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A Novel Missense Mutation, R279S, in *FRMD7* Gene in a Chinese Family with X-linked Infantile Nystagmus

Xiaojuan Wang[1,2,3], Ningdong Li[1,2*], Ming Ying[2], Suyan Li[3] and Kanxing Zhao[1,2]

[1]*Tianjin Medical University, Tianjin, People's Republic of China*
[2]*Tianjin Eye Hospital, Tianjin Eye Institute, Tianjin, People's Republic of China*
[3]*Xuzhou Eye Institute, the First Peoples' Hospital of Xuzhou, Xuzhou,Jiangsu Province, People's Republic of China*

Abstract

Purpose: To identify the gene mutations causing an X-linked Chinese Family with infantile nystagmus.

Methods: Families were ascertained and patients underwent complete ophthalmological examinations. Blood samples were collected and DNA was extracted. Four microsatellites were amplified by PCR reaction for linkage study. *FRMD7* gene was sequenced and mutations analyzed.

Results: A significant lod score of 2.4 was yielded at the microsatellite marker DXS1001. Sequencing of *FRMD7* gene showed a nucleotide change of c. 837G>C in the exon9 of *FRMD7* gene in the patients, which predicted to result in an R279S amino acid change. This novel mutation was absent in 100 normal Han Chinese controls.

Conclusions: We identified a novel mutation, c. 837G>C (p. R279S), in a Han Chinese family with Infantile nystagmus. This mutation expands the mutation spectrum of *FRMD7* and help to further study molecular pathogenesis of *FRMD7*.

Keywords: Infantile nystagmus; *FRMD7* gene; Mutation

Introduction

Infantile nystagmus (IN) is a group of clinically and genetically inheritable ocular motor disease. It usually presents at birth or develops within the first few months of life. IN has variable clinical features showing involuntary pendular or jerky conjugate ocular oscillations with or without compensatory head posture. Some patients may have reduced visual acuity, head oscillations and astigmism. Because the precise etiology is not known, IN also is referred to as congenital idiopathic nystagmus, idiopathic congenital nystagmus (ICN), or congenital "motor" nystagmus (CMN).

IN may be inherited as three Mendelian inheritable modes, of which the X-linked inheritance is believed to be the most common. [1] Thus far, five IN genetic loci and one IN disease gene (the FERM domain–containing 7 gene, *FRMD7* gene), have been identified.

FRMD7 gene is located on Xq26-q27, and mainly expressed in the midbrain where the center of the eye's movement is located[2,3]. Its protein has close homology to two other FERM domain containing proteins: FARP1 (NM_005766) and FARP2 (NM_014808). FARP1 is known to promote the dendritic growth of spinal motor neuron subtypes, while FARP2 has been shown to modulate the length and degree of neurite branching in developing cortical neurons.

To date, more than 40 mutations in the *FRMD7* gene have been reported worldwide in families with X-linked IN from various ethnic backgrounds. [3-12] Here, we reported a Chinese family with X-linked nystagmus. Clinical findings in these families are typical of congenital idiopathic nystagmus. Linkage analysis shows linkage to a region of chromosome Xq26-q27 including *FRMD7* gene. Sequencing of *FRMD7* shows a nucleotide change of c.837G>C, resulting in a R279S amino acid change in the protein.

Patient Ascertainment

A Han Chinese family with IN (the family NYS009) was recruited through the Ophthalmic Genetics Clinic of the Tianjin Eye Hospital, Tianjin, China. This study obtained IRB approval from Tianjin Eye Hospital, Tianjin, China, and conformed to the tenets of the Declaration of Helsinki. All participants underwent an ophthalmologic examination, including visual acuities; slit examination of the lens, examination of the vitreous, fundus, electroretinograms (ERGs), and visual evoked potentials (VEP) as well. Eye movements were recorded with the Eye Tracker system (Eyelink 2000; SR Research, Kanata, Ontario, Canada). The blood samples were collected from the affected and unaffected members after informed consent was obtained. DNA was extracted from blood lymphocytes according to the standard protocol (Roche Biochemical, Inc., Shanghai, China). Briefly, the white blood cells were separated from whole blood via a preferential red blood cell lysis and then lysed by a strong anionic detergent. After the proteins were removed by dehydration and precipitation, the purified DNA is subsequently recovered via ethanol precipitation.

