The roles of MYO5B in epithelial cells and the intestine

Leng, Changsen

DOI:
10.33612/diss.127906021

Chapter 2

Alimentary and pharmacological interventions in microvillus inclusion disease: where do we stand and where to go

Changsen Leng, Sven C. D. van IJzendoorn

Department of Biomedical Sciences of Cells and Systems, section Molecular Cell Biology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

Invited review, in preparation

SUMMARY

Microvillus inclusion disease (MVID) is a rare inherited and invariably fatal enteropathy, characterized by severe intractable secretory diarrhea and nutrient malabsorption. No cure exists and patients typically die during infancy because of treatment-related complications. The need for alternative treatment strategies is evident. Compared to other congenital enteropathies, MVID poses unique challenges regarding pharmacological strategies. Several pharmacological interventions with variable successes have been tried and reported for individual patients as part of their clinical care. Unfortunately, these interventions and their outcomes have remained hidden in case reports and have not been reviewed. Further, recent advances regarding MVID pathogenesis have shed new light on the outcomes of these pharmacological interventions and offer suggestions for future evidence-based interventions. Hence, an inventory of reported pharmacological interventions in MVID, their rationales and outcomes, and a discussion of these in the light of current knowledge is opportune. Together with a discussion on MVID-specific pharmacokinetic, -dynamic and -genetic concerns, we envision that this paper will aid researchers and clinicians in their efforts to develop pharmacological interventions to combat this devastating disease.

INTRODUCTION TO MICROVILLUS INCLUSION DISEASE

Clinical presentation

Microvillus inclusion disease (MVID; OMIM #251850) is an autosomal recessive congenital diarrheal disorder (1,2). From the start of their lives, patients with MVID suffer from unstoppable secretory diarrhea at complete bowel rest, often exceeding that seen in cholera-infected children but variable between patients. Oral or enteral feeding is not possible and causes massive osmotic diarrhea, resulting in patients' failure to thrive (1,2).

In order to provide a detailed description of the clinical presentation of MVID, we searched EMBASE and MEDLINE databases using the following search strings: ((microvill* inclusion disease) OR (microvill* atrophy)) AND case report) to collect all published MVID case reports. 83 valid case reports reporting on 131 MVID patients were retrieved (supplementary Table 1). From these, information on patient gender, gestation date at birth, bodyweight at birth, presence or absence of polyhydramnios, day of onset, stool output, fecal analyses and age at death, among others, were extracted and analysed (Tables 1 and 2).

Diagnosis

Intestinal biopsies reveal villus atrophy in the small intestine without signs of infection of inflammation (1). Villus enterocytes display the diagnostic intracellular accumulation of periodic acid-Schiff (PAS)-positive material and the brush border enzyme CD10 (3,4). Electron microscopy reveals microvillus brush border atrophy and the appearance of pathognomonic microvillus inclusions in the cytoplasm of some villus enterocytes (4). Bi-allelic mutations in the *MYO5B* (5) or *STX3* (6) gene confirm MVID diagnosis. All features of MVID can also present as part of familial hemophagocytic lymphohistiocytosis, an immune disorder caused by *STXBP2* gene mutations (7).

Pathogenesis

The proteins that are encoded by the three MVID-associated genes are functionally linked (8–10). *MYO5B, STX3 or STXBP2* mutation-driven loss of effective absorptive surface area causes malabsorption and chronic secretory diarrhea (10). The loss of absorptive surface area includes villus atrophy, microvillus atrophy, and the general mislocalization and/ or reduced expression of brush border proteins involved in *i)* dietary nutrient digestion or absorption (*e.g.*, sucrase-isomaltase, dipeptidyl peptidase IV, glucose/sodium symporter SGLT-1) (11,12), *ii)* water resorption (*e.g.*, aquaporins) (11) and *iii)* electrolyte transport across the brush border membrane (*e.g.*, the sodium/proton exchange protein SLC9A3 (NHE3), the bicarbonate/chloride

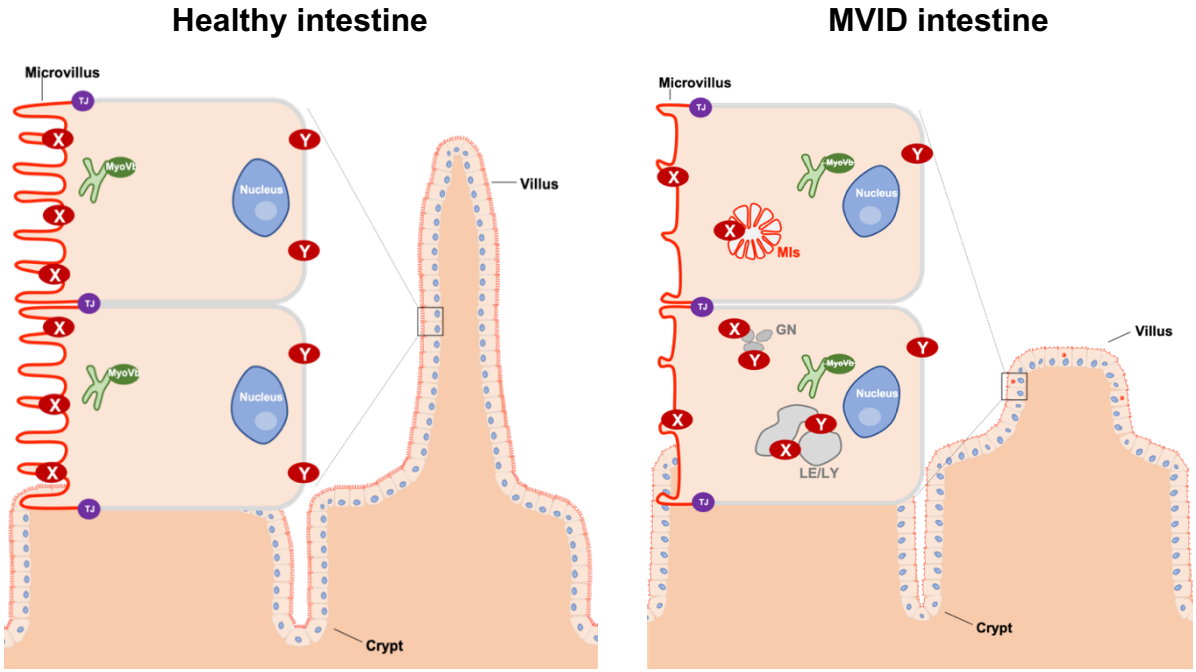

Figure 1. Schematic overview of tissue and cellular characteristics of healthy and MVID intestinal epithelium. In healthy enterocytes, the recycling endosome (Green) is mostly located sub-apically and plays an important role in transporting proteins to the plama membrane (in particular to the apical membrane). However, in MVID caused by the loss function of myosin Vb (MyoVb), the villi and microvilli are atrophic. Moreover, the proteins (marked as X and Y) in the plasma membrane are mislocalized in microvillus inclusions (MIs) or in the compartments of enlarged late endo/lysosomes (LE/LY) and granules (GN). **X** indicated CD10, DRA, GLUT5, NHE3, SGLT1, Sucrase-isomaltase (SI), 5'-nucleotidase (5'NT), Alkaline phosphatase (AP), AQP7, CD36, CFTR, and/or DPPIV. **Y** indicated Transferrin receptor (Tfr) and/or Na^+/K^+ ATPase. TJ: Tight junction. MIs: Microvillus inclusions. LE: Late endosomes. LY: Lysosomes. GN: Granules.

exchange protein SLC26A3 (DRA) and the chloride and bicarbonate transporter CFTR) (8,13,14). The loss of *MYO5B, STX3*, or *STXBP2* in intestinal cell or organoid cultures

or mice has been causally related to the reduced expression or mislocalization of brush border proteins involved in nutrient absorption and/or electrolyte exchange (6,9,11,13,15) and to the loss of microvilli (16,17). The relationship between the loss of these genes and the villus atrophy is less clear. The cellular defects appear less pronounced in the crypt area when compared to the villus area (18). This led to suggest that a defect in intestinal epithelial cell differentiation – and a resultant immature epithelium composed of predominantly secretory crypts and few absorptive villi - underlies the symptoms. For more detailed reviews on MVID pathogenesis the reader is referred to excellent recent review articles (19,20). A cartoon illustrating key tissue and cellular defects in MVID is presented in figure 1.

