Myosin 5B, Syntaxin 3 and Munc18-2 as central pillars for developing congenital microvillus atrophy

Abstract

Congenital microvillus atrophy (CMA) is a collection of disorders including microvillus inclusion disease (MVID), atypical MVID, and some forms of familial hemophagocytic lymphohistiocytosis type 5 (FHL5). CMA results in malabsorption of nutrients and water, triggering life-threatening conditions, and has the following characteristics found in enterocytes: microvillus atrophy, large granules, and in most cases microvillus inclusions. In humans, mutations in the genes coding for syntaxin 3 (Stx3), syntaxin binding protein 2 (Munc18-2), or myosin 5b (Myo5B) can result in the development of CMA. By finding functional and interactive links between these three proteins, we were able to develop an interactional model describing the connection between these proteins. Simplified, Stx3 forms a complex with Munc18-2, while Myo5B is responsible for the transportation of Stx3 to the apical membrane and facilitates the interaction between Stx3 and Rab8a/11a. In an attempt to explain why only some of the patients that develop FHL5 also develop CMA, we collected all known c.DNA changes known to cause FHL5. The c.DNA changes found in patients with diarrhea, a common symptom of CMA, were compared with the c.DNA changes found in patients without diarrhea. Unfortunately, most reported c.DNA changes only appear in one, two, or three patients, making determining if it causes CMA impossible. Furthermore, the situation that diarrhea sometimes also appears in FHL5 patients with incomplete genetic/medical documentation, further complicates the determining of CMA causation. Setting up a database that includes all newly discovered FHL5 patients, and promoting accurate reporting of patients and c.DNA changes, could help to reduce the effects caused by these problems, thereby giving insight into which mutations are responsible for developing CMA.

Introduction

Congenital microvillus atrophy (CMA) is a collection of rare inherited disorders, with lifelong dysfunctioning of the microvilli in epithelial cells in the intestine. These disorders include microvillus inclusion disease (MVID), some forms of familial hemophagocytic lymphohistiocytosis type 5 (FHL5) and atypical MVID. CMA results in malabsorption of nutrients and water, triggering life threatening conditions (Halac et al., 2011). Depending on the version, symptoms arise as early as a few days after birth to sometimes a couple of months after birth. An early survey found that most immediate symptoms are severe diarrhea, resulting in dehydration and metabolic imbalance, (Phillips & Schmitz, 1992). Because of the bowel failure, all nutrients and water are administered via parental uptake (TPN) (Ruemmele et al., 2006). Sadly, major complications are accompanied with TPN such as cholestasis (reduction or stoppage of bile flow), liver failure and infections (Ruemmele et al., 2006). This results in a survey finding that 15 of 23 patients died before reaching the age of 9 months (Phillips & Schmitz, 1992). A small bowel transplantation, often accompanied with a liver transplantation because of the liver failure, is the only curative solution available but there is some speculation on how successful this actually is (Ruemmele et al., 2004) (Halac et al., 2011). On a more cellular level, CMA has the following characteristics found in enterocytes, the predominant epithelial cells in the small intestine: microvillus atrophy, large granules and in most cases microvillus inclusions (Vogel et al., 2016) (Côte et al., 2009).

Mutations in Stx3, Munc18-2 and Myo5B are the cause of CMA

Most CMA patients have a mutation in their Myo5B gene, coding for myosin 5b (Myo5B). First described in 1982 (gene analysis in the 2000s,) it was thought to be the only gene causing this disease. Mutations in this gene are therefore thought to have an essential role in the development of CMA.

However, in 2013 there have been a few reported cases where mutations in the STXBP2 gene, coding for syntaxin binding protein 2 (Munc18-2), also cause CMA (Stepensky et al., 2013). Biopsies of the duodenum of these

patients show villous atrophy and crypt hyperplasia (increased cell proliferation). Exploration on a cellular level reveals decreased microvilli and large granules, but no microvilli inclusions (Stepensky et al., 2013) (Bin et al., 2015). This was undiscovered for a long time because mutations in STXBP2 was only known to cause familial hemophagocytic lymphohistiocytosis type 5 (FHL5), an autosomal recessive inheritable immune disorder (Côte et al., 2009). Characteristic of FHL5 are uncontrollable proliferation and activation of B, NK, T cells and macrophages resulting in an overactive immune response, which, in turn, often results in fever and damage to the liver and spleen (Henter et al., 2002). The disease is fatal if left untreated. By performing a bone marrow transplantation the FHL5 can often be cured. However, even after the FHL5 is cured the CMA remains present in some patients. It therefore appears that some STXBP2 mutations are causing both FHL5 and CMA.

One year later, mutations in the STX3 gene, coding for syntaxin 3 (Stx3), were identified to also be able to cause CMA (Wiegerinck et al., 2014). Although CMA was present in only two patients, both showed typical CMA symptoms and thereby clearly linking Stx3 to the development of CMA. Cellular examination showed slight differences to CMA found in Myo5B patients, thereby gaining the name atypical MVID.

These genes are up to date the only genes found in humans that are known to cause CMA. Therefore, in order to better understand the diseases and explain why these different patients develop the same symptoms, we tried to find functional and interactional links between these three proteins.

