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CASE REPORT

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Two cases of microvillous inclusion disease caused by novel mutations in *MYO5B* gene

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Key Clinical Message

Microvillous inclusion disease (MVID) typically appears with severe chronic diarrhea in the few days after birth and rapidly causes dehydration and metabolic acidosis. In this context, presenting two novel cases, we underline the crucial importance of mutation analysis for the diagnosis of this disease that may be easily misdiagnosed.

KEYWORDS

chronic diarrhea, congenital diarrheal disorders, defects of enterocyte structure, gene, microvillous inclusion disease, mutations

1 | INTRODUCTION

Microvillous inclusion disease (MVID) is a rare severe congenital diarrheal disorder due to the dysfunction of myosin Vb protein encoded by *MYO5B* gene. About 40 different mutations were described so far in patients with sporadic MVID. We describe two patients of Italian origin, bearing novel *MYO5B* mutations. The first one was homozygous for the novel mutation c.505A>G in exon 5 of *MYO5B* gene, and the second was compound heterozygous for the mutations c.1367A>G and c.2700delG (novel). Several bioinformatic tools were concordant on the pathogenicity of such mutations confirming that molecular analysis is a rapid and effective approach to specifically diagnose MVID among the myriad of conditions that cause severe diarrhea in early life.

Microvillous inclusion disease (MVID, OMIM 251850) is a rare congenital diarrheal disorder (CDD) inherited as an autosomal recessive trait.[1,2] It typically presents with severe chronic diarrhea in the few days after birth and rapidly causes dehydration and metabolic acidosis. No additional onset symptoms distinguishable from other CDDs are usually present in MVID patients born via normal delivery

after an uneventful pregnancy with no polyhydramnios.[3] A timely diagnosis is mandatory, and total parenteral nutrition is necessary for most MVID patients, even if it may cause cholestasis and liver failure. The outcome of the disease is usually fatal within 2-3 years[4] due to severe dehydration and electrolyte unbalance, even if intestinal transplantation may improve the outcome.[5] MVID has a better prognosis when the onset occurs within the first 3-4 months of life and there is less severe diarrhea with some residual intestinal functionality, which reduces the need for parenteral nutrition.[2] Forms of MVID with unusual onset have been also reported.[6]

Microvillous inclusion disease is classified within the subgroup of CDDs caused by defects of enterocyte polarization and differentiation[7] that are due to the dysfunction of myosin Vb protein, an actin filament-based motor protein that is involved in endosomal recycling and in cytoskeleton cell polarity determination. Small intestine biopsy shows the atrophy of villi with microvillus inclusion, crypt hyperplasia, and a mild (if any) inflammatory infiltration.[8–10] In fact, the main characteristic of MVID is the loss of the apical brush border and the formation of intracellular microvillus inclusions. The microvillus inclusions are usually observed in approximately 10% of enterocytes at the villus tips, whereas normal brush borders are often present on the enterocytes in the proximal part of the villus.[9,10] Focal or delayed alterations may be present in atypical MVID.[6]

Most patients with early-onset MVID display inactivating mutations in the *MYO5B* gene[11] encoding myosin Vb protein. *MYO5B* works as a dynamic tether for specific RAB-small GTPases (RAB8A, RAB10, and RAB11) maintaining these proteins at their appropriate subapical membrane localization. This interaction is crucial for a normal epithelial cell polarity, apical trafficking, and microvilli growth. Alterations in this interaction, deriving from mutations in *MYO5B*, lead to a mucosa with decreased absorptive pathways and a leaky epithelium at the villus tips, which causes alterations in both intercellular tight junctions and the ion transport pathways necessary for adaptation through trans-cellular pumps and channels.[12]

Once *MYO5B* is identified as the disease gene for MVID, molecular analysis strongly contributes to the unequivocal diagnosis of MVID[11–13] and even prenatal diagnosis.[14,15] A founder mutation was identified among Navajos[16] while about 60 different mutations were described so far in patients from other ethnic groups indicating the strong genetic heterogeneity of the disease. These findings were reviewed in the excellent papers by van der Velde[17] and Dhekne.[18] More recently, the *Syntaxin 3* gene was reported as responsible for an MVID variant.[19]

In the present paper, we describe two cases of MVID bearing novel *MYO5B* mutations and revise the pathogenic role of *MYO5B* mutations described in MVID patients so far.

