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REVIEW

Uncovering the Relationship Between Genes and Phenotypes
Beyond the Gut in Microvillus Inclusion DiseaseMingyue Sun,¹ Olena Pylypenko,² Zhe Zhou,¹ Mingqian Xu,¹ Qinghong Li,¹ Anne Houdusse,³ and Sven C. D. van IJzendoorn¹

¹Department of Biomedical Sciences of Cells and Systems, Center for Liver Digestive & Metabolic Diseases, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; ²Dynamics of Intra-Cellular Organization, Institute Curie, PSL Research University, CNRS UMR144, Paris, France; and ³Structural Motility, Institute Curie, PSL Research University, CNRS UMR144, Paris, France

SUMMARY

MVID, a rare digestive disorder, manifests with severe, uncontrollable diarrhea, nutrient malabsorption, and diverse life-threatening symptoms. Linked to gene variants (MYO5B, STX3, STXBP2, UNC45A), MVID's clinical complexity extends beyond the digestive system. Recent studies unveil correlations between gene variants and specific phenotypes, aiding healthcare professionals in personalized care.

Microvillus inclusion disease (MVID) is a rare condition that is present from birth and affects the digestive system. People with MVID experience severe diarrhea that is difficult to control, cannot absorb dietary nutrients, and struggle to grow and thrive. In addition, diverse clinical manifestations, some of which are life-threatening, have been reported in cases of MVID. MVID can be caused by variants in the MYO5B, STX3, STXBP2, or UNC45A gene. These genes produce proteins that have been functionally linked to each other in intestinal epithelial cells. MVID associated with STXBP2 variants presents in a subset of patients diagnosed with familial hemophagocytic lymphohistiocytosis type 5. MVID associated with UNC45A variants presents in most patients diagnosed with osteo-oto-hepato-enteric syndrome. Furthermore, variants in MYO5B or STX3 can also cause other diseases that are characterized by phenotypes that can co-occur in subsets of patients diagnosed with MVID. Recent studies involving clinical data and experiments with cells and animals revealed connections between specific phenotypes occurring outside of the digestive system and the type of gene variants that cause MVID. Here, we have reviewed these patterns and correlations, which are expected to be valuable for healthcare professionals in managing the disease and providing personalized care for patients and their families. (*Cell Mol Gastroenterol Hepatol* 2024;17:983–1005; <https://doi.org/10.1016/j.jcmgh.2024.01.015>)

Keywords: microvillus inclusion disease; MVID; PFIC10; MYO5B deficiency; cholestasis; MYO5B; malabsorption.

Microvillus inclusion disease (MVID) is a rare inherited condition characterized by severe diarrhea, inability to absorb dietary nutrients, and failure to thrive.^{1,2} In the majority of patients, diarrhea starts within the

first days after birth, but in some patients the diarrhea starts 2 months after birth. The diarrhea can be excessive and often mistaken for urine, with volume up to 150–300 mL/kg/day.

Currently, there is no cure for MVID, and available treatments focus on providing support to patients.³ Immediate rehydration is crucial, and lifelong reliance on total parenteral nutrition (TPN) is necessary in most cases. TPN involves intravenous administration of essential fluids, proteins, sugars, vitamins, and electrolytes. Many infants with MVID face life-threatening complications associated with TPN, including catheter-related bloodstream infections (sepsis) and liver failure. TPN-related complications are the major cause of death in MVID. In some cases, intestinal transplantation is considered,⁴ but the 5-year survival rate for pediatric intestinal transplantation is approximately 50%. TPN can also lead to non-fatal but debilitating complications such as weakened bones and increased susceptibility to fractures due to metabolic bone disease.⁵

Several medications have been tested to reduce diarrhea and promote intestinal maturation in MVID, but their effectiveness varies among patients, with most experiencing none or only moderate benefits.³ It is worth noting that certain drugs have received orphan drug designations for the treatment of MVID. These include a combination of retinyl palmitate, alistol, and Zn gluconate, which are believed to inhibit chloride secretion, inhibit diarrhea, and enhance nutrient absorption.⁶ In addition, crofelemer, an inhibitor of chloride secretion and diarrhea, has received orphan drug designation⁷ and has shown promise in inhibiting secretion in murine intestinal enteroid models of MVID.⁸

MVID Diagnosis and Pathogenesis

MVID primarily affects the intestinal lining,^{9,10} and its diagnosis is based on evaluating clinical phenotypes, ruling

Abbreviations used in this paper: FHL5, familial hemophagocytic lymphohistiocytosis type 5; HPO, Human Phenotype Ontology; MVID, microvillus inclusion disease; TPN, total parenteral nutrition.



Most current article

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out other causes of diarrhea, and analyzing intestinal biopsies.¹¹ When examining small intestinal biopsies from MVID patients using specific staining techniques, such as hematoxylin-eosin, and immunohistochemistry, certain abnormalities are typically observed. These abnormalities include shortened and distorted villi, as well as misplacement of proteins responsible for various functions in the brush border, which is the absorptive surface of the intestinal cells. The affected proteins can include enzymes (such as CD10, dipeptidyl peptidase IV, and intestinal alkaline phosphatase) and transporters responsible for moving nutrients and electrolytes⁴ (such as SGLT1, NHE3, and DRA). Interestingly, the distribution and function of the chloride transporter CFTR are generally unaffected,^{12,13} although it may be altered in some patients' enterocytes.¹⁴

Staining with periodic acid-Schiff reveals a thinning of the brush border on the surface of the enterocytes. Electron microscopy confirms the loss, reduced number, or disorganized structure of the microvilli on the surface of the intestinal cells. Pathognomonic microvillus inclusions can be seen within the cytoplasm of some enterocytes. The abnormalities are often more severe in the differentiated absorptive enterocytes located in the villi compared with the proliferative epithelial cells located in the crypts.¹⁵ Mouse MVID intestinal tissue further showed a decrease in the number of doublecortin-like kinase-positive tuft cells and trefoil factor 3-positive goblet cells and an increase in the number of lysozyme-positive Paneth cells.¹⁶ Combined, these abnormalities in cell differentiation severely limit the intestine's ability to absorb nutrients and promote excessive secretion of water.

Overall, these findings provide insights into the structural and cellular changes that occur in the small intestine of individuals with MVID, leading to its characteristic phenotypes.

MVID Genetics

MVID is inherited in an autosomal recessive manner and is associated with specific genes. Initially, MVID was linked to pathogenic variations in both copies of the *MYO5B* gene,¹⁷ followed by the discovery of pathogenic variations in both copies of the *STX3* gene.¹⁸ Notably, MVID patients with *STX3* gene variations tend to have a milder form of diarrhea.¹⁸ The *MYO5B* gene encodes a motor protein called myosin Vb, whereas the *STX3* gene encodes a fusion protein called syntaxin-3. These proteins play roles in the movement of proteins within the intestinal cells, specifically between recycling endosomes (RAB11A-positive) and the brush border membrane.¹⁹

The typical clinical and cellular characteristics of MVID were also found in some patients diagnosed with familial hemophagocytic lymphohistiocytosis type 5 (FHL5),²⁰ a rare hyperinflammatory immune disorder that clinically presents with fever, hepatosplenomegaly, and cytopenias. A subgroup of FHL5 patients, characterized by the absence of splice-site variants affecting exon 15 and an early onset of disease, experience severe congenital intractable diarrhea and failure to thrive and require TPN.²¹ Although a full hemopoietic stem cell transplantation can resolve the

