Expanding the phenotype of ATP6AP1 deficiency

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Abstract Vacuolar ATPases (V-ATPases) are large multisubunit proton pumps conserved among all eukaryotic cells that are involved in diverse functions including acidification of membrane-bound intracellular compartments. The ATP6AP1 gene encodes an accessory subunit of the vacuolar (V)-ATPase protein pump. Pathogenic variants in ATP6AP1 have been described in association with a congenital disorder of glycosylation (CDG), which are highly variable, but often characterized by immunodeficiency, hepatopathy, and neurologic manifestations. Although the most striking and common clinical feature is hepatopathy, the phenotypic and genotypic spectrum of ATP6AP1-CDG continues to expand. Here, we report identical twins who presented with acute liver failure and jaundice. Prenatal features included cystic hygroma, atrial septal defect, and ventriculomegaly. Postnatal features included pectus carinatum, connective tissue abnormalities, and hypospadias. Whole-exome sequencing (WES) revealed a novel de novo in-frame deletion in the ATP6AP1 gene (c.230_232delACT;p.Tyr77del). Although both twins have the commonly reported clinical feature of hepatopathy seen in other individuals with ATP6AP1-CDG-related disorder, they do not have neurological sequelae. This report expands the phenotypic spectrum of ATP6AP1-CDG-related disorder with both probands exhibiting unique prenatal and postnatal features, including fetal ventriculomegaly, umbilical hemia, pectus carinatum, micropenis, and hypospadias. Furthermore, this case affirms that neurological features described in the initial case series on ATP6AP1-CDG do not appear to be central, whereas the prenatal and connective tissue manifestations may be more common than previously thought. This emphasizes the importance of long-term clinical follow-up and variant interpretation using current updated recommendations.

INTRODUCTION

Congenital disorders of glycosylation (CDGs) are a rapidly growing group of genetic disorders that affect glycoprotein biogenesis. More than 130 types of CDG have been reported, and more than 140 genes are associated with different types of CDG that include symptoms ranging from mild to severe (Chang et al. 2018; Wilson and Matthijs 2021). A clinical diagnosis of CDG is often challenging because of the highly variable phenotype, broad effect on the function of multiple organs, and overall limited awareness of CDGs (Ng and Freeze 2018).

Vacuolar ATPase (V-ATPase) is a multisubunit rotary nanomotor in the Golgi-endosomal secretory pathway that is ubiquitously expressed (Nishi and Forgac 2002). V-ATPase is
essential for luminal acidification of secretory vesicles and to maintain homeostasis in the body (Casey et al. 2010; Pamarthy et al. 2018). Defects in V-ATPase complex and impairment in acidification of Golgi apparatus is predicted to modulate the Golgi-located glycosylation machinery that leads to defects in glycosylation (Guillard et al. 2009). Variants in causative genes (ATP6V0A2, ATP6V1A, ATP6V1E1, and ATP6AP2) encoding distinct subunits of V-ATPase are often associated with CGD (Kornak et al. 2008; Rujano et al. 2017; Van Damme et al. 2017; Ondruskova et al. 2020). In addition, a hypomorphic variant in the chaperone protein VMA21 that is required for V-ATPase assembly was recently reported in association with VMA21-CDG (Cannata Serio et al. 2020). ATP6AP1 is an accessory subunit of the V-ATPase, and pathogenic variants in ATP6AP1 have been reported to be associated with X-linked CDG, which was initially characterized by immunodeficiency with hepatopathy and neurological features (Jansen et al. 2016). Additional features that have been reported in case reports published since then include hepatomegaly, splenomegaly, pancreatic insufficiency, hearing loss, aortic dilation, and cutis laxa (Jansen et al. 2016; Dimitrov et al. 2018; Witters et al. 2018; Ondruskova et al. 2020; Song et al. 2020; Tvina et al. 2020). Here, we describe identical twin males with a novel hemizygous variant in the ATP6AP1 gene, exhibiting overlapping and additional clinical features in both prenatal and postnatal stages. We applied the point-based system for classification of this de novo variant based on current recommendations from The American College of Medical Genetics and Genomics (ACMG)/The Association for Molecular Pathology (AMP) and The Association for Clinical Genomic Science (ACGS) working group (SVI 2018; ACGS 2019).

RESULTS

Clinical Presentation and Family History

The probands are the product of monochorionic-diamniotic twin pregnancy from a father of Guyanese descent and a mother of Italian descent. Consanguinity was denied. There were no similarly affected family members.

