

Congenital Fibrosis of the Extraocular Muscles Type 2, an Inherited Exotropic Strabismus Fixus, Maps to Distal 11q13

S. M. Wang,¹ J. Zwaan,^{3,*} P. B. Mullaney,^{3,†} M. H. Jabak,³ A. Al-Awad,³ A. H. Beggs,¹ and E. C. Engle^{1,2}

¹Division of Genetics and ²Department of Neurology, Children's Hospital, Harvard Medical School, Boston; and ³King Khaled Eye Specialist Hospital, Riyadh, Kingdom of Saudi Arabia

Summary

The extraocular fibrosis syndromes are congenital ocular-motility disorders that arise from dysfunction of the oculomotor, trochlear, and abducens nerves and/or the muscles that they innervate. Each is marked by a specific form of restrictive paralytic ophthalmoplegia with or without ptosis. Individuals with the classic form of congenital fibrosis of the extraocular muscles (CFEOM1) are born with bilateral ptosis and a restrictive infraductive external ophthalmoplegia. We previously demonstrated that CFEOM1 is caused by an autosomal dominant locus on chromosome 12 and results from a developmental absence of the superior division of the oculomotor nerve. We now have mapped a variant of CFEOM, exotropic strabismus fixus ("CFEOM2"). Affected individuals are born with bilateral ptosis and restrictive ophthalmoplegia with the globes "frozen" in extreme abduction. This autosomal recessive disorder is present in members of three consanguineous Saudi Arabian families. Genetic analysis of 70 individuals (20 affected individuals) reveals linkage to markers on chromosome 11q13, with a combined LOD score of 12.3 at the single nonrecombinant marker, *D11S1314*. The 2.5-cM CFEOM2 critical region is flanked by *D11S4196/D11S4162* and *D11S4184/1369*. Two of the three families share a common disease-associated haplotype, suggesting a founder effect for CFEOM2. We hypothesize that CFEOM2 results from an analogous developmental defect to CFEOM1, one that affects both the superior and inferior divisions of the oculomotor nerve and their corresponding alpha motoneurons and extraocular muscles.

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Address for correspondence and reprints: Dr. E. C. Engle, Division of Genetics, Enders 5, The Children's Hospital, 300 Longwood Avenue, Boston, MA 02115. E-mail: engle@rascal.med.harvard.edu

*Present affiliation: Department of Ophthalmology, The Hospital for Sick Children, Toronto.

†Present affiliation: Department of Ophthalmology, University of Texas Health Science Center, San Antonio.

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Introduction

The extraocular fibrosis syndromes are congenital ocular-motility disorders that arise from dysfunction of the oculomotor, trochlear, and abducens nerves and/or the muscles that they innervate. The syndromes include Duane syndrome (MIM 126800) and congenital fibrosis of the extraocular muscles (CFEOM); each syndrome is manifested clinically by a specific form of restrictive paralytic ophthalmoplegia with or without ptosis (Brown 1950; Harley et al. 1978). Duane syndrome is typically sporadic, and affected individuals are born with restricted adduction and/or abduction with globe retraction on attempted adduction (Huber 1984). Two neuropathological studies of Duane syndrome found an absence of the abducens nerve and its corresponding abducens nucleus in the pons, with abnormalities of the lateral rectus muscle (which normally abducts the globe) (Hotchkiss et al. 1980; Miller et al. 1982).

Individuals with the classic form of congenital fibrosis of the extraocular muscles (CFEOM [MIM 135700]) are born with bilateral ptosis and restrictive external ophthalmoplegia, with their eyes partially or completely fixed in an infraducted (downward) and strabismic position. This disorder is inherited in an autosomal dominant fashion, and the causative gene has been mapped, in multiple families, to the centromere of chromosome 12 (Engle et al. 1994, 1995). We now refer to the expression of this clinical phenotype in members of autosomal dominant, fully penetrant pedigrees with genetic linkage to 12cen as CFEOM1 (MIM 135700). Neuropathological studies of an individual with CFEOM1 revealed an absence of the superior division of the oculomotor nerve and its corresponding alpha motoneurons in the midbrain, as well as marked abnormalities of the two muscles that this branch innervates—the superior rectus and the levator palpebrae superioris. These two muscles elevate the globe and the eyelid, respectively, and their functional absence results in the infraduction and ptosis found in CFEOM1 (Engle et al. 1997b). These findings suggest that the CFEOM1 and Duane syndrome genes are essential for the normal development and/or

axonal projection of a subset of human alpha motoneurons in the brain stem.

During the course of our studies, it became evident that there were families with forms of CFEOM that differ phenotypically and genotypically from CFEOM1. One of these variants is an autosomal recessive disorder that we have characterized in three consanguineous Saudi Arabian families. In contrast to the infraduction found in CFEOM1 patients, most of the affected individuals in these kindreds are born with bilateral exotropia and ptosis with the globes "frozen" in extreme abduction. Although there are cases of isolated bilateral restrictive exotropia or esotropia, referred to in the literature as "strabismus fixus" (Brown 1950; Apt and Axelrod 1978; Harley et al. 1978), this is the first genetic study of inherited restrictive exotropia. Here, we show that this disorder, designated "CFEOM2" (MIM 602078), is not allelic to CFEOM1 and maps to a locus on distal 11q13.

