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A novel *SLC5A6* homozygous variant in a family with multivitamin-dependent neurometabolic disorder: Phenotype expansion and long-term follow-up

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ABSTRACT

The sodium-dependent multivitamin transporter (hSMVT) encoded by the *SLC5A6* gene is required for the intestinal absorption of biotin, pantothenic acid and lipoate, three micronutrients essential for normal growth and development. Systemic deficiency of these elements, either occurring from nutritional causes or genetic defects, is associated with neurological disorders, growth delay, skin and hair changes, metabolic and immunological abnormalities. A few patients with biallelic variants of *SLC5A6* have been reported, exhibiting a spectrum of neurological and systemic clinical features with variable severity. We describe three patients from a single family carrying a homozygous p.(Leu566Valfs*33) variant of *SLC5A6* disrupting the frame of the C-terminal portion of the hSMVT. In these patients, we documented a severe disorder featuring developmental delay, sensory polyneuropathy, optic atrophy, recurrent infections, and repeated episodes of intestinal pseudo-obstruction. Two patients who did not receive multivitamin supplementation therapy died in early infancy. In a third patient, early supplementation of biotin and pantothenic acid stabilized the clinical picture changing the course of the disease. These findings extend genotype-phenotype correlations and show how a timely and lifelong multivitamin treatment may be crucial to reduce the risk of life-threatening events in patients with pathogenic variants of the *SLC5A6* gene.

1. Introduction

The sodium-dependent multivitamin transporter (hSMVT) catalyses the transport of water-soluble vitamins such as biotin and pantothenic acid, and of the metabolite lipoate in a Na^+-dependent manner (Quick and Shi, 2015). This transporter is highly conserved among species and ubiquitous, but most abundantly expressed in the intestinal epithelia, as well as in the brain capillary endothelial cells, where it is the major contributor to biotin and pantothenic acid transport across the blood–brain barrier (BBB) (Subramanian et al., 2009; Uchida et al., 2015). In humans, hSMVT activity is critical for proper growth and development, since neither biotin nor pantothenic acid are synthesized endogenously by mammalian cells but are both obtained through intestinal absorption from dietary and microbiota-generated sources (Neophytou and Pitsouli, 2022).

Biotin is an essential cofactor for multiple carboxylases involved in several essential metabolic pathways, including fatty acid biosynthesis, gluconeogenesis, and amino acid catabolism. It is also involved in energy metabolism, cellular oxidative stress response, and gene expression regulation, as well as in normal immune function and gut-microbiome interactions (Neophytou and Pitsouli, 2022; Sabui et al., 2018). Congenital disorders of biotin metabolism due to biallelic defects of the *BTD* (MIM #253260) or *HLCS* (MIM #253270) genes, respectively encoding for the biotinidase and holocarboxylase synthetase enzymes, may result in a wide spectrum of clinical manifestations including developmental delay, seizures, ataxia, brain atrophy, sensorineural

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hearing loss, peripheral and optic neuropathy, respiratory, intestinal, immunological, and cutaneous abnormalities. Biotin supplementation may prevent pre-symptomatic children from becoming symptomatic and improve clinical outcome in symptomatic patients (Elrefai and Wolf, 2014). Pantothenic acid is a precursor of coenzyme A and fatty acid synthase, and is important for carbohydrate, fat, and protein metabolism. Dietary deficiency of this essential micronutrient alters intermediary metabolism (Hauth et al., 2022). Lipoate is endogenously synthetized by mitochondria and is essential for redox reactions involved in energy and amino acid metabolism. Defective lipoate metabolism may result in encephalopathy and systemic abnormalities (Mayr et al., 2014).

The human SMVT protein consists of 635 amino acids and is encoded by the *SLC5A6* gene, in chromosome 2p23.3. Recently, biallelic variants of this gene have been identified in pediatric patients with developmental and growth delay, seizures, gastrointestinal and cutaneous abnormalities, and peripheral neuropathy (Hauth et al., 2022; Byrne et al., 2019; Schwantje et al., 2019; Subramanian et al., 2017; Holling et al., 2022). Timely multivitamin treatment may be crucial to reduce the risk of life-threatening events related to infections or severe metabolic alterations.

Here we describe three patients, exhibiting a severe neurometabolic disorder featuring developmental delay, hypotonia, optic atrophy, peripheral neuropathy, repeated episodes of intestinal pseudo-obstruction, and recurrent infections. Two patients died in early infancy. The only living patient experienced global clinical improvement after parenteral supplementation of hydro- and liposoluble vitamins was started at the age of 2 years and 6 months, before genetic diagnosis. In these patients, we identified a novel homozygous c.1694dup p.(Leu566Valfs*33) variant of the *SLC5A6* gene, disrupting the frame of the C-terminal portion of the protein.

2. Patients and methods

The family was referred to the Neuroscience Department of the Meyer Children's Hospital of Florence, Italy, for clinical assessment. We obtained from the family written informed consent to disclose clinical, neuroimaging and genetic findings. The study was approved by the Paediatric Ethics Committees of the Tuscany Region, Italy, in the context of the DESIRE FP7 EU project and its extension by the DECODE-EE project.

In Patient 2 (III-3 in Fig. 1A), and in her parents, we performed whole exome sequencing (WES) on DNA extracted from peripheral blood as reported elsewhere (Vetro et al., 2020). We annotated and filtered variants by the VarSeq software (Golden Helix, Inc v1.4.6, Bozeman, MT) focusing on exonic/splice-site single-nucleotide variants (SNVs) and coding insertions/deletions (InDels) with minor allele frequency (MAF) lower than 0.01 in the GnomAD database (v2.1, http://gnomad.broadinstitute.org/). We further excluded population-specific variants by interrogating our internal database (WES data from approximately 3000 patients with developmental and epileptic encephalopathies and their healthy relatives). Separately, we evaluated possible regions of autozygosity by the Agile VCF Mapper software (http://dna.leeds.ac.uk/autozygosity/). We performed variant validation and segregation analyses by Sanger sequencing in available family members (primers and conditions available on request).