First-trimester ultrasounds showed each twin had a cystic hygroma, dilated aorta, and an atrial septum defect. Chorionic villus sampling (CVS) was performed at an outside hospital, and a karyotype, chromosomal microarray, and Noonan syndrome panel were reported to be negative, although copies were not provided for our review. Second-trimester ultrasounds also showed cardiomegaly and borderline ventriculomegaly.

The probands were born at an outside institution at 36 weeks of gestation via cesarean section because of fetal malpositioning. Postnatally, both were noted to have pectus carinatum, umbilical and bilateral inguinal hernias, micro penis, and hypospadias, with twin B’s symptoms being more severe. Head ultrasounds and spinal magnetic resonance imagings (MRIs) were normal. Echocardiograms confirmed a septum primum aneurysm and mildly dilated ascending aorta. The twins developed jaundice but did not require phototherapy. After a 5-day neonatal intensive care unit (NICU) stay, they were discharged with close outpatient follow-up.

At 2 months of age they both had noticeable jaundice. Workup showed elevated bilirubin levels and abnormal liver function with ultrasounds concerning for gallstones, left liver lobe prominence, and ascites. Both probands were admitted to our institution for further evaluation. Plasma amino acids were within normal limits, whereas urine organic acids showed elevated levels of 4-hydroxy phenyllactic acid and 4-hydroxy phenylpyruvic acid. Twin B experienced encephalopathy with hyperammonemia. Given the above, a genetic consult was initiated. Because of the dysmorphic features, skin laxity around the epigastric region, flank, and groin (see Fig. 1A–F), and abnormal liver function, whole exome sequencing was initiated.
At 7 months, twin A underwent a liver transplant as well as inguinal and umbilical hernia repair. At 11 months, twin B underwent a liver transplant (complications included a right diaphragmatic hernia that was repaired at 14 months) and repair of the umbilical and inguinal hernias. Their abdomens remain prominent. At 21 months, twin A underwent a left-sided orchiopexy. At 2 years of age, both twins had atrial septum defect repair. They had multiple upper respiratory and ear infections even after bilateral myringotomy tubes were placed.

Both twins required early intervention services (physical therapy, occupational therapy, speech therapy, special instruction) because of mild developmental delays. By 3 years, all therapies were discontinued except for twin A’s speech therapy. Over time, their facial features grew coarser. The probands continue to have significant pectus carinatum, cutis laxa, and hypermobility, with twin B’s symptoms being more significant. However, no other remarkable clinical features were observed. Neither twin has developed signs of hearing loss.

**Genomic Analyses**

Postnatal findings of acute liver failure and jaundice led to suspicion of a mitochondrial hepatopathy, because hepatic involvement in childhood (primarily in the neonatal period) is a common feature in mitochondrial hepatopathies (Lee and Sokol 2007a,b). Mitochondrial DNA sequencing by next-generation sequencing (NGS) and mitochondrial DNA deletion analyses.
and rearrangement by Southern blot did not show any pathogenic variants, deletions, or rearrangements.

Whole-exome sequencing (WES) was performed for both twins and included parental control samples. Overall WES achieved average coverage of 99.1% for probands and 97.9% for the father and mother for the coding region. Trio-based exome analysis identified a de novo hemizygous in-frame deletion (Chr X: 153657460delCTA, GRCh37/hg19) in the ATP6AP1 gene present in both twins (Table 1). The variant (NM_001183.6: c.230_232delACT) is a 3-base pair deletion in exon 2 (out of 10 exons total) of the ATP6AP1 gene, which causes an in-frame deletion of tyrosine at position 77 out of 471 amino acids total (NP_001174.2: p.Tyr77del). This variant was absent in the Genome Aggregation Database (gnomADV2.1.1). In silico analysis predicted the change to be deleterious to protein structure and/or function (PROVEAN score = −10.6). Sanger sequencing confirmed the presence of this de novo hemizygous variant in both affected twins. The variant was absent in both parents, although we cannot rule out the possibility of low-level gonadal mosaicism.

| Genomic coordinates (hg19) | Reference allele | Alternative allele | Total reads | Variant allele fraction (VAF) | HGVS cDNA | HGVS protein (inheritance) | Zygosity | Variant type | gnomAD allele frequency | Variant classification |
|---------------------------|------------------|--------------------|-------------|------------------------------|-----------|---------------------------|----------|--------------|------------------------|------------------------|
| Chr X: 153657459 CCTACT  | CCT              | 120 100%           | c.230_232delACT | p.Tyr77del (de novo)         | Hemizygous In-frame deletion | Absent     | Likely pathogenic |
| Chr X: 153657459 CCTACT  | CCT              | 112 95%            | c.230_232delACT | p.Tyr77del (de novo)         | Hemizygous In-frame deletion | Absent     | Likely pathogenic |

The Refseq transcript used for annotation is NM_001183.6.

