 American Journal of Medical Genetics Part A 140A:1909–1914 (2006)

Mutation Analysis of the *FRAS1* Gene Demonstrates New Mutations in a Propositus With Fraser Syndrome

A. Slavotinek,[1]* C. Li,[2] E.H. Sherr,[3] and A.E. Chudley[2]

[1]Department of Pediatrics, Division of Clinical Genetics, University of California, San Francisco, California
[2]Department of Pediatrics and Child Health, Section Genetics and Metabolism, University of Manitoba, Winnipeg, Manitoba, Canada
[3]Department of Neurology, University of California, San Francisco, California

Received 17 March 2006; Accepted 19 June 2006

Fraser syndrome (OMIM 219000) is a rare, autosomal recessive condition with classical features of cryptophthalmos, syndactyly, ambiguous genitalia, laryngeal, and genitourinary malformations, oral clefting and mental retardation. Mutations causing loss of function of the *FRAS1* gene have been demonstrated in five patients with Fraser syndrome. However, no phenotype–genotype correlation was established and there was evidence for genetic heterogeneity. Fraser syndrome is rare and the *FRAS1* gene has 75 exons, complicating mutation screening in affected patients. We have screened two patients who fulfilled the diagnostic criteria for Fraser syndrome and three patients with related phenotypes (two patients with Manitoba oculotrichoanal syndrome and one patient with unilateral cryptophthalmos and labial fusion) for mutations in *FRAS1* to increase the molecular genetic data in patients with Fraser syndrome and related conditions. We report two new mutations in a patient with Fraser syndrome, a frameshift mutation and a deletion of two amino acids that we consider pathogenic as both alter the NG2-like domain of the protein. Although we are still unable to clarify a phenotype–genotype relationship in Fraser syndrome, our data add to the list of mutations associated with this syndrome.

Key words: Fraser syndrome; cryptophthalmos; MOTA syndrome; blebbed mutant

How to cite this article: Slavotinek A, Li C, Sherr EH, Chudley AE. 2006. Mutation analysis of the *FRAS1* gene demonstrates new mutations in a propositus with Fraser syndrome. Am J Med Genet Part A 140A:1909–1914.

INTRODUCTION

Fraser syndrome (FRS) is a rare, pleiotropic multiple congenital anomaly syndrome with major diagnostic features comprising cryptophthalmos (CO), syndactyly and ambiguous genitalia [Thomas et al., 1986; Gattuso et al., 1987; Boyd et al., 1988; Slavotinek and Tifft, 2002]. The *FRAS1* gene was cloned following the identification of a 1.5 cM region of autozygosity in a consanguineous family with FRS at chromosome 4q21 [McGregor et al., 2003] *FRAS1* encodes a protein that is widely expressed with a predicted length of 4007 amino acids and has sequence similarity to genes encoding a set of extracellular matrix (ECM) proteins [McGregor et al., 2003]. Homozygous mutations (four nonsense mutations and one frameshift mutation) resulting in premature protein termination and that were predicted to cause loss of function have been reported in five FRS patients mapped to 4q21 [McGregor et al., 2003], but there was no evidence of a phenotype–genotype correlation. A missense mutation, p.E1972K, was later demonstrated in two patients in *FREM2* that encodes a structurally similar protein to *FRAS1* [Jadeja et al., 2005]

Several phenotypes overlap with FRS but do not fulfill the diagnostic criteria. For example, six Manitoba Indian children were reported with unilateral eye anomalies including anophthalmia, upper eyelid colobomata, hypertelorism, and oculopalpebral synechia (Manitoba oculotrichoanal [MOTA] syndrome; OMIM 248450) [Marles et al., 1992] Additional findings in this variable condition were a low anterolateral hairline with a wedge of scalp hair extending from the frontotemporal region to the eyebrow (similar to the low anterior hairline documented in FRS), a grooved nasal tip, obstruction

Grant sponsor: REAC36248-524269; Grant sponsor: National Center for Research Resources; Grant number: 5 M01 RR-01271; Grant sponsor: U.S. Public Health Service.
*Correspondence to: A. Slavotinek, Department of Pediatrics, Division of Clinical Genetics, University of California, 533 Parnassus St., Room U585P, San Francisco CA 94143-0748. E-mail: slavotia@peds.ucsf.edu
DOI 10.1002/ajmg.a.31399

of the nasolacrimal ducts, omphalocele, anterior positioning of the anus, and anal stenosis requiring dilatation. Growth, development, and chromosome analysis have all been unremarkable in MOTA syndrome and the cause of this condition is unknown. The inheritance pattern is autosomal recessive because all six patients (three boys and three girls) were from four related families and several patients had consanguineous parents.

