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The clinical and molecular genetic features of idiopathic infantile periodic alternating nystagmus

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Periodic alternating nystagmus consists of involuntary oscillations of the eyes with cyclical changes of nystagmus direction. It can occur during infancy (e.g. idiopathic infantile periodic alternating nystagmus) or later in life. Acquired forms are often associated with cerebellar dysfunction arising due to instability of the optokinetic-vestibular systems. Idiopathic infantile periodic alternating nystagmus can be familial or occur in isolation; however, very little is known about the clinical characteristics, genetic aetiology and neural substrates involved. Five loci (NYS1-5) have been identified for idiopathic infantile nystagmus; three are autosomal (NYS2, NYS3 and NYS4) and two are X-chromosomal (NYS1 and NYS5). We previously identified the FRMD7 gene on chromosome Xq26 (NYS1 locus); mutations of FRMD7 are causative of idiopathic infantile nystagmus influencing neuronal outgrowth and development. It is unclear whether the periodic alternating nystagmus phenotype is linked to NYS1, NYS5 (Xp11.4-p11.3) or a separate locus. From a cohort of 31 X-linked families and 14 singletons (70 patients) with idiopathic infantile nystagmus we identified 10 families and one singleton (21 patients) with periodic alternating nystagmus of which we describe clinical phenotype, genetic aetiology and neural substrates involved. Periodic alternating nystagmus was not

detected clinically but only on eye movement recordings. The cycle duration varied from 90 to 280 s. Optokinetic reflex was not detectable horizontally. Mutations of the *FRMD7* gene were found in all 10 families and the singleton (including three novel mutations). Periodic alternating nystagmus was predominantly associated with missense mutations within the *FERM* domain. There was significant sibship clustering of the phenotype although in some families not all affected members had periodic alternating nystagmus. *In situ* hybridization studies during mid-late human embryonic stages in normal tissue showed restricted *FRMD7* expression in neuronal tissue with strong hybridization signals within the afferent arms of the vestibulo-ocular reflex consisting of the otic vesicle, cranial nerve VIII and vestibular ganglia. Similarly within the afferent arm of the optokinetic reflex we showed expression in the developing neural retina and ventricular zone of the optic stalk. Strong *FRMD7* expression was seen in rhombomeres 1 to 4, which give rise to the cerebellum and the common integrator site for both these reflexes (vestibular nuclei). Based on the expression and phenotypic data, we hypothesize that periodic alternating nystagmus arises from instability of the optokinetic-vestibular systems. This study shows for the first time that mutations in *FRMD7* can cause idiopathic infantile periodic alternating nystagmus and may affect neuronal circuits that have been implicated in acquired forms.

Keywords: periodic alternating nystagmus; *FRMD7*; optokinetic reflex; vestibulo-ocular reflex; *in situ* hybridization

Abbreviation: PAN = periodic alternating nystagmus

Introduction

Nystagmus is defined as the involuntary rhythmic oscillations of the eyes, with a reported prevalence of approximately 2.4 in 1000 (Sarvananthan *et al.*, 2009). There is significant negative social stigma and relatively poor visual function scores reported with this condition (Pilling *et al.*, 2005). In infantile nystagmus, these oscillations are horizontal and conjugate and are characterized by two components: (i) a slow drift of the eyes (called the slow phase); followed by (ii) a corrective fast eye movement (called the quick phase) that is responsible for realigning the fovea to the object of interest. In idiopathic infantile periodic alternating nystagmus (PAN), the direction of the quick phase and slow phase alternates periodically with time. This phenotype of nystagmus is distinct from other nystagmus forms due to the periodic time component. Acquired forms of PAN are also reported and arise due to instability of the vestibulo-optokinetic systems (Leigh *et al.*, 1981). Animal and mathematical models for acquired PAN have demonstrated how instability of the velocity storage mechanism for vestibular eye movements and an adaptive mechanism for this instability can result in a periodicity of oscillations of 4 min (Waespe *et al.*, 1985; Leigh and Khanna, 2006). It has also been shown that patients with acquired PAN have abnormalities of optokinetic nystagmus, with some patients having no optokinetic nystagmus response and the PAN cycle continues through the optokinetic nystagmus stimuli (Baloh *et al.*, 1976). The acquired

form of PAN often responds well to specific drugs such as baclofen (Halmagyi *et al.*, 1980).

Idiopathic infantile nystagmus is a genetically heterogeneous condition with X-linked, autosomal dominant and autosomal recessive modes of inheritance reported. To date, five chromosomal loci (NYS 1–5) have been described in the literature associated with idiopathic infantile nystagmus (Table 1). Within the NYS1 locus (Xq26.2), the *FRMD7* gene was identified, mutations of which cause X-linked idiopathic infantile nystagmus (Tarpey *et al.*, 2006; Schorderet *et al.*, 2007; Zhang *et al.*, 2007). Previous studies have shown that the penetrance among female carriers is ~50% (Tarpey *et al.*, 2006; Thomas *et al.*, 2008). Expression of *FRMD7* has been shown in neuronal tissue in the developing retina, mid and hind brain (Tarpey *et al.*, 2006; Betts-Henderson *et al.*, 2009), although it is not clear which specific gaze control systems are affected by mutations in the gene. Unaffected female carriers can have a subnormal optokinetic nystagmus gain (Thomas *et al.*, 2008). Recent studies in neuro-2A cells have demonstrated a role for *FRMD7* in neuronal outgrowth and development (Betts-Henderson *et al.*, 2009).

Idiopathic infantile PAN is considered to be a subtype of idiopathic infantile nystagmus; however, its diagnosis has different implications for pharmacological and surgical treatment (Reinecke, 1997). The first report of familial idiopathic infantile PAN was by Huygen and colleagues (1995), where both mother and daughter were reported to have PAN since birth.

Table 1 The nystagmus loci

Locus and name	OMIM™ number	Gene identified	Inheritance	Key publication
NYS 1 (Xq26.2)	310700	Yes (*FRMD7*)	X-linked	(Tarpey *et al.*, 2006)
NYS 2 (6p12)	164100	No	Autosomal dominant	(Kerrison *et al.*, 1996)
NYS 3 (7p11.2)	608345	No	Autosomal dominant	(Klein *et al.*, 1998)
NYS 4 (13q31-33)	193003	No	Autosomal dominant	(Ragge *et al.*, 2003)
NYS 5 (Xp11.4-p11.3)	300589	No	X-linked	(Cabot *et al.*, 1999)

OMIM™ = Online Mendelian Inheritance in Man.