Whole-exome sequencing (WES) was performed for both twins and included parental control samples. Overall WES achieved average coverage of 99.1% for probands and 97.9% for the father and mother for the coding region. Trio-based exome analysis identified a de novo hemizygous in-frame deletion (Chr X: 153657460delCTA, GRCh37/hg19) in the ATP6AP1 gene present in both twins (Table 1). The variant (NM_001183.6: c.230_232delACT) is a 3-base pair deletion in exon 2 (out of 10 exons total) of the ATP6AP1 gene, which causes an in-frame deletion of tyrosine at position 77 out of 471 amino acids total (NP_001174.2: p.Tyr77del). This variant was absent in the Genome Aggregation Database (gnomADV2.1.1). In silico analysis predicted the change to be deleterious to protein structure and/or function (PROVEAN score = −10.6). Sanger sequencing confirmed the presence of this de novo hemizygous variant in both affected twins. The variant was absent in both parents, although we cannot rule out the possibility of low-level gonadal mosaicism.

**Literature and Database Review**

To gain further insight into the spectrum of ATP6AP1 variants associated with ATP6AP1-CDG-related disorders, we reviewed the literature and publicly available databases. In the ClinVar database, a total of 20 single-nucleotide variants (SNVs) in the ATP6AP1 gene have been documented as pathogenic/likely pathogenic/variant of uncertain significance. Among them, 10 of the variants were reported to be associated with immunodeficiency 47 (OMIM #300972) ATP6AP1-CDG-related disorders. Of 20 reported single-nucleotide variants (SNVs), 19 are missense variants (95%) and one is a stop-gain variant (5%). The variants are distributed across all 10 exons and the predicted domains of the ATP6AP1 gene, and there is no mutational hotspot observed in the gene (Fig. 2A,B). The overall intolerance of the ATP6AP1 gene using all the seven missense variants reported in literature is plotted using missense tolerance ratio (MTR) viewer (Fig. 2C). We found that nine of the 10 (90%) disease-associated missense variants reside among the 50% most missense-intolerant sequence of the overall intolerant ATP6AP1 gene. The MTR plot is based on standing variation data in the gnomAD database, version 2.0 (Silk et al. 2019).

Among 20 individuals (Jansen et al. 2016; Dimitrov et al. 2018; Witters et al. 2018; Gumm et al. 2020; Ondruskova et al. 2020; Tvina et al. 2020; Yang et al. 2021) summarized in Table 2 (18 reported in the literature and two from the current study), 17 showed hepatomegaly/liver failure/cirrhosis, 16 showed recurrent infection, 13 showed splenomegaly, 8 showed neurological symptoms, 6 showed cutis laxa, 5 were reported to have cardiac abnormalities or bilateral inguinal hernias, and 3 had a congenital diaphragmatic hernia. None of the individuals reported in the literature with ATP6AP1-CDG have been reported with

**Table 1. ATP6AP1 variants identified in the individuals in this study, with relevant population frequencies, total reads, variant allele fraction, inheritance, and classification**

| Genomic coordinates (hg19) | Reference allele | Alternative allele | Total reads | Variant allele fraction (VAF) | HGVS cDNA | HGVS protein (inheritance) | Zygosity | Variant type | gnomAD allele frequency | Variant classification |
|---------------------------|------------------|--------------------|-------------|------------------------------|-----------|---------------------------|----------|--------------|------------------------|------------------------|
| Chr X: 153657459 CCTACT  | CCT              | 120 100%           | c.230_232delACT | p.Tyr77del (de novo)         | Hemizygous In-frame deletion | Absent     | Likely pathogenic |
| Chr X: 153657459 CCTACT  | CCT              | 112 95%            | c.230_232delACT | p.Tyr77del (de novo)         | Hemizygous In-frame deletion | Absent     | Likely pathogenic |
ventriculomegaly, umbilical hernia, pectus carinatum, micropenis, or hypospadias, which we found in the probands in this study. Of note, to avoid bias by double counting, as the probands reported by Gumm et al. (2020) and Tvina et al. (2020) are the same set of siblings from a single family, they are counted only once in the phenotypic description.

