cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Altered MYO5B Function Underlies Microvillus Inclusion Disease: Opportunities for Intervention at a Cellular Level

Deanna M. Bowman,[2] Izumi Kaji,[1,3,*] and James R. Goldenring[1,2,3,4,*]

[1]Section of Surgical Sciences, Vanderbilt University Medical Center, Nashville, Tennessee; [2]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee; [3]Epithelial Biology Center, Vanderbilt University School of Medicine, Nashville, Tennessee; and [4]Nashville VA Medical Center, Nashville, Tennessee

SUMMARY

Loss of functional Myosin Vb causes congenital diarrhea in microvillus inclusion disease (MVID). MVID models in cell lines, mice, pigs, and enteroid cultures have led to insights into the molecular pathology of MVID and possible treatment options.

Microvillus inclusion disease (MVID) is a congenital diarrheal disorder resulting in life-threatening secretory diarrhea in newborns. Inactivating and nonsense mutations in myosin Vb (MYO5B) have been identified in MVID patients. Work using patient tissues, cell lines, mice, and pigs has led to critical insights into the pathology of MVID and a better understanding of both apical trafficking in intestinal enterocytes and intestinal stem cell differentiation. These studies have demonstrated that loss of MYO5B or inactivating mutations lead to loss of apical sodium and water transporters, without loss of apical CFTR, accounting for the major pathology of the disease. In addition, loss of MYO5B expression induces the formation of microvillus inclusions through apical bulk endocytosis that utilizes dynamin and PACSIN2 and recruits tight junction proteins to the sites of bulk endosome formation. Importantly, formation of microvillus inclusions is not required for the induction of diarrhea. Recent investigations have demonstrated that administration of lysophosphatidic acid (LPA) can partially reestablish apical ion transporters in enterocytes of MYO5B KO mice. In addition, further studies have shown that MYO5B loss induces an imbalance in Wnt/Notch signaling pathways that can lead to alterations in enterocyte maturation and tuft cell lineage differentiation. Inhibition of Notch signaling leads to improvements in those cell differentiation deficits. These studies demonstrate that directed strategies through LPA receptor activation and Notch inhibition can bypass the inhibitory effects of MYO5B loss. Thus, effective strategies may be successful in MVID patients and other congenital diarrhea syndromes to reestablish proper apical membrane absorption of sodium and water in enterocytes and ameliorate life-threatening congenital diarrhea. *(Cell Mol Gastroenterol Hepatol 2022;14:553–565; https://doi.org/10.1016/j.jcmgh.2022.04.015)*

Keywords: Congenital diarrhea; Microvillus inclusion disease; MVID; Myosin VB; MYO5B; animal models; enteroids.

The History and Clinical Presentation of Microvillus Inclusion Disease

Microvillus inclusion disease (MVID) was first described in 1978 by Davidson et al[1] as congenital intractable diarrhea in neonates, which the authors originally named familial enteropathy. Antibiotics or steroids could not treat the patients with congenital diarrhea, and the symptoms persisted even on intravenous total parenteral nutrition (TPN), eventually leading to failure to thrive in many cases.[2] The designation of MVID was based on the observation in electron microscopy of large vacuoles (inclusions) in patient enterocytes that contained mature brush border microvilli. MVID diagnosis initially required the observance of inclusions along with the presence of periodic acid-Schiff (PAS)–positive granules in the apical cytoplasm of enterocytes.[3] Immunohistochemistry on MVID patient tissue also showed subapical staining of CD10, abnormal vesicle-like structures, and lysosomes with heterogeneous contents.[4–6]

In most neonatal patients with MVID, there is an uneventful pregnancy, but after birth, watery diarrhea often starts within the first few hours to days of life.[7] Diarrhea in MVID patients is so severe that it leads to dehydration, malnutrition, metabolic acidosis, and rapid weight loss.[1,8,9] Patients with MVID produce large volumes of stool, 150–300 mL/kg/d, and these stools usually demonstrate a high sodium content.[7,10] Other organs typically do not have any gross alterations, although cholestasis in the liver is common.[11–13] Different drugs were initially administered to treat patients with MVID, including steroids, antibiotics, loperamide, trifluoperazine, cholestyramine, and somatostatin, but none could treat the persistent diarrhea.[7] TPN is often used as a treatment because oral feeding increases the severity of diarrhea, but TPN itself can exacerbate

*Authors share co-corresponding authorship.

Abbreviations used in this paper: ATP, adenosine triphosphate; KO, knockout; LPA, lysophosphatidic acid; MVID, microvillus inclusion disease; NHE3, sodium-hydrogen exchanger 3; PAS, periodic acid-Schiff; PDK1, phosphoinositide-dependent protein kinase 1; PFIC, progressive familial intrahepatic cholestasis; SGLT1, sodium-glucose cotransporter 1; STX, syntaxin; TPN, total parenteral nutrition.

Most current article

2352-345X
https://doi.org/10.1016/j.jcmgh.2022.04.015

cholestasis and liver disease.[3,14,15] Unfortunately, TPN does not improve diarrhea, and many patients demonstrate a failure to thrive because of dehydration, malnutrition, and sepsis.[7] Lifetime TPN can be successful in these patients but can also lead to complications, so definitive treatment for MVID patients presently requires a small bowel transplant alone or in conjunction with a liver transplant.[14,16,17] Nevertheless, transplantation also carries attendant perioperative and chronic rejection-based morbidity and mortality,[18] indicating that less radical treatments options are clearly needed.

In 2008, two groups reported that mutations in MYO5B, the gene encoding the non-conventional motor protein myosin Vb, cause MVID.[19,20] Muller et al[19] identified nonsense and missense mutations in MYO5B in several MVID patients. Erikson et al[20] sequenced an MVID patient from the Navajo Nation, where there was a cluster of MVID cases within the population, with an incidence of 1 case per 12,000 births.[21–23] The mutation commonly found within the Navajo population is MYO5B-P660L (1977C>T) in exon 16.[20] Many different mutations in MYO5B can cause MVID as either homozygous or compound heterozygous mutations.[22] Regional increases in MVID frequency are most likely due to increased consanguinity, as expected in an autosomal recessive disease.[24,25] There are currently no prevalence data available for MVID. Genomic studies investigating common mutations in MYO5B still need to be performed to look for the prevalence of heterozygous mutations.

Myosin Vb and Trafficking

The myosin superfamily consists of approximately 12 classes, and 40 myosin genes have been identified in humans. All myosin motors generate force through adenosine triphosphate (ATP) hydrolysis.[26] Foundational work from many researchers from the 1930s to the 1950s established that muscle myosin was an ATPase responsible for muscle contraction.[27–32] Most 2-headed members have a similar organization, with a head domain that is responsible for ATP hydrolysis and actin-binding, a neck domain that allows for flexibility and the walking motion, a coiled-coil domain to allow for dimerization, and a globular tail domain to bind molecular cargoes.[33–37] Myosin motors can walk along actin in a hand-over-hand motion because of coupling of ATP hydrolysis with conformational changes in the motor domain, followed by the neck region.[38] For a myosin to transport cargo, it must be a processive motor. A motor is processive if it can undergo multiple catalytic cycles resulting in movement along the actin track. Processivity also depends on the duty ratio of the motor or the time a motor spends strongly bound to actin. Thus, a motor that forms a dimer (with 2 heads) like MYO5B must have a duty ratio of at least 0.5 (a motor head is bound to actin 50% of the time) to be processive. Each motor has variations in the motor domains and neck regions, resulting in significant differences in processivity, duty ratio, and enzymatic kinetics. Some myosin classes, eg, myosin II in muscle, act in large oligomer ensembles to create movement. Myosin V members are processive double-headed motors, with adenosine diphosphate release being the rate-limited step for the actin power stroke.[39,40] Single-molecule stepping kinetics have shown that there is only one rate-limiting step. The model for myosin V processivity is very different from other myosin family members, suggesting a unique function as a possible cargo delivery motor.[41] The yeast myosin V (Myo2p) is also regulated through head-to-tail interactions, and loss of this interaction leads to a constitutively active motor (Figure 1*A*).[42] Similar regulation has also been observed in kinesins, the motors that walk to the plus ends of microtubules.[42] It is likely that MYO5B may also be regulated in the same head-to-tail fashion, but there is currently no solved structure of the regulated conformation for MYO5B, and the amino acids involved have not been identified (Figure 1*A*).

Proper trafficking of cellular components is essential for the appropriate function of cells and cellular polarity. Microtubules are critical for polarized trafficking, but the actin cytoskeleton, brush border, and myosin motors are also essential for establishing cellular polarity.[43–45] MYO5B is an important motor in establishing polarity in epithelial cells.[46] There are 3 nonconventional mammalian class V myosins: MYO5A, MYO5B, and MYO5C. The myosin V members are involved in cell motility, endocytosis, vesicle trafficking, and protein localization.[47] MYO5A is well-characterized for its importance in trafficking in melanocytes.[48–50] Class V myosins establish their interaction with specific groups of trafficking vesicles through their cargo binding domains associated with Rab small guanosine triphosphatases that act as molecular zip codes to deliver intracellular cargoes.[51] RAB8A can interact with each of the isoforms of myosin V, whereas Rab11 family members (RAB11A, RAB11B, and RAB25) only interact with MYO5A and MYO5B.[51–53] RAB10 interacts with the alternatively spliced D exon of MYO5A and MYO5B and the homologous unspliced region in the MYO5C cargo binding region.[52] MYO5A and MYO5B also can interact with RAB6A.[54] MYO5A specifically interacts with RAB27A.[55,56] In addition, MYO5A and MYO5B interact with another RAB11-interacting protein RAB11-FIP2.[57] MYO5B forms a ternary complex with RAB11A and RAB11-FIP2 to stabilize interactions with recycling vesicles.[58]