Relation to other congenital diarrheal disorders

As a congenital diarrheal disorder, MVID displays characteristics of other diarrheal disorders. For example, the proteins DRA and NHE3 - which show reduced expression and/or are mislocalized in MVID enterocytes (11,14,21,22)– are mutated in congenital chloride diarrhea (CCD) and congenital sodium diarrhea (CSD), respectively. From all collected case reports (supplementary Table 1) we retrieved those that mentioned at least one data value regarding stool output, electrolytes, osmolarity or pH value (Table 1). Inspection of fecal electrolyte compositions from the retrieved MVID case reports (Table 2) indicate that the average MVID patient (fecal Na+ 89mEq/L, Cl- 75mEq/L) does not typically fulfil the criteria for the diagnosis of either CSD (fecal Na+ >100mEq/L, Cl- >40 mEq/L) or CCD (fecal Na+ >60mEq/L, Cl- >120 mEq/L). Further, unlike in CSD and CDD, polyhydramnios (in-utero diarrhea) is not common in MVID, although reported in seven out of 29 cases (24%; Table 1) for which the presence or absence of polyhydramnios was mentioned. Upon oral or enteral feeding, MVID resembles congenital osmotic diarrhea (characterized by high fecal sodium losses, *e.g.*, congenital glucose-galactose intolerance and congenital sucrase-isomaltase deficiency), presumably due to the reduced brush border localization of SGLT-1 and sucrase-isomaltase (SI). MVID thus presents as a mix of other congenital diarrheal and malabsorption disorders.

Table 1. Clinical details from published MVID case reports.

References	PMID#	Patients number	Gender	Gestation (week)	Birth body weight (g)	Poly hydra mnios	Onset (day)	Stool output (ml/kg/d)	Feces electrolyte (mmol/L)			Feces osmolarity (mOsm/kg)	Feces PH value	Dead/ Alive	Follow-up
									Na^+	Cl^-	K^+				
Lingaldinna et al. (2017)	28842815	1	Male	38	3500		3		78	64	7.3		7	Dead	1 month
		2	Female	34	2000		5						7	Dead	36 days
Schoen et al. (2017)	29546954	1	Male	36		No	10	190	120	67		30		Alive	36 months
Perry et al. (2014)	25111220	1	Female				150		15					Alive	132 months
Burgis et al. (2013)	23525737	1	Male	at term			6	150						Alive	168 months
Siahanidou et al. (2013)	23354788	1	Female	35	2330	Yes	1		85	78	22			Dead	7 months
AI-Sinani et al. (2012)	23226823	1	Female	at term		No	3	100						Dead	4 months
Thomas et al. (2012)	22318102	1	Female	35	2320	Yes	3		35	21				Dead	23 days
Vora et al. (2012)	22197941	1	Female	37	2900	Yes	1	100	78	42	40	11		Alive	4 days
Fuchs et al. (2011)	22152886	2	Male	36			4	148						Alive	41 months
Shahid et al. (2012)	21968248	1	Male	at term	2734	No	3	175	84	68	13			Alive	3 months
Khubchandani et al. (2011)	21299349	1	Male	35			3						6	Dead	2 months
Gathungu et al. (2008)	18277898	1	Male	34	2450	No	8		112	113	21.6	292		Alive	12 days
Amosu et al. (2007)	17418172	1	Male	31			3	100	139	105	4.7	279		Alive	12 days
Kucinskiene et al. (2004)	15456973	1	Female	37	2530		1	200						Dead	1.5 months
Mierau et al. (2001)	11783915	1	Male	at term			6	115	95	95	30	270	9	Alive	5 months
Ruemmele et al. (2001)	11414303	1	Male	36	2700	NO	2	200	100	60	17.5			Alive	4 months
Kennea et al. (2001)	11251929	1	Male	35	3720	Yes		300						Alive	3 days
Wilson et al. (2001)	11173328	1	Male	36	2740	No	3		76	79	39			Dead	6 months
Croft et al. (2000)	10941974	1	Female	at term	3510		11		108	55	11.9	330		Alive	39 months
Bunn et al. (2000)	10941971	1	Female	34	2100	No	6	175						Alive	24 months
Heinz-Erian et al. (1999)	9932857	1	Female	36			10	170				261		Alive	9 months
Pohl et al. (1999)	9880458	1	Male	39	3500		6	95	110	85	7			Alive	24 months
		2	Male	38	2600		6	135	81	44	2			Alive	96 months
		3	Male	36	3300		6	150	115	96	5			Alive	84 months
		4	Female	39	3160		4	175	107	84	18			Alive	48 months
		5	Female	37	2700		4	100	6		27			Dead	36 months

Kagitani et al. (1998)	9844114	1	Male	38	3300	No	1	75						Alive	132 months
Michail et al. (1998)	9822319	1	Male	36	3090	No	1	100	119	111	14		6.5	Alive	3 months
		2	Male				14	150	105	74	12	281		Alive	9 months
Randak et al. (1998)	9740207	1	Male	at term	3900	No	14	178	105	74	12.1	281	8	Dead	18 months
Beck et al. (1997)	9364305	1	Female	36	2700	No		175						Alive	3 months
Assmann et al. (1997)	9323563	1	Male	33	2950	Yes	1		99		12	240		Dead	7 months
Herzog et al. (1996)	8732907	1	Male	at term	3350	No	7	50						Alive	7 months
Raafat et al. (1994)	7959671	1	Female	at term	4100	No	14	60	91					Dead	39 months
		2	Male	at term	4200	No	14	50	100					Dead	5 months
		3	Male	at term	3800	No	7		95					Alive	58 months
Nathavitharana et al. (1994)	8067796	1	Male	38	3325	No	1	166	58	36	15	309		Dead	9 days
		2	Male	35	2880	No	2	200						Dead	4 months
Nizet et al. (1994)	8032396	1	Female	35	2810	No	3	120	104		19	240		Alive	7 months
Schofield et al. (1992)	1319670	1	Female	37	2700	No	4	200	6		27			Dead	37 months
Bell et al. (1991)	1660676	1	Male	at term	3530	Yes	1	150	103	89	19			Alive	72 months
		2	Male	37	3300	Yes	1	150	122	102	19.4			Alive	9 months
Couper et al. (1989)	2759484	1	Female	at term	2300	No	3	85	100	82	29			Alive	13 months
Phillips et al. (1985)	3977385	1	Female	37	2500	No	2		91					Dead	6 months
		2	Female	34	2200	No	4		93					Dead	6 months
Mendes et al. (2014)	25635218	1	Female	36	2800		1	120	83				8	Dead	9 months
Elena et al.		1	Female	36			7	100						Alive	13 months

The table includes patient data from the collected case reports (supplementary Table 1) which have at least one data value regarding stool output, feces of electrolyte or osmolarity or PH value. In this table, a total of 48 patients, including 27 males and 21 females (the ratio male/female is 1.29); Among of them, only 14 patients were at term of gestation, 32 patients were not at term (<39 weeks), and 2 patients were not reported; 7 patients were reported with polyhydramnios during pregnancy, pregnancy for 22 patients were not associated with polyhydramnios, and for 19 patients the presence or absence of polyhydramnios were not reported; The median of diarrhea onset was at 4 days, 37 patients' onsets (80.4%) are in the first week among the 46 patients reported; 19 patients were reported to have died and 29 patients were alive at the moment of reporting, the median time to death is 6 months. In addition, we summarized other data of minimum, maximum and average for the birth body weight, stool output and fecal characteristics in table 2.

Table 2. Summary of the clinical details from published MVID case reports.