3. Results

3.1. Clinical description

Patients 1 and 2 (III-2 and III-3) and their first-degree cousin Patient 3 (III-4) were products of marriages between two couples of siblings (Fig. 1A). Their respective parents were healthy and denied known consanguinity but were all originating from a small Italian village of approximately 3000 inhabitants.

Patient 1, a girl, came to our attention at six months of age due to global developmental delay, hypotonia, muscle wasting, strabismus, nystagmus, optic atrophy, alopecia, and perioral dermatitis. The child experienced recurrent episodes of diarrhoea and vomiting, mostly associated with infections, and concomitant intestinal pseudo-obstruction. Two brain MRI scans, performed at nine months and two years of age, were unrevealing. Metabolic investigations showed intermittent lactic acidosis and increased 3-hydroxyisovaleric acid in urine. Biochemical and immunohistochemical studies on muscle biopsy did not support the initial suspicion of a mitochondrial disorder. The girl died at three years of age due to complications of acute intestinal pseudo-obstruction. Her parents declined autopsy.

Patient 2, a girl, was born at the 40th week of an uncomplicated pregnancy. Birth weight (3400 gr; −0.05 SD) and length (50 cm; +0.2 SD) were in the normal range. Head circumference was 35.5 cm (+0.39 SD). Psychomotor development was normal in the first year of life. The girl experienced recurrent episodes of periorificial dermatitis and diarrhoea since the age of one year. Allergy tests were unrevealing. We first saw this patient when she was two years and six months, after acute onset of fever, abdominal distension, vomiting with severe dehydration, drowsiness, transient urinary bladder dysfunction, and facial dermatitis. Neurogenic pseudo-Hirschsprung disease was confirmed by abdominal X-ray and contrast enema. Her general conditions worsened abruptly, with respiratory failure, cardiomyopathy with severe left ventricular dysfunction and reduced state of consciousness, requiring hospitalization in the Intensive Care Unit for 28 days. Prolonged video-EEG recording was normal and brain MRI showed mild ventricular enlargement with posterior periventricular white matter hyperintensities in T2-weighted images. ^{1}H Magnetic Resonance Spectroscopy (^{1}HMRS) demonstrated a lactate peak within the hyperintensity areas. Immunohistochemical and biochemical investigations on muscle biopsy did not reveal signs of mitochondrial dysfunction. Laboratory blood and urine investigations documented hypertransaminasemia and cholestatic liver disease associated with coagulopathy, hypochromic normocytic anaemia, and hypoalbuminemia, as well as fluctuating increase in the levels of lactic acid in blood and urine.

Based on the clinical picture and family history resembling a biotinidase deficiency, before obtaining a genetic diagnosis, we initiated daily parenteral nutrition supplemented with thiamine (5 mg), riboflavin (7.2 mg), nicotinamide (80 mg), pyridoxine (8 mg), pantothenic acid (30 mg), ascorbic acid (200 mg), biotin (120 μg), folic acid (0.8 mg), and cyanocobalamin (10 μg) and oral administration of hydro- and liposoluble vitamins (ubidecarenone 100 mg, thiamine 100 mg, biotin 5 mg, riboflavin 240 mg, carnitine 1500 mg and ascorbic acid 1000 mg). This treatment led to overall clinical improvement with normalized heart function and gastrointestinal motility.

Since the age of three years and six months the girl developed progressive bilateral visual impairment, associated with optic atrophy and horizontal nystagmus. Visual evoked responses were mildly delayed.

Since the age of four years and six months, attempts of suspending parenteral nutrition or periods of poor adherence to therapy were followed by recurrent episodes of intestinal pseudo-obstruction and perioorificial skin dermatitis. Recurrent catheter-related infections associated with diarrhoea, hypoglycaemia, hyperammonaemia, pancytopenia, and subsequent alteration of haemostasis and icterus were reported during follow up.

After vitamin supplementation therapy was started, extensive immunological tests, including quantitative immunoglobulin (Ig) measurement, lymphocyte subset analysis and a bone marrow biopsy were unrevealing.

Neuropsychological evaluations, performed at nine years revealed mild cognitive impairment to borderline scores that had considerably improved at re-testing at 19 years (Wechsler Intelligence Scale for Children-Revised, WISC-R at nine years: Full IQ scale = 73; Performance IQ = 61; Verbal IQ = 88; Wechsler Adult Intelligence Scale-Fourth Edition, WAIS-IV at 19 years: Verbal Comprehension Index = 84;

