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ARTICLE

Novel homozygous, heterozygous and hemizygous *FRMD7* gene mutations segregated in the same consanguineous family with congenital X-linked nystagmus

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Congenital nystagmus (NYS) is characterized by bilateral, spontaneous, and involuntary movements of the eyeballs that most commonly presents between 2 and 6 months of life. To date, 44 different *FRMD7* gene mutations have been found to be etiological factors for the NYS1 locus at Xq26-q27. The aim of this study was to find the *FRMD7* gene mutations in a large eleven-generation Indian pedigree with 71 members who are affected by NYS. Mutation analysis of the entire coding region and splice junctions of the *FRMD7* gene revealed a novel missense mutation, c.A917G, predicts a substitution of Arg for Gln at codon 305 (Q305R) within exon 10 of *FRMD7*. The mutation was detected in hemizygous males, and in homozygous and heterozygous states in affected female members of the family. This mutation was not detected in unaffected members of the family or in 100 unrelated control subjects. This mutation was found to be at a highly conserved residue within the FERM-adjacent domain in affected members of the family. Structure prediction and energetic analysis of wild-type *FRMD7* compared with mutant (Q305R) revealed that this change in amino acid led to a change in secondary structure predicted to be an energetically unstable protein. The present study represents the first confirmation of *FRMD7* gene mutations in a multigenerational Indian family and expands the mutation spectrum for this locus.
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Keywords: nystagmus; *FRMD7* gene; mutation analysis

INTRODUCTION

Nystagmus (NYS), one of the most common neuro-ophthalmological disorders among live births, often seriously reduces visual acuity.[1] NYS could be physiological or pathological and the latter can be divided into acquired or congenital forms. Although congenital NYS is essentially present at birth, it can also occur early in the child's vision development. The estimated incidence of NYS in the general population was 2.4/1000.[2] Most cases of early onset NYS are hereditary and are transmitted in X-linked dominant, X-linked recessive, and autosomal dominant forms with incomplete penetrance and variable expressivity. Several family-based, genome-wide linkage studies showed evidence of linkage at seven NYS loci on Xq26.2 (NYS1 (MIM 310700)), 6p12 (NYS2 (MIM)), 7p11.2 (NYS3 (MIM 608345)), 13q31-q33 (NYS4 (MIM 193003)), Xp11.4 (NYS5 (MIM 300589)), Xp22.3 (NYS6 (MIM 300814)) and on 1q31-q32.2.[3]

We have analyzed a large consanguineous Indian family diagnosed as having NYS. Sequencing of the *FRMD7* gene, which is well established in its involvement in the pathogenesis of X-linked NYS, revealed a novel missense mutation, c.A917G, that predicts a substitution of Arg for Gln at codon 305 (Q305R) within exon 10 in the affected members of this family.

METHODS

Family Studies

This large eleven-generation pedigree (UR031) exhibiting isolated non-syndromic congenital NYS is from southern India. Experienced ophthalmologists (RS) performed detailed examinations on the 36 selected patients, testing visual acuities, slit lamp, color vision, intraocular pressure, transillumination defects of the iris, ocular oscillations, fundoscopy and performing visual evoked potentials as well. Angle of head turn was measured with the help of deviometer and electroretinogram testing was performed in selected affecteds.

Mutation analysis

Genomic DNA from 29 family members and 100 normal controls were PCR amplified for regions covering the entire coding sequence and splice junctions