MYO5B is distributed on plasma membrane recycling vesicles in non-polarized and polarized cells. The distribution of MYO5B, like that of RAB11A, is most characteristic of a microtubule-associated protein, and treatment with the microtubule inhibitor, nocodazole, disperses plasma membrane recycling membranes as well as RAB11A and MYO5B.[51] These findings have led to the suggestion that MYO5B may act as a processive anchor to the actin-cytoskeleton, resisting the movement of vesicles along microtubules. Indeed, knockdown of MYO5B or RAB11-FIP2 leads to more rapid directed movement of RAB11A-containing vesicles.[58] Nevertheless, motor activity in MYO5B is critical to recycling system function, because expression of the motorless tail of MYO5B in both non-polarized and polarized cells blocks plasma membrane recycling.[51] Importantly, MYO5B is a critical regulator of epithelial polarity and regulates the establishment of apical membrane recycling and apical trafficking.[59]

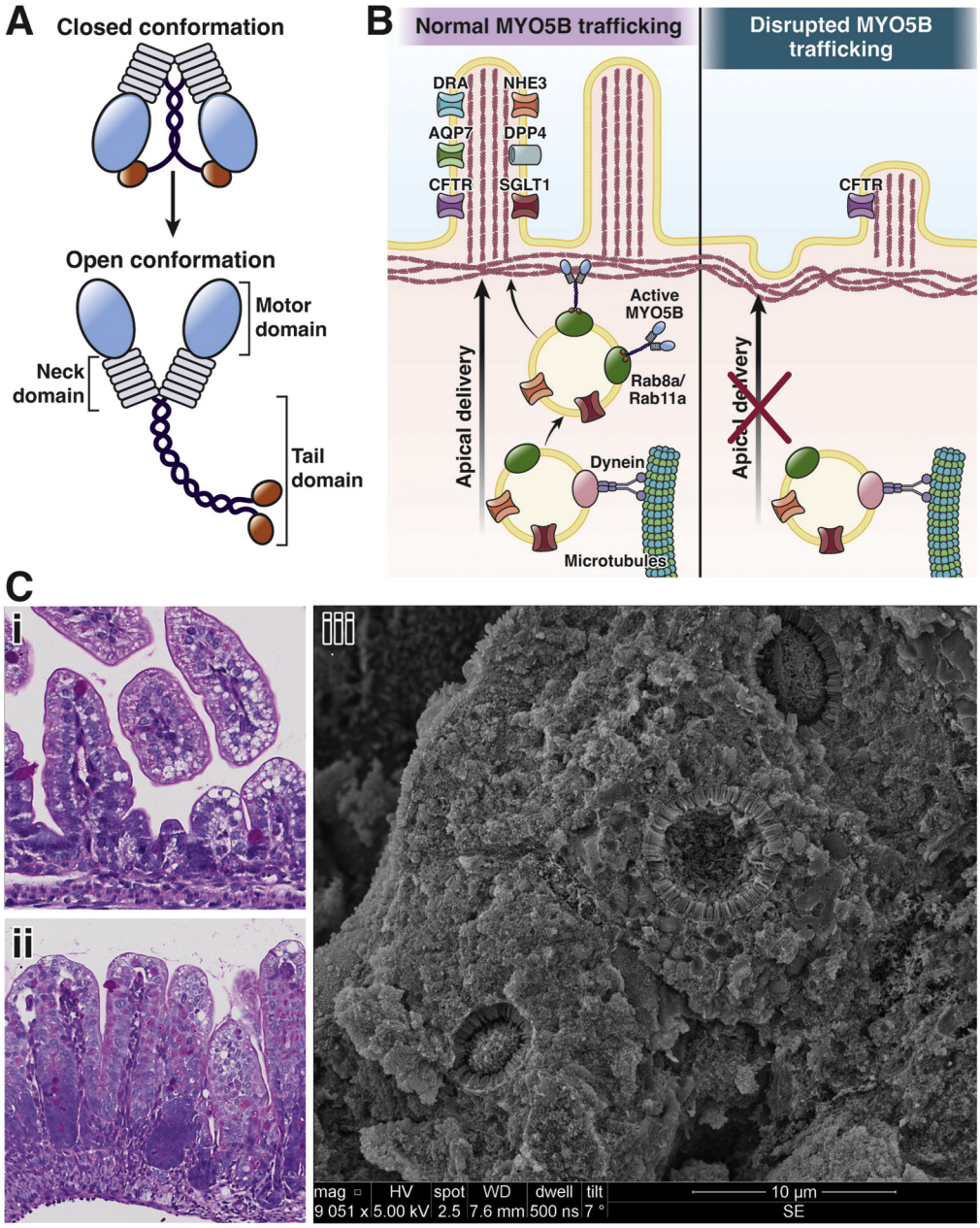

Figure 1. Mutations in MYO5B cause MVID. (*A*) MYO5B mutations and conformations. MYO5B consists of a motor domain, a neck domain that can bind 6 calmodulins to increase rigidity, and a tail domain that can bind Rab proteins. The open active conformation of MYO5B allows for ATP driven transport along actin. In the proposed closed inactive MYO5B conformation, the head and tail domains directly interact, and the motor cannot translocate along actin. (*B*) Model of MYO5B trafficking at the apical surface of normal and MVID affected enterocytes. (*Left*) In normal enterocytes, MYO5B works to apically traffic transporters, enzymes, and brush boarder components. (*Right*) In MVID, transporters, enzymes, and brush boarder components are not apically localized, but CFTR is retained at the apical surface, suggesting that it is delivered independently of MYO5B. Figure created using Biorender. (*C*) Abnormal PAS staining and inclusion formation in MYO5B knockout mice. (*i*) PAS staining in wild-type mice showing normal PAS-positive brush border and gross morphology. (*ii*) PAS staining in MYO5B knockout mouse showing blunted microvilli and accumulation of PAS stain below the apical surface. (*iii*) Fracture transmission electron microscopy showing microvillus inclusions formed on the interior of knockout mouse enterocytes.

Cell Biology of MVID

Applicable models to study MVID are essential to understanding the cellular defects within this disease. Access to MVID patient samples is limited, and genotyping has become a more efficient diagnostic method than biopsies. This section will examine different models used to study MVID and intestinal epithelial trafficking and polarity.

Evaluation of Enterocyte Function in Patient Tissue Studies

Patient tissue staining and cellular studies of MVID at a cellular level have been critical to the examination of how MYO5B mutations lead to trafficking defects (Figure 1*B*). Previous studies have established that loss of MYO5B disrupts the trafficking of apical recycling endosomes in enterocytes and hepatocytes.[11,60] Beyond the disruption of apical membrane trafficking, loss of MYO5B leads to aberrant localization of phosphoinositide-dependent protein kinase 1 (PDK1).[46] PDK1 is an activator of many apical proteins and is needed for exocytosis of sodium-hydrogen exchanger 3 (NHE3),[61] and the intracellular PDK1 localizes to RAB11-positive apical endosomes.[62] Initial investigations examined patient samples to determine how MVID affected the delivery of apical transporters in enterocytes.[63,64] In MVID patient tissue samples, the apical membrane proteins, such as sucrase isomaltase, alkaline phosphatase, NHE3, and cyclic guanosine monophosphate–dependent protein kinase, showed lower expression at the apical surface of enterocytes.[65,66] In MVID patients, cargo vesicle trafficking to the apical membrane is disrupted, causing defects in polarity, leading to the accumulation of apical transporters in RAB11A- and RAB8A-positive vesicles below the apical surface.[67] These studies using patient samples established that loss of MYO5B function leads to aberrant apical trafficking.

In Vitro Models of MVID

Several MVID-causing mutations identified in MYO5B are nonsense or mis-splicing, leading to protein truncation and loss, and other point mutations (eg, the P660L mutation in the Navajo tribe) produce a loss of motor function. Thus, the small interfering RNA knockdown of MYO5B allowed the development of other patient-relevant in vitro models for the disease. Much of this in vitro modeling was performed in the human colon adenocarcinoma-derived line, CaCo-2, which polarizes and elaborates a brush border on the apical surface.[68] In a CaCo-2 cell model with small interfering RNA knockdown of MYO5B, the protein expression levels of an MYO5B-binding partner, RAB8A, or MYO5A were not affected.[69] Cells with MYO5B knockdown exhibited microvillus inclusions, the hallmark of the disease in patient tissue. Although the protein expression of RAB8A was not affected, it was later shown that the localization of specific RAB guanosine triphosphatases was affected by the knockdown of MYO5B.[69,70] With MYO5B knockdown, RAB8A lost its apical localization, and RAB11A was accumulated in the perinuclear region instead of a broader vesicular distribution.[70] These studies showed that knockdown of MYO5B in a polarized cell line recapitulates the loss of brush border maintenance and apical trafficking.