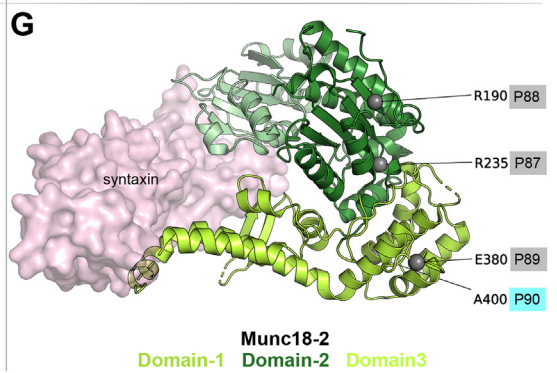
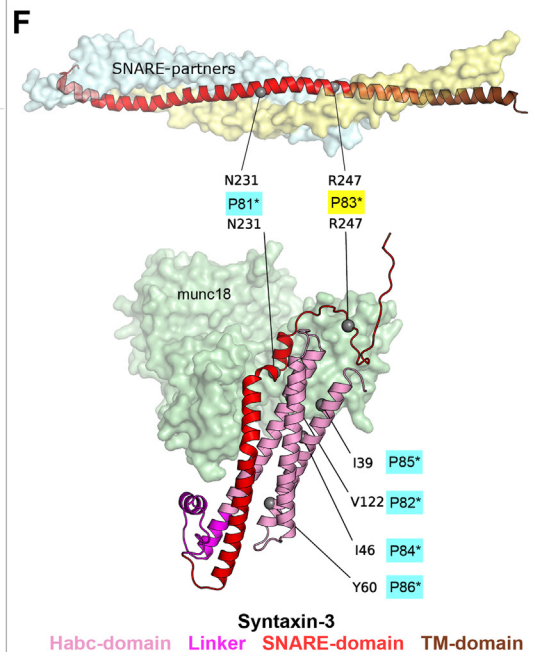
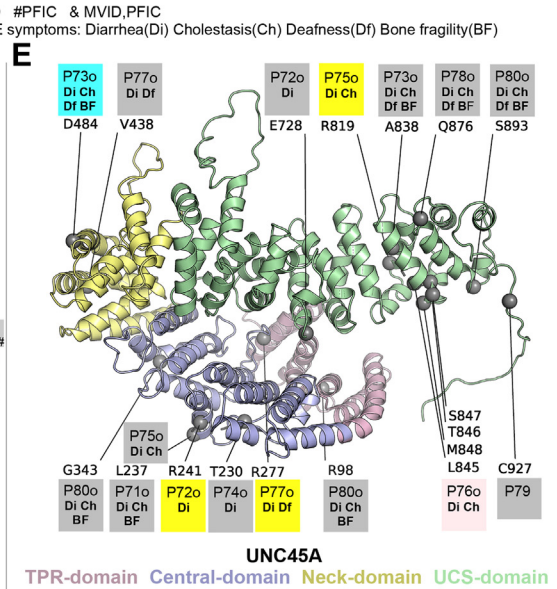
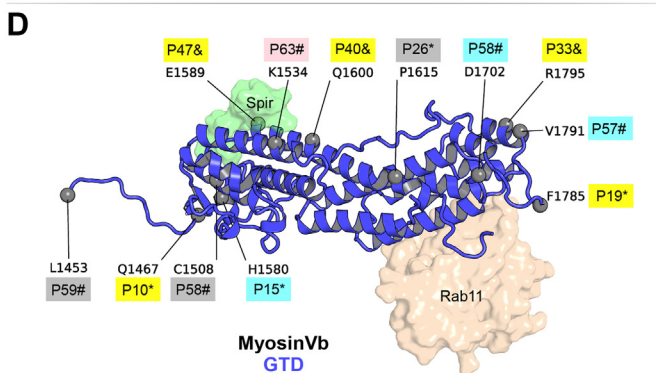
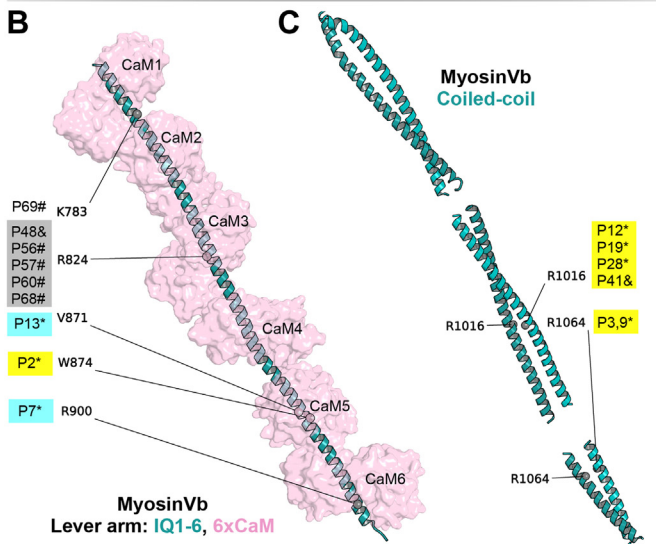
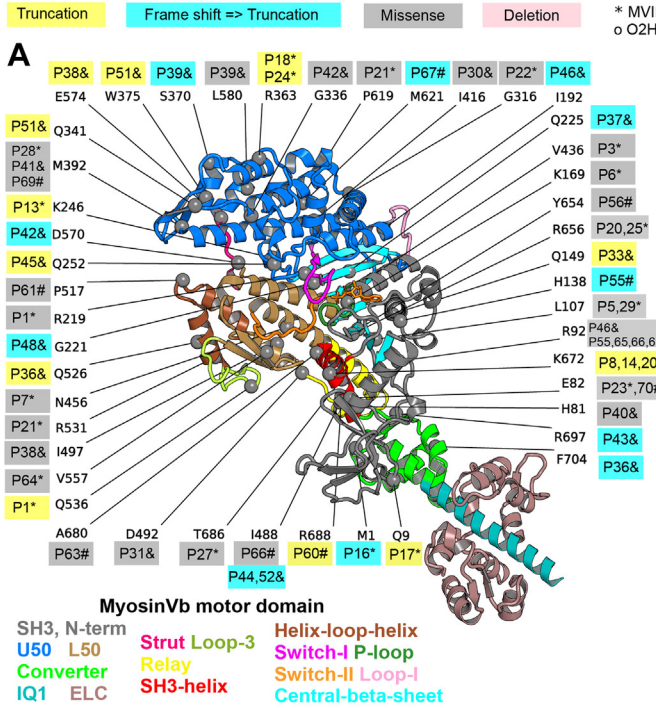
immune disorder, it does not alleviate the intestinal phenotypes. FHL5 is caused by bi-allelic variations in the *STXBP2* gene, which encodes a fusion protein called munc18-2 that interacts with syntaxin-3. Syntaxin-3 and munc18-2 were found to be abnormally localized in the intestinal cells of patients carrying variations in the *MYO5B* gene.^{22,23}

Most recently, variations in the *UNC45A* gene have been linked to MVID.²⁴ The *UNC45A* gene encodes a chaperone protein called UNC45A, which is required for the expression of myosin Vb^{24,25} and likely regulates other myosin proteins. When UNC45A is lost in intestinal epithelial cells, it leads to microvillus atrophy and misplacement of RAB11A-positive recycling endosomes.²⁵ Reintroducing wild-type myosin Vb can restore these cellular abnormalities.²⁵ Thus, the intestinal defects seen in MVID arise from disruptions in a molecular network involving myosin Vb and its chaperone UNC45A, as well as the fusion proteins syntaxin-3 and munc18-2 that are involved in the apical membrane fusion process.¹⁹ For excellent cartoons depicting the intricate interplay between UNC45A, myosin Vb, syntaxin-3, and munc18-2 in intestinal epithelial cells, we refer the reader to the studies by Vogel et al¹⁹ and Babcock et al.²⁶

The pathogenic variations in the *MYO5B*, *STX3*, and *STXBP2* genes have been analyzed using computational methods and reviewed in 2013²⁷ and 2018.²² In this study, we have compiled and analyzed all variants since then reported in these genes using available crystal structures of the encoded proteins.²⁸⁻³⁷ These included 88 pathogenic variants in the *MYO5B* gene and myosin Vb protein of 70 patients (Figure 1A-D), 16 pathogenic variants in the *UNC45A* gene and the UNC45A protein of 10 patients (Figure 1E), 7 pathogenic variants in the *STX3* gene and syntaxin-3 protein of 6 patients (Figure 1F), and 5 pathogenic variants in the *STXBP2* gene and munc18-2 protein of 4 patients (Figure 1G). We have predicted the potential impact on the encoded proteins (Table 1). Future studies are needed to mechanistically evaluate specific gene variants in correlation with functional or clinical implications in patients.

The Expanding Spectrum of MVID-Associated Phenotypes

MVID is a condition that exhibits a varying presentation over time.³⁸ The severity of diarrhea and dependence on TPN can fluctuate, and there have been reports of a small number of MVID patients successfully transitioning from TPN to enteral feeding.^{38,39} Because of the involvement of different genes, expressed not only in the intestine but also in other organs, the spectrum of phenotypes observed in patients with MVID continues to expand. Indeed, patients with MVID caused by *STX3*, *MYO5B*, *STXBP2*, or *UNC45A* variants have been frequently reported to experience vision loss,⁴⁰ cholestasis,^{41,42} immunologic impairment,^{20,21} or bone fragility, cholestasis, and hearing loss,^{24,41} respectively, likely reflecting the functions of these genes in different organs. These phenotypes often complicate the disease progression and some (eg, cholestasis) can even lead to fatality. Evaluating all phenotypes observed in patients with



MVID, ie, the MVID phenome, can help identify the underlying genetic cause. Conversely, the identification of the underlying gene variant can contribute to understanding the course of the disease.

