CFEOM3: A New Extraocular Congenital Fibrosis Syndrome that Maps to 16q24.2-q24.3

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PURPOSE. To define the clinical characteristics and determine the gene localization for a previously undescribed form of congenital fibrosis of the extraocular muscles (CFEOM), referred to as CFEOM type 5 (CFEOM3).

METHODS. A large family with CFEOM was identified, and participating individuals underwent ophthalmologic examination and donated blood for genetic analysis. The family's disorder was tested for linkage to the known CFEOM loci, followed by a genome-wide search and linkage refinement using polymorphic DNA markers.

RESULTS. Thirty-eight members of this Canadian family participated in the study. Affected individuals are born with a nonprogressive eye movement disorder characterized by variable expression of ptosis and restrictive external ophthalmoplegia. Severely affected individuals have ptosis, primary gaze fixed in a hypo- and exotropic position, and marked restriction of eye movement bilaterally. Mildly affected individuals have normally positioned globes with a limitation of vertical gaze. Moderately affected individuals have asymmetrical involvement with one eye severely and one eye mildly affected. The disorder is autosomal dominant with variable expression and probable incomplete penetrance. Genetic analysis reveals linkage to markers on 16q24.2-q24.3. A maximum lod score of 5.8 occurs at markers D16S3063 and D16S689, and the CFEOM3 disease gene is located within a ~5.6-cM region flanked by D16S486 and D16S671.

CONCLUSIONS. These data establish that CFEOM5 is a phenotypically variant and genomically distinct form of CFEOM with linkage to chromosome 16qter. The authors have previously demonstrated that CFEOM1 results from a developmental absence of the superior division of the oculomotor nerve. The authors hypothesize that CFEOM3 results from a defect analogous to, but distinct from, CFEOM1. (Invest Ophthalmol Vis Sci. 1999;40:1687-1694)

The congenital fibrosis syndromes are restrictive strabismic disorders that include Duane’s retraction syndrome and various forms of congenital fibrosis of the extraocular muscles (CFEOM).1,2 These disorders are characterized by congenital restrictive ophthalmoplegia with or without ptosis and are distinguished from each other on the basis of their specific clinical characteristics, genetic characteristics, or both.

Individuals with Duane’s syndrome (MIM 126800) are born with a horizontal motility defect and associated globe retraction.3 This disorder is typically sporadic and isolated but can occur as a dominant or recessive trait, often in association with other congenital anomalies.1 Neuropathologic examinations of isolated Duane’s syndrome patients revealed an absence of the abduces nerve (cranial nerve VI) and nucleus.4,5

Individuals with the classic form of CFEOM, CFEOM1 (MIM 135700), are born with bilateral ptosis and ophthalmoplegia, with their eyes partially or completely fixed in an infraducted (downward) position.6 Each CFEOM1 eye, in addition to being primarily infraducted, also may be eso- or exotropic. CFEOM1 is autosomal dominant, and the causative gene in multiple families has been mapped to the centromere of chromosome 12.7,8 Neuropathologic examination of an individual with CFEOM1 demonstrated an absence of the superior division of the oculomotor nerve (cranial nerve III) and corresponding oculomotor subnuclei.6

Individuals with CFEOM2 (MIM 602078) are born with bilateral ptosis and ophthalmoplegia, with their eyes partially or completely fixed in an exotropic (outward) position. Each CFEOM2 eye, in addition to being primarily exotropia, may also be mildly hyper- or hypotropic.9 CFEOM2 is autosomal recessive, and the causative gene in three consanguineous Saudi Arabian families has been mapped to chromosome 11q13.9

In the process of studying these disorders, we identified a family with an inherited form of congenital fibrosis syndrome that we refer to as CFEOM3. Here, we describe the clinical features of CFEOM3 and establish linkage of the causative gene to a locus near the telomere of the long arm of chromosome

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16. These data demonstrate that CFEOM3 is phenotypically variant and genotypically distinct from the other forms of CFEOM and that it represents a new congenital fibrosis syndrome.

MATERIALS AND METHODS

Pedigree Collection

A family with a congenital fibrosis syndrome was identified and family members agreed to participate in the study. Each participating individual was interviewed and examined by the authors (EJD, CTD, ECE), had photographs and video recordings taken of his or her eye movements, and donated a blood sample for DNA extraction. An individual was considered clinically affected if he or she had unilateral or bilateral limitation of globe movement. This study was approved by the Children's Hospital institutional review board, and all participants signed informed consent forms. Our methods adhered to the Declaration of Helsinki for research involving human subjects.