**Variant Classification**

We assessed this in-frame deletion using a point-based approach following ACMG/AMP criteria (Version 1.0) updated recommendations for classifying de novo variants (PS2/
| HPO#  | Family-1 | Family-2 | Family-3 | Family-4 | Family-5 | Family-6 |
|-------|----------|----------|----------|----------|----------|----------|
| Age/ethnicity | 20y/Caucasian | 12y/Caucasian | 34y/Caucasian | 14y/Caucasian | 8y/Druze | Died 4y/Druze | 23y/Caucasian | 18y/Caucasian | Died 12 mo | 3y/Tunisian | 4y/Irish |
| cDNA/protein consequence | c.1284G > A; p.Met428Ile | c.1284G > A; p.Met428Ile | c.1284G > A; p.Met428Ile | c.431T > C; p.Leu144Pro | c.1036G > A; p.Glu346Lys | c.1036G > A; p.Glu346Lys | c.1036G > A; p.Glu346Lys | c.1036G > A; p.Glu346Lys | c.938 > 4G; p.Tyr313Cys |
| Variant type | Missense | Missense | Missense | Missense | Missense | Missense | Missense | Missense | Missense | Missense |
| Investigation method | WES/Sanger sequencing | WES/Sanger sequencing | WES/Sanger sequencing | WES | WES | Sanger sequencing | WES | Sanger sequencing | WES | Sanger sequencing |
| Sex | Male | Male | Male | Male | Male | Male | Male | Male | Male | Male |
| Inheritance | Maternal | Maternal | Maternal | Maternal | Maternal | Maternal | Maternal | Maternal | Maternal | Maternal |
| Fetal cystic hygroma | HP:0010878 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Fetal atrial septal defect/dilated aorta/cardiomegaly | HP:0001631; HP:0034058; HP:0001640 | ND | ND | ND | ND | ND | ND | ND | ND |
| Fetal ventriculomegaly | HP:0010952 | ND | ND | ND | ND | ND | ND | ND | ND |
| Cutis laxa | HP:0000973 | ND | ND | ND | ND | ND | ND | ND | ND |
| Hepatomegaly/failure/cirrhosis | HP:0001392 | (+/-) | (-) | (-) | (-) | (+) | (+) | (+) | (+) |
| Splenomegaly | HP:0001744 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) |
| Neurological symptoms | HP:0000707 | (-) | (+/-) | (-) | (-) | (+) | (+) | (+) | (+) |
| Recurrent Infections | HP:0002719 | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) |
| Cardiac abnormalities | HP:0001627 | ND | ND | ND | ND | ND | ND | ND | ND |
| Bilateral inguinal hernias | HP:000023 | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) |
| Umbilical hernia | HP:0001537 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) |
| Diaphragmatic hernia | HP:0000776 | ND | ND | ND | ND | ND | ND | ND | ND |
| Pectus carinatum | HP:0000766 | ND | ND | ND | ND | ND | ND | ND | ND |
| Micropenis/hypospadias | HP:000054; HP:0000047 | ND | ND | ND | ND | ND | ND | ND | ND |

