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# Mutations in *FRMD7*, a newly identified member of the FERM family, cause X-linked idiopathic congenital nystagmus

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**Idiopathic congenital nystagmus is characterized 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 singleton cases of idiopathic congenital nystagmus (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 1,000. In ICN, visual function can be significantly reduced owing 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 (refs. 5,6). Others have proposed a 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 (ref. 8).

We screened 16 families with X-linked ICN using 17 markers extending from Xq26–Xq27 (**Supplementary Fig. 1** online)[6]. In these families, the disease was fully penetrant in males and ~50% penetrant in females. The phenotype was variable even within families (**Supplementary Fig. 2** and **Supplementary Videos 1**, **2** and **3** online). In all 16 families, marker haplotypes were compatible with linkage to Xq26–q27 (**Supplementary Methods** online). Recombinant events in affected males in family N1 refined the location of *NYS1* to a ~7.5-Mb interval between markers DXS1047 and DXS1041 (**Fig. 1a**,**b**).

The candidate interval contained >80 genes. We performed high-throughput DNA sequence analysis 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.

We detected mutations in *FRMD7* (FERM domain–containing 7, previously known as *LOC90167*) at Xq26.2 in 15/16 of the linked families after screening >40 genes by sequence analysis (**Fig. 1c**). *FRMD7* has 12 exons and encodes a previously unidentified member of the protein 4.1 superfamily (RefSeq accession number NM_194277). All mutations identified in *FRMD7* cosegregated with disease in the linked families and were absent from 300 male control chromosomes (**Table 1**). The nonsense mutations leading to 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

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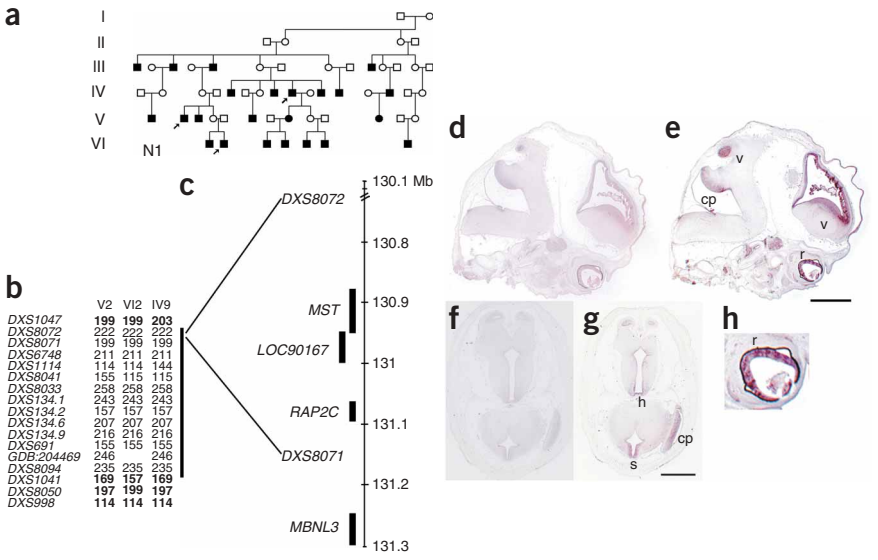


**Figure 1** Refinement of the linkage interval to Xq26 and the pattern of expression of *FRMD7* in brain of human embryos ~56 d.p.o. by *in situ* hybridization. (**a**) Pedigree of family N1. Arrow marks critical individuals whose haplotypes define the minimum critical linkage interval. (**b**) Allele size for marker *DXS1047* in individual IV9 is discordant compared with the other affected males, V2 and VI2, and the allele size at markers *DXS1041* and *DXS8050* in individual VI2 is discordant compared with individuals V2 and IV9. (**c**) Location of *LOC90167* (*FRMD7*) relative to the linkage markers *DXS8072* and *DXS8071*. (**d**–**h**) 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. **d** and **f** show *in situ* hybridization results using sense probes, and **e** and **g** use antisense probes in similar anatomical locations. **d** and **e** are sagittal sections showing the lateral ventricle (v), cerebellar peduncle (cp) and developing neural retina (r). **h** is an enlargement of **e**, illustrating the retina. **f** and **g** are transverse sections showing the spinal cord (s), hypothalamus (h) and cerebellar peduncle (cp). Scale bars: **e**, 2,000 μm; **g**, 1,800 μm. The study was approved by the University of Leicester Ethics Committee. Written informed consent was obtained from all study participants.

nonsense-mediated decay. In family N7, whose members had the mutation IVS2 +5G→A, we detected negligible amounts of transcript in the proband by amplification of exons 1–5 in lymphocytes compared with quantities in controls, suggesting that this is also disease associated (**Supplementary Fig. 3**, **Supplementary Methods** and **Supplementary Table 1** online). The silent variant, G252A (V84V in family N5), created a new splice acceptor site within exon 4 that resulted in the loss of transcript containing the sequence of exons 1–5 and the rare presence of a transcript with exon 4 skipped in lymphocytes (**Supplementary Fig. 3**).

