COLD SPRING HARBOR
Molecular Case Studies

RESEARCH REPORT

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 that could lead to mortality within the first year of life. We identify two 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 in 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. (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, the 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). 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.

RESULTS

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-yr-old, gravida 7, para 3. Her previous three daughters died in Syria at 7, 10, and 30 d of age, following intractable diarrhea of unknown diagnosis.

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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) 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 were unremarkable. Nonetheless, the patient had frequent hospital admissions because of dehydration and sepsis. During her disease, the patient also developed cholestasis with pruritus because of 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. The 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 postendoscopy. In this patient, the histopathology of the duodenal mucosa showed subtotal and total villous atrophy with slightly elongated crypts. There were no granuloma, dysplasia, nor malignancies seen. A vacuolated apical cytoplasm was noted in hematoxylin and eosin (H&E) sections and highlighted by special stains (PAS) that showed cytoplasmic staining of the luminal aspect of enterocytes. Immunohistochemical staining for polyclonal anti-CEA, CD10 stains illustrated microvillus abnormalities including patchy normal band-like brush border with the internalization of brush border in the 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 the *MYO5B* gene. Her clinical course and outcome are summarized in Table 1.

Nine months later, the mother became pregnant. Amniocentesis at 12 wk for genetic testing for the *MYO5B* mutation was positive; the parents chose to abort the baby. Six months later, the mother became pregnant again. Amniocentesis done at 13-wk gestation was negative, and 6 mo 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

Table 1. Demographic and clinical characteristics of the two patients with microvillus inclusion disease (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
Extraintestinal manifestations	Mild developmental delay; cholestasis with pruritus, low γ-glutamyl transferase, and high serum bile acids	Global developmental delay; intractable myoclonic seizures; medullary nephrocalcinosis; recurrent urinary tract infection
Outcome	Died at 19 mo following H1N1 infection	Relocated to a neighboring country at 17 mo and died at 23 mo of age

patient developed severe diarrhea associated with acidosis. Changing the feeds from breast milk to elemental formula (Neocate LCP, SHS) 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 mo, 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-2. Patient 2 also developed cholestasis with pruritis, in which liver enzymes level and serum bile acid levels were elevated most likely because of 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 a patchy distribution of the brush border evident in PAS stain as well as on CD10 (immunohistochemical [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 mo of age, the child died.

DISCUSSION

Geographically, there is a relatively high prevalence of MVID in the Mediterranean region—almost 50% of all the 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 report 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*. Because 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 delays, consistent with previously reported MVID cases (Phillips and Schmitz 1992; Halac et al. 2011). Patient 2 also developed intractable myoclonic seizures that needed three 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 extra-intestinal phenotypes may be iatrogenic or linked to the *MYO5B* mutations, and patient-specific disease models 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

Table 2. Variant table

Gene	Chr	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP/ dbVar ID	Genotype	ClinVar ID	Parent of origin
MYO5B	18	c.G4765T	p.Glu1589*	SNV	Substitution	rs762039116	Homozygous	VCV000620180	Both heterozygous

Table 3. 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

Syrian founder mutation. The utility of identifying causative mutations within a family allows for reliable genetic counseling, prenatal screening, and embryo selection by preimplantation genetic testing.

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 50× (Tables 2 and 3). 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). Candidate variants surviving this filtration were manually evaluated for genes with reasonable implications in disease by the literature and database searches.

Genetic Testing

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

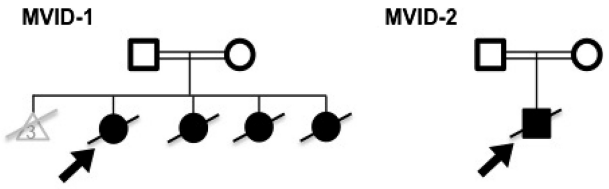

Figure 1. Family diagrams for the two patients identified with microvillus inclusion disease (MVID). In both cases, the index case (black arrow pointing to the sequenced index) and parents were collected and sequenced (thick boxes/circles). Shown also for family 1 are the four previous siblings born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

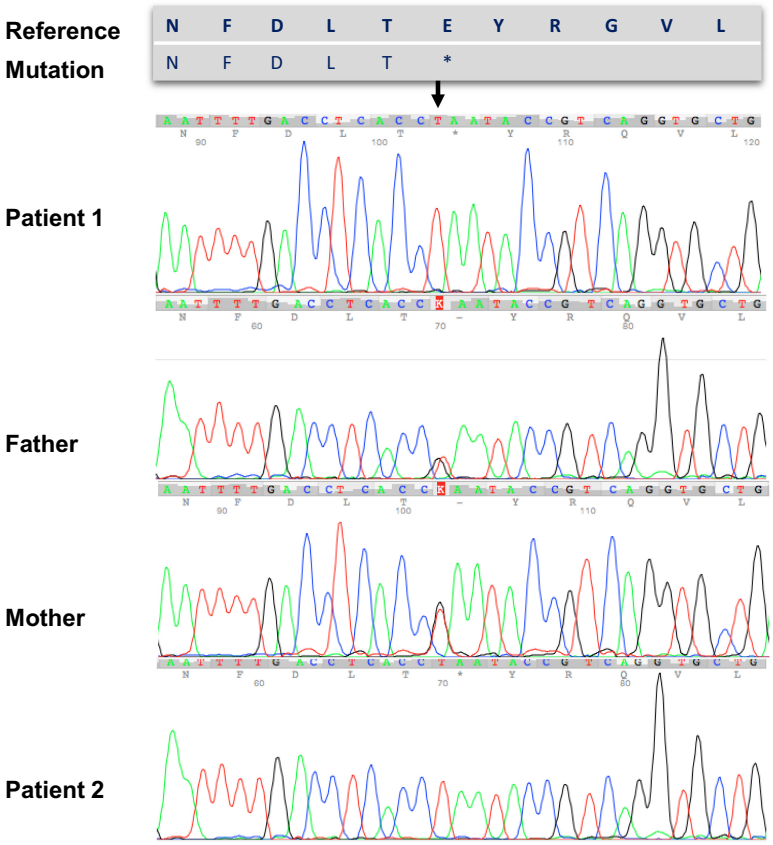

Figure 2. Sanger sequencing trace showing the glutamate to stop codon mutation at amino acid position 1589 in the *MYO5B* gene. This mutation is caused by a mutation from guanine (G) to thymine (T) at position 4765.

ADDITIONAL INFORMATION

Data Deposition and Access

The informed consent signed by the parents protected the privacy of study participants, and such data cannot be made publicly available. The novel variant has been deposited into ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession number VCV000620180.

Ethics Statement

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

Competing Interest Statement

The authors have declared no competing interest.

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