Shallo-Hoffmann *et al*. (1999, 2004) reported a case of idiopathic infantile PAN with an X-linked history of nystagmus. Hertle *et al*. (2005) described another family spanning three generations, with a phenotype of PAN in all four patients examined with eye movement recordings. Based on an X-linked mode of inheritance and the distinct phenotype consistently seen in all generations it was assumed that a unique locus for idiopathic infantile PAN was present on the X-chromosome. However, no molecular genetic studies were performed in these families to substantiate that a separate locus is present for idiopathic infantile PAN. There is some evidence for an additional locus for idiopathic infantile nystagmus on the X-chromosome. Cabot *et al*. (1999) reported a four generation French family with X-linked idiopathic infantile nystagmus. Linkage analysis showed mapping to Xp11.4-11.3 between the polymorphic markers DXS8015 and DXS1003, however, these investigators did not describe the type of nystagmus in this family. Due to the limited phenotypic data in the literature it is currently unknown whether idiopathic infantile PAN occurs in nystagmus with other inheritance patterns. In the family described by Huygen and colleagues (1995), the inheritance pattern is not clear. In this study we present evidence showing that mutations of the *FRMD7* gene can be associated with PAN. We report three novel mutations and eight previously described mutations of the *FRMD7* gene associated with PAN. Furthermore, we highlight the spectrum of variable phenotypes associated with *FRMD7* mutations and present evidence that expression of *FRMD7* correlates to neuronal circuits that have also been associated with acquired PAN.

Materials and methods

Patients and clinical examinations

Using eye movement recordings we examined a cohort of 31 families with X-linked idiopathic infantile nystagmus and 14 singletons (total of 70 patients). Fifteen of the families were previously described to have *FRMD7* mutations (Tarpey *et al*., 2006) and four families did not have *FRMD7* mutations. For the remaining 12 families with idiopathic infantile nystagmus, the genotype was not yet determined. Among the singletons, we included the previously identified three singletons with *FRMD7* mutations (Tarpey *et al*., 2006) and 11 singletons with idiopathic infantile nystagmus that were not yet sequenced. Within this cohort we identified 10 families (Fig. 1) and one singleton with idiopathic infantile PAN (total of 21 patients). In our study population, idiopathic infantile PAN occurred in 18 males and in three females. Detailed ophthalmic examination was performed in all patients. At least one affected patient within each family underwent electrodiagnostic examinations according to the International Society for Clinical Electrophysiology of Vision standards (visually evoked potentials and electroretinography) to exclude other infantile forms of nystagmus such as those associated with albinism or retinal diseases. Eye movement recordings were performed (EyeLink II, 500 Hz, SR Research, Toronto, Canada) using a central fixation task over a prolonged period of 5 min to detect idiopathic infantile PAN. Several members of most families and all the singletons underwent the prolonged fixation task (Fig. 1).

In addition, optokinetic nystagmus was tested in all affected patients with idiopathic infantile PAN. The optokinetic nystagmus stimulus consisted of square-wave contrast gratings of 2.2° cycle size and Michelson contrast 0.88 cd/m^2. Optokinetic nystagmus was tested at 20°/s velocity in both horizontal (stimulus direction: rightwards and leftwards) and vertical directions (upwards and downwards). For further details of the experimental setup see Thomas *et al*. (2008).

Informed consent was obtained from all subjects participating in this study. The study adhered to the tenets of the Declaration of Helsinki and was approved by the local Ethics Committee.

Sequencing and mutation analysis

Bidirectional sequence analysis of the exons and intron/exon junctions of the *FRMD7* gene were performed using DNA from the proband within each of the new families and singletons with idiopathic infantile nystagmus where no genetic test for FRMD7 had been performed previously. Primer details, expected product sizes and annealing temperature are shown in Table 2. Mutation analysis software Seqscape version 2.1.1 (Applied Biosystems) was used for base calling and alignment of the contigs. The Genbank file (NM_194277.2) was imported into Seqscape and used as the reference complementary DNA sequence for contig alignment. Base position + 1 corresponded to A of the translation initiation codon ATG. Intronic sequence changes were identified based on the *FRMD7* genomic sequence (NC_000023.10) and amino acid changes were identified based on the reference protein sequence (NP_919253.1). Allelic variations were assessed against the sequence data from 300 male controls (without nystagmus).

In situ hybridization experiments

Human embryonic and foetal tissues were obtained from the MRC-Wellcome Trust Human Developmental Biology Resource (www.hdbr.org; Lindsay and Copp, 2005), Institute of Human Genetics, Newcastle University. The samples were collected with appropriate maternal consents and ethical approval by the Newcastle and North Tyneside Research Ethics Committee. Tissue sections used embryos with a normal karyotype and morphology. Tissue sections from eight samples were analysed from Carnegie Stage 16 (~37 days post-conception; *n* = 1); Carnegie Stage 19 (~47 days post-conception; *n* = 3); Carnegie Stage 22 (~54 days post-conception; *n* = 2) and Carnegie Stage 23 (~56 days post-conception; *n* = 2). The stage of embryonic development was determined by assessment of external morphology as described in Bullen and Wilson (1997) and O'Rahilly and Muller (1987).