2 | METHODS

2.1 | Molecular analysis of the *MYO5B* gene

DNA was extracted from an EDTA blood sample with the Nucleon BACC2 kit (GE Healthcare Europe GmbH, Milan, Italy). Then, the DNA was amplified by PCR for all 40 exons of the *MYO5B* gene using primers and conditions available on request. A DHPLC procedure was used to exclude the presence of the novel *MYO5B* mutations in 100 alleles from 50 healthy subjects.

2.2 | Prediction of disease-associated variation

Protein sequence with the different amino acid variations was submitted to the Meta-SNP browser (https://snps.biofold.org/meta-snp/pages/methods.html), which integrates four existing methods for disease association prediction: PANTHER, PhD-SNP, SIFT, and SNAP.[20]

2.3 | Subjects

Case #1. A male infant of Caucasian origin with a history of diarrhea since the first week of life, hypernatremic dehydration, hyperchloremic metabolic acidosis, and failure to thrive as observed in a tertiary center for pediatric gastroenterology. He was born at 36 weeks of gestational age by cesarean delivery, from nonconsanguineous and healthy parents. Pregnancy was complicated by premature birth threats. Family history was negative for early-onset diarrhea. Birth weight was 2820 g. The patient required hospitalization from the first days of life because of the onset of severe watery diarrhea and dehydration associated with metabolic acidosis, hypernatremia, hyperchloremia, and hyperammonemia. At admission, he was 2 months old and he appeared sick, pale, and sleepy, with conserved consciousness. Body temperature, blood pressure, and cardiorespiratory function were within normal range for his age. There was a normal abdominal examination. Auxologic parameters were weight 2940 g, length 50 cm, and CC 36 cm. He assumed total parenteral nutrition since the first days of hospitalization. In order to investigate the etiology of chronic diarrhea, an extensive diagnostic workup was performed, including inflammatory biomarkers, stool microbiology, sweat test, food allergy screening tests, metabolic screening tests, and fecal calprotectin.[21–23] All these tests resulted negative. Mean electrolytes values and fecal osmolarity were as follows: Na+ 62 mEq/L; K+ 39 mEq/L; Cl- 69 mEq/L; Osm 262 mOsm/kg; anion gap 60. Duodenal histology showed intestinal villous atrophy (cod.T3a of Marsh classification modified by Oberhuber). Electron microscopy analysis of the duodenal mucosa described a

TABLE 1 MYO5B gene mutations found in patients with microvillous inclusion disease