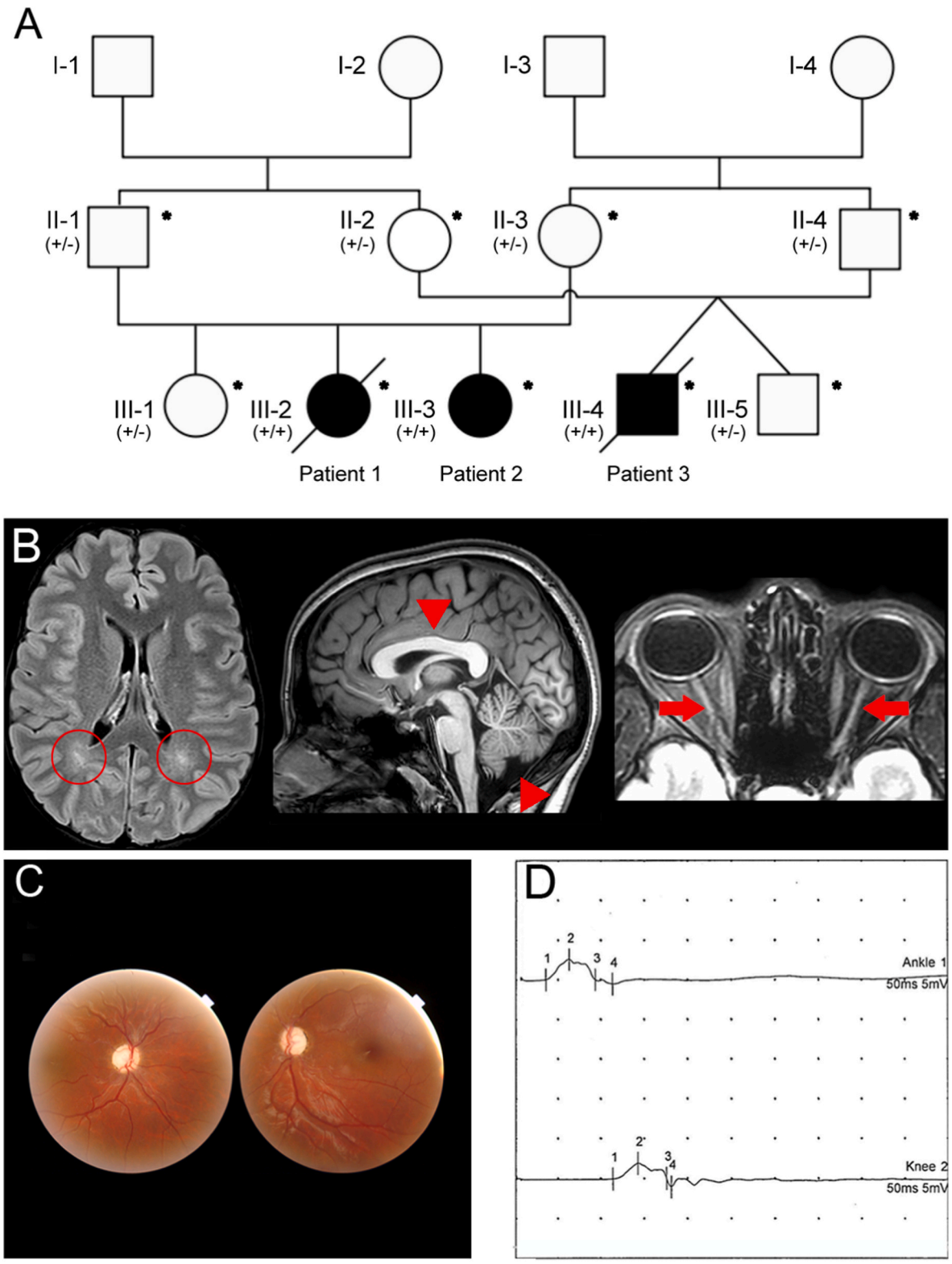

Fig. 1. Family tree and clinical features of Patient 2. A) Patients 1 (III-2) and 2 (III-3) were first-degree cousins of Patient 3 (III-4). Patients' 1 and 2 father (II-1) is the brother of Patient's 3 mother (II-2). Patients' 1 and 2 mother (II-3) is the sister of Patient's 3 father (II-4). No known consanguinity was reported between them. The asterisk indicates individuals for whom we had DNA available for testing, with the presence of the c.1694dup at the homozygous (+/+) or heterozygous (±) state indicated in brackets. B) Selected brain MRI sections at age 20 years show mild posterior white matter hyperintensity (left panel, axial T2 FLAIR, circles), thick corpus callosum and mild cerebellar vermis atrophy (middle panel, sagittal T2 FLAIR, arrowheads), and bilateral thinning of optic nerves highlighted by arrows (right panel, axial T2 FLAIR). C) Fundoscopic images showing bilateral optic disc pallor suggestive of optic atrophy (right and left eye) D) Nerve conduction study showing low amplitude compound muscle action potential (CMAP) (3.1 mV as measured peak to peak) over the left tibial nerve both on distal (at the ankle, upper trace) and proximal (at the knee, lower trace) site of stimulation, with slightly reduced motor conduction velocity (43 m/s). Marker: 1 Onset, 2 Negative peak, 3 Return to baseline, 4 Positive peak. Amplitude: peak to peak.

Perceptual Reasoning Index = 75; Working Memory Index = 103).

At the age of 20 years, brain MRI showed mild posterior white matter hyperintensity, thick corpus callosum, mild cerebellar vermis atrophy, and bilateral optic nerves atrophy (Fig. 1B), confirmed by fundoscopy (Fig. 1C). Neurophysiological investigations documented a sensory-motor polyneuropathy (Fig. 1D). Clinical evaluation demonstrated mild ataxic gait, *pes cavus*, absent deep tendon reflexes, kinetic and postural tremors, and horizontal nystagmus.

At the time genetic diagnosis as made, at age 20 years, nocturnal parenteral feeding was replaced by a specific oral therapy with high dosage of biotin (40 mg/day), lipoic acid (600 mg/day), and pantothenic acid (1000 mg/day).

After poor therapy compliance, at age 21 years the Patient experienced two episodes of mild ataxia and unresponsiveness lasting 3–4 min, followed by a generalized tonic-clonic seizure. EEG showed sharp waves in left centro-temporal region.

At last follow-up, at 24 years of age, the patient, still on vitamin supplementation therapy exhibited a stable clinical picture, featuring mild ataxia, nystagmus, tremor, and sensory polyneuropathy.

Patient 3, a boy, was born by caesarean section at the 33rd week of gestation, from a dizygotic twin pregnancy resulting from homologous in vitro fertilization (IVF). The twin brother was healthy. Birth weight was 1440 gr (−1.2 SD), length 39 cm (−1.7 SD) and head circumference 29.5 cm (−0.67 SD). Growth and psychomotor development were delayed in the first year of life. At one year of age, the child was hospitalized due to recurrent episodes of respiratory and urinary tract infections, in association with neutropenia, periorificial dermatitis, gastroesophageal reflux disease (GERD) and recurrent diarrhoea. Laboratory investigations for celiac disease were unrevealing. At 13 months of age, brain MRI showed mildly delayed myelination with enlarged lateral ventricles. The child died at the age of 17 months due to acute respiratory failure. Autopsy documented viral myocarditis and severe bronchopneumonia with diffuse alveolar damage.