In situ hybridizations were preformed as previously described (Moorman *et al*., 2001) with some modifications. Briefly, sections were dewaxed in xylene, gradually hydrated in decreasing ethanol concentrations before incubation in proteinase K (20 µg/ml) at room temperature, followed by fixation using 4% paraformaldehyde in phosphate buffered saline. Background was reduced by treating with 0.1 M triethanolamine pH 8. Sections were air dried and the sense or antisense probes [300 ng labelled probe per 100 µl of DIG Easy Hyb mix (Roche)] were added for hybridization at 68°C overnight. Next day sections were washed in 5 × followed by 2 × saline sodium citrate buffer at 60°C then incubated with anti-digoxigenin alkaline phosphatase Fab fragments (Roche) diluted 1:1000 at 4°C overnight. Sections were then washed and expression detected using NBT/BCIP (20 µl/ml; Roche) in 0.1 M Tris (pH 9.5)/0.1 M NaCl (Buffer 2) in the dark at room temperature. Developing was stopped by rinsing slides in Buffer 2 then distilled water. Sections were mounted using Aquamount and analysed using a Zeiss Axioplan 2 microscope. Images were captured with Zeiss Axiovision 4 imaging system.
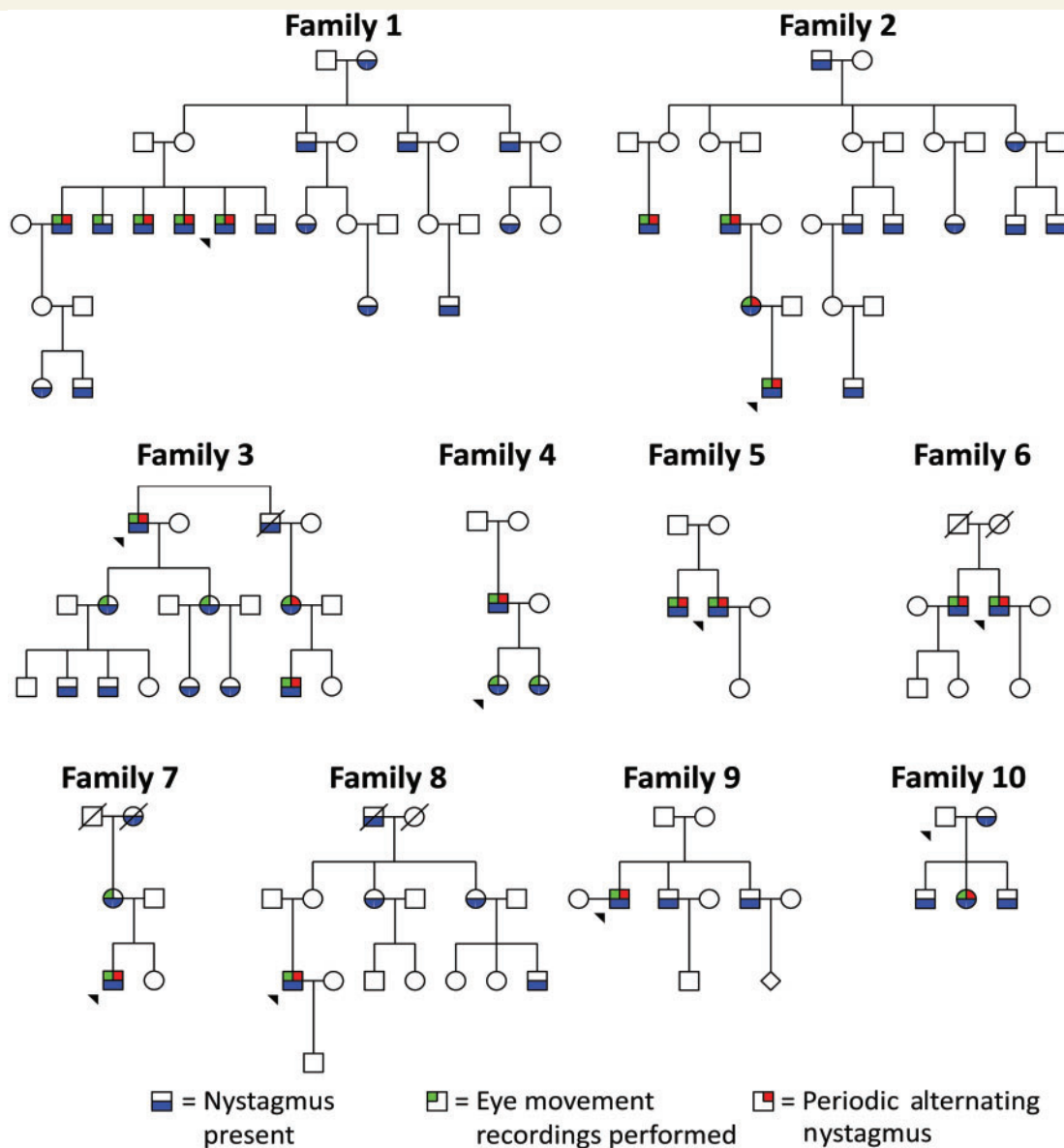
Figure 1 Families with idiopathic infantile PAN. In Families 1–4 eye movement recordings were performed in three or more affected individuals, whereas in Families 4–7 eye movement recordings were obtained in two or more affected individuals. In Families 8–10 eye movement recordings were only performed in one affected individual.

Results

Clinical characteristics and eye movement abnormalities

The pedigrees of patients diagnosed with idiopathic infantile PAN are shown in Fig. 1. Among the 10 families, we were able to perform eye movement recordings in 26 affected patients, of whom 20 had idiopathic infantile PAN (Fig. 1). The phenotypes in affected members of Family 2 have previously been described by Hertle *et al.* (2005).

The best-corrected visual acuity in our cohort of patients with PAN ranged from 0.0 LogMAR to 0.54 LogMAR with a median of 0.2 LogMAR. Three patients with idiopathic infantile PAN had a slight anomalous head posture between 5–10°. However, in only one patient was the head posture noticed to alternate to the right or left on two different examination days. None of the patients with idiopathic infantile PAN had strabismus. All had some degree of stereopsis; the range of stereoacuity was 85–550″ with a median stereoacuity of 150″.

Idiopathic infantile PAN was not diagnosed clinically in any of the families or the singletons. However, the use of eye movement recordings during a prolonged duration of fixation (5 min) aided the diagnosis of idiopathic infantile PAN. An example of original eye movement recordings from Family F3 is shown in Fig. 2. In Families F1, F3, F4 and F7 we observed phenotypic heterogeneity as not all the examined patients had idiopathic infantile

Table 2 Forward and reverse primer sequences, product sizes and melting temperature (T$_m$) used to amplify *FRMD7* exons

Exon	Primers 5′–3′	Product size (bp)	T$_m$ (°C)
Exon 1	GGGCTGTTCACAAATGACAA AAGAAGGCTAGAGGAAGTCCAG	389	59 58
Exon 2	AAACAAAGAGGGAGGACAAAAA CCAAGGTGGTTTGTTATAAGGC	500	57 60
Exon 3	TCTCAAAGCCCTTTTCTCCC TGGAGGGTTCAAATGTGGAG	500	58 58
Exon 4	CCCTTTGGATGATGAACACC GAAAGAGGGGTGGGAGAACT	500	58 60
Exon 5	TCCCCTGTAAACCCTAACACAG GCAGAAACCACCAGAGGATG	500	62 60
Exon 6	TGCCTCAAGAATGACATTTCC GAGGCTTTGGACTGCATTG	396	60 59
Exon 7	ATCACCTTGGGAAAGGACAC TCTGGCACAAACTCGGTACTT	500	58 59
Exon 8	GAGCTGCCCCATTTTCCTAT GCCTTCTTTGACCACAGCTC	500	58 60
Exon 9	CTTAGGTAAGGTGCCCCCAT TACAGGGAGCCAAGTGGAAA	500	60 58
Exon 10 + 11	TCAATTCATGGAAGCATTTGAA TGCCTGGTCCTTGAATAAGTG	500	55 59
Exon 12A	GACAGGGTCCATATAAATAAAAGGG TGTATACACTCAGAGTCTGGAAGTAGG	474	63 67
Exon 12B	CTTCATTGCAGTGGGCTCTA ACCAAAATGTGAATGGAGTGC	500	58 57
Exon 12C	AGAACTGCTGGCAACTCCTG CGGATGTGCCCTATATTCCT	481	60 58
Exon 12D	CCAATGAATATGTAGTAACCAAGAAAA AGCAAACCCCTAAAAGGTCC	500	61 58
Exon 12E	CCTTCCTGAGGCTGTAGGAG TGCTGAAGACAGGACTTCACTAAA	595	59 60
Exon 12F	CCTCCCTCCTTTTCCATGTT TGACTGTGTTGATCTTTGTGCTC	591	60 60

PAN (Fig. 2). Among the families with idiopathic infantile PAN, six of 26 subjects in which eye movements were performed did not have PAN. Overall, the time period for the idiopathic infantile PAN cycle varied between 90 and 260 s and the singleton had a periodicity of 280 s. All family (F1–F10) members with idiopathic infantile PAN had a jerk-related or dual jerk nystagmus. In family members without idiopathic infantile PAN (F1, III:3; F3, II:2 and 4; F4, III:1 and 2; F7, II:1;) the predominant nystagmus waveform was pendular.