(Continued on next page.)
| HPO#                     | Individual 12 | Individual 13 | Individual 14 | Individual 15 (Case 1) | Individual 16 | Individual 17 | Individual 18 | Twin A | Twin B | Consensus |
|-------------------------|---------------|---------------|---------------|------------------------|---------------|---------------|---------------|--------|--------|-----------|
| Age/ethnicity           | 10 y/ND       | 5 mo/ND       | 38 wk (neonatal)/ND | Died 3 mo/ Caucasian Czech | Died 11 mo/ Caucasian Czech | 8 mo/Chinese | 4 y/Guyanese;Italian | 4 y/Guyanese;Italian |
| cDNA/protein consequence| c.542T > G; p.Leu181Arg | c.649 T > A; p.Tyr217Ain | c.932T > A; p.Leu311Gln | c.221T > C; p.Leu74Pro | c.211T > C; p.Leu74Pro | c.932T > A; p.Leu311Gln | c.221T > C; p.Leu74Pro | c.230_232delACT; p.Tyr77del | c.230_232delACT; p.Tyr77del | - |
| Variant type            | Missense      | Missense      | Missense      | Missense              | Missense      | Missense      | Missense      | In-frame deletion | In-frame deletion | - |
| Investigation method    | WES           | Sanger        | WGS           | WES/Sanger sequencing | WES/Sanger sequencing | WES/Sanger sequencing | WES/Sanger sequencing | WES | WES/Sanger sequencing | WES/Sanger sequencing |
| Sex                     | Male          | Male          | Male          | Male                  | Male          | Male          | Male          | Male              | Male              | 20 of 20 (male) |
| Inheritance             | Maternal      | Maternal      | Maternal      | Maternal              | Maternal      | Maternal      | Maternal      | De novo          | De novo          | 4 of 20 (de novo) |
| Fetal cystic hygroma    | ND            | ND            | ND            | ND                    | ND            | ND            | ND            | ND                | ND                | 3 of 3 |
| Fetal atrial septal defect/ dilated aorta/ cardiomegaly | ND | ND | ND | ND | ND | ND | ND | ND | ND | 3 of 3 |
| Fetal ventriculomegaly  | ND            | ND            | ND            | ND                    | ND            | ND            | ND            | ND                | ND                | 2 of 2 |
| Cutis laxa              | HP:000973     | (+)           | (+)           | (-)                   | (-)           | (+)           | (+)           | (-)               | (+)               | 7 of 9 |
| Hepatomegaly/liver failure/ cirrhosis | HP:001392 | (+) | (+) | (+) | (+) | (+) | (+) | (+) | Liver failure | Liver failure | 17 of 20 |
| Splenomegaly            | HP:0001744    | (+)           | (+)           | (-)                   | (+)           | (+)           | (+)           | (+)               | (+)               | 13 of 20 |
| Neurological symptoms   | HP:0000707    | (-)           | (-)           | (-)                   | (-)           | (-)           | (-)           | (-)               | (-)               | 8 of 20 |
| Recurrent infections    | HP:0002719    | (+)           | (+)           | (-)                   | (-)           | (+)           | (+)           | (+)               | (+)               | 16 of 20 |
| Cardiac abnormalities   | HP:0001627    | (+)           | (+)           | (-)                   | (-)           | (-)           | (-)           | (-)               | (-)               | 5 of 7 |
| Bilateral inguinal hernias | HP:000023    | ND            | ND            | ND                    | ND            | ND            | ND            | ND                | ND                | 5 of 7 |
| Umbilical hernia        | HP:0001537    | ND            | ND            | ND                    | ND            | ND            | ND            | ND                | ND                | 2 of 7 |
| Diaphragmatic hernia    | HP:0000776    | ND            | ND            | ND                    | ND            | ND            | ND            | ND                | ND                | 3 of 4 |
| Pectus carinatum        | HP:0000766    | ND            | ND            | ND                    | ND            | ND            | ND            | ND                | ND                | 2 of 2 |
| Microcephaly/hydrocephalus | HP:000054 | ND | ND | ND | ND | ND | ND | ND | ND | 2 of 2 |

Unique features observed in this current study are highlighted in blue.

(WES) Whole-exome sequencing, (WGS) whole-genome sequencing, (ND) no data, (+) present, (-) absent, (+/-) indicated as such in the table outlining the clinical features of that individual in the original publication. Rows in gray highlight the unique features observer in the patients reported in this study.
PM6) published March 2018 (SVI 2018). Considering the overlapping features and consistent hepatic findings in all reported individuals, we used the strength level of strong, with a total of two points. Moreover as p.Tyr77del is a single-amino acid in-frame deletion, we downgraded the moderate level PM4 criteria to a supporting level of evidence following the ACGS framework (ACGS 2019). As this variant is absent in the population database, we implied the recommended supporting level evidence of PM2. Using these recommendations as a framework from various working groups, the c.230_232delACT;p.Tyr77del was classified as likely pathogenic.