Subjects and Methods

Pedigree Collection

The families affected with CFEOM2 were identified, and each participating individual signed a consent form and was interviewed and examined by one or more of the authors (J.Z., P.B.M., M.H.J., and/or A.A.). The study was approved by and performed with institutional-review-board approval from the King Khaled Eye Specialist Hospital (KKESH [Riyadh]) and Children's Hospital (Boston).

DNA Typing

Blood samples were collected from 70 individuals (20 affected individuals) from the three families, and lymphocyte DNA was extracted by use of a standard protocol (Kunkel et al. 1977). Chromosomal analysis of GTG-banded metaphase cells was performed on lymphocytes of affected individuals Z IV:1 and Z IV:4.

Linkage studies employed DNA microsatellite markers (di-, tri-, and tetranucleotides) from the Cooperative Human Linkage Center Human Screening Set/Weber version 6 (Research Genetics). Linkage refinement was conducted with additional markers from chromosomes 1 and 12, as described elsewhere (Engle et al. 1995, 1997a), as well as from Génethon and other published sources (Couillin et al. 1994; James et al. 1994; Dib et al. 1996; Guru et al. 1997; Merscher et al. 1997). All primer sequences are available from either Genome Database or these publications. Primers were purchased from Research Genetics, and Genosys Biotechnologies. Amplification and analysis of each repeat polymorphism

were performed as reported elsewhere (Engle et al. 1994, 1995).

Linkage Analysis

LOD scores were calculated with the Fastlink version 3.0 package of programs (Lathrop and Lalouel 1984; Lathrop et al. 1984; Cottingham et al. 1993; Schäffer et al. 1994), under the assumption of autosomal recessive inheritance with full penetrance. Data on the population incidence of the mutation(s) are unavailable; for purposes of LOD-score calculations, we used a disease incidence of 1/1,000,000 births and 10 marker alleles of equal frequency. Alteration of this incidence by $\pm 1,000$ -fold had negligible effects on the maximum LOD scores.

Results

Clinical Description

The study is based on three Saudi Arabian families—BD, BB, and Z—as shown in figures 1–3. Thirty-seven members (9 affected) from pedigree BD, 24 members (8 affected) from pedigree BB, and 9 members (3 affected) from pedigree Z participated in the study. In all three families, the disorder is inherited as an autosomal recessive trait. There are two affected individuals (BD IV:3 and BB IV:5) who have affected offspring, demonstrating a pseudodominant inheritance pattern. Members of each family identified loops of consanguinity resulting from first-cousin marriages—one loop in Z, two loops in BB, and six loops in BD. Pedigrees BD and BB are from separate families/tribes within the same geographical area of eastern Saudi Arabia. Pedigree Z's family/tribe is from western Saudi Arabia and extends to central Saudi Arabia. None of the families is aware of an ancestral relationship to either of the other two.

A manuscript describing the clinical examinations and surgical findings of the affected members of the three families is in preparation (J. Zwann, P. B. Mullaney, M. H. Jabak, and A. Al-Awad, unpublished data). In brief, all affected individuals were born with nonprogressive bilateral upper lid ptosis and restrictive ophthalmoplegia. Most remarkably, in 18 of 20 affected individuals, the globes were fixed in abduction with a ~ 50 – 90 -prism diopter exotropia bilaterally (fig. 4). In addition, 10 of these individuals had much less remarkable deviations in the vertical plane—6 (Z IV:1, BB V:12, BD V:4, BD V:5, BD V:14, and BD VI:6) had mild unilateral hypertropia, and 4 (BB IV:5, BB V:13, BD V:17, and BD V:18) had mild unilateral hypotropia. Two of the 20 affected individuals did not have exotropia: the eyes of BD IV:3 were fixed in the neutral position; and the right

