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Unequal effects of *MYO5B* mutations in liver and intestine determine the clinical presentation of low-GGT cholestasis

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Abstract

Mutations in the *MYO5B* gene cause in some patients low gamma-glutamyltransferase (low-GGT) cholestatic liver disease (CLD) and in other patients microvillus inclusion disease (MVID, a congenital diarrheal and malabsorption disorder). Overlap of symptoms occurs but more MVID patients present cholestasis than CLD patients present diarrhea. Clinical observations indicate that *MYO5B* mutations can cause but also protect against CLD. This complicates family counseling and therapeutic decisions. Here we have reviewed the literature on *MYO5B* mutations in relation to CLD. It appears that variations in the clinical presentation of low-GGT CLD can be attributed to the coincident expression but unequal effects of *MYO5B* mutations in hepatocytes versus enterocytes, two cell types that jointly constitute the core of the enterohepatic circulation. Therefore, contrasting other low-GGT CLDs, those associated with *MYO5B* mutations should be viewed as a disease of the enterohepatic circulation rather than solely of the liver.

***MYO5B*-associated cholestatic liver disease**

Cholestatic liver disease (CLD) is characterized by an increase in the serum concentrations of compounds that are normally excreted with bile, such as bile acids and bilirubin (1). CLD clinically manifest with cholestasis, jaundice and pruritis. Diagnosis involves evaluation of the patient's clinical manifestations, exclusion of common causes of childhood cholestasis, analyses of blood biochemistry and liver histology (1). One blood marker that aids in the differential diagnosis of liver diseases is gamma-glutamyltransferase (GGT), a liver enzyme which is typically elevated in serum upon liver damage. The best known CLDs characterized by low/normal GGT are benign recurrent of progressive forms of familial intrahepatic cholestasis (BRIC/PFIC), which are caused by mutations in the ATPase phospholipid transporting 8B1 (*ATP8B1)* gene encoding the ATP8B1 protein (BRIC/PFIC1) or in the ATP-binding cassette family B member 11 (*ABCB11)* gene encoding the canalicular bile salt export pump (BSEP) (BRIC/PFIC2) (2,3). In hepatocytes, ATP8B1 and BSEP are localized to the apical bile canalicular domain where they contribute to biliary secretion. When mutated as in PFIC1/2 patients, the respective proteins are less expressed, mislocalized and/or display impaired activity, leading to defective biliary secretion and, consequently, cholestasis (2,3).

Since 2017 four independent reports identified in total 30 different *MYO5B* mutations in 22 non-MVID patients who were diagnosed with intermittent, recurrent or progressive cholestasis presented as jaundice, pruritus, hepatomegaly and with low/normal serum levels of GGT (Figure 1, supporting Table 1). These patients tested negative for mutations in other PFIC-associated genes (4-7). *MYO5B* mutations may account for approximately 20% of pediatric patients with idiopathic low-GGT intrahepatic cholestasis (24). Based on microscopy analyses of liver biopsies, a reduced expression or mislocalization of BSEP was reported (4,5), but not in all patients (7).

MYO5B mutations were earlier reported to cause microvillus inclusion disease (MVID) (8). MVID is a severe congenital enteropathy, characterized by severe intractable secretory diarrhea from the moment of birth and the inability to absorb any nutrients (9). Diagnosis involves evaluation of the patient's clinical manifestations, exclusion of common causes of diarrhea and inspection of small intestinal biopsies. MVID small intestinal tissue reveals (near-)total villus atrophy and, at the cellular level, microvillus atrophy and mislocalization of many apical brush border proteins including the diagnostic brush border protein CD10 (9,10). The presence of microvillus inclusion bodies in the enterocytes by electron microscopy confirms the diagnosis. Tissue and cellular

defects are seen throughout the intestine (11) and have been causally related to the loss of function of the encoded myosin Vb protein. For their survival, MVID patients require life-long total parenteral nutrition (TPN) and, in some cases, intestinal transplantation (12,13).

