Prolonged Pursuit by Optokinetic Drum Testing in Asymptomatic Female Carriers of Novel FRMD7 Splice Mutation c.1050 +5 G>A

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Objective: To determine the genotype underlying suspected X-linked infantile nystagmus in a family and to correlate genotype with clinical examination in potential female carriers.

Methods: Ophthalmic examination (ophthalmic, orthoptic, optokinetic [OKN] drum, and electrophysiologic when possible) and candidate gene analysis.

Results: Two affected brothers had infantile nystagmus with no evidence of associated visual or neurological disease. The symptomatic maternal aunt had infantile nystagmus in addition to congenital fibrosis of the extracocular muscles (CFEOM) (bilateral hypotropia, exotropia, ptosis, almost complete ophthalmoplegia, and poorly reactive pupils). A sister, the mother, and the maternal grandmother—all 3 of whom were asymptomatic—had delayed corrective saccades (prolonged pursuit) during OKN drum testing. A brother and the father—both of whom were asymptomatic—had unremarkable examination findings. A FRMD7 splice variant (c.1050 +5 G>A) was identified in the 2 affected brothers and in the 3 asymptomatic women only. Allele sharing analysis further confirmed that the aunt’s phenotype was not related to the FRMD7 variant, which was absent in 246 ethnic controls. Her phenotype was also not related to mutation in known CFEOM genes (KIF21A, PHOX2A, TUBB3).

Conclusions: Prolonged pursuit responses during OKN drum testing in asymptomatic female carriers is consistent with the concept of infantile nystagmus being an abnormally increased pursuit oscillation. Further studies are required to determine the reproducibility of this potential female carrier sign. Rather than being FRMD7-related, nystagmus in the maternal aunt represented a second disease in this family, likely related to CFEOM.

Clinical Relevance: Clinicians can use the OKN drum to assess obligate female carriers in a family suspected of having X-linked nystagmus.

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Nystagmus is an eye movement control system abnormality characterized by rhythmic involuntary to-and-fro ocular oscillations that can occur with or without associated visual or neurological disease. Although nystagmus is most accurately characterized by waveform analysis, such analysis is not readily available to most ophthalmologists. Clinicians can describe nystagmus as jerk (with a fast phase and a slow phase) or pendular (with apparent equal velocity of the to-and-fro movements). In jerk nystagmus, directionality (right, left, upbeat, downbeat) are based on the fast phase, although it is the slow phase that is considered pathological. For familial idiopathic infantile nystagmus without associated ocular or neurological disease, X-linked inheritance is the most common inheritance pattern. Since mutation in FRMD7 (FERM domain-containing 7; OMIM 300628) was identified as a cause for X-linked infantile nystagmus (OMIM 310700) in 2006, at least 40 causative mutations have been described, although the specific function of the gene remains unknown. As is true for X-linked disease in general, FRMD7-related nystagmus tends to occur in men. Affected women have been reported more commonly in pedigrees with nontruncating mutations as opposed to truncating mutations, possibly owing to a dominant-negative effect of the abnormal protein in the former and nonsense-mediated mRNA decay (and therefore lack of abnormal protein product) in the latter. In addition, skewed X-inactivation toward the mutant allele can sometimes be observed in af-

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The family of 2 affected male siblings with possible X-linked infantile nystagmus was invited to participate in this prospective genetic study, which was approved by our institutional review boards. Family history was significant for similar “shaking eyes” in a maternal aunt and uncle, one of whom (the aunt) also had large-angle obvious congenital strabismus with limited voluntary eye movements since birth. Available family members (Figure 1) participated after informed consent: the 2 affected brothers (III:2, III:4), the symptomatic maternal aunt (II:9), and the unaffected sister (II:1), an unaffected brother (III:3), the 2 unaffected parents (II:4, II:9), and the unaffected mater-

**Figure 1.** Family pedigree of FRMD7 showing 3 generations and allele-sharing results. The mutant allele is in red, normal alleles are in black. Arrows point to the individuals who participated in this study.