DISCUSSION

There are 18 individuals reported in the literature with ATP6AP1-CDG-related disorder with missense variants in the ATP6AP1 gene (Jansen et al. 2016; Dimitrov et al. 2018; Witters et al. 2018; Ondruskova et al. 2020; Song et al. 2020; Tvina et al. 2020). The most striking clinical features shared among all reported cases is liver disease that varies from increased transaminase to steatosis, cirrhosis, or/and cholestasis. This is similar to other CDGs reported in the literature (Starosta et al. 2021). Immunodeficiency is also commonly reported. Although the initial case series in 2016 reported neurological symptoms as a component of the phenotype, the neurological features were only present in individuals with one specific variant (p.Glu346Lys), and no cases reported since, including this one, have included neurological features. However, there have been multiple cases, including our own, that have noted cutis laxa as a significant feature of ATP6AP1-CDG-related disorder. Both twins in this case study presented with significant connective tissue features, including velvety soft skin with laxity, especially in the epigastric and groin/flank regions, pectus carinatum, inguinal and umbilical hernias, and a dilated ascending aorta. Furthermore, the prenatal features seen in these twins are consistent with the previously reported prenatal phenotype of dilated ascending aorta (Tvina et al. 2020); however, our patients also presented with cystic hygroma, atrial septal defect, and ventriculomegaly prenatally. Thus, our case further affirms that although the connective tissue and prenatal findings are likely more common than previously assessed, neurological features do not appear to be central in ATP6AP1-CDG-related disorder.

Similar to a patient recently reported (Gumm et al. 2020; Tvina et al. 2020), the probands presented in this study did not demonstrate any defects in glycosylation, which was performed after liver transplantation. Indeed, Gumm et al. reported that although the same variant was present in two siblings, it showed an abnormal glycosylation in one of the probands, whereas it was normal in the other proband. The observation of normal glycosylation patterns in two independent studies may indicate that although a clinical indication could be remarkable, not all patients with a significant variant in the ATP6AP1 gene (which functions primarily in luminal acidification of the secretory vesicle) will necessarily exhibit abnormal glycosylation patterns. This may also indicate that in the case of a strong clinical suspicion for ATP6AP1-CDG-related disorder, sequencing should be included as part of the evaluation, even in patients with a normal glycosylation profile. Indeed, variability in glycosylation patterns in patients with ATP6AP1-CDG have also been reported by Jansen et al. and diagnosis of CDG with a normal glycosylation pattern has been reported in other known types of CDG in the literature (Zuhlsdorf et al. 2015). Although cutis laxa with minimum neurological findings is not enough to differentiate ATP6AP1-GDG from other V-ATPases, these findings, along with hepatic dysfunction, should raise enough suspicion to think of the particular condition regardless of transferrin glycosylation studies.

Excluding this case, all previously reported SNVs associated with ATP6AP1-CDG-related disorders were missense variants. Although a functional study on patient fibroblasts harboring a missense (c.542T > G, p.Leu181Arg) variant has excluded apoptosis as a cause, there
was significant elongation of doubling time and reduced proliferation of fibroblasts observed (Dimitrov et al. 2018). Our patients have a 3-bp in-frame deletion that eliminates a single tyrosine residue and leads to a clinical phenotype consistent with ATP6AP1-CDG-related disorder.

When WES was done initially in 2016, the de novo in-frame deletion (c.230_232delACT; p.Tyr77del) identified in our patients was classified as variant of uncertain significance. This classification was based on the data and evidence available at that point of time. However, clinical reevaluation of these two siblings in 2020, together with updated recommendations on variant interpretation (SVI 2018; ACGS 2019) and additional evidence of case-level data from recent literature, showed an overlapping phenotype associated with ATP6AP1-CDG, which resulted in reclassification of this variant to likely pathogenic. Our results support the importance of long-term clinical follow-up of individuals with rare diseases and reinterpretation of rare variants based on emerging evidence and updated guidelines/recommendations, which has the potential to alter overall management. Although it is a limitation in this study, in the future, determination of the consequences of the tyrosine deletion on overall structure and folding of the ATP6AP1 might reveal additional information on the potential function of this subunit on the V-ATPase complex.

Conclusion
In conclusion, we present two additional individuals with ATP6AP1-CDG-related congenital disorder of glycosylation due to a novel de novo in-frame deletion. Our case extends the knowledge of clinical manifestations in the prenatal and postnatal stages, which increases the understanding and knowledge about this rare disorder. It further affirms that neurological features do not seem to be central to this condition, and that cutis laxa as well as other connective tissue features are more prevalent. Moreover, it highlights the impact of variant reinterpretation with implementation of updated guidelines/recommendations and its clinical implication on long-term patient care and genetic counseling.