In this review, we advocate for a MVID- and patient-centric view wherein the dominant archetypical phenotype of MVID is intricately linked to several genes, rather than considering the enteric phenotype as part of 4 distinct genetic diseases. Indeed, in patients with bi-allelic variants in the *MYO5B*, *STX3*, *STXBP2* (without splice variants affecting exon 15), or *UNC45A* gene, a consistent symptom, intractable diarrhea, along with the characteristic hallmarks of microvillus atrophy, villus atrophy, aberrant localization of brush border proteins, and microvillus inclusions, is observed. Additional symptoms related to other organ systems typically manifest later or concurrently in these patients. This temporal pattern suggests a unified disease entity with a core set of enteric symptoms preceding or coinciding with other manifestations. Furthermore, in the absence of knowledge of the underlying gene defect, patients presenting with intractable diarrhea and failure to thrive and displaying the archetypical combination of enteric symptoms and cellular hallmarks receive the diagnosis of MVID. This diagnostic unity supports the idea of a single disease entity with varied additional symptomatology based on the underlying gene defect. This unity extends to patients with *STXBP2* variants (without splice-site variants affecting exon 15) who received the diagnosis FHL5. Indeed, OMIM (www.omim.org) renamed FHL5 (OMIM# 613101) to FHL5 with or without MVID, underscoring the relationship between *STXBP2* variants and MVID diagnosis. The shared main cause of death in patients with variants in *STXBP2* (without splice-site variants affecting exon 15) being enteropathy-/TPN-related further reinforces the commonality. This presented perspective not only facilitates a unified diagnosis but also aids in predicting and preparing for the

diverse spectrum of symptoms beyond the intestine that may develop. For both families and physicians, understanding the underlying genetic defect proves valuable in anticipating and managing associated symptoms, thus enhancing patient care and prognosis.

To collect and categorize all reported phenotypes in MVID, we conducted a comprehensive review of 107 articles presenting patient case reports encompassing in total 211 patients diagnosed with MVID (see methods section for search strategy). These include cases in which the causative gene identified (mostly after 2008) and cases for which the causative gene was unknown. These reports were thoroughly screened to identify any mention of phenotypes or associated diseases. The resulting phenotypes were annotated to Human Phenotype Ontology (HPO) terms (hpo.jax.org)⁴³ (Figure 2A). By analyzing the reported phenotypes, we identified 184 unique phenotypes, each linked to unique HPO identifier codes. Of these, 42 unique phenotypes were reported at least 5 times and are depicted in Figure 2A. The HPO database has a hierarchical structure with variable number of branches and subbranches depending on the number of unique phenotypes. By tracing the unique phenotypes back to their first common-ancestor terms, the 184 unique phenotypes clustered into 24 distinct HPO groups that each consisted of at least 1 unique phenotype (Figure 2B). As expected, some of the largest clusters included diarrhea, malnutrition, growth failure (abdominal symptoms cluster), or the direct consequences thereof, including dehydration and acidosis in the abnormal metabolism/homeostasis cluster and weight loss and growth delay in the growth abnormalities cluster (Figure 2C). Another major cluster was abnormalities of the immune system, which included mostly sepsis (Figure 2C), the main cause of death and likely related to TPN line infections. Extraintestinal phenotypes clustered into 7 main HPO groups, involving liver function, the urinary system, the respiratory system, prenatal development or

Figure 1. (See previous page). *MYO5B*, *UNC45A*, *STX3*, and *STXBP2* variants mapped on the corresponding protein models are shown as grey spheres, corresponding patient number, phenotype (*MID; #PFIC; &MVID, PFIC; oO2HE), and variant type are indicated next to mutated residue name (in one letter code) and number according to Table 1. Protein domains and key structural elements color code are assigned below the models. (A) *MYO5B* variants mapped on the myosin Vb motor domain homology model based on the myosin Va crystal structure (PDB ID 1W7J).²⁸ (B) *MYO5B* variants mapped on the myosin Vb lever arm model composed of a long helix with 6 IQ motives and 6 calmodulin light chains. (C) *MYO5B* variants mapped on the myosin Vb coiled-coil domain, a dimerization unit of the motor. B and C panel models were generated on basis of the myosin Va cryo-EM structure (PDB ID 7YV9).²⁸ (D) *MYO5B* variants mapped on the myosin Vb globular tail domain (GTD) crystal structure in complex with Rab11 (PDB ID 4LX0).³² RAB- effector-binding site on GTD is indicated with the model ed Spir peptide (based on the crystal structure PDB ID 5JCY).³⁴ (E) *UNC45A* variants mapped on the human *UNC45A* protein homology model based on PDB ID 4I2Z crystal structure.²⁸ A myosin binding surface is in the UCS domain groove formed by the stacked ARM repeat superhelix twisting; Hsp90 and Hsp70 chaperones bind to a cavity on the surface of TPR domain.^{29,30} (F) *STX3* variants mapped on the homology models of syntaxin-3 bound to a syntaxin-binding-protein (lower part) based on the crystal structure of syntaxin-1a bound to Munc18a (PDB ID 4JEH).²⁷ Syntaxin SNARE-domain can reversibly engage in an intramolecular interaction with the 3-helix bundle Habc-domain, resulting in a tetrameric helical bundle not competent in the formation of complexes with cognate SNARE proteins. Syntaxin “open” conformation in which the SNARE motif is released from the bundle and extended allows its engagement in interactions with cognate SNAREs to form a SNARE complex of a 4-helix bundle arrangement as shown in the upper part: a homology model of *STX3* in a context of a SNARE complex based on crystal structure of SNARE complex composed of syntaxin 1A, SNAP-25, and synaptobrevin 2 (PDB ID 3HD7).^{27,31,35} (G) *STXBP2* variants mapped on the model of Munc18-2 protein (PDB ID 4CCA),³⁷ a bound homology model of syntaxin (in pink) positioned based on syntaxin-1/munc18-1 crystal structure (PDB ID 4JEU).³⁷ Munc18-2 domains 1 and 3 create a main syntaxin binding surface; domain 2 is important for the protein structural integrity and the relative orientations of domains 1 and 3.

Table 1. Gene Variants and Their Predicted Consequences for the Encoded Protein Structure or Function

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/ function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/ function 2	PubMed ID
MYO5B								
1	MVID	c.656G > A	p.(Arg219His)	Drastic lack of motor function of Myo5	c.1606C > T	p.(Gln536X)	No motor and no targeting due to premature stop codon	34816459
2	MVID	c.2621G > A	p. (Trp874*)	No motor and no targeting due to premature stop codon	exons 11-12 deletion (GRCh37 (chr18: 47488636-47489400) × 1)		?	34816459
3	MVID	c.1306 G > T	p.(Val436Phe)	Drastic lack of motor function of Myo5	c.3190 C > T	p.Arg1064*	No targeting due to premature stop codon	34503561
4	MVID	c.3277-2A>G		?	deletion of exons 24–27		?	33544913
5	MVID	c.320T>C	p.(Leu107Pro)	Drastic lack of motor function of Myo5	c.947-2A>G		?	33120470
6	MVID	c.505A>G	p.(Lys169Glu)	Drastic lack of motor function of Myo5	SAME	SAME	Drastic lack of motor function of Myo5	30564347
7	MVID	c.1367A>G	p.(Asn456Ser)	Drastic lack of motor function of Myo5	c.2700delG	p. (Arg900Sfs*4)	No motor targeting due to premature stop codon	
8	MVID	c.2014A > T	p.(Lys672*)	No motor and no targeting due to premature stop codon	SAME	SAME	No motor and no targeting due to premature stop codon	33525641
9	MVID	c.3190C > T	p.(Arg1064*)	No motor targeting due to premature stop codon	c.3514C > T	p.(Gln1172*)	No motor targeting due to premature stop codon	33525641
10	MVID	c.4399C > T	p.(Gln1467*)	No motor targeting due to premature stop codon	SAME	SAME	No motor targeting due to premature stop codon	33525641
11	MVID	c.1323-2A > G	?	?	SAME	SAME	?	33525641
12	MVID	c.3046C > T	p.(Arg1016*)	No targeting due to premature stop codon	SAME	SAME	No targeting due to premature stop codon	33525641
13	MVID	c.736C > A	p.(Lys246*)	No motor and no targeting due to premature stop codon	c.2612del	p. (Val871Glyfs* 33)	No targeting due to premature stop codon	33525641

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/function 2	PubMed ID
14	MVID	c.2014A > T	p.(Lys672*)	No motor and no targeting due to premature stop codon	SAME	SAME	No motor and no targeting due to premature stop codon	33525641
15	MVID	c.838 + 1G > A	?	?	c.4740_4741del	p. (His1580Glnfs*1)	No targeting due to premature stop codon, possibly unfolded and easily degraded	33525641
16	MVID	c.2T > A	p.(Met1?)	No motor and no targeting due to premature stop codon	c27 + 2T > A	?	No motor and no targeting due to premature stop codon	33525641
17	MVID	c.25C > T	p.(Gln9*)	No motor and no targeting due to premature stop codon	exon 1del	start removal	?	33525641
18	MVID	c.1087C > T	p.(Arg363*)	No motor and no targeting due to premature stop codon	SAME	SAME	No motor and no targeting due to premature stop codon	33525641
19	MVID	c.3046C > T	p.(Arg1016*)	No targeting due to premature stop codon	c.5354_5355del	p.(Phe1785*)	Possible impairment of motor targeting and regulation	33525641
20	MVID	c.1966C > T	p.(Arg656Cys)	Drastic lack of motor function of Myo5	c.2014A > T	p.(Lys672*)	No motor and no targeting due to premature stop codon	33525641
21	MVID	c.1591C > T	p.(Arg531Trp)	Drastic lack of motor function of Myo5	c.1856C > T; c.5395-2A > G	p.(Pro619Leu)	Possible impairment of motor function	33525641
22	MVID	c.947G > T	p.(Gly316Val)	Possible lack of motor function of Myo5	c.4082T > C	p.(Leu1361Pro)	No structural model to describe exactly the role of this mutation	33525641
23	MVID	c.244G > A	p.(Glu82Lys)	Drastic lack of motor function of Myo5	SAME	SAME	Drastic lack of motor function of Myo5	33525641
24	MVID	c.1087C > T	p.(Arg363*)	No motor and no targeting due to premature stop codon	exon 18del	?	?	33525641