A significant number of MVID patients present CLD with low/normal serum GGT levels, manifesting as jaundice (associated with elevated levels of conjugated serum bilirubin), pruritus and hepatomegaly (12,14). The prevalence of CLD in MVID patients has been estimated at ~30% in a local cohort of 28 patients (14). We have extracted data from all published MVID case reports until 2020 (reporting on 133 MVID patients) (supporting Table 1) and calculated the prevalence of MVID-associated CLD to be 37%. When we restricted the analyses to only MVID patients with reported *MYO5B* mutations the prevalence was 54%. It is possible that this is an underestimation as MVID patients that were still alive at the moment of publication of their case report could have developed CLD later on.

Microscopy analyses of liver biopsies of MVID patients with *MYO5B* mutations showed a reduced expression and/or the mislocalization of BSEP, which is considered a major factor in the severity of cholestasis (15). Further, a reduced expression and/or mislocalization of the multidrug resistance protein and bilirubin and bile acid transporter MRP2 was shown in some (16) but not all (13) MVID patients. Mutations in the MRP2-coding gene, ATP-binding cassette family C member 2 (*ABCC2)*, cause Dubin-Johnson syndrome associated with elevated conjugated bilirubin levels in serum (17,18). The expression or canalicular localization of ATP8B1 in liver biopsies of MVID patients has not been studied. The mislocalization of MRP2, which is not typically observed in patients with *ABCB11* mutations, indicates that *MYO5B* mutations can affect multiple canalicular transporters in parallel.

Because *MYO5B* mutations disrupt the intracellular trafficking of brush border proteins in the intestine (19,20), it has been assumed that the same *MYO5B* mutations similarly disrupt the trafficking of the canalicular bile acid- and bilirubin-transporting proteins in the liver (8). While this seems plausible and is supported by earlier cell culture studies (21), not all data are in support of this. For example, in other MVID patients with *MYO5B* mutations and presenting with CLD no mislocalization of these canalicular transporters were reported, and CLD later resolved (22). In another non-MVID patient diagnosed with CLD and *MYO5B* mutations no mislocalization of BSEP was observed (7). Furthermore, five MVID patients showed reduction of cholestasis and pruritus when soybean oil-base lipids in the TPN were replaced by fish oil-based lipids, indicating

TPN as a contributing factor in cholestasis and pruritus in at least these MVID patients (23-25). The prevalence of TPN-associated CLD in pediatric cohorts was estimated to be between 20 (<1 month of TPN) and 70% (>2 months of TPN) (26,27). Given their life-long TPN dependency (6), a prevalence of CLD of 37-54% in MVID patients seems rather low. Clearly, with these prevalence numbers at hand it is difficult to conclude whether CLD in TPN-dependent MVID patients is caused by the TPN or the patient's *MYO5B* mutations. Nonetheless, it appears that pathogenic *MYO5B* mutations may not always affect bile canalicular protein localization and/or cause CLD.

Correlations between MYO5B genotype and CLD

Similar to 37-54% of patients diagnosed with MVID presenting symptoms of CLD, 21% of patients diagnosed with low/normal GGT CLD were reported to present loose stools or diarrheal episodes (4,5,7). Overlap of symptoms thus occurs but more MVID patients present cholestasis than CLD patients present diarrhea. An outstanding question is why *MYO5B* mutations cause either CLD or MVID with or without symptoms of MVID or CLD, respectively. Possibly the type of *MYO5B* mutation may play a role. While the many private *MYO5B* mutations running in the affected families complicates a detailed explanation, some generalizations can be made. For example, it was suggested that in patients with isolated CLD their *MYO5B* mutations did not result in sufficient loss of myosin Vb protein function to cause intestinal failure. This was supported by *in silico* analyses based on the myosin Vb homology protein structure. These revealed that the compound heterozygous patients with isolated CLD often carried in one allele a mutation that corresponded to more peripheral residues of the motor domain that were predicted to be less damaging for the protein's motor function (28). Further, several *MYO5B* mutations associated with isolated CLD give rise to amino acid substitutions that normally occur in other species, suggesting that these do not significantly affect the protein function. Thus, CLD in non-MVID patients appears a manifestation of relatively mild *MYO5B* mutations (4,28). Why relatively mild *MYO5B* mutations that cause a liver defect would not cause intestinal defects is not known. Possibly, the higher expression level of *MYO5B* in the intestine (https://www.gtexportal.org/home/gene/MYO5B) may compensate for a lower per protein activity.

