Novel MYO5B Mutation in Microvillous Inclusion Disease of Syrian Ancestry

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Abstract

Microvillus inclusion disease (MVID) is a rare autosomal recessive condition characterized by a lack of microvilli on the surface of enterocytes, resulting in severe, life-threatening diarrhea which could lead to mortality within the first year of life. We identify 2 unrelated families, each with one child presenting with severe MVID from birth. Using trio whole-exome sequencing, we observed that the two families share a novel nonsense variant (Glu1589*) in the MYO5B gene, a type Vb myosin motor protein in which rare damaging mutations were previously described to cause MVID. This founder mutation was very rare public databases and is likely specific to patients of Syrian ancestry. We present a detailed account of both patients' clinical histories to fully characterize the effect of this variant and expand the genotype-phenotype databases for MVID patients from the Middle East.

Introduction

Microvillus inclusion disease (MVID, MIM# 251850), also known as congenital microvillus atrophy, was first described by Davidson et al. (Davidson et al. 1978) in 1978. It is a rare autosomal recessive disease that presents with an intractable life-threatening watery diarrhea either within the first days of life (early-onset form) or at several months of life (late-onset form) (Ruemmele et al. 2006). The hallmarks of MVID are a lack of microvilli on the surface of villous enterocytes, occurrence of microvillous inclusions and the cytoplasmic accumulation of periodic acid-schiff-positive vesicles (Davidson et al. 1978; Cutz et al. 1989; Ruemmele et al. 2010). In 2008, Muller et al (Müller et al. 2008) showed that mutations in MYO5B (MIM # 606540), encoding the unconventional type Vb myosin motor protein, were associated with MVID in an extended Turkish kindred. Since then, more mutations were described in different populations (Dhekne et al. 2018). In this report we describe a novel mutation in two unrelated Syrian patients with MVID.

Patient 1 (MVID-1)

A Syrian girl was born at 36 wk gestation to consanguineous parents originally from the eastern part of Syria. The mother was 22 years old, gravida 7, para 3. Her previous 3 daughters died in Syria at 7, 10 and 30 days of age, following intractable diarrhea of unknown diagnosis.

Since the first day of life, the patient was started on breast milk but developed severe diarrhea leading to dehydration and acidosis. Changing the feeds to elemental formula (Neocene LCP, SHS, Liverpool, UK) made no difference to her symptoms. The oral feeding was discontinued and she was started on total parenteral nutrition (TPN). Examinations of other organ involvement such as cardiovascular system, lungs, and kidney was unremarkable. Nonetheless, the patient had frequent hospital admissions due to dehydration and sepsis. During her disease, the patient also developed cholestasis with pruritus due to prolonged TPN use. Her Serum bile acids and liver enzymes were high, and she was treated with ursodeoxycholic acid to improve cholestasis.

Investigations for infection, cystic fibrosis, metabolic and immunodeficiency disorders were all unremarkable. Patient was also seen and investigated extensively by the endocrinology team to rule out congenital adrenal hyperplasia (CAH) as a cause of diarrhea.

Histological analysis of multiple gastrointestinal biopsies was obtained post endoscopy. In this patient, the histopathology of the duodenal mucosa showed subtotal and total villous atrophy with slightly elongated crypts. There were no granuloma, dysplasia or malignancies seen. A vacuolated apical cytoplasm was noted in H&E sections and highlighted by special stains (PAS) that showed cytoplasmic staining of luminal aspect of enterocytes. Immunohistochemical staining for polyclonal anti-CEA, CD10 stains illustrated microvillus abnormalities including patchy normal band- like brush border with internalization of brush border in cytoplasm. The overall morphological and immunohistochemical appearances were broadly compatible with the clinical

impression of MVID. Microscopic examination of fragments of the large bowel mucosa and the stomach revealed no significant histological abnormality.

Whole exome sequence (WES) identified a novel mutation in MYO5B gene (see Results). Her clinical course and outcome are summarized in Table I.

Nine months later the mother became pregnant. Amniocentesis at 12 wk for genetic testing for MYO5B mutation was positive; the parents chose to abort the baby. Six months later the mother became pregnant again. Amniocentesis done at 13 weeks gestation was negative and 6 months later the mother gave birth to a healthy baby.

Patient 2 (MVID-2)

A Syrian boy was born at 34 wk gestation to consanguineous parents originally from the western part of Syria. This was their first baby and there was no history of abortion. The patient developed severe diarrhea associated with acidosis. Changing the feeds from breast milk to elemental formula (Neocate LCP, SHS, Liverpool, UK) made no difference and he had to be started on (TPN). Investigations for infection, cystic fibrosis, metabolic and immunodeficiency disorders were all unremarkable. At the age of 6 months the patient developed Acute disseminated encephalomyelitis (ADEM) – an immune-mediated inflammatory demyelinating condition that predominately affects the white matter of the brain and spinal cord. Convulsive seizures occur around the onset of ADEM in as many as 35% of cases as well as long tract signs such as clonus and increased muscle stretch reflexes that can occur in as many as 85% of cases. ADEM was only present in MVID-Patient 2. Patient 2 also developed cholestasis with pruritis, where liver enzymes level and serum bile acid levels were elevated most likely due to the prolonged TPN use. This was also present in Patient 1.

