A mild case of sodium-dependent multivitamin transporter (SMVT) deficiency illustrating the importance of treatment response in variant classification

Ingeborg Hauth,[1] Hans R. Waterham,[2,3] Ronald J.A. Wanders,[2,3] Saskia N. van der Crabben,[3,4] and Clara D.M. van Karnebeek[3,4,5]

[1]Department of Pediatrics (Metabolic Diseases), Amalia Children's Hospital, Radboud University Medical Center, 6525 GA Nijmegen, The Netherlands; [2]Laboratory Genetic Metabolic Diseases, Department of Clinical Chemistry, Amsterdam University Medical Centers—University of Amsterdam, 1105 AZ Amsterdam, The Netherlands; [3]United for Metabolic Diseases, 1105 AZ Amsterdam, The Netherlands; [4]Department of Human Genetics, University Medical Centers, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands; [5]Department of Pediatrics, Emma Children's Hospital, Amsterdam University Medical Centers, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands

Abstract Sodium-dependent multivitamin transporter (SMVT) deficiency is a recently described multivitamin-responsive inherited metabolic disorder (IMD) of which the phenotypic spectrum and response to treatment remains to be elucidated. So far, four pediatric patients have been described in three case reports with symptoms ranging from severe neurodevelopmental delay to feeding problems and failure to thrive, who demonstrated significant improvement after initiation of enhancement of targeted multivitamin treatment (biotin, pantothenic acid, and lipoic acid). We describe a fifth case of a patient presenting at the relatively mild end of the phenotypic spectrum with failure to thrive, frequent vomiting and metabolic acidosis with hypoglycemia, and mild osteopenia, who was diagnosed with SMVT deficiency due to compound heterozygous variants in *SLC5A6*. Additional genetic testing of variants of unknown significance (VUSs) as well as the clinical improvement in all aspects of the patient's disease upon initiation of treatment with biotin and pantothenic acid (plus lipoate as antioxidant) aided in the confirmation of this diagnosis. This case report aims to enhance recognition of the broad phenotypic spectrum of SMVT deficiency due to *SLC5A6* mutations and discusses the different treatment strategies. It demonstrates how combining biochemical and genetic testing with the evaluation of (early) treatment response (i.e., using a "diagnostic therapeuticum") can influence confirmation of pathogenicity of genomic variants.

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Corresponding author:
c.d.vankarnebeek@
amsterdamumc.nl

INTRODUCTION

Multivitamin-Responsive IMDs

Inherited metabolic disorders (IMDs) comprise a continuously expanding class of rare genetic disorders, of which more than 1500 have been identified since the first IMD alkaptonuria was proposed in 1902 by Sir Archibald Garrod (Garrod 1902; www.iembase.org). Because of

the rapidly progressing developments in the field of genetics, with expanding knowledge of the underlying pathophysiology caused by the impairment in the metabolic pathway(s) involved, Ferreira et al. (2019, 2021) proposed an up-to-date nosology (International Classification of Inherited Metabolic Disorders [ICIMD]; www.icimd.org), consisting of a hierarchical, group-based classification of all currently known IMDs. Within this classification, the disorders of vitamin and cofactor metabolism account for 73 IMDs, encompassing a relatively large, potentially treatable group of IMDs because vitamin supplementation is usually readily available and safe and has been proven to be very efficient, especially if given early on in the disease course (Ferreira et al. 2019, 2021; Mandia et al. 2021). Therefore, timely recognition and knowledge of treatment strategies is paramount. Reviewing the treatment strategies of these 73 IMDs shows that, thus far, only a few of those IMDs are known to be multivitamin-responsive—that is, treatable by supplementation of two or more vitamins. One example of such an IMD is biotin-thiamine-responsive basal ganglia disease (BTBGD), which is characterized by (subacute) encephalopathy, seizures and other neurological symptoms, presenting in childhood, early infancy, or adulthood (Tabarki et al. 2013; Ortigoza-Escobar et al. 2014). Biotin and thiamine are given as early in the disease course as possible and are continued lifelong. Here, we present an illustrative case vignette of another such treatable multivitamin-responsive IMD: the recently reported sodium-dependent multivitamin transporter (SMVT) deficiency. The phenotype, genotype, and treatment response of our patient are compared to other cases described in literature. By sharing knowledge and the diagnostic process of single cases affected by this rare disease and comparing treatment strategies, we aim to pave the way for future recognition and treatment of SMVT-deficient patients. Moreover, it demonstrates the importance of phenotype enhanced genotyping.

