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FRMD7 mutations in Chinese families with X-linked congenital motor nystagmus

Qingjiong Zhang, Xueshan Xiao, Shiqiang Li, Xiangming Guo

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China

Purpose: To identify mutations causing X-linked congenital motor nystagmus (XL-CMN) in Chinese families.
Methods: Genomic DNA was prepared from peripheral blood leukocytes. Cycle sequencing was used to detect the sequence variation of the *FERM domain containing 7* (*FRMD7*) gene, where mutations have been identified recently to associate with XL-CMN.
Results: Sequencing of the coding and the adjacent intron regions of *FRMD7* identified mutations in four families with XL-CMN, c.41-43delAGA (p.Lys14del) in exon 1, c.70G>A (p.Gly24Arg) in exon 2, c.436C>T (p.Arg146Trp) in exon 6, and c.685C>T (p.Arg229Cys) in exon 8, respectively, where the last two were novel. These mutations were not detected in 196 normal controls. In the two families with X-linked recessive CMN, females carrying a heterozygous mutation in *FRMD7* did not have any sign of nystagmus.
Conclusions: Our results provide additional evidence for mutations in *FRMD7* as a common cause of XL-CMN and expand its mutation spectrum. CMN in a Chinese family with pure X-linked recessive pattern, previously mapped to Xq23-q27, is associated with the c.41-43delAGA mutation in *FRMD7*.

Congenital motor nystagmus (CMN), or congenital idiopathic nystagmus (CIN), is an isolated form of nystagmus, which is not associated with known ocular or systemic diseases. CMN is most frequently observed as an X-linked trait although autosomal dominant and autosomal recessive traits have been reported [1-3]. Incomplete penetrance and variable expression has been observed for X-linked CMN, where some pedigrees shown a pattern of X-linked dominant [1,3,4] but other presented as a pure X-linked recessive pattern [5,6]. X-linked CMN has been mapped to Xp11.4-p11.3 and Xq26-q27, respectively [1,3-6]. Recently, mutations in the *FERM domain containing 7* (*FRMD7*) gene have been identified to be responsible for X-linked CMN mapped to Xq26-q27 (OMIM 310700, 300628) [7], where large pedigrees shown a X-linked dominant mode of transmission.

We have mapped CMN of X-linked recessive pattern to the Xq23-q27 [5], which was further refined to Xq24-q26.3 by Self et al. [6]. *FRMD7* is located in the linked region. In this study, four mutations in FRMD7 were identified in four Chinese families, including the large family with X-linked recessive CMN mapped to Xq23-q27.

METHODS

Patients: The procedure for collecting patients and obtaining informed consent is the same as previously described [8]. This study followed the tenets of the Declaration of Helsinki. Ophthalmological examination was performed by ophthalmologists (Q.Z. and X.G.). Criteria for CMN are the same as previously described [5]. Genomic DNA was prepared from venous blood. Pedigrees for the four families with *FRMD7* mutations were shown in Figure 1.

Mutation detection: DNA fragments encompassing the coding and adjacent intron regions of *FRMD7* (human genome build 36.2, NC_000023 for genomic DNA, NM_194277 for mRNA, and NP_919253 for protein) were amplified by polymerase chain reaction, using 14 pairs of primers (Table 1). The DNA sequences were identified with ABI BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 Genetic Analyzer. Sequencing results from patients as well as *FRMD7* consensus sequences from the NCBI Human Genome Database (NC_000023) were imported into the SeqManII program of the Lasergene package (DNAStar Inc., Madison, WI) and then aligned to identify variations. Each mutation was confirmed by bi-directional sequencing. Mutation description followed the recommendation of the Human Genomic Variation Society (HGVS) [9]. Any variation detected in *FRMD7* was further evaluated in available family members as well as 196 controls.