None of the patients with idiopathic infantile PAN showed an optokinetic response for either horizontal stimulus directions (rightwards and leftwards). The PAN cycle continued through the optokinetic nystagmus testing and was not changed by the optokinetic nystagmus stimuli (Fig. 3). Vertical optokinetic nystagmus was seen in all patients in both directions (upwards and downwards).

Genetic analysis

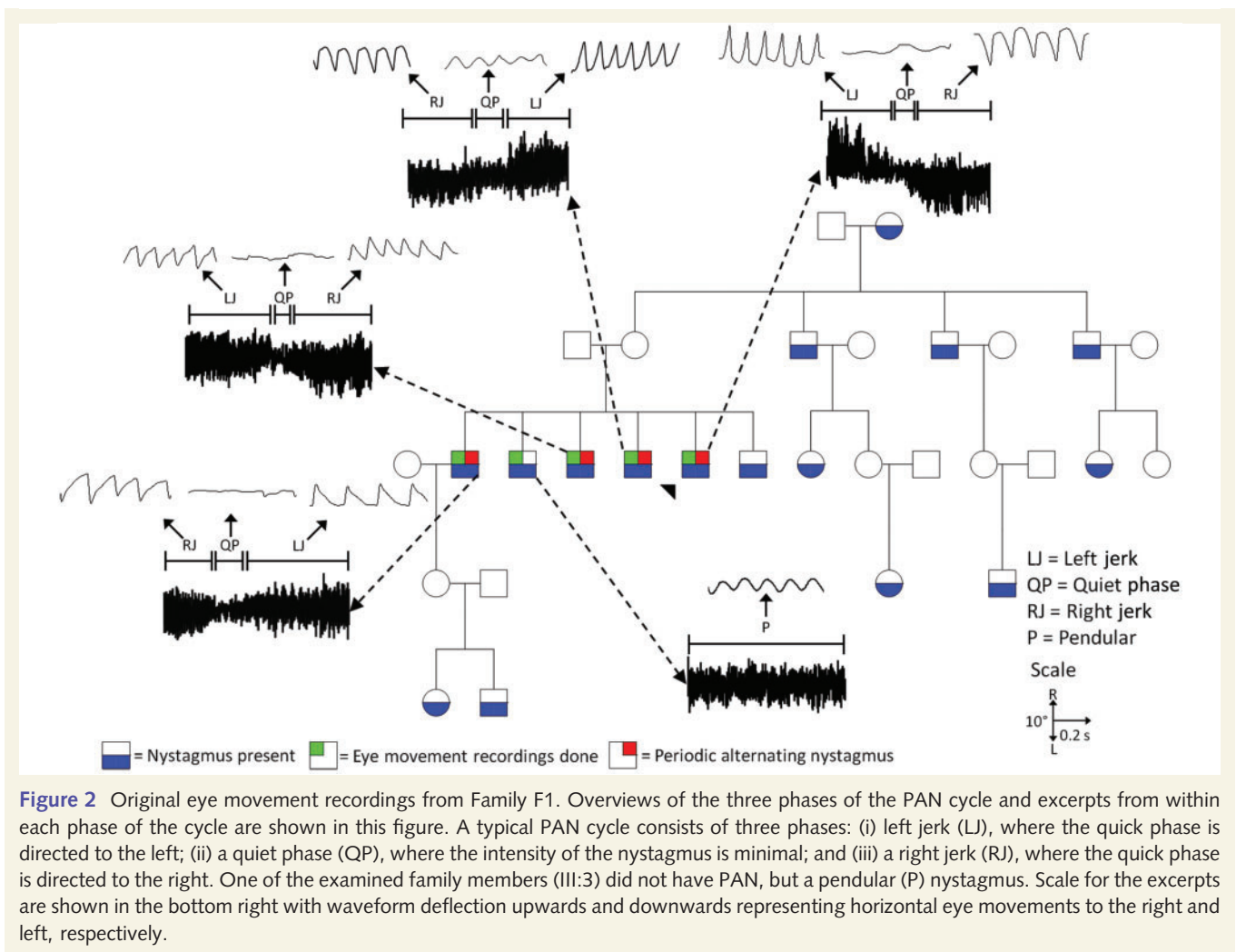Among the 12 families with idiopathic infantile nystagmus we identified mutations in nine out of 12 previously uncharacterized families; similarly within the 11 singletons we identified mutations in two out of 11 singletons. Thus, the total cohort consisted of a total of 24 families and five singletons with an *FRMD7* mutation; the remaining seven families and nine singletons had no such mutations. In all 10 of the idiopathic infantile PAN families and the singletons with idiopathic infantile PAN, there were mutations of the *FRMD7* gene, three of which were novel. A summary of the mutations and domains affected are shown in Fig. 4.

The three novel mutations were in Family F8, F9 and singleton S1. Family F8 had a missense mutation (c.47T > C) in exon 1, resulting in the substitution of phenylalanine by serine at position 16 (p.F16S). Sequence analysis of the proband in family F9 revealed a splice-site mutation (c.58-1G > A) at the 3′-end of intron 1. This results in the loss of the conserved splice acceptor residue. The effects of the mutation were predicted using the alternative splice-site predictor (Wang and Marín, 2006), and considered pathological due to exon skipping resulting in a messenger RNA transcript with exon 2 missing. The singleton S1, had a missense mutation (c.811T > A) in exon 9 resulting in a substitution of cysteine to serine at position 271 (p.C271S). Both missense mutations at amino acid positions 16 and 271 were considered pathological as they involved residues that were identical within invariant blocks in the species *Mus*, *Gallus*, *Xenopus* and *Tetraodon*. Furthermore, the structural effects of these mutations were elucidated based on the known crystal structure of the closest orthologue (PDB Accession ID: 1GG3) and secondary structure prediction of the FRMD7 protein sequence using Emboss Garnier (Garnier *et al.*, 1978). Finally the stability of the mutant protein was assessed using I-Mutant (v2.0) (Capriotti *et al.*, 2005) and Coot (v0.6) (Emsley and Cowtan, 2004). The amino acid change C271S would disrupt a large alpha-helical domain in the wild-type structure. Similarly, the amino acid change F16S is likely to disrupt adjacent secondary structure. Consequently, both missense mutations, F16S and C271S, decrease the stability of the mutant protein (due to a decrease in the free energy value).