An additional correlation between *MYO5B* genotype and isolated CLD has been observed. That is, isolated CLD did not involve any patient with bi-allelic nonsense or frameshift mutations in *MYO5B* that induce a premature termination codon (PTC) and affect the C-terminal rab11a-binding domain (4,28). A molecular mechanism to explain this genotype-phenotype correlation in CLD was recently proposed (29). Thus, the expression of a MVID- and CLD-associated *MYO5B* missense mutation (c.1979C>T), but not nonsense mutations, in a *MYO5B*-depleted human hepatoma cell line caused the mislocalization of MRP2 and other bile canalicular transporter proteins. The depletion of *MYO5B* in these cells or in the mouse liver as such did not result in the mislocalization of these canalicular transporters. The discordant effect of missense versus PTC-inducing *MYO5B* mutations suggested that the mutant myosin Vb protein exerted a gain-of-toxic function. It was shown that this gain-of-toxic function required the carboxyl-terminus of the mutant myosin Vb protein. Protein-truncating mutations cause the loss of the myosin Vb protein or the synthesis of a protein that lacks this carboxyl-terminus which explains, at least from the perspective of the studied canalicular transporters, why isolated CLD does not appear to correlate well with PTC-inducing *MYO5B* mutations (29).

The relationship between PTC-inducing *MYO5B* mutations and the occurrence of CLD in MVID patients has not been studied. Therefore, we examined the published MVID case reports (supporting Table 1) and the international MVID registry (www.mvid-central.org) and extracted information about the mutation and presence of CLD. Details regarding the mutations and the presence or absence of CLD were reported for 22 MVID patients with bi-allelic *MYO5B* mutations (Table 1). One of the 11 patients (18%) with at least one PTC-inducing mutation and nine of the 11 patients (82%) without a PTC mutation developed CLD. There was a significant relationship between the two variables (*Chi* square (1, N=22)=8.9, $p<.01$). There was also a significant relationship between the two variables when omitting multiple patients with the same mutation (*Chi* square (1, N=17)=4.5, $p<.05$). While the inclusion of more patients will tell whether this relationship holds, the current numbers suggest that patients with PTC-inducing *MYO5B* mutations have a reduced risk of developing CLD. Conceivably, with *MYO5B* expression abolished on one allele, the c.3163-3165dup (p.Leu1055dup) mutation (a common single nucleotide polymorphism (rs200219597) (gnomad.broadinstitute.org) in the *MYO5B* gene on the other allele (Table 1) may not provide a strong enough effect to cause CLD.

MYO5B mutations can protect against cholestatic liver disease in MVID patients

Intriguingly, three out of a group of eight MVID patients with *MYO5B* mutations that underwent intestinal transplantation presented with post-transplantation onset of CLD and others showed exacerbation of preexisting CLD (12,14). Also, in our medical center, a MVID patient developed cholestasis and pruritus after receiving an intestine transplant. His CLD resolved following rejection and subsequent removal of the graft. In addition, a late-onset MVID patient presented with CLD after birth which resolved at the moment when his enteral symptoms appeared. CLD did not return following a combined liver and intestine transplantation. These observations led to suggest that the onset or severity of CLD may be inversely related to intestinal function (14,28).

Because *MYO5B* mutations cause total malabsoption due to villus and brush border atrophy and the mislocalization of many brush border proteins (9,10), a reduced bile acid absorption capacity in the ileum (via the solute carrier family 10 member 2 (*SLC10A2)*-encoded apical sodium-bile acid transporter (ASBT) (30,31) can be expected. The consequently expected reduction of recirculating bile acids may (partially) diminish the clinical CLD symptoms. Indeed, the interruption of the bile acid circulation between liver and intestine, via either intestinal graft removal, ileal exclusion, nasobiliary drainage or partial external bile diversion, led to a (partial) remission of CLD in MVID patients (14), which is in line with the effectivity of these treatments in other PFIC patients (32). Thus, *MYO5B* mutations that reduce the bile acid absorption capacity of the intestine may provide some protection against CLD (14). Future studies are needed to address the bile acid pool and bile flow dynamics in the various patients with *MYO5B* mutations.