Cellular studies have also led to insights on the role of MYO5B in the maintenance of intestinal epithelial polarity. Knockdown of MYO5B in CaCo-2 cells led to PDK1 delocalization from the apical surface, which could impact downstream signaling that activates water absorption in the cells.[46] Cellular studies have also investigated MYO5B's interaction with the apical recycling RAB proteins. Our group established that RAB8A and RAB11A are critical for typical vesicle trafficking in enterocytes.[71] Using MYO5B knockdown in CaCo-2BBE cells that were rescued with MYO5B mutants, which were only able to interact with either RAB8A or RAB11A, we determined that RAB8A interaction with MYO5B mediates the establishment of the functional apical brush border, whereas the loss of RAB11A interaction with MYO5B led to inclusion formation.[66] Proper localization of RAB8A and RAB11A is critical for maintaining the apical domain in enterocytes and for generating lumens by directing CDC42-dependent apical exocytosis.[72] Although MYO5B disruption causes mislocalization of many apical proteins, some proteins are retained at the cell surface. Studies using CaCo-2 cells as villus-like cells and T84 cells as a model for the crypt cells determined that CFTR was preserved at the apical surface of MYO5B knockdown cells.[73] The authors proposed that the preserved apical localization of CFTR reduced the functional expression of NHE3 and DRA, contributing to Cl^- and Na^+ loss in stool.[73] One quandary that remains is why the impact of MYO5B loss is so focused on the intestines and liver, especially because MYO5B shares most of its capabilities and Rab protein interactions with MYO5A. This major impact on these organs may accrue from the unusually low expression of MYO5A in liver and intestine compared with other organs (eg, kidney and brain).[52]

Animal Models of MVID

To study MVID, several mouse models have been created that rely on the inactivation of the MYO5B gene. In 2015, Carton-Garcia et al[74] reported the first germline MYO5B knockout (KO) mice. These mice died within 12 hours after birth and had characteristic intracellular PAS accumulation and mislocalization of ALP, 5′-nucleotidase, ezrin, and transferrin receptor in the intestinal epithelial cells. Importantly, microvillus inclusions were found in enterocytes. Our group has created multiple mouse MVID models including a germline KO, an intestine-specific KO (Villin-Cre), and a tamoxifen-inducible intestine-specific KO (Villin-Cre-ERT2) of MYO5B.[75] Germline KO mice crossed onto an out-bred CD-1 background survived for 4–5 days postnatally but ultimately succumbed to severe diarrhea and demonstrated microvillus inclusions, villus blunting, and prominent loss of apical transporters. The intestine-specific KO model showed an intestinal phenotype similar to the germline KO, with inclusions in enterocytes, shortened and fused villi, and elongated crypts (Figure 1*C*). The germline KO of MYO5B leads to expansion of the crypt length and increased Paneth cell numbers, suggesting that MYO5B plays a role in intestinal cell proliferation.[75] Beyond the effects of MYO5B deficiency on epithelial cell morphology, we investigated the distribution of RAB11A and RAB8A distribution in MYO5B KO enterocytes. RAB11A was frequently associated with the inclusions, whereas RAB8A was mislocalized throughout the cytoplasm, consistent with our previously published research using CaCo-2 cells.[66,75]

Two groups have developed tamoxifen-inducible intestine-specific MYO5B KO mouse models.[75,76] When the inducible intestine-targeted mice received tamoxifen to knockout MYO5B, rapid weight loss was noted, which resulted in the euthanizing of mice by day 4 because of severe dehydration.[75] These mice demonstrated shortened microvilli, microvillus inclusions, and loss of sodium transporters at the apical surface, as seen in the germline KO mice.[74–76] Schneeberger et al[76] reported more frequent inclusion formation than observed in our inducible intestine-specific mice.[75] This disparity could be due to the age of mice and level of development of the intestine when Cre recombinase was induced with tamoxifen. Mice in earlier developmental stages may be more inclined to form inclusions in response to the loss of MYO5B than more mature mice. This idea is substantiated by evidence from experiments with CaCo-2 cells and experiments looking at germline MYO5B inactivation, intestine-specific germline MYO5B inactivation, and adult inducible mouse models.[66,75] Schneeberger et al[76] also examined early time points after tamoxifen induction to determine the effect of MYO5B loss. Subapical accumulation of granules in enterocytes was observed in the lower parts of villi before inclusion formation, suggesting that MYO5B is essential for brush border formation in enterocytes. The disparity between MYO5B KO mouse models and the number of inclusions observed indicate that the inclusions may not cause diarrhea or mistrafficking of apical proteins. The mislocalization of apical enzymes and transporters responsible for water absorption in the intestines is likely an independent event from the inclusion formation.[77] We have established that with the loss of MYO5B in tamoxifen-induced mice, many transporters and enzymes were lost from the apical surface, including NHE3, sodium-glucose cotransporter 1 (SGLT1), aquaporin 7, sucrase isomaltase, alkaline phosphatase, and Cdc42. However, there was a retention of CFTR at the apical surface, which is seen in MVID patient tissue.[78] Maintenance of CFTR at the apical membrane could exacerbate the lack of water absorption in the intestines by continued secretion of chloride ions into the lumen of the intestines.[78]

Evaluation of the Origin of Microvillus Inclusions

For a number of years, investigators believed that microvillus inclusions formed through arrest of exocytosis. This belief was primarily supported by the impression of an accumulation of PAS-positive granules in the apical cytoplasm of MVID enterocytes. However, these PAS-positive granules did not coincide with inclusions. Most notably, Reinshagen et al[64] reported that enterocytes in biopsies from MVID patients showed apical uptake of cationic ferritin into microvillus inclusions, suggesting an apical endocytic mechanism. Our lab has studied how inclusions are formed in MVID through examination of mouse models and derived enteroids (Figure 2). In support of the initial findings of Reinshagen et al, we demonstrated that germline MYO5B KO mice showed uptake of mother's milk into inclusions.[75] Previous investigations had shown that Pacsin 2 (syndapin 2) is an F-bar adapter protein involved in curvature-sensing and endocytosis.[79,80] We demonstrated that MYO5B;Pacsin 2 double KO mice lost microvillus inclusions but still presented with life-threatening diarrhea. These findings demonstrated that microvillus inclusions were not the major determinant of diarrheal disease. We also determined that VAMP4 was a cargo in microvillus inclusions. The effects of Pacsin 2 loss and the presence of VAMP4 suggested similarities with the process of "activity-dependent bulk endocytosis" reported in hyperstimulated brain synapses.[81–83] In brain, activity-dependent bulk endocytosis uses syndapin 1 (Pacsin 1). The incorporation of 70-kD FITC-dextran from the apical side of MYO5B KO mouse intestine tissue explants into microvillus inclusions confirmed that the inclusions were formed at the apical surface (Figure 2*A*).[77]

Enteroids, cultures of intestinal crypts that form 3-dimensional mini-guts, from MYO5B KO mice formed intracellular microvillus inclusions (Figure 2*B*). Live cell imaging studies of MYO5B KO enteroids demonstrated that inclusions formed from invaginations of the apical membrane over a 2- to 3-hour period.[77] Treating enteroids with Dyngo, a dynamin inhibitor, caused arrest of inclusion formation, and many forming bulk endosomes remained attached to the apical membrane.[77] All of these studies have supported the conclusion that microvillus inclusions form by a process of apical bulk endocytosis in MYO5B-deficient enterocytes. Using the same enteroid models, we have subsequently determined that formation of apical bulk endosomes is associated with the relocalization of tight junction proteins, such as Zonula occludens-1, occludin, and claudin-2, to the orifice regions of the apical bulk endosomes.[85] Members of the Par complex, which is involved in establishment of polarity, and Crumbs 3, which is involved in cell polarity and apical junctions, were mislocalized and also associated with inclusions.[85] These findings suggest that formation of microvillus inclusions in MYO5B-deficient enterocytes requires a coordinated apical bulk endocytosis mechanism, which also recruits tight junction and apical polarity proteins to interact with dynamin in completing scission of apical bulk endosomes.

Other genetically engineered mouse models have evaluated the roles of Rab proteins that interact with MYO5B in regulating the trafficking pathways that control cell polarity and apical microvillus formation. Many of these mice show shortened microvilli but do not completely phenocopy MYO5B loss. A mouse model with germline KO of Rab8a showed shortened microvilli, enlarged lysosomes, and microvillus inclusions.[86] These findings are similar to those in MYO5B KO mice, but the Rab8a KO mice showed less prominent apical deficits and diarrhea. Intestine-targeted KO mouse models of Cdc42, a critical factor for cell polarity, showed alterations in intestinal enterocyte populations and inclusions in enterocytes, but the mice did not die of diarrhea at an early age.[87,88] Rab11a intestine-specific KO mouse models developed short microvilli and deficits in apical trafficking with occasional microvillus inclusions.[89,90] In addition, microvilli were seen forming at the lateral membranes of enterocytes.[89] Dual intestine-targeted KO for both Rab11a and Rab8a induced a more prominent