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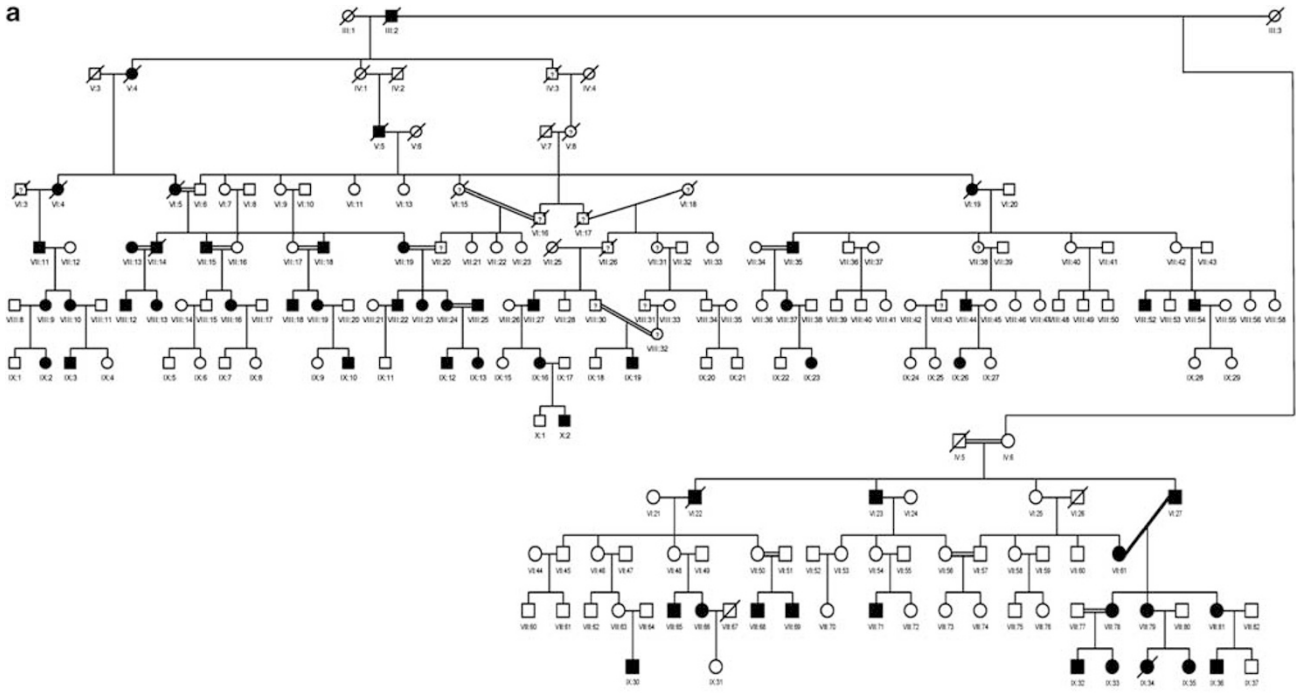
of the *FRMD7* gene. Detailed methodology is described in Supplementary Methods.

Protein modeling

The secondary structure of human wild-type and mutant *FRMD7* was predicted using I-TASSER,[4] and the results saved in PDB file format. The PDB files obtained for the two samples were then used by Jmol to visualize the structures of these proteins.[5] The effect of amino-acid replacement on structure or function was predicted by the PolyPhen-2 program,[6] which calculates position-specific independent counts and computes the difference between profile scores of both allelic variants in the polymorphic position. Larger positive values of this difference are indicative that the studied substitution is rarely or never observed in the protein family. The model for evaluating rare alleles, dense mapping of regions identified by genome-wide association studies, and analysis of natural selection were used to obtain the confidence score. Finally, the energy profiles were generated using ANOLEA program to identify the likely stability of the mutant protein structure as compared with its wild type.[7]

RESULTS

The pattern of inheritance in this 444-member family (200 males and 244 females) is consistent with X-linked dominant mode of inheritance with reduced penetrance. The age distribution of affecteds is from 6 to 90 years and onset was between 3 and 6 months of age. The family is highly consanguineous with 19 visible consanguineous marriages, and the phenotype was present in 71 members of the pedigree. The partial pedigree structure is shown in Figure 1a. All the individuals examined showed involuntary asymmetric pendular eye movements with unidirectional jerky NYS. Head tilt was observed in 20 out of 36 affecteds. No other anomalies were found apart from NYS including visual acuity, color vision, optic nerves, and retina.



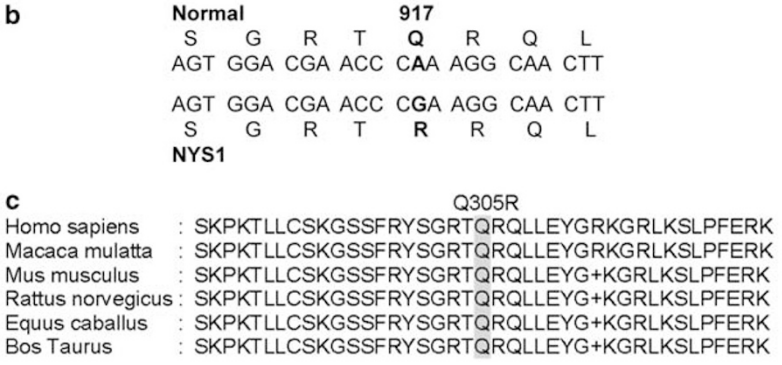


Figure 1 (**a**) Partial pedigree of UR031 with NYS1. Affected individuals are shown with blackened symbols, and normal individuals are shown with clear symbols. Individuals indicated by a cross are deceased. Deceased and individuals with question mark were not examined. (**b**) Comparison of normal and derived amino acid sequences due to the missense mutation. (**c**) Conservation of amino acids is altered by missense mutations in the highly conserved residue of FERM-adjacent (FA) domain. Amino-acid sequence comparison in several related proteins. The six proteins depicted are human, *Macaca mulatta*, *Mus musculus*, *Rattus norvegicus*, *Equus caballus,* and *Bos Tauru.* The *FRMD7* mutation is indicated above the aligned sequence, with the amino acid shaded in the alignment.