Conclusions and future perspectives

MYO5B is a new kid on the block in CLD. Different mutations in *MYO5B* display seeming contradictory effects on the clinical presentation of CLD. It appears that variations in the clinical presentation of *MYO5B*-related low-GGT CLD can be attributed to the coincident expression but unequal effects of *MYO5B* mutations in enterocytes versus hepatocytes (Figure 1). Thus, (a) (combination of) *MYO5B* mutations with a predicted mild effect on myosin Vb protein function

can cause canalicular transport defects in hepatocytes but do not cause (serious) problems in enterocytes, resulting in a BRIC/PFIC-like CLD. *MYO5B* missense mutations that have a more profound impact on myosin Vb protein function can cause similar (or stronger) canalicular transport defects in hepatocytes. However, these additionally cause absorptive defects in the intestine and, in case of an expected mislocalization of ASBT, likely reduce ileal bile acid recirculation and thereby diminish the clinical presentation of CLD. PTC-inducing mutations cause absorptive defects in the intestine but, as such, no apparent canalicular defects.

Of interest, the amount of bile acids that is absorbed in the intestine likely affects the bile acid synthesis and pool size via FXR-FGF19 signaling (33). Future studies should address the size of the bile acid pool and bile flow dynamics in patients with *MYO5B* mutations.

For most MVID patients it remains uncertain whether CLD results from TPN and/or their *MYO5B* mutations. Current prevalence data of CLD in MVID (this study) versus that of non-MVID pediatric TPN cohorts suggest that *MYO5B* mutations in general do not predispose to TPN-associated CLD. Changing to a TPN formula with fish oil-based lipids may provide insight in the contribution of the TPN to MVID-associated CLD. Knowledge as to whether given *MYO5B* mutations disrupt canalicular transport in hepatocytes will aid family counseling and therapeutic decision making. Currently, a combined bowel-liver transplantation is considered for children with MVID to reduce the risk of post-transplantation exacerbation or onset of CLD (12,14). However, if not necessary, the scarce donor liver may be used for another patient in need. Immunohistochemistry analyses of the localization of bile canalicular transporters in patients' liver biopsies may prove informative. Patient stem cell-derived hepatocytes (34) and liver-gut on-a-chip models (35) may provide powerful preclinical tools to assess the impact of the patient´s mutation(s) on bile canalicular transport. As a cautionary note, however, it should be emphasized that gene mutations are not likely to be the sole determinant of CLD, as siblings with the same *MYO5B* mutations showed different disease courses (4).

In contrast to familial CLDs that are caused by predominantly liver-specific genes, *i.e.*, *ABCB11* (https://www.gtexportal.org/home/gene/ABCB11) and ATP-binding cassette family B member 4 (*ABCB4)* (https://www.gtexportal.org/home/gene/ABCB4) (32), *MYO5B*-associated CLD should be viewed as a disease of the enterohepatic circulation, rather than of solely the liver. We expect that this may also apply to other familial CLDs caused by genes that are expressed in both liver and intestine (*e.g.*, *ATP8B1,* or nuclear receptor subfamily 1 group H member 4 (*NR1H4)*(32)).

Clearly, that a given (set of) *MYO5B* mutation(s) unequally affect two organs which cooperatively control bile flow complicates the disease presentation. Therefore, to understand the development and natural course of the disease as well as post-intervention outcomes, a personalized patient/mutation-specific multi-organ system approach will be required.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed with the search terms "MYO5B", "cholestasis", "microvillus inclusion disease", and "liver" from earliest records until May 2020. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review.