Ancestry	G.	Mutations	E/I	Protein	Effect	Ref.
Italian	M	c.505A>G (Hom)	5	p.Lys169Glu	Missense	This work
Italian	M	c.1367A>G	11	p.Asn456Ser	Missense	[13]
		c.2700delG	21	p.Arg900SerfsX4	Frameshift	This work
Algerian Arabic	F	c.866C>A	8	p.Ser289X	Nonsense	[11]
		c.4840C>T	36	p.Qln1614X	Nonsense	
Turkish	M	c.502G>A (Hom)	5	p.Gly168Arg	Missense	[11]
Algerian Arabic	M	c.4667_4668TT>GC (Hom)	35	p.Leu1556Arg	Missense	[11]
Italian	F	c.1202G>A (Hom)	10	p.Arg401His	Missense	[11]
French	F	c.1303G>A	10	p.Gly435Arg	Missense	[11]
		Unknown				
French	M	c.42G>A	2	p.Trp14X	Nonsense	[11]
		c.428C>A	4	p.Ala143Glu	Missense	
Kosovo	M	c.28-2A>G			Splicing	[11]
		c.1202G>A	10	p.Arg401His	Missense	
Portuguese	F	c.1110_1113delTCAG	10	p.Ser370ArgfsX27	Frameshift	[11]
		c.4755_4576dupT	36	p.Asp1586X	Stop codon	
Italian	F	c.2003-2A>G (Hom)	I 16		Splicing	[11]
French	M	c.557C>A	5	p.Ser186X	Nonsense	[11]
		c.1A>G	1	p.Met1?		
Turkish	M	c.947-1G>A (Hom)	I 8		Splicing	[11]
Navajo (9 cases)		c.1979C>T (Hom)	16	p.Pro660Leu	Missense	[16]
Hispanic	M	c.946G>A (Hom)	8	p.Gly316Arg	Missense	[13]
Hispanic	F	c.2330delG (Hom)	19	p.Gly777AspfsX6	Frameshift	[13]
Caucasian	F	c.2245C>T	19	p.Arg749X	Nonsense	[13]
		Unknown				
Polish	F	c.28?_1545+?del	2-12			
		c.1367A>G	11	p.Asn456Ser	Missense	
Moroccan	M	c.4366C>T (Hom)	33	p.Gln1456X	Nonsense	[13]
Dutch	M	c.4460-1G>C	I 33		Splicing	[13]
		c.1540T>C	12	p.Cys514Arg	Missense	
French	F	c.2671C>T	21	p.Q891X	Nonsense	[12]
		Unknown				
Turkish	M	c.656G>A (Hom)	6	p.R219H	Missense	[12]
Irish	M	c.3046C>T	23	p.R1016X	Nonsense	[12]
		Unknown				
Turkish	M	c.5392C>T (Hom)	39	p.R1795X	Nonsense	[12]
Turkish	M	c.1966C>T (Hom)	16	p.R656C	Missense	[12]
Turkish	M	c.1125G>A (Hom)	10	p.W375X	Nonsense	[12]
Turkish	M	c.323T>G (Hom)	4	p.V108G	Missense	[12]
Turkish	M	c.1323-2A>G (Hom)	I 10		Splicing	[12]
Turkish	F	c.1362insAGTTCTGTA (Hom)	11	p.Cys454insKFC	Insertion	[12]
Taiwan	M	c.445C>T	4	p.Gln149X	Nonsense	[14]
		c.1021C>T	9	p.Gln341X	Nonsense	
Caucasian	M	c.1087C>T	10	p.Arg363X	Nonsense	[17]
		Unknown				

(Continues)

TABLE 2 (Continued)

Ancestry	G.	Mutations	E/I	Protein	Effect	Ref.
Turkish	M	c.3163_3165dupCTC	24	p.Leu1055dup	Duplication	[17]
		Unknown				
Dutch	F	c.5616-2A>G	40		Splicing	[17]
		c.1591C>T	13	p.Arg531Trp	Missense	
		c.1856C>T	15	p.Pro619leu	Missense	

E/I, exon/intron, F, female, G, Gender, (Hom), patient homozygous for the indicated mutation, M, male.

submicroscopic pattern characteristic of MVID, characterized by intestinal mucosa with partial villous atrophy, enterocyte-depleted microvillous (which were not well oriented and absent in some sections), several intracytoplasmatic microvillar inclusions, and dense granules.

The clinical history was characterized by watery diarrhea; metabolic acidosis; failure to thrive; relapsing sepsis; bilateral congenital cataract; retinitis pigmentosa; hypopigmentation of skin and hair; recurrent urinary tract infections; delayed psychomotor development. The patient was dependent on total parenteral nutrition. The patient died at the age of 23 months during a severe sepsis with a clinical picture of macrophage activation syndrome (MAS) unsuccessfully treated with cyclosporine and steroids.

