A Novel KIF21A Mutation in a Patient With Congenital Fibrosis of the Extraocular Muscles and Marcus Gunn Jaw-Winking Phenomenon

Koki Yamada, MD, PhD; David G. Hunter, MD, PhD; Caroline Andrews, MS; Elizabeth C. Engle, MD

Objective: To determine whether congenital fibrosis of the extraocular muscles (CFEOM) with Marcus Gunn jaw-winking phenomenon (MG) can result from mutations in the KIF21A gene encoding a kinesin motor protein.

Methods: An individual with CFEOM1 (classic autosomal dominant CFEOM) and MG underwent a comprehensive ophthalmic examination. He and his healthy parents underwent screening for mutations in the KIF21A gene by direct DNA sequencing. The clinical records of our previously described patients with CFEOM and KIF21A mutations were reviewed for evidence of more extensive dysinnervation.

Results: A de novo and novel KIF21A mutation 2840T→C (M947T) was present in the proband. In addition, among our previously described patients with CFEOM and KIF21A mutations, 3 individuals had MG and 1 had hypertropia during toothbrushing.

Conclusions: This report introduces a new CFEOM1 KIF21A mutation and is, to our knowledge, the first report of a genetic defect associated with MG. The combination of CFEOM1 with MG supports a primary neurogenic etiology of CFEOM resulting from KIF21A mutations.

Clinical Relevance: These findings will increase understanding of the etiology of CFEOM and increase awareness of the affiliation of CFEOM with MG.


Recent genetic and neuropathological studies have suggested that a group of congenital neuromuscular disorders likely results from developmental errors in innervation of the ocular and facial muscles.1-5 We now refer to these disorders, including congenital fibrosis of the extraocular muscles (CFEOM), Duane syndrome, Möbius syndrome, congenital ptosis, and congenital facial palsy, as the congenital cranial dysinnervation disorders.6

The various forms of CFEOM are congenital paralytic strabismus syndromes primarily affecting vertical eye movement, in contrast to Duane syndrome, which is characterized by a primary defect in horizontal eye movement.7 We have identified the following 3 CFEOM phenotypes: classic autosomal dominant (CFEOM1), nonclassic autosomal recessive (CFEOM2), and nonclassic autosomal dominant (CFEOM3).8

Classic autosomal dominant CFEOM (online Mendelian Inheritance in Man No. 135700) is the most common form of CFEOM, and individuals with CFEOM1 have a characteristic phenotype referred to as classic CFEOM. Key features of CFEOM1 are bilateral nonprogressive ophthalmoplegia, bilateral ptosis, and an infraducted (downward) primary position of each eye with limited supraduction.9 Patients with CFEOM1 lack binocular vision and amblyopia can develop. The CFEOM1 pedigrees demonstrate autosomal dominant inheritance with full penetrance and minimal variation in expression. A neuropathological study from our group demonstrated that CFEOM1 is associated with abnormal development of the oculomotor axis, primarily affecting the superior division of the oculomotor nerve, corresponding alpha motoneurons in the midbrain, and the target extraocular muscles, the levator and superior rectus muscles.9 Researchers from our group mapped CFEOM1 to the centromeric region of chromosome 12 (referred to as the FEOM1 locus)9-12 and recently demonstrated that CFEOM1 results from heterozygous mutations in the KIF21A gene encoding a kinesin motor protein.3

Author Affiliations: Division of Genetics (Drs Yamada and Engle and Ms Andrews) and Departments of Ophthalmology (Dr Hunter) and Neurology (Dr Engle), Children’s Hospital Boston, and Departments of Neurology (Drs Yamada and Engle and Ms Andrews) and Ophthalmology (Dr Hunter) Harvard Medical School (Drs Yamada, Hunter, and Engle and Ms Andrews), Boston, Mass.
identified 6 different pathogenic KIF21A missense mutations, altering only 4 amino acids, in 44 of 45 CFEOM1 probands. Five of the mutations alter 3 amino acids within the same coiled-coil region of the KIF21A stalk, and one of these mutations occurred in 32 (72%) of the 45 probands.3