What Do We Know about SMVT Deficiency Currently?

Among the disorders of vitamin metabolism, SMVT deficiency is a recently described inherited metabolic disorder with so far a broad phenotypic spectrum ranging from feeding problems, failure to thrive, metabolic acidosis, and mild to severe neurological deficits. Responses to treatment differ between cases. SMVT is encoded by *SLC5A6* located on Chromosome 2p23.3. This transmembrane protein transports the water-soluble vitamins pantothenic acid (vitamin B5) and biotin (vitamin B7) and the metabolite lipoate in the presence of sodium in both the digestive system and across the blood–brain barrier (Balamurugan et al. 2003; Sabui et al. 2018). Having recessive variants in *SLC5A6* causing SMVT deficiency is a recently described inherited metabolic disorder. Clinically, the disease mimics biotinidase deficiency with a broad phenotypic spectrum ranging from feeding problems, failure to thrive, metabolic acidosis, and mild to severe neurological deficits (Schwantje et al. 2019).

Biotin is an essential micronutrient, vital in normal cellular metabolism, growth, and development, as well as acting as an essential cofactor in the different carboxylase enzymes known to be active in various metabolic pathways, including gluconeogenesis (pyruvate carboxylase), catabolism of amino acids (propionyl-CoA carboxylase and 3-methylcrotonyl-CoA carboxylase), and fatty acid synthesis (acetyl-CoA carboxylase) (McMahon 2002; Said 2012). In addition, biotin plays a role in energy metabolism, cellular oxidative stress regulation, and gene expression, as well as enabling normal immune functioning (Rodriguez-Melendez and Zempleni 2003; Madsen et al. 2015). Deficiency of biotin can lead to a variety of clinical symptoms including failure to thrive, neurological disorders such as ataxia, developmental delay and seizures, and impairment in bone development, as well as dermatological features (Mock et al. 1981; Wolf 2012; Ghosal et al. 2013).

Pantothenic acid is also an essential micronutrient, functioning as a precursor of coenzyme A, which in turn plays an indispensable role in fatty acid oxidation, and to a lesser extent

in carbohydrate and protein metabolism (Lederer et al. 1971; Said 2011). Pantothenic acid deficiency leads to disturbed intermediary metabolism; that is, it impairs CoA biosynthesis, stimulates polyol-pathway activity, impairs glycolysis and tricarboxylic acid cycle activity, and modifies urea metabolism, as evident on organic acid, amino acid, and acylcarnitine profiles. Lipoate is one of the cofactors in the glycine cleavage system and the pyruvate dehydrogenase, branched chain ketoacid dehydrogenase, and ketoglutarate dehydrogenase complexes. It catalyzes redox reactions in the mitochondrial energy production, enabling oxidative decarboxylation reactions of amino acids and keto acids, as well as providing antioxidative, and anti-inflammatory effects (Morikawa et al. 2001). Lipoate deficiency is associated with encephalopathy and other neurological disorders as well as low bone density (Mayr et al. 2011; Roberts and Moreau 2015).

Ghosal et al. (2013) found that mice with intestine-specific deletion of *SMVT* either died prematurely or displayed significant growth retardation, decreased bone density and length, and lethargic behavior compared to controls. Sabui et al. (2018) showed that intestinal-specific SMVT knockout of the mouse was associated with growth retardation. They also developed spontaneous and severe inflammation causing early death. All clinical features were completely reversed by biotin and pantothenic acid supplementation.

CASE VIGNETTE OF SMVT DEFICIENCY

Case Report

Our case is a currently 5-yr-old girl. She was born as the first child to healthy, nonconsanguineous parents after an uncomplicated term pregnancy. She was noted as small for gestational age (SGA) at 2650 grams (<P5), but further physical examination revealed no dysmorphic features or microcephalia. Soon after birth, feeding difficulties became apparent with frequent vomiting and failure to thrive. Antacid treatment was started for suspected gastroesophageal reflux disease, with temporary positive effect. Her psychomotor development was age-appropriate, albeit she was described as somewhat clumsy. She was easily fatigued apparently because of a lack of energy and had very dry, eczematous skin. Laboratory findings showed mild osteopenia for which supplements of vitamin D and calcium were started. She was frequently hospitalized the first 2 years of her life because of persistent vomiting, diarrhea, and insufficient oral intake resulting in dehydration and ketotic hypoglycemia requiring nasogastric (NG) or intravenous rehydration. From the age of 1.5 to 2 yr old, she was fed through an NG and nasoduodenal (ND) tube. At 2 yr of age she was admitted to the pediatric intensive care unit (PICU) with severe metabolic decompensation (pH 7.10, lactate 3 mmol/L, glucose 1.7 mmol/L, ketones 6 mmol/L) in the course of a gastrointestinal viral infection. She recovered from this episode but suffered from subsequent periods of cyclic vomiting with a tendency to ketoacidosis. Extensive additional testing was done, with high suspicion of an underlying metabolic disorder.