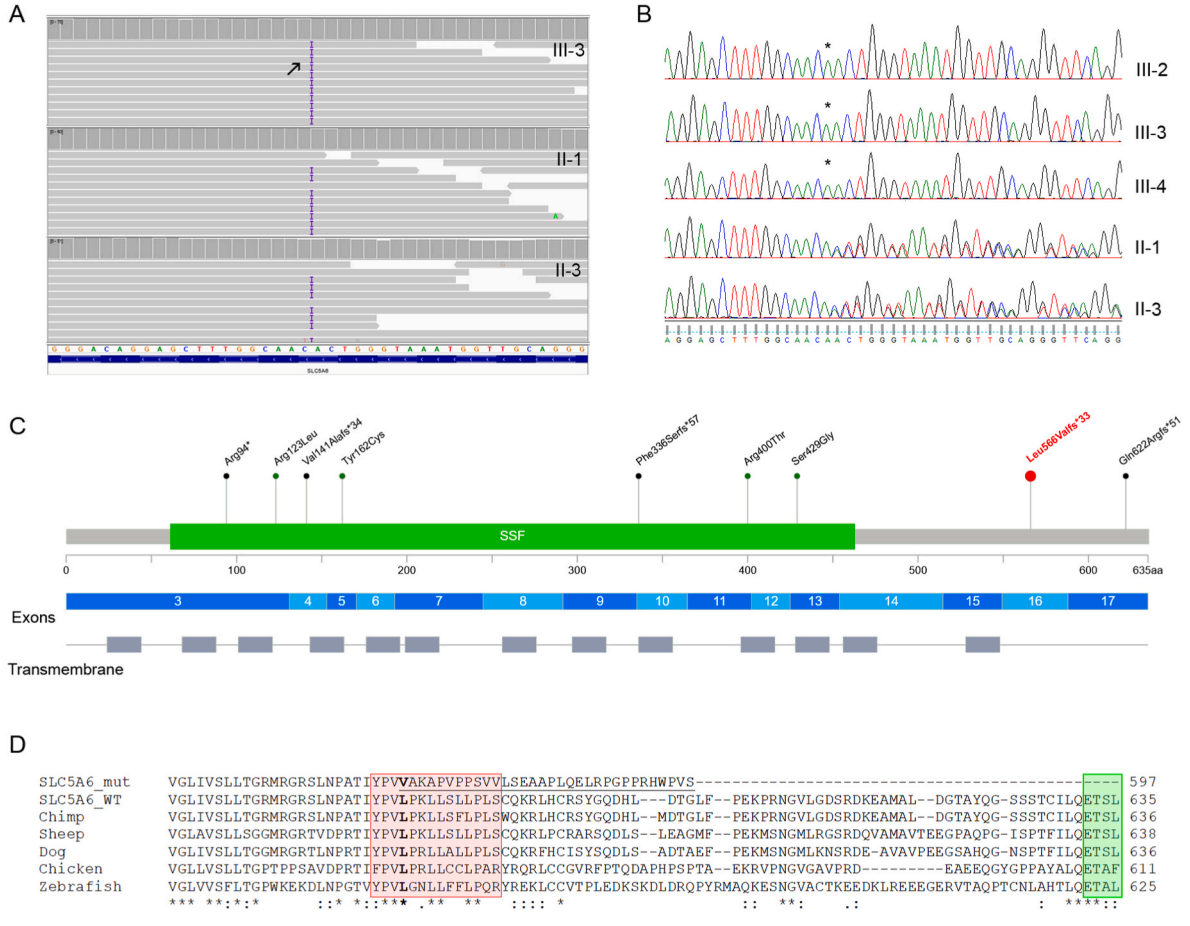

Fig. 2. Genetic findings. A) Screenshot of a 48-bp window (chr2:27423916-27423963, hg19) as visualized by Integrative Genomics Viewer (https://software.broadinstitute.org/software/igv/). Horizontal bars represent the mapped reads aligned to the reference genome, whose sequence is shown below. The coverage track is displayed for each sample. The upper panel shows the homozygous single A base duplication at chr2:27423936 (arrow) in Patient 2, heterozygous in both parents (middle and lower panels). B) Sanger sequencing results in the family: the variant (asterisks) is homozygous in the DNA of the three affected individuals (upper panels) and heterozygous in that of the parents of Patients 1 and 2. Sanger sequencing in the parents of Patient 3 and in the healthy siblings of Patients 1–3, all heterozygous carriers for the variant, is not shown. The reference sequence is shown below. C) The lollipop-diagram shows the distribution of *SLC5A6* reported variants in the linear sequence of the protein, the gene exons, and transmembrane protein regions according to MutationMapper (http://www.cbioportal.org/mutation_mapper). The p.(Leu566Valfs*33) variant is in red and previously reported missense and truncating variants are in green and black, respectively. The green bar represents the Pfam-classified SSF domain (Sodium:solute symporter family, amino acids 61–463; PF00474). D) Alignment of the COOH-terminal amino acid sequence of SLC5A6 in vertebrates. The mutated human SLC5A6 is on the top, with the aberrant amino acid sequence underlined. The Leucine corresponding to the human residue 566 is in bold in all species. The alignment shows the significant conservation of the region between amino acids 563 and 575 (red box) containing a putative endocytosis motif (YXXL) as well as a polyproline core (PXXP) and 2 sequential dileucine (LL) motifs responsible for protein mis-localization when removed (Subramanian et al., 2009). The alignment also shows the PDZ binding motif (ETSL; green box) in the C-terminal tail (Nabokina et al., 2011). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Molecular findings