**METHODS**

The family of 2 affected male siblings with possible X-linked infantile nystagmus was invited to participate in this prospective genetic study, which was approved by our institutional review boards. Family history was significant for similar “shaking eyes” in a maternal aunt and uncle, one of whom (the aunt) also had large-angle obvious congenital strabismus with limited voluntary eye movements since birth. Available family members (Figure 1) participated after informed consent: the 2 affected brothers (III:2, III:4), the symptomatic maternal aunt (II:9), and the unaffected sister (II:1), an unaffected brother (III:3), the 2 unaffected parents (II:4, II:9), and the unaffected mater-

There is higher incidence of strabismus in patients with FRMD7-related nystagmus than the general population; however, the cranial dysinnervation disorder congenital fibrosis of the extraocular muscles (CFEOM) is not associated with FRMD7 mutation, and infantile nystagmus is not associated with CFEOM. Congenital fibrosis of the extraocular muscles is a rare form of congenital incontinent strabismus caused by orbital dysinnervation. Congenital fibrosis of the extraocular muscles type 1 (OMIM 135700), due to heterozygous mutation in KIF21A (kinesin family member 21A; OMIM 608283), is characterized by bilateral ptosis, exotropia, hypotropia, and almost complete ophthalmoplegia. Congenital fibrosis of the extraocular muscles type 3 (OMIM 600638) is an asymmetric variable form of CFEOM that typically does not involve the pupils and can be due to heterozygous mutation in TUBB3 (Tubulin beta-3; OMIM 602661) or KIF21A. There are rare reports of patients with CFEOM with obvious neurological disease but none to our knowledge with documented constant nystagmus.

The purpose of this study is (1) to determine the underlying genotype for apparent X-linked infantile nystagmus in a family with 2 affected brothers and 1 symptomatic maternal aunt and (2) to correlate the genotype with clinical examination and clinical OKN drum testing in potential female carriers.

**CLINICAL EVALUATIONS**
nal grandmother (I:2). Participants underwent comprehensive ophthalmic and orthoptic examination by a pediatric ophthalmologist; the examination included detailed slitlamp examination to exclude signs of albinism and foveal hypoplasia. Visual acuity was measured using a +10 diopter occluder over the contralateral eye. Pursuit and saccades were specifically assessed by OKN drum testing (Model 450; Ophthalmic Research Ltd, Winchester, England) at near horizontally and vertically in both directions. Full-field electroretinography and visual evoked potentials were performed in the affected brothers and the unaffected mother according to International Society for Clinical Electrophysiology of Vision standards.16

DNA SEQUENCING AND ALLELE SHARING

DNA from family members and 246 controls was extracted from 3 mL of whole blood using a Genetra Puregene Blood kit (Qia- gen Inc, Valencia, California), according to manufacturer conditions. The coding exons of FRMD7 (RefSeq NM_194277) were amplified in family members (primers and conditions outlined in the eTable; http://www.archophthalmol.com). The opening reading frames of KIF21A (RefSeq NM_017641), PHOX2A (RefSeq NM_005169), TUBB3 (RefSeq NM_006086), and coding exons were amplified in the affected maternal aunt who also had CFEOM (patient II.5 in Figure 1) using previously described primers and conditions.9,17

Polymerase chain reactions were performed in a 25-µL reaction volume containing 2.5 µL of 10X reaction buffer (15-mM MgCl2), 0.2-mM deoxyribonucleotide triphosphates, 10-µM primers, 1 U of HotStarTaq polymerase (Qiagen, Dusseldorf, Germany), and 10 ng of genomic DNA. Cycling parameters were 94°C for 10 minutes, 30 to 35 cycles of 94°C for 45 seconds, annealing at 59°C for 45 seconds, and 72°C for 45 seconds followed by a final elongation step at 72°C for 10 minutes. Products were visualized on GelStar (Lonza, Maine)—stained agarose gels. Polymerase chain reaction products from all exons of FRMD7, KIF21A, PHOX2A, and TUBB3 were sequenced using an ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, California), as described by the manufacturer. Results were exported in one of several formats for visualization and sequence was analyzed using SeqMan 6.1 (Lasergene 6 software package; DNASTAR Inc, Madison, Wisconsin).

Allele sharing was performed using 6-carboxyfluorescein (FAM)–labeled primers for the 2 microsatellite markers DXS1049 and DX691 flanking the (FAM)–labeled primers for the 2 microsatellite markers DXS1049 (RefSeq NM_006086) and FRMD7 (RefSeq NM_194277) using the software package; DNASTAR Inc, Madison, Wisconsin).

Identified variants were assayed in 246 ethnically matched controls by automated sequencing.

RESULTS

CLINICAL MANIFESTATIONS

The pedigree of the family was compatible with X-linked inheritance (Figure 1). There was no history of consanguinity, although the spouses were from the same tribal region. No individual had evidence of neurologic or ophthalmic disease other than nystagmus, CFEOM, or delayed-onset reflex saccades.