	Birth body weight (g) n=37	Stool output (ml/kg/d) n=35	Feces electrolyte (mmol/L)			Feces osmolarity (mOsm/kg) n=12	Feces PH value n=7
			Na^+ n=34	Cl^- n=23	K^+ n=25		
Minimum	2000	50	6	21	2	11	6
Maximum	4200	300	139	113	40	330	9
Average	2987	140.2	88.8	75.0	17.8	235.3	7.36

This table shows the minimum, maximum and average values of birth body weight, stool output and fecal characteristics from table 1.

Current treatment of MVID

Treatment options are limited. First treatment addresses immediate life-threatening dehydration and metabolic acidosis. Oral rehydration solution (glucose-mediated sodium absorption) is ineffective because of the absence of brush border SGLT-1. MVID patients typically require life-long total parenteral nutrition (TPN), also known as hyperalimentation, to compensate for fecal fluid and salt losses and provide nutrients to support growth (23,24). Intestinal transplantation is an option (25) but because of its lower 5-year survival rate (~60%) only for specific cases.

ALIMENTARY INTERVENTIONS IN MVID

While life-saving at first, TPN/hyperalimentation does not stop the diarrhea and most patients die during infancy because of TPN-associated complications (26). Most importantly, TPN-related complications are the predominant cause of death in MVID. The most frequent TPN-related complication that causes death of MVID patients is catheter-related sepsis. Another life-threatening complication of long-term TPN in patients is liver failure (cholestasis and pruritus progressing to cirrhosis) (27,28). Interestingly, for five MVID patients a reduction of liver symptoms was reported when soybean oil-based lipids in the TPN were replaced by fish oil-based lipids (29,30). Nevertheless, TPN does not cure the malabsorption and diarrhea and better treatment for patients with MVID is clearly needed.

PHARMACOLOGICAL INTERVENTIONS IN MVID

Several pharmacological treatments have been tried with individual MVID patients and reported in published case reports. In order to review these, each of the collected case studies (supplementary Table 1) was manually screened for reports of non-routine pharmacological intervention. This yielded 15 articles reporting in total 35 patient treatments involving in total eight different pharmacological interventions (Table 3). The reported pharmacological interventions could be divided from a conceptual point of view into three categories: *i*) drugs that stimulate enterocyte proliferation and/ or differentiation, *ii*) anti-diarrheal drugs that modulate ion balance across the enterocytes' brush border membrane and *iii*) other anti-diarrheal drugs. For all reported treatments their rationales and outcomes are discussed below.

Drugs that stimulate the proliferation and- or differentiation of enterocytes

Epidermal growth factor (EGF) - EGF is a naturally secreted peptide that binds to the EGF receptor on the basal surface of the enterocytes, resulting in the activation of signaling pathways that stimulate cell proliferation and maturation. Given the severe villus atrophy in the small intestine of MVID patients, the rationale for use in MVID was that EGF may stimulate the proliferation of cells in the crypt and in this way yield more enterocytes to repopulate and thereby regenerate absorptive villi.

Drumm and colleagues (31) reported two MVID patients that received continuous intravenous infusion at 100 ng/kg/hr. The patients then received the same dosage of EGF for five days (case 1) and for 21 days (case 2) followed by the same dose for 21 days intravenously (case 1) or by continuous enteral infusion (case 2). Outcome measures were 24h stool collections, disaccharidase activity in jejunal biopsy homogenates, and mucosal epithelial morphometry. An increase in crypt cells proliferation indicated that EGF displayed functionally activity. However, EGF did not result in increased villus length or clinical improvement. Similarly, Walker-Schmitz and colleagues (32) reported that one patient received dosage of 100 ng/kg/hr EGF for two 6 days with a 5 days rest period between two courses. Outcome measures were stool volume, small-bowel mucosal morphometry and epithelial cell kinetics. However, only an increase in crypt cells proliferation was found without other

Table 3. Summary of pharmacological interventions reported in MVID.

Drug name	Protocol	Outcome measures	Result	Patients Number	References	PMID#
EGF	100 ng/kg/h for two 6-day with a 5-day rest period between two courses	Stool volume, small-bowel mucosal morphometry and epithelial cell kinetics	No effect except mitotic index in duodenal crypt increased	1	Walker-Smith et al. (1985)	2866310
EGF	100 ng/kg/h (IV) for 5 days, then followed by same dose for 21 days intravenously	24h stool collections, disaccharidase activity in jejunal biopsy homogenates and mucosal epithelial morphometry	No effect except mitotic index in duodenal crypt increased	1	Drumm et al. (1988)	2891946
EGF	100 ng/kg/h (IV) for 21 days, then followed by same dose for 21 days continuous enteral infusion	24h stool collections, disaccharidase activity in jejunal biopsy homogenates and mucosal epithelial morphometry	No effect except mitotic index in duodenal crypt increased	1	Drumm et al. (1988)	2891946
EGF	100 ng/kg/h (IV) for 2 weeks	Stool volume and small-bowel mucosal morphometry	No effect except population of microvilli increased	1	Beck et al. (1997)	9364305
Somatostatin	100 μg (SC) Bid for 21 days	Stool volume	Decreased from 210 ml/kg/day to 150 ml/kg/day	1	Couper et al. (1989)	2759484
Somatostatin	100 μg (SC) Bid for 14 days	Stool volume	Decreased from 275 ml/kg/day to 161 ml/kg/day	1	Couper et al. (1989)	2759484
Somatostatin	n.r.	Stool volume	No effect	1	Bell et al. (1991)	1660676
Somatostatin	n.r.	Stool volume	Mild decreased	1	Schofield et al. (1992)	1319670
Somatostatin	n.r.	n.r	No effect	1	Cegla et al. (1993)	8114773
Somatostatin	n.r.	n.r.	No effect	1	Assmann et al. (1997)	9323563
Somatostatin	n.r.	n.r.	No effect	1	Pohl et al. (1999)	9880458
Octreotide	n.r.	n.r.	No effect	1	Rhoads et al. (1991)	1993505
Octreotide	n.r.	n.r.	No effect	2	Raafat et al. (1994)	7959671
Octreotide	4 μg/kg/day	Stool volume	No effect	2	Beck et al (1997)	9364305
Octreotide	n.r.	n.r.	No effect	1	Ukarapol et al. (2001)	11800313
Octreotide	n.r.	n.r.	No effect	1	Mendes et al. (2014)	25635218
Loperamide	1 mg/kg/day	n.r.	No effect	1	Phillips et al. (1985)	3977385
Loperamide	0.1 mg/kg/day	Stool volume	Decreased remarkably	1	Phillips et al. (1985)	3977385
Loperamide	n.r.	Stool volume	No effect	1	Bell et al. (1991)	1660676
Loperamide	n.r.	n.r.	No effect	1	Raafat et al. (1994)	7959671
Loperamide	0.2 mg/kg Qid	Stool frequency, Bristol stool chart	No effect	1	Tran et al. (2017)	27682357
Steroid	n.r.	Stool volume	No effect	1	Bell et al. (1991)	1660676
Steroid	n.r.	n.r.	No effect	1	Raafat et al. (1994)	7959671

Steroid	2 mg/kg/d for 3 weeks	Stool volume	No effect	1	Beck et al. (1997)	9364305
Prednisolone	n.r.	n.r.	No effect	1	Phillips et al. (1985)	3977385
Dexamethasone	Oral	Stool volume	No effect	1	Phillips et al. (1985)	3977385
Adrenocorticotrophic hormone	n.r.	n.r.	No effect	1	Phillips et al. (1985)	3977385
Hydrocortisone	IV for 4-week	Stool volume	No effect	1	Drumm et al. (1988)	2891946
Glucocorticosteroids	n.r.	n.r	No effect	1	Cegla et al. (1993)	8114773
Cholestyramine	n.r.	Stool volume	No effect	1	Bell et al. (1991)	1660676
Cholestyramine	n.r.	Stool volume	Decreased from 150 mg/kg/day to 50 mg/kg/day	1	Beck et al. (1997)	9364305
Cholestyramine	n.r.	n.r.	No effect	1	Ukarapol et al. (2001)	11800313
Pentagastrin	n.r.	n.r	No effect	1	Cegla et al. (1993)	8114773
Racecadotril	1.5 mg/kg Tid	Stool frequency, Bristol stool chart	The mean daily number of stools fell from 6.5 to 2.1 and stool consistency improved to Bristol type 6.	1	Tran et al. (2017)	27682357
Mesenchymal stem cells	$1*10^6$ U transduodenal and $2*10^6$ U (IV)	Fluid and electrolyte requirements	No effect except blood stream infections were reduced	1	Ozge et al. (2019)	

This table includes all the patients who were treated with pharmacological interventions from the case reports. This yielded 15 articles reporting in total of 35 patient treatments involving in total eight different pharmacological interventions. (IV: intravenous injection; SC: subcutaneous injection; Bid: two times per day; Tid: three times per day; Qid: four times per day; n.r.: not reported.)

improvements. In a later study, Beck and colleagues (34) tried EGF (100 ng/kg/h for two weeks) with one MVID patient and reported some microvillus restoration but no reduction is stool volume.