In Patient 2 and her parents, trio exome sequencing identified an average of 454 rare coding exonic, splice-site variants and coding InDels per individual, spanning approximately 370 different genes. The analysis also identified multiple small ROH (region of homozygosity) regions suggesting remote parental consanguinity. Family-based filtering under the hypothesis of a autosomal recessive disorder narrowed the number of candidates to two homozygous variants [NM_016239.3:c.3460T > C p.(Trp1154Arg) of *MYO15A* and NM_021095.2:c.1694dup p.(Leu566-Valfs*33) of *SLC5A6*]. WES did not highlight any additional potentially pathogenic variant. We tested the *MYO15A* and *SLC5A6* variants by Sanger sequencing in the DNA of additional family members, including Patients 1 and 3. Segregation analysis showed that the *SLC5A6* c.1694dup homozygous variant was shared by the three patients, and was heterozygous in both parents of Patients 1 and 2 (Fig. 2A and B), as well as in those of Patient 3, and in the healthy siblings of Patients 1–3 (data not shown). We excluded the *MYO15A* c.3460T > C variant from further investigations, as it was heterozygous in the DNA of Patient 1. The *SLC5A6* c.1694dup variant was not reported in either public allele frequency databases or our internal exome database and was located within a 7.3 Mb ROH of chromosome 2p23. The c.1694dup introduced a frame shift in the exon 16 resulting in a premature termination codon located 33 codons downstream. The variant was predicted to be damaging by MutationTaster (Steinhaus et al., 2021), and to escape nonsense-mediated decay (NMD) according to NMDEscPredictor (https://nmdprediction.shinyapps.io/nmdescpredictor/) and Variant Effect Predictor (McLaren et al., 2016). Since the *SLC5A6* gene is not expressed in accessible tissues like skin and blood, according to the gene expression databases GTEx Portal (https://gtexportal.org) and Human Protein Atlas (https://www.proteinatlas.org), we could not test *SLC5A6* mRNA in biological samples from our Patients. We submitted the variant's details to the public database DECIPHER (https://www.deciphergenomics.org; ID 510204).

4. Discussion

Systemic biotin deficiency may occur from nutritional causes or from genetic defects of enzymes involved in biotin homeostasis such as biotinidase or holocarboxylase synthetase (Elrefai and Wolf, 2014). Affected patients exhibit neurological disorders, growth delay, skin and hair changes, metabolic and immunological abnormalities, with a variable level of severity, partly depending on residual enzymatic activity. The symptoms of pantothenic acid and lipoate deficiency largely overlap with those of biotin deficiency, as these nutrients participate in the same pathways, and include growth delay, neurodegeneration, immune and intestinal defects, and loss of bone density (Hauth et al., 2022; Mayr et al., 2014).

Clinical findings of the patients with biallelic variants of *SLC5A6*, including our Patients, are summarized in Table 1. The clinical spectrum comprises mild to moderate developmental delay, epilepsy, and peripheral neuropathy. Systemic manifestations overlap those of vitamin defects-associated disorders, including growth delay, microcephaly, feeding and gastrointestinal problems, hair, and skin abnormalities, and immunodeficiency associated with recurrent infections. Severe metabolic decompensation with acidosis are potential life-threatening complications precipitated by infections. (Schwantje et al., 2019), as observed in Patient 3 who died at 17 months due to complications of a viral respiratory infection.

Most patients (8/13, 62%) manifested a spectrum of gastrointestinal dysfunctions, including GERD and haemorrhages, leading to early death in two of those who had not received vitamin supplementation therapy (Byrne et al., 2019). In patients with defects of *SLC5A6,* gastrointestinal dysfunctions might be caused by the reduced capacity of hSMVT in maintaining normal intestinal health and mucosal integrity. In the mice model, the intestinal-specific knock-out of *Slc5a6,* with consequently reduced biotin availability, caused alterations of the structure and permeability of intestinal epithelium, with shortened and dysplastic intestinal villi and chronic inflammation of the cecum (Ghosal et al., 2013; Sabui et al., 2016).

In two of our patients (Patients 1 and 2), gastrointestinal dysfunction manifested as multiple episodes of pseudo-obstruction due to severe impairment of propulsive motility within the gastrointestinal tract, further reducing nutrient absorption. Patient 3, instead, manifested GERD and recurrent diarrhoea.

Skin abnormalities, alopecia, and recurrent dermatitis represent a frequent finding in hSMVT deficiency (7/11, 64%). In Patient 2, perioricial dermatitis worsened coincidentally to periods of poor therapy adherence. In this patient, we also documented peripheral neuropathy, as previously reported in 6 individuals with biallelic *SLC5A6* variants (Byrne et al., 2019; Holling et al., 2022). Axonal irregularity and patchy denervation atrophy in peripheral nerves, demonstrated in a previously reported patient, led to the suggestion that reduced biotin, pantothenic acid and/or lipoate levels may be neurotoxic (Byrne et al., 2019; Holling et al., 2022).

In Patients 1 and 2, we documented optic atrophy, a finding not previously described in association with defects of the hSMVT but known to occur in approximately 13% of those with biotinidase deficiency due to *BTD* mutations (Salbert et al., 1993) in whom reduced biotin availability in turn causes reduced carboxylases activity with accumulation of organic acids in the retinal ganglion cells and consequent apoptosis (Huizing et al., 2005).

In Patient 2, we started early parenteral nutrition and intravenous supplementation of biotin and pantothenic acid on an empirical basis since we suspected a multivitamin-dependent disorder, as later supported by genetic testing. At last evaluation (age 24 years), the clinical picture was characterized by mild intellectual disability, gait disturbance, nystagmus, and tremor. This patient is the oldest reported to date carrying pathogenic *SLC5A6* variants and the only one with a follow-up lasting longer than twenty years.

Differences in phenotype severity in the reported patients might be determined by multiple variables, including the variants' functional effects, the age of ascertainment, and the timing of therapy initiation and adherence to it. Although only nine different variants of *SLC5A6* have been reported (Fig. 2C), it has been suggested that the most severe phenotype associated with life-threatening or fatal events is most likely caused by a null allele (e.g., an early truncation associated with NMD) *in trans* with a second allele producing a partially functioning hSMVT. Four of the five patients manifesting the mildest phenotype carry a homozygous missense p.(Ser429Gly) variant (Holling et al., 2022), possibly sparing most of the hSMVT vitamin transporter function. These individuals were ascertained in their adolescence because of motor neuropathy but had normal psychomotor development (Holling et al., 2022).