RESULTS

Upon complete analysis of the coding and the adjacent intron regions of *FRMD7*, four mutations were identified in 4 of 14 families with CMN. Each of the four families had one of the following mutations, i.e., c.41-43delAGA (p.Lys14del) in exon 1, c.70G>A (p.Gly24Arg) in exon 2, c.436C>T (p.Arg146Trp) in exon 6, and c.685C>T (p.Arg229Cys) in exon 8 (Figure 1). Of the four, c.41-43delAGA and c.70G>A were known mutations, and c.436C>T and c.685C>T are novel mutations. In two families with available family members, the mutations cosegregated with affected males and obligate female carriers

Correspondence to: Qingjiong Zhang, MD, PhD, Ophthalmic Genetics & Molecular Biology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 Xianlie Road, Guangzhou 510060, China; Phone:. Phone: (+86)-20-87330422; FAX: (+86)-20-87333271; email: qingjiongzhang@yahoo.com

(i.e., male patients had hemizygous mutation and unaffected obligate female carriers had heterozygous mutation [Table 2]). When used as a marker in linkage analysis, the mutation itself yields a LOD score of 2.41 at θ=0 in family A (Figure 1), which was comparable to the LOD score obtained in previous linkage analysis [5] and established a linkage for CMN on the X chromosome according to the conventional criteria for X-linked disease. All four mutations were not detected in unaffected males in the families and were not present in 196 normal controls. A χ^2 test showed that the association of *FRMD7* mutations with CMN was statistically significant (χ^2=57.09, p=4.2x10^{-14}).

Patients with mutations in *FRMD7* had nystagmus since infancy. All patients showed horizontal pendular oscillation