Steroids – Steroids, namely (gluco)corticosteroids such as prednisolone or hydrocortisone, have been reported to have beneficial effects on the maturation and function of the small intestine in animal models (35). Six studies reported treatment of in total eight MVID patients with steroids but reported no beneficial effects on stool volume (31,34,36-39).

Anti-diarrheal drugs that modulate electrolyte transport across the brush border membrane

Somatostatin / octreotide - Somatostatin is a naturally-occurring hormone. Somatostatin binds to somatostatin receptors, which are G_i-protein-coupled transmembrane receptors that, when activated, inhibit adenylate cyclase (AC) (40). AC is an enzyme that, downstream of G_α-coupled receptors at the cell surface, produces cAMP. cAMP, in turn, can stimulate the activity and brush border abundance of the CFTR protein and inhibit the function of the NHE3 and DRA proteins (41). Inhibition of adenylate cyclase, therefore, may be expected to reduce the secretion of chloride and stimulate sodium transport across the brush border surface. Because somatostatin is rapidly degraded by peptidase enzymes in cells and plasma and is poorly absorbed in the gut, continuous intravenous infusion is required but impractical for long-term management. Therefore, the hydrophilic octapeptide octreotide, which is a long-acting somatostatin analogue, has also been used for stool output reduction in patients with high-output secretory diarrhea of various causes.

Couper and colleagues (42) reported a 40% stool volume decline and reductions in the output of stool electrolytes following subcutaneous octreotide injections in one MVID patient. In contrast, Schofield and colleagues (43) reported no beneficial effect of somatostatin in one MVID patient. Six studies reported octreotide as intervention with eight MVID patients. Beck and colleagues (34) and Raafat and colleagues (37) found no effect of octreotide on stool volume in two MVID patients per study. Three other

studies mentioned octreotide been tried with one MVID patient per study and reported no beneficial effects (44-46). Unfortunately, in these studies the study protocol and outcome measures were not specified. No follow-ups of any of these patients have been reported.

Racecadotril - Racecadotril (acetorphan) is a lipophilic thiorphan derivative and rapidly converted to thiorphan, a potent inhibitor of enkephalinase (47). Inhibition of enkephalinase prevents the degradation - and thereby increases the biological half-life - of enkephalins, which are endogenous opioid peptides secreted by myenteric and submucosal neurons in the digestive tract. These enkephalins bind and activate delta-opioid receptor on the basal surface of enterocytes, thereby inhibiting AC activity and reducing cAMP levels. As for somatostatin and octreotide, a resultant reduction in cAMP levels was expected to inhibit CFTR-mediated chloride output and stimulate sodium absorption and thereby to reduce fluid secretion. Tran and colleagues (48) treated one MVID patient with racecadotril and used stool frequency and Bristol stool chart indications as outcome measures. Mean daily number of stools were reduced and stool consistency improved. Cessation of nocturnal stooling, improved sleep and appetite was reported by the patient's parents. Withdrawal resulted in frequent watery stools, which was once again improved after reintroduction of the drug. Since this report there have been no additional studies reporting on racecadotril in MVID.

Loperamide - Loperamide is an opioid-receptor agonist and acts on the μ-opioid receptors in the myenteric plexus of the large intestine, thereby decreasing the activity of the myenteric plexus and consequently intestinal motility (49,50). This results in healthy people in reduced transit time, allowing more time for water homeostasis and stool thickening. Loperamide can be prescribed for adults and children above 2 years of age for treatment of (chronic) diarrhea. Four studies mentioned loperamide intervention with five MVID patients at varying doses (0.1-1.0 mg/kg/day). One patient was reported to display a - non-specified - significant reduction in stool volume (38) whereas no effect was observed in the other patients (36,38,48,51).

Other anti-diarrheal drugs

Cholestyramine - Cholestyramine is a bile acid sequestrant used in the clinic for the symptomatic control of bile acid-induced diarrhea due to short bowel syndrome (52). One study reported a three-fold reduction (from 150 to 50 mg/kg/day) in stool volume upon treatment of a MVID patient with cholestyramine (34). Two other studies with each one MVID patient found no effect (36,45).

DISCUSSION AND FUTURE PERSPECTIVES

Rational approaches for pharmacological treatment of MVID patients

Variable but overall little if any treatment response with regard to reducing stool volume has been observed in different MVID patients treated with AC-inhibiting compounds (Table 3). Recent analyses of ion transporter deficits in MVID patient tissue and MVID cell line and animal models (11,14,53), in conjunction with new insights in the cAMP-mediated regulation of these transporters (54,55), provide mechanistic explanations for the variable and overall limited effectiveness of these compounds.

For example, cAMP stimulates the endocytosis of NHE3 from the brush border surface, thereby inhibiting sodium absorption and contributing to diarrhea (56). The thus expected inhibitory effect of AC-inhibiting compounds on the endocytosis of NHE3 and resultant stimulation of sodium absorption (56), however, will not occur in MVID enterocytes because the majority of NHE3 is not present at the surface but in cytoplasmic vesicles. Furthermore, the inhibitory effect of cAMP on NHE3 requires active ezrin (57), which is inhibited in MVID (17). Likewise, the expected effects of AC-inhibiting compounds on DRA will be minimal because DRA expression is severely reduced in MVID enterocytes (14). Loss of DRA activity and elevated luminal chloride will also further reduce NHE3 function, as is seen in CCD patients. Conversely, in the absence of NHE3, DRA functions become more dependent on CFTR (58), the remaining target of AC-inhibiting drugs. CFTR has been reported to maintain its brush border localization in some patients (14), but not in other patients (9,59). MVID patients of which enterocytes show mislocalization of CFTR to the cytoplasm may therefore not at all respond to AC-inhibiting compounds. Concluding, AC-inhibiting drugs may be effective in situations where (pathogen-induced) diarrhea is triggered by an increase in cAMP levels and resultant effects on DRA, NHE3 and CFTR at the brush border

surface of otherwise normal enterocytes, but not in MVID where basal DRA, NHE3 and possibly CFTR expression and/ or trafficking is impaired.

Novel anti-diarrheal drugs targeted against specific brush border transporter proteins are being developed (60,61), but thus far have not been tried in MVID. For MVID, the effectivity of CFTR-targeting drugs will depend on the amount of functional CFTR present at the brush border surface, which varies between patients (13,14,59). Similarly, the effectivity of NHE3-activating peptides will depend on the amount of NHE3 at the cell surface, which is limited in most MVID cases where this protein was investigated. The effectivity of drugs or probiotics - such as Lactobacillus acidophilus - aimed at stimulating NHE3 or DRA gene expression (62,63) will be limited by the inability of newly synthesized NHE3 and DRA proteins to maintain sufficient localization at the brush border due to the *MYO5B* mutations (13,64). Nonetheless, in MVID patients that show residual brush border localization an increase in total amount of these transporters may be beneficial.