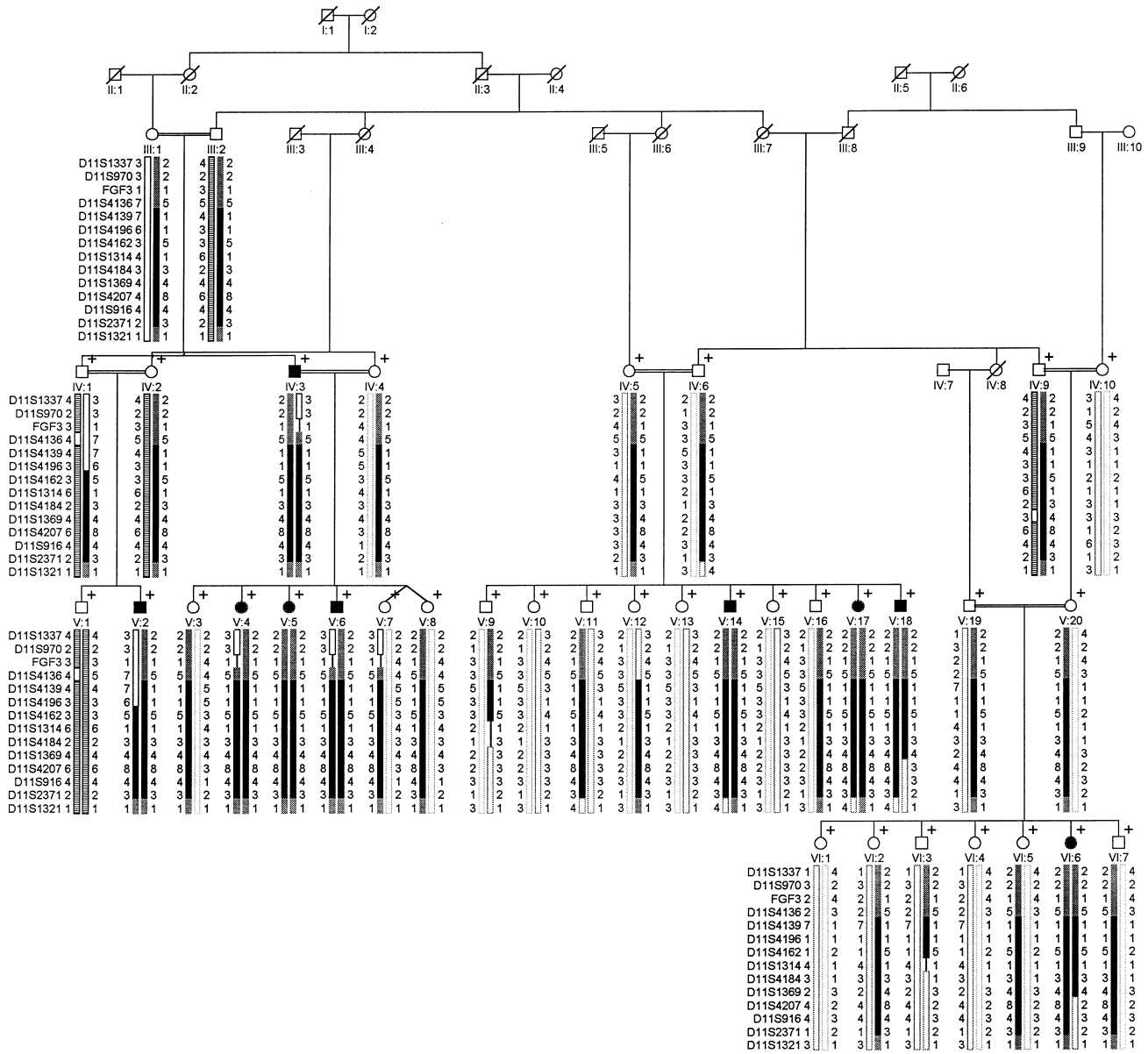


Figure 1 Haplotype analysis of family BD, segregating CFEOM2. Blackened symbols denote those individuals who are clinically affected with CFEOM 2; a diagonal slash (/) through a symbol denotes that the individual is deceased; a double horizontal line (=) denotes consanguinity; and a plus sign (+) denotes that the individual participated in the study. Genotyping data and schematic haplotype bars for chromosome 11 markers are shown below the symbol for each individual and indicate regions of crossover. Gray-shaded bars denote disease-associated regions of homozygosity within a family, and blackened bars denote subregions of haplotype identity between families. Horizontally hatched bars highlight the inheritance of specific non-disease-associated haplotypes, and vertical lines denote noninformative regions adjacent to critical recombination events.

eye of BB IV:5 was fixed in the neutral position, with minimal residual down gaze, and the left eye was slightly hypotropic with only mild limitation of horizontal gaze. In all affected individuals, voluntary globe movements were absent or consisted only of minimal residual abduction, forced duction testing was positive (restricted), and binocular vision was absent.

Most affected individuals had poor visual acuity, and many had severe amblyopia, particularly in eyes in which the pupil was covered by a ptotic lid (fig. 4). In many of the older affected individuals, the pupils were myotic and fixed or only sluggishly reactive to light or pupillary dilators. Anterior segments were normal, and the disks were normal or pale. Affected individuals BB

