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Mutations in a novel member of the FERM family, *FRMD7* cause X-linked idiopathic congenital nystagmus (NYS1)

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URLs
Online Mendelian Inheritance in Man and BLAST are found at http://wwww.ncbi.nlm.nih.gov/. The HUGO nomenclature site is at http://www.gene.ucl.ac.uk/nomenclature/). The electronic expression profile of LOC90167 is at http://symatlas.gnf.org/SymAtlas/). LOC90167 RefSeq DNA: NM_194277, FARP1 AB018336 and FARP2 AB008430.

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Abstract

Idiopathic congenital nystagmus (ICN) is characterised by involuntary, periodic, predominantly horizontal, oscillations of both eyes. We identified 22 mutations in *FRMD7* in 26 families with X-linked idiopathic congenital nystagmus. Screening of 42 ICN singleton cases (28 male, 14 females) yielded three mutations (7%). We found restricted expression of *FRMD7* in human embryonic brain and developing neural retina suggesting a specific role in the control of eye movement and gaze stability.

The prevalence of idiopathic congenital nystagmus (ICN) is estimated to be 1 in 1000. In ICN visual function can be significantly reduced due to constant eye movement, but the degree of visual impairment varies[1,2]. The disease is likely to be due to abnormal development of areas in the brain controlling eye movements and gaze stability[3]. ICN is distinct from other hereditary causes of nystagmus and ocular pathology, including ocular albinism, congenital stationary night blindness, achromatopsia, blue cone monochromatism and sensory visual defects of early childhood such as congenital cataract, retinitis pigmentosa, cone-rod dystrophy and optic nerve hypoplasia [4].

ICN is usually inherited as an X-linked trait with incomplete penetrance in females. Most families map to Xq26-q27 and the locus (known as *NYS1*) has previously been mapped to a 12 Mb interval between markers DXS9909 and DXS1211[5,6]. Zhang et al (2005) proposed further reduction of the candidate region to an interval between DXS8033 and DXS8043 based on a recombination event in a clinically unaffected female[7]. X-linked genetic heterogeneity has been suggested on the basis of a single ICN family that is reported to map to Xp11.4- Xp11.3[8].

We screened 16 families with X-linked ICN using 17 markers extending from Xq26-Xq27 (see supplementary figure 1) [6]. In these families the disease was fully penetrant in males and 50 % penetrant in females. The phenotype was variable even within families (see supplementary figure 2 and supplementary video 1,2 and 3). In all 16 families marker haplotypes were compatible with linkage to Xq26-q27. Recombinant events in affected males in family N1 refined the location of *NYS* to a 7.5 Mb interval between markers DXS1047 and DXS1041 (see figure 1a and 1b).

The candidate interval contained >80 genes and high throughput DNA sequence analysis was performed of all coding exons of all genes within this interval [9]. DNA from one affected male individual from each of the 16 linked families was screened for mutations.

Mutations were detected in 15/16 of the linked families in *FRMD7* (FERM domain containing 7, previously known as *LOC90167*) at Xq26.2 having screened >40 genes by sequence analysis (see figure 1c). *FRMD7* has 12 exons and encodes a novel member of the protein 4.1 superfamily (RefSeq DNA: NM_194277), (http://www.gene.ucl.ac.uk/nomenclature/). All mutations identified in *FRMD7* co-segregated with disease in the linked families and were absent from 300 male control chromosomes (see table 1). The nonsense mutations Q201X and R335X predict truncated proteins containing 28% and 47% of the protein respectively. Four of the five splice site mutations were at conserved splice donor residues (position +1 and +2) and are thus predicted to be pathological by classical exon skipping and nonsense mediated decay. For

the mutation IVS2 +5G>A, in family N7, compared to controls negligible amounts of transcript could be detected through amplification of exons 1-5 in lymphocytes suggesting that this is also disease-associated (see supplementary figure 3a). The silent variant, G252A, V84V in family N5, created a novel splice acceptor site within exon 4 which results in loss of transcript containing exon 1-5 sequence and the rare presence of a transcript with exon 4 skipped in lymphocytes (see Figure 3a and b supplementary data).

The seven missense mutations at amino acid positions 24, 142, 231, 266, 271 and 301 in the linked families not only involve highly conserved residues that are invariant in *Rattus, Mus, Gallus, Xenopus* but are also located within invariant blocks of highly conserved residues suggesting that mutations at these locations are critical to the normal function of the protein. Residues at position 142, 231, 271 and 301 are further conserved in *Tetraodon*. Furthermore, with the exception of L231V the mutations are largely non-conservative in function: G24R, L142R, A266P, C271Y, Y301C. We modelled the effects of these mutations on the three dimensional structure of the protein by mapping them onto the closest orthologue of known structure which is the core domain of the cytoskeletal protein 4.1R (1GG3 in the Protein Data Bank)[10,11]. The crystal structure extends from residues 1-279 and therefore all but Y301C of the missense mutations in *FRMD7* in the linked families could be mapped. The structural environment of the disease-associated missense mutations was inspected using the program Coot, which allows the identity and conformation of residues to be manipulated easily[12]. Although the effect on the structure of L231V is not clearly apparent, mutations G24R, L142R and C271 are likely to destabilise the protein by the introduction of larger amino acids within restricted areas of the protein and the introduction of a proline residue at position 266 (A266P) will disrupt a helical domain in the wild-type structure.