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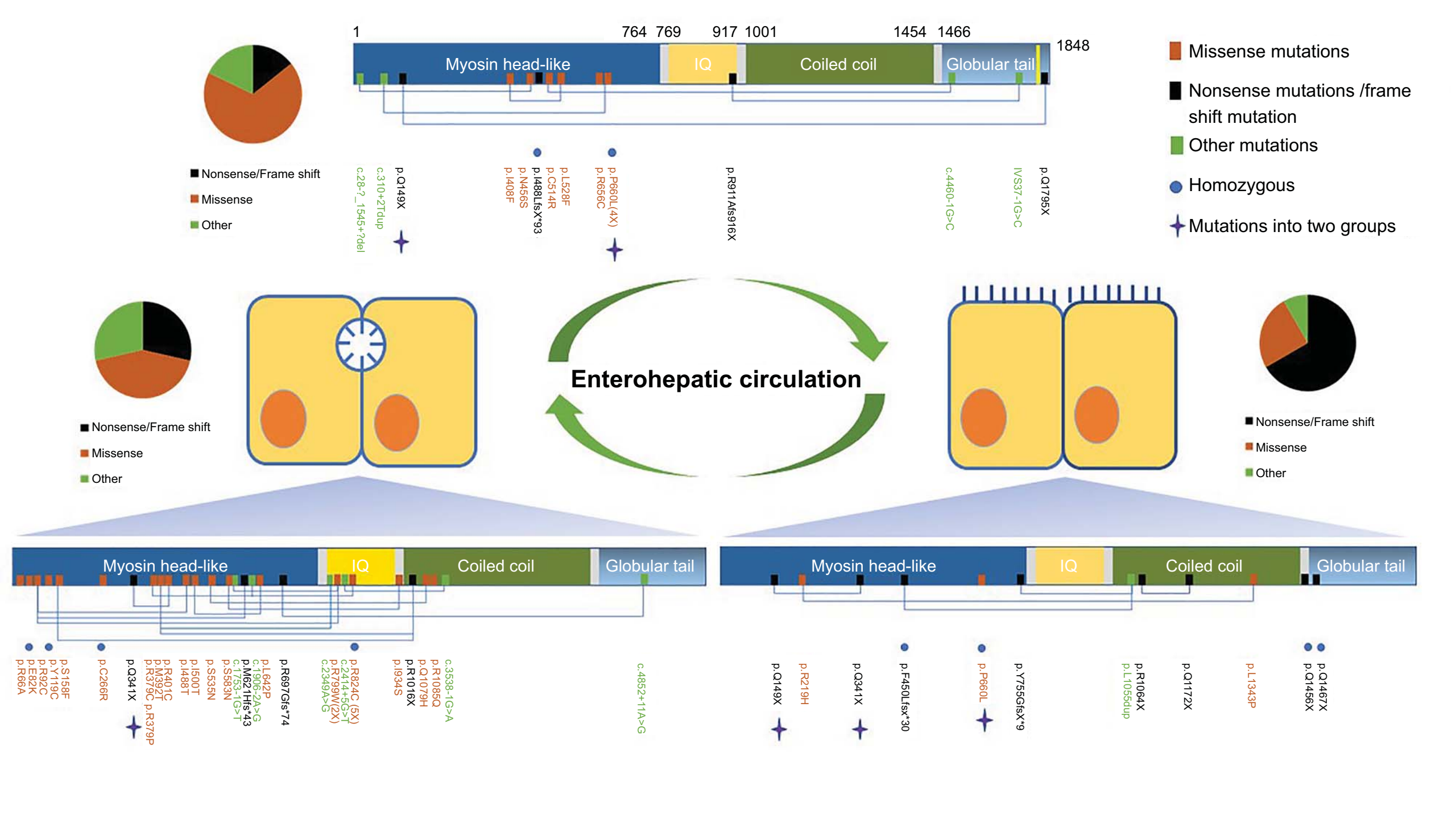
Figure Legends

Figure 1. *MYO5B* mutations associated with isolated CLD, isolated MVID or MVID + CLD. Cartoon showing MYO5B mutations associated with isolated CLD, isolated MVID or MVID + CLD.