Diagnostic Confirmation and Treatment

Metabolic screening performed in plasma and urine showed nonspecific subtle elevations of various organic acids including 3-hydroxyisovaleric acid, lactic acid, and 3-hydroxybutyrate. Elevated lactate levels as observed in plasma and urine of the patient points to the impaired degradation of pyruvate and is a characteristic finding in patients with biotinidase deficiency as well as in patients with a defect in the biosynthesis of lipoic acid and, in fact, patients with a defect in the biosynthesis of coenzyme A as in PKAN2 deficiency. Furthermore, 3-hydroxyisovaleric acid is one of these typical metabolites that is elevated in biotinidase deficiency. Chromosomal microarray did not reveal pathogenic copy-number variants. Trio-based

Table 1. Variant table

Gene	Chromo-some	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP/dbVar ID	Genotype	ClinVar ID	Parent of origin	Observed effect (if shown to be different from predicted effect)
SLC5A6	2	c.1005+1C>T (NM_021095.4)		Splice donor	Splicing defect	Rs565711489	Heterozygous	SCV002097395	Mater-nal	Retention of 61 bp of intron 9 resulting in p.(Phe336Serfs*57)
SLC5A6	2	c.1865_1866del (NM_021095.4)	p.(Gln622Argfs*51)	Deletion	Deletion	Rs774193816	Heterozygous	SCV002097396	Pater-nal	NA

whole-exome sequencing (trio-WES) was performed in the EN-ISO 15189:2012 certified clinical DNA diagnostics laboratory Amsterdam UMC Genome Diagnostics (AGDx). This revealed compound heterozygous potential pathogenic variants in *SLC5A6* (NM_021095.4): c.1005 + 1G > A, predicted to affect splicing, and c.1865_1866del, introducing a shift of the reading frame (p.(Gln622Argfs*51)) (Table 1). Subsequent *SLC5A6* cDNA analysis, performed on reverse transcription (RT)-transcribed mRNA isolated from cultured primary skin fibroblasts of the patient, confirmed the incorrect splicing of intron 9 because of the c.1005 + 1G > A variant resulting in retention of 61 bp of intron 9 and predicted to result in a nonfunctional protein (p.(Phe336Serfs*57)). In addition, the mis-spliced allele was less abundant, which may be because of nonsense-mediated mRNA decay (see Supplemental Fig. S1). This variant was reported 1/152142 in gnomAD v3.1.2 (https://gnomad .broadinstitute.org/) and 1/996 in the Genome of the Netherlands (GoNL) database (https:// www.nlgenome.nl/). Based on these findings we classify the variant as pathogenic (PVS1, PS3_Supporting, PM3_Supporting, PM2_Supporting, PP4) (Richards et al. 2015). The c.1865_1866del variant results in a shift of reading frame as a consequence of which the carboxy-terminal 13 amino acids will be substituted by 50 other amino acids (p.(Gln622Argfs*51)). The carboxyl terminus of SMVT is not well-conserved among species so the consequences of this variant for protein functioning remain unclear. Because it was previously reported in another patient with SMVT deficiency (Schwantje et al. 2019), we considered this variant likely pathogenic (PM3, PM4, PM2_Supporting, PP4) (Richards et al. 2015). Its frequency was reported 15/152128 in gnomAD v3.1.2.

Based on this diagnosis, our patient was started on oral supplementation of biotin, pantothenic acid, and, as antioxidant, lipoic acid, which were gradually increased to once daily at doses of 15, 300, and 300 mg, respectively. On this targeted multivitamin treatment, vomiting ceased. There have been no more metabolic decompensations or hospital admissions, even with subsequent infections. The patient's growth and development have improved and normalized, as have her overall condition and exercise tolerance. The positive response on vitamin supplementation confirms the clinical and genetic diagnosis.

Comparison to Other Described SMVT Deficiency Cases

To the best of our knowledge, only three other case reports have appeared in which SMVT deficiency was reported due to pathogenic *SLC5A6* variants (see Table 2 for a detailed comparison). Subramanian et al. (2017) describe a severely affected patient with significant developmental delay, failure to thrive, severe gastroesophageal reflux, variable immunodeficiency, and osteoporosis. After oral supplementation was started and eventually optimized, the infant improved in growth and verbal and motor development and his immune status normalized.