METHODS

Whole-Exome Sequencing (WES) and Analysis
WES was performed after written informed consent was obtained, at the Laboratory of Personalized Genomic Medicine at Columbia University Irving Medical Center on DNA obtained from peripheral blood of both twins as well as their parents. Paired-end sequencing was performed on the Illumina HiSeq 2500 platform using Agilent SureSelectXT (Human All Exon v.5 + UTRs) capture kit following manufacturers’ protocol. NextGENe (version 2.3; SoftGenetics, LLC) software was used to align (hg19) and annotate the sequence data. Variants were filtered and annotated using a New York State–approved in-house-developed pipeline. Variants were reviewed as part of the workflow for constitutional clinical exome sequencing in the Laboratory of Personalized Genomic Medicine at Columbia University Medical Center as previously described (Kurtz et al. 2021).

ADDITIONAL INFORMATION

Data Deposition and Access
The variants were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession number VCV001686802.1.
Ethics Statement
Clinical genetic testing was performed with informed consent. Parental consent was obtained for publication of clinical features and photographs of the twins, probands (P) 1 and 2. Clinical testing does not require IRB approval.

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Author Contributions
S.Ba. analyzed genomic WES data, investigated, wrote the original draft, and reviewed and edited the manuscript. S.Be. performed genetic counseling, data extraction from the medical records, and reviewed and edited the manuscript. E.M.P. performed the data extraction from the medical records, examined and followed-up with the probands, supervised the project, and wrote, reviewed and edited the manuscript. V.J. performed formal analysis of the genomic WES data, supervised the project, and reviewed and edited the manuscript. All authors revised, contributed intellectually, and approved the final version of the manuscript.

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REFERENCES
ACGS Best Practice Guidelines for Variant Classification. 2019. https://www.acgs.uk.com/quality/best-practice-guidelines/
Cannata Serio M, Graham LA, Ashikov A, Larsen LE, Raymond K, Timal S, Le Meur G, Ryan M, Czarnowska E, Jansen JC, et al. 2020. Mutations in the V-ATPase assembly factor VMA21 cause a congenital disorder of glycosylation with autophagic liver disease. Hepatology 72: 1968–1986. doi:10.1002/hep.31218
Casey JR, Grinstein S, Orlowski J. 2010. Sensors and regulators of intracellular pH. Nat Rev Mol Cell Biol 11: 50–61. doi:10.1038/nrm2820
Chang IJ, He M, Lam CT. 2018. Congenital disorders of glycosylation. Ann Transl Med 6: 477. doi:10.21037/atm.2018.10.45
Dimitrov B, Himmelreich N, Hipgrave Edeveen AL, Luchtenborg C, Okun JG, Breuer M, Hutter AM, Carl M, Guglielmi L, Hellwig A, et al. 2018. Cutis laxa, exocrine pancreatic insufficiency and altered cellular metabolomics as additional symptoms in a new patient with ATP6AP1-CDG. Mol Genet Metab 123: 364–374. doi:10.1016/j.ymgme.2018.01.008
Guillard M, Dimopoulou A, Fischer B, Morava E, Lefeber DJ, Kornak U, Wevers RA. 2009. Vacuolar H+-ATPase meets glycosylation in patients with cutis laxa. Biochim Biophys Acta 1792: 903–914. doi:10.1016/j.bbadis.2008.12.009
Kornak U, Reynders E, Dimopoulou A, van Reeuwijk J, Fischer B, Rajab A, Budde B, Nurnberg P, Foulquier F, Group AD-tS, et al. 2008. Impaired glycosylation and cutis laxa caused by mutations in the vesicular H+-ATPase subunit ATP6V0A2. Nat Genet 40: 32–34. doi:10.1038/ng.2007.45
Kurtz J, Fernandes JA Jr, Mansukhani M, Copeland WC, Nairn AB. 2021. Whole-exome sequencing identifies a novel POLG frameshift variant in an adult patient presenting with progressive external ophthalmoplegia and mitochondrial DNA depletion. Case Rep Genet 2021: 9969071. doi:10.1155/2021/9969071
Lee WS, Sokol RJ. 2007a. Liver disease in mitochondrial disorders. Semin Liver Dis 27: 259–273. doi:10.1055/s-2007-985071
Lee WS, Sokol RJ. 2007b. Mitochondrial hepatopathies: advances in genetics and pathogenesis. Hepatology 45: 1555–1565. doi:10.1002/hep.21710
Ng BG, Freeze HH. 2018. Perspectives on glycosylation and its congenital disorders. Trends Genet 34: 466–476. doi:10.1016/j.tig.2018.03.002
Nishi T, Forgac M. 2002. The vacuolar (H+)-ATPases—nature’s most versatile proton pumps. Nat Rev Mol Cell Biol 3: 94–103. doi:10.1038/nrm729