DNA Typing

Blood for DNA extraction was collected from participating family members. Lymphocyte DNA was extracted using the Puregene kit (Gentra, Research Triangle Park, NC). Chromosomal analysis of GTG-banded metaphase cells was performed on lymphocytes of affected individual II:2.

The initial linkage studies were based on analysis of DNA from 11 affected and 7 unaffected individuals and were conducted using DNA microsatellite markers from selected regions of chromosomes 1, 10, chromosomes 11, 19 and chromosome 12 and, subsequently, using polymorphic markers from the Cooperative Human Linkage Center (CHLC) Human Screening Set/Weber Version 6a (Research Genetics, Huntsville, AL). Linkage refinement was based on analysis of DNA from all 38 participants and was conducted using additional di-, tri-, and tetranucleotide polymorphic markers from Genethon (http://www.genethon.fr/genethon_en.html)11 and the Marshall Center for Human Genetics (http://www.marshmed.org/genetics).12 All primer sequences are available from the Genome Database (http://gdbwww.gdb.org) or these publications. Primers were purchased from Genosys Biotechnologies (http://www.genosys.com). Genotypes were determined by 30 cycles of polymerase chain reaction amplification of 10-μl reaction volumes containing 40 to 60 ng of genomic DNA, 40 ng of each primer, 200 μM each of dATP, dCTP, dGTP, and dTTP, 1μCi α-32P-dCTP (3000 Ci mmol−1), and 0.5 U Taq polymerase (Perkin-Elmer). The polymerase chain reaction products were separated on 6% denaturing polyacrylamide sequencing gels, and the alleles were visualized by autoradiography.7

Linkage Analysis

Logarithm of the odds of linkage (lod) scores were calculated using the Fastlink version 3.0 package of programs,13 assuming autosomal dominant inheritance with incomplete penetrance and 10 marker alleles of equal frequency. For lod score calculations, an individual was scored as affected based on the consensus of the examining physicians before genotyping, or if an individual had both an affected parent and an affected offspring. Because we did not know whether individual II:2 carries a de novo mutation, we calculated lod scores using two different affection status assignment methods: method 1 scored the affection status of I:1, I:2, II:8, II:10, and III:20 as unknown; method 2 scored I:1 as affected and I:2, II:8, II:9, II:10, and III:20 as unaffected. Lod scores were calculated for each assignment with 80%, 85%, 90%, and 95% penetrance. Data on the population incidence of the CFEOM5 mutation are unavailable; for purposes of lod score calculations we used a disease incidence of 1/1,000,000 births. Alteration of this incidence by ±1000-fold had negligible effect on the maximum lod scores.

RESULTS

Clinical Description

Thirty-eight individuals (31 family members and 7 spouses) from a New Brunswick, Canada, family participated in the study (Fig. 1). One affected individual (IV:11) chose not to participate. The family is of Anglo-Saxon descent and segregates a congenital fibrosis syndrome as an autosomal dominant trait, with male-to-male transmission (ruling out X-linked and mitochondrial inheritance), and no clinical evidence of anticipation. Clinically affected individuals are born with a nonprogressive eye movement disorder with varying degrees of ophthalmoplegia, ptosis, and abnormal residual eye movements. The primary horizontal position of gaze of each affected eye is on or hypotropic to the horizontal midline. The primary vertical position of gaze is on or exotropic to the vertical midline. In addition, extraocular movements are limited to some degree in all affected individuals. The disorder is variably expressed, and examination findings of affected individuals can range from severe to mild. These findings are described below, diagrammed in Table 1 and photographically documented by severity group in Figure 2 and by family group in Figure 3.

Nine individuals are severely affected (group 1 of Table 1; Figs. 2A, 2B, 3A, 3D). The eyes of these individuals are fixed bilaterally hypo- and exotropic (II:2, III:8, III:12, III:14, IV:2, IV:13, IV:15) or one eye hypo- and exotropic and the other eye only exotropic (IV:7, IV:10). These individuals have complete restriction of up-gaze and marked restriction of down-gaze, and they demonstrate minimal lateral eye movement. Seven of these individuals have severe and two have moderate degrees of bilateral ptosis. All have a compensatory chin elevation and head turn. Individuals III:14, IV:2, IV:7, and IV:13 have undergone strabismus, lid surgery, or both and individual II:2 has had bilateral cataract surgery. An operative report is available from individual IV:2, and forced duction testing confirmed restriction of globe movement.

Two individuals are moderately affected (group 2 of Table 1, Figs. 2C, 2D). Both of these individuals, III:19 and IV:9, have one eye fixed in a hypotropic position and one eye in a straight position, and the hypotropic eye has greater restriction of movement and a greater degree of ptosis. Neither individual displays a compensatory posturing of the head.