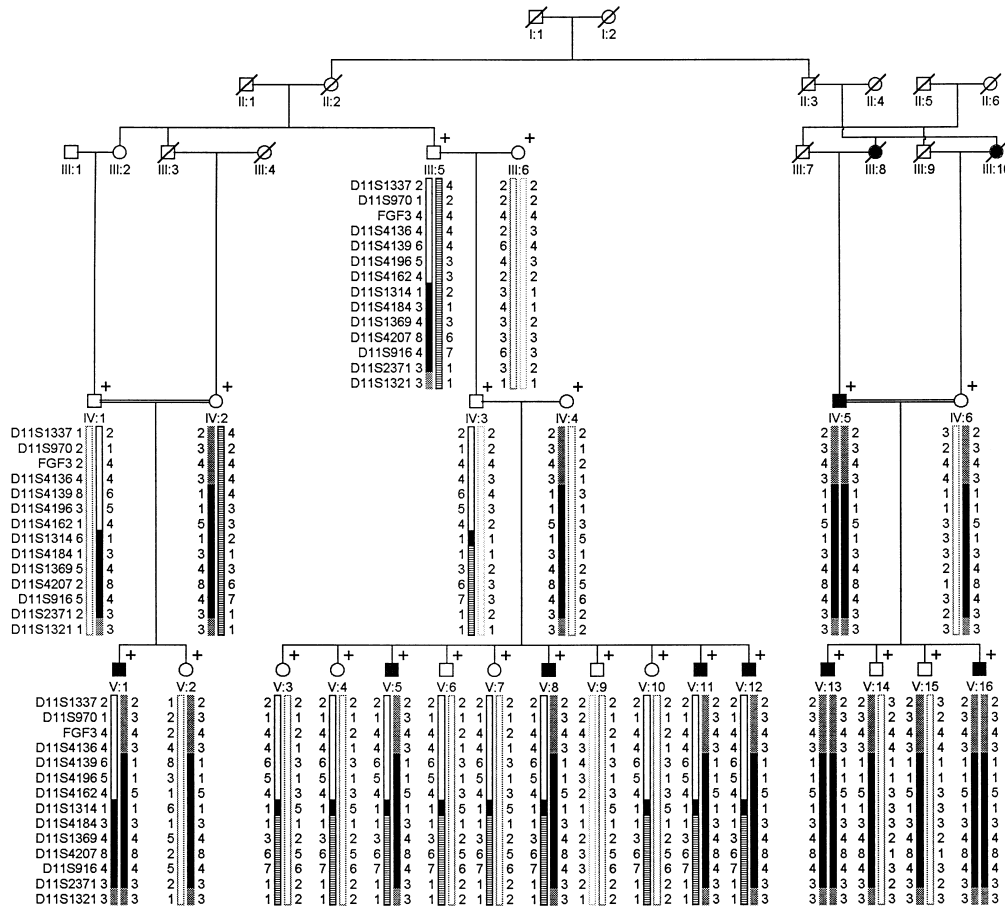


Figure 2 Haplotype analysis of family BB, segregating CFEOM2. Symbols are as defined in the legend to figure 1.

IV:5, BB V:13, and BB V:16 had extremely poor vision, complete myosis, and electroretinogram (ERG) recordings that revealed a generalized reduction in amplitude. Further ERG investigations are currently underway to determine if these findings are independent or coinherited with the fibrosis syndrome. Results of the remaining cranial-nerve and general neurological examinations were normal, although the affected children and some affected adults appeared to have mild facial weakness.

Linkage of CFEOM2 to Distal Chromosome 11q13

Cytogenetic analysis of chromosomes from affected individuals Z IV:1 and Z IV:4 appeared normal at >400-band level. The CFEOM1 locus on chromosome 12 (Engle et al. 1994, 1995) and the congenital ptosis locus on chromosome 1 (Engle et al. 1997a) were analyzed, and linkage was excluded at both loci as follows. At the 3-cM critical region surrounding the CFEOM1 locus, LOD scores < -2.0 were obtained in pedigrees BB and Z, at marker D12S61, with a recombination frequency (θ) of .08 and .05, and at markers D12S345 and

D12S1090, with $\theta = .07$ and .04, respectively. A LOD score < -2 was obtained in pedigree BD, at marker D12S1668, with $\theta = .11$. At the 3-cM critical region surrounding the congenital ptosis locus, LOD scores < -2.0 were obtained in pedigrees BD, BB, and Z, at marker D1S2134, with $\theta = .07$, .05, and .02, respectively, and in pedigree Z, at markers D1S2733 and D1S1616, with $\theta = .03$ and .01, respectively. A genome-wide search for linkage within family BD then was performed, and 64 loci were ruled out successfully (data not shown) before linkage to marker D11S2371, with a LOD score of 5.8 at $\theta = .05$, was found (table 1). Pedigrees BB and Z were then tested, and the disease gene in these kindreds was shown to cosegregate with D11S2371 (table 1).

To define the CFEOM2 critical region, additional polymorphic markers in the region were tested for linkage (table 1). On the basis of the structure and consanguinity loops found in the pedigrees, we hypothesized that the mutation was introduced on a single allele by individual I:1 or individual I:2 in families BD and Z and

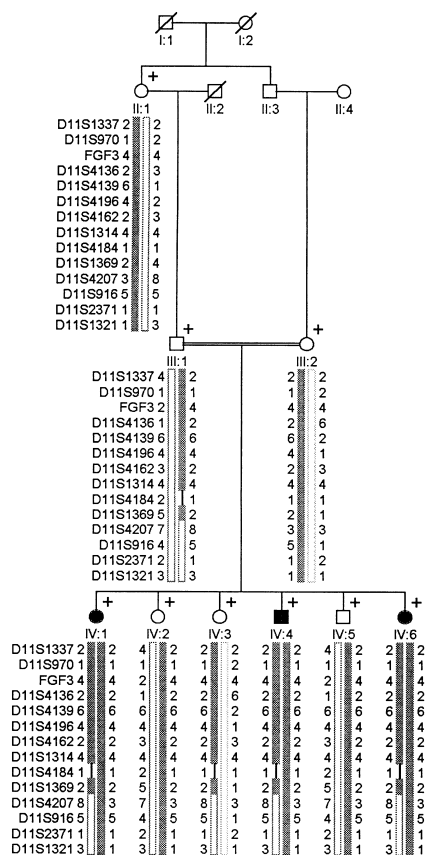


Figure 3 Haplotype analysis of family Z, segregating CFEOM2. Symbols are as defined in the legend to figure 1.