Investigation of sections from the small intestine showed loss of villous architecture with mild crypt hyperplasia. There also appeared to be "internalization" with patchy distribution of the

brush border evident in PAS stain as well as on CD10 (IHC stains done with appropriate controls). Both the villous atrophy and "internalization of brush border" were suggestive of MVID.

Investigation with WES revealed the same mutation as in patient 1. Trials of even small amounts of different formulae caused significant diarrhea and electrolyte disturbances. His clinical course is summarized in Table 1. At 23 months of age the child died.

Methods

WES of proband and parents was done using Agilent's SureSelect v5 platform and sequenced on Illumina's HiSeq 2000. Reads were mapped to the human reference genome GRCh37/hg19 using BWA 0.5.9 and variants called using GATK Best Practices (as described in Fakhro et al. 2019). Each exome was verified to have a minimum average depth of 50X (Table II, III). Causative variants were identified by searching for high-quality (quality score > 100), rare (minor allele frequency < 0.1%), recessive mutations with a predicted pathogenic impact (loss of function mutation, and/or GERP score > 2, PolyPhen score > 0.9, CADD score > 20; GERP, Polyphen, CADD). Candidate variants surviving this filtration were manually evaluated for genes with reasonable implication in disease by literature and database searches.

Genetic Testing

The exomes of all available family members were sequenced to a minimum depth of 54X (mean depth 73.5X) and variant calls according to best practices (see Methods). Within the framework of reported consanguinity, variants were filtered based on being recessive (homozygous in index and heterozygous in the parents), rare, and predicted damaging by a range of evolutionary scores (see Methods). Both sets of patients were found to have the nonsense mutation p.Glu1589* in the MYO5B gene (Figures 1, 2). This protein-truncating mutation has a reported global allele frequency of < 0.001%, and is predicted to cause loss of gene function.

Discussion

Geographically, there is a relatively high prevalence of MVID in the Mediterranean region; almost 50 % of all patients with reported geographical information (Halac et al. 2011; van der Velde et al. 2013). There have been no reported MVID cases from Syria, and the two patients reported in this paper, are the first MVID cases of Syrian ancestry. Although both families were not closely related and hailed from two different areas in Syria, they both carried the same *MYO5B* novel mutation Glu1589*. Since this variant is globally very rare, its presence in two families from the same country suggests that this is a geographically-specific founder mutation. As expected from the predicted loss-of-function effect on the protein, the clinical presentation showed severe disease in both patients who could hardly tolerate oral feeding and were completely dependent on (TPN). Both patients had developmental delay, consistent with previously reported MVID cases (Phillips and Schmitz 1992; Halac et al. 2011). Patient 2 also developed intractable myoclonic seizures that needed 3 anti-epileptic medications to control; this complication was never reported in previous MVID cases. Patient 1 developed pruritus and cholestasis consistent with progressive familial intrahepatic cholestasis-like illness, which has been previously described in MVID (Girard et al. 2014). It is unclear to what extent these extraintestinal phenotypes may be iatrogenic or linked to the *MYO5B* mutations, and patient-specific disease model are eagerly awaited (van der Velde et al. 2013).

In conclusion, this is the first report of MVID in Syrian patients. The rarity of this mutation in public databases yet being homozygous in two unrelated Syrian patients suggests this is a Syrian founder mutation. The utility of identifying causative mutations within a family allows for reliable genetic counseling, prenatal screening and in embryo selection by pre-implantation genetic testing.

Additional Information

Ethics Statement: This study was performed with Institutional Review Board approval for Human Subjects Research at Hamad Medical Corporation and at Weill Cornell Medicine-Qatar. Research participants were recruited using IRB-approved protocols and informed consents.

Data Deposition and Access: The informed consents signed by the parents protected the privacy of study participants and such data cannot be made publically available. The novel variant has been deposited into the Clinvar repository under accession number VCV000620180.

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Conflict of interest: The authors declare that there are no conflicts.