Case #2. A male infant of Caucasian origin with a history of diarrhea since the first 10 days of life, metabolic acidosis, and failure to thrive as observed in a tertiary center for pediatric gastroenterology. He was born at 37 weeks of gestational age by cesarean delivery, from nonconsanguineous and healthy parents. The pregnancy was complicated by mild polyhydramnios. Birth weight was 3280 g. His older sister died at one month of age because of severe dehydration, metabolic acidosis, hyperammonemia, and bowel perforation. From the first days of life, he was hospitalized for the onset of severe diarrhea and dehydration associated with metabolic acidosis, hypernatremia, hyperchloremia, and hyperammonemia. At admission, he was 37 days old and he appeared sick, pale and with abdominal distension. Auxologic parameters were: weight 3090 g, length 51 cm, CC 35.8 cm. He assumed total parenteral nutrition from the first days of hospitalization. In order to investigate the etiology of chronic diarrhea, the same extensive diagnostic workup previously described was performed,[21–23] but all tests resulted negative. Mean electrolytes values and fecal osmolarity were as follows: Na+ 94 mEq/L; K+ 16 mEq/L; Cl- 78 mEq/L; Osm 268 mOsm/kg; anion gap 48. Duodenal histology showed villous atrophy (cod. T3a of Marsh classification modified by Oberhuber). Electron microscopy revealed MVID features with villous atrophy, with microvillus inclusion bodies within the cytoplasm of enterocytes with rarefied microvilli and secretory granules.

The clinical history was characterized by metabolic acidosis requiring frequent daily NaHCO3 oral intake; ammonium urate stone urinary excretion; neutrophilic leukocytosis without overt signs of inflammation and/or infection; presence of PAS-positive vacuoles at muscle biopsy; failure to thrive; thin and inelastic skin with prominent cheekbones; and thin lips. The patient did not tolerate nutritional support with minimal enteral feeding and was dependent on total parenteral nutrition.

The patient died at the age of 7 months following a drug-resistant epilepsy with respiratory distress.

All the participants (guardians in the case of minors) provided written informed consent to anonymously use a DNA sample and clinical data for research purposes.

3 | RESULTS

Case #1: Molecular analysis revealed the homozygous mutation c.505A>G in exon 5 of the *MYO5B* gene. To exclude the presence of a heterozygous deletion, we analyzed the parents, which resulted in both being heterozygous for the c.505A>G mutation. The mutation was novel not previously described in MVID patients. It is a missense mutation that causes the change of the lysine (Lys, K) with glutamic acid (Glu, E) at the codon 169 (p.Lys169Glu) (see Table 1).

Case #2: Molecular analysis revealed the heterozygous mutations c.1367A>G and c.2700delG *in trans* (Table 1), as confirmed by the analysis of the parents. The novel mutation c.2700delG changes the arginine (Arg, R) with serine (Ser, S) at codon 900 causing a frameshift from the codon 900 (p.Arg900Serfs*4). The missense mutation c.1367A>G causes the change in the asparagine (Asn, N) with serine (Ser, S) at the codon 456 (p.Asn456Ser). This has already been found in a patient with MVID.[13]

4 | DISCUSSION

Severe chronic diarrhea in the newborn is a serious challenge because it may be a potentially life-threatening condition and because a differential diagnosis is required among a myriad of different conditions.[7] This is particularly true for MVID considering that: (a) a rapid and specific diagnosis is mandatory in order to immediately start the parenteral nutrition, followed, when possible, by intestinal transplantation[3]; (b) the diagnosis of MVID may be complex due to the heterogeneity of the clinical phenotype,[3,4] and histologic diagnosis is invasive and

sometimes challenging[17]; and (c) cases of MVID with atypical presentation were described.[6] In this scenario, molecular genetics may significantly contribute to rapid diagnosis reducing the need for invasive procedures. However, sequencing analysis may identify novel mutation for which several approaches to assess their causative role are required.[24]