Schwantje et al. (2019) presented a case of a then 3-yr-old girl with a delay in gross motor development, frequent vomiting, chronic diarrhea, and failure to thrive in the first years of

Table 2. Clinical features and treatments

Clinical features	Subramanian et al. 2017	Schwantje et al. 2019	Byrne et al. 2019 I-1	Byrne et al. 2019 I-2	This study
Neurological					
Neurocognitive regression	Yes, onset infantile	No	Yes, onset 14 months	Yes, onset 12 months	No
Microcephaly	Yes	No	Yes, relative	Yes, relative	No
Spasticity	Yes	No	No	No	No
Gross motor development	Profound delay	Delayed	Profound delay	Profound delay	Normal, but clumsy
Seizures	NR	No	No	Yes	No
Peripheral neuropathy	NR	No	NR	Yes, mixed demyelinating and axonal sensorimotor polyneuropathy	No
Neuroimaging (MRI)	Cerebral atrophy, brainstem (pontine), atrophy, thin corpus callosum	No abnormalities	No cerebral atrophy, right cerebellar hemorrhagic foci, T2/FLAIR signal hyperintensity (periventricular and parieto-occipital white matter)	Cerebral atrophy (progressive), cerebellar atrophy (progressive), brainstem (pontine) atrophy, thin corpus callosum, T2/FLAIR signal hyperintensity (central segmental tract and peritrigonal regions), mega cisterna magna	Not done
Electroencephalogram (EEG)	Normal	Not done	Not done	Background slowing (encephalopathy), epileptiform activity: generalized and multifocal spike wave (2–3 Hz)	Not done
Histopathology	Skeletal muscle biopsy: normal	NR	Central nervous system: axonal spheroids; peripheral nervous system: undefined thickening; skeletal muscle biopsy: denervation atrophy	Cutaneous biopsy: membranous cytoplasmic inclusions	Not done
Gastrointestinal					
Feeding difficulties/ failure to thrive	Yes	Yes	Yes, bulbar dysfunction	Yes, bulbar dysfunction	Yes
Nasogastric tube/ gastrostomy feeding	Yes	Yes	Yes	Yes	Yes
GI hemorrhage	Yes	Yes	Yes	Yes	No

(Continued on next page.)

Table 2. (*Continued*)

Clinical features	Subramanian et al. 2017	Schwantje et al. 2019	Byrne et al. 2019 I-1	Byrne et al. 2019 I-2	This study
Other	NR	GORD	GORD	Cyclical vomiting, GORD	Cyclical vomiting, GORD
Other					
Hypogammaglobulinemia	Yes, IgG/IgA deficiency	NR	NR	Yes, isolated IgG deficiency	No
Osteopenia	Yes	No	No	No	Yes
Birthweight	Normal	Normal	Normal	Normal	SGA
Metabolic acidosis with hypoglycemia	No	Yes	No	No	Yes
Easily fatigued	NR	NR	NR	NR	Yes
Dry eczematous skin	NR	NR	NR	NR	Yes
Genetic mutation *SLC5A6*					
	c.280C > T, p.(Arg94Ter) and c.368 G > T, p.(Arg123Leu)	c.422_423del, p.(Val141fs) and c.1865_1866del, p.(Gln622Argfs*51)	c.422_423del, p.(Val141fs) and c.1199G > C, p.(R400T)	c.422_423del, p.(Val141fs) and c.1199G > C, p.(R400T)	c.1005 + 1G > A, p.(?) and c.1865_1866del, p.(Gln622Argfs*51)
Treatment					
	Biotin (oral) 10–30 mg/day, pantothenic acid (oral) 250–500 mg/day, α-lipoic acid (oral) 150–300 mg/day	Biotin (oral) 10 mg twice a day, panthothenic acid (oral) 250 mg once a day	No (deceased)	Biotin (i.m.) 10 mg weekly, dexpanthenol (i.m.) 250 mg weekly, α-lipoic acid (i.v.) 300 mg weekly	Biotin (oral) 15 mg once a day, pantothenic acid (oral) 300 mg once a day, lipoic acid (oral) 300 mg once a day

(MRI) Magnetic resonance imaging, (GI) gastrointestinal, (GORD) gastroesophageal reflux disease, (NR) not reported, (SGA) small for gestational age.

life, accompanied by a delay in gross motor development. Oral treatment resulted in somatic growth normalization and resolution of diarrhea. However, so far her delay in motor development persisted.