AFFECTED BROTHERS (III.2, III.4)

For both brothers, there was no evidence of strabismus or pupillary defect. Findings of slitlamp examination, intraocular pressure measurement, fundus examination, and cycloplegic refraction were unremarkable. Findings of electroretinography and visual evoked potentials were within reference limits. Patient III.2 was a 10-year-old boy with 20/40 visual acuity in either eye. He preferred a moderate right face turn, mild left head tilt, and mild chin depression. Moderate amplitude conjugate pendular nystagmus was observable in the forced primary position (Video 1; http://www.archophthalmol.com) and other gaze positions without significant change, although the preferred head position suggested dampening with that head position. The nystagmus was variable in direction (mostly horizontal, sometimes vertical/torsional) and frequency (mostly moderately rapid as shown, at times faster). Delayed corrective saccades (prolonged pursuit) were evident during OKN testing (Video 2). Patient III.4 was a 4-year-old boy with 20/40 visual acuity in either eye and occasional head nodding. He preferred a mild left face turn and chin depression. Large amplitude conjugate pendular nystagmus was observable in forced primary position and other gaze positions without significant change, although the preferred head position suggested dampening with that head position (Video 3). The nystagmus was moderately rapid and mostly in the horizontal plane, although at times it would become more rapid and have small vertical/torsional components. Delayed-corrective saccades (prolonged pursuit) were evident by OKN testing.

SYMPTOMATIC MATERNAL AUNT (II.5)

The patient was a 32-year-old woman with 20/60 visual acuity in the right eye and 20/30 in the left eye. Findings of ophthalmic examination were significant for low-amplitude, high-frequency variable (pendular/jerk, vertical/torsional) constant nystagmus, bilateral ptosis, large-angle exotropia, bilateral hypotropia, and mid-dilated, poorly reactive pupils (Video 4). There was virtually complete ophthalmoplegia other than the nystagmus. There was no convergence or other aberrant eye movement with attempted upgaze. Small, visually insignificant vacuoles were present in the lens nucleus of both eyes. Results of intraocular pressure measurement, fundus examination, and cycloplegic refraction were unremarkable. Electroretinography and visual evoked potentials were attempted but aborted because the patient could not keep her head in a comfortable position for the examination.

ASYMPTOMATIC WOMEN (III.1, II.4, I.2)

All 3 asymptomatic women had delayed corrective saccades (prolonged pursuit) by OKN testing (Video 5). Other than the OKN abnormality, findings of ophthalmic evaluation were unremarkable except for that of patient I.2, who had an acquired corneal scar following childhood infection in her left eye, with hand-motion visual acuity in that eye and a sensory exotropia. None had obvious clinical nystagmus.

ASYMPTOMATIC MEN (III.3, II.9)

Both asymptomatic men had unremarkable findings on examination.
MUTATION AND VARIANTS IDENTIFIED IN FRMD7

In FRMD7, an intronic variant c.1050+5 G>A (intron 11) was identified in several family members (Figure 2). Affected brothers (III:2 and III:4) were hemizygous for this variant allele, while the unaffected mother (II:4) and unaffected sister (III:1) were heterozygous carriers. The unaffected father (II:9) and brother (III:3) were hemizygous for the normal allele. This variant was absent in the symptomatic aunt (II:5) and it was not present in any of the 246 controls. Both affected brothers and the 3 asymptomatic female carriers for this variant had abnormalities in results of OKN testing, as documented. In silico analysis predicts that this splice variant would affect the splice donor site, resulting in a 345-base inclusion of intron 11, a resultant frame shift, and a truncated protein of 372 amino acids (rather than 714 amino acids).18,19 This variant was not found in Human Gene Mutation Database Professional version on November 14, 2009.6 In addition, consensus nucleotide frequency patterns strongly support the expected pathogenicity of the variant’s G>A +5 intronic change.20

The previously reported nonpathogenic FRMD7 variants, c.842C>T (S281L, rs5977625) and c.1533 T>C (I511, rs5977623), were also identified in family members (Figure 1).

ALLELE SHARING ANALYSIS FOR FRMD7

Allele sharing was traced in family members using 2 microsatellite markers flanking FRMD7 gene and the 3 variants identified in this gene in family members. Results confirmed that only asymptomatic women with oculomotor carrier signs by OKN drum testing and the brothers with infantile nystagmus shared the allele that is in linkage with the c.1050+5 G>A mutation. The asymptomatic aunt (II:5) did not share this allele, confirming that her phenotype is not related to the FRMD7 mutation (Figure 1).

CFEOM ANALYSIS IN PATIENT II:5

No pathogenic variants were found in KIF21A, PHOX2A, or TUBB3 in patient II:5.