The c.1694dup p.(Leu566Valfs*33) observed in our patients, is located in the penultimate exon of *SLC5A6*, introducing a late frame shift and a premature termination codon. Due to its location, the variant is unlikely triggering NMD (Lindeboom et al., 2016), but is rather predicted to cause the loss of the COOH-terminal tail of the protein (Fig. 2D). Such domain bears conserved motifs that are necessary for trafficking the SLC5A6 protein to the apical cell membrane (Subramanian et al., 2009). Subramanian et al. (2009) demonstrated that removing the entire cytoplasmic tail (amino acids 553–635) completely abrogated the functionality of hSMVT, whereas the deletion of up to 15 amino acids (621–635) was sufficient to decrease the biotin transport by 20–30%. These authors also showed that SLC5A6 deletion mutants lacking the last 60–65 amino acids were retained within the endoplasmic reticulum, and identified a region approximately located between amino acids 563–575, including signals for membrane targeting in other transporter proteins (Subramanian et al., 2009). In the more distal C-terminal tail of the hSMVT, a PDZ-binding domain with the ETSL sequence (amino acids 632–635), allows the interaction of the

Table 1
Molecular and clinical features in patients with biallelic variants of *SLC5A6*. Abbreviations: NA, Not Available/Not Applicable; NR, Not Reported; GERD, gastro-oesophageal reflux disease; GI, gastro-intestinal; m, months; w, weeks; y, years.

Patients/Families	Subramanian et al., 2017 (Subramanian et al., 2017)	Schwantje et al., 2019 (Schwantje et al., 2019)	Byrne et al., 2019 (Family 1, Patient II-1) (Byrne et al., 2019)	Byrne et al., 2019 (Family 1, Patient II-2) (Byrne et al., 2019)	Holling et al., 2022 (Family 1) (Holling et al., 2022)
Variants [NM_021095.4]	c.[280C > T]; [368G > T], p.[(Arg94*)]; [(Arg123Leu)]	c.[422_423delTG]; [1865_1866del], p.[(Val141Alafs*34)]; [(Gln622Argfs*51)]	c.[422_423delTG]; [1199G > C], p.[(Val141Alafs*34)]; [(Arg400Thr)]	c.[422_423delTG]; [1199G > C], p.[(Val141Alafs*34)]; [(Arg400Thr)]	c.[1285A > G]; [1285A > G], p.[(Ser429Gly)]; [(Ser429Gly)]
Sex	M	F	F	M	F
Age at clinical onset	1m	10m	1y 2m	1y	13y 6m
Age last follow-up	2y 9m	3y	2y 7m	10y 5m	15y 8m
Microcephaly	Yes	NR	Yes	Yes	No
DD	Yes	Yes	Yes	Yes	No
Other neurological features	Nystagmus	NR	Ataxia, dyskinetic movements	Ataxia, dyskinetic movements, nystagmus	No
Seizures	No	No	No	Yes	No
Peripheral neuropathy	NR	No	NR	Mixed demyelinating and axonal sensorimotor polyneuropathy	Focal demyelination in the right upper extremity
Brain MRI (age, findings)	12m, thin corpus callosum, cerebraland brainstem (pontine) atrophy	17m, suspected mild ischemic changes	2y, right cerebellar haemorrhagic foci, T2/FLAIR signal hyperintensity (periventricular and parieto-occipital white matter)	7y 5m, cerebral and cerebellar atrophy (progressive), brainstem (pontine) atrophy, thin corpus callosum, T2/FLAIR signal hyperintensity (central segmental tract & peritrigonal regions), mega cisterna magna	NA
Recurrent infections	Yes	No	NR	NR	No
Immunodeficiency	Yes	No	NR	Yes, isolated IgG deficiency	NR
Feeding problems/GI dysfunctions	Yes, feeding difficulties requiring nasogastric tube	Yes, frequent vomiting and GI haemorrhage (Mallory–Weiss tear)	Yes, GERD and GI haemorrhage	Yes, frequent vomiting, GERD and GI haemorrhage	NR
Hair and skin abnormalities/ Dermatitis	No	No	No	No	Dry skin
Age at initiation of therapy	1y 7m	1y 5m	NA	7y 1m	15y 1m
Therapy – max dosage	Biotin 30 mg/day, pantothenic acid 500 mg/day, lipoic acid 300 mg/day	Biotin 10 mg twice a day, pantothenic acid 250 mg/day	NA	Biotin (intramuscular) 10 mg weekly; Dexpanthenol (intramuscular) 250 mg weekly; lipoic acid (intravenous) 300 mg weekly	Biotin 100 mg/day; pantothenic acid 500 mg/day; lipoic acid 300 mg/day
Clinical outcome	Significant growth, motor, and language improvements	Improving of growth and feeding problems; DD unchanged	Deceased at 2y 7m due to acute gastro-intestinal haemorrhage following perforation of a duodenal ulcer	Motor and cognitive improvement, seizure free, resolution of peripheral neuropathy, reduction of vomiting	Motor improvement (hand grip and finger strength)