FRS is genetically heterogeneous, but the results of mutation analyses in the *FRAS1* and *FREM2* genes have so far been confined to the original papers because of the rarity of this condition and the large size of the genes. We chose to sequence *FRAS1* in five probands, two with FRS, two with MOTA syndrome, and one patient with microphthalmia and labial fusion, to attempt to identify further mutations in this gene. We now report two new mutations in the *FRAS1* gene in a patient with FRS.

CLINICAL REPORTS

The clinical features of the patients are summarized and compared to the published incidence of phenotypic features in FRS in Table I. The first patient was born to a healthy Caucasian couple

TABLE I. Clinical Features of Patients With Fraser Syndrome and Related Phenotypes

Clinical features	Case 1	Case 2	Case 3	Case 4		
Diagnosis	FRS	FRS	MOTA	MOTA	Case 5	FRS[a]
Ophthalmology						
Cryptophthalmos	+	+				88%
Anophthalmia		+				6%
Microphthalmia					+	21%
Eyelid coloboma			+	+		18%
Hypertelorism				+		21%
Digital features						
Cutaneous syndactyly						62%
Genital features						
Bicornuate uterus	+					9%
Fusion of labia					+	9%
Small penis	N/A	N/A				15%
Otolaryngeal features						
Laryngeal stenosis/webs	+	+				31%
Hearing impairment		+				6%
Inner ear dysplasia	+					Rare
Microtia/dysplastic ears		+				16%/54%
Orofacial clefting						
Cleft lip and palate		+				4%
Dysmorphology						
Anterior tongue of hair	+	+		+		34%
Nasal hypoplasia		+				13%
Grooved/bifid nasal tip			+	+		15%
Facial asymmetry	+					Rare
Low-set umbilicus						11%
Urinary tract features						
Renal agenesis						45%
Hydronephrosis	+					Rare
Bladder hypoplasia						17%
Gastrointestinal features						
Omphalocele			+	+		8%
Anal stenosis				+		Unknown
Pulmonary features						
CAML						Unknown
Nervous system features						
Microcephaly					+	Unknown
Agenesis corpus callosum		+				Unknown
Gyral abnormalities		+				Rare
Seizures		+				Rare
Skeletal features						
Craniosynostosis		+				Rare
Talipes						9%
Height and weight	Normal	<3rd	Normal	Normal	Normal	Variable
Developmental delay	None	Severe	None	None	Mild	Variable
Karyotype			46,XX	46, XY		Normal
Survival	A 2 years	A 13 years	A 15 years	A 2 years	A 2 years	N/A

CAML, cystadenomatoid malformation of the lung; N/A, not applicable; A, alive.
[a]Taken from Slavotinek and Tifft [2002].

following a normal pregnancy. She had unilateral cryptophthalmos with a tongue of hair extending from the anterior scalp hairline to the eyebrow (Fig. 1), asymmetry of the nares, stridor with minor webbing of the vocal cords, hearing impairment, hydronephrosis, and a bicornuate uterus. There was no syndactyly and her development at age 1 year was apparently normal.

The second patient (Fig. 2) was born to a 14-year-old mother at 42 weeks of gestation. There was no known history of consanguinity or exposures during the pregnancy. Birthweight was 3,685 g and length was 20.5 inches (both measurements appropriate for gestational age). The baby had cryptophthalmos with small and fused palpebral fissures bilaterally, bilateral anophthalmia and bilateral cleft lip and palate. There was an extension of the anterior hairline across the lateral forehead and the nares were hypoplastic with a groove to the left of the midline of the nasal tip and a right preauricular pit. Examination of the extremities and genitalia were normal. A magnetic resonance imaging (MRI) scan showed absence of the corpus callosum with abnormal folding of the gyri, partial fusion of the thalami, dysplasia of the hippocampi and lateral ventricles, and prominent caudate heads. Abdominal and renal ultrasound scans and an echocardiogram were normal. Her medical history has included craniosynostosis with fusion of the right coronal suture and both lambdoid sutures, a 25–30 dB conductive hearing loss and generalized tonic clonic seizures from 10 years of age requiring medications. At 13 years of age, she had severe developmental delay and was able to walk with support but had no purposeful language. Her weight was 26.3 kg (<3rd centile; 50th centile for 8 to 9 years) and height was 120 cm (<3rd centile; 50th centile for 8 years).