Interactional links between Myo5B, Munc18-2 and Stx3

A simple overview of Stx3, Munc18-2, and Myo5B functions in protein transport

Proteins must be transported to the apical membrane in order for the cell to form microvilli. Myo5B is one of the proteins that is responsible for this by grabbing onto vesicles from the apical recycling endosome and moving over actin filament to the apical membrane. Myo5B is an actin based motor protein that is able to grab vesicles from the apical recycling endosome (ARE) and transport those through the cell by binding to actin in order to deliver the vesicle to the apical membrane (van der Velde et al., 2013). Once at the target location, the apical membrane, the vSNARE (soluble N-ethylmaleimide-sensitive fusion factor attachment protein receptors), connects with the tSNARE, in which Stx3 is a component, into a SNARE-complex (Tadokoro et al, 2007). Munc18-2 is the mediator and regulator of this process. (Tadokoro et al, 2007). Munc18-2 also regulates vesicle exocytosis after forming the SNARE-complex.

Myo5B is connected to Stx3 via Rab11a and Rab8a

Myo5B is able to bind the small GTPases Rab8a, Rab11a and Rab10, although the binding site for Rab10 on Myo5b (on exon D) is often spliced away and no information into the connection between Myo5B and Rab10 on the development of CMA is reported (Roland et al., 2009).

Rab8a and Rab11a have distinct binding sites on the carboxyl-terminal tail of Myo5B (Roland et al., 2009) (Roland et al., 2011). Rab8a and Rab11a are bound to ARE vesicles (then called Rab11a/8a positive vesicles), and by binding either of these two proteins Myo5B is able to transport the vesicle to the apical target (Knowles et al., 2014). In CaCo2 cells, if the interaction between Rab11a and Myo5B cannot be induced, the cell will form CMA like symptoms (Knowles et al., 2014). This is because transportation is essential for multiple proteins that are necessary for proper microvilli formation and an inability to transport these proteins results in microvillus atrophy. For example, mammalian sterile 20 like kinase 4, an ezrin-phosphorylator, is transported via this route. Disruption of the transport then also disrupts ezrin-phosphorylation required for proper microvillus formation and this has been suggested to be partly responsible for microvillus atrophy (Shillingford et al., 2015) (Dhekne et al., 2014).

The Rab11a/8a transportation route is also responsible for transporting one of our proteins of interest, namely Stx3. In CaCo2-BBE Rab11a-KD cells, Stx3 was localized away from the apical surface indicating a failure in transportation (Knowels et al., 2015). However, in Rab8a-KD cells Stx3 was located in vesicles under the apical

membrane suggesting that transport was not inhibited but that exocytosis was (Knowels et al., 2015). This can be explained by the finding that Rab8a and Rab11a interact with the T-Snare Stx3 at the apical membrane and this interaction is therefore most likely needed for correct vesicle exocytosis (Vogel et al., 2015). Interestingly, Myo5B binding with Rab11a is a prerequisite for the Rab11a/Stx3 interaction and it is hinted at that the same goes for the Rab8a and the Rab8a/Stx3 interaction (Vogel et al., 2015).
Concluding, Myo5B is connected to Stx3 via the Rab8a/11a cascade in two different ways. Myo5B is required for the transportation of Stx3 from the ARE to the apical membrane, and Myo5B binding with Rab8a/11a is essential for Rab8a/11a binding with Stx3. Interestingly, this also means that Stx3 is transported via a route in which Stx3 is already required to be at the apical membrane.

The inability of Myo5B to form a complex with Stx3 as a cause of CMA

In FHL5 patients with CMA, CD10, a common marker for CMA, appears to remain inside vesicles close to the apical membrane instead of lining along the apical surface, indicating an error in the exocytosis from these vesicles, which is most likely due to the inability of Munc18-2 to facilitate this (Stepensky et al., 2013).
A possible explanation why only some FHL5 patients develop CMA can be derived when looking at the interactional partners Munc18-2 has, which varies between tissues. Munc18-2 is found to interact with different T-SNARE forming proteins depending on the type of cell. In cytotoxic T lymphocytes and natural killer cells Munc18-2 is favouring complex forming with syntaxin 11 (Stx11) and disrupting this complex forming is known to be a cause of FHL5 (Hackmann et al., 2013). However in enterocytes, Munc18-2 is forming complexes with Stx3 and not with Stx11 (Riento et al., 2000). Therefore, mutations that only inhibit Munc18-2/Stx11 complex forming and function, and not Munc18-2/Stx3 complex, can explain why a part of the FHL5 patients do not develop CMA. In support of this, we could find no FHL4 patients (patients with a syntaxin 11 mutation) with CMA like symptoms. So far it is unknown what the difference precisely is between the interaction domains of Munc18-2 with Stx3 and Munc18-2 with Stx11. In mast cells, the Lys46 and Glu59 of domain 1 of Munc18 proteins are essential in the chaperoning of closed Stx3 in order to facilitate exocytosis (Bin et al., 2015). Exploring if these two areas are also important in Stx11 chaperoning could further support or oppose this hypothesis. None of the reported patients had a c.DNA change close to Lys46 or Glu59 indicating that there must be other parts of Munc18 that are essential for Stx3 binding.
Another hypothesis comes from a measurement in FHL5 patients which showed a reduction and sometimes a complete absence of expression of Munc18-2 in lymphocytes (Cote et al., 2009). This is suggested to be responsible for developing FHL5 in a subset of the patients. It is possible that Munc18-2 is also not expressed in enterocytes in these patients resulting in developing CMA. Reporting on Munc18-2 expression in lymphocytes is however too scarce and measuring the Munc18-2 expression of FHL5 patients in enterocytes has never been done to our knowledge, which is why we can make no conclusions about this as a cause of CMA. However, some effector pathways of Munc18-2 in apical membrane trafficking are independent of Stx3 interactions (Riento et al., 2000). Loss of these effector pathways might result in cellular changes that are additional to the normal CMA symptoms. Examining bowel samples of these patients for abnormal symptoms might clarify this hypothesis.