Byrne et al. (2019) described two siblings with a profound neurodevelopmental delay during infancy, with progressive ataxia, dyskinesia, and epilepsy. Severe reflux and failure to thrive were also present. At 2 yr of age, one of the siblings died of acute gastrointestinal hemorrhage following perforation of a duodenal ulcer. After initiating treatment of the second sibling, both neurological and gastrointestinal symptoms improved.

DISCUSSION AND FUTURE DIRECTIONS

Diagnostic Difficulties and Treatment Strategies

Confirmation of diagnosis in our case was established through a combination of strategies. RNA analysis was performed showing aberrant splicing of one of the *SLC5A6* variants. Initiation of therapy by targeted supplementation of biotin, pantothenic acid and lipoic acid led to significant improvement of growth, development, and overall condition, comparable to the other described cases. Although several enzymes require covalently bound

lipoamide, free lipoate is not capable of participating in this modification, at least in mammals. Indeed, the phenotype of the SVMT-deficient c-KO mouse is rescued by pantothenate and biotin without lipoate (Sabui et al. 2018). Oral administration of these supplements is well-tolerated and safe, and certainly less invasive; parenteral administration is indicated in case of vomiting, encephalopathy, and/or possible metabolic decompensation.

Our patient also had no further metabolic decompensations. In case of patients with a suspected inherited metabolic disorder but with only variants of unknown significance and/or likely pathogenic variants, in addition to performing further additional genetic testing, treating physicians could think about starting treatment in case potential benefits of treatment might outweigh the possible side effects of treatment. In case treatment diminishes clinical symptoms, preferably in different, but similar affected, not-related patients, we suggest this might be added to the classification of the identified genetic variants, in accordance with the paper by Shen et al. (2020). Importantly, such early initiation of treatment can prevent further, irreversible, organ damage.

Comparison to Other Described SMVT Deficiency Cases

When comparing all five cases, all of the patients suffered from feeding difficulties, frequent vomiting, and failure to thrive. The patients of Subramanian et al. and Byrne et al. were severely impaired neurologically, whereas our patient and that of Schwantje et al. (2019) were only described as being somewhat clumsy or with mild delays in gross motor development. This milder presentation may be related to the associated genetic variants (Byrne et al. 2019). The c.1865_1866del (p.(Gln622Argfs*51)) variant, shared by both patients, is predicted to only affect the extreme carboxyl terminus of the encoded protein and thus likely results in a protein with residual catalytic activity. Unfortunately, the activity of the protein cannot be easily assessed to confirm this. All patients except one, the first and deceased sibling described by Byrne et al., have received treatment since diagnosis. Dosages were based on the initial case description by Subramanian et al. with some variation. Subramanian et al. suggest that in case of impaired intestinal absorption or cellular uptake of biotin and pantothenic acid (and lipoic acid), as in SMVT deficiency, uptake of these nutrients by different cells at high supraphysiologic concentrations occurs via simple diffusion, thereby (at least partially) ameliorating the SMVT system. It is notable that the reported cases that were mild in nature—that is, the patient reported by Schwantje et al. (2019) and the current patient—experienced metabolic decompensations, whereas the (other) more severe cases did not. There are several possible explanations including variability in environmental triggers and severe intercurrent illness, as well as a difference in available clinical history data—that is, decompensations may have been missed.

METHODS

Medical and laboratory records were searched for data collection and extraction. DNA extraction and RNA analysis were performed according to the methods described by Maxit et al. (2017).

ADDITIONAL INFORMATION

Data Deposition and Access

Patient consent to deposit raw requencing data was not granted. Data on the variants and phenotype are deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession numbers SCV002097395 and SCV002097396.

Ethics Statement

Parents of the patient have provided written consent for publication of this case report. The Medical Ethics Board at Amsterdam UMC does not require separate study approval for the publication of single case studies.

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Author Contributions

I.H. collected clinical and genetic data, wrote the first draft, and revised and finalized the manuscript. S.N.v.d.C contributed to patient phenotyping and diagnosis and supervised data collection and drafting of the manuscript. H.R.W. performed the genetic variant interpretation and RNA studies and contributed to drafting and revision of the manuscript. R.J.A.W. performed the biochemical and metabolic data interpretation and contributed to drafting and revision of the manuscript. C.D.M.v.K. designed the study, supervised patient diagnostics, treatment, and follow-up, and supervised data collection and drafting and revision of the manuscript.

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