FIG. 1. The facial appearance of the first patient showed unilateral cryptophthalmos. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

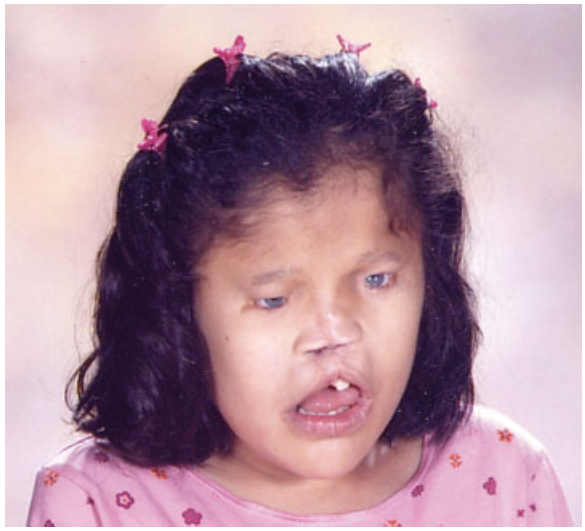

FIG. 2. The facial appearance of the second patient showed facial asymmetry, nasal hypoplasia, and repaired cleft lip. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The third and fourth patients had MOTA syndrome. These two patients are full sibs. The fourth patient was a female, the first patient to be born in a sibship of five. She was noted to have ocular hypertelorism, a grooved nasal tip, and a small omphalocele. There was no aberrant anterior hairline, no coloboma of the eyelids, no anal anomalies, and no syndactyly. Her younger brother had a wedge-shaped anterior hairline extending into the eyebrows, ocular hypertelorism, a grooved nasal tip, an omphalocele, and anal stenosis. The remaining of the examination was normal in both siblings. Both patients were born at term after uneventful pregnancies. At the age of 15 and 2 years, both have developed normally in regard to their cognitive, motor and social adaptive abilities and have normal vision and hearing. Abdominal ultrasound, cranial ultrasounds, cranial radiographs, and karyotypes have all been normal.

The fifth patient was a 15-month-old female who had unilateral microphthalmia, microcephaly, and fusion of the labia minora treated by estrogen creams. A developmental assessment at 11 months of age showed gross and fine motor development compatible with 6 months of age and language and social/emotional skills ranging from 7 to 9 months of age. A pelvic ultrasound scan showed a normal uterus but ovaries were not clearly identified. Her examination showed microcephaly with head circumference of 42.3 cm (<5th centile) but her height and weight were within the normal range. There was a high anterior hairline and a tall forehead with relative prominence of the metopic suture. Her left palpebral fissure was small and contained an ocular prosthesis. The palate was high and narrow and there was a wide diastema between the first and second upper incisors. The external genitalia were normal.

TABLE II. *FRAS1* Mutations in Fraser Syndrome

Mutation	Exon	Predicted effect on protein	Protein domain	Reference
p.Q1266X	29	Truncation Aa1266	NG2-like	McGregor et al. [2003]
p.S1423X	31	Truncation Aa1423	NG2-like	McGregor et al. [2003]
c.5446delTCTTTC	40	Deletion Aa1816-1817	NG2-like	This paper
c.5605insT	41	Premature truncation	NG2-like	McGregor et al. [2003]
c.6992insGG	49	Truncation Aa 2336	NG2-like	This paper
p.Q2863X	58	Truncation Aa2863	CALX-β	McGregor et al. [2003]
p.Q3000X	61	Truncation Aa3000	CALX-β	McGregor et al. [2003]

Aa, amino acid.

Her hands showed mildly tapering fingers with minor hypoplasia of the toenails and fifth finger clinodactyly.