Five members of the family are mildly affected and have both eyes in a straight primary position (group 3 of Table 1, Figs. 2E, 2F, 3B, 3C). The three of the five (III:9, IV:8, IV:21) have restriction of vertical gaze and mild unilateral ptosis. III:9, who considers himself affected and has two affected sons, has almost complete restriction of up-gaze. Although IV:8 and IV:21 were considered unaffected by their families, clinical examination demonstrated that IV:8 had marked restriction of vertical gaze (Figs. 2E, 2F) and IV:21 had mild
restriction of vertical gaze. Two of the five mildly affected individuals, III:2 and IV:1, had no ptosis and minimal restrictions of ocular motility, and both considered themselves to be clinically unaffected. Their subtle restrictions of motility (Table 1, Figs. 3B, 3C) were not fully appreciated until a post-genotyping review of the video recordings (denoted by half-filled symbols in Fig. 1). The variable expression of the CFEOM3 phenotype within a nuclear family is demonstrated by the spectrum of clinical findings of individuals II:2, III:2, IV:1, and IV:2 (Table 1, Fig. 3). The grandmother, II:2, is severely affected. Her daughter, III:2, is mildly affected. Of the daughter’s two sons, one is mildly affected (IV:1) and one is severely affected (IV:2). Thus, one cannot predict an individual’s phenotype based on their affected parent or sibling’s phenotype. In addition, because individual III:2 is minimally affected and has a severely affected mother (II:2) and son (IV:2), she provides essential evidence that the CFEOM3 disease mutation can be minimally expressed and may potentially be nonpenetrant (Fig. 3).

In addition to limited ocular motility, some affected members of the family also have abnormal residual eye movements. IV:1 (Fig. 3C) has right globe retraction with palpebral fissure narrowing on leftward gaze. IV:7 has bilateral divergence on attempted lateral gaze. IV:8 (Figs. 2E, 2F) has down-shooting of the right adducted eye on left gaze, and III:19 has down-shooting of the left adducted eye on right gaze. IV:2 (Fig. 3D) has bilateral divergence on attempted down-gaze and bilateral independent horizontal nystagmoid movements with both fixation and attempted saccades.

Pupillary function, slit-lamp examination of the anterior segment, and fundoscopic examination were normal in all affected individuals. Severely affected individuals have amblyopia associated with strabismus and ptosis. In addition, these individuals tend to have myopia and astigmatism (Table 1). Two (IV:10, IV:15) were refractive to conventional amblyopic treatment in early childhood. Visual acuity is less affected in patients with less severe motility disturbances. Severely affected individuals also appear to have mild facial weakness (Figs. 2A, 2B, 3A, 3D). No other congenital anomalies were found.

No abnormalities of eye position or movement were detected on examination of the remaining participating family members. II:2’s two living siblings, II:8 and II:10, and one of II:10’s three children (III:20) participated in the study and had normal examinations. In addition, II:2’s parents, deceased sib-
lings, and all the siblings’ descendants are reportedly unaffected.

**CFEOM3 Linkage Analysis**

Twelve individuals (9 severe, 2 moderate, and 1 mild) were affected both by their own report and by our examination and were therefore scored as affected for linkage analysis. Fourteen offspring of affected individuals considered themselves to be unaffected. Of these 14, 4 were found on examination to have abnormalities of ocular motility (II:2, IV:1, IV:8, IV:21; Table 1, group 3). Because IV:8 and IV:21 were judged affected by our pregenotyping examinations, they were scored as affected for linkage analysis. Although III:2’s minimal limitations of gaze were not appreciated until a post-genotyping review of video recordings, she was scored as affected because her mother (II:2) and one of her sons (IV:2) are affected, and thus she is an

<table>
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<th>VA (corrected)</th>
<th>Refraction</th>
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<td>os: 20/40</td>
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<td>−7.00 + 2.50×120</td>
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<td>os: 20/25</td>
<td>−4.75</td>
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<td>od: 20/200</td>
<td>os: 20/40</td>
<td>−2.25 (with prisms)</td>
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<td>os: 20/60</td>
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<td>plano + 2.75×90</td>
<td>−0.50 + 3.00×180</td>
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</table>

**Group 2—moderate**

| III:19        | Left mild | od: 20/25 | os: 20/40 | −0.25 + 0.25×180 | −0.50 + 0.75×110 |
| IV:9          | Right mod | od: 20/30 | os: 20/22 | −0.5 + 0.50×90 | plano |

(continues)
obligate carrier of the CFEOM3 gene mutation. Because IV:1’s minimal limitations of gaze were not appreciated until a post-genotyping review of video recordings and because we did not know if the globe retraction found on the pre-genotyping examination was a consequence of the CFEOM3 mutation, he was scored as clinically unaffected for linkage analysis.

