Published in final edited form as:
Am J Med Genet A. 2013 March ; 161(3): 473–478. doi:10.1002/ajmg.a.35736.

Novel *FREM1* Mutations Expand the Phenotypic Spectrum Associated with Manitoba-Oculo-Tricho-Anal (MOTA) Syndrome and Bifid Nose Renal Agenesis Anorectal Malformations (BNAR) Syndrome

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Abstract

Loss of function mutations in *FREM1* have been demonstrated in Manitoba-oculo-tricho-anal (MOTA) syndrome and Bifid Nose Renal Agenesis and Anorectal malformations (BNAR) syndrome, but the wider phenotypic spectrum that is associated with *FREM1* mutations remains to be defined. We screened three probands with phenotypic features of MOTA syndrome. In one severely affected infant who was diagnosed with MOTA syndrome because of bilateral eyelid colobomas, a bifid nasal tip, hydrometrocolpos and vaginal atresia, we found two nonsense mutations that likely result in complete loss of *FREM1* function. This infant also had renal dysplasia, a finding more consistent with BNAR syndrome. Another male who was homozygous for a novel stop mutation had a extensive eyelid colobomas, corneopalpebral synechiae and unilateral renal agenesis. A third male child diagnosed with MOTA syndrome because of corneopalpebral synechiae and eyelid colobomas had a homozygous splice site mutation in *FREM1*. These cases illustrate that disruption of the *FREM1* gene can produce a spectrum of clinical manifestations encompassing the previously described MOTA and BNAR syndromes, and that features of both syndromes may be seen in the same individual. The phenotype of FREM1-related disorders is thus more pleiotropic than for MOTA and BNAR syndrome alone and more closely resembles the widespread clinical involvement seen with Fraser syndrome. Moreover, our first case demonstrates that vaginal atresia may be a feature of FREM1-related disorders.

Keywords

Manitoba-oculo-tricho-anal (MOTA) syndrome; Bifid nose renal agenesis anorectal malformations (BNAR) syndrome; *FREM1*; vaginal atresia

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INTRODUCTION

The extracellular matrix (ECM) contains a specialized basement membrane (BM) that underlies layers of epithelial and endothelial cells. Disruption of BM assembly can compromise normal morphogenesis and elicit a variety of developmental defects and diseases [for reviews, see Yurchenco and Patton, 2009; Van Agtmael and Bruckner-Tuderman, 2010; Wiradjaja et al., 2010]. The FRAS/FREM complex, comprising the secreted proteins FRAS1, FREM1 and FREM2, is critical for the normal adherence of the BM to the epidermis during embryogenesis [Short et al., 2007; Pavlakis et al., 2011]. In humans, failure of normal FRAS/FREM complex formation causes several multiple congenital anomaly syndromes – Fraser syndrome, Manitoba-oculo-tricho-anal (MOTA) syndrome and bifid nose renal agenesis and anorectal malformations (BNAR) syndrome.

Fraser syndrome [OMIM 219000] is a pleiotropic condition that encompasses malformations in almost all body systems, including the eye, urogenital tract, heart, lungs, gastrointestinal tract and brain [Slavotinek and Tifft, 2002; Van Haelst et al., 2007]. Fraser syndrome is caused by mutations in *FRAS1* and *FREM2* in approximately 50% of cases [Van Haelst et al., 2008]; mutations in *GRIP1*, a FRAS1-interacting protein, have also recently been described [Vogel et al., 2012]. MOTA syndrome [OMIM 248450] is a milder condition that has substantial phenotypic overlap with Fraser syndrome and is caused by loss of function mutations in *FREM1* [Li et al., 2011; Slavotinek et al., 2011; Mateo et al., 2012]. Homozygous deleterious sequence alterations in *FREM1* can also result in BNAR syndrome [OMIM 608980] [Alazami et al., 2009], a condition that overlaps with MOTA syndrome because of shared craniofacial malformations and anal defects. Finally, heterozygous mutations of *FREM1* have been found to be associated with isolated metopic cranioysnostosis [Vissers et al., 2011]. Although all of the gene mutations in these conditions are predicted to disrupt FRAS/FREM complex stability and alter basement membrane adherence, the causes of phenotypic variability associated with mutations in each gene remains unclear and the full extent of the phenotypic findings associated with *FREM1* loss-of-function has yet to be determined. Here, we present four individuals diagnosed with MOTA syndrome who had novel mutations within *FREM1* that highlight the phenotypic spectrum seen with disruption of the gene and illustrate that features of MOTA and BNAR may be seen in the same patient.