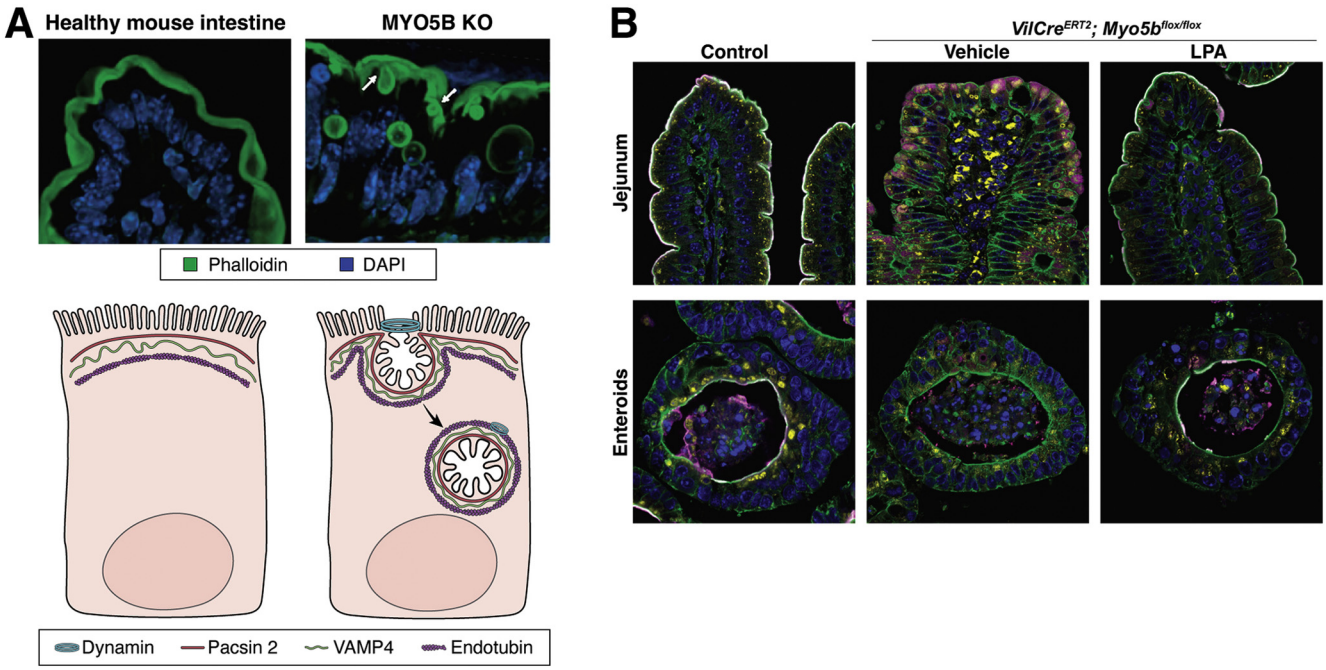

Figure 2. Loss of MYO5B induces apical bulk endocytosis and deficits in apical transporter trafficking: a target for therapeutic intervention. (*A*) Molecular mechanism of microvillus inclusion formation through apical bulk endocytosis induced by MYO5B loss. (*Upper panels*) F-actin (phalloidin, *green*) staining shows uniform brush borders in healthy control mouse intestine. Microvillus inclusions form from invaginations of the apical membranes of enterocytes through a process of apical bulk endocytosis in the MYO5B KO mouse (*white arrows*). Microvillus inclusions that are attached to the apical membrane demonstrate the process of apical bulk endocytosis. *Lower diagram* shows the topologies of intracellular proteins that are involved in apical bulk endocytosis and microvillus inclusion formation (adapted from Engevik et al[77]). (*B*) LPA treatment ameliorates brush border structure and SGLT1 localization in vivo and in mouse enteroids. Immunostaining for SGLT1 (*magenta*), ACTG1 (*green*), and LAMP1 (*yellow*) indicate localization of the Na-glucose co-transporter, cell membrane and brush borders, and lysosomes, respectively. Mature brush borders containing SGLT1 are shown in *white* in control tissues. Tamoxifen-induced MYO5B KO mice with vehicle treatment show expanded lysosomes and disrupted brush border. Both mouse tissues and enteroids treated with LPA partly recover brush border structure and SGLT1 localization on the apical membrane (adapted from Kaji et al[84]). It is notable that LPA treatment does not alter inclusion formation, suggesting that apical bulk endocytosis and trafficking defects are separable.

phenotype with early postnatal mortality, demonstrating loss of apical transporters, short apical microvilli, and lateral microvilli.[91] All of these results suggest that the severe phenotype seen in MYO5B loss or inactivation may accrue from the broad impact of MYO5B on multiple Rab small guanosine triphosphatase–dependent trafficking pathways.

Porcine Model of MVID

Although mouse models are valuable for investigating inactivating mutations of proteins, larger animal models provide insights into physiology that is more similar to humans. A porcine model was developed with a mutation that mimics the P660L point mutation found in the Navajo population.[92] The piglets were phenotypically similar to previous mouse MYO5B KO models with blunted villi, extensive inclusion formation in the small intestines, and loss of NHE3 and SGLT1 from the apical surface with maintenance of CFTR at the apical membrane.[92] This porcine model also showed an altered localization of the bile salt export pump from its usual location at the bile canaliculi in the liver, providing more evidence that mutations in MVID also directly impact the liver.[92] The altered localization of bile salt export pump has not been previously reported for MYO5B KO mouse models but has been observed in human patient samples.[11] The porcine model has been used to evaluate whether the localization of tight junction proteins to inclusions was a mouse model-specific phenomenon. In the porcine model, claudin-2 was observed covering the inclusions, indicating relocalization of tight junction components to the orifice of forming apical bulk endosomes is not mouse-specific.[85] This porcine model of the point mutation causing MVID in patients from the Navajo Nation demonstrates strong similarity to findings in human patients and supports the conclusion that results in MYO5B KO mice generally reflect MVID pathogenesis.

Other Mutations That Manifest MVID Hallmarks

In 2014, two patients who had been diagnosed with MVID did not demonstrate mutations in MYO5B but rather were found to have mutations in syntaxin 3 (STX3).[93] Both patients had inclusions and atypical PAS staining. Interestingly, the enterocytes in patients with STX3 mutations look

phenotypically similar to Rab11a deletion mouse models with prominent lateral microvilli.[89,93] Mutations in the syntaxin-binding protein MUNC18 lead to an MVID phenotype that presented as part of familial hemophagocytic lymphohistiocytosis.[94] It is important to note that MYO5B, STX3, and MUNC18 are functionally linked with RAB8A and RAB11A-dependent trafficking.[95] Congenital diarrhea has also been observed in infants with mutations in UNC45A, a myosin co-chaperone that facilitates folding and stability of myosin motors.[96,97] UNC45A mutations may impact myosin V stability. Thus, treatment strategies that can reestablish apical sodium and water transport may ameliorate diarrheal disease in patients with a range of congenital mutations.

Evaluating Strategies to Bypass Epithelial Deficits Elicited by MYO5B Loss of Function

Because intestinal transplant represents the only definitive treatment for MVID presently, it was critical to evaluate whether any strategies might bypass the blockade in apical trafficking seen in MVID enterocytes. Our previous studies had demonstrated that CFTR trafficking to the apical membrane was not affected by MYO5B loss, indicating that multiple trafficking pathways to the apical must exist, some dependent on MYO5B and others independent. We first examined the effect of lysophosphatidic acid (LPA) administration in mice lacking MYO5B. Prior research showed that the activation of the G protein–coupled LPA receptor, LPAR2, inhibits CFTR-dependent secretory diarrhea, whereas another subtype, LPAR5 stimulates NHE3 trafficking to increase water absorbance.[98–100] LPA is also involved in gastrointestinal repair and protection from apoptosis.[101–105] We observed that LPA treatment alleviated gross morphologic changes attendant with MYO5B loss by reducing villus blunting and inhibiting abnormal large lysosome formation (Figure 2*B*).[84] Using electrophysiological assays with Üssing chambers, LPA-treated mice showed increased SGLT1 activity and decreased CFTR activity in the jejunum.[84] Consistent with the previous report, CFTR inhibition was mediated by LPAR2, but an LPAR2 agonist did not recapitulate SGLT1 activation. In addition, we confirmed that MYO5B-deficient mouse intestines do not exhibit junctional defects or a leaky mucosa phenotype. This indicates that leaky gut syndrome or sepsis in MVID patients might be more associated with long-term TPN rather than the primary effect of MYO5B loss.

LPA treatment in mice partially restored the brush border height and apical localization of SGLT1 and NHE3 in the entire small intestine. Furthermore, these LPA effects were recapitulated in enteroids, suggesting that LPA can promote the differentiation of enterocytes and functional maturation of brush borders through epithelial LPA receptor(s). We found that LPA treatment increased the tuft cell population in healthy and tamoxifen-induced MYO5B KO mice.[106] Although previous studies have suggested that the loss of MYO5B may impact intestinal stem cell differentiation, this was not investigated until recently. Our recent work identified that the loss of MYO5B affected stem cell characteristics and differentiated epithelial cell lineage choice.[106] Tamoxifen-induced intestine-targeted MYO5B KO mice showed disrupted stem cell marker expression, a decrease in goblet cells, and a loss of tuft cells that were correlated with the imbalance of Wnt/Notch signaling pathways (Figure 3).[106] MYO5B loss significantly decreased the transcription levels of Wnt ligands but did not alter Notch signaling molecules. As noted in germline KO mice in our previous work,[75,78] an increase in Paneth cells was seen along with an expanded progenitor zone that expressed OLFM4 suggested that Paneth cells are a Notch provider for stem cells of crypts.[106] Indeed, treatment with a Notch signaling inhibitor, DBZ, reversed goblet and tuft cell population losses and improved enterocyte apical transporter presentation in the tamoxifen-induced MYO5B KO mice.[106] These observations suggest that MYO5B is likely involved in promotion of Wnt signaling pathway and that Wnt/Notch imbalance due to MYO5B loss alters enterocyte maturation and lineage production in the intestine.[106] In addition, this work supports the concept that rebalancing of Wnt/Notch signaling and/or LPA receptor activation may successfully treat the severe diarrheal symptoms in MVID. Future work is necessary to establish effective and sustained delivery of LPA receptor ligands and/or Notch inhibitors to intestinal stem cells, which could promote functional maturation of enterocytes in the face of MYO5B loss (Figure 3).

Liver-Specific Mutations in MYO5B

Originally it was thought that MIVD was specific to only the intestines, but most patients with MVID also develop intrahepatic cholestasis.[13,107] Although this cholestasis was once thought to be caused by the consistent use of TPN, more recent research suggests that mutations in MYO5B may be a primary cause of cholestasis (progressive familial intrahepatic cholestasis [PFIC]6), although certainly parenteral nutrition may exacerbate liver dysfunction.[108,109] In 2005, Wakabayashi et al[110] reported that RAB11A and MYO5B are needed for bile canalicular formation in WIF-B9 cells, suggesting that RAB11A and MYO5B-mediated trafficking is critical for the polarization of hepatocytes. Girard et al[11] found that there was disruption of polarity in MVID patient hepatocytes and mislocalization of MYO5B, RAB11A, and BSEP in the liver. This work suggested that the cholestasis found in MVID patients might be due to MYO5B mutations and not a secondary effect of treatments. A recent study has determined that MYO5B is required to establish hepatocyte polarity and ATP7B trafficking, implicating MYO5B dysfunction in non-Wilsonian disease.[111] It was also reported that some patients with biallelic or suspected biallelic mutations in MYO5B developed cholestasis but not diarrhea.[112] These patients with PFIC develop liver disease without any history of apparent intestinal symptoms. The mutations in MYO5B that are associated with PFIC occur at sites distinct from those that induce MVID with congenital diarrhea. These mutations have been reported as both homozygous as well as compound heterozygous mutations. These PFIC patients with associated MYO5B mutations often demonstrate alterations in bile salt export pump expression.[112,113] Although the mutations tend to be clustered