Holling et al., 2022 (Family 1) (Holling et al., 2022)	Holling et al., 2022 (Family 1) (Holling et al., 2022)	Holling et al., 2022 (Family 2) (Holling et al., 2022)	Holling et al., 2022 (Family 3) (Holling et al., 2022)	Hauth et al., 2022 (Hauth et al., 2022)	This study, Patient 1	This study, Patient 2	This study, Patient 3	Summary
c.[1285A > G]; [1285A > G], p. [(Ser429Gly)]; [(Ser429Gly)]	c.[1285A > G]; [1285A > G], p. [(Ser429Gly)]; [(Ser429Gly)]	c.[280C > T]; [485A > G], p. [(Arg94*)]; [(Tyr162Cys)]	c.[1285A > G]; [1285A > G], p. [(Ser429Gly)]; [(Ser429Gly)]	c.[1005+1G > A]; [1865_1866del], p. [(Phe336Serfs*57)]; [(Gln622Argfs*51)]	c.[1694dup]; [1694dup], p. [(Leu566Valfs*33)]; [(Leu566Valfs*33)]	c.[1694dup]; [1694dup], p. [(Leu566Valfs*33)]; [(Leu566Valfs*33)]	c.[1694dup]; [1694dup], p. [(Leu566Valfs*33)]; [(Leu566Valfs*33)]	
F	M	F	F	F	F	F	M	4M,9F
10y	6y 9m	8y 5m	8y	1w	6m	2y 6m	1y	1w-13y 6m (5.2y average)
13y 6m	8y 6m	10y	13y 9m	5y	3y	24y	1y 5m	6m-24y (8.7y average)
No	No	No	No	Yes	NR	No	No	4/11
No	No	Yes	No	No	Yes	Yes	Yes	8/13
No	No	No	Mild cerebellar signs including gaze evoked nystagmus, dysdiadochokinesia	No	No	Nystagmus, kinetic and postural tremor	No	5/12
No	No	No	No	No	No	Yes	No	2/13
Motor neuropathy	Demyelinating and axonal features in the upper extremities	Mild motor neuropathy	Axonal and demyelinating motor findings	No	NA	Sensory polyneuropathy	NA	7/9
NA	NA	NA	NA	NA	9m and 2y, normal	20y, thinning of optic nerves, bilaterally	13m, mildly delayed myelination	6/7 mild aspecific abnormalities
No	No	No	No	No	Yes	Yes	Yes	4/11
NR	NR	NR	NR	No	No	No	Neutropenia	3/7
NR	NR	NR	NR	Frequent vomiting, GERD, diarrhoea	Recurrent vomiting and diarrhoea, intestinal pseudoobstruction	Intestinal pseudoobstruction	Yes, GERD, recurrent diarrhoea	8/13
Premature greying of hair (4m)	Dry skin	NR	NR	Dry, eczematous skin	Alopecia and dermatitis	Alopecia and dermatitis	Dermatitis	7/11
12y 10m	7y 10m	9y 3m	13y 6m	2y	NA	2y 6m	NA	1y 5m-13y 6m (7.3y average)
Biotin 100 mg/day; pantothenic acid 500 mg/day; lipoic acid 300 mg/day	Biotin 100 mg/day; pantothenic acid 500 mg/day; lipoic acid 300 mg/day	Biotin 10 mg twice a day; pantothenic acid 250 mg twice a day; lipoic acid 150 mg twice a day	Biotin 10 mg three times a day; lipoic acid 100 mg twice a day	Biotin 15 mg; pantothenic acid 300 mg; lipoic acid 300 mg	NA	Before genetic diagnosis: biotin 105 mg/day, pantothenic acid 30 mg/day, lipoic acid 600 mg/day. After genetic diagnosis (20y): biotin 40 mg/day, pantothenic acid 1000 mg/day, lipoic acid 600 mg/day	NA	
Motor improvement (hand grip and finger strength)	No significant improvements	Improvement in weight, height, balance, muscle strength, and exercise tolerance	Improvement in gait and coordination	Improvement of growth and development, no further metabolic decompensations and vomiting	Deceased at 3y due to complications of an acute intestinal pseudo-obstruction episode	Stable clinical picture: mild ataxia, nystagmus, tremor, and sensory polyneuropathy	Deceased at 1y 5m due to complications of a viral respiratory infection	3/13 deceased (no therapy); 10/13 improved/stabilized outcome (on therapy)

transporter with the PDZD11 protein, which is important for delivery and/or retention of the hSMVT at the plasma membrane (Nabokina et al., 2011). The c.1694dup variant disrupts the reading frame from Leucine 566, removing such motifs (Fig. 2D) and possibly impairing membrane targeting and transport activity of the hSMVT. Although we could not test the *SLC5A6* mRNA in biological samples from our Patients, the clinical overlap with previously reported patients with hSMVT deficiency, including the response to vitamin supplementation therapy, segregation in the family, and exclusion of further pathogenic/likely pathogenic variants in WES data, all support a pathogenic role of the homozygous variant.

Defects of hSMVT are overlooked by the most widely applied screening protocols for biotinidase deficiency (Heard et al., 1984). Although no clear biochemical marker is available for the screening of hSMVT defects, we observed intermittent lactic acidosis and increased urinary 3-hydroxyisovaleric acid in Patient 1, and fluctuating hyperalaninaemia and hyperlactacidemia in Patient 2. In Patient 2 the Acylcarnitine profile was normal, but parenteral supplementation of biotin and pantothenic acid was already provided at the time of the test. Metabolic abnormalities in urine and plasma suggested a defect of the biotin metabolism in two previously reported patients with pathogenic biallelic *SLC5A6* variants (Hauth et al., 2022; Schwantje et al., 2019).

In conclusion, the family we have described further extends the phenotypic spectrum associated with *SLC5A6* biallelic variants. In the surviving Patient 2, carrying the same homozygous c.1694dup variant of her deceased sister (Patient 1) and first-degree cousins (Patient 3), supplementation of biotin and pantothenic acid radically changed the natural clinical course of the disorder. Targeted vitamin supplementation with biotin, pantothenic acid, and lipoic acid seems to be the most effective treatment in patients with defects of *SLC5A6*, as it can partially rescue both growth and psychomotor outcome in younger patients and stabilize the clinical picture in older patients. The transient clinical worsening observed in Patient 2 in relation to poor therapy compliance confirms the vitamin-dependent nature of the disorder.

As for other disorders of biotin metabolisms, early diagnosis of *SLC5A6* defects is crucial, as it allows early initiation of a specific supplementation therapy that can prevent acute life-threatening events and the irreversible damage to auditory, visual, or central nervous system functions.