Six of the eight remaining families (F1, 2, 3, 4, 7 and 10) all revealed missense mutations of the *FRMD7* gene. These mutations have been reported previously and their translational effects have been described (Tarpey *et al.*, 2006). Family F5 and F6 were the only families that had a nonsense mutation (c. 1003C > T) resulting in a premature stop codon at amino acid position 335 (p. R335X). Family F2 was previously reported with an idiopathic infantile PAN phenotype and an X-linked inheritance (Hertle *et al.*, 2005). Sequence analysis of this family revealed a missense mutation (c.70G > A) resulting in hemizygous replacement of glycine with arginine at position 24 (p. G24R). The amino acid changes as a result of the missense mutations in families F1 (L231V), F2 (G24R), F3 (C271Y), F4 (A266P), F7 (A226T) and F10 (S340L) are associated with a decrease in the free energy value that is likely to destabilize the mutant protein.

Expression of *FRMD7*

In situ hybridization experiments showed strong hybridization signals from the structures involved in setting up the vestibulo-ocular reflex and optokinetic reflex arc, this included the developing cerebellum, vestibular apparatus and developing neural

Figure 2 Original eye movement recordings from Family F1. Overviews of the three phases of the PAN cycle and excerpts from within each phase of the cycle are shown in this figure. A typical PAN cycle consists of three phases: (i) left jerk (LJ), where the quick phase is directed to the left; (ii) a quiet phase (QP), where the intensity of the nystagmus is minimal; and (iii) a right jerk (RJ), where the quick phase is directed to the right. One of the examined family members (III:3) did not have PAN, but a pendular (P) nystagmus. Scale for the excerpts are shown in the bottom right with waveform deflection upwards and downwards representing horizontal eye movements to the right and left, respectively.

retina (Fig. 5A–E). Expression is detected in the ventricular zone of the neural retina and optic stalk (Fig. 5B). In structures of the vestibulo-ocular reflex, *FRMD7* is expressed in the otic vesicle and vestibulocochlear ganglion (Fig. 5C). The vestibular nuclei, which arise partly from the ventricular zone of rhombomeres 2, 3 and 4, form the horizontal neural integrator, which is an important structure in the vestibulo-ocular reflex and optokinetic reflex arcs. *FRMD7* expression is seen in the ventricular zone of rhombomeres 2, 3 and 4 (Fig. 5D) as well as in the developing cerebellum (Fig. 5E). The cerebellum arises entirely from rhombomere 1 and its ventricular zone gives rise to neuroblasts that migrate on radial glia to develop into the cerebellar nuclei and Purkinje cells in the cerebellar cortex. *FRMD7* is expressed in differentiating and migrating neurons as well as in the ventricular zone, for example in the cerebellum and rhombomeres 3 and 4 in the hindbrain (Fig. 5D and E) but also in the subpallium in the forebrain (data not shown).

Discussion

In this study we show for the first time that idiopathic infantile PAN can be associated with mutations of the *FRMD7* gene. Idiopathic infantile PAN was only detected using eye movement

recordings and most patients had relatively good visual acuity (median: 0.2 LogMAR) and stereoacuity (median: 150''). Fewer females were affected since *FRMD7*-related infantile nystagmus represents a disorder associated with variable penetrance in females (Tarpey et al., 2006; Thomas et al., 2008). The PAN cycle length varied from 90–280 s and none of the patients with idiopathic infantile PAN had a horizontal optokinetic reflex. We show that *FRMD7* is expressed within developing vestibulo-ocular reflex and optokinetic reflex arcs, which identifies the likely neural substrates involved in idiopathic infantile PAN and in *FRMD7*-related infantile nystagmus. We identified 11 mutations in 10 families and one singleton and describe both the phenotypic and translational effects of these mutations. In our cohort of families, the predominant class of mutation associated with this phenotype were missense mutations (8/11) though both truncating (2/11) and splice-site (1/11) mutations are also seen. Ten of the 11 mutations resulted in amino acid changes within functionally significant domains (FERM-N, FERM-C and FA).

From a clinical point of view PAN is typically under-diagnosed (Abadi and Pascal, 1994; Gradstein et al., 1997; Abadi and Bjerre, 2002) as it can often only be identified on eye movement recordings during an extended fixation task to demonstrate the
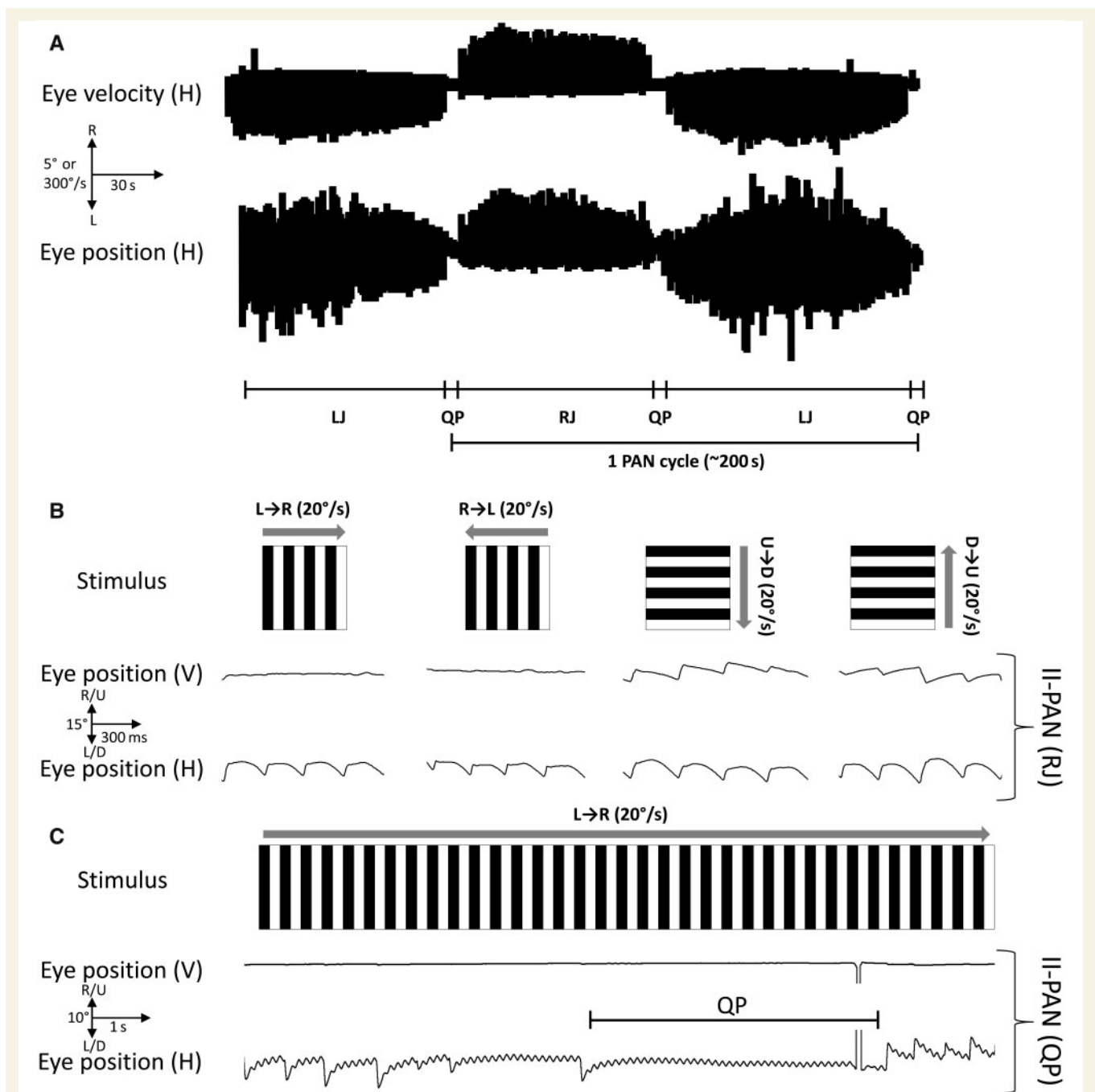
Figure 3 Compressed eye movement recordings showing an overview of the various phases of the PAN cycle (**A**). In the above example one cycle consists of right jerk (RJ) followed by a quiet phase (QP), left jerk phase (LJ) and another quiet phase (QP). Upward deflection of the horizontal (H) position and velocity trace represents right-beating nystagmus and downward deflection represents left-beating nystagmus. The optokinetic response was measured for optokinetic nystagmus stimuli (**B**) moving in the horizontal [rightwards (L→R) and leftwards (R→L)] and in the vertical direction [downwards (U→D) and upwards (D→U)]. The patient (II-PAN) shows no horizontal optokinetic response to the stimulus; the nystagmus is unchanged in the right jerk phase during optokinetic nystagmus testing. However for the vertical optokinetic nystagmus stimuli, a vertical optokinetic response is seen in the vertical trace (V) for the patient. The idiopathic infantile PAN cycle was not changed by the horizontal optokinetic nystagmus stimuli as shown in (**C**). The transition (QP) between left jerk and right jerk is seen during an extended horizontal optokinetic nystagmus task.