was likely to be on a common background within family BB. We also considered it quite likely that the mutation in all three families arose from a common founder individual. Therefore, we tested 44 polymorphic markers between *D11S1985* and *D11S1321*, in an attempt to identify recombination events as well as regions of homozygosity within families and allelic association between families. This analysis revealed that, of the 44 markers, the distal 14 markers spanned the CFEOM2 critical region (figs. 1–3). Regions of overlapping homozygosity were identified within each family and, in figures 1–3, are represented by the gray-shaded portions of the schematic haplotype bars. In addition, within these regions of homozygosity within each family, there is a 3.6-cM subregion of haplotype identity found in all nonrecombinant affected individuals of pedigrees BD and BB. This shared disease-associated haplotype (1-1-5-1-3-4-8-4-3) extends from *D11S4139* to *D11S2371* and, in figures 1 and 2, is highlighted as the blackened portions of the haplotype bars. The affected members of pedigree Z do not share with BD or BB a common disease haplotype at the markers tested. The disease haplotype shared between pedigrees BD and BB helps to

determine phase and to identify ancestral recombination events. The recombination events within each family are described below.

Pedigree BD.—Affected individuals V:5, V:14, and V:17 share a region of allele homozygosity extending from above *D11S1337* to *D11S2371* (data shown in fig. 1, as gray-shaded/blackened haplotypes). Recombination events between *D11S4184/D11S1369* and *D11S4207* in affected individuals V:18 and VI:6, however, establish *D11S4207* as the telomeric flanking marker for the disease gene in this family. In addition, the disease alleles carried by the two brothers IV:1 and IV:3 each contain a centromeric recombination. This recombination occurs between *D11S4196* and *D11S4162* in IV:1. Although the Génethon map places *D11S4162* as 0.1 cM centromeric to *D11S4196* (Dib et al. 1996), we have provisionally reversed the order of these two markers, to avoid the necessity of positing a double recombination in individual IV:1. As a result, we refer to the flanking centromeric marker as “*D11S4196/D11S4162*,” until the markers are definitively ordered. Of note, tracing the inheritance of an unaffected haplotype (shown, in fig. 1, as horizontally hatched haplotypes) suggests that individuals III:3 and III:8 are related to their spouses, although these relationships have not been determined. In addition, our data support a spontaneous mutation of

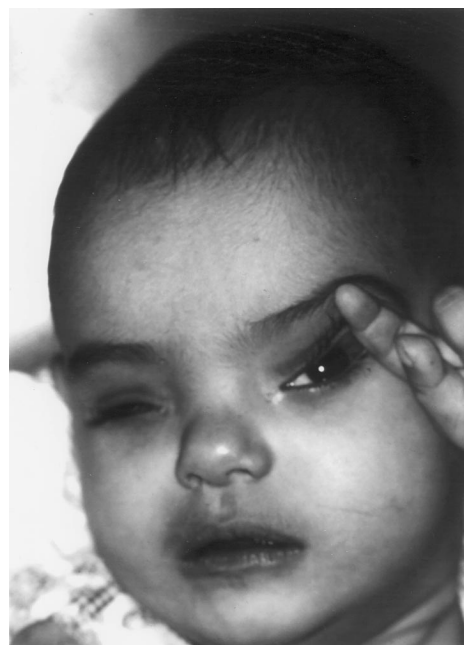


Figure 4 Photograph of individual BB V:12, showing the clinical features of CFEOM2. The toddler has bilateral ptosis and 60–80-prism-diopter exotropia, right hypertropia, and marked limitation of adduction, elevation, and depression. He fixes with his exotropic left eye by deviating his head to the right and elevating his left lid with his left index finger.