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/function 2	PubMed ID
25	MVID	c.[1966C > T;	p.(Arg656Cys)	Drastic lack of motor function of Myo5, drastic mutation in the CBD - regulation affected	SAME	SAME	Drastic lack of motor function of Myo5, drastic mutation in the CBD - regulation affected	33525641
26		4844C > T]	p.(Pro1615Leu)					
27	MVID	c.2057C > T	p.(Thr686Met)	Drastic lack of motor function of Myo5	SAME	SAME	Drastic lack of motor function of Myo5	33525641
28	MVID	c.1175T > C	p.(Met392Thr)	Drastic lack of motor function of Myo5	c.3046C > T	p.(Arg1016*)	No targeting due to premature stop codon	33525641
29	MVID	c.320T>C	p.(Leu107Pro)	Drastic lack of motor function of Myo5	c.947-2A>G	?	?	33120470
30	MVID, PFIC	c.1247T > A	p.(Ile416Asn)	Possible impairment of motor function	SAME	SAME	Possible impairment of motor function	33525641
31	MVID, PFIC	c.1475A > G	p.(Asp492Gly)	Possible impairment of motor function	SAME	SAME	Possible impairment of motor function	33525641
32	MVID, PFIC	c.3131-2A > G	?	?	SAME	SAME	?	33525641
33	MVID, PFIC	c.445C > T	p.(Gln149*)	No motor and no targeting due to premature stop codon	c.5383C > T	p.(Arg1795*)	Possible impairment of motor targeting and regulation	33525641
34	MVID, PFIC	c.1323-2A > G	?	?	SAME	SAME	?	33525641
35	MVID, PFIC	c.5395-1C > G	?	?	SAME	SAME	?	33525641
36	MVID, PFIC	c.1576C > T	p.(Gln526*)	No motor and no targeting due to premature stop codon	c.2111del	p. (Phe704Sfs*67)	No motor and no targeting due to premature stop codon	33525641
37	MVID, PFIC	c.672_673del	p. (Gln225Dfs*4)	No motor and no targeting due to premature stop codon	SAME	SAME	No motor and no targeting due to premature stop codon	33525641
38	MVID, PFIC	c.1489A > T	p.(Ile497Phe)	Possible impairment of motor function	c.1720G > T	p.(Glu574*)	No motor and no targeting due to premature stop codon	33525641
39	MVID, PFIC	c.1739T > C	p.(Leu580Pro)	Possible impairment of motor function	c.1110_1113del	p.(Ser370Argfs* 27)	No motor and no targeting due to premature stop codon	33525641

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/ function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/ function 2	PubMed ID
40	MVID, PFIC	c.242A > G	p.(His81Arg)	Possible impairment of motor function	c.4798C > T	p.(Gln1600*)	No targeting due to premature stop codon	33525641
41	MVID, PFIC	c.1175T > C	p.(Met392Thr)	Drastic lack of motor function of Myo5	c.3046C > T	p.(Arg1016*)	No targeting due to premature stop codon	33525641
42	MVID, PFIC	c.1708dup	p. (Asp570Glyfs*5)	No motor and no targeting due to premature stop codon	c.1006G > A	p.(Gly336Arg)	Likely lack of motor function of Myo5	33525641
43	MVID, PFIC	c.2090+3A > T	p.(Arg697fs*47)	No motor and no targeting due to premature stop codon	SAME	SAME	No motor and no targeting due to premature stop codon	35706451
44	MVID, PFIC	c.1462del	p. (Ile488Leufs93)	No motor and no targeting due to premature stop codon	SAME	SAME	No motor and no targeting due to premature stop codon	31559144
45	MVID, PFIC	c.C1550T	p.(Pro517Leu)	Drastic lack of motor function of Myo5	c.C754T	p.(Gln252X)	No motor and no targeting due to premature stop codon	35129155
46	MVID, PFIC	c.574delA	p. (Ile192Serfs*47)	No motor and no targeting due to premature stop codon	c.C274T	p.(Arg92Cys)	Possible impairment of motor function or its regulation	35129155
47	MVID, PFIC	c.G4765T	p.(Glu1589*)	No targeting due to premature stop codon	SAME	SAME	No targeting due to premature stop codon	34815247
48	MVID, PFIC	c.662del	p. (Gly221Alafs19)	No motor and no targeting due to premature stop codon	c.2470C>T	p.(Arg824Cys)	May affect regulation and function of the motor - mutation in the lever arm / Cam Binding	34811877
49	MVID, PFIC	c.2812-1G>A	?	?	c.3340C>T	p.(Pro1114Ser)	May affect regulation or targeting? Unclear as this is in the coiled coil	34811877
50	MVID, PFIC	c.2090+3A>T	?	?	c.2090+3A>T	?	?	34811877

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/ function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/ function 2	PubMed ID
51	MVID, PFIC	c.1021C>T	p.(Gln341*)	No motor and no targeting due to premature stop codon	c.1125G>A	p.(Trp375*)	No motor and no targeting due to premature stop codon	34811877
52	MVID, PFIC	c.1462del	p.(Ile488Leufs*93)	No motor and no targeting due to premature stop codon	SAME	SAME	No motor and no targeting due to premature stop codon	31559144
53	MVID, PFIC	c.1966C > T	p.(Arg656Cys)	Drastic lack of motor function of Myo5	c.310+2Tdup	?	?	28899465
54	PFIC	c.3045+3A>T		?	SAME	SAME	?	34900494
55	PFIC	c.412dupC	p.(His138Profs*17)	No motor and no targeting due to premature stop codon	c.274C>T	p.(Arg92Cys)	Possible impairment of motor function or its regulation	32459745
56	PFIC	c.C2470T	p.(Arg824Cys)	May affect regulation and function of the motor - mutation in the lever arm / Cam Binding	c.A1961G	p.(Tyr654Cys)	Possible impairment of motor function or its regulation	35129155
57	PFIC	c.2470C>T	p.(Arg824Cys)	May affect regulation and function of the motor - mutation in the lever arm / Cam Binding	c.5370del	p.(Val1791Trpfs*22)	Possible impairment of motor targeting and regulation	34811877
58	PFIC	c.5104delG	p.(Asp1702Thrfs*15)	Possible impairment of motor targeting and regulation	c.4524C>G	p.(Cys1508Trp)	Possible impairment of motor targeting and regulation	34811877
59	PFIC	c.656G>A	p.(Arg219His)	Drastic lack of motor function of Myo5	c.4357C>T	p.(Leu1453Phe)	May affect regulation of the motor - mutation in the linker prior to CBD	34811877
60	PFIC	c.2062C>T	p.(Arg688*)	No motor and no targeting due to premature stop codon	c.2470C>T	p.(Arg824Cys)	May affect regulation and function of the motor - mutation in the lever arm / Cam Binding	34811877
61	PFIC	c.1549C>T	p.(Pro517Ser)	Drastic lack of motor function of Myo5	c.2470C>T	p.(Arg824Cys)	May affect regulation and function of the motor - mutation in the lever arm / Cam Binding	34811877
62	PFIC	c.1322+5G>A	?	?	c.839-1G>A	?	?	34811877