Subsequently, we identified KIF21A mutations in 2 of the 22 CFEOM3 probands in our database, showing that KIF21A mutations can rarely result in CFEOM3.13 Although the specific function of the KIF21A protein and its stalk are yet to be determined, the mouse ortholog, Kif21a, was found to be an anterograde microtubule-based motor protein expressed predominantly in neuronal tissues.14 We found that human KIF21A is expressed most abundantly in developing neuronal tissues, suggesting that it plays an important role in neuronal development consistent with the CFEOM1 phenotype.7 Because KIF21A expression also has been observed in other tissues, including skeletal muscle, it remains to be determined whether the fundamental pathology of CFEOM resulting from KIF21A mutations is in the nervous system (neurogenic) and/or the extraocular muscles (myopathic). For this reason, we sought to determine whether a subset of individuals with CFEOM resulting from KIF21A mutations had clinical evidence of more diffuse dysinnervation extending beyond the oculomotor, trochlear, and abducens nuclei. This finding would support a primary neurogenic cause for CFEOM resulting from KIF21A mutations.

Several cases with CFEOM accompanied by the Marcus Gunn jaw-winking phenomenon (MG; online Mendelian Inheritance in Man No. 154600) have been reported.15,16 Marcus Gunn jaw-winking phenomenon is categorized clinically as an ocular miswiring syndrome.17 Affected individuals have ptosis accompanied by elevation of the ptotic eyelid on movement of the lower jaw. This syndrome is proposed to result from misdirection of axons intended to travel within the motor branch of the trigeminal nerve to innervate the ipsilateral pterygoid muscle. Instead, these axons aberrantly innervate myofibers of the levator palpebrae superioris muscle, which is normally innervated by a branch of the oculomotor nerve.18 Although the familial occurrence of MG has occasionally been reported,19-21 no specific genes have been associated with this phenomenon. A second example of the extensive dysinnervation in individuals with CFEOM is the unilateral hypertropia that was observed during toothbrushing in 1 CFEOM1 case.22 This phenomenon likely results from aberrant innervation between branches of the trigeminal and oculomotor nerves.

We herein report the clinical features and underlying genetic defect of a male proband with CFEOM1 and MG. We also identify 4 additional patients with CFEOM1 and reported KIF21A mutations who have clinical evidence of aberrant innervation extending beyond the extraocular muscles.

METHODS

The study was approved by the institutional review board of the Children’s Hospital Boston, Boston, Mass. Informed consent was obtained from all study participants. The study design adhered to the Declaration of Helsinki for research involving human subjects.

The proband (JR01) underwent a comprehensive ophthalmologic examination, including visual acuity test; documentation of compensatory head position; palpebral fissure size measurement; pupil evaluation; a sensorimotor evaluation; evaluation for aberrant movements including synergistic convergence and divergence, globe retraction, and MG; slitlamp examination; and dilated fundus examination. Forced-duktion testing was performed during surgery.

Blood samples were obtained from the affected proband (JR01) and his unaffected parents (JR02 and JR03). High-molecular-weight genomic DNA was extracted directly from the blood samples and subjected to haplotype and mutation analysis. Haplotype analysis was conducted using the following 6 locus-specific polymorphic microsatellite markers: D12S1648, D12S1692, D12S539, D12S1067, D12S1048, and D12S1668. Fluorescently labeled primers were purchased from Research Genetics (Huntsville, Ala), and primer sequences are available from the UniSTS in NCBI (available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unisnists). Fluorescently labeled products were made by means of polymerase chain reaction amplification of genomic DNA using each fluorescent primer set, and the products were analyzed using a DNA analyzer (ABI Prism 3730; Applied Biosystems, Foster City, Calif) following the manufacturer’s specifications.

The KIF21A exons 8, 20, and 21 (in which all mutations have been detected in CFEOM1/CFEOM3-affected individuals to date) underwent screening according to our current protocol. These KIF21A exons and corresponding exo-intron boundaries were amplified by polymerase chain reaction from genomic DNA and subjected to analysis by direct DNA sequencing as described previously.3 The sequence from the patient was compared with that of control individuals and the published gene sequence. One hundred five controls recruited from unaffected participants who had married into pedigrees segregating dominant disorders underwent screening for putative mutations. These individuals were of varied ethnicity and from multiple regions, including North and South America, Europe, the Middle East, Japan, India, and Australia. To identify additional patients with CFEOM and evidence of more diffuse dysinnervation, we reviewed the clinical records of the patients with CFEOM and known KIF21A mutations in our database.