CLINICAL REPORTS

In the first proband, born to healthy nonconsanguineous Filipino parents (Table I, Patient 1; Fig. 1), ultrasonography performed at 33 weeks gestation due to size-dates discrepancy revealed oligohydramnios and a large cystic abdominal mass. Fetal magnetic resonance imaging (MRI) demonstrated that this “mass” consisted of a markedly distended vagina and fluid-filled uterus. Additional prenatal imaging findings included bilaterally dilated ureters and pelvicaliectasis, a severely decompressed bladder, dysplastic changes within both kidneys and a small chest with low lung volumes. No other major structural abnormalities were identified. The infant was born via elective cesarean at 34 1/7 weeks gestation with APGARs of 4 at 1 minute and 7 at 5 minutes. She weighed 2390 g (50^{th} centile) at birth. Physical exam was notable for bilateral upper eyelid colobomas that included the medial third of the palpebral fissure, hypertelorism, prominent epicanthic folds and a bifid nasal tip (Fig. 1). Both eyes had bilateral epibulbar lesions lateral to the cornea. The palate was high. The anus was small and anteriorly placed. She had hydrometrocolpos and vaginal atresia. Respiratory distress was present immediately after birth, and progressively worsened over a period of several hours, due to pulmonary hypoplasia secondary to oligohydramnios and poor lung development resulting from an early ureteral obstruction sequence. A decision

was made with the family to pursue supportive care options, and the infant succumbed to respiratory failure from pulmonary hypoplasia at several hours of life.

An autopsy was performed, which demonstrated a large pelvic mass underneath the umbilical vein that extended to the liver edge. The pelvic mass was caused by hydrometrocolpos. The dilated uterus and cervix contained 200 mL of watery and mucinous fluid. Examination of the perineum showed a cystic structure at the introitus without a palpable vaginal canal, and a transverse urogenital septum. Sectioning of the kidneys showed pelvicaliectasis with ureteral hypertrophy and fibrosis. The remainder of the urinary tract was compressed, but patent. There was pulmonary hypoplasia, with a combined lung weight of 19.81 g (expected mean weight for 25 weeks gestation; normal weight for 28–36 weeks gestation, 44.91g +/− 19.34 g; De Paepe et al., 2005). The gastrointestinal tract was patent, and there were no anterior abdominal wall defects. The anus appeared small and anteriorly placed, but was patent. Megalencephaly was present upon examination of the brain. Single-nucleotide polymorphism (SNP) microarray analysis, carried out using the Omni1 BeadChip (Illumina, San Diego, CA) was unremarkable.

The second proband (Patient 2, Table I; Figs. 2A and 2B) was born to healthy, second cousin, Uzbekistani parents. This male infant had ocular hypertelorism, corneopalpebral synechiae and upper eyelid colobomas. The nose was wide, with a bifid nasal tip. The anterior hairline extended from the temple regions bilaterally and the lateral margins of the eyebrows were sparse on both sides. He also has a pilonidal dimple. His older sister (Patient 3, Table I) had a similar bifid nasal tip and a depressed and hypoplastic columella but was without eye findings. Both children were otherwise healthy and had normal development.

The last proband (Patient 4, Table I; Fig. 3) is a 1-year 3-month-old male born to first cousins of British Pakistani heritage. There were no problems in the pregnancy. He was noted to have structural eye anomalies at birth with the eyelids fused to the cornea. He had large, bilateral upper eyelid colobomas with corneopalpebral synechiae, a broad, flattened and deficient nasal tip and bilateral, wedge-shaped extensions of the anterior hairline from the temple region to each ipsilateral eye. Examination of the hands, feet, genitalia and anus were normal. There was unilateral renal agenesis with normal renal function. He had a small atrial septal defect. Growth parameters at 6 months of age were length 69 cm (50th–75th centile), weight 7.5 kg (25th – 50th centile) and occipital-frontal-circumference (OFC) 45cm (91st centile), and at 1 year 3 months, OFC was 48.2 cm (25–50th centile), height 81.2 cm (75–91st centile) and weight 10.15 kg (25–50th centile). He was able to sit with support at 6 months and was taking steps with help at 1 year 3 months. His vision was limited to light appreciation. His mother was pleased with his general development and he had 10 words at age 15 months.