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/ function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/ function 2	PubMed ID
63	PFIC	c.2039C>T	p.(Ala680Val)	Prediction of mild motor impairment	c.4599_4601del	p. (Lys1534del)	No targeting due to premature stop codon	34811877
64	PFIC	c.1669G > T	p.(Val557Leu)	Prediction of mild motor impairment	SAME	SAME	Prediction of mild motor impairment	33525641
65	PFIC	c.274C > T	p.(Arg92Cys)	Possible impairment of motor function or its regulation	c.4123C > T	p.(Gln1375*)	No targeting due to premature stop codon	33525641
66	PFIC	c.274C>T	p.(Arg92Cys)	Possible impairment of motor function or its regulation	c.1463T>C	p.(Ile488Thr)	No targeting due to premature stop codon	32304554
67	PFIC	c.1860dupT	p. (Met621Hisfs*43)	No motor and no targeting due to premature stop codon	c.274C>T	p.(Arg92Cys)	Possible impairment of motor function or its regulation	32304554
68	PFIC	c.2470C>T	p.(Arg824Cys)	May affect regulation and function of the motor - mutation in the lever arm / Cam Binding	SAME	SAME	May affect regulation and function of the motor - mutation in the lever arm / Cam Binding	32304554
69	PFIC	c.1175T>C	p.(Met392Thr)	Drastic lack of motor function of Myo5	c.2349A>G	p.(Lys783Lys)	No change in function?	32304554
70	PFIC	c.244G>A	p.(Glu82Lys)	Possible impairment of motor function	SAME	SAME	Possible impairment of motor function	32304554
<i>UNC45A</i> isoform 1								
71	O2HE (diarr, chol, bone)	c.710T>C	p.(Leu237Pro)	The mutation in the central domain, compatible with the structure; however, it might result in a local structural rearrangement, the protein level is similar to the WT (35575086)	SAME	SAME		35575086

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/function 2	PubMed ID
72	O2HE (diarr)	c.2182G>A	p.(Gly728Lys)	USC domain surface residue mutation compatible with the protein structure, might result in a local structural rearrangement, the protein level is similar to WT (35575086)	c.721T>C	p.(Arg241*)	Protein truncated at the central domain, protein expression is inhibited (35575086) - unstable	35575086
73	O2HE (diarr, chol, bone, deaf, blind: right, anophthalmia, Peters anomaly & left pyramidal cataract /severe ID & behavioral disorder)	c.1452delinsGC	p. (Asp484Glu*fs)	Truncation at the middle of the central domain will result in no chaperone activity, protein expression is inhibited (35575086), the protein is unstable	c.2512G>C	p.(Ala838Pro)	The Pro residue might introduce a kink in the UCS domain helix destabilizing it; however, the protein level is similar to WT (35575086)	35575086
74	O2HE (diarr)	c.689G>C	p.(Thr230Arg)	In the central domain, introducing the long side chain of an Arg at the position of the Thr might result in steric hindrance with surrounding residues destabilizing the protein, the protein level is slightly reduced (35575086)	SAME	SAME		35575086
75	O2HE (diarr, chol)	c.2455C>T	p.(Arg819*)	The truncation results in an incomplete UCS domain, an unstable protein, inhibiting protein expression	c.710T>C	p.(Leu237Pro)	Protein compatible with the structure; however, it might result in local structural rearrangement	36587802

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/function 2	PubMed ID
76	O2HE (diarr, chol)	c.292C>T	p.(Arg98Trp)	The bulky Trp side chain is not compatible with the neighboring helices positioned within the TPR domain, it might affect the HSP90/70 binding site within the TPR domain	c.2534-2545del	p. (Leu845-Met848-del)	The deletion of 4 residues makes the USC 15H- helix shorter and also shortens a linker to the next helix, it may destabilize the domain structural integrity and change the conformation of the myosin binding groove	36699472
<i>UNC45A</i> isoform 2								
77	O2HE (diarr, deaf)	c.784C>T	p.(Arg262*) [Arg277*] ^a	The truncation results in a protein with a short (unstable) central domain, also missing the neck and USC domains	c.1268T>A	p.(Val423Asp) [Val438Asp] ^a	In the neck domain, substitution of the hydrophobic Val with the charged Asp in the hydrophobic core of the protein may destabilize the protein	29429573
78	O2HE (chol, bone, deaf, diarr)	c.2633C>T	p.(Ser878Leu) [Ser893Leu] ^a	Mild consequences to the structure. The mutation of the polar surface Ser to the hydrophobic Leu eliminates some stabilizing interactions and changes the surface properties, making it less polar	c.2584C>T	p.(Gln861*) [Gln876*] ^a	The truncation at the position 876 results in a short, probably unstable USC domain. This would destabilize fully the myosin binding groove	29429573
79		c.2734T>G	p.(Cys912Gly) [Cys927Gly] ^a	The substitution of the Cys by a Gly introduces some flexibility in the main chain of the helix; however, it is compatible with the protein structure				

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/function 2	PubMed ID
80	O2HE (diarr, chol, bone)	c. 247C>T	p.(Arg83Trp) [Arg98Trp] ^a	The bulky Trp side chain is not compatible with the neighboring helices positioned within the TRP domain, it might affect the HSP90/70 binding site within the TPR domain	c.983G>T	p.(Gly328Val) [Gly343Val] ^a	The residue substitution is compatible with the protein structural integrity	29429573
<i>STX3</i>								
81	MVID	chr11:59562859: T > TA	p. (Asn231Leufs Terfs19)	No membrane anchoring due to the missing TM domain, no membrane fusion can be supported. The truncated protein might still be able to bind and sequester syntaxin-binding-proteins and cognate SNARE proteins.	SAME	SAME	SAME	35769957
82	MVID	c.363_366delinsGA	p.(Val122fs14)	Truncated STX3 protein corresponding to the N-terminal 122 residue with incomplete regulatory domain extended by 14 residues of irrelevant sequence. Not functional.	SAME	SAME	SAME	30909251
83	MVID	c. 739C>T	p.(Arg247*)	No membrane anchoring due to the missing TM domain, no membrane fusion can be supported. The truncated protein might still be able to bind and sequester syntaxin-binding-proteins and cognate SNARE proteins.	SAME	SAME	SAME	29282386

Table 1. Continued

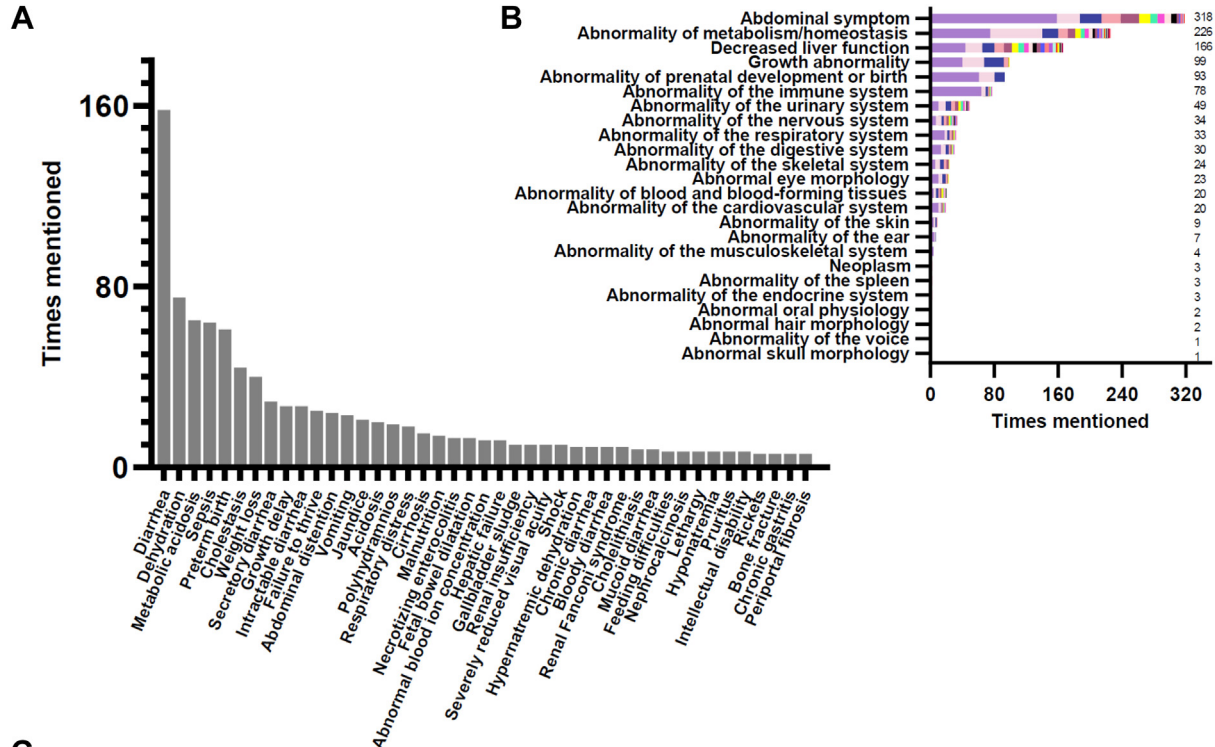
Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/function 2	PubMed ID
84	MVID	c.138del	p. (Ile46Metfs*8)	N-terminal Peptide of 46 residues. Not functional.	SAME	SAME	SAME	33974130
85	MVID	c.115-2A>G	?		r.115_214del	p.(Ile39Leufs*6)	N-terminal peptide of 39 residues with an extension of 6 residues of STX3 irrelevant sequence. Not functional.	33974130
86	MVID	c.177_178delCT	p.(Tyr60Qfs*16)	N-terminal peptide of 60 residues with an extension of 16 residues of STX3 irrelevant sequence. Not functional.	SAME	SAME	SAME	33974130
<i>STXBP2</i>								
87	FHL5, diarrhea	c.1247-1G>C		Splicing acceptor site is affected	c.704G>A	p.(Arg235Gln)	The conserved Arg, buried in the core of the domain2, the side chain makes several H-bonds to the surrounding residues, including a Glu546 also in the protein core, the Agr-Glu bridge in the protein core is stabilizing, the substitution of the Arg with a Gln does not allow to compensate the negatively charged Glu residue in the core potentially reducing the protein stability	34249802