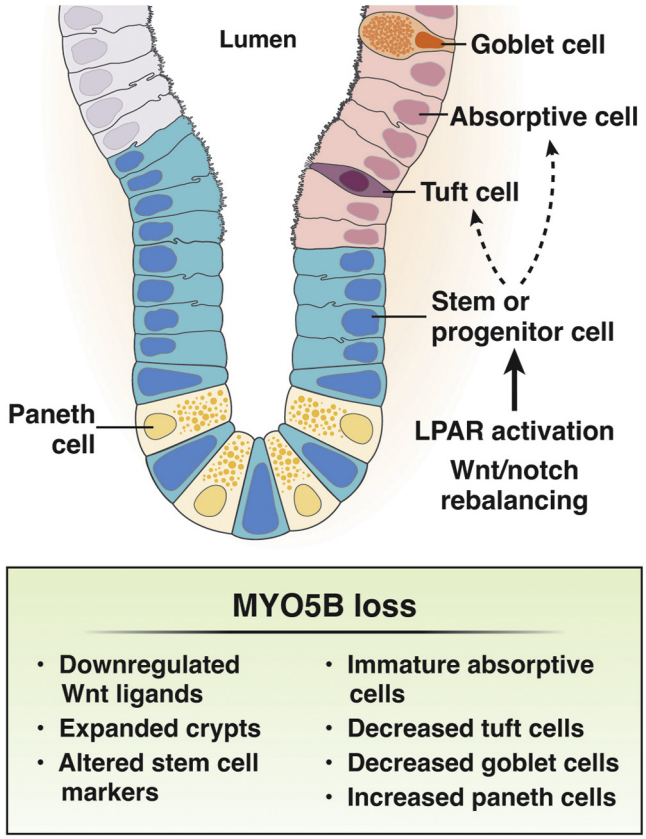

Figure 3. Summary of cell differentiation and maturation deficits in MYO5B-deficient intestine and treatment strategy. Functional MYO5B loss disrupts stem cell differentiation and enterocyte maturation. LPA receptor (LPAR) activation and/or rebalancing of Wnt/Notch signaling may rescue proper differentiation and maturation and consequently improve nutrient absorption.

around the motor head domain, no data have defined how these mutations would induce liver-specific phenotypes. Possible explanations include tissue-specific instability of mutant MYO5B in the liver versus the intestine or loss of liver-specific protein interactions.

Remaining Cell Biological Questions in the Pathophysiology of MVID

Although previous studies have determined that microvillus inclusions form by apical bulk endocytosis in MYO5B-deficient enterocytes, the significance of apical bulk endocytosis in normal enterocytes is unclear. Our previous studies in adult inducible MYO5B KO mice, as well as observations in older MVID patients, have suggested that the observation of microvillus inclusions is far more prominent in neonates before weaning and is also more prominent in the duodenum compared with the distal regions of small intestine.[75] Neonatal enterocytes demonstrate an expanded subapical endosomal processing system that is enriched in endotubin, a protein that regulates post-endocytotic trafficking.[114–117] Interestingly, endotubin was localized around internalizing apical bulk endosomes in neonatal MYO5B KO mice,[77] and endotubin KO mice demonstrate microvillus inclusions in their enterocytes.[116] Thus, neonatal enterocytes may be especially adapted to use of a bulk endocytic process to "drink" components of maternal milk as a source of nutrition. This pathway may use apical bulk endocytosis to rapidly internalize proteins and then process them through endosomes for degradation in the lysosomes. The remarkable size of apical bulk endosomes in MYO5B-deficient enterocytes may accrue from the lack of processing into lysosomes. Alternatively, apical bulk endocytosis may represent a process linked to the lack of maturation observed in MYO5B-deficient enterocytes. Indeed, in neonatal inducible MYO5B KO mice treated with tamoxifen, induced inclusions are observed in enterocytes as they emerge from the crypts.[75] Furthermore, studies are necessary to evaluate the role of apical bulk endocytosis in normal neonatal enterocyte physiology and to discern initiating signals that stimulate the process.

In terms of the concept of personalized cell biology, one open question is how particular MVID-causing mutations in MYO5B affect the physiological function of MYO5B. Although several mutations could impact motor function directly, other mutations can affect the movement of the lever arm or association with specific cargo domain interactions.[66] Alternatively, mutations may alter the ability of MYO5B to autoinhibit motor function through intramolecular head-to-tail interaction and auto-inactivation.[42,118] Other mutations may produce loss of MYO5B by early termination mutation or altering protein folding and promoting rapid degradation as is seen for point mutations in DGAT1.[119,120] Depending on the impact of the mutation on MYO5B function, the ability to impact diarrhea may be variable. Detailed characterization of the molecular impact of MYO5B mutations with clinical presentations and outcomes in the future can lead to more personalized decisions for treatment. Although most data suggest that MYO5B loss and MYO5B mutations that cause diarrheal disease also induce slower but significant cholestatic changes in the liver, it is clear that some mutations (eg, C266R) can certainly cause liver disease without inducing diarrheal disease. The underlying mechanism for these liver-specific MYO5B mutations remains unclear, suggesting that liver-specific interactions with liver proteins may exist. Similarly, it remains unclear whether other mutations in MYO5B may lead to less apparent disease that would be manifested in non-definitive alterations in MYO5B function that could be revealed later in life in the setting of comorbid changes (eg, gastroenteritis).

Our recent studies importantly show that it is possible to allay many of the sequalae of functional inactivation or loss of MYO5B and return sodium and water transporters to the apical membrane of enterocytes. These studies have also supported the concept that loss of MYO5B leads to a defect in the completion of enterocyte maturation after leaving the crypts in the small intestine under the influence of a balance between Wnt and Notch signaling. This incomplete maturation of enterocytes can be ameliorated with reestablishment of the correct balance between Wnt and Notch

signaling (Figure 3). Thus, development of therapeutic interventions targeting these signaling pathways as well as LPA-dependent signaling and their metabolic consequences may lead to definitive treatments for MVID as well as other congenital diarrheal diseases.

References

1. Davidson GP, Cutz E, Hamilton JR, Gall DG. Familial enteropathy: a syndrome of protracted diarrhea from birth, failure to thrive, and hypoplastic villus atrophy. Gastroenterology 1978;75:783–790.
2. Cutz E, Rhoads JM, Drumm B, Sherman PM, Durie PR, Forstner GG. Microvillus inclusion disease: an inherited defect of brush-border assembly and differentiation. N Engl J Med 1989;320:646–651.
3. Phillips AD, Jenkins P, Raafat F, Walkersmith JA. Congenital microvillous atrophy: specific diagnostic features. Arch Dis Child 1985;60:135–140.
4. Koepsell SA, Talmon G. Light microscopic diagnosis of microvillus inclusion disease on colorectal specimens using CD10. Am J Surg Pathol 2010;34:970–972.
5. Iancu TC, Mahajnah M, Manov I, Shaoul R. Microvillous inclusion disease: ultrastructural variability. Ultrastruct Pathol 2007;31:173–188.
6. Groisman GM, Amar M, Livne E. CD10: a valuable tool for the light microscopic diagnosis of microvillous inclusion disease (familial microvillous atrophy). Am J Surg Pathol 2002;26:902–907.
7. Phillips AD, Schmitz J. Familial microvillous atrophy: a clinicopathological survey of 23 cases. J Pediatr Gastroenterol Nutr 1992;14:380–396.
8. Al-Daraji WI, Zelger B, Zelger B, Hussein MR. Microvillous inclusion disease: a clinicopathologic study of 17 cases from the UK. Ultrastruct Pathol 2010;34:327–332.
9. Croft NM, Howatson AG, Ling SC, Nairn L, Evans TJ, Weaver LT. Microvillous inclusion disease: an evolving condition. J Pediatr Gastroenterol Nutr 2000; 31:185–189.
10. Rhoads JM, Vogler RC, Lacey SR, Reddick RL, Keku EO, Azizkhan RG, Berschneider HM. Microvillus inclusion disease: in vitro jejunal electrolyte transport. Gastroenterology 1991;100:811–817.
11. Girard M, Lacaille F, Verkarre V, Mategot R, Feldmann G, Grodet A, Sauvat F, Irtan S, Davit-Spraul A, Jacquemin E, Ruemmele F, Rainteau D, Goulet O, Colomb V, Chardot C, Henrion-Caude A, Debray D. MYO5B and bile salt export pump contribute to cholestatic liver disorder in microvillous inclusion disease. Hepatology 2014;60:301–310.
12. Golachowska MR, van Dael CM, Keuning H, Karrenbeld A, Hoekstra D, Gijsbers CF, Benninga MA, Rings EH, van Ijzendoorn SC. MYO5B mutations in patients with microvillus inclusion disease presenting with transient renal Fanconi syndrome. J Pediatr Gastroenterol Nutr 2012;54:491–498.
13. Aldrian D, Vogel GF, Frey TK, Ayyildiz Civan H, Aksu AU, Avitzur Y, Boluda Ramos E, Cakir M, Demir AM, Deppisch C, Duba HC, Duker G, Gerner P, Hertecant J, Hornova J, Kathemann S, Koeglmeier J, Koutroumpa A, Lanzersdorfer R, Lev-Tzion R, Lima R, Mansour S, Meissl M, Melek J, Miqdady M, Montoya JH, Posovszky C, Rachman Y, Siahanidou T, Tabbers M, Uhlig HH, Unal S, Wirth S, Ruemmele FM, Hess MW, Huber LA, Muller T, Sturm E, Janecke AR. Congenital diarrhea and cholestatic liver disease: phenotypic spectrum associated with MYO5B mutations. J Clin Med 2021;10.
14. Ruemmele FM, Jan D, Lacaille F, Cezard JP, Canioni D, Phillips AD, Peuchmaur M, Aigrain Y, Brousse N, Schmitz J, Revillon Y, Goulet O. New perspectives for children with microvillous inclusion disease: early small bowel transplantation. Transplantation 2004; 77:1024–1028.
15. Koseesirikul P, Chotinaruemol S, Ukarapol N. Incidence and risk factors of parenteral nutrition-associated liver disease in newborn infants. Pediatr Int 2012;54:434–436.
16. Herzog D, Atkison P, Grant D, Paradis K, Williams S, Seidman E. Combined bowel-liver transplantation in an infant with microvillous inclusion disease. J Pediatr Gastroenterol Nutr 1996;22:405–408.
17. Kumpf VJ. Parenteral nutrition-associated liver disease in adult and pediatric patients. Nutr Clin Pract 2006; 21:279–290.
18. Halac U, Lacaille F, Joly F, Hugot JP, Talbotec C, Colomb V, Ruemmele FM, Goulet O. Microvillous inclusion disease: how to improve the prognosis of a severe congenital enterocyte disorder. J Pediatr Gastroenterol Nutr 2011;52:460–465.
19. Muller T, Hess MW, Schiefermeier N, Pfaller K, Ebner HL, Heinz-Erian P, Ponstingl H, Partsch J, Rollinghoff B, Kohler H, Berger T, Lenhartz H, Schlenck B, Houwen RJ, Taylor CJ, Zoller H, Lechner S, Goulet O, Utermann G, Ruemmele FM, Huber LA, Janecke AR. MYO5B mutations cause microvillus inclusion disease and disrupt epithelial cell polarity. Nat Genet 2008;40:1163–1165.
20. Erickson RP, Larson-Thome K, Valenzuela RK, Whitaker SE, Shub MD. Navajo microvillous inclusion disease is due to a mutation in MYO5B. Am J Med Genet A 2008;146A:3117–3119.
21. Schlegel C, Weis VG, Knowles BC, Lapierre LA, Martin MG, Dickman P, Goldenring JR, Shub MD. Apical membrane alterations in non-intestinal organs in microvillus inclusion disease. Dig Dis Sci 2018; 63:356–365.
22. van der Velde KJ, Dhekne HS, Swertz MA, Sirigu S, Ropars V, Vinke PC, Rengaw T, van den Akker PC, Rings EH, Houdusse A, van Ijzendoorn SC. An overview and online registry of microvillus inclusion disease patients and their MYO5B mutations. Hum Mutat 2013; 34:1597–1605.
23. Pohl JF, Shub MD, Trevelline EE, Ingebo K, Silber G, Rayhorn N, Holve S, Hu D. A cluster of microvillous inclusion disease in the Navajo population. J Pediatr 1999; 134:103–106.
24. Comegna M, Amato F, Liguori R, Berni Canani R, Spagnuolo MI, Morroni M, Guarino A, Castaldo G. Two cases of microvillous inclusion disease caused by novel mutations in MYO5B gene. Clin Case Rep 2018; 6:2451–2456.