METHODS

We performed Sanger sequencing for the 37 coding exons of *FREM1* with polymerase chain reaction (PCR) using GoTaq® polymerase master mix (Promega, Madison WI) and betaine (20% v:v; Sigma-Aldrich, St Louis, MO). PCR products were treated with Shrimp Alkaline Phosphatase (Affymetrix/USB, Santa Clara, CA) and Exonuclease I (Fermentas, Inc., Glen Burnie, MD) prior to sequencing with appropriate primers. Mutation analysis was done with the computer software Sequencher (GeneCodes Corporation, Ann Arbor, MI). MutationTaster [Schwarz et al. 2010] was used to predict the effect of the mutations and the database of Single Nucleotide Polymorphisms (dbSNP; http://www.ncbi.nlm.nih.gov/projects/SNP/) and the Exome variant server (http://evs.gs.washington.edu/EVS/) were used to determine if the sequence alterations were present in unaffected individuals.

RESULTS

A summary of the sequencing results for these patients can be found in Table I. The first proband had two nonsense mutations in *FREM1* - c.2148G>T, predicting p.Glu717X (See Supporting Information online eFig. S1. A) in exon 13 inherited from her mother and c. 3820G>T, predicting p.Glu1274X (See Supporting Information online eFig Fig. S1. B) in exon 22 that was inherited from her father (numbering according to transcript FREM1-202 ENST0000042223 with A from ATG = 1; http://www.ensembl.org/index.html). Both of these mutations were predicted to be deleterious because of nonsense-mediated decay and were not listed in dbSNP or the Exome variant server (data not shown). This is the first report of an individual with compound heterozygosity for two nonsense mutations in *FREM1* and the predicted total loss of function is consistent with the severe phenotype.

The second proband and his sister were both homozygous for the deletion of a single G nucleotide from a pair of two G nucleotides situated at the last base pair of intron 35 and the first base pair of exon 36 at chr9:14,746,466–14,746,467 (See Supporting Information online eFig S1. C; Genome Build hg19), c.6139delG, predicting p.Asp2047Met*fs*X21. The deletion was also predicted to abolish the splice acceptor site [Mutation Taster; Schwarz et al., 2010]. Both parents were heterozygous for the same mutation (See Supporting Information online eFig S1. C).

The fourth patient was homozygous for a further novel nonsense mutation, c.5648C>G, predicting p.Ser1883X in exon 32 of *FREM1* (See Supporting Information online eFig S1. D) and loss of function due to nonsense-mediated decay. Sequencing of the mother revealed that she was a heterozygote, but no sample was available from the father. There were no entries for this mutation in dbSNP or the Exome variant server (data not shown).

DISCUSSION

We have reported on a neonate with classical findings of MOTA syndrome, including a bifid and depressed nasal tip, upper eyelid colobomas, hypertelorism, and an anteriorly placed anus with small opening. This child was found to have two novel nonsense mutations on different alleles of *FREM1*, p.Glu717X and p.Glu1274X. These two mutations occur relatively early in the protein and both are expected to result in nonsense-mediated decay and complete insufficiency for *FREM1*.

The infant also had hydrometrocolpos that was caused by vaginal atresia. Vaginal atresia has previously been reported as a manifestation of MOTA syndrome in one other female patient who was homozygous for a frameshift mutation in *FREM1*, p.Lys699Asn*fs*X10 [Fryns, 2001; Slavotinek et al., 2011; Patient 7]. This individual did not manifest hydrometrocolpos, but showed numerous dysmorphic features including bilateral upper eyelid colobomas, a high forehead with a frontal upsweep of hair, maxillary hypoplasia, small nasal alae with nasal colobomas and a bifid nasal tip, a short philtrum, a thin upper lip, and relative microstomia [Fryns, 2001].

In the first proband, the characteristic findings of MOTA syndrome led to this diagnosis, although Fraser syndrome could also have been considered. However, the presence of bilateral epibulbar dermoids also led to consideration of Goldenhar syndrome, although this diagnosis was considered less likely because of the eyelid and nasal findings. The first proband also had ureteral obstruction sequence leading to cystic renal dysplasia, a complication not noted to date in individuals with MOTA syndrome but recognized as a manifestation of BNAR syndrome [Alazami et al., 2009]. It is likely that ascertainment bias complicated the initial delineation of both MOTA and BNAR and the presence of findings from both conditions argues for a single, more inclusive syndrome name for these ‘FREM1-

related' disorders [Slavotinek et al., 2011]. The phenotype of the 'FREM1-related' disorders is thus more pleiotropic than for MOTA and BNAR syndromes alone and more closely resembles the widespread clinical involvement seen with Fraser syndrome.

