu, X., Zhang, L., Tong, P., Xun, G., Su, W., Xiong, Z., Zhu, T., Zheng, Y., Luo, S., Pan, Y., Xia, K., & Hu, Z. (2013). Exome sequencing identifies UPF3B as the causative gene for a Chinese non-syndrome mental retardation pedigree. *Clinical Genetics*, *83*, 560–564. https://doi.org/10.1111/cge.12014

Yavarna, T., Al-Dewik, N., Al-Mureikhi, M., Ali, R., Al-Mesaifri, F., Mahmoud, L., Shahbeck, N., Lakhani, S., AlMulla, M., Nawaz, Z., Vitazka, P., Alkuraya, F. S., & Ben-Omran, T. (2015). High diagnostic yield of clinical exome sequencing in middle eastern patients with mendelian disorders. *Human Genetics*, *134*(9), 967–980. https://doi.org/10.1007/s00439-015-1575-0. Epub 2015 Jun 16.

Zhang, Y., Kong, W., Gao, Y., Liu, X., Gao, K., Xie, H., & Jiang, Y. (2015). Gene mutation analysis in 253 Chinese children with unexplained epilepsy and intellectual/developmental disabilities. *PLoS One*, *10*(11), e0141782.

Zhu, L., Li, L., Qi, Y., Yu, Z., & Xu, Y. (2019). Cryo-EM structure of SMG1-SMG8-SMG9 complex. *Cell Research*, *29*(12), 1027–1034. https://doi.org/10.1038/s41422-019-0255-3. Epub 2019 Nov 15.

How to cite this article: Romano, F., Haanpää, M. K., Pomianowski, P., Peraino, A. R., Pollard, J. R., Di Feo, M. F., Traverso, M., Severino, M., Derchi, M., Henzen, E., Zara, F., Faravelli, F., Capra, V., & Scala, M. (2024). Expanding the phenotype of *UPF3B*-related disorder: Case reports and literature review. *American Journal of Medical Genetics Part A*, e63534. https://doi.org/10.1002/ajmg.a.63534