Genotyping and Linkage Analysis

Linkage analysis was performed by using four fluorescently labeled microsatellite markers, DXS1214 and DXS993 Xp11.4-p11.3, and DXS1001 and DXS1047 on Xq26-q27. The purpose of selecting the markers DXS1214 and DXS993 was to exclude the possibility of the disease linking with the other known IN locus on Xp11.4-p11.3[13]. After amplification in a 10μl reaction volume containing 50ng of genomic DNA, 1×PCR buffer, 2.0mM MgCl2, 0.2 mmol/L of each

***Corresponding author:** NingDong Li, Tianjin Medical University, Tianjin Eye Hospital, Tianjin, 300022, People's Republic of China, Tel: +86-22-27313336-83045; Fax: +86-22-27313095; E-mail: Ind68@yahoo.com

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dNTP, 5 pmol/L of each primer each of forward and reverse primers, and 0.2U of Ampli Taq Gold DNA polymerase, the PCR products from each DNA sample were pooled and mixed with a loading cocktail containing HD-400 size standards (PE Applied Biosystems, Foster City, CA) and loading dye. The resulting PCR products were separated on an ABI3130 sequencer, and analyzed with GENEMAPPER 3.7 (PE Applied Biosystems, Foster city, CA).

Two-point Lod scores were calculated with SuperLink v 1.4 program in the EasyLinkage plus v4.0 beta software assuming an X-linked dominant trait with an affected allele frequency of 0.001.

Mutation Analysis

Mutation screening of *FRMD7* gene in the family NYS009 was carried out by direct DNA sequence analysis. Individual exons of *FRMD7* were amplified by PCR as previously described.[9] The PCR products were extracted using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). DNA sequencing analysis was performed using the BigDye Terminator Cycle Sequencing V3.1 kit on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Sequencing results were assembled and analyzed with the Seqman program of DNASTAR software (DNASTAR Inc, Madison, WI). Multiple sequence alignment was performed using the Clustal W algorithm in the software package. The reference cDNA sequence was obtained from Genebank (NM_194277.1) and +1 corresponds to the A of the ATG translation initiation codon.

Results

The family NYS009 was from Henan province, and included 3 affected males, 4 affected females and 5 female carriers (Figure 1). The IV2 was a proband in this family. She developed nystagmus at ages of 3 to 4 months after birth and had a horizontal jerk ocular oscillation at distance with the best corrected visual acuity of 0.8 in both eyes at the ages of 12. Her eye movement recordings in a primary gaze of the right and left eyes showed horizontal nystagmus with slow leftward drift and fast beats to the right (Figure 2). The ocular oscillation could be dampened at left gaze of 10°, and also could be achieved at a viewing distance of 33 cm (Figure 2). Other patients in this family had various reduced visual acuity, ranging from 0.2 to 1.0 with a horizontal jerk ocular oscillation. None of the patients in this family had a compensatory face turn.

Linkage study showed that a significant LOD scores of 2.4 was yielded at the polymorphic marker DXS1001 (θ =0) and a positive lod score of 1.50 at DXS1047, and the negative Lod scores were produced at both DXS1214 and DXS 993 (Table 1). Sequencing of *FRMD7* gene showed a single nucleotide change of c.837G>C in exon9, which would predict to result in a wild type amino acid of Arginine (R) substituted by a mutant type amino acid of Serine (S) at codon 279(Figure 3.). This nucleotide change cosegregated with the disease phenotype in all affected male and female members in this family and was absent in 100 normal controls after sequencing of *FRMD7* gene. Multiple sequence alignment of FRMD7 protein shows that R279S is conserved among Homo sapiens, Pongo abelii, Rattus norvegicus, Mus musculus, Canis familiaris, Gallus gallus, Xenopus and Danio rerio (Figure 4.). A PISC (Position-**S**pecific **I**ndependent **C**ounts) score of 2.05 was yielded after using the POLYPHEN program (http://coot.embl.de/PolyPhen/) to predict the functional and structural change of the amino acid substitution.

Discussion

In this study, we found a novel mutation (p. R279S) in exon 9 of the *FRMD7* gene in a Han Chinese pedigree with X-linked IN. The c.837G>C (p. R279S) should be regarded as a novel gene mutation rather than a polymorphism nucleotide change due to the following facts: 1) It is absent in 100 normal controls. 2) The c.837G>C change would predict to result in a Arginine to Serine change at codon 279 where it is located within highly conserved regions that are invariant in Homo sapiens, Pongo abelii, Rattus norvegicus, Mus musculus, Canis familiaris, Gallus gallus, Xenopus and Danio rerio, which suggested that the Arginine at codon 279 is critical to the normal function of the protein. 3) A PISC score of 2.05 predicted by POLYPHEN program is suggested that the substitution of Arginine to Serine at codon 279 would be probably damaging to the protein structure and/or the protein function.