We found that significant variations have been reported in stool output (50-300 ml/kg/day) and/or in fecal electrolyte compositions between patients (Tables 1 and 2). Whether there is a relationship between the severity of diarrhea and treatment response is not known. The cause of interpatient variations in stool output, stool electrolyte composition and the precise relative expression and localization pattern of the different ion transporters at the brush border is also not known. Possibly, these reflect the patients' *MYO5B* mutation. More than 60 unique *MYO5B* mutations have been identified, with each family carrying distinct sets of *MYO5B* mutations (www.mvid-central.org) (10,16). These can differently affect the encoded myosin Vb protein and, conceivably, brush border protein localization and drug response. A genotype-dependency for butyrate therapeutic efficacy was recently reported in CCD patients (65). Future genotype-phenotype correlation studies may shed light on and help predicting treatment responses. There is much interest in the use of patient-specific intestinal organoids for studying genotype-phenotype correlations (66). However, care should be taken as cultured patient organoids may not accurately reflect rapidly changing brush border transporter (including NHE3) profiles during early childhood development.

It should be emphasized that MVID is both a diarrheal and malabsorption disorder. The prevention of diarrhea in MVID, as such, is likely to improve the management of electrolyte supplementation and reduce the risk of metabolic decompensation. However, it is not expected to reduce patients' dependency on TPN and therewith associated mortality. Indeed, chronic diarrhea or malabsorption and villus atrophy are not typically correlated, as evidenced by the normal intestinal architecture in CSD, CCD and congenital glucose-galactose malabsorption patients. Resolving diarrhea may therefore not be expected to normalize intestinal architecture (*i.e.*, restore villus length).

The remaining villus atrophy precludes an optimal absorptive epithelium to accommodate oral or enteral nutrient intake. Therefore, in conjunction with the search for effective antidiarrheal treatment, efforts should be directed at restoring villus length. Thus far, drugs such as EGF could stimulated enterocyte proliferation and some differentiation but proved ineffective to ameliorate clinical symptoms in all MVID patients studied. It was proposed that the villus atrophy in MVID reflects (in part) a degeneration triggered by a hitherto unknown factor (31). A recent study in germline *Myo5b* knockout mice, which showed normal intestinal villi before birth and villus atrophy within days after birth (67), suggests a postnatal factor. The further identification of these postnatal factors may unlock new territories for pharmacological interventions.

Practical aspects of pharmacological treatment of MVID patients

Thus far, most drugs have been provided via intravenous or subcutaneous injections. This is not the most practical for children with MVID as they likely require life-long treatment. Orally administered drugs would be more practical. However, oral bioavailability of pharmacological drugs, in particular hydrophilic drugs that require active trans-mucosal transport, is a critical parameter especially in MVID. Indeed, *MYO5B* mutations cause the downregulation and/or mislocalization of a wide variety of brush border transporters. It should therefore be anticipated that brush border transporters for the uptake of hydrophilic drugs such as SLC15A1 (PepT1) may show reduced brush border membrane expression or, in case of proton-dependent

transporters, show reduced activity due to the lack of NHE3- or v-ATPase-mediated proton secretion in MVID enterocytes. Furthermore, cell surface expression of drug efflux transporters such as the multidrug resistance protein ABCC2 (MRP2) has been shown to be inhibited by *MYO5B* mutations in the liver of MVID patients (68,69). While these drug-transporting cell surface proteins have not been studied in the MVID intestine, alterations in these may have consequences for pharmacokinetics and optimal dug dosage. Convectional washout of drugs because of the continuous severe diarrhea poses an additional pharmacokinetic hurdle for the oral and enteral administration of both hydrophilic and lipophilic drugs.

With regard to treatment duration and timing, it is recommended that treatment duration is at least 5 days because of the relatively high turnover time of enterocyte. An unsuccessful intervention in a MVID patient with mesenchymal stem cells was attributed to a too short treatment (70). The timing of pharmacological interventions aimed at specific brush border transporters in young children is important as such transporters show changes in expression during postnatal development, thereby affecting drug efficacy. In this regard, therapeutic targets identified based on results of patient biopsy analyses that were taken prior to the diagnosis may no longer reflect the situation in the patient´s intestine at (later) moments of drug intervention.

Suggestions for future reporting

MVID is a very rare disease and large randomized controlled trials are not foreseen. Crossover placebo-controlled n-of-1 trials may be an appropriate choice given the limited number of available MVID patients and the chronicity of the condition, provided that the expected treatment response is stable, the onset of the treatment effect is quick, and that carryover effects (depending on drug elimination half-life) are controlled.

Likely, reports of pharmacological interventions thus will continue to typically involve one or two patient per published report. Treatment recommendations will be largely be based on clinical experience and anecdotal reporting in the medical literature. To facilitate future meta-analyses of these single patient studies, it is recommended that publications include more systematic descriptions of the study protocols (*e.g.*, dosage,

intervention duration, administration route, outcome measures) and clinical details (*e.g.*, early/late onset MVID, age, mutation details, baseline measures (*e.g.*, volume, frequency ad consistency of diarrhea, fecal electrolytes)).

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. november 1978;75(5):783–90.

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. 9 maart 1989;320(10):646–51.

3. Phillips AD, Szafranski M, Man LY, Wall WJ. Periodic acid-Schiff staining abnormality in microvillous atrophy: photometric and ultrastructural studies. J Pediatr Gastroenterol Nutr. januari 2000;30(1):34–42.

4. 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. juli 2002;26(7):902–7.

5. Müller T, Hess MW, Schiefermeier N, Pfaller K, Ebner HL, Heinz-Erian P, e.a. MYO5B mutations cause microvillus inclusion disease and disrupt epithelial cell polarity. Nat Genet. oktober 2008;40(10):1163–5.

6. Wiegerinck CL, Janecke AR, Schneeberger K, Vogel GF, van Haaften-Visser DY, Escher JC, e.a. Loss of syntaxin 3 causes variant microvillus inclusion disease. Gastroenterology. juli 2014;147(1):65-68.e10.

7. Stepensky P, Bartram J, Barth TF, Lehmberg K, Walther P, Amann K, e.a. Persistent defective membrane trafficking in epithelial cells of patients with familial hemophagocytic lymphohistiocytosis type 5 due to STXBP2/MUNC18-2 mutations. Pediatr Blood Cancer. juli 2013;60(7):1215–22.

8. Vogel GF, Janecke AR, Krainer IM, Gutleben K, Witting B, Mitton SG, e.a. Abnormal Rab11-Rab8-vesicles cluster in enterocytes of patients with microvillus inclusion disease. Traffic. 2017;18(7):453–64.

9. Vogel GF, van Rijn JM, Krainer IM, Janecke AR, Posovszky C, Cohen M, e.a. Disrupted apical exocytosis of cargo vesicles causes enteropathy in FHL5 patients with Munc18-2 mutations. JCI Insight. 20 juli 2017;2(14).

10. Dhekne HS, Pylypenko O, Overeem AW, Zibouche M, Ferreira RJ, van der Velde KJ, e.a. 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(3):333–44.

11. Engevik AC, Kaji I, Engevik MA, Meyer AR, Weis VG, Goldstein A, e.a. Loss of MYO5B Leads to Reductions in Na+ Absorption With Maintenance of CFTR-Dependent Cl- Secretion in Enterocytes. Gastroenterology. 2018;155(6):1883-1897.e10.

12. Knowles BC, Roland JT, Krishnan M, Tyska MJ, Lapierre LA, Dickman PS, e.a. Myosin Vb uncoupling from RAB8A and RAB11A elicits microvillus inclusion disease. J Clin Invest. juli 2014;124(7):2947–62.

13. Vogel GF, Klee KMC, Janecke AR, Müller T, Hess MW, Huber LA. Cargo-selective apical exocytosis in epithelial cells is conducted by Myo5B, Slp4a, Vamp7, and Syntaxin 3. J Cell Biol. 9 november 2015;211(3):587–604.