Table 1
Two-Point LOD Scores between the CFEOM2 Locus and 11q Markers

MARKER AND FAMILY	LOD SCORE AT $\theta =$					
	.00	.05	.10	.20	.30	.40
D11S4136:						
BD	$-\infty$	6.5	6.0	4.7	3.1	1.4
BB	-1.4	3.0	2.8	2.1	1.2	.4
Z	<u>2.6</u>	<u>2.4</u>	<u>2.1</u>	<u>1.5</u>	<u>1.0</u>	<u>.5</u>
Total	$-\infty$	11.9	10.9	8.3	5.3	2.3
D11S4139:						
BD	$-\infty$	6.3	5.8	4.4	2.8	1.2
BB	-2.2	1.5	1.6	1.3	.8	.3
Z	<u>1.1</u>	<u>1.0</u>	<u>.9</u>	<u>.7</u>	<u>.4</u>	<u>.2</u>
Total	$-\infty$	8.8	8.3	6.4	4.0	1.7
D11S4196:						
BD	$-\infty$	4.8	4.4	3.3	2.0	.7
BB	-4.6	-.6	-.2	.1	.2	.2
Z	<u>1.1</u>	1.0	.9	.7	.4	.2
Total	$-\infty$	5.2	5.1	4.1	2.6	1.1
D11S4162:						
BD	9.5	8.5	7.6	5.6	3.5	1.4
BB	-.5	2.9	2.8	2.2	1.3	.5
Z	<u>2.1</u>	<u>1.8</u>	1.5	1.0	.5	.1
Total	11.1	13.2	11.9	8.8	5.3	2.0
D11S1314:						
BD	7.1	6.4	5.7	4.1	2.6	1.1
BB	4.9	4.3	3.8	2.7	1.6	.6
Z	<u>.3</u>	<u>.2</u>	<u>.2</u>	<u>.1</u>	<u>.0</u>	<u>.0</u>
Total	12.3	10.9	9.7	6.9	4.2	1.7
D11S4184:						
BD	5.1	4.5	4.0	2.8	1.7	.8
BB	-2.9	1.6	1.6	1.3	.7	.3
Z	<u>1.2</u>	<u>1.1</u>	<u>1.0</u>	<u>.8</u>	<u>.5</u>	<u>.3</u>
Total	3.4	7.2	6.6	4.9	2.9	1.4
D11S1369:						
BD	5.8	5.2	4.7	3.5	2.3	1.1
BB	-1.5	4.2	3.8	2.8	1.8	.7
Z	<u>2.6</u>	<u>2.4</u>	<u>2.1</u>	<u>1.5</u>	<u>1.0</u>	<u>.5</u>
Total	6.9	11.8	10.6	7.8	5.1	2.3
D11S4207:						
BD	$-\infty$	6.3	6.1	4.8	3.2	1.5
BB	-1.5	4.2	3.8	2.8	1.7	.7
Z	<u>-4.4</u>	<u>.3</u>	<u>.5</u>	<u>.6</u>	<u>.4</u>	<u>.3</u>
Total	$-\infty$	10.8	10.4	8.2	5.3	2.5
D11S916:						
BD	$-\infty$	3.4	3.5	2.9	2.0	1.0
BB	-1.5	4.2	3.8	2.8	1.7	.7
Z	<u>2.4</u>	<u>2.2</u>	<u>1.9</u>	<u>1.4</u>	<u>1.0</u>	<u>.5</u>
Total	$-\infty$	9.8	9.2	7.1	4.7	2.2
D11S2371:						
BD	$-\infty$	5.8	5.5	4.3	2.7	1.1
BB	-1.5	4.2	3.8	2.8	1.7	.7
Z	<u>2.4</u>	<u>2.2</u>	<u>1.9</u>	<u>1.4</u>	<u>1.0</u>	<u>.5</u>
Total	$-\infty$	12.2	11.2	8.5	5.4	2.3

the non-disease-associated allele of marker *D11S4136* in individual IV:1. This result was reproduced several times and is the only spontaneous mutation found in the study, and it does not influence the results. Thus, the flanking markers for the CFEOM2 disease gene in ped-

igree BD are *D11S4196/D11S4162* and *D11S4207*. The maximum LOD score for pedigree BD is 9.5 ($\theta = 0$) and occurs at marker *D11S4162* (table 1).

Pedigree BB.—Affected individual IV:5 and his two affected sons (V:13 and V:16) share a region of homozygosity extending from above *D11S1337* to below *D11S1321* (shown, in fig. 2, as gray-shaded/blackened haplotypes). This disease haplotype was introduced into the family not only by either I:1 or I:2 but also by II:4, either II:5 or II:6, and IV:4, suggesting additional unidentified loops of consanguinity. This disease haplotype accounts for one of the two disease alleles in affected individuals V:1, V:5, V:8, V:11, and V:12. The second haplotype inherited by these five individuals contains a centromeric recombination and, in all but V:1, a telomeric recombination. The centromeric recombination, between *D11S4196/D11S4162* and *D11S1314*, was most likely introduced by II:1 (suggesting another unidentified loop), since it is inherited by III:5 and IV:1. As a result of this recombination, affected individual V:1 shares haplotype identity with V:13 and V:16, in the region from *D11S1314* through *D11S1321*. The telomeric recombination occurs between *D11S1314* and *D11S4184/D11S1369* in unaffected individual IV:3. Therefore, the paternally inherited disease allele of affected individuals V:5, V:8, V:11, and V:12 defines the centromeric and telomeric flanking markers for the CFEOM2 gene as being *D11S4162/D11S4162* and *D11S4184/D11S1369*, respectively. The maximum LOD score for pedigree BB is 4.9 ($\theta = 0$) and occurs at the single nonrecombinant marker, *D11S1314* (table 1).

Pedigree Z.—Affected siblings IV:1, IV:4, and IV:6 share a region of homozygosity extending from above *D11S1337* to *D11S1369* (shown, in fig. 3, as gray-shaded haplotypes). A recombination event in individual III:1, between *D11S4184/D11S1369* and *D11S4207*, establishes *D11S4207* as the telomeric flanking marker in this family. The maximum LOD score obtainable in this smaller pedigree is 2.6 ($\theta = 0$) and occurs at the maximally informative markers, *D11S4136* and *D11S1369* (table 1).

Pooling the recombination data from each of the families results in the definition of an ~2.5-cM critical region for the CFEOM2 disease gene, a region that falls within distal 11q13 (Merscher et al. 1997). The critical region is flanked by *D11S4196/D11S4162* and *D11S4184/D11S1369* (figs. 1-3 and table 1), and only one non-recombinant marker, *D11S1314*, currently lies within it.