From our study we conclude that mutations in *FRMD7* are a major cause of familial X-linked congenital motor nystagmus. We then assessed the prevalence of mutations in *FRMD7* in smaller families where linkage data was not available and in a cohort of males and females with ICN but with no family history of the condition. We screened 14 families with two or more affected individuals of either sex and found mutations in 8/14 (57%). We also identified mutations in 3/42 (7%) patients without a family history that had undergone careful clinical and electrophysiological investigation to exclude other causes of inherited congenital nystagmus (see Table 1). Mutations were identified in 1/14 female singletons and 2/28 male singletons and none of the novel mutations were found in 300 male control samples. The results suggest that mutation analysis of *FRMD7* may be considered of diagnostic value even in isolated cases of either sex.

Expression analysis of *FRMD7* shows that the mRNA is present in most tissues but at low levels (http://symatlas.gnf.org/SymAtlas/). This was confirmed by RT-PCR with expression detected in human adult kidney, liver, pancreas and at low levels in heart and brain (data not shown). In human fetal tissue the transcript was only detectable in kidney by this method.

We then performed *in situ* hybridisation experiments in human embryo brain to investigate whether expression of *FRMD7* was localised or restricted. In embryos 56 dpo there is expression in the ventricular layer of the forebrain, midbrain and cerebellar primordium, spinal cord and in the developing neural retina. In earlier embryos (37 dpo) the expression is restricted to the mid and hind-brain, regions known to be involved in motor control of eye movement (see figure 1d).

The functional role of FRMD7 protein is unknown but BLAST (http://www.ncbi.nlm.nih.gov/) search analysis detected close amino acid sequence

homology to FARP1 (FERM, RhoGEF and pleckstrin domain protein 1; chondrocyte-derived ezrin-like protein) (AB018336) and FARP2 (AB008430). The homology is concentrated at the N terminus of the protein where a B41 and a FERM-C domain are present. The B41 domain extends from residues 1-182 and the FERM-C domain is located from residue 175-268 in FRMD7. The location of mutations relative to these domains is shown in supplementary figure 3c. The homologous protein FARP2 modulates the length and the degree of branching of neurites in rat embryonic cortical neurons and reorganises the cytoskeleton. Overexpression of FARP2 results in increased numbers of lateral growth cones extending from neurites and associated decrease in total length of the neurites per neuron [13,14]. Whether the function of FRMD7 is similar to FARP2 in specialised neuronal pathways governing integration and coordination of eye movements remains to be proved. The hypothesis that null mutations in *FRMD7*, as found in families with X-linked congenital motor nystagmus, alter the neurite length and degree of branching of neurons as they develop in the midbrain, cerebellum and retina is a plausible explanation of how defects in the protein coded for by *FRMD7* causes disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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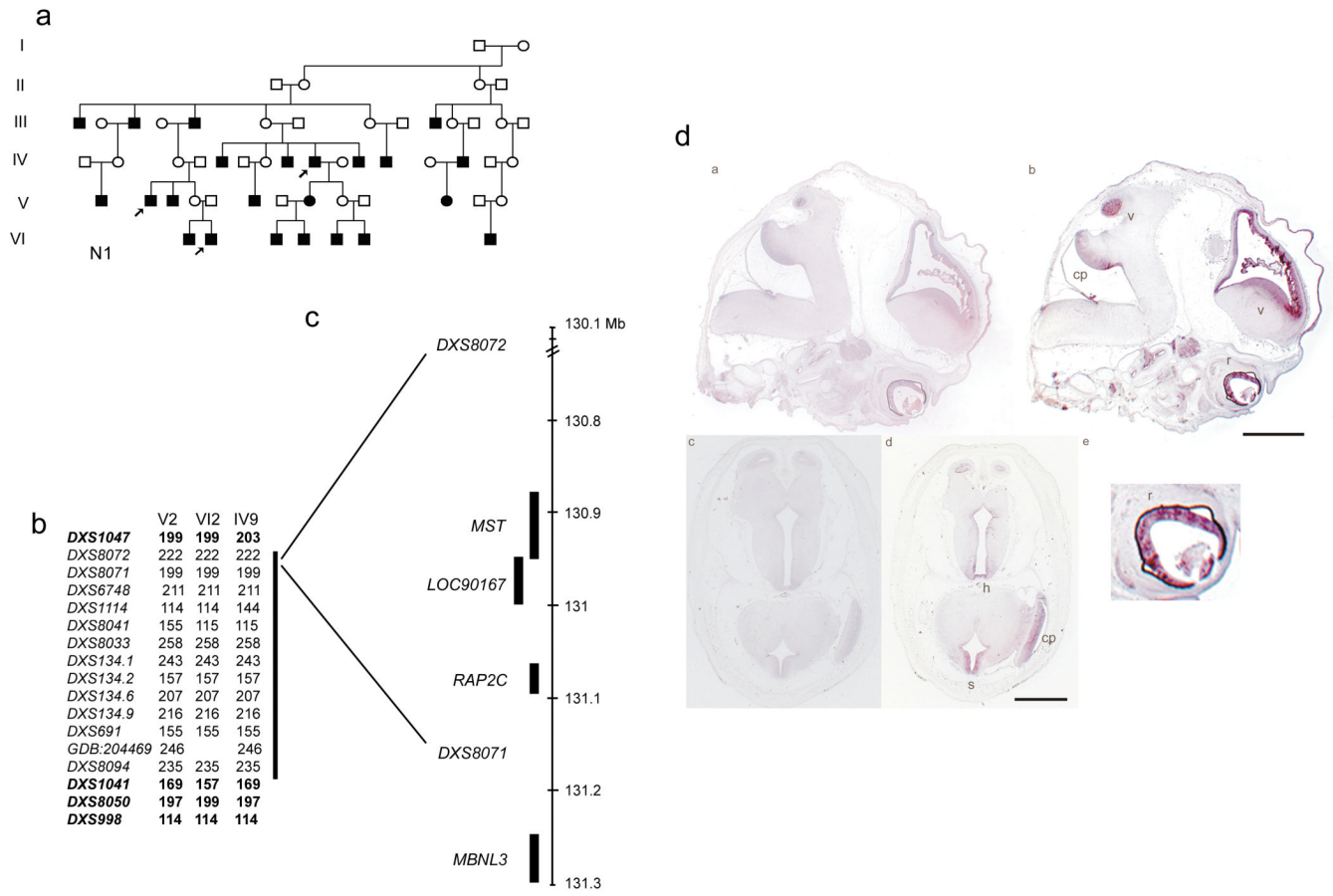