Rare Stx3 mutations can cause atypical MVID

In a recent study, two MVID patients (now known to be atypical MVID) were detected to have STX3 mutations (Wiegerinck et al., 2014). Patient 1 had a homozygous nonsense mutation (c.739C>T) in exon 9 and patient 2 a homozygous frame-shifting 2-bp intersection (c.372_373dup) in exon 6. Both patients developed identical symptoms to other CMA patients. Also the apical side of the enterocytes in these two patients showed typical CMA like symptoms confirming the assumption that Stx3 is heavily involved in the development of CMA. What is surprising is that unlike the FHL5 patients, these two patients also developed bilateral inclusions and microvillic incisions. This suggests that those symptoms develop independent of the Stx3/Munc18-2 complex. The Stx3/SNAP-23 complex, another T-SNARE, is spatially separated from the Stx3/Munc18-2 complex and is therefore a likely candidate to be

the cause of these symptoms (Pombo et al., 2003). However, Stx3 might have other interactional partners in enterocytes which it can form a T-SNARE with that are unknown up to date.

Interestingly, another study found three patients, 2 brothers and a sister, who also had Stx3 mutations. This time it was a homozygote Stx3 missense mutation (c.122A>G) in exon 3 (Chograni et al., 2015). However these patients developed congenital cataracts and had intellectual disabilities and never developed any like CMA symptoms. The mutation is found in the domain that is involved in SNAP interactions (Chograni et al., 2015). Stx3 has different interactional partners depending on the tissue. In the brain and in the retina, Stx3 is interacting with SNAP-25, however, in non-neural tissue this interaction is formed with SNAP-23 (Xu, et al., 2013) (Mazelova et al., 2009) (Pombo, et al., 2003). This could mean that this mutation only hinders Stx3/SNAP-25 and not Stx3/SNAP-23 interactions. Another possibility is that the Stx3/SNAP-23 interaction is hindered, but that this interaction is not vital for non-neural tissue. We however do not favour this hypothesis because it is at odds with explanation that Stx3/SNAP-23 complex inhibition is responsible for non-FHL5 CMA symptoms such as basolateral microvilli and microvilli inclusions.

Concluding

By combining the information put forward in this paper we were able to produce a simple model describing the connectional relations between Myo5B, Stx3 and Mun18-2 (Fig. 1). Following this model, it appears that Stx3 plays a key role in the development of CMA. However, the only evidence are the rare Stx3 mutations found in two patients with all CMA characterizing symptoms and localization variations in different knock-downs. We therefore suggest exploring the effect of various STX3 gene mutations, for example the ones found in the atypical MVID patients, in order to create a better understanding of the disease.

FHL5 causing StxBP2 mutations and their effect on developing CMA

Because the symptoms develop by some FHL5 patients are nearly identical to MVID and atypical MVID, finding out why only some FHL5 patients develop these symptoms could help to understand what main factors are involved in developing CMA. We therefore collected all reported DNA changes and patient data that were connected to FHL5. By comparing the patients that develop CMA with patients that do not develop CMA, we might be able to determine the effect of certain changes on developing CMA.

Method

By searching the PubMed database using the search terms 'familial hemophagocytic lymphohistiocytosis type 5', 'FHL5', 'StxBP2' and 'Munc18-2' all publications of FHL5 patients that reported the c.DNA change where gathered (total of 8 papers). This includes one patient report and seven research articles. Papers that didn't report the c.DNA change were omitted, however, patients without a known c.DNA change described in a paper that did report the c.DNA change patients were admitted.

The patients (n=74) were split into four groups: patients with diarrhea (n=19) (table 3A), without

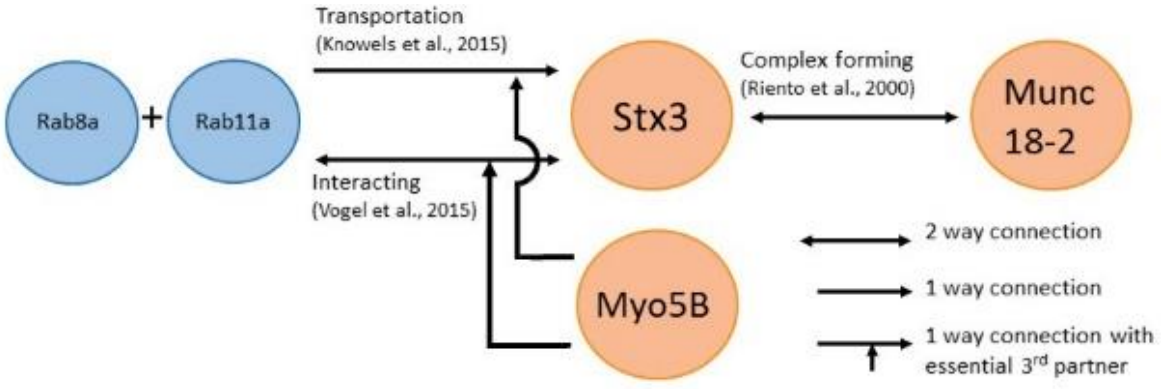

Figure 1. Simplified schematic overview of our proteins of interest (orange), secondary proteins (blue) and the connections between them (arrows).

diarrhea (n=25) (table 3B), unknown diarrhea status (n=25) (table 3C) and unknown c.DNA change (n=5) (table 3D). Chronic diarrhea was chosen as a marker between groups because it is the only symptom associated with CMA that was also repeatedly reported on.