25. Nathavitharana KA, Green NJ, Raafat F, Booth IW. Siblings with microvillous inclusion disease. Arch Dis Child 1994;71:71–73.
26. Hartman MA, Spudich JA. The myosin superfamily at a glance. J Cell Sci 2012;125(Pt 7):1627–1632.
27. Polis BD, Meyerhof O. Studies on adenosinetriphosphatase in muscle; concentration of the enzyme on myosin. J Biol Chem 1947;169:389–401.
28. Von Muralt AL, Edsall JT. Studies in the physical chemistry of muscle globulin: IV—the anisotropy of myosin and double refraction of flow. J Biol Chem 1930; 89:351–386.
29. Weber A, Winicur S. The role of calcium in the superprecipitation of actomyosin. J Biol Chem 1961; 236:3198–3202.
30. Engelhardt VA, Ljubimova MN, Meitina RA. Chemistry and mechanics of the muscle studied on myosin threads. Compt Rend De l'acad Sci de l'USSR 1941; 30:644.
31. Engelhardt VA, Lyubimova MN. Myosin and adenosinetriphosphatase. Nature 1939;144:668–669.
32. Lohmann K. Über die enzymatische ausspaltung der kreatinephosphorsaure; zugleich ein beitrag zum chemismus der muskelkontraktion. BioChem 1934;271:264–277.
33. Lodish H, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. Molecular cell biology. 4th ed. New York: W H Freeman, 2000.
34. Sun Y, Goldman YE. Lever-arm mechanics of processive myosins. Biophys J 2011;101:1–11.
35. Purcell TJ, Morris C, Spudich JA, Sweeney HL. Role of the lever arm in the processive stepping of myosin V. Proc Natl Acad Sci U S A 2002;99:14159–14164.
36. Sakamoto T, Yildez A, Selvin PR, Sellers JR. Step-size is determined by neck length in myosin V. Biochemistry 2005;44:16203–16210.
37. Uyeda TQ, Abramson PD, Spudich JA. The neck region of the myosin motor domain acts as a lever arm to generate movement. Proc Natl Acad Sci U S A 1996; 93:4459–4464.
38. Kodera N, Yamamoto D, Ishikawa R, Ando T. Video imaging of walking myosin V by high-speed atomic force microscopy. Nature 2010;468:72–76.
39. De La Cruz EM, Wells AL, Rosenfeld SS, Ostap EM, Sweeney HL. The kinetic mechanism of myosin V. Proc Natl Acad Sci U S A 1999;96:13726–13731.
40. Mehta AD, Rock RS, Rief M, Spudich JA, Mooseker MS, Cheney RE. Myosin-V is a processive actin-based motor. Nature 1999;400:590–593.
41. Rief M, Rock RS, Mehta AD, Mooseker MS, Cheney RE, Spudich JA. Myosin-V stepping kinetics: a molecular model for processivity. Proc Natl Acad Sci U S A 2000; 97:9482–9486.
42. Donovan KW, Bretscher A. Head-to-tail regulation is critical for the in vivo function of myosin V. J Cell Biol 2015;209:359–365.
43. Achler C, Filmer D, Merte C, Drenckhahn D. Role of microtubules in polarized delivery of apical membrane proteins to the brush border of the intestinal epithelium. J Cell Biol 1989;109:179–189.
44. Gilbert T, Rodriguez-Boulan E. Induction of vacuolar apical compartments in the Caco-2 intestinal epithelial cell line. J Cell Sci 1991;100(Pt 3):451–458.
45. Peterson MD, Mooseker MS. Characterization of the enterocyte-like brush border cytoskeleton of the C2BBe clones of the human intestinal cell line, Caco-2. J Cell Sci 1992;102(Pt 3):581–600.
46. Kravtsov D, Mashukova A, Forteza R, Rodriguez MM, Ameen NA, Salas PJ. Myosin 5b loss of function leads to defects in polarized signaling: implication for microvillus inclusion disease pathogenesis and treatment. Am J Physiol Gastrointest Liver Physiol 2014; 307:G992–G1001.
47. Tomic TT, Olausson J, Rehammar A, Deland L, Muth A, Ejeskar K, Nilsson S, Kristiansson E, Wassen ON, Abel F. MYO5B mutations in pheochromocytoma/paraganglioma promote cancer progression. PLoS Genet 2020;16:e1008803.
48. Hume AN, Ushakov DS, Tarafder AK, Ferenczi MA, Seabra MC. Rab27a and MyoVa are the primary Mlph interactors regulating melanosome transport in melanocytes. J Cell Sci 2007;120(Pt 17):3111–3122.
49. Van Gele M, Geusens B, Schmitt AM, Aguilar L, Lambert J. Knockdown of myosin Va isoforms by RNAi as a tool to block melanosome transport in primary human melanocytes. J Invest Dermatol 2008;128:2474–2484.
50. Rodriguez OC, Cheney RE. Human myosin-Vc is a novel class V myosin expressed in epithelial cells. J Cell Sci 2002;115(Pt 5):991–1004.
51. Lapierre LA, Kumar R, Hales CM, Navarre J, Bhartur SG, Burnette JO, Provance DW Jr, Mercer JA, Bahler M, Goldenring JR. Myosin vb is associated with plasma membrane recycling systems. Mol Biol Cell 2001; 12:1843–1857.
52. Roland JT, Lapierre LA, Goldenring JR. Alternative splicing in class V myosins determines association with Rab10. J Biol Chem 2009;284:1213–1223.
53. Roland JT, Kenworthy AK, Peranen J, Caplan S, Goldenring JR. Myosin Vb interacts with Rab8a on a tubular network containing EHD1 and EHD3. Mol Biol Cell 2007;18:2828–2837.
54. Lindsay AJ, Jollivet F, Horgan CP, Khan AR, Raposo G, McCaffrey MW, Goud B. Identification and characterization of multiple novel Rab-myosin Va interactions. Mol Biol Cell 2013;24:3420–3434.
55. Strom M, Hume AN, Tarafder AK, Barkagianni E, Seabra MC. A family of Rab27a-binding proteins: melanophilin links Rab27a and myosin Va functions in melanosome transport. J Biol Chem 2002;277:25423–25430.
56. Wu X, Rao K, Bowers MB, Copeland NG, Jenkins NA, Hammer JA. Rab27a enables myosin Va-dependent melanosome capture by recruiting the myosin to the organelle. J Cell Sci 2001;114:1091–1100.
57. Hales CM, Griner R, Hobdy-Henderson KC, Dorn MC, Hardy D, Kumar R, Navarre J, Chan EK, Lapierre LA, Goldenring JR. Identification and characterization of a family of Rab11-interacting proteins. J Biol Chem 2001; 276:39067–39075.
58. Schafer JC, Baetz NW, Lapierre LA, McRae RE, Roland JT, Goldenring JR. Rab11-FIP2 interaction with

