A novel mutation in FRMD7 causing X-linked idiopathic congenital nystagmus in a large family

Xiang He,1,2 Feng Gu,1,2 Yujing Wang,3 Jinting Yan,1,2 Meng Zhang,1,2 Shangzhi Huang,2,4 Xu Ma1,2,5

1Department of Genetics, National Research Institute for Family Planning, 2Peking Union Medical College, 3Dezhou Woman and Child Hospital, 4Department of Medical Genetics, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China; 5Department of Reproductive Genetics, WHO Collaborative Center for Research in Human Reproduction, Beijing, China

Purpose: To identify the gene responsible for causing an X-linked idiopathic congenital nystagmus (XLICN) in a six-generation Chinese family.

Methods: Forty-nine members of an XLICN family were recruited and examined after obtaining informed consent. Affected male individuals were genotyped with microsatellite markers around the FRMD7 locus. Mutations were comprehensively screened by direct sequencing using gene specific primers. An X-inactivation pattern was investigated by X chromosome methylation analysis.

Results: The patients showed phenotypes consistent with XLICN. Genotype analysis showed that male affected individuals in the family shared a common haplotype with the selected markers. Sequencing FRMD7 revealed a G>T transversion (c.812G>T) in exon 9, which caused a conservative substitution of Cys to Phe at codon 271 (p.C271F). This mutation co-segregated with all affected individuals and was present in the obligate, non-penetrant female carriers. However, the mutation was not observed in unaffected familial males or 400 control males. Females with the mutant gene could be affected or carrier and they shared the same inactivated X chromosome harboring the mutation in blood cells, which showed there is no clear causal link between X-inactivation pattern and phenotype.

Conclusions: We identified a novel mutation in FRMD7 and confirmed the role of this mutation in the pathogenesis of X-linked congenital nystagmus.

Congenital nystagmus (CN) is a common oculomotor disorder (frequency of 1/1,500 live births) characterized by bilateral involuntary, periodic, predominantly ocular oscillations. CN onset typically occurs at birth or within the first few months of life [1] and occurs secondary to the genetic ocular diseases such as albinism, achromatopsia, and Leber congenital amaurosis (OMIM 204000).

CN can be an idiopathic disease or associated with various diseases as a syndrome [2]. The inheritance model is mainly X-linked idiopathic congenital nystagmus (XLICN), but autosomal recessive (OMIM 257400) and autosomal dominant (OMIM 164100,608345,193003) forms have been described.

Some studies indicated that two disease loci of XLICN were mapped to Xq26-q27 and Xp11.4- Xp11.3 [1,3]. Recently, Tarpey et al. [4-6] identified several mutations in FRMD7 (OMIM 300628), a gene localizing to Xq26-q27 and responsible for a major part of XLICN.

In this study, 49 members in a Chinese XLICN family were recruited and examined. Male affected members were genotyped with microsatellite markers at FRMD7, and direct sequencing identified a mutation. Using one female patient, two carriers, and other family members in this large family (Figure 1), we assessed the correlation between X-inactivation pattern and phenotype.

METHODS

Clinical evaluations and DNA specimens: This study followed the tenets of the Declaration of Helsinki, and the protocol was approved by the Ethics Committee at the National Research Institute for Family Planning. Informed consent was obtained from all family members participating in this study. The family, originating from Shandong province in China, contained 21 affected individuals within a six-generation pedigree (Figure 2). In total, 49 members in this family were recruited, including 15 affected individuals (5 males and 10 females) and 34 unaffected individuals or spouses (Figure 2). Ophthalmologists confirmed the diagnosis of CN and there was no history of other ocular or systemic abnormalities in the family. A 5 ml venous blood sample was drawn into an ethylenediamine tetraacetic acid (EDTA) sample tube from every subject.

Genotyping and allele-sharing analysis: Genotyping was performed as described previously [7]. The primer sequences were taken from GDB. Allele-sharing analysis was performed with two microsatellite markers, DXS1047 and DXS691, which were linked with FRMD7 [4] on five affected males individuals. The physical locations of DXS1047-FRMD7-DXS691 are 128.9 Mb, 131.0 Mb, and 135.2 Mb, respectively.