Table 1. Continued

Patient no.	Symptoms	cDNA variant 1	Protein variant 1	Predicted consequences on the protein structure/function	cDNA variant 2	Protein variant 2	Predicted consequences on the protein structure/function 2	PubMed ID
88	FHL5, diarrhea	c.568C>T	p.(Arg190Cys)	The mutated protein expression level is not affected, the mutant binds to STX11 (34630398). R190 is a buried residue, located in a beta strand of domain2 and makes multiple polar contacts with residues from a surface loop of the protein, supporting the loop conformation. The substitution by a Cys results in the loss of the stabilizing H-bonds and local destabilization.				34630398
89	FHL5, diarrhea	c.1139A>G	p.(Gln380Arg)	Gln380 is a surface loop residue in domain3, the substitution with an Arg is not expected to change the protein structural integrity, but it introduces a positively charged residue and locally changes the charge distribution on the protein surface.	SAME	SAME	SAME	31807395
90	FHL5, diarrhea	c.1197delC	p.(Ala400fs)	No functional protein: the missing 125 residues contribute to the domain2 and domain3 structural integrity.				36503158

NOTE. The predicted consequences on protein structure/function were made by the structural analysis of the protein residues localization and involvement in the intramolecular interactions or in the interactions with partner proteins.
chol, cholestasis; diarr, diarrhea.

^aUNC45A Isoform 1 corresponding numbering is in the square brackets; the UNC45A residue numbering reported in 29429573 corresponds to isoform2 (the sequence difference is 1-17 MTVSGPGTPEPRPATPG → MT).



C

Abdominal symptom (HP:0011458) (n=318)	Growth abnormality (HP:0001507) (n=99)	Abnormality of the nervous system (HP:0000707) (n=34)
Unique phenotypes	Unique phenotypes	Unique phenotypes
HPO.ID	HPO.ID	HPO.ID
n	n	n
Diarrhea HP:0002014 158	Weight loss HP:0001824 40	Lethargy HP:0001254 7
Secretory diarrhea HP:0005208 29	Growth delay HP:0001510 27	Intellectual disability HP:0001249 7
Intractable diarrhea HP:0002041 27	Failure to thrive HP:0001508 25	Hypokinesia HP:0002375 3
Abdominal distention HP:0003270 24		
Vomiting HP:0002013 23		
Malnutrition HP:0004395 14		

Abnormality of metabolism or homeostasis (HP:0001939) (n=226)	Abnormality of prenatal development or birth (HP:0001197) (n=93)	Abnormality of the digestive system (HP:0025031) (n=30)
Unique phenotypes	Unique phenotypes	Unique phenotypes
HPO.ID	HPO.ID	HPO.ID
n	n	n
Dehydration HP:0001944 75	Premature birth HP:0001622 61	Necrotizing enterocolitis HP:0033165 13
Metabolic acidosis HP:0001942 65	Polyhydramnios HP:0001561 19	Chronic gastritis HP:0005231 6
Acidosis HP:0001941 20	Fetal bowel dilatation HP:4000140 13	Malabsorption HP:0002024 4
Abnormal blood ion concentration HP:0003111 12		
Hypertremic dehydration HP:0004906 9		
Hyponatremia HP:0002902 7		

Abnormality of the liver (HP:0001392) (n=166)	Abnormality of the immune system (HP:0002715) (n=78)	Abnormality of the skeletal system (HP:0000924) (n=24)
Unique phenotypes	Unique phenotypes	Unique phenotypes
HPO.ID	HPO.ID	HPO.ID
n	n	n
Cholestasis HP:0001396 44	Sepsis HP:0100806 64	Rickets HP:0002748 6
Jaundice HP:0000952 21	Recurrent infections HP:0002719 5	Bone fracture HP:0020110 6
Cirrhosis HP:0001394 15	Hypothyroidism HP:0000821 3	Bone fragility HP:0002659 5
Hepatic failure HP:0001399 12		
Gallbladder sludge HP:0001081 8		
Cholelithiasis HP:0001081 8		

Abnormality of the urinary system (HP:0000079) (n=49)	Abnormality of the respiratory system (HP:0002086) (n=33)	Abnormal eye morphology (HP:0012372) (n=23)
Unique phenotypes	Unique phenotypes	Unique phenotypes
HPO.ID	HPO.ID	HPO.ID
n	n	n
Renal insufficiency HP:0000083 10	Respiratory distress HP:0002098 18	Severely reduced visual acuity HP:0001141 10
Renal Fanconi syndrome HP:0001994 9	Nonspecific interstitial pneumonia HP:0033584 3	Nystagmus HP:0000639 5
Nephrocalcinosis HP:0000121 7	Respiratory failure HP:0002878 3	Blindness HP:0000618 4

Table 2. The Top-4 Phenotypes Reported in Cases with *MYO5B*, *STX3*, *STXBP2*, or *UNC45A* Variants

	Gene			
	<i>MYO5B</i> N (%)	<i>STX3</i> N (%)	<i>STXBP2</i> (without exon 15 splice site variants) N (%)	<i>UNC45A</i> N (%)
Top 4 HPO phenotypes	Diarrhea HP:0002014 86 (100)	Diarrhea HP:0002014 11 (100)	Diarrhea HP:0002014 23 (92)	Diarrhea HP:0002014 11 (92)
	Abnormality of prenatal development or birth 25 (29)	Severely reduced visual acuity HP:0001141 10 (91)	Failure to thrive HP:0001508 22 (88)	Cholestasis HP:0001396 8(67)
	Metabolic acidosis HP:0001942 22 (26)	Nystagmus HP:0000639 3(27)	Fever HP:0001945 25 (100)	Bone fragility HP:0002659 5(42)
	Cholestasis HP:0001396 15 (17)	Metabolic acidosis HP:0001942 4 (36)	Hepatosplenomegaly HP:0001433 19 (76)	Jaundice HP:0000952 3 (25)

NOTE. Data are presented as number of cases in the specific category (N) and percentage of all cases in that category (%).

birth, the nervous system, the skeletal system, and the eye (Figure 2C).

For the cases in which the causative gene has been identified, we have summarized the top 4 unique HPO phenotypes per gene in Table 2, yielding 11 unique phenotypes. The top 4 unique HPO terms related to *UNC45A* were diarrhea, cholestasis, bone fragility, and jaundice; for *STX3* they were diarrhea, vision loss, nystagmus, and metabolic acidosis; for *MYO5B* they were diarrhea, metabolic acidosis, prenatal signs of abnormality, and cholestasis; and for *STXBP2* (without splice-site variants affecting exon 15) they were chronic diarrhea, failure to thrive, fever, and hepatosplenomegaly. Table 3 shows the (co-)occurrence of the 11 unique phenotypes in cases associated with any of the 4 genes, indicating that some phenotypes are positively or negatively correlated with variants in specific genes. The exact causes of many of the extraintestinal phenotypes are still unknown. Some may be related to medical interventions (iatrogenic), may be secondary to the intestinal phenotypes, may be a direct result of the specific pathogenic gene variants, or may be combinations of these. Gaining a comprehensive understanding of the etiology and impact of these extraintestinal phenotypes is crucial for informed clinical decision-making and effective counseling of patients and their families.

Correlations Between Pathogenic Gene Variants and Extraintestinal Phenotypes in MVID

Extraintestinal phenotypes that have been clearly correlated with specific MVID-associated gene variants include cholestasis, severely reduced visual acuity, and premature birth. Here we will discuss these separately.

Intrahepatic Cholestasis in Patients With MVID Correlates With MYO5B and UNC45A Variants

Cholestasis associated with low to normal levels of gamma-glutamyl transferase is a frequently observed extraintestinal phenotype in patients with MVID.^{41,42} The occurrence of cholestasis in MVID patients has been thought to be iatrogenic and attributed to the long-term use of TPN. It has been found that soybean-based lipids in TPN formulas can have damaging effects on the liver.⁴⁴ Interestingly, switching to TPN formulas with fish oil-based lipid emulsion has been shown to alleviate liver phenotypes in some MVID patients,^{45,46} indicating the involvement of TPN in the development of intrahepatic cholestasis. It is worth noting that the occurrence of cholestasis in 54% of MVID cases is lower compared with the prevalence of long-term TPN-

Figure 2. (See previous page). **MVID-associated phenotypes.** (A) Presentation of all unique phenotypes with HPO ID (<https://hpo.jax.org/>) reported at least 5 times in the collection of all cases of MVID. The x-axis shows the unique phenotypes, and the y-axis shows the times each phenotype was mentioned in the case reports. (B) Unique phenotypes aggregate in 24 distinct HPO clusters. All unique HPO phenotypes were traced back in the hierarchy tree to their first common-ancestor terms, which resulted in their clustering in 24 distinct HPO groups that each consisted of at least 1 unique phenotype. Different colors in the horizontal bars represent unique HPO phenotypes and correspond to the unique HPO phenotypes per cluster as displayed in C. (C) The most mentioned (minimally 20 counts) unique HPO phenotypes for each of the 12 largest clusters. Colors used correspond to colors used in B.