14. Kravtsov DV, Ahsan MK, Kumari V, van Ijzendoorn SCD, Reyes-Mugica M, Kumar A, e.a. Identification of intestinal ion transport defects in microvillus inclusion disease. Am J Physiol Gastrointest Liver Physiol. 01 2016;311(1):G142-155.

15. Ruemmele FM, Müller T, Schiefermeier N, Ebner HL, Lechner S, Pfaller K, e.a. 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. mei 2010;31(5):544–51.

16. van der Velde KJ, Dhekne HS, Swertz MA, Sirigu S, Ropars V, Vinke PC, e.a. An overview and online registry of microvillus inclusion disease patients and their MYO5B mutations. Hum Mutat. december 2013;34(12):1597–605.

17. Dhekne HS, Hsiao N-H, Roelofs P, Kumari M, Slim CL, Rings EHHM, e.a. Myosin Vb and Rab11a regulate phosphorylation of ezrin in enterocytes. J Cell Sci. 1 maart 2014;127(Pt 5):1007–17.

18. Thoeni CE, Vogel GF, Tancevski I, Geley S, Lechner S, Pfaller K, e.a. Microvillus inclusion disease: loss of Myosin vb disrupts intracellular traffic and cell polarity. Traffic. januari 2014;15(1):22–42.

19. Jayawardena D, Alrefai WA, Dudeja PK, Gill RK. Recent advances in understanding and managing malabsorption: focus on microvillus inclusion disease. F1000Res. 2019;8.

20. Ruemmele FM, Schmitz J, Goulet O. Microvillous inclusion disease (microvillous atrophy). Orphanet J Rare Dis. 26 juni 2006;1:22.

21. Engevik AC, Goldenring JR. Trafficking Ion Transporters to the Apical Membrane of Polarized Intestinal Enterocytes. Cold Spring Harb Perspect Biol. 02 2018;10(1).

22. Michail S, Collins JF, Xu H, Kaufman S, Vanderhoof J, Ghishan FK. Abnormal expression of brush-border membrane transporters in the duodenal mucosa of two patients with microvillus inclusion disease. J Pediatr Gastroenterol Nutr. november 1998;27(5):536–42.

23. Thoeni CE, Vogel GF, Tancevski I, Geley S, Lechner S, Pfaller K, e.a. Microvillus inclusion disease: loss of Myosin vb disrupts intracellular traffic and cell polarity. Traffic. januari 2014;15(1):22–42.

24. Phulware RH, Gahlot GPS, Malik R, Gupta SD, Das P. Microvillous Inclusion Disease as a Cause of Protracted Diarrhea. Indian J Pediatr. 2019;86(9):854–6.

25. Ruemmele FM, Jan D, Lacaille F, Cézard J-P, Canioni D, Phillips AD, e.a. New perspectives for children with microvillous inclusion disease: early small bowel transplantation. Transplantation. 15 april 2004;77(7):1024–8.

26. van der Velde KJ, Dhekne HS, Swertz MA, Sirigu S, Ropars V, Vinke PC, e.a. An overview and online registry of microvillus inclusion disease patients and their MYO5B mutations. Hum Mutat. december 2013;34(12):1597–605.

27. Halac U, Lacaille F, Joly F, Hugot J-P, Talbotec C, Colomb V, e.a. Microvillous inclusion disease: how to improve the prognosis of a severe congenital enterocyte disorder. J Pediatr Gastroenterol Nutr. april 2011;52(4):460–5.

28. Girard M, Lacaille F, Verkarre V, Mategot R, Feldmann G, Grodet A, e.a. MYO5B and bile salt export pump contribute to cholestatic liver disorder in microvillous inclusion disease. Hepatology. juli 2014;60(1):301–10.

29. Fuchs J, Fallon EM, Gura KM, Puder M. Use of an omega-3 fatty acid-based emulsion in the treatment of parenteral nutrition-induced cholestasis in patients with microvillous inclusion disease. J Pediatr Surg. december 2011;46(12):2376–82.

30. Anez-Bustillos L, Dao DT, Potemkin AK, Perez-Atayde AR, Raphael BP, Carey AN, e.a. An Intravenous Fish Oil-Based Lipid Emulsion Successfully Treats Intractable Pruritus and Cholestasis in a Patient with Microvillous Inclusion Disease. Hepatology. maart 2019;69(3):1353–6.

31. Drumm B, Cutz E, Tomkins KB, Cook D, Hamilton JR, Sherman P. Urogastrone/epidermal growth factor in treatment of congenital microvillous atrophy. Lancet. 16 januari 1988;1(8577):111–2.

32. Walker-Smith JA, Phillips AD, Walford N, Gregory H, Fitzgerald JD, MacCullagh K, e.a. Intravenous epidermal growth factor/urogastrone increases small-intestinal cell proliferation in congenital microvillous atrophy. Lancet. 30 november 1985;2(8466):1239–40.

33. Walker-Smith JA, Unsworth DJ, Hutchins P, Phillips AD, Holborow EJ. AUTOANTIBODIES AGAINST GUT EPITHELIUM IN CHILD WITH SMALL-INTESTINAL ENTEROPATHY. The Lancet. 6 maart 1982;319(8271):566–7.

34. Beck NS, Chang YS, Kang IS, Park WS, Lee HJ, Suh YL. Microvillus inclusion disease in two Korean infants. J Korean Med Sci. oktober 1997;12(5):452–6.

35. Black HE. The effects of steroids upon the gastrointestinal tract. Toxicol Pathol. 1988;16(2):213–22.

36. Bell SW, Kerner JA, Sibley RK. Microvillous inclusion disease. The importance of electron microscopy for diagnosis. Am J Surg Pathol. december 1991;15(12):1157–64.

37. Raafat F, Green NJ, Nathavitharana KA, Booth IW. Intestinal microvillous dystrophy: a variant of microvillous inclusion disease or a new entity? Hum Pathol. november 1994;25(11):1243–8.

38. Phillips AD, Jenkins P, Raafat F, Walker-Smith JA. Congenital microvillous atrophy: specific diagnostic features. Arch Dis Child. februari 1985;60(2):135–40.

39. Cegla M, Lohner M, Schaefer HE. [Congenital villous atrophy. Disease picture of congenital chronic diarrhea with poor prognosis]. Monatsschr Kinderheilkd. december 1993;141(12):925–7.

40. Siperstein AE, Levin KE, Gum ET, Clark OH. Effect of somatostatin on adenylate cyclase activity in normal and neoplastic thyroid tissue. World J Surg. augustus 1992;16(4):555–60; discussion 560-561.

41. Li C, Naren AP. CFTR Chloride Channel in the Apical Compartments: Spatiotemporal Coupling to its Interacting Partners. Integr Biol (Camb). 7 april 2010;2(4):161–77.

42. Couper RT, Berzen A, Berall G, Sherman PM. Clinical response to the long acting somatostatin analogue SMS 201-995 in a child with congenital microvillus atrophy. Gut. juli 1989;30(7):1020–4.

43. Schofield DE, Agostini RM, Yunis EJ. Gastrointestinal microvillus inclusion disease. Am J Clin Pathol. juli 1992;98(1):119–24.

44. Rhoads JM, Vogler RC, Lacey SR, Reddick RL, Keku EO, Azizkhan RG, e.a. Microvillus inclusion disease. In vitro jejunal electrolyte transport. Gastroenterology. maart 1991;100(3):811–7.

45. Ukarapol N, Chotinaruemol S, Lertprasertsuk N, Wongsawasdi L. Microvillus inclusion disease as a cause of severe protracted diarrhea in infants. J Med Assoc Thai. september 2001;84(9):1356–60.

46. Mendes C, Figueiredo C, Mansilha H, Proença E, Oliveira D, Lima R, e.a. A case of Protracted Diarrhea in a Newborn: a Diagnostic Challenge. Pediatr Rep. 12 augustus 2014;6(3):5596.