DNA sequencing: Mutations in FRMD7 (NM_194277) were screened by direct sequencing. Polymerase chain reac-
tion (PCR) products of the 12 exons and flanking intron se-
quences of FRMD7 were sequenced on an ABI A3730 Au-
mated Sequencer (Applied Biosystem, Foster City, CA) [5].

Denaturing HPLC: Wave DHPLC (Transgenomic, San
Jose, CA) was used to screen exon 9 of FRMD7 from pa-
tients, carriers, family members, and 400 normal, unrelated,
male individuals. DHPLC was performed according to
the protocols described previously [7] with initial concentra-
tions of buffer A (0.1 M triethylammonium acetate-TEAA) at 53%
and 47% for buffer B (0.1 M TEAA containing 25% acetoni-
trile) while maintaining the procedure at 58.7 °C.

X-Inactivation assay: Genomic DNA (1 µg) from a nor-
mal male (III:10, as negative control), two male patients (IV:29
and IV:30, as the positive controls), a female patient (V:20),
and two obligate non-penetrant carriers (III:9 and III:11) was
incubated overnight with and without 1 U HpaII in a total
volume of 20 µl. After a 1:2 dilution with water, 2 µl of the
diluted digest was amplified by PCR to detect the polymor-
phic CAG repeat of the androgen receptor (AR). PCR prod-
ucts were separated on a 6% polyacrylamide denaturing gel
for 1.5 h using constant voltage (600 V) and were subsequently
detected by silver stain.

RESULTS

Clinical data: In this family, there were nine normal carriers
and 11 affected females (Figure 2), who have a milder phenol-
type than the affected males. All affected individuals had nys-
tagmus in early childhood. Night blindness or photophobia
were not observed in any of the affected individuals in the
family nor were there any incidences of systemic or other ocu-
lar anomalies. The X-linked disease was transmitted from fe-
male carriers/patients to afflicted sons yet there was no evi-
dence of male-to-male transmission in this family.

The proband was a 10-year-old female (V:20, Figure 2).
Her visual acuity was about 20/25. While visual acuity of her
father (36-year-old, IV:29, also affected) is 20/32 (right/left).
Myopia was also identified in her father (right/left, 27.50 mm/
27.20 mm).

Allele-sharing analysis: Allele-sharing analysis con-
firmed the linkage of the disease in the family with the mu-
tation in FRMD7 (data not shown).

Mutation detection in FRMD7: Sequence analysis re-
vealed a G>T transversion in exon 9 (Figure 3) that caused a
conservative substitution of Cys to Phe at codon 271
(p.C271F). No sequence change was detected in the remain-
ing coding sequence of FRMD7. DHPLC analysis confirmed
this mutation and found the mutation to co-segregate with all
affected individuals and obligate carriers in the family. No
mutation was detected in any of the unaffected male family
members or in any of the 400 normal unrelated male indi-
viduals.

Figure 1. Analysis of methylation of HpaII sites in the human andro-
gen-receptor locus. The different alleles at the X androgen-receptor
locus are shown. The upper is the results of DNA without treatment
of HpaII. The bottom shows that only genomic DNA of the female
affected individuals (V:20) and two carriers (III:9 and III:11) were
digested by HpaII. As to affected female (V:20), after digestion, only
an amplified band of AR allele from her mother has been achieved
and she may inherit the mutant allele of FRMD7 on the active X
chromosome (unmethylated) from the father. While, carrier (III:11)
and her sister (III:9, carrier) hold a different active X chromosome.
Affected male individual (IV:29) and his affected brother (IV:31)
herited different allele on the AR locus from their mother (III:11).
Multiple-sequence alignment and mutation analysis: Using the National Center for Biotechnology Information (NIH) websites, a multiple-sequence alignment of the FRMD7 proteins in various species (Homo sapiens, Canis familiaris, Mus musculus, Rattus norvegicus, and Gallus gallus) was obtained. This alignment was compared to homologous proteins (FERM, RhoGEF, FARP2 protein) using DNAMAN biosoftware (Lynnon Biosoft, Quebec, Canada). The mutation, p.C271F, occurred within a highly conserved region of the gene. The online bioinformatics software SIFT (Sorting Intolerant, Tolerant) algorithm [8] predicted whether the amino acid substitution in FRMD7 would have a phenotypic effect. The software determined that the substitution of Cys to Phe at position 271 was deleterious.