MYO5B regulates movement of Rab11a-containing recycling vesicles. Traffic 2014;15:292–308.
59. Roland JTE, Bryant DM, Datta A, Itzen A, Mostov KE, Goldenring JR. Rab GTPase-Myo5B complexes control membrane recycling and epithelial polarization. Proc Natl Acad Sci U S A 2011;108:2789–2794.
60. Szperl AM, Golachowska MR, Bruinenberg M, Prekeris R, Thunnissen AM, Karrenbeld A, Dijkstra G, Hoekstra D, Mercer D, Ksiazyk J, Wijmenga C, Wapenaar MC, Rings EH, van ISC. Functional characterization of mutations in the myosin Vb gene associated with microvillus inclusion disease. J Pediatr Gastroenterol Nutr 2011;52:307–313.
61. Lin R, Murtazina R, Cha B, Chakraborty M, Sarker R, Chen TE, Lin Z, Hogema BM, de Jonge HR, Seidler U, Turner JR, Li X, Kovbasnjuk O, Donowitz M. D-glucose acts via sodium/glucose cotransporter 1 to increase NHE3 in mouse jejunal brush border by a Na+/H+ exchange regulatory factor 2-dependent process. Gastroenterology 2011;140:560–571.
62. Mashukova A, Forteza R, Wald FA, Salas PJ. PDK1 in apical signaling endosomes participates in the rescue of the polarity complex atypical PKC by intermediate filaments in intestinal epithelia. Mol Biol Cell 2012; 23:1664–1674.
63. Phillips A, Fransen J, Hauri HP, Sterchi E. The constitutive exocytotic pathway in microvillous atrophy. J Pediatr Gastroenterol Nutr 1993;17:239–246.
64. Reinshagen K, Naim HY, Zimmer KP. Autophagocytosis of the apical membrane in microvillus inclusion disease. Gut 2002;51:514–521.
65. Ameen NA, Salas PJ. Microvillus inclusion disease: a genetic defect affecting apical membrane protein traffic in intestinal epithelium. Traffic 2000;1:76–83.
66. Knowles BC, Roland JT, Krishnan M, Tyska MJ, Lapierre LA, Dickman PS, Goldenring JR, Shub MD. Myosin Vb uncoupling from RAB8A and RAB11A elicits microvillus inclusion disease. J Clin Invest 2014; 124:2947–2962.
67. Vogel GF, Janecke AR, Krainer IM, Gutleben K, Witting B, Mitton SG, Mansour S, Ballauff A, Roland JT, Engevik AC, Cutz E, Muller T, Goldenring JR, Huber LA, Hess MW. Abnormal Rab11-Rab8-vesicles cluster in enterocytes of patients with microvillus inclusion disease. Traffic 2017;18:453–464.
68. Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 1989;96:736–749.
69. Ruemmele FM, Muller T, Schiefermeier N, Ebner HL, Lechner S, Pfaller K, Thoni CE, Goulet O, Lacaille F, Schmitz J, Colomb V, Sauvat F, Revillon Y, Canioni D, Brousse N, de Saint-Basile G, Lefebvre J, Heinz-Erian P, Enninger A, Utermann G, Hess MW, Janecke AR, Huber LA. Loss-of-function of MYO5B is the main cause of microvillus inclusion disease: 15 novel mutations and a CaCo-2 RNAi cell model. Hum Mutat 2010; 31:544–551.
70. Thoeni CE, Vogel GF, Tancevski I, Geley S, Lechner S, Pfaller K, Hess MW, Muller T, Janecke AR, Avitzur Y, Muise A, Cutz E, Huber LA. Microvillus inclusion disease: loss of Myosin vb disrupts intracellular traffic and cell polarity. Traffic 2014;15:22–42.
71. Roland JT, Bryant DM, Datta A, Itzen A, Mostov KE, Goldenring JR. Rab GTPase-Myo5B complexes control membrane recycling and epithelial polarization. Proc Natl Acad Sci U S A 2011;108:2789–2794.
72. Bryant DM, Datta A, Rodriguez-Fraticelli AE, Peranen J, Martin-Belmonte F, Mostov KE. A molecular network for de novo generation of the apical surface and lumen. Nat Cell Biol 2010;12:1035–1045.
73. Kravtsov DV, Ahsan MK, Kumari V, van Ijzendoorn SC, Reyes-Mugica M, Kumar A, Gujral T, Dudeja PK, Ameen NA. Identification of intestinal ion transport defects in microvillus inclusion disease. Am J Physiol Gastrointest Liver Physiol 2016;311: G142–G155.
74. Carton-Garcia F, Overeem AW, Nieto R, Bazzocco S, Dopeso H, Macaya I, Bilic J, Landolfi S, Hernandez-Losa J, Schwartz S Jr, Ramon y, Cajal S, van Ijzendoorn SC, Arango D. Myo5b knockout mice as a model of microvillus inclusion disease. Sci Rep 2015;5: 12312.
75. Weis VG, Knowles BC, Choi E, Goldstein AE, Williams JA, Manning EH, Roland JT, Lapierre LA, Goldenring JR. Loss of MYO5B in mice recapitulates microvillus inclusion disease and reveals an apical trafficking pathway distinct to neonatal duodenum. Cell Mol Gastroenterol Hepatol 2016;2:131–157.
76. Schneeberger K, Vogel GF, Teunissen H, van Ommen DD, Begthel H, El Bouazzaoui L, van Vugt AH, Beekman JM, Klumperman J, Muller T, Janecke A, Gerner P, Huber LA, Hess MW, Clevers H, van Es JH, Nieuwenhuis EE, Middendorp S. An inducible mouse model for microvillus inclusion disease reveals a role for myosin Vb in apical and basolateral trafficking. Proc Natl Acad Sci U S A 2015;112:12408–12413.
77. Engevik AC, Kaji I, Postema MM, Faust JJ, Meyer AR, Williams JA, Fitz GN, Tyska MJ, Wilson JM, Goldenring JR. Loss of myosin Vb promotes apical bulk endocytosis in neonatal enterocytes. J Cell Biol 2019; 218:3647–3662.
78. Engevik AC, Kaji I, Engevik MA, Meyer AR, Weis VG, Goldstein A, Hess MW, Muller T, Koepsell H, Dudeja PK, Tyska M, Huber LA, Shub MD, Ameen N, Goldenring JR. Loss of MYO5B leads to reductions in Na(+) absorption with maintenance of CFTR-dependent Cl(-) secretion in enterocytes. Gastroenterology 2018;155:1883–1897 e10.
79. Senju Y, Suetsugu S. Possible regulation of caveolar endocytosis and flattening by phosphorylation of F-BAR domain protein PACSIN2/Syndapin II. Bioarchitecture 2015;5:70–77.
80. Plomann M, Wittmann JG, Rudolph MG. A hinge in the distal end of the PACSIN 2 F-BAR domain may contribute to membrane-curvature sensing. J Mol Biol 2010;400:129–136.
81. Cousin MA. Activity-dependent bulk synaptic vesicle endocytosis: a fast, high capacity membrane retrieval mechanism. Mol Neurobiol 2009;39:185–189.

82. Clayton EL, Cousin MA. The molecular physiology of activity-dependent bulk endocytosis of synaptic vesicles. J Neurochem 2009;111:901–914.
83. Nicholson-Fish JC, Kokotos AC, Gillingwater TH, Smillie KJ, Cousin MA. VAMP4 is an essential cargo molecule for activity-dependent bulk endocytosis. Neuron 2015;88:973–984.
84. Kaji I, Roland JT, Watanabe M, Engevik AC, Goldstein AE, Hodges CA, Goldenring JR. Lysophosphatidic acid increases maturation of brush borders and SGLT1 activity in MYO5B-deficient mice, a model of microvillus inclusion disease. Gastroenterology 2020; 159:1390–1405.e20.
85. Engevik AC, Krystofiak ES, Kaji I, Meyer AR, Weis VG, Goldstein A, Coutts AW, Melkamu T, Saqui-Salces M, Goldenring JR. Recruitment of polarity complexes and tight junction proteins to the site of apical bulk endocytosis. Cell Mol Gastroenterol Hepatol 2021;12:59–80.
86. Sato T, Mushiake S, Kato Y, Sato K, Sato M, Takeda N, Ozono K, Miki K, Kubo Y, Tsuji A, Harada R, Harada A. The Rab8 GTPase regulates apical protein localization in intestinal cells. Nature 2007;448:366–369.
87. Sakamori R, Das S, Yu S, Feng S, Stypulkowski E, Guan Y, Douard V, Tang W, Ferraris RP, Harada A, Brakebusch C, Guo W, Gao N. Cdc42 and Rab8a are critical for intestinal stem cell division, survival, and differentiation in mice. J Clin Invest 2012;122:1052–1065.
88. Melendez J, Liu M, Sampson L, Akunuru S, Han X, Vallance J, Witte D, Shroyer N, Zheng Y. Cdc42 coordinates proliferation, polarity, migration, and differentiation of small intestinal epithelial cells in mice. Gastroenterology 2013;145:808–819.
89. Knowles BC, Weis VG, Yu S, Roland JT, Williams JA, Alvarado GS, Lapierre LA, Shub MD, Gao N, Goldenring JR. Rab11a regulates syntaxin 3 localization and microvillus assembly in enterocytes. J Cell Sci 2015; 128:1617–1626.
90. Sobajima T, Yoshimura S, Iwano T, Kunii M, Watanabe M, Atik N, Mushiake S, Morii E, Koyama Y, Miyoshi E, Harada A. Rab11a is required for apical protein localisation in the intestine. Biol Open 2014; 4:86–94.
91. Feng Q, Bonder EM, Engevik AC, Zhang L, Tyska MJ, Goldenring JR, Gao N. Disruption of Rab8a and Rab11a causes formation of basolateral microvilli in neonatal enteropathy. J Cell Sci 2017;130:2491–2505.
92. Engevik AC, Coutts AW, Kaji I, Rodriguez P, Ongaratto F, Saqui-Salces M, Medida RL, Meyer AR, Kolobova E, Engevik MA, Williams JA, Shub MD, Carlson DF, Melkamu T, Goldenring JR. Editing myosin VB gene to create porcine model of microvillus inclusion disease, with microvillus-lined inclusions and alterations in sodium transporters. Gastroenterology 2020; 158:2236–2249 e9.
93. Wiegerinck CL, Janecke AR, Schneeberger K, Vogel GF, van Haaften-Visser DY, Escher JC, Adam R, Thoni CE, Pfaller K, Jordan AJ, Weis CA, Nijman IJ, Monroe GR, van Hasselt PM, Cutz E, Klumperman J, Clevers H, Nieuwenhuis EE, Houwen RH, van Haaften G, Hess MW, Huber LA, Stapelbroek JM, Muller T, Middendorp S. Loss of syntaxin 3 causes variant microvillus inclusion disease. Gastroenterology 2014;147:65–68 e10.
94. Stepensky P, Bartram J, Barth TF, Lehmberg K, Walther P, Amann K, Philips AD, Beringer O, Zur Stadt U, Schulz A, Amrolia P, Weintraub M, Debatin KM, Hoenig M, Posovszky C. Persistent defective membrane trafficking in epithelial cells of patients with familial hemophagocytic lymphohistiocytosis type 5 due to STXBP2/MUNC18-2 mutations. Pediatric Blood & Cancer 2013;60:1215–1222.
95. Dhekne HS, Pylypenko O, Overeem AW, Zibouche M, Ferreira RJ, van der Velde KJ, Rings E, Posovszky C, van der Sluijs P, Swertz MA, Houdusse A, van ISCD. MYO5B, STX3, and STXBP2 mutations reveal a common disease mechanism that unifies a subset of congenital diarrheal disorders: a mutation update. Hum Mutat 2018; 39:333–344.
96. Esteve C, Francescatto L, Tan PL, Bourchany A, De Leusse C, Marinier E, Blanchard A, Bourgeois P, Brochier-Armanet C, Bruel AL, Delarue A, Duffourd Y, Ecochard-Dugelay E, Hery G, Huet F, Gauchez P, Gonzales E, Guettier-Bouttier C, Komuta M, Lacoste C, Maudinas R, Mazodier K, Rimet Y, Riviere JB, Roquelaure B, Sigaudy S, Stephenne X, Thauvin-Robinet C, Thevenon J, Sarles J, Levy N, Badens C, Goulet O, Hugot JP, Katsanis N, Faivre L, Fabre A. Loss-of-function mutations in UNC45A cause a syndrome associating cholestasis, diarrhea, impaired hearing, and bone fragility. Am J Hum Genet 2018;102:364–374.
97. Escalante SG, Brightmore JA, Piper PW, Millson SH. UCS protein function is partially restored in the Saccharomyces cerevisiae she4 mutant with expression of the human UNC45-GC, but not UNC45-SM. Cell Stress Chaperones 2018;23:609–615.
98. Li C, Dandridge KS, Di A, Marrs KL, Harris EL, Roy K, Jackson JS, Makarova NV, Fujiwara Y, Farrar PL, Nelson DJ, Tigyi GJ, Naren AP. Lysophosphatidic acid inhibits cholera toxin-induced secretory diarrhea through CFTR-dependent protein interactions. J Exp Med 2005; 202:975–986.
99. Lin S, Yeruva S, He P, Singh AK, Zhang H, Chen M, Lamprecht G, de Jonge HR, Tse M, Donowitz M, Hogema BM, Chun J, Seidler U, Yun CC. Lysophosphatidic acid stimulates the intestinal brush border Na(+)/H(+) exchanger 3 and fluid absorption via LPA(5) and NHERF2. Gastroenterology 2010;138:649–658.
100. Jenkin KA, He P, Yun CC. Expression of lysophosphatidic acid receptor 5 is necessary for the regulation of intestinal Na(+)/H(+) exchanger 3 by lysophosphatidic acid in vivo. Am J Physiol Gastrointest Liver Physiol 2018;315:G433–G442.
101. Sturm A, Zeeh J, Sudermann T, Rath H, Gerken G, Dignass AU. Lisofylline and lysophospholipids ameliorate experimental colitis in rats. Digestion 2002; 66:23–29.
102. Sturm A, Dignass AU. Modulation of gastrointestinal wound repair and inflammation by phospholipids. Biochim Biophys Acta 2002;1582:282–288.
103. Funk-Archuleta MA, Foehr MW, Tomei LD, Hennebold KL, Bathurst IC. A soy-derived antiapoptotic