47. Matheson AJ, Noble S. Racecadotril. Drugs. 1 april 2000;59(4):829–35.

48. Tran LC, Lazonby G, Ellis D, Goldthorpe J, Iglesias N, Steele J, e.a. Racecadotril May Reduce Diarrhoea in Microvillous Inclusion Disease. J Pediatr Gastroenterol Nutr. 2017;64(1):e25–6.

49. Giagnoni G, Casiraghi L, Senini R, Revel L, Parolaro D, Sala M, e.a. Loperamide: evidence of interaction with mu and delta opioid receptors. Life Sci. 1983;33 Suppl 1:315–8.

50. Ooms LA, Degryse AD, Janssen PA. Mechanisms of action of loperamide. Scand J Gastroenterol Suppl. 1984;96:145–55.

51. Raafat F, Green NJ, Nathavitharana KA, Booth IW. Intestinal microvillous dystrophy: a variant of microvillous inclusion disease or a new entity? Hum Pathol. november 1994;25(11):1243–8.

52. Barkun A, Love J, Gould M, Pluta H, Steinhart AH. Bile acid malabsorption in chronic diarrhea: Pathophysiology and treatment. Can J Gastroenterol. november 2013;27(11):653–9.

53. Engevik AC, Coutts AW, Kaji I, Rodriguez P, Ongaratto F, Saqui-Salces M, e.a. Editing Myosin VB Gene to Create Porcine Model of Microvillus Inclusion Disease, With Microvillus-lined Inclusions and Alterations in Sodium Transporters. Gastroenterology. 26 februari 2020;

54. Singh V, Yang J, Chen T, Zachos NC, Kovbasnjuk O, Verkman AS, e.a. Translating molecular physiology of intestinal transport into pharmacologic treatment of diarrhea: stimulation of Na+ absorption. Clin Gastroenterol Hepatol. januari 2014;12(1):27–31.

55. Van Ree JM, Verhoeven WM, De Wied D. Gamma-type endorphins: neurolepticum-like and antipsychotic action. Prog Neuropsychopharmacol Biol Psychiatry. 1985;9(5–6):561–7.

56. Musch MW, Arvans DL, Wang Y, Nakagawa Y, Solomaha E, Chang EB. Cyclic AMP-mediated endocytosis of intestinal epithelial NHE3 requires binding to synaptotagmin 1. Am J Physiol Gastrointest Liver Physiol. februari 2010;298(2):G203-211.

57. Hayashi H, Tamura A, Krishnan D, Tsukita S, Suzuki Y, Kocinsky HS, e.a. Ezrin is required for the functional regulation of the epithelial sodium proton exchanger, NHE3. PLoS ONE. 2013;8(2):e55623.

58. Tse C-M, Yin J, Singh V, Sarker R, Lin R, Verkman AS, e.a. cAMP Stimulates SLC26A3 Activity in Human Colon by a CFTR-Dependent Mechanism That Does Not Require CFTR Activity. Cell Mol Gastroenterol Hepatol. 2019;7(3):641–53.

59. Ameen NA, Salas PJ. Microvillus inclusion disease: a genetic defect affecting apical membrane protein traffic in intestinal epithelium. Traffic. januari 2000;1(1):76–83.

60. Thiagarajah JR, Ko E-A, Tradtrantip L, Donowitz M, Verkman AS. Discovery and development of antisecretory drugs for treating diarrheal diseases. Clin Gastroenterol Hepatol. februari 2014;12(2):204–9.

61. Thiagarajah JR, Donowitz M, Verkman AS. Secretory diarrhoea: mechanisms and emerging therapies. Nat Rev Gastroenterol Hepatol. augustus 2015;12(8):446–57.

62. Singh V, Raheja G, Borthakur A, Kumar A, Gill RK, Alakkam A, e.a. Lactobacillus acidophilus upregulates intestinal NHE3 expression and function. Am J Physiol Gastrointest Liver Physiol. 15 december 2012;303(12):G1393-1401.

63. Kumar A, Anbazhagan AN, Coffing H, Chatterjee I, Priyamvada S, Gujral T, e.a. Lactobacillus acidophilus counteracts inhibition of NHE3 and DRA expression and alleviates diarrheal phenotype in mice infected with Citrobacter rodentium. Am J Physiol Gastrointest Liver Physiol. 01 2016;311(5):G817–26.

64. 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 USA. 15 februari 2011;108(7):2789–94.

65. Canani RB, Terrin G, Elce A, Pezzella V, Heinz-Erian P, Pedrolli A, e.a. Genotype-dependency of butyrate efficacy in children with congenital chloride diarrhea. Orphanet J Rare Dis. 19 december 2013;8:194.

66. Chen KG, Zhong P, Zheng W, Beekman JM. Pharmacological analysis of CFTR variants of cystic fibrosis using stem cell-derived organoids. Drug Discov Today. 2019;24(11):2126–38.

67. Cartón-García F, Overeem AW, Nieto R, Bazzocco S, Dopeso H, Macaya I, e.a. Myo5b knockout mice as a model of microvillus inclusion disease. Sci Rep. 23 juli 2015;5:12312.

68. Schlegel C, Weis VG, Knowles BC, Lapierre LA, Martin MG, Dickman P, e.a. Apical Membrane Alterations in Non-intestinal Organs in Microvillus Inclusion Disease. Dig Dis Sci. 2018;63(2):356–65.

69. Overeem AW, Li Q, Qiu Y-L, Carton-Garciá F, Leng C, Klappe K, e.a. A molecular mechanism underlying genotype-specific intrahepatic cholestasis resulting from MYO5B mutations. Hepatology. 21 november 2019;

70. Onay OS, Tekin AN, Gunes D, Aydemir O, Artan S, Aydemir Y. GP248 Mesenchymal stem cell therapy in microvillus inclusion disease. Archives of Disease in Childhood. 1 juni 2019;104(Suppl 3):A133–A133.

Supplementary table 1. Clinical details from published MVID case reports.

References	PMID#	Patients number	Gender	Gestation (week)	Birth body weight (g)	Poly hydra mnios	Onset (day)	Stool output (ml/kg/d)	Feces electrolyte (mmol/L)			Feces osmolarity (mOsm/kg)	Feces PH value	Dead/ Alive	Follow-up
									Na^+	Cl^-	K^+				
Lingaldinna et al. (2017)	28842815	1	Male	38	3500		3		78	64	7.3		7	Dead	1 month
		2	Female	34	2000		5						7	Dead	36 days
Schoen et al. (2017)	29546954	1	Male	36		No	10	190	120	67		30		Alive	36 months
Perry et al. (2014)	25111220	1	Female				150		15					Alive	132 months
Burgis et al. (2013)	23525737	1	Male	at term			6	150						Alive	168 months
Siahanidou et al. (2013)	23354788	1	Female	35	2330	Yes	1		85	78	22			Dead	7 months
AI-Sinani et al. (2012)	23226823	1	Female	at term		No	3	100						Dead	4 months
Thomas et al. (2012)	22318102	1	Female	35	2320	Yes	3		35	21				Dead	23 days
Vora et al. (2012)	22197941	1	Female	37	2900	Yes	1	100	78	42	40	11		Alive	4 days
Fuchs et al. (2011)	22152886	1	Male	36			4	148						Alive	41 months
Shahid et al. (2012)	21968248	1	Male	at term	2734	No	3	175	84	68	13			Alive	3 months
Khubchandani et al. (2011)	21299349	1	Male	35			3						6	Dead	2 months
Gathungu et al. (2008)	18277898	1	Male	34	2450	No	8		112	113	21.6	292		Alive	12 days
Amosu et al. (2007)	17418172	1	Male	31			3	100	139	105	4.7	279		Alive	12 days
Kucinskiene et al. (2004)	15456973	1	Female	37	2530		1	200						Dead	1.5 months
Mierau et al. (2001)	11783915	1	Male	at term			6	115	95	95	30	270	9	Alive	5 months
Ruemmele et al. (2001)	11414303	1	Male	36	2700	NO	2	200	100	60	17.5			Alive	4 months
Kennea et al. (2001)	11251929	1	Male	35	3720	Yes		300						Alive	3 days
Wilson et al. (2001)	11173328	1	Male	36	2740	No	3		76	79	39			Dead	6 months
Croft et al. (2000)	10941974	1	Female	at term	3510		11		108	55	11.9	330		Alive	39 months
Bunn et al. (2000)	10941971	1	Female	34	2100	No	6	175						Alive	24 months
Heinz-Erian et al. (1999)	9932857	1	Female	36			10	170				261		Alive	9 months
Pohl et al. (1999)	9880458	1	Male	39	3500		6	95	110	85	7			Alive	24 months
		2	Male	38	2600		6	135	81	44	2			Alive	96 months
		3	Male	36	3300		6	150	115	96	5			Alive	84 months
		4	Female	39	3160		4	175	107	84	18			Alive	48 months
		5	Female	37	2700		4	100	6		27			Dead	36 months