The six missense mutations resulting in amino acid substitutions at positions 24, 142, 231, 266, 271 and 301 in the linked families not only involve highly conserved residues that are invariant in *Rattus norvegicus*, *Mus musculus*, *Gallus gallus* and *Xenopus tropicalis* 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 nigroviridis*. Furthermore, with the exception of L231V, the mutations lead to changes in amino acids that are largely nonconservative in function (G24R, L142R, A266P, C271Y and Y301C). We modeled the effects of these mutations on the three-dimensional structure of the protein by mapping them onto the closest ortholog of known structure: the core domain of the cytoskeletal protein 4.1R (1GG3 http://www.pdb.org/pdb/navbarsearch.do?=All&inputQuickSearch=1gg3 in the Protein Data Bank)[10,11]. The crystal structure extends from residues 1–279, and therefore we were able to map all the changes in amino acids resulting from missense mutations in *FRMD7* except the one leading to Y301C in the linked families. We inspected the structural environment of the disease-associated missense mutations 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 leading to G24R, L142R and C271 are likely to destabilize 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 the results above, we concluded 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 in which linkage data was not available and in a cohort of males and females with ICN but without a 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%) individuals without a family history who had undergone careful clinical and electrophysiological investigation to exclude other causes of inherited congenital nystagmus (**Table 1**). We identified mutations in 1/14 female singletons and 2/28 male singletons, and none of the new mutations was found in samples from 300 male control individuals. 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 at low levels (http://symatlas.gnf.org/SymAtlas/). We confirmed this by RT-PCR, detecting expression in human adult kidney, liver, pancreas and, at low levels, heart and brain (data not shown). Using this method in human fetal tissue, we detected the transcript only in kidney.

We then performed *in situ* hybridization experiments in human embryonic brain to investigate whether expression of *FRMD7* was localized or restricted. In embryos ~56 d post-ovulation (d.p.o.), there is expression in the ventricular layer of the forebrain, midbrain, cerebellar primordium, spinal cord and the developing neural retina. In earlier embryos (~37 d.p.o.) the expression is restricted to the mid- and hindbrain, regions known to be involved in motor control of eye movement (**Fig. 1d–h**).

The functional role of FRMD7 protein is unknown, but we detected close amino acid sequence homology to FARP1 (FERM, RhoGEF and pleckstrin domain protein 1; chondrocyte-derived ezrin-like protein; NM_005766) and FARP2 (NM_014808) by BLAST search analysis (http://www.ncbi.nlm.nih.gov/). The homology is concentrated at the N terminus of the protein, where B41 and FERM-C domains are present. The B41 domain is located at residues 1–192, and the FERM-C domain is located at residues 186–279 in FRMD7. The location of mutations relative to these domains is shown in **Supplementary Figure 3**. The homologous protein FARP2 modulates the length and the degree of branching of neurites in rat embryonic cortical neurons

**Table 1 Mutations in *FRMD7* and variations in FRMD7**

| Sample | Class | Mutation/variation | Origin |
|---|---|---|---|
| N15 | M | G70A, G24R | Ireland |
| N7 | T | IVS2+5G→A | England |
| N4 | T | IVS3+2T→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 |
| N3 | 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 |

Families where linkage was performed are prefixed by N; those that are familial but for which no linkage data were available are prefixed F. Singleton males are prefixed by 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 variation starting from the first methionine at position 1. The reference sequence for the genomic sequence is AL49792.

and reorganizes 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 specialized 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.

*Note: Supplementary information is available on the Nature Genetics website.*

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**AUTHOR CONTRIBUTIONS**

This study was designed by I.G., R.C.T., F.L.R., M.R.S., S.T. and N.S.; phenotype assessment was performed by I.G., S.T., N.S., E.O.R., M.A., M.S., R.J.M., R.D.R., A.L., S.L., M.K., G.W., R.P.G., C.D., K.D., I.A., A.A.Z., C.P., O.C.B., S.J., M.C.B., D.G.H. and R.W.H.; DNA extraction, linkage analysis, sequencing, *in situ* hybridization and molecular modeling were performed by F.L.R., P.T., S.T., R.C.T., U.M., S.L., C.J.T., E.O.R., M.S., C.D.V., R.D.M., L.B., C.S.C., R.J.R., S.E., S.O., A.P., C.S., J.T., R.W. and P.A.F. and the paper was written by F.L.R., I.G., P.T., M.R.S. and S.T.

**COMPETING INTERESTS STATEMENT**

The authors declare that they have no competing financial interests.

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1. Stayte, M., Reeves, B. & Wortham, C. *Br. J. Ophthalmol.* **77**, 228–232 (1993).
2. Pilling, R.F., Thompson, J.R. & Gottlob, I. *Br. J. Ophthalmol.* **89**, 1278–1281 (2005).
3. Jacobs, J.B. & Dell'Osso, L.F. *J. Vis.* **4**, 604–625 (2004).
4. Gottlob, I.Nystagmus. *Curr. Opin. Ophthalmol.* **12**, 378–383 (2001).
5. Kerrison, J.B., Vagefi, M.R., Barmada, M.M. & Maumenee, I.H. *Am. J. Hum. Genet.* **64**, 600–607 (1999).
6. Kerrison, J.B., Giorda, R., Lenart, T.D., Drack, A.V. & Maumenee, I.H. *Ophthalmic Genet.* **22**, 241–248 (2001).
7. Zhang, B. *et al. Hum. Genet.* **116**, 128–131 (2005).
8. Cabot, A. *et al. Am. J. Hum. Genet.* **64**, 1141–1146 (1999).
9. Tarpey, P. *et al. Am. J. Hum. Genet.* **75**, 318–324 (2004).
10. Berman, H., Henrick, K. & Nakamura, H. *Nat. Struct. Biol.* **10**, 980 (2003).
11. Han, B.G., Nunomura, W., Takakuwa, Y., Mohandas, N. & Jap, B.K. *Nat. Struct. Biol.* **7**, 871–875 (2000).
12. Emsley, P. & Cowtan, K. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132 (2004).
13. Kubo, T. *et al. J. Neurosci.* **22**, 8504–8513 (2002).
14. Toyofuku, T. *et al. Nat. Neurosci.* **8**, 1712–1719 (2005).