In fact, our patient #1 was homozygous for the c.505A>G (p.Lys169Glu) novel mutation, while patient #2 was compound heterozygous for the c.2700delG (p.Arg900Serfs*4), a novel frameshift mutation (thus with a clear pathogenic role), and the c.1367A>G (p.Asn456Ser), already found in a Polish MVID patient who was compound heterozygous for the p.Asn456Ser mutation.[13] Thus, we followed several criteria to define the pathogenic effect of either the p.Lys169Glu or the p.Asn456Ser missense mutations: (a) No other mutations were found in the whole coding regions of the *MYO5B* gene in the patients (including exon-intron boundaries); (b) p.Lys169Glu mutation changed a neutral with an acidic amino acid within the ATP-binding regulatory domain of the protein[11]; (c) both mutations were absent in 100 alleles from 50 healthy subjects from the same ethnic group of the patients (southern Italy) tested by DHPLC; moreover, the novel mutations were not reported in the Exome Variant Server (https://evs.gs.washington.edu/EVS/) (August 2018) and in the 1000 genome browser (https://phase3browser.1000genomes.org/index.html). Both browsers consider more than 8000 subjects; and (d) several tools[20] predicted a high damaging probability of both mutations (Table S1).

The described mutations of our two patients should be added to cases[17] previously described in patients affected by MVID (Table 1), and such list warrants some comments. MVID has a higher frequency among Navajos, where a single mutation (ie, the c.1979C>T) was found in the nine cases described so far,[13,14] due to the high incidence of consanguineous marriages. However, a strong genetic heterogeneity was found in sporadic cases reported so far (Table 1). In fact, considering the MVID patients homozygous for a mutation (mostly born to consanguineous parents) and the patients that were compound heterozygous, causative mutations spread in all coding regions of the gene. Once excluded, of the two affected siblings described by Szperl et al, who were homozygous for the same mutation, only one mutation was found in each MVID patient: The c.1202G>A was found homozygous in a MVID patient of Italian origin and compound heterozygous in a patient from Kosovo. The c.1367A>G was found compound heterozygous in two patients (one Polish and the other Italian described in the present study). All the other mutations were found in single MVID patients. Finally, 62 different mutations (including the c.1979C>T that is peculiar to Navajos) were found in the MVID patients studied so far.[18] Several of these mutations have a clear pathogenic effect (ie, nonsense, splicing, or frameshift), while 24 were missense. We revised 15 of them by using several bioinformatic tools [20] and predicted the pathogenicity (Table S1) in all cases with the exception of the c.1856C>T mutation, which was found in a Dutch patient with MVID that had two other pathogenic mutations (Table 1). Of Course, functional studies are needed to prove their putative pathogenicity.

Noteworthy, among the 33 sporadic cases tested by gene sequencing, two causative mutations were found in 27 cases (81.8%) while only a single mutation was found in six cases, showing a mutation detection rate of 90.9%, which is similar to other sequencing analysis (ie, hemophilia A and cystic fibrosis).[20,25] It is possible that mutations in noncoding regions of the gene, like the promoter or 3'UTR (that are not currently analyzed), would be present in patients bearing undetected mutations in the coding regions of the *MYO5B* gene, as shown for other genetic diseases.[26,27] It is also possible that other genes have a role.[19] Finally, in contrast to other congenital diarrhea, in which molecular analysis may help to predict the outcome of the disease or to guide the therapy,[28] in MVID there is not a clear genotype-phenotype correlation because the prognosis of the disease is strictly dependent on early and effective management, including intestinal transplantation.[2,3,17]

To conclude, our data confirm the relevance of molecular analysis to rapidly diagnose MVID and underline the importance to update the database with novel mutations found in patients to help other laboratories that perform molecular analysis.[17] The absence of the mutation in normal subjects tested with scanning procedures like DHPLC,[29] and the use of prediction tools may contribute to defining the role of novel mutations (mainly missense) for which a pathogenic role may be difficult to establish.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

MC and RL: performed the molecular analysis of MYO5B gene. RBC, MIS, MM, and AG: involved in patient management and data collection. FA: performed bioinformatic prediction. FA and GC: planned the study and wrote the manuscript. All authors critically reviewed the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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