Results

General genetic findings

A total of 32 mutations associated with FHL5 were found, of which 12 were seen only in patients with diarrhea, 15 only in patients without diarrhea and 4 in both types of patients (table 1). One last mutation was only found in a patient with an unknown diarrhea status (table 2). Most mutations were only present in one (n=21, 66%) two (n=4, 13%) or three (n=4, 13%) patients. The remaining four mutations that are more common in patients are: c.1214G>A (p.Arg405Gln) (n=4), c.1247-1G>C (Splice Error) (n=26), c.1430C>T (p.Pro477Leu) (n=11) and c.1621G>A (p.Gly541Ser) (n=12). Having a homozygote mutation, rather than 2 heterozygote mutations, results in a more than three times higher change of developing CMA (65% to 19%). Despite the low frequency of most c.DNA changes, the rarity of c.DNA change overlap between the diarrhea and non-diarrhea groups suggests that in most cases the c.DNA change is the direct cause of the CMA and that other factors, which are patient variable, do not have a large influence on the outcome.

Specific c.DNA changes in patients with and without diarrhea

The changes c.1247C>T (Splice error) and c.1621G>A (p.Gly541Ser) are both often present in patients without diarrhea (14 and 7 times

A

c.DNA change	Location	Patients
c.37+5GA Splice error	Exon 2	1 (0)
c.87+2T>G Spice Error	Exon 2	1 (0)
c.116G>C p.Arg39Pro	Exon 3	1 (0)
c.del224_227ATTT p.Tyr75CysfsX2	Exon 4	1 (0)
c.260delT p.Leu87ArgfsX32	Exon 5	1 (0)
c.310A >T p.Ile104Phe	Exon 5	1 (0)
c.626T>C p.Leu209Pro	Exon 8	1 (0)
c.706delG p.Ala236GlnfsX24	Exon 9	1 (0)
c.del769>771 p.Leu257del	Exon 9	1 (1)
c.875G>A p.Arg292His	Exon 10	1 (1)
c.902+5GA Splice error	Exon 11	3 (0)
c.1066_1074del p.His356_Lys358del	Exon 13	2 (0)
c.1247-G>C Splice Error*	Exon 15	14 (4)
c.1294C>A p.Gln432X	Exon 15	1 (0)
c.1356+1G>A Splice Error	Exon 15	1 (0)
c.1430C>T p.Pro477Leu*	Exon 16	2 (2)
c.1621G>A p.Gly541Ser*	Exon 18	7 (0)
c.1634C>T p.Ser545Leu	Exon 19	1 (0)
c.1724_1729delGCTTCC p.Arg575_Phe576del*	Exon19	1 (0)
Total		42 (8)

Table 1. c.DNA changes in patients groups without (left, A) and with chronic diarrhea (right, B). Location on the StxBP2 gene, the number of patients with that change in either of his/her allele and the number of those patients that had a homozygote change (in brackets). *Change present in both groups. #Present in confirmed CMA patients

B

c.DNA change	Location	Patients
c.279delG p. Thr94ProfsX25	Exon 5	1 (1)
c.326-24del 8bp	Intron 5	1 (0)
c.474del10bpins G>A p.C237WfsX76	Exon 7	1 (0)
c.502_dupC p.Gln168ProFsX71	Exon 7	3 (3)
c.693_695delGAT p.Ile232del	Exon 9	3 (3)
c.901+6T>G#	Intron 10	1 (0)
c.1099_1107 del9bp p.V367_Q369del#	Intron 13	1 (0)
c.1213C>T p.Arg405Trp	Exon 14	2 (1)
c.1214G>A p.Arg405Glu#	Exon 14	2 (2)
c.1247-G>C Splice Error*	Exon 15	1 (0)
c.1430C>T p.Pro477Leu*	Exon16	1 (1)
c.1601C>T p.Leu534Pro	Exon 18	1 (0)
c.1621G>A p.Gly541Ser*	Exon18	1 (0)
c.1696+1G>A Splice Error	Exon 18	1 (1)
c.1697G>A p.Gly566Asp	Exon 18	1 (1)
c.1727delT p.Phe576SerfsX4*	Exon 19	2 (2)
Total		23 (15)

c.DNA change	Location	Patients
c.395A>C p.Glu132Ala	Exon 6	1 (1)
c.1214G>A p.Arg405Gln	Exon 14	2 (2)
c.1247-1G>C Splice Error	Exon 15	9 (7)
c.1430C>T p.Pro477Leu	Exon 16	8 (8)
c.1621G>A p.Gly541Ser	Exon 18	4 (2)
c.1696+1G>A Splice Error	Exon 18	1 (1)
Total		25 (21)

Table 2. c.DNA changes in unknown diarrhea patients. Location on the StxBP2 gene, the number of patients with that change in either of his/her allele total and the number of patients with a homozygote change (brackets).