References

- Cutz E, Rhoads JM, Drumm B, Sherman PM, Durie PR, Forstner GG. 1989. Microvillus inclusion disease: an inherited defect of brush-border assembly and differentiation. *N Engl J Med* **320**(10): 646-651.
- Davidson GP, Cutz E, Hamilton JR, Gall DG. 1978. Familial enteropathy: a syndrome of protracted diarrhea from birth, failure to thrive, and hypoplastic villus atrophy. *Gastroenterology* **75**(5): 783-790.
- Dhekne HS, Pylypenko O, Overeem AW, Zibouche M, Ferreira RJ, van der Velde KJ, Rings E, Posovszky C, van der Sluijs P, Swertz MA et al. 2018. MYO5B, STX3, and STXBP2 mutations reveal a common disease mechanism that unifies a subset of congenital diarrheal disorders: A mutation update. *Hum Mutat* **39**(3): 333-344.
- Fakhro KA, Robay A, Rodrigues-Flores JL, Mezey JG, Al-Shakaki AA, Chidiac O, Stadler D, Malek JA, Imam AB, Sheikh A et al. 2019. Point of Care Exome Sequencing Reveals Allelic and Phenotypic Heterogeneity Underlying Mendelian disease in Qatar. *Hum Mol Genet* **28**(23): 3970-3981.
- Girard M, Lacaille F, Verkarre V, Mategot R, Feldmann G, Grodet A, Sauvat F, Irtan S, Davit-Spraul A, Jacquemin E et al. 2014. MYO5B and bile salt export pump contribute to cholestatic liver disorder in microvillous inclusion disease. *Hepatology* **60**(1): 301-310.
- Halac U, Lacaille F, Joly F, Hugot JP, Talbotec C, Colomb V, Ruemmele FM, Goulet O. 2011. Microvillous inclusion disease: how to improve the prognosis of a severe congenital enterocyte disorder. *J Pediatr Gastroenterol Nutr* **52**(4): 460-465.
- Müller T, Hess MW, Schiefermeier N, Pfaller K, Ebner HL, Heinz-Erian P, Ponstingl H, Partsch J, Röllinghoff B, Köhler H et al. 2008. MYO5B mutations cause microvillus inclusion disease and disrupt epithelial cell polarity. *Nat Genet* **40**(10): 1163-1165.
- Phillips AD, Schmitz J. 1992. Familial microvillous atrophy: a clinicopathological survey of 23 cases. *J Pediatr Gastroenterol Nutr* **14**(4): 380-396.
- Ruemmele FM, Müller T, Schiefermeier N, Ebner HL, Lechner S, Pfaller K, Thöni CE, Goulet O, Lacaille F, Schmitz J et al. 2010. 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* **31**(5): 544-551.
- Ruemmele FM, Schmitz J, Goulet O. 2006. Microvillous inclusion disease (microvillous atrophy). *Orphanet J Rare Dis* **1**: 22-22.
- van der Velde KJ, Dhekne HS, Swertz MA, Sirigu S, Ropars V, Vinke PC, Rengaw T, van den Akker PC, Rings EH, Houdusse A et al. 2013. An overview and online registry of microvillus inclusion disease patients and their MYO5B mutations. *Hum Mutat* **34**(12): 1597-1605.

Table I. Demographic and Clinical Characteristics of the Two Patients with MVID

Parameter	MVID-1	MVID-2
Sex	Female	Male
Ancestry	Syrian, Eastern region	Syrian, Western region
Consanguinity	Yes	Yes
MYO5B mutation	c.G4765T Glu1589* : stop-gained	c.G4765T p.GLlu1589* : stop-gained
Presentation	Early onset severe diarrhea	Early onset severe diarrhea
Extra-intestinal manifestations	- Mild Developmental delay - Cholestasis with pruritus, low Y-Glutamyl transferase and high serum bile acids	- Global developmental delay - Intractable myoclonic seizures - Medullary nephrocalcinosis - Recurrent urinary tract infection
Outcome	Died at 19 months follow- ing H1N1 infection	Relocated to a neighboring coun- try at 17 months and died at 23 months of age

Table II. Variant Table

Gene	Chr	HGVS DNA Ref- erence	HGVS Protein Reference	Variant Type	Predicted Ef- fect (substitu- tion, deletion, etc.)	dbSNP/dbVar ID	Genotype (het- erozygous/ ho- mozygous)	ClinVar ID	Parent of Origin
MYO5B	18	c.G4765T	p.Glu1589*	SNV	Substitution	rs762039116	Homozygous	620180	Both heterozygous

Table III. Sequencing Coverage Table

Sample	Mean Depth of Coverage
MVID-1-Index	78.2
MVID-1-Mother	67.3
MVID-1-Father	54.4
MVID-2-Index	93.3
MVID-2-Mother	64.9
MVID-2-Father	83.1
Average Depth	73.5

Figure Legends

Figure 1. Figure 1. Family Diagrams for the 2 patients identified with MVID. In both cases, the index case (black arrow pointing to the sequenced index) and parents were collected and sequenced (thick boxes/circles). Shown also for family 1 are the 4 previous siblings born with MVID who died perinatally.

Figure 2. Sanger sequencing trace showing the glutamate to stop codon mutation at amino acid position 1598 in the Myo5B gene. This mutation is caused by a mutation from guanine (G) to thymine (T) at position 4765.

Figure 1

Figure 2

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