Discussion

Our data localize the causative gene for CFEOM2, an exotropic variant of the congenital fibrosis syndromes, to an ~2.5-cM region on chromosome 11q13 flanked

by polymorphic markers *D11S4196/D11S4162* and *D11S4184/D11S1369*. The marker order supported by our linkage data is generally consistent with that previously published (James et al. 1994; Dib et al. 1996; Merscher et al. 1997), although we provisionally have reversed the order of *D11S4196* and *D11S4162*. In addition, the CFEOM2-flanking telomeric marker remains ambiguous, since *D11S4184* and *D11S1369* have not been mapped relative to one another and cannot be ordered on the basis of our data.

The CFEOM2 critical region begins ~6 cM telomeric to *D11S913* and the *MEN1* region (Guru et al. 1997) and may fall partially or completely within the 5.5-Mb high-resolution integrated map of distal 11q13 constructed by Merscher et al. (1997). Determining whether *D11S4184*, *D11S1314*, and the CFEOM2-centromeric recombinant markers fall within the 1,600 kb of the Merscher map that are centromeric to *D11S1369* will better define the physical boundaries of the critical region. A physical map will provide the framework needed to correctly order the flanking markers, to identify additional polymorphic markers, and to allow us to include or exclude four potential candidate genes mapped to the region: *KRN1*, *NUMA1*, *FOLR1*, and *FOLR2* (Merscher et al. 1997). In addition, since all three CFEOM2 families are of similar ethnic and geographic origin, all demonstrate consanguinity, and BD and BB share a common disease-associated haplotype across the critical region, it seems likely that their disorder arose from a single founder mutation. Pedigree Z does not share, at the markers studied, a common haplotype with BD and BB; however, this family is from a different region of Saudi Arabia and may be more distantly related. Therefore, there might be, around marker *D11S1314*, a small region that displays a haplotype shared by all three families. Generation of additional polymorphic markers around *D11S1314* will allow us to search for such a region and to refine further the CFEOM2 locus by homozygosity mapping.

Autosomal recessive CFEOM has been reported previously in the Saudi Arabian population (Traboulsi et al. 1993; Assaf 1997). However, to the best of our knowledge, these families represent the most thoroughly investigated group of patients with exotropic strabismus fixus. Virtually all of the CFEOM2 individuals examined have extreme exotropia with little or no deviation of the globe in the vertical plane. Since the eyes of two affected individuals are not horizontally deviated, however, bilateral exotropia cannot be considered the defining feature of CFEOM2. Despite this, all affected members of the three families are clinically distinguishable from individuals with CFEOM1, since none have significant bilateral infraduction. CFEOM2 and CFEOM1 are genetically distinguishable by their mode of inheritance (recessive vs. dominant) and by the chromosomal lo-

calization of the mutated gene (11q13 vs. 12cen). The population incidence of mutations in the CFEOM2 gene is unknown, and it remains to be determined whether additional patients will be identified. Once the CFEOM2 gene is cloned, it will be possible to look for pathogenic mutations in those individuals with the more common, sporadic exotropic strabismus fixus (Brown 1950; Apt and Axelrod 1978; Harley et al. 1978), as well as in patients with sporadic and, as yet, unclassified forms of CFEOM.

The neuropathology underlying CFEOM2 has not been elucidated. We hypothesize, however, that it may be analogous to the defects found in individuals with CFEOM1 and Duane syndrome and that it may result from a selective absence of a pool of midbrain alpha motoneurons and their corresponding axons. The abnormal development of the entire motor division of the oculomotor (and, possibly, the trochlear) nucleus and/or nerve would leave the abducens-innervated lateral rectus muscle unopposed, resulting in ptosis and restrictive globe abduction. Thus, while CFEOM1 appears to be due to a defect in the development of the superior division of the oculomotor nerve, CFEOM2 is predicted to result from a defect in the development of both its superior and inferior branches.

There are many possible functions for the CFEOM1 and CFEOM2 genes, such as motoneuron growth and differentiation factors and axonal navigation and pathfinding mediators. Theoretical candidates include such molecules as the downstream targets of the *Wnt-1* and *Engrailed-1* gene products, which are critical for the development of oculomotor and trochlear motoneurons in mice (Wurst et al. 1994; Fritsch et al. 1995; Porter and Baker 1997), and a relative of *semaphorin III/D*, which, when knocked out in mice, results in errors of axonal projection of cranial nerves V, VII, and IX–XI but not of the oculomotor nerve (Taniguchi et al. 1997). Although none of the currently known genes in the CFEOM2 critical region are known to have similar function, they remain viable candidates on the basis of their position, and it is likely that future studies will identify additional transcription units in this region.