The predicted p.C271F substitution represented a conservative amino-acid change with the uncharged polar sulphydryl side group of Cys replaced by the nonpolar group on Phe. This change is likely to destabilize the protein by inducing structural changes by placing a larger amino acid within a restricted space of the protein. Additional changes associated with losing the free sulfhydryl group may disturb the disulfide bond formation between inter- or intra-molecular residues.

Overall, the cosegregation of the G-T transversion is only found within the affected and obligate, non-penetrant carriers in the pedigree. These results suggest that the p.C271F substitution was a causative mutation.

X-Inactivation assay: Affected female individuals carried the mutated X chromosome. Genes in this affected X chromosome were active and unmethylated because the DNA could be digested with HpaII (V:20, the allele from her father IV:29 is cut by HpaII). As a result, only methylated DNA on the X chromosome was used as a PCR template (Figure 1, V:20 and the allele from her mother, IV:30). The X chromosome from carrier III:9 was methylated and contained the mutated FRMD7. Additionally, carrier III:11 had an unmethylated X chromosome containing the FRMD7 mutation. Take together, carrier (III:11) and her sister (III:9, carrier) held a different active X chromosome. Meanwhile, affected male individual (IV:29) and his affected brother (IV:31) inherited different allele on the AR locus from their mother (III:11).

DISCUSSION

Congenital nystagmus is a clinically and genetically heterogeneous disease that causes visual impairment in childhood. Clinically, the congenital nystagmus is divided into idiopathic and nystagmus-related syndromes. Idiopathic congenital nystagmus is thought to represent an abnormal development of the ocular motor areas of the brain that control fixation. As these patients may have normal visual acuity, it is presumed that the nystagmus represents a primary defect in the parts of the brain responsible for ocular motor control.

Genetically, at least four loci have been proposed for familial idiopathic congenital nystagmus [1-6,9-12]. Two loci have been identified for XLICN with one being mapped to Xq26-q27 by Kerrison and the other to Xp11.4-p11.3 by Cabot [1,3].

In this family, it was obvious that the trait is X-linked because there was no male-to-male transmission, but there was frequent female-to-male transmission. The estimated penetrance among obligate female carriers was estimated to be 55% [11 of 20] with no age dependent affect. The penetrance in this family is lower than that found in a family studied by Schorderet and colleagues [5]. The different penetrance in different XLICN families is consistent with previous studies by Waardenburg. He felt there was no reason to separate an X-linked recessive from an X-linked dominant form as some have attempted (OMIM 310700). In some families, the disorder is recessive in one line and dominant in another. The explanation could be that the mutation is identical, but a series of “wildtype” isoalleles have different effects on penetrance of the mutation in the heterozygous female.

Meanwhile, X chromosome inactivation may be one mechanism for the variation in penetrance. Inactivation is a methyluation-dependent phenomenon and consists of transcriptional silencing of one of the two alleles on X chromosomes in mammalian females [13]. Most females are mosaics with a mixture of cells expressing either their mothers or fathers X-linked genes. Often, cell mosaicism is advantageous by ameliorating the deleterious effects of X-linked mutations and contributing to physiologic diversity [14]. Yet, in some cases, females carrying the mutant gene have clinical manifestation as observed in this study.

Figure 3. DNA sequence chromatograms. DNA sequence chromatograms of the affected members (A), carriers (B), and unaffected members (C) in an X-linked idiopathic congenital nystagmus family is shown. There is a single base G>T transversion in exon 9 of FRMD7 that causes a conservative substitution of Cys to Phe at codon 271 (p.C271F). This mutation co-segregated with all affected individuals and was present in the obligate, non-penetrant carrier.
To uncover the correlation between the X-inactivation pattern and phenotype, we assayed for the pattern in one female patient, two carriers, and other family members in this large family. Because the affected IV:29 and IV:31 patients inherited different alleles of CAG repeats at AR from their mother (III:11), the results showed that there is at least one recombination event in these nuclear families. Since physical distance from the FRMD7 to the AR gene is 64.2 Mb (131.0 Mb, 66.8 Mb for FRMD7, AR, respectively), a marker closer to the FRMD7 locus is required to assay the methylation status of the wild-type and mutant allele.