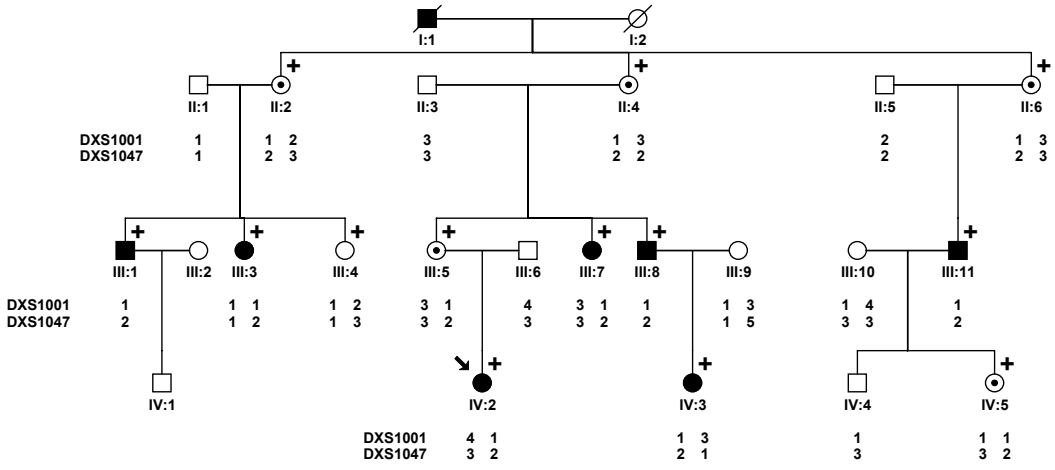

Figure 1: A Chinese family with X-linked infantile nystamus. Squares are males, circles are females, filled symbols are affected individuals, dotted circles are female carriers, a diagonal line through a symbol indicates a deceased family member, and the arrow indicates the proband. The plus sign indicates individuals who underwent DNA sequencing. The number of the microsatellite used indicates the alleles at each locus. The family is inherited as the x-linked trait with non-complete penetrance because the female individual III3,III7,IV2,and IV3 are the affected females while their mothers, individual II2,II4, and III5, are the female carriers.

The FRMD7 protein is structurally composed of five domains, including B41, FERM-N, FERM-M, FERM-C, and FA domains. The B41 domain is located between residues 1–192, and the FERM-C domain is located between residues 186–279. Both of them are critical to the function of the FRMD7 protein since the conserved domains are concentrated at the B41 and FERM-C domains.

To date, approximately 23 missense mutations have occurred in FRMD7,[3] [6-9], [11], [12], with 14 of these mutations located in the FERM-C domain. The mutation of R279S happened at the final amino acid in the FERM-C domain and is close to other three known mutations (H275P, C271Y and C271F) in the same domain. We compared the characteristics of these four mutations (R279S, H275P, C271Y and C271F) using The Sequence Profile Model (http://www.snps3d.org/modules), and found that a SVM (Support Vector Machines) score of -3.5 was obtained at H275P, -3.16 at R279S, -2.31 at C271Y and -1.97 at C271F. A positive SVM score indicates an animo acid change classified as non-deleterious, and a negative score indicates a deleterious case. The larger the score, the more confident is the classification. So, the mutation of R279S is predicted to be more deleterious to the stability of the FRMD7 structure than either C271Y or C271F, and less than the mutation of H275P, according to the SVM score. Further study on the protein function should be helpful to prove this prediction.

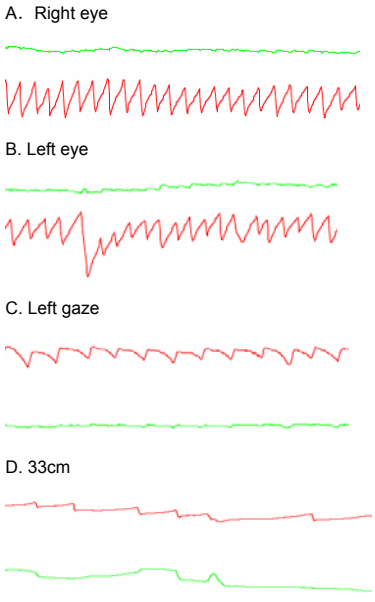

Figure 2: Eye movement recording of the proband. Eye movement recordings in primary gaze of the right and left eyes of the proband showed horizontal nystagmus (A and B). The ocular oscillation can be dampened at left (C) gaze of 10 degree , and also can be done at 33cm viewing distance (D). The vertical component of eye movement is denoted in green color, and the horizontal in red color.