Sequence analysis of all affecteds and carriers in family (UR031) revealed a missense mutation, c.A917G, in exon 10, which would result in substitution of Arg for Gln at codon 305 (Q305R) (Figure 1b). The same mutation was not observed in unaffected males/females nor in the 100 normal controls from the same geographic region.

The structure prediction of human wild-type and mutant *FRMD7* has revealed that the single nucleotide change (A>G) at position 305 results in the substitution of the amino acid glutamine (Q) in the wild type by arginine (R) in the mutant phenotype. This change in amino acid led to a change in secondary structure from helix to a coil (Figures 2a–d). Additionally, PolyPhen-2 prediction of functional effect(s) of nsSNPs showed a position-specific independent count score of 0.824. The confidence score obtained with the sensitivity value of 0.81 and specificity 0.90 predicted this mutation to be damaging to protein structure and/or function of the mutant. The stability of a protein structure can be predicted from energy calculations for each amino acid in a protein chain. If the energy of each amino acid in a given protein chain is negative, the protein is considered to be structurally stable. Based on the energy calculation using ANOLEA, a favorable energy environment would be damaged by this amino-acid substitution.

As the modeled protein structure of the mutant containing R in place of Q would be energetically unstable (Figures 2e–f), the mutation at position 305 changes the energy environment of the protein. The amino acids in the mutated protein tend to have positive energy values as compared with the wild-type protein, thereby disrupting the structural stability of the mutated protein.[8]

DISCUSSION

FRMD7 mutations account for almost 50% of Western families with NYS and ~5% of the sporadic cases.[9] To date, 44 *FRMD7* gene mutations have been identified in various NYS families suggesting that this gene has an important role in the NYS development.[8–24] The majority of these mutations were clustered in the highly conserved FERM-C domain in exons of 7–9 (Table 1).

The present novel c.A917G (Q305R) mutation was observed as hemizygous in affected males ($n=9$), and homozygous ($n=8$) or heterozygous ($n=3$) in affected females. Additionally, the obligate female carriers ($n=5$) without disease had a heterozygous mutation. The disease was fully penetrant in all males with the mutation. All the affected females with homozygous mutation showed complete expression of the phenotype, including head tilt and NYS. As some of the obligate carrier females are not showing the phenotype, we are