respectively) but both are also found once as a heterozygote mutation in the patients with diarrhea. This large difference suggests that both changes are not CMA causing changes but that they also cannot always compensate for other c.DNA changes. By performing bowel biopsies the change c.1214G>A (p.Arg405Glu) is confirmed to cause CMA (Stepensky et al., 2013). This is in accord with our findings as that change was not present the patients without diarrhea group and present in one other patient with diarrhea. Interestingly, two patients developed diarrhea with a c.1213C>T mutation causing the same protein change (p.Arg405Trp) further supporting that this location is essential for the correct functioning of Munc18-2 in enterocytes. Another confirmed CMA patient had two different heterozygous changes in the StxBP2 gene: c.1099_1107del9bp (p.V367_Q369del) and c.901+6T>C (in intron 10) (Stepensky et al., 2013). Because both were only present in this patient, it is impossible to determine if one is responsible for the chronic diarrhea and the other one is unable to compensate, or that both changes are chronic diarrhea inducers.

Discussion

To our knowledge, we were the first to collect all known FHL5 associated c.DNA changes and compare CMA with non-CMA causing genes. By this we were able to identify changes that are more likely to cause CMA. However, there are several problems that makes definitively determining which mutations cause CMA very difficult. First, chronic diarrhea is a marker that not only applies to CMA but also to other diseases and can even be a result of infections, which are more frequent in FHL5 patients. This may, for example, be the case in patient 19 who had chronic diarrhea but also had a gastro infection which often results in diarrhea (table 3A). Combined with the infrequent reporting of gastrointestinal problems like gastro infections it is impossible to determine how large this effect is on the found results. Secondly, although it is hinted at, it is unknown if a certain c.DNA change always has the same outcome (developing or not developing CMA) or that individual variables have a sizable effect on the results. If the latter is true, the low sample sizes render most found results obsolete.

Another very real possibility is that in a heterozygote patient, a non-CMA causing change can compensate for a CMA causing one. This would entail that some of the c.DNA changes found in patients without diarrhea (table 1a) are wrongly categorized and should be reported as CMA causing mutations. This compensation could explain why patients with diarrhea have a three times higher change of being homozygote.

Setting up a database that included all newly discovered FHL5 patients and promoted accurate reporting could help to reduce the effect caused by these problems.

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A

ID	Gender	Ancestry	Consang-uinity	Relative with FHL5	c.DNA change	Location	Homozygote/ Heterozygote	Munc18-2 expression*	Age at first FHL5 symptoms	HSCT	Other gastrointestinal symptoms	Diarrhea	Microvillus retention pattern	Other symptoms	Reference
19	Female	Pakistani	Yes	Yes	c.1213C>T p.Arg405Trp	Exon 14	Heterozygote		4 Years	No	Inflammation; Gastro infection	Yes		No	Meeths et al., 2016
19	Female	Pakistani	Yes	Yes	c.1247-G>C Splice Error	Exon 15	Heterozygote		4 Years	No	Inflammation; Gastro infection	Yes		No	Meeths et al., 2016
30	Female	Turkish	Yes	No	c.279delG p.Thr94ProfsX25	Exon 5	Homozygote		2 Months	No		Yes			Pagel et al., 2016
32	Female	Turkish	Yes	Yes	c.502_dupC p.Gln168ProFsX71	Exon 7	Homozygote		2 Months	No		Yes			Pagel et al., 2016
33	Female	Turkish	Yes	Yes	c.502_dupC p.Gln168ProFsX71	Exon 7	Homozygote		11 Months	Yes		Yes			Pagel et al., 2016
34	Female	Turkish	Yes	Yes	c.502_dupC p.Gln168ProFsX71	Exon 7	Homozygote		1 Months	No		Yes			Pagel et al., 2016
35	Female	Turkish	Yes	No	c.693_695delGAT p.Ile232del	Exon 9	Homozygote		2 Months	No		Yes			Pagel et al., 2016
36	Male	Turkish	Yes	Yes	c.693_695delGAT p.Ile232del	Exon 9	Homozygote		2 Months	Yes		Yes			Pagel et al., 2016
38	Female	Turkish	Yes	Yes	c.1214G>A p.Arg405Glu	Exon 14	Homozygote		3 Months	Yes		Yes		No	Pagel et al., 2016
40	Male	Turkish	Yes	No	c.1213C>T p.Arg405Trp	Exon 14	Homozygote		9 Months	No		Yes		No	Pagel et al., 2016
45	Male	United Arab Emirates	Yes	Yes	c.1430C>T p.Pro477Leu	Exon 16	Homozygote		1 Months	Yes		Yes		No	Pagel et al., 2016
46	Female	Austrian		Yes	c.1696+1G>A Splice Error	Exon 18	Homozygote		2 Months	Yes		Yes			Pagel et al., 2016
48	Male	Danish	No	Yes	c.1601C>T p.Leu534Pro	Exon 18	Heterozygote		2 Months	No		Yes			Pagel et al., 2016
48	Male	Danish	No	Yes	c.1621G>A p.Gly541Ser	Exon18	Heterozygote		2 Months	No		Yes			Pagel et al., 2016
49	Male	Sri Lankan	No	Yes	c.1697G>A p.Gly566Asp	Exon 9	Homozygote		2 Months	Yes		Yes		No	Pagel et al., 2016
51	Female	Lebanese	Yes	Yes	c.1727delT p.Phe576SerfsX4	Exon 19	Homozygote		2 Months	Yes		Yes		No	Pagel et al., 2016
52	Female	Lebanese	Yes	Yes	c.1727delT p.Phe576SerfsX4	Exon 19	Homozygote		1 Months	Yes		Yes		No	Pagel et al., 2016
67	Male	Turkish	Yes	Brother; Sister	c.693_695del3bp p.I232del	Exon 9	Homozygote		3 Weeks	No		Yes			Stepensky et al., 2013
69	Female	Jewish Ashkenazi	No	No	c.474del10bpinsG >A p.C237WfsX76	Exon 7	Heterozygote		12 Months	Yes	No	Yes		Neurological; Hearing	Stepensky et al., 2013
69	Female	Jewish Ashkenazi	No	No	c.326-24del 8bp	Intron 5	Heterozygote		12 Months	Yes	No	Yes		Neurological; Hearing	Stepensky et al., 2013
70	Male	United Arab Emirates	No	No	c.1099_1107 del9bp p.V367_Q369del	Exon 13	Heterozygote		6 Weeks	Yes	No	Yes	Yes	No	Stepensky et al., 2013
70	Male	United Arab Emirates	No	No	c.901þ6T>G	Intron 10	Heterozygote		6 Weeks	Yes	No	Yes	Yes	No	Stepensky et al., 2013
71	Female	Pakistani	No	No	c.1214G>A p.Arg405Glu	Exon 14	Homozygote		4.5 Months	Yes	No	Yes	Yes	Neurological; Endocrine	Stepensky et al., 2013