MATERIALS AND METHODS

Consent was obtained for all samples using a protocol approved by the Committee for Human Research at the University of California, San Francisco (H41842-22157-03A). DNA was obtained from peripheral blood lymphocytes using standard methods (Qiagen, Valencia, CA). The genomic sequence of *FRAS1* was obtained from GenBank and primers were designed using Vector NTI software. The 75 exons of *FRAS1* were sequenced using 71 amplicons in all probands. ABI BigDye v3.1 terminator sequencing chemistries were used for the sequencing reactions and the products were separated using a fluorescent sequencing analyzer (ABI PRISM 3700). The ABI sequence analysis version 3.7 program was used for basecalling and the sequences were read by Sequencher 4.1.4 software (Gene Codes Corporation, Ann Arbor, MI). The first two sequence alterations were verified as mutations by sequencing more than 180 control chromosomes from CEPH families.

RESULTS

We have summarized the detected mutations together with previously reported mutations in *FRAS1* in Table II. We found three new sequence alterations in the *FRAS1* gene in the first patient. The first alteration was c.5446delTTCTCT in exon 40 (Fig. 3A) and was inherited from her mother (data not shown). This sequence change results in the deletion of two amino acids from the predicted *FRAS1* protein, F at residue 1,816 and S at residue 1,817. The second alteration was a two base pair insertion, c.6992insGG in exon 49 (Fig. 3B) and was inherited from her father (data not shown). This small insertion is predicted to cause a frameshift at amino acid 2,331 and a premature stop codon at residue 2,336. Neither of these alterations was demonstrated in more than 180 control chromosomes (data not shown). We hypothesize that the second alteration is likely to be important in the pathogenesis of the anomalies in this patient because it predicts premature protein truncation. However, the deletion of two amino acids resulting from the first sequence alteration would not cause premature protein termination, although it is located in exon 40, close to a previously reported frameshift mutation in exon 41 with both of these sequence changes affecting the NG2-like domain of the FRAS protein [McGregor et al., 2003]. Our conclusion is that these first two sequence changes are mutations and pathogenic in this patient. Both the patient and her mother also had an additional sequence alteration, c.T10178G, predicting an amino acid substitution, p.Y3391D (data not shown) that lies outside the functional domains of the protein. This sequence alteration was present in more than 10% of 50 Caucasian control chromosomes and can therefore be considered to be a polymorphism (data not shown).

The second patient had c.G3095A, predicting p.G1032E in heterozygous form in exon 24 (data not shown). Unfortunately, no parental DNA was

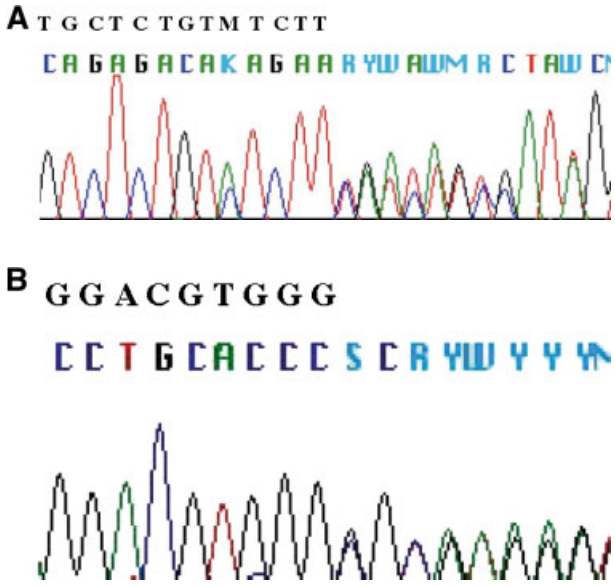

FIG. 3. **A**: Electropherogram showing c.5446delTCTTTC in the first patient, predicted to cause loss of two amino acids at positions 1,816 and 1,817. **B**: Electropherogram showing c.6992insGG in exon 49 in the first patient, predicted to cause premature protein termination at amino acid residue 2,336. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

available from this adopted patient and further sequence alterations were not identified. Control chromosomes were not sequenced for this alteration as the absence of the change from control chromosomes would not necessarily imply pathogenicity and the interpretation of this alteration remains unclear. No mutations were found in the two patients with MOTA syndrome or in the fifth patient. However, both the second patient and one of the patients with MOTA syndrome were heterozygous for a polymorphism predicting an amino acid change, p.V3571I (data not shown). The nucleotide sequence resulting in this polymorphism has been detected in cDNA clones of the *FRAS1* gene (data not shown).