periodicity of the nystagmus and its three phases. A recent study estimated that ~15% of all infantile nystagmus syndrome patients have PAN (Hertle *et al*., 2009). In contrast, Shallo-Hoffmann *et al*. (1999) showed that a higher proportion of ~39% of congenital

nystagmus patients have PAN. Interestingly, in the idiopathic infantile PAN incidence study by Shallo-Hoffmann and colleagues (1999, 2004), a family with X-linked PAN was described though no genetic diagnosis was provided. We noticed the occurrence of
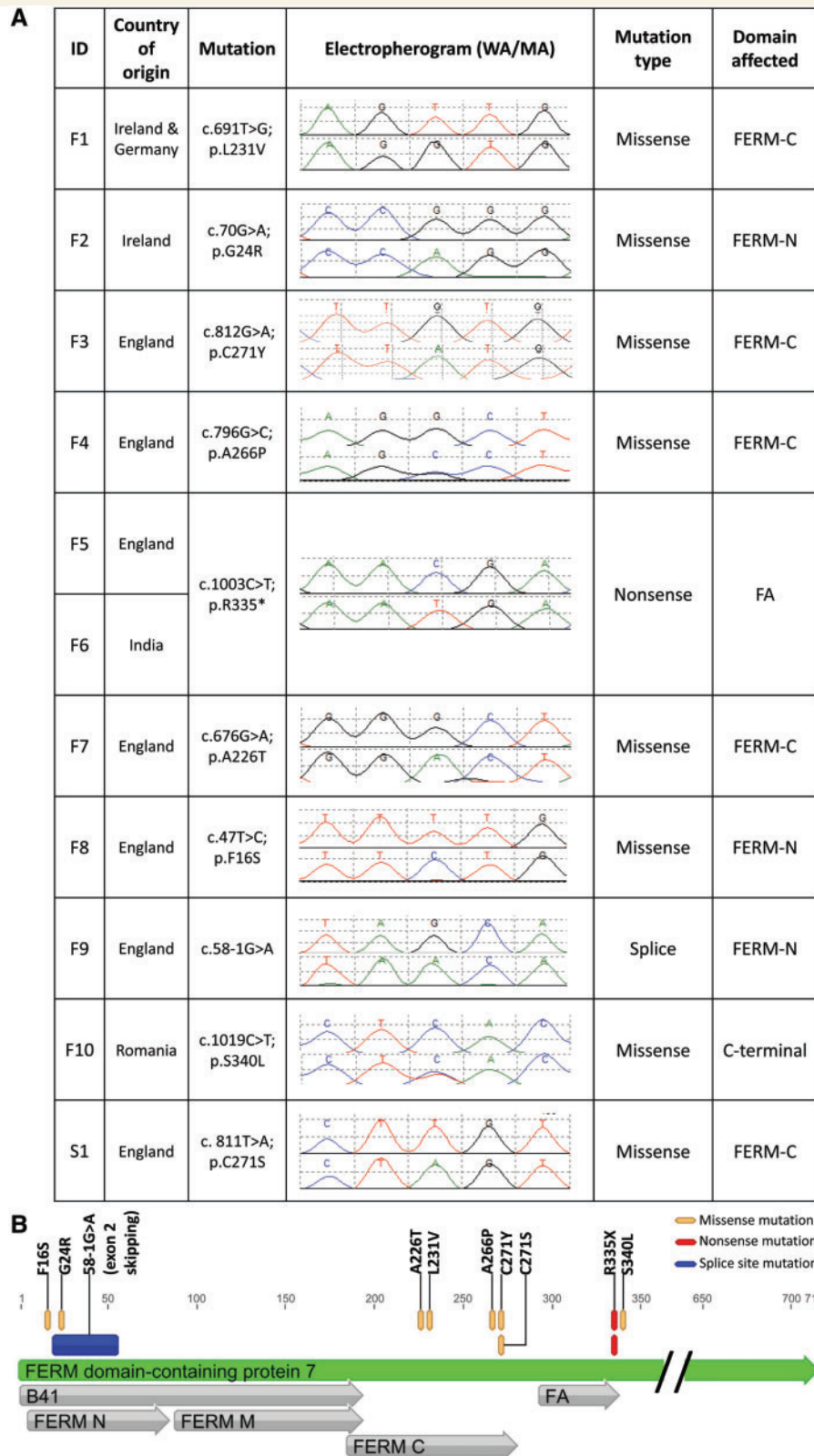
A