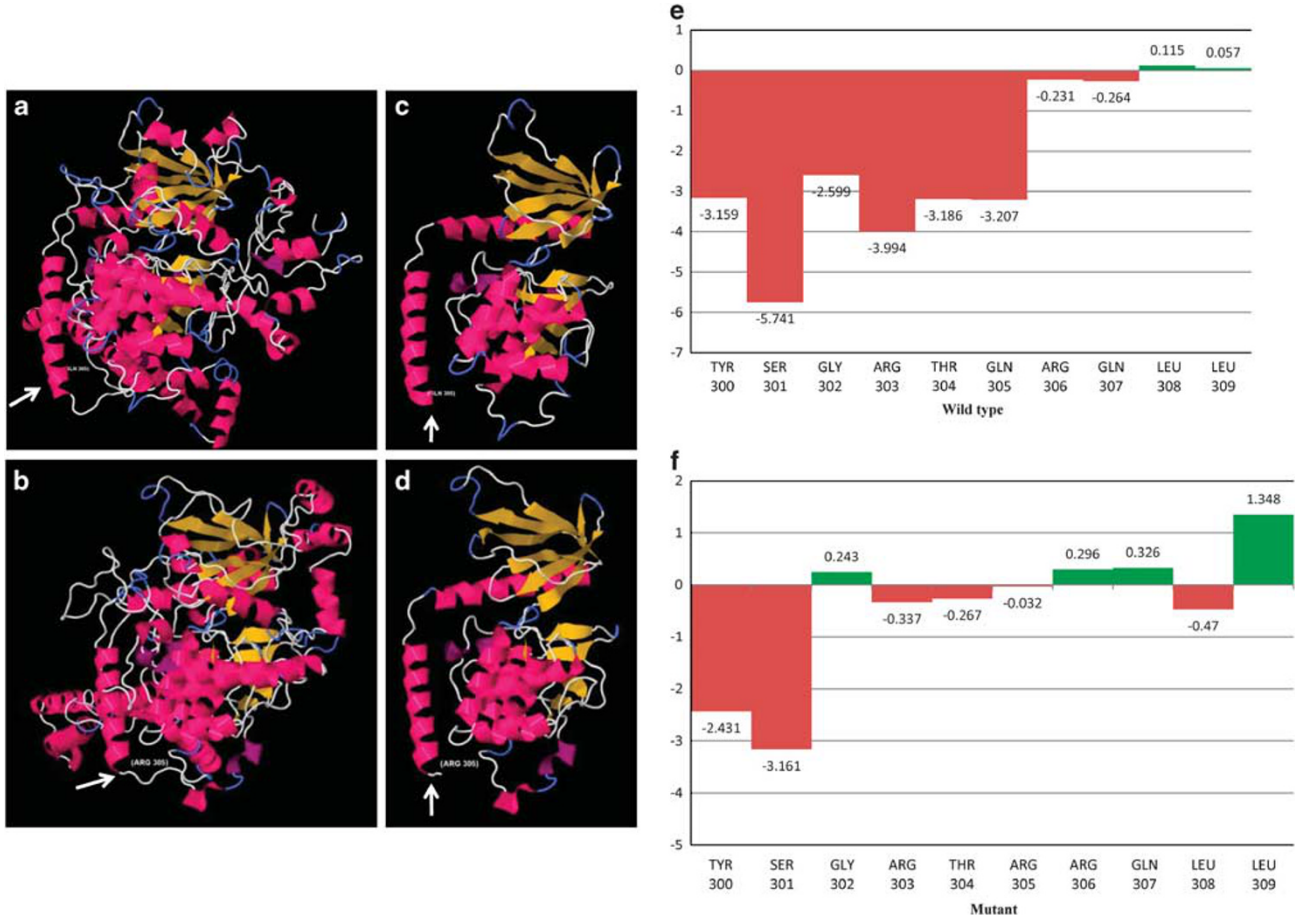


Figure 2 (**a**–**f**) Change in the secondary structure of *FRMD7* as result of mutation of amino acid in position 305. (**a**) Wild type; (**b**) mutant; (**c**) residue 1–305 of wild-type protein (**d**): Residue 1–305 of mutant. The area indicated by the arrow head shows that the helical structure of the residue 306 is changed to coil. Mean force potential plot for the protein of human wild-type (**e**) and mutant (**f**) *FRMD7*. The *y*-axis represents the energy for each amino acid of the protein at the location given in the *x*-axis. The negative energy (shown in red) shows favorable energy enviornment, whereas positive energy (shown in green) represents unfavorable enviornment. A favorable energy environment tends to be damaged because of Q to R change at 305.