COMMENT

The OKN drum testing showed prolonged pursuit in asymptomatic female relatives (patients III:1, II:4, I:2) who harbored the splice FRMD7 variant associated with infantile nystagmus in 2 brothers (patients III:2, III:4). This clinical finding was a female carrier sign for X-linked nystagmus in the family. The OKN drum is an easily accessible tool for practicing clinicians and may be useful in assessing similar families suspected of having X-linked nystagmus.

A major mechanistic theory of infantile nystagmus is that it represents abnormally increased oscillation of the pursuit system.21 This theory is consistent with our observation of prolonged pursuit during OKN testing of the asymptomatic female FRMD7 mutation carriers. The OKN drum sign can easily be tested for by practicing clinicians; however, it is unclear how frequently this potential female carrier sign is clinically observable in families with X-linked nystagmus. Although the literature suggests that the penetrance of clinical signs in women with FRMD7 mutation is 64% in nontruncating mutations and 31.9% in truncating mutations,3 prior studies did not assess asymptomatic carrier women for oculomotor abnormality by OKN drum testing. In the one prior article that addressed the subject of OKN testing of asymptomatic female carriers, testing was done using screen stimuli and eye movement recording analysis.10 The authors described poor OKN gain in a subset of 14 asymptomatic obligate female carriers who were tested. However, because OKN gain abnormalities identified by eye movement recording were on a continuum with normal OKN gain values, the authors could not quantify how many of the 14 had abnormal values; they stated that some had abnormal values, while others did not.19 In addition, the type of FRMD7 mutations were not provided for these 14 individuals.10 Our study reports clinical OKN abnormality and the associated FRMD7 variant for 3 asymptomatic female carriers; however, we are limited by the inclusion of only 3 such individuals.

Although the pathophysiology of FRMD7 mutation in infantile nystagmus syndrome remains to be elucidated, the gene’s FERM domain suggests involvement in signal transduction between cellular plasma membrane and cytoskeleton.22,23 Recent evidence suggests that FRMD7 plays an important role in the regulation of neurite outgrowth and specification and that FRMD7 mutation can

Figure 2. Variants of the c.1050+5 G>A mutation identified in FRMD7, showing the sequence chromatogram of a mutant hemizygous (A), a normal wild type homozygous (B) and a heterozygous carrier (C). Arrows point to the site of mutation.
affect neurite length and neuron branching. In situ hybridization applied to human embryonic brain tissue at day 56 after ovulation reveals FRMD7 expression in the ventricular layer of the forebrain, midbrain, cerebellar primordium, spinal cord, and developing neural retina. At day 37, expression was restricted to the midbrain and hindbrain, regions known to be involved in motor control of eye movement. Murine models have shown an frmd7 expression pattern that mirrors the pattern of genes involved in synapse formation and function as well as genes related to axon growth and guidance.

Rather than being FRMD7-related, nystagmus in the symptomatic maternal aunt with CFEOM (II:5) represented phenocopy in the context of the 2 brothers' nystagmus. Although the family pedigree suggested that the symptomatic maternal aunt may have had FRMD7-related nystagmus, in fact, the FRMD7 variant was excluded in her both by sequencing and allele-sharing analysis and was likely related to her CFEOM. Her phenotype can be considered CFEOM3 but differs from the reported CFEOM3 phenotypes with a known genetic basis (ie, from heterozygous mutation in KIF21A or TUBB3). Results of analysis for known candidate genes for CFEOM—KIF21A, PHOX2A, and TUBB3—were negative. The symptomatic maternal aunt's unique phenotype deserves further analysis; however, she is currently unwilling to consent to further studies.

In summary, clinical OKN drum testing in this family revealed prolonged pursuit in asymptomatic female carriers with an FRMD7 variant associated with infantile nystagmus in men. Prolonged pursuit response during OKN testing was an easily observable carrier sign in this family that is consistent with infantile nystagmus being an increased pursuit oscillation. Further studies are needed to assess the reproducibility of this potential carrier sign for other similar families.

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Author Contributions: Dr Khan had access to all data, confirms the integrity of all data, and confirms the accuracy of data analysis.

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REFERENCES

Correction

Error in Abstract. In the Ophthalmic Molecular Genetics article titled "Prolonged Pursuit by Optokinetic Drum Testing in Asymptomatic Female Carriers of Novel FRMD7 Splice Mutation c.1050 + 5 G>A" by Khan et al, published in the July issue of the Archives (2011; 129[7]:936-940), there is an error in the "Results" section of the abstract. The sentence, "A brother and the father—all 3 of whom were asymptomatic—had unremarkable examination findings" should have read, "A brother and the father—both of whom were asymptomatic—had unremarkable examination findings." This article was corrected online.