Table 3. All reported patient data and c.DNA changes in FHL5 patient. A, Patients with diarrhea. B, Patients without diarrhea. C, Patients with an unknown diarrhea status. D, Patients without a c.DNA change reported. * Measured in lymphocytes. #Detailed patient report without the mention of diarrhea.

B

ID	Gender	Ancestry	Consang-uinity	Relative with FHL5	c.DNA change	Location	Homozygote/ Heterozygote	Munc18-2 expression*	Age at first FHL5 symptoms	HSCT	Other gastrointestinal symptoms	Diarrhea	Microvillus retention pattern	Other symptoms	Reference
5	Male	Caucasian		No	c.1247-G>C Splice Error	Exon 15	Heterozygote		5-6 Years	Yes	No	No#		Severe chronic active Epstein-Barr virus	Cohen et al., 2016
5	Male	Caucasian		No	c.1621G>A p.Gly541Ser	Exon 18	Heterozygote		5-6 Years	Yes	No	No#		Severe chronic active Epstein-Barr virus	Cohen et al., 2016
18	Female	Russian	No	No	c.1294C>A p.Gln432X	Exon 15	Heterozygote		17 Years	No	Inflammation	No		Facial Palsy; Thrombocytopenic	Meeths et al., 2016
18	Female	Russian	No	No	c.1634C>T p.Ser545Leu	Exon 16	Heterozygote		17 Years	No	Inflammation	No		Facial Palsy; Thrombocytopenic	Meeths et al., 2016
21	Male	Pakistani	Yes	No	c.del769>771 p.Leu257del	Exon 9	Homozygote		6 Months	Yes	Gastroesophageal reflux; no symptoms after treatment	No		No	Meeths et al., 2016
22	Male	Dutch	No	No	c.del224_227ATTT p.Tyr75CysfsX2	Exon 4	Heterozygote		8.5 Months	Yes	No	No		No	Meeths et al., 2016
22	Male	Dutch	No	No	c.1247-1GC Splice Error	Exon 15	Heterozygote		8.5 Months	Yes	No	No		No	Meeths et al., 2016
23	Male	Dutch	No	No	c.116G>C p.Arg39Pro	Exon 3	Heterozygote		19 Months	Yes	Inflammation	No		No	Meeths et al., 2016
23	Male	Dutch	No	No	c.1247-1GC Splice Error	Exon 15	Heterozygote		19 Months	Yes	Inflammation	No		No	Meeths et al., 2016
24	Female	Norwegian	N.R		c.37+5GA Splice error	Exon 2	Heterozygote		11 Months	Yes	No	No		No	Meeths et al., 2016
24	Female	Norwegian	N.R		c.1621G>A p.Gly541Ser	Exon 16	Heterozygote		11 Months	Yes	No	No		No	Meeths et al., 2016
26	Male	Danish	No	Yes	c.902+5GA Splice error	Exon 11	Heterozygote		12 Years	Yes	Abdominal Pain; Loss of appetite; Recovered after transplantation	No		No	Meeths et al., 2016
26	Male	Danish	No	Yes	c.1247-1GC Splice Error	Exon 15	Heterozygote		12 Years	Yes	Abdominal Pain; Loss of appetite; Recovered after transplantation	No		No	Meeths et al., 2016
27	Male	Danish	No	Yes	c.902+5GA Splice error	Exon 11	Heterozygote		2 Months	Yes	No	No		No	Meeths et al., 2016
27	Male	Danish	No	Yes	c.1066_1074del p.His356_Lys358del	Exon 13	Heterozygote		2 Months	Yes	No	No		No	Meeths et al., 2016
28	Female	Danish	No	Yes	c.902+5GA Splice error	Exon 11	Heterozygote		No symptoms	No	No	No		No	Meeths et al., 2016
28	Female	Danish	No	Yes	c.1066_1074del p.His356_Lys358del	Exon 13	Heterozygote		No symptoms	No	No	No		No	Meeths et al., 2016
29	Female	Swiss	No	Yes	c.87+2T>G Spice Error	Exon 2	Heterozygote		2 Months	Yes		No		Sensory Loss	Pagel et al., 2016
29	Female	Swiss	No	Yes	c.1621G>A p.Gly541Ser	Exon 18	Heterozygote		2 Months	Yes		No		Sensory Loss	Pagel et al., 2016
31	Female	German	No	No	c.310A >T p.Ile104Phe	Exon 5	Heterozygote		1 Months	Yes		No		Sensory Loss	Pagel et al., 2016
31	Female	German	No	No	c.1621G>A p.Gly541Ser	Exon 18	Heterozygote		1 Months	Yes		No		Sensory Loss	Pagel et al., 2016