ID	Country of origin	Mutation	Electropherogram (WA/MA)	Mutation type	Domain affected
F1	Ireland & Germany	c.691T>G; p.L231V		Missense	FERM-C
F2	Ireland	c.70G>A; p.G24R		Missense	FERM-N
F3	England	c.812G>A; p.C271Y		Missense	FERM-C
F4	England	c.796G>C; p.A266P		Missense	FERM-C
F5	England	c.1003C>T; p.R335*		Nonsense	FA
F6	India				
F7	England	c.676G>A; p.A226T		Missense	FERM-C
F8	England	c.47T>C; p.F16S		Missense	FERM-N
F9	England	c.58-1G>A		Splice	FERM-N
F10	Romania	c.1019C>T; p.S340L		Missense	C-terminal
S1	England	c. 811T>A; p.C271S		Missense	FERM-C

B

Figure 4 Country of origin, mutations of the *FRMD7* gene in the families (F1–10) and singleton (S1) with idiopathic infantile PAN are shown in (**A**). The electropherograms from the respective families and singleton are shown with the wild-type allele (WA) represented on top of the mutant allele (MA). All mutant electropherograms show hemizygous mutations of the *FRMD7* gene except for the female probands in Families F4 and F10, where a heterozygous mutation is shown. The type of mutation and domain affected is shown in (**B**). Missense mutations were the most common and changes to amino acid at positions 271 and 335 occurred in two families (271: F3 and S1; 335: F5 and F6). F1 = Family 1; S1 = Singleton 1; B41 = Band 4.1; FA = FERM adjacent domain.
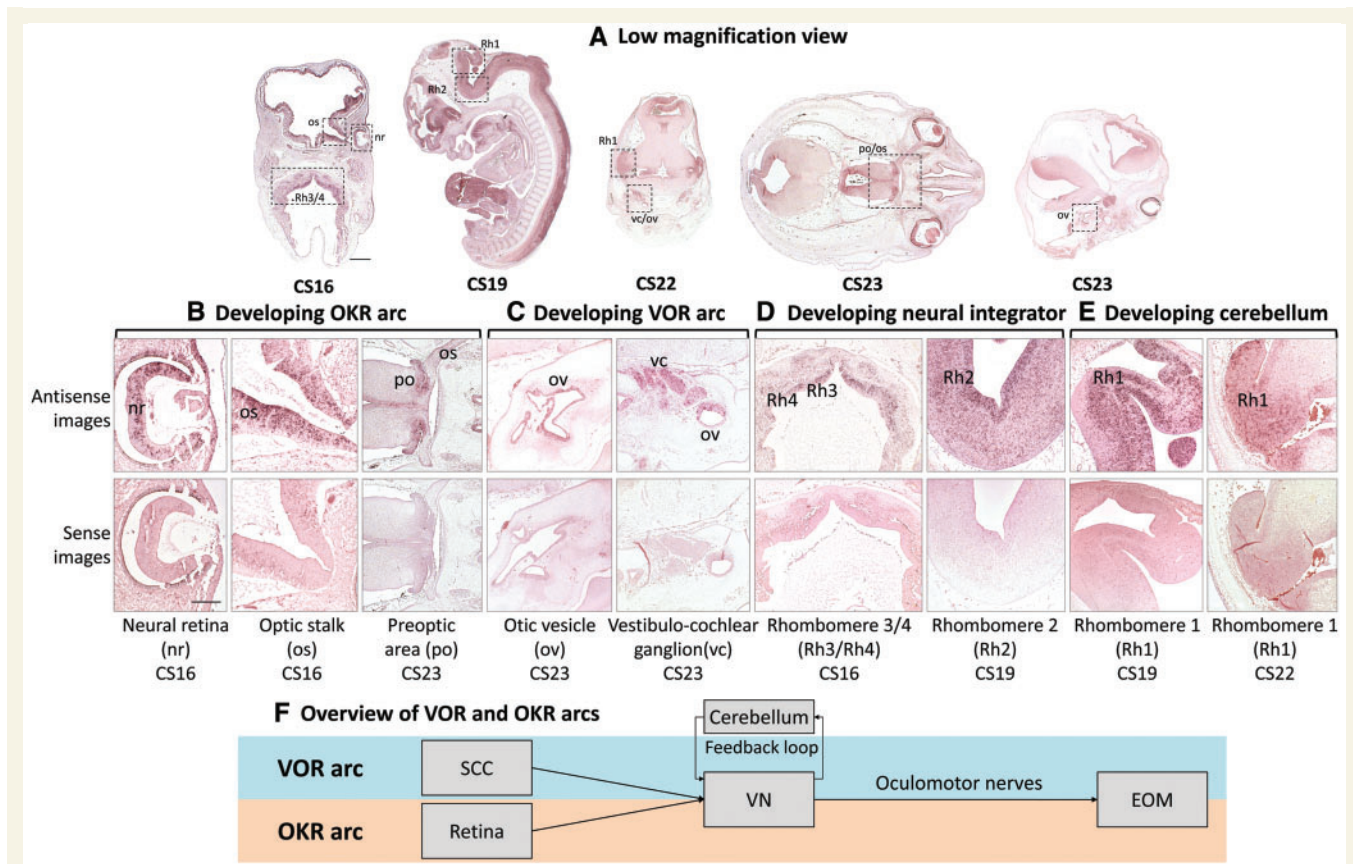
Figure 5 FRMD7 expression in developing human brain. Panel **A** shows low magnification views of the sections from which higher magnification views are shown in (**B–E**). (**F**) gives a simplified overview of the vestibulo-ocular reflex (VOR) and optokinetic reflex (OKR) arcs indicating the afferent arm of the reflex arc starting at the semicircular canals (SCC) and retina followed by the cranial nerves (CN) involved in the respective arcs. The neural signal is integrated at the vestibular nucleus (VN) that is subject to the feedback loop through the cerebellum. The efferent arm of the reflex consists of the oculomotor nerves innervating the effector organ i.e. the extraocular muscles (EOM). (**A**) shows the following images from left to right: Carnegie Stage 16 section through forebrain and hindbrain; Carnegie Stage 19 whole embryo sagittal section; Carnegie Stage 22 section through midbrain and hind brain; Carnegie Stage 23 section through midbrain, hindbrain and forebrain; and Carnegie Stage 23 head sagittal section. In (**C–F**) images of sections hybridized with antisense probes (signal detected as purple stain) are shown above corresponding sections hybridized to sense control probes (no signal detected). Scale bar for the low magnification images (**A**) represents 1 mm for all images except the Carnegie Stage 16 image where the scale bar is 0.5 mm. For all high magnification images (**B–E**) the scale bar represents 0.3 mm except the preoptic image (Carnegie Stage 23) where it represents 0.6 mm. nr = neural retina; os = optic stalk; po = preoptic area; ov = otic vesicle; vc = vestibulocochlear ganglion; Rh1–4 = rhombomere 1–4.

idiopathic infantile PAN, using eye movement recordings, in at least one family member with an *FRMD7* mutation in 10/31 (32%) families and 1/14 (7%) singletons. This suggests that most patients with idiopathic infantile PAN are likely to have a family history of nystagmus and it is important to screen for *FRMD7* mutations. Diagnosing PAN is important since it has different therapeutic implications compared with other forms of infantile nystagmus. The Kestenbaum procedure is used in idiopathic infantile nystagmus to correct anomalous head posture if it is constantly directed towards one side. However it is inappropriate in patients with PAN since it does not correct anomalous head posture which can alternate to both sides as in idiopathic infantile PAN or may even accentuate the head position to one side (Gradstein *et al.*, 1997). Abadi and Bjerre (2002) showed that among the cohort of patients with PAN, 18% were idiopathic

infantile nystagmus whereas 82% were associated with albinism. The incidence of PAN with both albinism and *FRMD7* mutations suggests that there may be a common mechanism in the occurrence of PAN in these two disorders.