fraction decreases methotrexate toxicity in the gastrointestinal tract of the rat. Nutr Cancer 1997;29:217–221.
104. Deng W, Balazs L, Wang DA, Van Middlesworth L, Tigyi G, Johnson LR. Lysophosphatidic acid protects and rescues intestinal epithelial cells from radiation- and chemotherapy-induced apoptosis. Gastroenterology 2002;123:206–216.
105. Deng W, Wang DA, Gosmanova E, Johnson LR, Tigyi G. LPA protects intestinal epithelial cells from apoptosis by inhibiting the mitochondrial pathway. Am J Physiol Gastrointest Liver Physiol 2003;284:G821–G829.
106. Kaji I, Roland JT, Rathan-Kumar S, Engevik AC, Burman A, Goldstein AE, Watanabe M, Goldenring JR. Cell differentiation is disrupted by MYO5B loss through Wnt/Notch imbalance. JCI Insight 2021;6:e150416.
107. Siahanidou T, Koutsounaki E, Skiathitou AV, Stefanaki K, Marinos E, Panajiotou I, Chouliaras G. Extraintestinal manifestations in an infant with microvillus inclusion disease: complications or features of the disease? Eur J Pediatr 2013;172:1271–1275.
108. van IJzendoorn SCD, Li Q, Qiu YL, Wang JS, Overeem AW. Unequal effects of myosin 5B mutations in liver and intestine determine the clinical presentation of low-gamma-glutamyltransferase cholestasis. Hepatology 2020;72:1461–1468.
109. Vinayagamoorthy V, Srivastava A, Sarma MS. Newer variants of progressive familial intrahepatic cholestasis. World J Hepatol 2021;13:2024–2038.
110. Wakabayashi Y, Dutt P, Lippincott-Schwartz J, Arias IM. Rab11a and myosin Vb are required for bile canalicular formation in WIF-B9 cells. Proc Natl Acad Sci U S A 2005;102:15087–15092.
111. Gupta A, Schell MJ, Bhattacharjee A, Lutsenko S, Hubbard AL. Myosin Vb mediates Cu+ export in polarized hepatocytes. J Cell Sci 2016;129:1179–1189.
112. Qiu YL, Gong JY, Feng JY, Wang RX, Han J, Liu T, Lu Y, Li LT, Zhang MH, Sheps JA, Wang NL, Yan YY, Li JQ, Chen L, Borchers CH, Sipos B, Knisely AS, Ling V, Xing QH, Wang JS. Defects in myosin VB are associated with a spectrum of previously undiagnosed low gamma-glutamyltransferase cholestasis. Hepatology 2017;65:1655–1669.
113. Hess MW, Krainer IM, Filipek PA, Witting B, Gutleben K, Vietor I, Zoller H, Aldrian D, Sturm E, Goldenring JR, Janecke AR, Muller T, Huber LA, Vogel GF. Advanced microscopy for liver and gut ultrastructural pathology in patients with MVID and PFIC caused by MYO5B mutations. J Clin Med 2021;10.
114. McCarter SD, Johnson DL, Kitt KN, Donohue C, Adams A, Wilson JM. Regulation of tight junction assembly and epithelial polarity by a resident protein of apical endosomes. Traffic 2010;11:856–866.
115. Wu MH, Padilla-Rodriguez M, Blum I, Camenisch A, Figliuolo da Paz V, Ollerton M, Muller J, Momtaz S, Mitchell SAT, Kiela P, Thorne C, Wilson JM, Cox CM. Proliferation in the developing intestine is regulated by the endosomal protein endotubin. Dev Biol 2021;480:50–61.
116. Cox CM, Lu R, Salcin K, Wilson JM. The endosomal protein endotubin is required for enterocyte differentiation. Cell Mol Gastroenterol Hepatol 2018;5:145–156.
117. Wilson JM, Whitney JA, Neutra MR. Identification of an endosomal antigen specific to absorptive cells of suckling rat ileum. J Cell Biol 1987;105:691–703.
118. Nascimento AF, Trindade DM, Tonoli CC, de Giuseppe PO, Assis LH, Honorato RV, de Oliveira PS, Mahajan P, Burgess-Brown NA, von Delft F, Larson RE, Murakami MT. Structural insights into functional overlapping and differentiation among myosin V motors. J Biol Chem 2013;288:34131–34145.
119. Haas JT, Winter HS, Lim E, Kirby A, Blumenstiel B, DeFelice M, Gabriel S, Jalas C, Branski D, Grueter CA, Toporovski MS, Walther TC, Daly MJ, Farese RV Jr. DGAT1 mutation is linked to a congenital diarrheal disorder. J Clin Invest 2012;122:4680–4684.
120. Schlegel C, Lapierre LA, Weis VG, Williams JA, Kaji I, Pinzon-Guzman C, Prasad N, Boone B, Jones A, Correa H, Levy SE, Han X, Wang M, Thomsen K, Acra S, Goldenring JR. Reversible deficits in apical transporter trafficking associated with deficiency in diacylglycerol acyltransferase. Traffic 2018;19:879–892.

Received February 20, 2022. Accepted April 29, 2022.

Correspondence
Address correspondence to: James R. Goldenring, MD, PhD, Epithelial Biology Center, Vanderbilt University Medical Center, MRB IV Room 10435G, 2213 Garland Avenue, Nashville, Tennessee 37232. e-mail: jim.goldenring@vumc.org; fax: (615) 343-1591. Izumi Kaji, PhD, Epithelial Biology Center, Vanderbilt University Medical Center, 2213 Garland Avenue, MRB IV Room 10465H, Nashville, Tennessee 37232. e-mail: izumi.kaji@vumc.org.

CRediT Authorship Contributions
Deanna M. Bowman (Conceptualization: Equal; Writing – original draft: Lead; Writing – review & editing: Equal)
Izumi Kaji (Conceptualization: Equal; Funding acquisition: Equal; Investigation: Equal; Supervision: Equal; Writing – review & editing: Supporting)
James R. Goldenring, MD, PhD (Conceptualization: Equal; Funding acquisition: Equal; Investigation: Equal; Supervision: Equal; Writing – review & editing: Equal)

Conflicts of interest
The authors disclose no conflicts.

Funding
Dr Goldenring is supported by RC2 DK118640 and R01 DK48370 from the National Institutes of Health (NIH) and a gift from the Christine Volpe Fund. Dr Kaji is supported by NIH R01 DK128190 and a Vanderbilt Digestive Diseases Research Center Pilot and Feasibility Grant (P30 058404). Ms Bowman is supported by T32 GM07628.