B

ID	Gender	Ancestry	Consang-uinity	Relative with FHL5	c.DNA change	Location	Homozygote/ Heterozygote	Munc18-2 expression*	Age at first FHL5 symptoms	HSCT	Other gastrointestinal symptoms	Diarrhea	Microvillus retention pattern	Other symptoms	Reference
37	Female	Turkish	Yes	Yes	c.875G>A p.Arg292His	Exon 10	Homozygote		2 Months	Yes		No			Pagel et al., 2016
41	Male	United Arab Emirates	Yes	Yes	c.1430C>T p.Pro477Leu	Exon 16	Homozygote		2 Months	Yes		No		No	Pagel et al., 2016
42	Male	United Arab Emirates	Yes	Yes	c.1430C>T p.Pro477Leu	Exon 16	Homozygote		6 Months	Yes		No			Pagel et al., 2016
53	Male	German		No	c.260delT p.Leu87ArgfsX32	Exon 5	Heterozygote		1.3 Years	Yes		No			Pagel et al., 2016
53	Male	German		No	c.1247-G>C Splice Error	Exon 15	Heterozygote		1.3 Years	Yes		No			Pagel et al., 2016
54	Female	German		No	c.626T>C p.Leu209Pro	Exon 8	Heterozygote		2.3 Years	No		No		Yes	Pagel et al., 2016
54	Female	German		No	c.1247-G>C Splice Error	Exon 15	Heterozygote		2.3 Years	No		No		Yes	Pagel et al., 2016
55	Male	Czech		No	c.706delG p.Ala236GlnfsX24	Exon 9	Heterozygote		1.3 Years	Yes		No		No	Pagel et al., 2016
55	Male	Czech		No	c.1247-G>C Splice Error	Exon 15	c.1247-G>C Splice Error		1.3 Years	Yes		No		No	Pagel et al., 2016
56	Male	Turkish	Yes	Yes	c.1247-G>C Splice Error	Exon 15	Homozygote	Reduced	2.4 Years	Yes		No		No	Pagel et al., 2016
59	Male	Turkish	Yes	No	c.1247-G>C Splice Error	Exon 15	Homozygote	Reduced	9 Years	No		No		No	Pagel et al., 2016
60	Male	German		No	c.1247-G>C Splice Error	Exon 15	Homozygote	Reduced	3 Years	No		No		Yes	Pagel et al., 2016
61	Male	German	No	Yes	c.1247-G>C Splice Error	Exon 15	Homozygote	Reduced	14 Years	Yes		No			Pagel et al., 2016
62	Female	German	No	No	c.1247-G>C Splice Error	Exon 15	Heterozygote		7 Years	Yes		No			Pagel et al., 2016
62	Female	German	No	No	c.1621G>A Splice Error	Exon 18	Homozygote		7 Years	Yes		No			Pagel et al., 2016
63	Female	German	No	No	c.1247-G>C Splice Error	Exon 15	Heterozygote		19 Years	Yes		No			Pagel et al., 2016
63	Female	German	No	No	c.1621G>A Splice Error	Exon 18	Heterozygote		19 Years	Yes		No			Pagel et al., 2016
64	Female	German/ Indonesian	No	No	c.1247-G>C Splice Error	Exon 15	Heterozygote		6 Years	Yes		No		Yes	Pagel et al., 2016
64	Female	German/ Indonesian	No	No	c.1724_1729delGCTTCC p.Arg575_Phe576del	Exon 19	Heterozygote		6 Years	Yes		No		Yes	Pagel et al., 2016
65	Female	German	No	No	c.1356+1G>A Splice Error	Exon 15	Heterozygote		13 Years	Yes		No		Yes	Pagel et al., 2016
65	Female	German	No	No	c.1621G>A p.Gly541Ser	Exon 18	Heterozygote		13 Years	Yes		No		Yes	Pagel et al., 2016

