Rapid Publication
Navajo Microvillous Inclusion Disease Is Due to a Mutation in *MYO5B*

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Microvillous Inclusion Disease (MID) is a rare, autosomal recessive gastrointestinal disease of increased frequency among the Navajos. Previous work has shown a deficiency of RAB8 in one Japanese patient, while homozygous mutations in *MYO5B* were found in 7 of 10 mostly Middle Eastern families. We have identified a shared homozygous mutation in *MYO5B* in seven affected Navajos with the expected heterozygosity in five parents. We have developed a simple restriction enzyme based assay that allows for rapid screening for this mutation. © 2008 Wiley-Liss, Inc.

Key words: secretory diarrhea; apical transport; enterocytes; genetic bottlenecks; Ras-associated proteins

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INTRODUCTION

The Navajo, an Athabaskan-speaking tribe of the Southwestern part of the United States, have been through three genetic bottlenecks: (1) the crossing of the Bering strait, later than other Amerinds, shared with other members of the Na-Dene linguistic group (to which Athabaskan belongs), (2) a migration along the Rocky Mountains to the Southwest, and (3) forced internment in a concentration camp at Bosque Redondo [Erickson, 1999]. Similar to other groups that have been through bottlenecks, they have an increased frequency of rare autosomal recessive disorders, among which is microvillous inclusion disease (MID) [Pohl et al., 1999].

MID is a gastrointestinal disorder that causes an intractable secretory diarrhea with usual onset near birth. All the Navajo patients that were involved in our study had the severe form of MID with onset at birth. The diarrhea associated with this form of MID is extremely watery; at birth it is frequently thought to be urine and only later does the realization occur that it is feces. The pathology shows shortened microvilli and villous atrophy with an increased number of secretory granules within enterocytes and membrane-bound inclusions [Phillips and Schmitz, 1992]. Studies of specific transporters have indicated that apical, but not basolateral, membrane transport systems are defective [Michail et al., 1998]. The finding that Rab8 (a small GTP-binding protein involved in localizing apical proteins in intestinal epithelial cells) deficiency in mice causes a pathological picture nearly identical to MID, but only at weaning, further implicated intracellular transport pathways [Sato et al., 2007]. Interestingly, although this group found absent *RAB8* mRNA and protein in one MID patient's biopsy specimen, they did not find mutations in *RAB8* in this or two other patients [Sato et al., 2007].

Recently, mutations in myosin Vb (*MYO5B*) were found in 9 out of 10 separate families with MID-affected members. Most of the patients were homozygous for one of eight different mutations, but of note, only heterozygous mutations were found in two early onset patients while a homozygous mutation was found in one late onset case who is managed with more than 50% enteral nutrition, that

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is, a mild case [Müller et al., 2008]. Thus, mutations in other genes, for example, RAB8, may be causal for MID in some cases. Here we report a mutation in *MYO5B* causing severe, early onset MID in Navajos.

MATERIALS AND METHODS

DNA Extraction

Genomic DNA was extracted from peripheral blood lymphocytes of seven patients and six parents using standard protocols. The diagnosis was established when a peroral, small intestinal biopsy disclosed classic pathological findings of villous atrophy with secretory inclusions. Blood was collected after obtaining informed consent from all participating family members. These human studies were approved by our institutional review board.

Mutation Analysis

Genomic sequences and annotations were obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov. ezproxy1.library.arizona.edu/). Primers for mutation screening were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Amplification of the 249 bp region was carried out using the forward primer 5′- caataac-taccgccacccata-3′ and reverse primer 5′- cctgttccacct-gaacctct-3′ in a 25 µl reaction containing 50–100 ng DNA, 0.2 mM dNTPs, 0.25 µM sense and antisense primers, 2.5 µl 10× BIOLASE PCR buffer, 2.5 µl 25 mM magnesium and 0.25 U Taq DNA polymerase (BIOLASE, http://www.bioline.com). Thermocycling conditions consisted of an initial denaturation step of 95°C for 3 min, followed by 35 cycles of 95°C for 30 sec, 57°C for 30 sec, and 72°C for 45 sec, with a final extension step of 72°C for 6 min. Dideoxy terminator sequencing was performed on both strands at the University of Arizona, Biotechnology facility.

We developed a restriction enzyme based assay for more rapid, and less expensive, genotyping. The above PCR products were digested with *Alu*I restriction endonuclease (New England Biolabs, http://www.neb.com). Affected patients yielded products of 163, 71, and 15 bp, while unaffected yielded products of 178 and 71 bp. Results were visualized on 3% ultrapure agarose gel stained with ethidium bromide.