Figure 1. Refinement of the linkage interval to Xq26 and the pattern of expression of *FRMD7* in brain of human embryos 56 dpo by *in situ* hybridization

(a) shows the pedigree N1 with the critical individuals marked with an arrow whose haplotype define the minimum critical linkage interval (b) the allele size for marker *DXS1047* is discordant in individual IV9 with the other affected males, V2 and VI2 and the allele size at markers *DXS1041* and *DXS8050* in individual VI2 is discordant as compared to individuals V2 and IV9 (c) the location of *LOC90167* is shown relative to the linkage markers *DXS8072* and *DXS807* (d) A 599 bp and a 681 bp probe from unique sequence in the 3 UTR of *FRMD7* were used as probes for *in situ* hybridization. Panels a and c show in situ hybridization results using sense probes, b and d use antisense probes in similar anatomical locations. Panels a and b are sagittal sections showing the lateral ventricle(v), cerebellar peduncle (cp) and developing neural retina(r). Panel e is an enlargement of panel b to illustrate the retina. Panels c and d are transverse sections showing the spinal cord (s), hypothalamus (h) and cerebellar peduncle. In panel b the scale bar = 2000μm and in panel d =1800μm.

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Table 1

Mutations in *FRMD7*

Families where linkage was performed are prefixed by N, those that are familial but no linkage data was available are prefixed F, and singleton males are prefixed SM and singleton females SF. The class of mutation is categorized as T=truncating, M= missense, S=silent and del= deletion. The country of origin is denoted. All mutations identified were not found in 300 control male chromosomes. The reference cDNA sequence NM_194277 is used as a basis for numbering the nucleotide of the mutation. All mutations are located relative to the A of the first coding ATG at position 179. The reference protein sequence NP_919253 is used as the basis for numbering the amino acid mutation starting from the first methionine at position 1. The reference sequence for the genomic sequence is AL49792.

Sample	Class	Mutation	Origin
N15	M	G70A, G24R	Ireland
N7	T	IVS2+5G>A	England
N4	T	IVS3+2 T>G	England
N5	S	G252A, V84V	England
N1, F26	T	IVS4+1G>A	England, England
N16	M	T425G, L142R	Ireland
N13	T	IVS7+1G>C	Madagascar
N2	T	C601T, Q201X	Italy-Germany
N 3	M	T691G, L231V	Ireland -Germany
N6, SF21	M	G796C, A266P	England, England
N11	M	G812A, C271Y	Scotland
N14	T	887delG, G296fs	Austria
N12	M	A902G, Y301C	England
N10, F24, SM08	T	C1003T, R335X	England, India, England
N9	T	IVS11+1G>C	Germany
F31	del	41_43delAGA, 14delI	England
F21	M	G71A, G24E	Austria
F28	T	479insT, 160fs	England
F15	M	A661G, N221D	England
F16	M	G676A, A226T	England
F20	M	C1019T, S340L	Romania
SM10	T	1262delC, 421fs	England