Table 1. Individual non-transplanted MVID patients with reported MYO5B variations and presence or absence of CLD

	Variations of the patient	**with CLD**
MVID patients with at least one PTC-inducing *MYO5B* mutation	c.5139-1G>C(het[1])/c.2731delC (p.Arg911Alafs*6, het)[2]	No
	c.445C>T (p.Gln149Ter, het)/c.5383C>T (p.Arg1795Ter, het)[2]	Yes
	c.3163_3165dup (p.Leu1055dup, het)[3]/ c.2259_2262dup (p.Tyr755Glyfs*9, het)[2]	No
	c.3163_3165dup (p.Leu1055dup, het)[3]/ c.2259_2262dup (p.Tyr755Glyfs*9, het)[2]	No
	c.4399C>T (p.Gln1467Ter, hom[1])	No
	c.1347delC (p.Phe450Leufs*30, het)2/c.3163_3165dup (p.Leu1055dup, het)[3]	No
	c.1347delC (p.Phe450Leufs*30, het)2/c.3163_3165dup (p.Leu1055dup, het)[3]	No
	c.445C>T (p.Gln149Ter, het)[2]/c.1021C>T (p.Gln341Ter, het)[2]	No
	c.1390C>T (p.Arg1064Ter, het)[2]/c.3514C>T (p.Gln1172Ter, het)[2]	No
	c.4366C>T (p.Gln1456Ter, hom)[2]	No
	c.4399C>T (p.Gln1467Ter, hom)	No
MVID patients without PTC-inducing *MYO5B* mutations	c.1222A>T (p.Ile408Phe, het)/c.1582C>T (p.Leu528Phe, het)	Yes
	c.1222A>T (p.Ile408Phe, het)/c.1582C>T (p.Leu528Phe, het)	Yes
	c.310+2dupT, het/c.1966C>T (p.Arg656Cys,het)	Yes
	c.1540T>C (p.Cys514Arg, het)/c.4460-1G>C, het	Yes
	c.28-?_1545+?del, het/c.1367A>G (p.Asn456Ser, het)	Yes
	c.1979C>T (p.Pro660Leu)	Yes
	c.1979C>T (p.Pro660Leu)	Yes
	c.1979C>T (p.Pro660Leu)	Yes
	c.1979C>T (p.Pro660Leu)	Yes
	c.656G>T (p.Arg219His, het)/c.4028T>C (p.Leu1343Pro, het)	No
	c.1979C>T (p.Pro660Leu, hom)	No

[1]hom: homozygous mutation; het: heterozygous mutation. [2]This mutation is expected to result in the expression of a truncated protein that retained its rab8 and rab11a-bindig sites. [3]This variation is a common single nucleotide polymorphism (rs200219597).


1
764
769
917
1001
1454
1466
1848
Myosin head-like
IQ
Coiled coil
Globular tail
Missense mutations
Nonsense mutations /frame shift mutation
Other mutations
Homozygous
Mutations into two groups
Nonsense/Frame shift
Missense
Other
p.Q149X
c.310+2Tdup
c.28-?_1545+?del
p.I488LfsX*93
p.N456S
p.I408F
p.L528F
p.C514R
p.P660L(4X)
p.R656C
p.R911Afs916X
c.4460-1G>C
IVS37-1G>C
p.Q1795X
Enterohepatic circulation
Nonsense/Frame shift
Missense
Other
Myosin head-like
IQ
Coiled coil
Globular tail
p.R824C (5X)
c.2414+5G>T
p.R799W(2X)
c.2349A>G
p.I934S
p.R1016X
p.R1085Q
p.Q1079H
c.3538-1G>A
c.4852+11A>G
p.R697Gfs*74
p.M621Hfs*43
c.1753-1G>T
p.S583N
p.S535N
p.I500T
p.I488T
p.R401C
p.M392T
p.R379C p.R379P
p.L642P
c.1906-2A>G
p.Q341X
p.C266R
p.S158F
p.Y119C
p.R92C
p.E82K
p.R66A
Myosin head-like
IQ
Coiled coil
Globular tail
p.Q149X
p.R219H
p.Q341X
p.F450Lfsx*30
p.P660L
p.Y755GfsX*9
p.R1064X
p.L1055dup
p.Q1172X
p.L1343P
p.Q1456X
p.Q1467X