Cytogenetic analysis of chromosomes from individual II:2 was normal at greater than the 400-band level. Analysis of genetic markers surrounding the existing loci excluded linkage of the CFEOM3 disease gene to the chromosome 12 CFEOM1 locus, the chromosome 11 CFEOM2 locus, and the chromosome 1 congenital ptosis locus.7–10 Therefore, a complete genome-wide search was undertaken to map the CFEOM3 gene, and 162 polymorphic markers spanning the human genome were analyzed for linkage. Of the markers in the initial screen, only one marker had a maximum lod score greater than 1 (lod $= 1.5$ at $\theta = 0.10$). This marker, D16S539, is located approximately 10 cM from 16qter. Analysis of 22 additional markers in this region established linkage of CFEOM3 to the region telomeric to D16S539 (Table 2). A maximum lod score of 5.8 at $\theta = 0.00$ was obtained at two nonrecombinant markers, D16S486 and D16S476 in individual III:2 (Fig. 1 and Table 2). The resultant recombinant disease-associated haplotype carried by III:2 was inherited by both of her sons, of whom one is severely affected (IV:2) and one is minimally affected (IV:1; Fig. 3). The telomeric flanking marker, D16S671, is defined by recombination events in affected individuals IV:7, IV:8, and IV:10 between markers D16S671 and D16S303/D16S3121 (Fig. 1 and Table 2; relative order of D16S303 and D16S121 not known). Two clinically unaffected sisters of II:2 participated in the study, and one (II:6) inherited the disease-associated haplotype. We do not know if the CFEOM3 mutation carried by II:2 arose de novo or was inherited. Therefore, we do not know if II:6 inherited the common disease haplotype without the mutation, or if she is an unaffected obligate carrier of the disease mutation. Identifying the CFEOM3 disease gene will allow us to address this question.

**DISCUSSION**

Our findings establish CFEOM3 as a phenotypically variant and genotypically unique form of the congenital fibrosis syndromes. This disorder presents as a congenital nonprogressive, autosomal dominant ocular motility disorder with variable expression. Family members may be bilaterally or unilaterally affected, and their oculomotility defects range from complete ophthalmoplegia (with the eyes fixed in a hypo- and exotropic position), to mild asymptomatic restrictions of ocular move-
Ptosis, refractive error, amblyopia, and compensatory head positions are associated with the more severe forms of the disorder.

CFEOM3 is variably expressed and can result in a phenotype that overlaps with that of CFEOM1 or CFEOM2. This adds a level of complexity to the clinical distinction of the specific congenital fibrosis syndromes. Given our current knowledge, the clinical evaluation of an affected patient (without the benefit of linkage analysis) may or may not be sufficient to distinguish CFEOM3 from CFEOM1 or CFEOM2. For example, the members of the CFEOM3 pedigree who have bilateral ptosis and globe exotropia with significant infraduction are indistinguishable from some individuals with CFEOM1. Similarly, the members of the CFEOM3 pedigree who have ptosis and globe exotropia and mild unilateral hypotropia are indistinguishable from some individuals with CFEOM2. On the other hand, individuals with CFEOM3 who have unilateral affection or subtle motility defects, individuals with CFEOM1 who have infraduction and esotropia, and individuals with CFEOM2 who have exotropia and supraduction can be distinguished clinically from one another, and their findings do not currently overlap with each of the other syndromes. The clinical distinctions, or possible lack of distinction, between the genetically defined congenital fibrosis syndromes will become more clear as additional families are identified.

Identifying additional CFEOM3 families may also help determine whether CFEOM3 is incompletely penetrant, or if all individuals who inherit a mutation have some phenotypic expression of the disease. Although we have found deficits of ocular motility in all family members who carry the CFEOM3 haplotype, we are not sure whether the minimal abnormalities identified during the post-genotype review of videotapes of individuals III:2 and IV:1 (Figs. 3B, 3C) are consequences of the CFEOM3 genotype or whether they reflect normal variation in the population. If they are a consequence of the genotype, then CFEOM3 is completely penetrant in this family. Using pregenotyping examination results, however, the penetrance is calculated to be 83% to 88%. One of the six individuals in generation III with affected offspring is not affected (II:2), yielding a penetrance based on skipped generations of 83%. Combining the examination results with genotype data revealed that 14 of 16 individuals carrying the disease haplotype are clinically affected, yielding an observed penetrance of 88%. Thus, the data presented in Table 2 are calculated assuming a 90% penetrance.