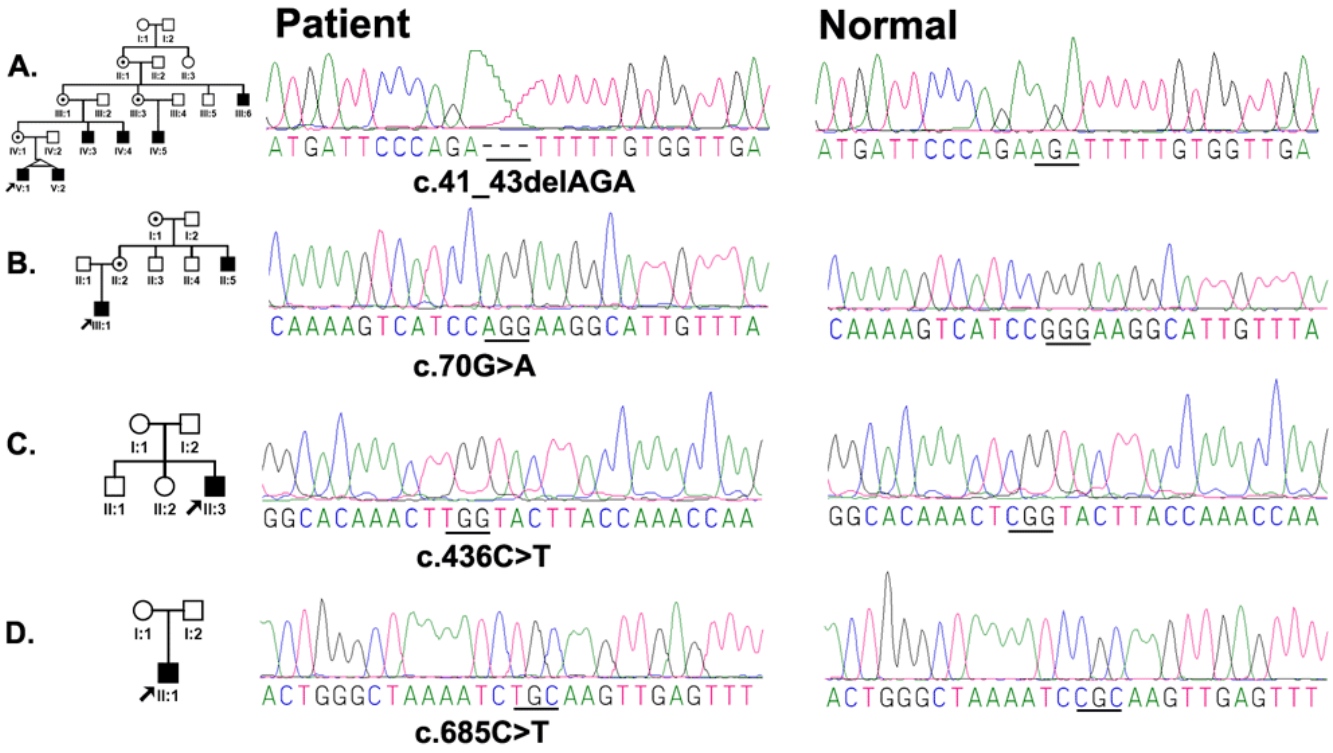

Figure 1. Mutations in the *FERM domain containing 7* gene identified in four families with congenital motor nystagmus. Mutations in *FRMD7* identified in four families with CMN (A, B, C, and D). Each column from left to right represents pedigree of each family, mutant *FRMD7* sequence, and corresponding normal sequence. The exact mutation was labeled under each sequence according to the nomenclature recommended by HGVS. Arrow indicates the proband in each family.

Table 1. Primers used to amplify and to sequence the *FERM domain containing 7* gene

Exon	Forward primer	Reverse primer	Size of PCR products (bp)	Annealing temperature (°C)
1	5'-AGGAAGTCCAGTTAGATTTG-3'	5'-ACAGTCCTCCTTCATTCAGT-3'	428	60
2	5'-ATGCAGGTCCTCTAAACAGT-3'	5'-GGAATTGAACCCTACATACC-3'	320	62
3	5'-GAAAATATAAGGGGGCAGAT-3'	5'-TGGATGTATGAAGGGTTGAA-3'	368	54
4	5'-GAGGGGACGGAAGAGGAGA-3'	5'-TGAGAATGGCCAGAAGCACT-3'	287	64
5	5'-CCCCAAAAAGGCATCTGA-3'	5'-TCTCCCCTGTAAACCCTAAC-3'	339	60
6	5'-GATGGAGGACAAGGGTATGC-3'	5'-GCCACTGAAAGGGGAAAGAA-3'	393	60
7	5'-AGCAAGCCCTTAAACCTGAG-3'	5'-CCCTTTCTGGCTGGTGATAA-3'	391	60
8	5'-AATGCCTTCTTTGACCACAGC-3'	5'-GCCAGCCGGCTTTTACAAT-3'	365	60
9	5'-AGTGGCCCTGTCTATTCCTC-3'	5'-GGTGCCCCCATCTTCCTC-3'	551	60
10-11	5'-CTGCCTGGTCCTTGAATAAG-3'	5'-TCCCCAGGAAGCTAACCTA-3'	563	60
12a	5'-ATGGATCTTGTTAAATGACTT-3'	5'-ACCAACCTGCTGACCTGTA-3'	541	54
12b	5'-TCCCACATTGCTACATCAGTC-3'	5'-CAAATTTGGGTCTTCCTCTTC-3'	520	60
12c	5'-ATGTGCCCTATATTCCTTGTA-3'	5'-ATGGGTGACCTTATTTCTTTG-3'	592	60
12d	5'-TCCCAGAGCCAATCAGACAT-3'	5'-TTTCTGCCTAAGTCGGTAACA-3'	435	60

Primer sequences, size of PCR products, and the annealing temperature for amplification were listed.

of both eyes. Most patients with the mutation had mild to moderate reduced visual acuity (Table 2). Normal color vision was recorded in four patients from one family (family A) who received color vision evaluation. All patients from the four families examined had normal fundus appearance, including normal macular architecture. None of the females with *FRMD7* mutations had any type of nystagmus (Table 2).

DISCUSSION

Mutations in *FRMD7* have been recently identified in families with X-linked CMN, but none of the pedigrees presented a clearly recognizable X-linked recessive mode of transmission pattern [7]. It is well known that mutations in the same gene may cause both dominant and recessive inherited disease [10-15] but it has also been established that dominant and recessive disease may be caused by different genes (RetNet). The same disease may be caused by mutations in different genes even if this disease is mapped to the same chromosome region [15]. Both X-linked dominant and X-linked recessive CMN have been mapped to Xq26-q27 region [3-6,16]. It whould be interesting to know whether these two forms are caused by mutations in the same gene or in different genes [6].