The FRMD7 protein is homologous to the FARP1 and FARP2 proteins; particularly at the N-terminus. Previous studies have shown that FARP1 and FARP2 are involved in neurite outgrowth and branching (Toyofuku *et al.*, 2005; Zhuang *et al.*, 2009). Recently it has been demonstrated that knockdown of *FRMD7* in neuro-2A cells results in shorter neurites, suggesting a role in axonogenesis or dendritogenesis (Betts-Henderson, *et al.*, 2009). We found expression of *FRMD7* within the developing vestibulo-ocular reflex and optokinetic reflex arcs. In this study we observed that all patients with PAN and *FRMD7* mutations had no optokinetic nystagmus response. Previous phenotypic

studies in *FRMD7*-related infantile nystagmus have shown that the optokinetic nystagmus gain is lower or no optokinetic nystagmus response is detected in affected individuals with *FRMD7* mutations (Self *et al.*, 2007). There are also reports of reversal of optokinetic nystagmus in patients with congenital nystagmus (Halmagyi *et al.*, 1980), albino rabbits (Collewijn *et al.*, 1978) and achiasmatic fish (Huang *et al.*, 2006), findings suggested by the authors to result from miswiring within the optokinetic nystagmus arcs. In unaffected carriers of *FRMD7* mutation a subnormal optokinetic nystagmus gain has been reported (Thomas *et al.*, 2008). This suggests that the optokinetic system is involved in this disorder and we have now provided substantial evidence from the expression studies that *FRMD7* is expressed within the neural substrates in the developing optokinetic nystagmus and vestibulo-ocular reflex arcs. However the expression is not restricted to these tissues (e.g. expression is also detected in the midbrain, Fig. 5A). This provides general evidence of the neuronal networks involved in *FRMD7*-related infantile nystagmus. Based on the *in vitro* assays in FRMD7 and studies from homologous proteins (FARP1 and FARP2) there may be miswiring of the developing optokinetic nystagmus and vestibulo-ocular reflex systems, thus predisposing to the phenotypes of PAN and *FRMD7*-related infantile nystagmus. Phenotypic data (not confined to PAN) from patients with albinism also suggest that vestibulo-ocular reflex and optokinetic nystagmus systems are affected (Yee *et al.*, 1980; Demer and Zee, 1984). The higher prevalence of PAN in patients with albinism (as suggested by Abadi and Bjerre in 2002) may be due to the misrouting of the retinogeniculate fibres, which may predispose to instability within the optokinetic reflex arc. Therefore the PAN phenotype may represent a part of the spectrum of infantile nystagmus forms depending on the degree of instability of the vestibulo-optokinetic systems. However, the presence of sibship clustering (seen in the larger families F1, F2 and F3) and familial involvement of the PAN phenotype may suggest that certain mutations of the *FRMD7* gene predispose to the PAN phenotype. For example the R335X mutation was seen in two families of different descent, similarly mutations at amino acid position 271 (F3: C271Y and singleton C271S) in one family and the singleton resulted both in idiopathic infantile PAN phenotype. The differences in phenotype between patients could possibly be due to variable expressivity, which could arise as a result of involvement of disease modifying genes and environmental influence that may affect the post-natal development of the oculomotor system. We have previously also reported intra-familial variability in the type of nystagmus (Thomas *et al.*, 2008).

The aetiology of acquired PAN has been associated with dysfunction of the cerebellum, including cerebellar degenerations, cerebellar tumours, multiple sclerosis and other mass lesions involving the cerebellum (Leigh *et al.*, 1981; Furman *et al.*, 1990; Matsumoto, *et al.*, 2001; Hashimoto *et al.*, 2003). Leigh and colleagues (1981) hypothesized that acquired PAN arises as a result of instability within the optokinetic-vestibular system. Phenotypic data from patients with acquired PAN also suggest that the patients had no optokinetic function and the optokinetic nystagmus stimuli did not perturb the PAN cycle (Baloh *et al.*, 1976; Leigh *et al.*, 1981). The time period for one PAN cycle in acquired forms varies from 200–240 s (Baloh *et al.*, 1976; Leigh *et al.*,

1981; Furman *et al.*, 1990). In this study we observed that the time period for idiopathic infantile PAN varies from 90–280 s. Only one of our patients had a time period of 90 s, whereas the remaining had a time period >190 s. Thus, there is some similarity in the periodicity of the idiopathic infantile PAN cycle when compared with the acquired PAN. The neuronal substrates implicated in acquired PAN and phenotypic data from patients with acquired PAN are closely related to the phenotypic and expression results highlighted in this study. This also suggests some similarity in the aetiological mechanisms between infantile and acquired PAN.

Acquired forms of PAN have been treated successfully using baclofen (a GABA agonist). Baclofen suppresses the velocity-storage mechanism possibly by reinforcing the action of the inhibitory GABAergic Purkinje cells from the nodulus to the vestibular nuclei. Some congenital forms respond occasionally to baclofen (Solomon *et al.*, 2002; Comer *et al.*, 2006). It would therefore be interesting to see whether this subset of a phenotypically homogenous population has a different therapeutic response compared with the other phenotypes encountered with both *FRMD7* patients and non-*FRMD7* patients (Thomas *et al.*, 2008).

In conclusion, we have shown that mutations in the *FRMD7* gene form the genetic basis of idiopathic infantile PAN. Expression and phenotypic data suggest that congenital PAN arises from instability of the optokinetic-vestibular systems.

Web resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
Primer3, http://frodo.wi.mit.edu/primer3/
NCBI, http://www.ncbi.nlm.nih.gov/sites/entrez
I-Mutant2.0, http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant2.0/I-Mutant2.0.cgi
Multiple sequence alignment program for DNA or proteins (ClustalW), http://www.ebi.ac.uk/Tools/clustalw2/index.html
The European Molecular Biology Open Software Suite (EMBOSS), http://emboss.sourceforge.net/index.html
Drummond AJ, Ashton B, Buxton S, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2010) Geneious v4.8, Available from http://www.geneious.com

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