C

ID	Gender	Ancestry	Consang-uinity	Relative with FHL5	c.DNA change	Location	Homozygote/ Heterozygote	Munc18-2 expression*	Age at first FHL5 symptoms	HSCT	Other gastrointestinal symptoms	Diarrhea	Microvillus retention pattern	Other symptoms	Reference
1	Male	Italian	Yes		c.395A>C p.Glu132Ala	Exon 6	Homozygote		4 year	No		Unknown			Cetica et al., 2016
2	Female	Pakistani	Yes		c.1214G>A p.Arg405Gln	Exon 14	Homozygote		4.5 months	Yes		Unknown			Cetica et al., 2016
3	Female	Kuwaiti	No		c.1430C>T p.Pro477Leu	Exon 16	Homozygote		7 months	Yes		Unknown			Cetica et al., 2016
4	Female	United Kingdom			c.1621G>A p.Gly541Ser	Exon 18	Homozygote		7 months	Yes		Unknown			Cetica et al., 2016
6	Female	Saudi Arabian	Yes	Cousin 1st degree	c.1430C>T p.Pro477Leu	Exon 16	Homozygote		3.5 Months			Unknown			Cote et al., 2009
7	Female	Saudi Arabian	Yes	Sister	c.1430C>T p.Pro477Leu	Exon 16	Homozygote		1.5 Months			Unknown			Cote et al., 2009
8	Female	Saudi Arabian	Yes	Sister	c.1430C>T p.Pro477Leu	Exon 16	Homozygote		1 Months			Unknown			Cote et al., 2009
9	Male	Saudi Arabian	Yes	Brother	c.1430C>T p.Pro477Leu	Exon 16	Homozygote	No	1 Months			Unknown			Cote et al., 2009
10	Male	Saudi Arabian	Yes	Brother	c.1430C>T p.Pro477Leu	Exon 16	Homozygote	No	2 Months			Unknown			Cote et al., 2009
11	Male	Palestinian	No	No	c.1247-1G>C Splice Error	Exon 15	Homozygote		8 Years	Yes		Unknown		Epstein-Barr virus	Cote et al., 2009
12	Male	Turkish	Yes	Brother	c.1247-1G>C Splice Error	Exon 15	Homozygote	Reduced	10.5 Years	No		Unknown		Epstein-Barr virus	Cote et al., 2009
13	Male	Turkish	Yes	Brother	c.1247-1G>C Splice Error	Exon 15	Homozygote	Reduced	Asymptomatic	No		Unknown			Cote et al., 2009
14	Male	Iranian	Yes	Sister	c.1247-1G>C Splice Error	Exon 15	Homozygote		12.5 Years			Unknown			Cote et al., 2009
15				Unknown	c.1621G>A p.Gly541Ser	Exon 18	Heterozygote					Unknown			Kostova et al., 2015
15				Unknown	c.1247-1G>C Splice Error	Exon 15	Heterozygote					Unknown			Kostova et al., 2015
16				Sibling	c.1247-1G>C Splice Error	Exon 15	Homozygote					Unknown		Prolonged bleeding	Meeths et al., 2016
17				Sibling	c.1247-1G>C Splice Error	Exon 15	Homozygote					Unknown		Hypotonia; Thrombocytopenic	Meeths et a., 2016
39	Female	Turkish	Yes	Yes	c.1214G>A p.Arg405Glu	Exon 14	Homozygote		2 Months	Yes		Unknown			Pagel et al., 2016
43	Female	Saudi Arabian	Yes	Yes	c.1430C>T p.Pro477Leu	Exon 16	Homozygote		4 Months	Yes		Unknown			Pagel et al., 2016
44	Male	Saudi Arabian	Yes	Yes	c.1430C>T p.Pro477Leu	Exon 16	Homozygote		1 Months	Yes		Unknown			Pagel et al., 2016
47	Male	Austrian		Yes	c.1696+1G>A Splice Error	Exon 18	Homozygote		5 Days	No		Unknown			Pagel et al., 2016
57	Female	Turkish	Yes	Yes	c.1247-G>C Splice Error	Exon 15	Homozygote	Reduced	1.3 Years	Yes		Unknown			Pagel et al., 2016
58	Female	Turkish	Yes		c.1247-G>C Splice Error	Exon 15	Homozygote	Reduced	1.6 Years	Yes		Unknown			Pagel et al., 2016
72				Sibling	c.1621G>A p.Gly541Ser	Exon 18	Homozygote	No		Yes		Unknown			Zhao et al., 2016
73					1247-1G>G Splice Error	Exon 15	Homozygote					Unknown			Zhao et al., 2016
74					c.1621G>A p.Gly541Ser	Exon 18	Heterozygote	No				Unknown			Zhao et al., 2016
74					1247-1G>G Splice Error	Exon 15	Heterozygote	No				Unknown			Zhao et al., 2016

D

ID	Gender	Ancestry	Consang-uinity	Relative with FHL5	c.DNA change	Location	Homozygote/ Heterozygote	Munc18-2 expression	Age at first FHL5 symptoms	HSCT	Other gastrointestinal symptoms	Diarrhea	Microvillus retention pattern	Other symptoms	Reference
20	Male	Pakistani	Yes	Yes	Unknown				4 Months	No	No	Yes		No	Meeths et al., 2016
25	Female	Danish	No	Yes	Unknown				7 Years		No	Abdominal Pain	No		Meeths et al., 2016
50	Male	Sri Lankan	No	Yes	Unknown				3 Days			Unknown			Pagel et al., 2016
66	Female	Turkish	Yes	2 Brothers	Unknown				2 Weeks	No	Metabolic Acidosis	Yes			Stepensky et al., 2013
68	Male	Turkish	Yes	Brother; Sister	Unknown				3 Weeks	Yes	Metabolic Acidosis	Yes	Yes	Neurological; Renal; Endocrine; Hearing	Stepensky et al., 2013