Kagitani et al. (1998)	9844114	1	Male	38	3300	No	1	75						Alive	132 months
Michail et al. (1998)	9822319	1	Male	36	3090	No	1	100	119	111	14		6.5	Alive	3 months
		2	Male				14	150	105	74	12	281		Alive	9 months
Randak et al. (1998)	9740207	1	Male	at term	3900	No	14	178	105	74	12.1	281	8	Dead	18 months
Beck et al. (1997)	9364305	1	Female	36	2700	No		175						Alive	3 months
Assmann et al. (1997)	9323563	1	Male	33	2950	Yes	1		99		12	240		Dead	7 months
Herzog et al. (1996)	8732907	1	Male	at term	3350	No	7	50						Alive	7 months
Raafat et al. (1994)	7959671	1	Female	at term	4100	No	14	60	91					Dead	39 months
		2	Male	at term	4200	No	14	50	100					Dead	5 months
		3	Male	at term	3800	No	7		95					Alive	58 months
Nathavitharana et al. (1994)	8067796	1	Male	38	3325	No	1	166	58	36	15	309		Dead	9 days
		2	Male	35	2880	No	2	200						Dead	4 months
Nizet et al. (1994)	8032396	1	Female	35	2810	No	3	120	104		19	240		Alive	7 months
Schofield et al. (1992)	1319670	1	Female	37	2700	No	4	200	6		27			Dead	37 months
Bell et al. (1991)	1660676	1	Male	at term	3530	Yes	1	150	103	89	19			Alive	72 months
		2	Male	37	3300	Yes	1	150	122	102	19.4			Alive	9 months
Couper et al. (1989)	2759484	1	Female	at term	2300	No	3	85	100	82	29			Alive	13 months
Phillips et al. (1985)	3977385	1	Female	37	2500	No	2		91					Dead	6 months
		2	Female	34	2200	No	4		93					Dead	6 months
Mendes et al. (2014)	25635218	1	Female	36	2800		1	120	83				8	Dead	9 months
Elena et al.		1	Female	36			7	100						Alive	13 months
Sadiq et al. (2019)	31559144	1	Female	35	2445	No	1							Alive	1 month
Comegna et al. (2018)	30564347	1	Male	36	2820		1							Dead	23 months
		2	Male	37	3280	Yes	1							Dead	7 months
Khalsi et al. (2018)	30364420	1	Male	35	3030	No	5							Dead	3 months
Alsaleem et al. (2017)	29282386	1	Male	at term		No	3							Alive	1 month
Bulut et al. (2017)	28707991	1	Male	at term	3300	No	2							Alive	5 months
Tran et al. (2017)	27682357	1	Male											Alive	48 months
Perry et al. (2014)	25111220	1	Female	36	2950	No	2							Alive	36 months
		2	Male	at term	3290		7							Alive	24 months
		3	Male	at term	3570	Yes	42							Alive	12 months

		4	Male	at term	3360	No	5	Alive	144 months
		5	Male	40	2350	No		Alive	156 months
		6	Male	31	1645		420	Alive	288 months
		7	Male				150	Alive	336 months
Wiegerinck et al. (2014)	24726755	1	Female				2	Alive	12 months
		2	Male				14	Alive	18 months
Oatman et al. (2014)	23648791	1	Male				30	Alive	132 months
Chiang et al. (2015)	23608388	1	Male	36			1	Dead	5 months
Golachowska et al. (2012)	22441677	1	Male	at term			3	Alive	60 months
		2	Male				60	Alive	60 months
Fuchs et al. (2011)	22152886	1	Male	37			1	Alive	48 months
		2	Male	37			3	Alive	48 months
Chen et al. (2011)	22030065	1	Female	23	634	No		Dead	6 months
Chen et al. (2010)	21199752	1	Male	36	3355	Yes	1	Dead	6 months
Rund et al. (2006)	16454574	1	Male	35			1	Alive	1.5 months
Weeks et al. (2003)	14708724	1	Female					Alive	8 months
Gambarara et al. (2003)	14697977	1	Male				2	Alive	165 months
		2	Male					Alive	36 months
		3	Female				2	Alive	132 months
		4	Male				1	Alive	13 months
Martinez et al. (2002)	12028658	1	Female	35	2110		1	Alive	4 months
Goldman et al. (2002)	11903944	1	Male				30	Alive	36 months
Levental et al. (2002)	11883547	1	Female				21	Alive	60 months
Ameen et al. (2000)	11208062	1	Female	at term		No		Alive	36 months
Kaneko et al. (1999)	10484813	1	Male	35	2805		1	Dead	37 months
		2	Male	37	3308		2	Alive	132 months
		3	Female	35	2922		2	Dead	7 months
		4	Male	34	2558		2	Dead	4 months
Beck et al. (1997)	9364305	1	Male	39	3000	No	3	Alive	8 months
Roggero et al. (1997)	9142307	1	Male	at term	3570	No	2	Dead	29 months
Drumm et al. (1988)	2891946	1	Female	33	1900		1	Dead	11 months
		2	Female	39			1	Dead	22 months

Walker-Smith et al. (1985)	2866310	1	Female					Alive	5 months
Phulware et al. (2019)	31049800	1	Male					Alive	3 months
Cheng et al. (2017)	28899465	1	Female	39	2850		2	Alive	1 month
Ozge et al. (2019)		1	Male	36			2	Alive	6 months
Cegla et al. (1993)	8114773	1							
Rhoads et al. (1991)	1993505	1							
Ukarapol et al. (2001)	11800313	1	Male						
Mao et al. (2016)	27984607	1							
Van Hoeve et al. (2016)	27477384	1	Male						
Paulus et al. (2015)	26057766	1	Male	at term					
Thoeni et al. (2013)	24138727	1							
Poley et al. (2006)	17784640	1							
Morroni et al. (2006)	16609911	1							
		2							
Youssef et al. (2004)	15785408	1							
Lopez et al. (2001)	11339115	1	Male						
Acar et al. (1999)	10770118	1	Female						
Gambarara et al. (1997)	9142308	1	Male						
		2	Male						
		3	Female						
Steininger et al. (1997)	9065586	1							
		2							
Oliva et al. (1994)	8119548	1	Female	at term	2870	No		Alive	53 months
Mendes et al. (2014)	25635218	1	Female	36	2800		3		
Al-Daraji et al. (2010)	21070163	1	Male					Alive	3 months
		2	Famale					Alive	2 months
		3	Female						
		4	Female						
		5	Female					Alive	2 months
		6	Female					Dead	11 months
		7	Female					Alive	1 month

8	Female		
9	Male		
10	Male		
11	Male	Alive	1 month
12	Male		
13	Male	Alive	2 months
14	Female	Alive	5 months
15	Male	Alive	4 months
16	Female	Alive	2 months
17	Male	Alive	1 month

We searched EMBASE and MEDLINE databases using the following search strings: ((microvill* inclusion disease) OR (microvill* atrophy)) AND case report) to collect all published MVID case reports. Totally, 83 valid case reports reporting on 131 MVID patients were retrieved.