In a sib pair also diagnosed with MOTA syndrome, we detected homozygosity for a single nucleotide deletion predicted to result in abolition of the exon 36 splice acceptor site and a frameshift. The proband from this family had eyelid colobomas and corneopalpebral synechiae, a combination of anomalies that has been interpreted as a form of abortive cryptophthalmos [Nouby, 2002; Li et al., 2011]. No other anomalies besides the bifid nasal tip and aberrant hairline were apparent. His sister was more mildly affected with reportedly normal ocular structures and a mildly grooved nasal tip. This family illustrates the previously observed intrafamilial variability that can be present with identical *FREM1* mutations [Slavotinek et al., 2011; Families 1 and 2].

The third proband was found to be homozygous for a novel nonsense mutation, p.Ser1883X, in exon 32 of *FREM1* (Fig. S1. D.). FREM1 has two isoforms – a longer isoform 1 (NM_144966.5 → NP_659403.4) encoding a 2179 amino acid protein and a short isoform 2 (NM_001177704.1 → NP_001171175.1), encoding a 715 amino acid protein that has a different N-terminus. A phenotype genotype correlation has not been established for *FREM1* mutations and the number of reported mutations is still low. Although we first hypothesized that a milder phenotype may result if one FREM1 isoform was unaffected, similar to the situation in the second proband and his sister, the data do not support this and it is possible that modifying factors unrelated to genotype may influence phenotype.

In the patients in this report, craniofacial malformations with upper eyelid coloboma, corneopalpebral synechiae, aberrant temporal hairlines and bifid nasal tip were most frequent and it is possible that this combination of findings provide strong indication that a *FREM1* mutation is likely, although not invariable, in view of *FREM1* mutation-negative patients [Yeung et al., 2009].

There has been no obvious mutation 'hot spot' to date, although in the protein, the chondroitin sulfate proteoglycan (CSPG) repeats, cadherin repeat-like repetitive units originally identified in NG2, a cell-surface chondroitin sulfate proteoglycan [Kiyozumi et al., 2007], have frequently been affected. These repeats may be responsible for targeting the protein to the basement membrane, with disruption therefore predicted to significantly impair FRAS/FREM complex formation [Kiyozumi et al., 2006; Kiyozumi et al., 2007].

Finally, none of the parents were known to show signs of an abnormal metopic suture [Vissers et al., 2011] despite heterozygosity for *FREM1* loss of function mutations. The frequency of craniosynostosis with a bifid nasal tip in FREM1-related conditions has yet to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Anne Slavotinek was funded by a K08 grant K08HD053476-01A1 from The Eunice Kennedy Shriver National Institute of Child Health, National Institutes of Health.

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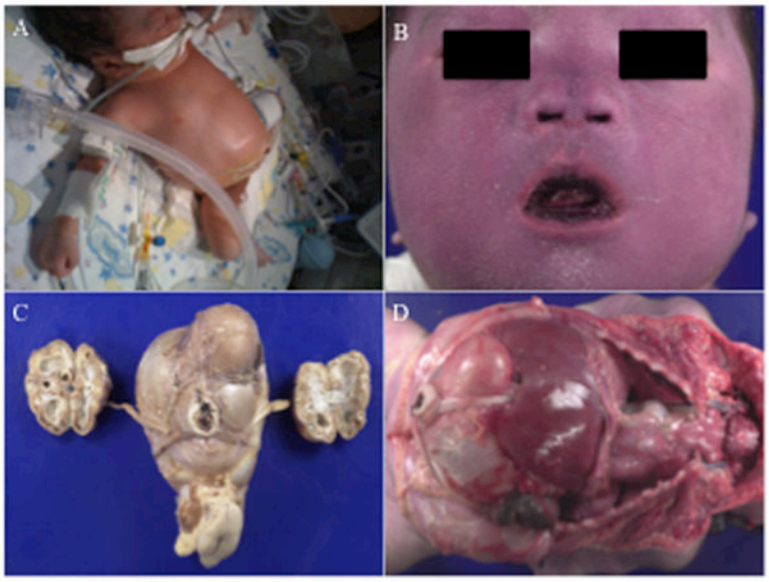

Fig. 1.

Fig. 1A. View of the abdomen of the first proband in the neonatal period, showing gross abdominal distension caused by hydrometrocolpos.

Fig. 1B. Facial view of the first proband at autopsy, showing a bifid nose.