Table 3. The (Co-)Occurrence of the 11 Top-4 Phenotypes in Cases With Known Underlying Gene Defect

	<i>MYO5B</i> N (%)	<i>STX3</i> N (%)	<i>STXBP2</i> (without exon 15 splice site variants) N (%)	<i>UNC45A</i> N (%)
Diarrhea	86 (100)	11 (100)	23 (92)	11 (92)
Metabolic acidosis	22 (26)	4(36)	4 (16)	0
Failure to thrive	8 (9)	3 (27)	22 (88)	2 (17)
Cholestasis	15 (17)	0	0	8 (67)
Abnormality of prenatal development or birth	25 (29)	1 (9)	0	1 (8)
Severely reduced visual acuity	0	10 (91)	0	0
Nystagmus	0	3 (27)	0	0
Fever	0	0	25 (100)	0
Hepatoplenomegaly	0	0	19 (76)	0
Bone fragility	0	0	0	5 (42)
Jaundice	9 (10)	0	0	3 (25)

NOTE. Data are presented as number of cases in the specific category (N) and percentage of all cases in that category (%).

associated liver phenotypes in a general pediatric cohort without MVID.⁴⁷ Some MVID patients have developed cholestasis only after undergoing a bowel transplant, and cholestasis resolved after subsequent interruption of the enterohepatic circulation, eg, after removal of the intestinal graft, ileal exclusion, or nasobiliary drainage.⁴² This suggests a potential inverse relationship between intestine- and liver-related phenotypes.⁴² Clearly, the presentation of cholestasis varies among MVID patients, and some individuals may even be protected against TPN-associated liver disease phenotypes.⁴⁷

So far, cholestasis has been reported in MVID patients with pathogenic *MYO5B* or *UNC45A* gene variants, and not in MVID patients with pathogenic *STX3* or *STXBP2* gene variants. This indicates a strong correlation between cholestasis and the specific pathogenic gene. In fact, variants of the *MYO5B* or *UNC45A* gene can also cause cholestasis without significant intestinal phenotypes.^{48–51} When comparing clinical data of patients with bi-allelic *MYO5B* gene variants, 3 distinct groups emerged: (1) those with intestinal phenotypes but no cholestasis, (2) those with intestinal phenotypes and cholestasis, and (3) those with cholestasis and minimal or no intestinal phenotypes^{46,50} (Table 1). Analysis of these groups revealed correlations between the type and combination of *MYO5B* variant(s) and the specific clinical presentation. First, patients with isolated cholestasis were found to have relatively mild variants in the *MYO5B* gene of at least 1 allele. These variants typically affect surface residues of the myosin Vb protein or involve variants observed in other species.²² On the other hand, patients presenting with secretory diarrhea and malabsorption have more severe *MYO5B* variants on both alleles. These severe variants affect highly conserved residues in critical parts of the protein or truncate or eliminate the protein. Our analyses of all *MYO5B* variants reported since 2018 in patients presenting with isolated diarrhea, isolated cholestasis, or a mix of both partly support this conclusion (Figure 1A, Table 1).

Our analysis expands the spectrum of *MYO5B* variants associated with isolated cholestasis with those that may affect only the regulation of the motor protein (Table 1). Notably, 2 patients with isolated cholestasis presented with bi-allelic splicing variants that are predicted to result in the loss of a motor-containing protein (Table 1), although this has not been experimentally verified. It has been proposed that more severe *MYO5B* variants may impact the apical sodium-dependent bile acid transporter-mediated bile acid reabsorption by enterocytes in the small intestine.^{47,51} Correlating with the impact on bile acid reabsorption, the resulting reduction in bile acid load in the liver may explain why some MVID patients with severe *MYO5B* variants do not develop cholestasis.^{47,51} Supporting this hypothesis, some MVID patients only developed cholestasis after receiving an intestine transplant, which restored intestinal function.⁴² It may be expected that apical sodium-dependent bile acid transporter inhibitors that are currently being developed for treatment of cholestasis⁵² may be effective to treat cholestasis in MVID patients, most of whom are currently treated with ursodeoxycholic acid. Second, bi-allelic variants in the *MYO5B* gene that generate a premature termination codon and/or result in the loss of the C-terminal RAB11A-binding site in the mutant myosin Vb protein have not been found in patients with isolated cholestasis.^{47,49–51} In addition, MVID patients with such variants in the *MYO5B* gene less frequently present with cholestasis.⁴⁷ Cell- and animal-based studies have shown that loss of *MYO5B* expression or expression of a mutated *MYO5B* variant that cannot bind RAB11A does not affect bile canaliculi in hepatic cells.⁵³ However, the expression of *MYO5B* carrying bi-allelic missense variants, such as the Navajo P660L variant, negatively impacts bile canaliculi, ABCC2 localization, and recycling endosome (RAB11A-positive) positioning.⁵³ This effect is not observed when cells co-express a dominant-negative mutant of RAB11A that cannot bind myosin Vb.⁵³ On the basis of these

findings, it has been proposed that *MYO5B*-associated cholestasis involves a gain-of-toxic function of the expressed mutant myosin Vb protein through its interaction with RAB11A.⁵³

An important question that remains to be answered is how *MYO5B* variants contribute to the development of cholestasis. Because myosin Vb plays a role in regulating the trafficking of proteins to the apical plasma membrane, a likely explanation is that mutated myosin Vb disrupts the trafficking of canalicular bile salt transporters, similar to its effects on apical brush border proteins in enterocytes. In vitro studies have demonstrated that the expression of mutated myosin Vb proteins interferes with the proper localization of ABCC2 and ANO6 in human hepatocellular HepG2 cells and human pluripotent stem cell-derived hepatocytes.⁵³ Furthermore, liver biopsies from both MVID and non-MVID patients with pathogenic *MYO5B* variants have shown abnormal localization of ABCC2 and ABCB11.^{42,53} However, in some studies this abnormal localization is not unambiguous, and other studies have reported normal localization of ABCB11 and ABCC2 in the canaliculi in liver biopsies of patients with pathogenic *MYO5B* variants,^{54,55} highlighting the presence of heterogeneity in these observations without a clear explanation for the discrepancy. There is a high probability that factors other than *MYO5B* variants play a role in the observed variability. This is supported by the observation that the expression of mutant myosin Vb protein can differ among hepatocytes within the same biopsy, indicating differences between cells in the same tissue in how they handle the mutant protein.^{49,54} In addition, the clinical manifestation of cholestasis can vary among siblings who carry identical *MYO5B* variants,⁴⁷ suggesting the involvement of non-genetic factors.

The other gene associated with cholestasis in MVID is *UNC45A*.^{24,48} However, the causality between *UNC45A* variants and cholestasis has not been established in animal or cellular models, and the precise mechanism by which pathogenic *UNC45A* variants may lead to cholestasis remains unclear. Eighty percent of the reported patients with bi-allelic pathogenic *UNC45A* variants presented with cholestasis. We analyzed all reported *UNC45A* variants and found no correlation between the (set of) variants, the position of the variants in the *UNC45A* protein domains, and the clinical presentations (ie, diarrhea, cholestasis, deafness, and bone fragility) (Figure 1E, Table 1). It is worth noting that in most patients with pathogenic *UNC45A* variants, cholestasis was transient and resolved within years after birth. Western blot analysis of expressed mutant proteins showed that the majority of these variants resulted in the loss of the mutant *UNC45A* protein.⁴⁸ When *UNC45A* was knocked out in cellular models, including hepatocellular HepG2 cells, a significant reduction in myosin Vb expression was observed.²⁵ However, similar to the knockout of *MYO5B* in HepG2 cells, there were no defects observed in the appearance of bile canaliculi, the localization of ABCC2, or the peri-canalicular distribution of RAB11A.²⁵ It is also unlikely that the remaining, potentially misfolded myosin Vb proteins behaved as motor mutant proteins, because this

would have resulted in the loss of bile canaliculi and mis-localization of ABCC2.⁵³