RESULTS

PHENOTYPE

The proband (JR01) was first examined at the Department of Ophthalmology at Children’s Hospital Boston at 7½ years of age, complaining of persistent exotropia, ptosis, upgaze limitation, and an anomalous head posture. The only known family member with strabismus was a paternal uncle with amblyopia and a history of eye muscle surgery. Both parents (JR02 and JR03) and a younger brother were unaffected.

We reviewed records from an outside hospital and family photographs. Briefly, the proband was examined by a pediatric ophthalmologist at 4 months of age when bilateral but asymmetric ptosis, an intermittent exotropia, upgaze limitation, and dyskinetic eye movements with possible synergistic divergence and MG were documented. At 11 months of age, results of
forced-duction testing were positive for restriction in both eyes, most severe in the inferior rectus and superior oblique muscles. He was treated with 4.0-mm bilateral inferior rectus recessions. He subsequently underwent 3 additional procedures before 3 years of age, including 2 additional inferior rectus weakening procedures, bilateral lateral rectus recessions, and bilateral superior oblique tenotomies. He also had bilateral levator resections for ptosis.

At his examination at Children’s Hospital Boston, his best-corrected visual acuity was 20/50 OD and 20/30 OS. A chin-up head posture with a left head tilt was observed. Bilateral, asymmetric ptosis was present, with the palpebral fissures 4.5 mm on the right and 3.0 to 11.0 mm on the left, depending on the mouth position (Figure 1A). He controlled his left eyelid position using his mouth position (Figure 1B). The mouth movements did not change the vertical misalignment.

Sensorimotor evaluation disclosed a left eye fixation preference with manifest latent nystagmus. At rest, each eye was hypotropic and exotropic. Abduction was full. Adduction was limited symmetrically to approximately 10° bilaterally. Elevation was limited to the midline on the right and to just below the midline on the left; depression was symmetrically limited to approximately 20° below the midline bilaterally. A convergence-type movement was noted in attempted upgaze. Binocular alignment was variable, with a 25-prism diopter (PD) exotropia in the distance and at near with correction, changing to esotropia of 20 PD with accommodative and fusional efforts. Stereopsis was not detectable. Results of slit-lamp and fundus examinations were unremarkable.
At 7½ years of age, eye muscle surgery was performed using adjustable sutures. On forced-duction testing, the lateral rectus muscles were remarkably tight; all vertical rectus muscles were also tight, whereas the medial rectus muscles abducted without restriction. The previously recessed lateral rectus muscles underwent additional recession to a location 16 mm posterior to the limbus (after suture adjustment). Initially, adduction improved and the corneal light reflexes were centered; however, after 2 to 3 months, the exotropia recurred. Eye muscle surgery was repeated 5 months later. Once again, the lateral rectus muscles were remarkably tight. The lateral rectus muscles were placed 18 mm posterior to the limbus, resulting in an esotropia of more than 20 PD in the early postoperative period, but the exotropia recurred again after several months. Such clinically significant late recurrence of strabismus is often observed when an antagonist extraocular muscle is paretic. In this case, there was clinical adduction of each eye preoperatively, indicating that the medial rectus muscle was not completely paretic. At last follow-up, the residual exotropia was managed using botulinum toxin injections, but definitive surgery may require complete inactivation of the lateral rectus muscles by extirpation or by suturing the muscle ends to the orbital rim.

**A NOVEL KIF21A MUTATION**

We identified a novel heterozygous 2840T→C transition mutation at the second nucleotide position of codon 947 in exon 20, resulting in a methionine-to-threonine substitution (M947T) in the proband, JR01 (Figure 2). The unaffected parents (JR02 and JR03) did not harbor the mutation, nor did 105 control individuals of mixed ethnicity. Haplotype analysis of polymorphic markers surrounding the KIF21A gene showed that the patient carries 1 maternal and 1 paternal allele (Table 1), supporting the de novo occurrence of the KIF21A mutation.