RESULTS

We sequenced a number of exons of *MYO5B* and found homozygous c.1977C > T (P660L) in exon 16. This substitution of leucine for proline is highly nonconservative; the substitution of the bulky aliphatic for the small, fixed-turn amino acid. This position, as is most of the protein sequence, is highly
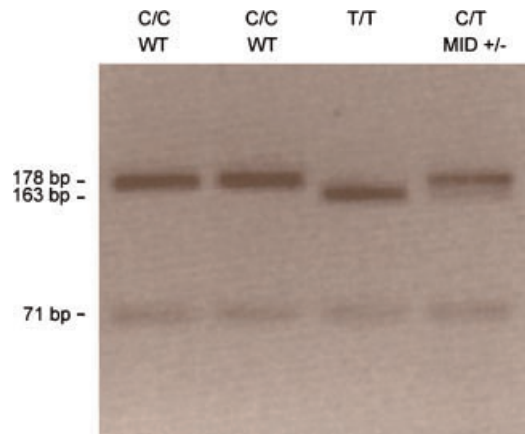

Fig. 1. Electrophoresis of *Alu*I digested PCR products on 3% gel. Lanes 1 and 2, normal; Lane 3, homozygous affected; and Lane 4, heterozygous carrier. Sizes of bands indicated on left.

conserved with both mouse and rat. Not surprisingly, this does not correspond to a SNP in HapMap (HapMap.org). This mutation creates a new *Alu*I site in exon 16, allowing a restriction enzyme assay for the mutation (Figure 1). Using this test, we found homozygosity for this mutation in six more affected samples while five obligate carriers were heterozygous. Eight other unrelated Navajos were negative for the mutation. These 16 chromosomes are insufficient to establish a carrier frequency, but we are limited in our access to Navajo DNA samples.

DISCUSSION

MYO5B was a good candidate for MID since it is required for polarization of hepatocytes by delivery of rab11a—myosin 5b—containing membranes to the apical surface of a hepatic epithelial cultured cell line [Wakabayashi et al., 2005]. It was located in a 17 Mb region of homozygosity in first cousin cases in a Turkish kindred [Müller et al., 2008]. However, only heterozygous mutations were found in two unrelated cases and no mutation was found in a third [Müller et al., 2008]. Thus, other genetic causes of MID are possible. There may be genetic heterogeneity or even digenic inheritance. Of note, despite a similarity in name, MYO5A is a motor protein primarily involved in organelle transport [Shiroguchi and Kinosita, 2007] and defects in it cause Gricelli syndrome Type I [Pastural et al., 1997], a disorder of hypopigmentation (due to melanocyte clumping), hypotonia, and mental retardation [Bahadoran et al., 2003].

Navajo MID patients have very early onset and require total parental nutrition or small bowel transplant to survive. Our finding of homozygous P660L mutation three codons away from the homozygous R656L mutation reported by Müller et al. [2008] in an early onset case requiring bowel transplant may indicate that this region of exon 16

encodes a critical folding domain of the protein. To date, one homozygous mutation each has been found in exons 4, 6, 10, 21, 23, and 39 while two homozygous mutations each have been found in exons 11 and 16. The mutations in exons 21 and 23 were nonsense but were only found as heterozygous changes [Müller et al., 2008]. While a deletion or mutation in the *MYO5B* regulatory region cannot be excluded, this may suggest digenic inheritance with one mutation in *MYO5B* and another in an interacting partner, possibly *RAB11*. Clearly more mutations will need to be found to fully establish the mutational spectrum.

Although there are a number of disorders caused by genetic defects in vesicle-mediated trafficking [reviewed in Ikonen, 2008], only chylomicron retention disease (CMRD; also known as Anderson disease) primarily involves the intestine. CMRD is an autosomal recessive disease of chronic diarrhea with malabsorption, failure to thrive, and hypocholesterolemia due to hypobetalipoproteinemia [Anderson et al., 1961; Roy et al., 1987]. Histology reveals microvacuolization of enterocytes due to retention of chylomicrons and lipid vacuoles in the cytoplasm [Boldrini et al., 2001]. The causative gene, *SARIB,* codes a small GTPase associated with COPII vesicles [Jones et al., 2003]. The COPII (coat protein) carriers are involved in endoplasmic reticulum to Golgi transport [reviewed in Duden, 2003] and SARIB and Sec23 are particularly involved in chylomicron and LDL transport [Shoulders et al., 2004].

While anyone of three bottlenecks could have led to an increased frequency of this mutation in the Navajo, the lack of MID cases among the Apache or Alaska/Northwest Territories Na-Dene speaking tribes would suggest that the Navajo-specific bottleneck was involved. However, there are many more Navajos than members of these other tribes and cases of MID may not yet have been ascertained among the latter. Future research may allow us to estimate the date of occurrence of this founder mutation—the founder mutation for oculocutaneous albinism type 2 among the Navajo is estimated to have originated only 400–1,000 years ago [Yi et al., 2003].

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