Four mutations in *FRMD7*, two novel (c.436C>T and c.685C>T) and two known (c.41- 43delAGA and c.70G>A), were identified in four Chinese families with CMN. Three of the mutation would affect the B41 domain of the encoded protein and another would affect the FERM-C domain. These results not only expand the mutation spectrum of *FRMD7* but also indicate that mutations in *FRMD7* are a common cause of X-linked CMN in different ethnic groups. By analyzing a large Chinese family (family A in Figure 1) mapped to this region previously [5], our results demonstrate that mutations in *FRMD7* are also responsible for X-linked recessive CMN. The c.41-43delAGA (p.Lys14del) mutation identified in the family A (Figure 1) is the same as that found in an English family with X-linked dominant CMN [7]. This indicates that the nystagmus in females is most likely due to skewed X inactivation rather than to a mutation-specific phenotype.

For those X-linked families without a *FRMD7* mutation identified, it is not impossible that some might be caused by *FRMD7* mutations localized in yet undetermined functional regions as seen in other genes [17,18]. Alternatively, some might be caused by mutations in other genes since X-linked genetic heterogeneity of CMN has been suggested: (1) Another locus for CMN has been mapped to the Xp11.4-Xp11.3 region [1], and (2) *FRMD7* is outside the linked region of an X-linked dominant CMN mapped to Xq26.3-q27.1 region [4]. Further analysis on these families as well as additional families may help to resolve this mystery.

In summary, our results not only reveal that mutations in *FRMD7* are associated with X-linked recessive CMN but also expand the mutation spectrum of *FRMD7*. Further studies on functional properties of the FRMD7 protein, especially by generating Knockin and knockout animal models, will help to unravel how *FRMD7* mutations alter molecular interactions, still largely unknown, involved in the generation of motor nystagmus. Most importantly, the discovery of these interactions should help to discover the neuro-anatomical circuits involved in the generation of the motor nystagmus.

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Table 2. *FERM domain containing 7* mutations detected in Chinese patients with congenital motor nystagmus

				FRMD7 mutation detected					Visual acuity	
Family	ID#	Sex	Age	DNA	Protein	Status	Exon	CMN	OD; OS	Fundus
A	II:1	F	75	c.41_43delAGA	p.Lys14del	Heterozygous	1	No	0.6; 0.8	Normal
	II:3	F	83	Normal				No	HM; HM	N/A*
	III:1	F	53	c.41_43delAGA	p.Lys14del	Heterozygous	1	No	0.9; 0.8	Normal
	III:2	M	52	Normal				No	0.8; 1.0	Normal
	III:3	F	48	c.41_43delAGA	p.Lys14del	Heterozygous	1	No	1.2; 1.2	Normal
	III:5	M	40	Normal				No	1.0; 1.0	Normal
	III:6	M	32	c.41_43delAGA	p.Lys14del	Hemizygous	1	Yes	0.5; 0.5	Normal
	IV:1	F	30	c.41_43delAGA	p.Lys14del	Heterozygous	1	No	1.0; 1.0	Normal
	IV:2	M	33	Normal				No	1.0; 1.0	Normal
	IV:3	M	27	c.41_43delAGA	p.Lys14del	Hemizygous	1	Yes	0.9; 0.9	Normal
	IV:4	M	25	c.41_43delAGA	p.Lys14del	Hemizygous	1	Yes	1.0; 0.9	Normal
	IV:5	M	14	c.41_43delAGA	p.Lys14del	Hemizygous	1	Yes	0.8; 0.8	Normal
	V:1	M	5	c.41_43delAGA	p.Lys14del	Hemizygous	1	Yes	0.3; 0.4	Normal
	V:2	M	5	c.41_43delAGA	p.Lys14del	Hemizygous	1	Yes	0.4; 0.4	Normal
B	II:1	M	28	Normal				No	N/A	N/A
	II:2	F	27	c.70G>A	p.Gly24Arg	Heterozygous	2	No	1.0; 1.0	Normal
	III:1	M	4	c.70G>A	p.Gly24Arg	Hemizygous	2	Yes	0.3; 0.2	Normal
C	II:1	M	10	c.436C>T	p.Arg146Trp	Hemizygous	6	Yes	0.1; 0.1	Normal
D	II:1	M	5	c.685C>T	p.Arg229Cys	Hemizygous	8	Yes	0.3; 0.3	Normal

The hemizygous mutation was present in male patients while the heterozygous mutation was present in unaffected obligate female carriers. The asterisk indicates that we were unable to observe the fundus due to senile cataracts.

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