Fig. 1C. Dissection of the abdomen of the first proband at autopsy, showing renal cystic dysplasia with dilatation of the internal female genitalia.

Fig. 1D. Dissection of the abdomen of the first proband at autopsy showed severe pulmonary hypoplasia secondary to early ureteral obstruction sequence.

Fig. 2.

Fig. 2A. Facial view of the second proband, showing colobomas affecting the medial third of the upper eyelid, aberrant anterior hairlines, grooved nasal tip and corneopalpebral synechiae between the upper eyelid and cornea.

Fig. 2B. Facial view of the second proband following surgery.

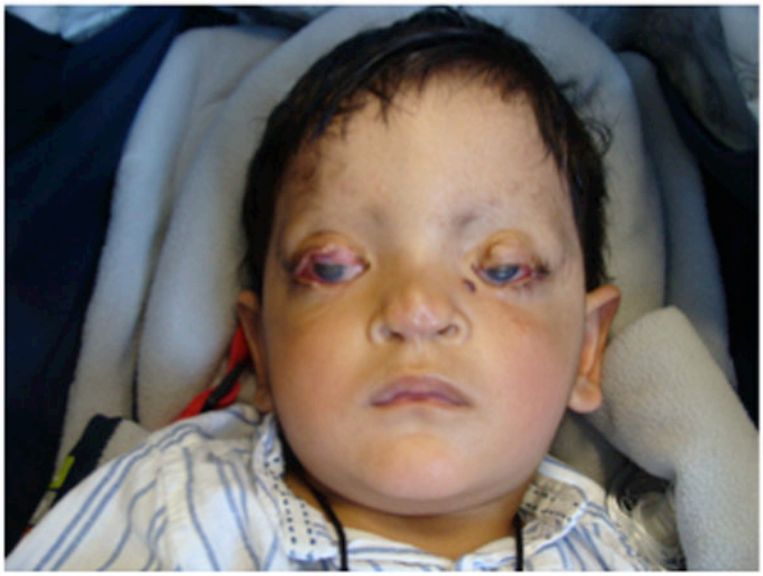

Fig. 3.
Facial view of the third proband showing extensive upper eyelid colobomas, aberrant anterior hairlines and a flattened and bifid nasal tip.

Table I

Phenotypic features of patients with MOTA syndrome and BNAR syndrome with published *FREM1* mutations

	Case 1	Case 2	Case 3	Case 4	MOTA patients with *FREM1* mutations[a]	BNAR patients with *FREM1* mutations[b]	BNAR patients with *FREM1* mutations[c]
Cranial findings							
Metopic synostosis	−	−	−	−	−	−	U[d]
Eye findings							
Corneopalpebral synechiae	−	+	−	+	0/8	0/4	U
Anophthalmia/microphthalmia	−	−	−	−	1/8	0/4	U
Eyelid coloboma(s)	+	+	−	+	2/8	0/4	U
Aberrant hairline	−	+	−	+	6/8	0/4	U
Hypertelorism	+	+	−	+	3/8	0/4	U
Nasal findings							
Bifid nasal tip	+	+	+	+	7/8	4/4	5/5
GIT findings							
Omphalocele	−	−	−	−	3/8	0/4	U
Anterior anus	+	−	−	−	2/8	3/3	0/5
Anal stenosis	+/−	−	−	−	2/8	3/3	0/5
Rectal atresia/fistula[e]	−	−	−	−	0/8	1/4	0/5
GUT findings							
Renal cystic dysplasia	−	U	U	−	0/8	U	U
Renal agenesis	−	U	U	+	0/8	4/4	2/5
Renal pelviectasis	+	U	U	−	1/8	U	U
Vaginal atresia	+	NA[f]	−	NA	1/6	0/2	U
Development	NA	U	Normal	?mild delays	Normal in 7/8	Normal in 3/3	U
FREM1 mutation(s)	p.Glu717X p.Glu1274X	c.6139delG p.Asp2047 Met*fs*X21 (HZ)[g]	c.6139delG p.Asp2047 Met*fs*X21 (HZ)	p.Ser1883X(HZ)	Various	c.2721delG p.Val908 Ser*fs*X17	Various

[a] from Slavotinek et al., 2011, 8 cases;

[b] from Al-Gazali et al., 2002, 4 cases;

[c] Alazami et al., 2009, 5 cases;

[d] Unknown;

[e] Rectal atresia with rectovaginal fistula;

[f] Not Applicable;

[g] Homozygous.