Carriers III:9 and III:11 showed different methylation patterns for the X chromosome, implying that a molecular basis for variable methylation might not be involved in the dissimilar penetrance in this family. Female heterozygotes contain two cell types where the proportion of cells departs from equality following cell selection at the tissue or organism level [15]. Willemesen et al. [16] reported that monozygotic twin sisters with the fragile X mutation could have different phenotypes resulting from skewed X-inactivation. Furthermore, other results have proven that familial non-random inactivation is linked to the X-inactivation center in heterozygotes that manifest hemophilia A, one of the inherited blood diseases [17]. While the patterns of X-inactivation in blood may not necessarily reflect those in tissue, the methylation status of FRMD7 in the pathologic tissue could be different from that of blood. Taken together, no correlation between the X-inactivation pattern and phenotype were observed in blood analyzed in this study. This finding is consistent with a recent study [18] and as such, further studies need to investigate the X-inactivation pattern and phenotype in this family.

Since only two loci have been mapped on the X chromosome and the results of genotyping show that the affected individuals shared the same allele with the FRMD7 locus, we sequenced the coding and flanking intron sequences of FRMD7, the only reported gene responsible for XLICN. This data led us to conclude that the family in this study contains a novel mutation.

Tarpey et al. [4] identified 22 novel mutations in FRMD7 in 26 families with XLICN. Schorderet et al. [5] identified five novel mutations in six families with XLICN. Among these 22 mutations, a missense mutation of C271Y in exon 9 was detected in a Scottish family. In our study, the p.C271F mutation is responsible for this disease. Schorderet et al. [5] identified another mutation (H275P) in exon 9. These studies suggested that the coding region of exon 9 is critical to the normal function of the FRMD7 protein.

FRMD7 encodes a member of the FERM-domain (Band 4.1 family) containing proteins. The FERM domain is found in cytoskeletal-associated proteins such as ezrin, moesin, radixin, 4.1R, and merlin. Proteins of the FERM-domain family are thought to be involved in cytoskeletal attachment to the plasma membrane [19,20]. The mouse genome encodes at least 50 FERM proteins, but the functions of only a few, including the actin-binding proteins ezrin (also known as Vili), radixin, moesin, NF2 (also known as merlin), and the erythrocyte protein band 4.1, have been characterized. Therefore, from the function of these FERM proteins, FRMD7 may have functions relevant to cell structure, cell migration, normal cell growth, cellular differentiation, and signal transduction. FRMD7 is expressed in early human embryos at about 56 days post-ovation in the ventricular layer of the forebrain, midbrain, cerebellar primordium, spinal cord, and the developing neural retina [4]. This restricted expression suggests a specific role in the control of eye movement and gaze stability.

This study confirmed that FRMD7 plays an important role in motor control of eye movement and provided additional information on gene mutations in exon 9 of FRMD7 that lead to mutations causing XLICN. Therefore, FRMD7 is an ideal candidate for XLICN mutation screening. When no mutation has been identified, it is suggested to scan the whole X chromosome to mapping the responsible gene. While the role of FRMD7 in disease genesis is still unclear, further studies need to provide insights into the molecular pathology of XLICN.

ACKNOWLEDGEMENTS

The authors thank the family for their participation in this project. We are indebted to Professor Yi Tong and Dr. Juhua Yang (Fujian University of Medical Sciences) for helpful reading and discussions on the manuscript. This work is partly supported by the National “973” Basic Research Funding Scheme of China (2007CXB5119005) and National Infrastructure Program of Chinese Genetic Resources (2006DKA21300).