Ondruskova N, Honzik T, Vondrackova A, Stranecky V, Tesarova M, Zeman J, Hansikova H. 2020. Severe phenotype of ATP6AP1-CDG in two siblings with a novel mutation leading to a differential tissue-specific ATP6AP1 protein pattern, cellular oxidative stress and hepatic copper accumulation. J Inherit Metab Dis 43: 694–700. doi:10.1002/jimd.12237

Pamarthy S, Kulshrestha A, Katara GK, Beaman KD. 2018. The curious case of vacuolar ATPase: regulation of signaling pathways. Mol Cancer 17: 41. doi:10.1186/s12943-018-0811-3

Rujano MA, Cannata Serio M, Panasyuk G, Peanne R, Reunert J, Rymen D, Hauser V, Park JH, Freisinger P, Souche E, et al. 2017. Mutations in the X-linked ATP6AP2 cause a glycosylation disorder with autophagic defects. J Exp Med 214: 3707–3729. doi:10.1084/jem.20170453

Silk M, Petrovski S, Ascher DB. 2019. MTR-Viewer: identifying regions within genes under purifying selection. Nucl Acids Res 47: W121–W126. doi:10.1093/nar/gkz457

Song Q, Meng B, Xu H, Mao Z. 2020. The emerging roles of vacuolar-type ATPase-dependent lysosomal acidification in neurodegenerative diseases. Transl Neurodegener 9: 17. doi:10.1186/s40035-020-00196-0

Starosta RT, Boyer S, Tahata S, Raymond K, Lee HE, Wolfe LA, Lam C, Edmondson AC, Schwartz IVD, Morava E. 2021. Liver manifestations in a cohort of 39 patients with congenital disorders of glycosylation: pin-pointing the characteristics of liver injury and proposing recommendations for follow-up. Orphanet J Rare Dis 16: 20. doi:10.1038/s13023-020-01630-2

SVI Recommendation for De Novo Criteria (PS2 & PM6)—Version 1.0. https://clinicalgenome.org/working-groups/sequence-variant-interpretation/

Tvina A, Thomsen A, Palatnik A. 2020. Prenatal and postnatal phenotype of a pathologic variant in the ATP6AP1 gene. Eur J Med Genet 63: 103881. doi:10.1016/j.ejmg.2020.103881

Van Damme T, Gardeitchik T, Mohamed M, Guerrero-Castillo S, Freisinger P, Guillemyn B, Kariminejad A, Dalloyaux D, van Kraaij S, Lefeber DJ, et al. 2017. Mutations in ATP6V1E1 or ATP6V1A cause autosomal-recessive cutis laxa. Am J Hum Genet 100: 216–227. doi:10.1016/j.ajhg.2016.12.010

Wilson MP, Matthijs G. 2021. The evolving genetic landscape of congenital disorders of glycosylation. Biochim Biophys Acta Gen Subj 1865: 129976. doi:10.1016/j.bbagenen.2021.129976

Witters P, Breckpot J, Fouquier F, Preston G, Jaeken J, Morava E. 2018. Expanding the phenotype of metabolic cutis laxa with an additional disorder of N-linked protein glycosylation. Eur J Hum Genet 26: 618–621. doi:10.1038/s41431-017-0044-8

Yang X, Lv Z-L, Tang Q, Chen X-Q, Huang L, Yang MX, Lan L-C, Shan Q-W. 2021. Congenital disorder of glycosylation caused by mutation of ATP6AP1 gene (c.1036G>A) in a Chinese infant: case report. World J Clin Diseases 9: 7876–7885. doi:10.12988/wjcc.v9.i26.7876

Zuhlsdorf A, Park JH, Wada Y, Rust S, Reunert J, DuChesne I, Gruneberg M, Marquardt T. 2015. Transferrin variants: pitfalls in the diagnostics of congenital disorders of glycosylation. Clin Biochem 48: 11–13. doi:10.1016/j.clinbiochem.2014.09.022

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