**ADDITIONAL CFEOM CASES WITH EXTENSIVE DYSINNERVATION ASSOCIATED WITH KIF21A MUTATIONS**

Three additional individuals with CFEOM1 and MG (AL03, EO01, and EX01) and 1 individual with unilateral hypertropia during toothbrushing (FG01) were identified from our records of patients with CFEOM and previously reported KIF21A mutations (Table 2). Individual AL03 harbors a 3029T→C mutation (I1010T). The remaining 3 patients (EO01, EX01, and FG01) have the 2860C→T mutation, leading to the common R954W. Both individuals EO01 and EX01 have relatives with CFEOM1 without MG who harbor the identical mutation. Individuals AL03 and FG01 carried de novo mutations.

**COMMENT**

We identified a novel de novo 2840T→C KIF21A mutation in a proband with CFEOM and unilateral left MG. Although the original appearance cannot be observed because of the previous surgeries, it is possible to classify his CFEOM on the basis of previous records and photographs. A review of previous photographs and the observation that bilateral inferior rectus recessions were performed at 11 months of age strongly support severe, bilateral upgaze restriction. Considering the early inferior rectus surgery, the congenital bilateral ptosis, the bilateral severe restrictive ophthalmoplegia, and the exo-

*Figure 2. Sequence chromatographs of the family. The healthy parents have normal sequences (top), whereas the proband with congenital fibrosis of the extraocular muscles and Marcus Gunn jaw-winking phenomenon (bottom) harbors a de novo heterozygous 2840T→C transition mutation in KIF21A.*
tropic, infraducted primary position of each eye, the clinical diagnosis in this case is CFEOM1 (rather than CFEOM3). The novel de novo \textit{KIF21A} mutation \(2840T \rightarrow C\) found in the proband is predicted to result in a M947T amino acid substitution. Our group previously reported 3 different single-base substitutions at codon 947,5,13 including a heterozygous \(2839A \rightarrow G\) transition mutation at the first position of codon 947 (M947V) in a CFEOM1 proband, a heterozygous \(2840T \rightarrow G\) transversion at the second position of codon 947 (M947R) in a CFEOM1 proband, and a heterozygous \(2841G \rightarrow A\) transition at the third position of codon 947 (M947I) in a CFEOM3 proband. The \(2840T \rightarrow C\) (M947T) mutation identified in our proband is the fourth mutation our group has identified in codon 947 and the eighth \textit{KIF21A} mutation we have identified overall, further supporting codon 947 as a frequent site of \textit{KIF21A} mutations that cause CFEOM. Codon 947 in \textit{KIF21A} is an evolutionally conserved methionine residue and is located at the A position of a heptad repeat within the coiled-coil region of the \textit{KIF21A} stalk. The findings of this study reinforce the importance of this residue in the biological function of the \textit{KIF21A} protein.

To support our hypothesis that the association of CFEOM1 and MG in this patient was not by chance, we searched the clinical records of our patients with CFEOM harboring \textit{KIF21A} mutations and identified 3 additional participants with CFEOM1 and MG. All 4 individuals were male and had left-sided MG. Two are members of dominant pedigrees and have relatives with CFEOM1 without MG who harbor the same mutation; 2 share the most common \textit{KIF21A} CFEOM mutation with many other individuals who have CFEOM1 but not MG. Therefore, the MG phenotype appears to occur at a low frequency with \textit{KIF21A} mutations. Sidedness and sex predilection have not been found in larger studies of patients with MG,23,24 and we do not know the significance of the sex and sidedness findings in these 4 patients. It is interesting, however, to contrast this with Duane syndrome studies, in which a predominance of female patients and left eye involvement has been reported.25,26 One case of left-sided Duane syndrome with MG has been reported in a female patient.27

To our knowledge, this is the first report of a genetic association for MG and expands our knowledge of the genetic and phenotypic heterogeneity of CFEOM. It remains unclear whether MG in the setting of CFEOM1 arises as a primary result of \textit{KIF21A} dysfunction or from more random secondary miswiring. All of the 4 individuals with CFEOM1 and MG also had facial weakness and/or delays in motor and/or speech development, findings that are present at a lower frequency in our patients with CFEOM1 without MG. Together, these signs and symptoms suggest aberrant innervation extending be-