The loss of *UNC45A* function may affect other myosin proteins localized to the bile canaliculus that are involved in bile canalicular dynamics in hepatocytes, such as non-muscle myosin II.^{56–59} Previous studies have shown that non-muscle myosin II is an *UNC45A* client in other cell types.⁶⁰ Further investigations using liver-specific knockout or knock-in mice, induced pluripotent stem cell- or patient liver tissue-derived liver organoids are necessary to determine the causal relationship and underlying mechanism of *UNC45A*-related cholestasis. The reason why cholestasis is not observed in patients with *STX3* or *STXBP2* variants (Table 3) remains unknown. Although both genes are expressed in hepatocytes, there may be redundancy in the vesicle fusion machinery at the bile canalicular plasma membrane of hepatocytes compared with other epithelial cell types.⁶¹

Abnormalities of the Eye in Patients With MVID Correlates With *STX3* Variants

Abnormalities of the eye, including severely reduced visual acuity, have been observed in patients diagnosed with MVID carrying nonsense or frameshift variants in *STX3*,⁴⁰ whereas patients with *MYO5B*, *STXBP2*, or *UNC45A* variants do typically not exhibit such pathology (Table 3). In one case, a patient with *STX3* variants presented with autosomal recessive congenital cataract and intellectual disability but without intestinal phenotypes.⁶² Recent studies in mice lacking *Stx3* expression in the retina have demonstrated the causal role of *STX3* in retinal pathology.^{40,63} Whether MVID patients with *STX3* variants develop retinal pathology depends on the specific *STX3* variant involved.⁵⁹ *STX3* undergoes alternative splicing, resulting in different isoforms (*STX3A* and *STX3B*) with specific organ distributions, including the retina (*STX3B*). Therefore, only *STX3* variants that are predicted to affect the *STX3B* splice variant expressed in the retina are associated with retinal pathology.⁴⁰ Mechanistically, it has been proposed that loss of *STX3B* function causes defects in the intracellular trafficking of outer membrane proteins, synapses, or more general cellular functions, resulting in the degeneration of photoreceptors.⁴⁰ Detailed information regarding the specific *STX3* variant(s) will greatly aid in understanding the disease's progression and facilitate counseling for parents and patients.

Idiopathic Late Premature Birth in Patients With MVID Correlates With *MYO5B* Variants

Idiopathic late preterm birth (delivery between 34-0/7 to 36-6/7 weeks of gestation) has been reported in MVID cases,¹⁰ and a recent comprehensive case report study identified it as a bona fide phenotype in patients diagnosed with MVID.⁶⁴ Because the intestine grows rapidly between 32 and 40 weeks of gestation, the immature intestine of late-preterm children may have consequences for post-natal intestinal function. Notably, idiopathic late preterm birth in MVID was found exclusively in patients with *MYO5B*

variants and did not align with known risk factors for preterm birth.⁶⁴ Intriguingly, prenatal signs of bowel dysfunction, as detected by ultrasound (such as polyhydramnios or dilated bowels), did not consistently precede late preterm birth in MVID.⁶⁴ This in line with the observation that the occurrence of prenatal signs of bowel dysfunction did not correlate with specific MVID-causing genes. In addition, late preterm birth was not commonly observed in other congenital diarrheal disorders caused by variants in other genes.¹⁰ These findings suggest that in contrast to prenatal signs of bowel dysfunction, the occurrence of late preterm birth in MVID is not directly linked to intestinal pathology. It is plausible that *MYO5B* variants may impact other processes or tissues involved in fetal development, such as the transporting epithelium of the placenta where *MYO5B* exhibits high expression. Late preterm birth in MVID was associated with neonatal respiratory distress, higher postnatal stool volumes, and shorter average survival time.⁶⁴ Therefore, variants in *MYO5B* may contribute to postnatal phenotypes and a less favorable prognosis through their effects on prenatal development. Consequently, it is important to consider the gestational age at birth in cases of MVID.

Phenotypes in Patients With MVID Without Clear Genotype Correlation

For other MVID-associated phenotypes, ie, those belonging to the HPO clusters involving the urinary system, the respiratory system, the nervous system, and the skeletal system, no clear correlations with genotypes could be established. Renal Fanconi syndrome (one of the main unique phenotypes in the urinary system cluster) has been linked to defects in RAB11A-mediated apical trafficking in renal tubular epithelial cells,⁶⁵ but no defects in apical plasma membrane morphology, apical protein localization of RAB11A positioning was observed in kidney biopsies of MVID patients presenting with renal Fanconi syndrome.^{66,67} Respiratory distress, the main unique phenotype in the respiratory system cluster, has been associated with preterm birth in MVID.⁶⁴ A role of myosin Vb in the lung epithelium has not been reported. The main unique phenotype in the nervous system cluster, lethargy, has been co-mentioned with the term *diarrhea* in the medical literature (source: MEDLINE), suggesting a relationship between these phenotypes. Bone-related phenotypes (skeletal system cluster) are frequently, but not exclusively, mentioned in cases associated with pathogenic *UNC45A* variants. The term *bone fragility* was mentioned most frequently in patients with *UNC45A* variants (Figure 2B), whereas other terms in this cluster (ie, bone fracture, osteoporosis, rickets) were mentioned also for patients with pathogenic *MYO5B* variants. Bone fragility, fractures, rickets, and osteoporosis are also a well-described complication of TPN-mediated metabolic bone disease.⁵ On a cautionary note, like MVID-associated cholestasis was long thought to simply be a TPN-mediated complication, it cannot be formally excluded that MVID-causing gene variants may also contribute to other extraintestinal phenotypes.

Patient Enteroids and Organoids for the Study of Genotype-Phenotype Correlations

Intestinal cell models generated from patients' adult stem cells (termed *enteroids*) or pluripotent stem cells (termed *organoids*) have successfully replicated key cellular characteristics of MVID in patients and/or animal models with pathogenic genetic variants in the *MYO5B*, *STX3*, *STXBP2*, or *UNC45A* genes.^{8,17-19,68-70} These reproduced characteristics include microvillus atrophy, appearance of cytoplasmic microvillus inclusions, aberrant localization of brush border proteins, and defective signaling pathways but does not include the villus atrophy because organoids or enteroids do not typically develop villi unless transplanted into mice. Enteroids from intestine-specific *Myo5b* knockout mice also showed reduced differentiation of tuft cells, recapitulating the reduced number of tuft cells seen in the *in vivo* intestine of these mice.⁸ Enteroids from patients with variants in *MYO5B* have also been applied in two-dimensional culture to address defects of electrolyte absorption and secretion.⁸ The advantage of patient-specific pluripotent stem cells is their potential to differentiate into various organ tissues, allowing the study of specific variants in multiple organs. The use of enteroids and organoids opens up possibilities for investigating personalized drug screening, as demonstrated in other diseases such as cystic fibrosis, where enteroids have been used to evaluate the effects of CFTR-modulating drugs for personalized treatment.⁷¹ Enteroids derived from patients presenting with MVID and carrying specific *MYO5B* variants have been used to assess the efficacy of antisecretory and other compounds.⁸ If the results obtained from patient enteroids can be validated in clinical studies, this approach holds promise for the development of personalized treatments to address diarrhea in MVID, which has thus far seen limited success with conventional drugs.³ Furthermore, it will be of interest to use animal model- or patient-derived induced pluripotent stem cell lines to generate gut and liver tissue together on microfluidic chips to study how specific gene variants expressed in either or both tissues co-determine disease outcome.

Concluding Remarks and Future Perspectives

The number of genotypes and phenotypes observed in patients with MVID is gradually expanding, with various phenotypes and phenotypes impacting the course of the disease including morbidity and mortality. Thus far, no MVID patients with combined pathogenic variants in the *MYO5B*, *STX3*, *STXBP2*, or *UNC45A* gene have been reported, indicating that MVID is a monogenic disease with pleiotropic phenotypes.

To make informed clinical decisions and provide expert counseling to patients and their families, it is crucial to comprehend the relationships between genotypes and phenotypes in patients diagnosed with MVID. The publication and sharing of detailed clinical and phenotypic

information in scientific literature are essential for this purpose. In addition, experimental model systems such as molecularly engineered mice with patient-specific gene variants⁶⁶ and patient-specific intestinal enteroids and organoids will contribute to improving our understanding of genotype-phenotype relationships.

Methods

To collect all published MVID case reports from MEDLINE, the search engine PubMed was used with {microvillus inclusion disease OR microvillus atrophy}, {MYO5B}, {STX3}, {UNC45A}, and {STXBP2} as search strings. Resulting articles were manually inspected for the presence of case reports. This resulted in 107 articles presenting 211 cases. From these case reports patient-specific information including gene variants and reported symptoms and phenotypes were extracted and used for analyses. The extracted symptoms and phenotypes were annotated as HPO terms⁴³ (www.hpo.jax.org; version v2023-10-09).

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Correspondence

Address correspondence to: Sven C. D. van IJzendoorn, PhD, University Medical Center Groningen, Department of Biomedical Sciences of Cells and Systems, Antonius Deusinglaan 1, 9713AV Groningen, Netherlands. e-mail: s.c.d.v.an.ijzendoorn@umcg.nl.

Conflicts of interest

The authors disclose no conflicts.

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