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CRediT authorship contribution statement

Martino Montomoli: Conceptualization, Formal analysis, Investigation, Writing – original draft. **Annalisa Vetro:** Conceptualization, Formal analysis, Investigation, Writing – original draft. **Flavia Tubili:** Investigation. **Maria Alice Donati:** Investigation, Writing – original draft. **Marta Daniotti:** Investigation. **Francesca Pochiero:** Investigation. **Francesca Rivieri:** Investigation. **Salvatore Girlando:** Investigation. **Renzo Guerrini:** Conceptualization, Funding acquisition, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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References

Byrne, A.B., Arts, P., Polyak, S.W., et al., 2019. Identification and targeted management of a neurodegenerative disorder caused by biallelic mutations in SLC5A6. npj Genomic Med. 4 (1). https://doi:10.1038/s41525-019-0103-x.

Elrefai, S., Wolf, B., 2014. Disorders of Biotin Metabolism. Elsevier Inc.

Ghosal, A., Lambrecht, N., Subramanya, S.B., Kapadia, R., Said, H.M., 2013. Conditional knockout of the Slc5a6 gene in mouse intestine impairs biotin absorption. Am. J. Physiol. Gastrointest. Liver Physiol. 304 (1), 64–71. https://doi:10.1152/ajpgi.00379.2012.

Hauth I, Waterham H, Wanders RJ, van der Crabben S, van Karnebeek CD. A mild case of SMVT deficiency illustrating the importance of treatment response in variant classification Mol. Case Stud. [published online ahead of print: 2022]; https://doi:10.1101/mcs.a006185.

Heard, G.S., Secor McVoy, J.R., Wolf, B., 1984. A screening method for biotinidase deficiency in newborns. Clin. Chem. 30 (1). https://doi:10.1093/clinchem/30.1.125.

Holling, T., Nampoothiri, S., Tarhan, B., et al., 2022. Novel biallelic variants expand the SLC5A6-related phenotypic spectrum. Eur. J. Hum. Genet. 30 (4), 439–449. https://doi:10.1038/s41431-021-01033-2.

Huizing, M., Brooks, B.P., Anikster, Y., 2005. Optic atrophies in metabolic disorders. Mol. Genet. Metabol. 86 (1–2). https://doi:10.1016/j.ymgme.2005.07.034.

Lindeboom, R.G.H., Supek, F., Lehner, B., 2016. The rules and impact of nonsense-mediated mRNA decay in human cancers. Nat. Genet. 48 (10). https://doi:10.1038/ng.3664.

Mayr, J.A., Feichtinger, R.G., Tort, F., Ribes, A., Sperl, W., 2014. Lipoic acid biosynthesis defects. J. Inherit. Metab. Dis. 37 (4), 553–563. https://doi:10.1007/s10545-014-9705-8.

McLaren, W., Gil, L., Hunt, S.E., et al., 2016. The ensembl variant effect predictor. Genome Biol. 17 (1). https://doi:10.1186/s13059-016-0974-4.

Nabokina, S.M., Subramanian, V.S., Said, H.M., 2011. Association of PDZ-containing protein PDZD11 with the human sodium-dependent multivitamin transporter. Am. J. Physiol. Gastrointest. Liver Physiol. 300 (4). https://doi:10.1152/ajpgi.00530.2010.

Neophytou, C., Pitsouli, C., 2022. Biotin controls intestinal stem cell mitosis and host-microbiome interactions. Cell Rep. 38 (10), 110505. https://doi:10.1016/j.celrep.2022.110505.

Quick, M., Shi, L., 2015. The sodium/multivitamin transporter. A multipotent system with therapeutic implications. In: Vitamins and Hormones.

Sabui, S., Bohl, J.A., Kapadia, R., et al., 2016. Role of the sodium-dependent multivitamin transporter (SMVT) in the maintenance of intestinal mucosal integrity. Am. J. Physiol. Gastrointest. Liver Physiol. 311 (3), G561–G570. https://doi:10.1152/ajpgi.00240.2016.

Sabui, S., Kapadia, R., Ghosal, A., et al., 2018. Biotin and pantothenic acid oversupplementation to conditional SLC5A6 KO mice prevents the development of intestinal mucosal abnormalities and growth defects. Am. J. Physiol. Cell Physiol. 315 (1), C73–C79. https://doi:10.1152/ajpcell.00319.2017.

Salbert, B.A., Astruc, J., Wolf, B., 1993. Ophthalmologic findings in biotinidase deficiency. Ophthalmologica 206 (4). https://doi:10.1159/000310387.

Schwantje, M., de Sain-van der Velden, M., Jans, J., et al., 2019. Genetic defect of the sodium-dependent multivitamin transporter: a treatable disease, mimicking biotinidase deficiency. JIMD Rep. 48 (1), 11–14. https://doi:10.1002/jmd2.12040.

Steinhaus, R., Proft, S., Schuelke, M., et al., 2021. MutationTaster 2021. Nucleic Acids Res. 49 (W1). https://doi:10.1093/nar/gkab266.

Subramanian, V.S., Marchant, J.S., Boulware, M.J., Ma, T.Y., Said, H.M., 2009. Membrane targeting and intracellular trafficking of the human sodium-dependent multivitamin transporter in polarized epithelial cells. Am. J. Physiol. Cell Physiol. 296 (4), 663–671. https://doi:10.1152/ajpcell.00396.2008.

Subramanian, V.S., Constantinescu, A.R., Benke, P.J., Said, H.M., 2017. Mutations in SLC5A6 associated with brain, immune, bone, and intestinal dysfunction in a young child. Hum. Genet. 136 (2), 253–261. https://doi:10.1007/s00439-016-1751-x.

Uchida, Y., Ito, K., Ohtsuki, S., et al., 2015. Major involvement of Na+-dependent multivitamin transporter (SLC5A6/SMVT) in uptake of biotin and pantothenic acid by human brain capillary endothelial cells. J. Neurochem. 134 (1), 97–112. https://doi:10.1111/jnc.13092.

Vetro, A., Pisano, T., Chiaro, S., et al., 2020. Early infantile epileptic-dyskinetic encephalopathy due to biallelic PIGP mutations. Neurol. Genet 6 (1). https://doi:10.1212/NXG.0000000000000387.