			Lod Score					
Marker	Position	0	0.1	0.2	0.3	0.4	Z_{max}	θ_{max}
DXS1214	33.54cM	-9.54	-0.07	0.36	0.41	0.27	0.41	0.3
DXS993	42.50M	-7.51	0.26	0.62	0.57	0.31	0.62	0.2
DXS1001	75.79cM	2.40	2.03	1.62	1.16	0.63	2.40	0
DXS1047	82.07cM	1.50	1.27	1.01	0.72	0.39	1.50	0

Table 1: Two-point LOD score with polymorphic DNA markers on chromosome Xp11.4-p11.3 and Xq26-q27.

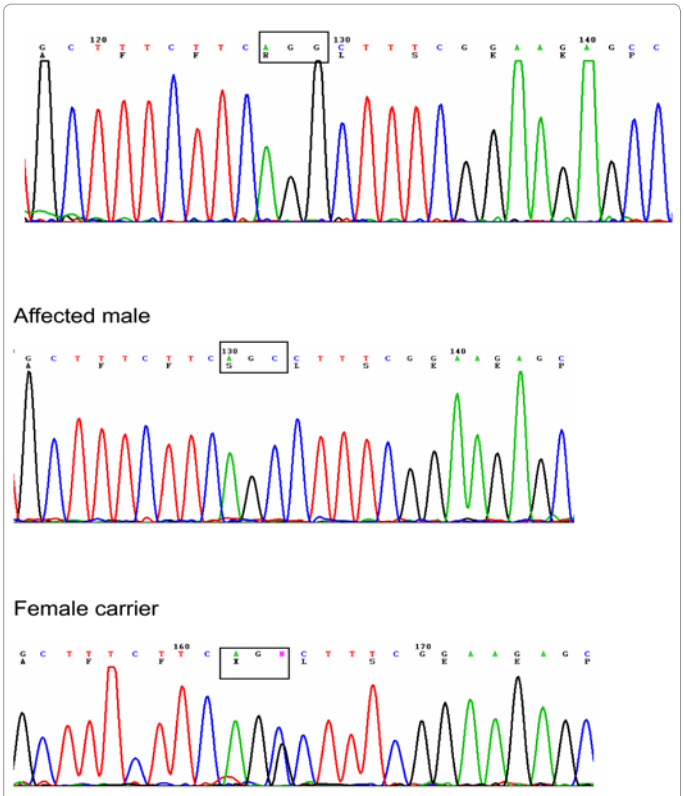

Figure 3: Identification of the 837G>C (p. R279S), in *FRMD7* gene. Sequencing chromatograms from a normal individual (wild type, top), an affected male (mutant type, middle) and an affected female (bottom), showing a G to C change in exon9.

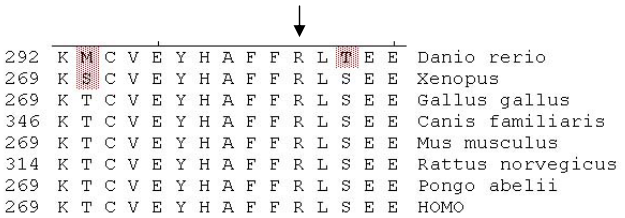

Figure 4: Alignment of FRMD7 amino acids. The alignment of amino acids around p.R279 (denoted by the black arrow) of FRMD7 revealed evolutionary conservation of the Arginine among Homo sapiens, Pongo abelii, Rattus norvegicus, Mus musculus, Canis familiaris, Gallus gallus, Xenopus and Danio rerio.

FRMD7 has been shown to regulate the adhesion and morphogenesis of cells by modulating changes in the cytoskeleton[14,15]. However, the precise role of either the wild type or the mutant type of the FRMD7 protein in the pathogenesis of ICN is not clear. The mutant FRMD7 protein might disrupt the development of nerve cells in some areas controlling the eye movement in the brain. Abnormal development of these nerve cells likely causes the involuntary side-to-side eye movements that are characteristic of X-linked infantile nystagmus.

In summary, we investigated the gene mutations in a Han Chinese

pedigree with infantile nystagmus and identified a novel gene mutation, c. 837G>C (p. R279S), in *FRMD7* gene in this pedigree. This mutation would be expanding the mutation spectrum of *FRMD7* and help to further study molecular pathogenesis of *FRMD7* gene.

Acknowledgement

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