Table 1 FRMD7 Mutations in NYS Families

Exons	*DNA change*	*Amino acid change*	*Mutation type*	*Family history*	*Reference*
Ex 1	41_43delAGA	K14del	Deletion	England/Chinese	Tarpey *et al*[9]; Zhang *et al*[22]
Ex 2	58C>T	Q20X	Truncating	German	Schorderet *et al*[17]
Ex 2	70G>T	G24W	Missense	Chinese	Li *et al*[16]
Ex 2	70G>A	G24R	Missense	Chinese/Ireland	Tarpey *et al*[9]; Zhang *et al*[22]
Ex 2	71G>A	G24E	Missense	Austria	Tarpey *et al*[9]
Ex 4	252G>A	V84V	Silent	England	Tarpey *et al*[9]
Ex 6	425T>G	L142R	Missense	Ireland/American-2	Tarpey *et al*[9]; Shiels *et al*[19]
Ex 6	436C>T	R146W	Missense	Chinese	Zhang *et al*[22]
Ex 6	479insT	H160fs	Truncating	England	Tarpey *et al*[9]
Ex 7	601C>T	Q201X	Truncating	Italy–Germany	Tarpey *et al*[9]
Ex 7	623A>G	H208R	Missense	Chinese	Li *et al*[8]
Ex 8	661A>G	N221D	Missense	England	Tarpey *et al*[9]
Ex 8	673T>G	W225G	Missense	Switzerland	Schorderet *et al*[17]
Ex 8	676G>A	A226T	Missense	England	Tarpey *et al*[9]
Ex 8	685C>T	R229C	Missense	Chinese	Zhang *et al*[22]
Ex 8	686C>G	R229G	Missense	Turkish	Kaplan *et al*[13]
Ex 8	689-690delAG	S232del	Deletion	Chinese	Li *et al*[16]
Ex 8	691T>G	L231V	Missense	Ireland–Germany	Tarpey *et al*[9]
Ex 9	781C>G	A261G	Missense	Chinese	Zhang *et al*[22]
Ex 9	782G>A	R260Q	Missense	Chinese-2	Li *et al*[16]
Ex 9	796G>C	A266P	Missense	England-2	Tarpey *et al*[9]
Ex 9	880insA	293fs	Truncating	England	Self *et al*[18]
Ex 9	812G>A	C271Y	Missense	Scotland	Tarpey *et al*[9]
Ex 9	812G>T	C271F	Missense	Chinese	Li *et al*[16]; He *et al*[12]
Ex 9	824A>C	H275P	Missense	American	Schorderet *et al*[17]
Ex 9	886G>C	G296A	Missense	Chinese	Zhang *et al*[22]
Ex 9	887delG	G296fs	Truncating	Austria	Tarpey *et al*[9]
Ex 9	902A>G	Y301C	Missense	England	Tarpey *et al*[9]
Ex 10	910C>T	R303X	Truncating	Chinese	Li *et al*[16]
Ex 10	917A>G	Q305R	Missense	Indian	Present Study
Ex 11	1003C>T	R335X	Truncating	England-2/Indian/Chinese	Tarpey *et al*[9]; Zhang *et al*[22]
Ex 11	1019C>T	S340L	Missense	Romania	Tarpey *et al*[9]
Ex 12	1262delC	P421fs	Truncating	England	Tarpey *et al*[9]
Ex 12	1274-1275delTG	426 to 429	Deletion	Chinese	He *et al*[12]
Ex 12	1486-1489delTTTT	F497fs26X	Deletion	Chinese	Du *et al*[24]
In 1	IVS2+5G>A		Truncating	England	Tarpey *et al*[9]
In 1	57+5G>A		Splice	German	Schorderet *et al*[17]
In 11	IVS11+1G>C		Truncating	Germany	Tarpey *et al*[9]
In 3	IVS3+2T>G		Truncating	England	Tarpey *et al*[9]
In 4	IVS4+1G>A		Truncating	England-2	Tarpey *et al*[9]; Self *et al*[18]
In 6	676-2A>G		Splice	American	Schorderet *et al*[17]
In 7	IVS7+1G>C		Truncating	Madagascar	Tarpey *et al*[9]
Deletion	Exons 2–4del		Truncating	American	Fingert *et al*[10]
Variant	1050+5G >A		Splice	Saudi Arabia	Khan *et al*[23]

concluding that this could be due to incompletely penetrant dominant gene. This is consistent with a previously published report.[25] The clinical significance of hemi/homozygous as compared with heterozygous mutation is not yet known, although Kaplan *et al*[13] found a missense mutation, R229G, in the *FRMD7* gene in heterozygous and homozygous condition in members of a large X-linked NYS family without phenotypic variation.

The location of the present residues is highly conserved in multiple species (Figure 1c) suggesting that the mutation at codon 305 is likely to have a significant effect on FRMD7 protein functionality. As the present reported mutation is located in a highly conserved residue of the FERM-adjacent domain and possessed similarity to other protein kinase substrates, we also hypothesized that this genetic region has regulatory functions. Further, the FERM-C domain was a mutation-rich region, suggesting that this region should be treated as the most important candidate region when screening for mutations. Prediction and energetic analysis of the structure of mutant and wild-type proteins revealed a change in the secondary structure from helix to a coil that led to an energetically unstable protein. Similarly, Li *et al*[8] reported a predicted unstable target protein of human *FRMD7* with a missense mutation (H208R) in a different location within the gene.

To our knowledge, this is the first report of homozygous, hemizygous, and heterozygous *FRMD7* mutations in the same multigenerational family with isolated non-syndromic NYS with many consanguineous loops. The present data extend the mutation spectrum of *FRMD7* gene mutations to a novel functional domain downstream from the FERM-C domain of the protein.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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WEB RESOURCES

Ensembl, http://www.ensembl.org/Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for *FRMD7, NYS1, NYS2, NYS3, NYS4, NYS5, and NYS6*)

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)