<table>
<thead>
<tr>
<th>Marker</th>
<th>Distance, kb*</th>
<th>Father (JR02)</th>
<th>Mother (JR03)</th>
<th>Proband (JR01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D12S1648</td>
<td>1803</td>
<td>10.9</td>
<td>5.1</td>
<td>10.5</td>
</tr>
<tr>
<td>D12S1692</td>
<td>1005</td>
<td>3.3</td>
<td>1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>D12S59</td>
<td>3716</td>
<td>7.7</td>
<td>2.1</td>
<td>7.2</td>
</tr>
<tr>
<td>D12S1067</td>
<td>372</td>
<td>1.2</td>
<td>3.3</td>
<td>1.3</td>
</tr>
<tr>
<td>KIF21A</td>
<td>1190</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D12S1048</td>
<td>177</td>
<td>2.1</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>D12S1668</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Abbreviations: FEOM1, classic autosomal dominant fibrosis of the extraocular muscles; kb, kilobase.
*Indicates physical distance to the next marker or gene.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Sex</th>
<th>Origin</th>
<th>Mutated Nucleotide</th>
<th>Altered Amino Acid</th>
<th>Phenotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>JR01</td>
<td>M</td>
<td>United States</td>
<td>2840T→C</td>
<td>M947T</td>
<td>CFEOM1, left-sided MG, and mild facial weakness</td>
<td>Current report</td>
</tr>
<tr>
<td>AL03</td>
<td>M</td>
<td>United States</td>
<td>3029T→C</td>
<td>I1010T</td>
<td>CFEOM1, left-sided MG, mild facial weakness, and mild gross motor delay</td>
<td>Yamada et al,5 2003</td>
</tr>
<tr>
<td>EO01</td>
<td>M</td>
<td>United States</td>
<td>2860C→T</td>
<td>R954W</td>
<td>CFEOM1, left-sided MG, and mild speech delay*</td>
<td>Yamada et al,5 2003</td>
</tr>
<tr>
<td>EX01</td>
<td>M</td>
<td>United States</td>
<td>2860C→T</td>
<td>R954W</td>
<td>CFEOM1, left-sided MG, and speech delay</td>
<td>Yamada et al,5 2003; Brodsky,15 1998</td>
</tr>
<tr>
<td>FG01</td>
<td>F</td>
<td>England</td>
<td>2860C→T</td>
<td>R954W</td>
<td>CFEOM1 and right hypertropia during toothbrushing</td>
<td>Yamada et al,5 2003; Gottlob et al,20 2002</td>
</tr>
</tbody>
</table>

Abbreviations: CFEOM, congenital fibrosis of the extraocular muscles; CFEOM1, classic autosomal dominant CFEOM; MG, Marcus Gunn jaw-winking phenomenon.
*He and his unaffected fraternal brother have mild speech delay.
yond cranial nerves III, IV, and VI and support a potentially broader role for KIF21A in human axonal development and neuromuscular innervation.

Submitted for Publication: June 21, 2004; final revision received November 15, 2004; accepted January 3, 2005.

Correspondence: Elizabeth C. Engle, MD, Division of Genetics, Children’s Hospital Boston, 300 Longwood Ave, Boston, MA 02115 (engle@enders.tch.harvard.edu).

Financial Disclosure: None.

Funding/Support: This study was supported by grants R01-EY12498 and R01-EY13583 from the National Eye Institute, National Institutes of Health, Bethesda, Md (Dr Engle) and P30-HD18655 (Mental Retardation Research Center, Children’s Hospital Boston) from the National Institutes of Health.

Acknowledgments: We thank Joseph Calhoun, MD; James Katowitz, MD; Scott M. Goldstein, MD; Michael Brodsky, MD; and Irene Gottlob, MD, for referring patients for the study, and the patients and families for their participation.

REFERENCES

9. Engle EC, McIntosh N, Yamada K, et al. CFEOM1, the classic familial form of congenital fibrosis of the extraocular muscles, is genetically heterogeneous but does not result from mutations in ARIX. BMC Genet. 2002;3:3.