The neuropathologic findings in the two congenital fibrosis syndromes that have been studied, Duane’s syndrome and CFEOM1, suggest that these disorders do not result from primary muscle fibrosis but, instead, from maldevelopment of specific cranial motoneuron pools. Neuropathologic examinations of individuals with Duane’s syndrome demonstrated an absence of the abducens nerve (cranial nerve VI) and nucleus, and partial innervation of the lateral rectus muscle by branches from the oculomotor nerve. Neuropathologic examination of an individual with CFEOM1 identified the absence of the superior division of the oculomotor nerve (cranial nerve III),
specific loss of the nerve’s corresponding levator palpebrae superioris and superior rectus motoneurons (with sparing of other subpopulations of motoneurons in the cranial nerve III nucleus), and marked abnormalities of the levator palpebrae superioris and superior rectus muscles, which elevate the eyelid and the globe, respectively." These findings suggest that the CFEOM1 and Duane’s syndrome genes are essential for the normal development and/or axonal projection of a subset of human alpha motoneurons in the brain stem.

Neither extraocular muscle biopsies nor neuropathologic studies from individuals with CFEOM3 are available. However, CFEOM3 shares many features with CFEOM1 and Duane’s syndrome, and, by analogy, we propose that CFEOM3 may result from a similar anatomic defect. The fixed hypo- and exotropic positions of the eyes, ptosis, and pupillary sparing found in the severely affected CFEOM3 family members are most consistent with dysfunction of the entire somatic motor component of the oculomotor nerve (both the superior and inferior branches), with sparing of its visceral motor (parasympathetic) component. Therefore, we hypothesize that the gene mutated in CFEOM3 may play a role in the development of both the superior and inferior somatic motor divisions of the oculomotor nerve and corresponding oculomotor subnuclei.

The CFEOM3 disease gene is not allelic to CFEOM1, CFEOM2, or congenital ptosis and maps to chromosome 16q24.2-q24.3. Recombination events in affected family members define a disease gene region of approximately 5.6 cM flanked by markers D16S486 and D16S671 and correspond to a physical distance of approximately 3.7 megabases. The marker order established by the family’s recombination data support the published order with one exception. Because individual III:2 is recombinant for D16S486 but is not recombinant for D16S476 or D16S3063, we have provisionally altered the Marshfield marker order to reflect this.

At least 20 genes and 30 partial transcripts (expressed sequence tags) have been physically mapped close to, or within, the CFEOM3 critical region (National Center for Biotechnology Information database, http://www.ncbi.nlm.nih.gov). Among these are the disease genes for Fanconi anemia, mucopolysaccharidosis IVA and spastic paraplegia-5B, and genes for cytochrome c oxidase subunit IV, adenine phosphoribosyltransferase, and an inward rectifying potassium channel. Based on our speculations, none of the genes are clear CFEOM3 disease gene candidates. Many of the expressed sequence tags are expressed in the brain, and a few have been isolated only from infant brain libraries and, therefore, might be good candidates. However, because the current region is still very large and contains many genes, the best approach may be to identify additional CFEOM3 families through whom we may refine the critical region further by genetic analysis.

To identify additional CFEOM3 pedigrees it will be important to carefully examine family members of congenital fibrosis patients, because the oculomotility defect in relatives of individuals with CFEOM3 can be subtle. There are several previously published pedigrees that are phenotypically similar to the reported CFEOM3 family.15–17 These families’ diseases may be caused by mutations in the CFEOM3 gene. If they are linked to the 16qter locus, they will help further define the penetrance and clinical spectrum of CFEOM3 and, possibly, contribute to refining the genetic localization. The identification of the CFEOM3 disease gene should elucidate the etiology of this disorder and may help to determine the basis of the phenotypic variability among affected individuals. In addition, our eventual ability to study the various CFEOM and ptosis gene products should provide an understanding of the molecular basis of this spectrum of ocular motility disorders and could lead to new insight into cranial nerve development.

Acknowledgments

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References


Table 2. Lod Scores of CFEOM3 and Chromosome 16qter Markers

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</tbody>
</table>

<sup>a</sup> Maximum likelihood estimate of the lod score.

<sup>b</sup> Maximum likelihood estimate of θ.

<sup>c</sup> Lod scores were calculated on the assumption of 90% penetrance and the affection assignment method 1 in the Methods section. Each marker’s maximum lod score varied, at most, by 0.7 when calculated with each of the four penetrances using each of the affection assignments.

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