DISCUSSION

We report the results of mutation analysis of the *FRAS1* gene in two patients with FRS, two with MOTA syndrome, and one patient with microphthalmia and labial fusion. Five mutations that predict loss of function (four nonsense and one frameshift) have previously been reported in homozygous form in FRS patients (Table II) [McGregor et al., 2003]. Mutations in *FRAS1* cause disruption to the integrity of epithelial cells in-utero, resulting in external malformations such as the eyelid and limb defects because of mechanical disruption to the development of those structures [Darling and Gossler, 1994]. However, the internal malformations in FRS (for example, renal agenesis) are not explained by this mechanism and loss of function for *FRAS1* is thought to produce a defective extracellular matrix (ECM) at midgestation that disrupts signaling by the TGF-β family or other signal transduction pathways, compromising normal developmental processes, such as metanephric differentiation in kidney formation [McGregor et al., 2003].

The FRAS1 protein showed the highest sequence identity (32%) to the ECM3 protein, a component of the ECM fibers that are important in gastrulation in the sea urchin [Hodor et al., 2000]. FRAS1 contains a domain with sequence similarity to the chondroitin sulfate proteoglycan NG2, a transmembrane protein that is known to interact with other molecules important in growth signaling including bFGF and PDGF-AA [Goretzki et al., 1999]. Three mutations affecting the NG2-like domain in FRAS1 have previously been described and we report two further sequence alterations affecting this protein domain in the first proband, a frameshift mutation in exon 49 and a deletion of six nucleotides in exon 40 (Table II; Fig. 3A,B). We consider that the frameshift mutation is likely to be pathogenic, but the significance of the deletion of two amino acids in the first patient is harder to establish in the absence of a functional assay. However, these two amino acids are conserved in the mouse (data not shown) and the mutation occurs in an exon that encodes the NG2-like domain of the FRAS1 protein and thus could conceivably affect growth signaling in the same way as mutations causing premature protein truncation [McGregor et al., 2003]. Evidence for the importance of the NG2-like domain can also be gleaned from a mouse model of FRS generated by homologous recombination [Vrontou et al., 2003]. Homozygous mutant mice had absent staining for collagen VI in the dermis [Vrontou et al., 2003]. This implies an inability of *fras1* to interact collagen VI in the mutant mice as it is known that the chondroitin sulfate proteoglycan core protein NG2 interacts with collagen types V and VI in vitro [Tillet et al., 1997; Vrontou et al., 2003].

The first patient had CO and abnormal genitalia with a bicornuate uterus, two of the major diagnostic criteria for FRS, together with several minor features in FRS such as laryngeal webbing and anomalies of the nose and ears [Thomas et al., 1986]. However, her presentation was relatively mild and she is apparently making good developmental progress. It is possible that the second mutation in this patient permits partial functioning of the FRAS1 protein and accounts for her milder phenotype. However, a phenotype–genotype correlation has not yet been established for this condition and is clearly complicated by the rarity of the syndrome and the size and difficulty in screening the gene. The detection of mutations in this patient may mean that mutation detection should be offered to more mildly affected probands.

We did not detect two mutations in the second patient with FRS, but genetic heterogeneity has already been demonstrated for FRS [McGregor et al., 2003]. The absence of mutations in the two probands with MOTA syndrome does not support allelism to FRS for MOTA syndrome.

We have sequenced the *FRAS1* gene in two patients with FRS, two patients with MOTA syndrome, and one patient with microphthalmia and labial fusion. We detected two new mutations in a patient with FRS, a frameshift mutation and a deletion of two amino acids. Although we are still unable to clarify a phenotype–genotype relationship in Fraser syndrome, our data augment the known *FRAS1* mutations and do not support allelism for MOTA syndrome with FRS.

ACKNOWLEDGMENTS

This work was supported by REAC grant number 36248-524269, University of California, San Francisco. These studies were carried out in part in the Pediatric Clinical Research Center, Moffitt Hospital, University of California, San Francisco, with funds provided by the National Center for Research Resources, 5 M01 RR-01271, U.S. Public Health Service. We are grateful to Dr. Marles and Dr. Greenberg

for their clinical assessment of MOTA syndrome patients and to Dr. Scambler for reading the manuscript.

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