REFERENCES

1. Kerrison JB, Vagefi MR, Barnada MM, Maumenee IH. Congenital motor nystagmus linked to Xq26.q27. Am J Hum Genet 1999; 64:600-7.
2. Kerrison JB, Arnould VJ, Barnada MM, Koenenroop RK, Schmeckpeper BJ, Maumenee IH. A gene for autosomal dominant congenital nystagmus localizes to 6p12. Genomics 1996; 33:523-6.
3. Cabot A, Rozet JM, Gerber S, Perrault I, Ducroq D, Smahi A, Souied E, Munnich A, Kaplan J. A gene for X-linked idiopathic congenital nystagmus (NYSL) maps to chromosome Xp11.4-p11.3. Am J Hum Genet 1999; 64:141-6.
4. Tarpey P, Thomas S, Sarvananthan N, Mallya U, Lligo S, Talbot C, Roberts EO, Awan M, Surenrendan M, McLean RJ, Reinecke RD, Langmann A, Lindner S, Koch M, Jain S, Woodruff G, Gale RP, Degg C, Droukas K, Asproudis I, Zubeck AA, Pieh C, Veal CD, Machado RD, Backhouse OC, Baumber L, Constantinescu CS, Brodsky MC, Hunter DG, Hertle RW, Read RJ, Edkins S, O’Meara S, Parker A, Stevens C, Teague J, Wooster R, Futrelal PA, Tremath RW, Raymond FL, Goltob I. Mutations in FRMD7, a newly identified member of the FERM family, cause X-linked idiopathic congenital nystagmus. Nat Genet 2006; 38:1242-4.
5. Schorderet DF, Tiab L, Gaillard MC, Lorenz B, Klaingutti G, Kerrison JB, Trebuoli ES, Munier FL. Novel mutations in FRMD7 in X-linked congenital nystagmus. Mutation in brief #963. Online. Hum Mutat 2007; 28:525.
6. Zhang Q, Xiao X, Li S, Guo X. FRMD7 mutations in Chinese families with X-linked congenital motor nystagmus. Mol Vis 2007; 13:1375-8.
7. Gu F, Li R, Ma XX, Shi LS, Huang SZ, Ma X. A missense mutation in the gammax-D-crystallin gene CRYGD associated with...
autosomal dominant congenital cataract in a Chinese family. Mol Vis 2006; 12:26-31.
8. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003; 31:3812-4.
9. Gutmann DH, Brooks ML, Emanuel BS, McDonald-McGinn DM, Zackai EH. Congenital nystagmus in a (46,XX/45,X) mosaic woman from a family with X-linked congenital nystagmus. Am J Med Genet 1991; 39:167-9.
10. Guo X, Li S, Jia X, Xiao X, Wang P, Zhang Q. Linkage analysis of two families with X-linked recessive congenital motor nystagmus. J Hum Genet 2006; 51:76-80.
11. Klein C, Vieregge P, Heide W, Kemper B, Hagedorn-Grewe M, Hagenah J, Vollmer C, Breakefield XO, Kompf D, Ozelius L. Exclusion of chromosome regions 6p12 and 15q11, but not chromosome region 7p11, in a German family with autosomal dominant congenital nystagmus. Genomics 1998; 54:176-7.
12. Ragge NK, Hartley C, Dearlove AM, Walker J, Russell-Eggitt I, Harris CM. Familial vestibulocerebellar disorder maps to chromosome 13q31-q33: a new nystagmus locus. J Med Genet 2003; 40:37-41.
13. Raynaud M, Dessay S, Ronce N, Opitz J, Pembeve M, Romano C, Moraine C, Briault S. Skewed X chromosome inactivation in carriers is not a constant finding in FG syndrome. Eur J Hum Genet 2003; 11:352-6.
14. Migeon BR. The role of X inactivation and cellular mosaicism in women’s health and sex-specific diseases. JAMA 2006; 295:1428-33.
15. Lyon MF. X-chromosome inactivation and human genetic disease. Acta Paediatr Suppl 2002; 91:107-12.
16. Willemsen R, Olmer R, De Diego Otero Y, Oostra BA. Twin sisters, monozygotic with the fragile X mutation, but with a different phenotype. J Med Genet 2000; 37:603-4.
17. Bicocchi MP, Migeon BR, Pasino M, Lanza T, Bottini F, Boeri E, Molinari AC, Corsolini F, Morerio C, Acquila M. Familial non-random inactivation linked to the X inactivation centre in heterozygotes manifesting haemophilia A. Eur J Hum Genet 2005; 13:635-40.
18. Self JE, Shawkat F, Malpas CT, Thomas NS, Harris CM, Hodgkins PR, Chen X, Trump D, Lotery AJ. Allelic variation of the FRMD7 gene in congenital idiopathic nystagmus. Arch Ophthalmol 2007; 125:1255-63.
19. Bretscher A, Edwards K, Fehon RG. ERM proteins and merlin: integrators at the cell cortex. Nat Rev Mol Cell Biol 2002; 3:586-99.
20. Mangeat P, Roy C, Martin M. ERM proteins in cell adhesion and membrane dynamics. Trends Cell Biol 1999; 9:187-92.