It has been recognized that there is a considerable degree of homology between chromosome 11 and chromosome 12. Examples of paralogous genes mapped to chromosome 11 and chromosome 12, respectively, include insulinlike growth-factor 2 (*IGF2*) and insulinlike growth-factor 1 (*IGF1*), parathyroid hormone (*PTH*) and parathyroid hormone-like hormone (*PTH LH*), and Harvey rat sarcoma viral oncogene homologue (*HRAS*) and Kirsten rat sarcoma 2 viral oncogene homologue (*KRAS2*) (OMIM). The members of each of these pairs of genes share high degrees of homology, suggesting a common evolutionary origin. Similarly, although they come from regions of chromosome 11 and chromosome

12 that have no known paralogous pairs of genes, the CFEOM2 and CFEOM1 genes may well have parallel functions in the development of the oculomotor axis. Therefore, it is possible that they arose from a common evolutionary origin and are homologous genes. If this is true, then the identification of one may assist in the cloning of the second.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, <http://www.genethon.fr>

Genome Database, <http://gdbwww.gdb.org> (for primer sequences)

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for Duane syndrome [MIM 126800], CFEOM1 [MIM 135700], and CFEOM2 [MIM 602078])

References

- Apt. L, Axelrod RN (1978) Generalized fibrosis of the extraocular muscles. *Am J Ophthalmol* 85:822-829
- Assaf A (1997) Bilateral congenital vertical gaze disorders: congenital muscle fibrosis or congenital central nervous abnormalities. *Neuroophthalmology* 17:23-30
- Brown HW (1950) Congenital structural muscle anomalies. In: Allen JH (ed) *Strabismus ophthalmic symposium*. CV Mosby, St Louis, pp 205-236
- Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. *Am J Hum Genet* 53:252-263
- Couillin P, Le Guern E, Vignal A, Fizames C, Ravise N, Delportes D, Reguigne I, et al (1994) Assignment of 112 microsatellite markers to 23 chromosome 11 subregions delineated by somatic hybrids: comparison with the genetic map. *Genomics* 21:379-387
- Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152-154
- Engle EC, Castro AE, Macy ME, Knoll JHM, Beggs AH (1997) *aA* gene for isolated congenital ptosis maps to a 3-cM region within 1p32-p34.1. *Am J Hum Genet* 60:1150-1157
- Engle EC, Goumernov B, McKeown CA, Schatz M, Johns DR, Porter JD, Beggs AH (1997) *bO* oculomotor nerve and muscle abnormalities in congenital fibrosis of the extraocular muscles. *Ann Neurol* 41:314-325
- Engle EC, Kunkel LM, Specht LA, Beggs AH (1994) Mapping a gene for congenital fibrosis of the extraocular muscles to the centromeric region of chromosome 12. *Nat Genet* 7:69-73
- Engle EC, Marondel I, Houtman WA, de Vries B, Loewenstein A, Lazar M, Ward DC, et al (1995) Congenital fibrosis of the extraocular muscles (autosomal dominant congenital external ophthalmoplegia): genetic homogeneity, linkage refinement, and physical mapping on chromosome 12. *Am J Hum Genet* 57:1086-1094
- Fritzsch B, Nichols D, Echelard Y, McMahon A (1995) Development of midbrain and anterior hindbrain ocular motoneurons in normal and Wnt-1 knockout mice. *J Neurobiol* 27:457-469
- Guru SC, Olufemi SE, Manickam P, Cummings C, Gieser LM, Pike BL, Bittner ML, et al (1997) A 2.8-Mb clone contig of the multiple endocrine neoplasia type 1 (MEN1) region of 11q13. *Genomics* 42:436-445
- Harley RD, Rodrigues MM, Crawford JS (1978) Congenital fibrosis of the extraocular muscles. *J Pediatr Ophthalmol Strabismus* 15:346-358
- Hotchkiss MG, Miller NR, Clark AW, Green WG (1980) Bilateral Duane's retraction syndrome: a clinical-pathological case report. *Arch Ophthalmol* 98:870-874
- Huber A (1984) Duane's retraction syndrome: consideration on pathophysiology and etiology. In: Reinecke R (ed) *Strabismus II*. Grune & Stratton, Orlando, pp 345-361
- James MR, Richard CW, Schott J-J, Yousry C, Clark K, Bell J, Terwilliger JD, et al (1994) A radiation hybrid map of 506 STS markers spanning human chromosome 11. *Nat Genet* 8:70-76
- Kunkel LM, Smith KD, Boyer SH, Borgaonkar DS, Wachtel SS, Miller OJ, Breg WR, et al (1977) Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci USA* 74:1245-1249
- Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 36:460-465
- Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 81:3443-3446
- Merscher S, Bekri S, De Leeuw B, Pedoutour F, Grosgeorge J, Shows TB, Mullenbach R, et al (1997) A 5.5-Mb high-resolution integrated map of distal 11q13. *Genomics* 39:340-347
- Miller NR, Kiel SM, Green WR, Clark AW (1982) Unilateral Duane's retraction syndrome (type 1). *Arch Ophthalmol* 100:1468-1472
- Porter JD, Baker RS (1997) Absence of oculomotor and trochlear motoneurons leads to altered extraocular muscle development in the Wnt-1 null mutant mouse. *Brain Res Dev Brain Res* 100:121-126
- Schäffer AA, Gupta SK, Shriram K, Cottingham RW Jr (1994)

- Avoiding recomputation in linkage analysis. *Hum Hered* 44: 225–237
- Taniguchi M, Yuasa S, Fujisawa H, Naruse I, Saga S, Mishina M, Yagi T (1997) Disruption of *Semaphorin III/D* gene causes severe abnormality in peripheral nerve projection. *Neuron* 19:519–530
- Traboulsi E, Jaafar M, Kattan H, Parks M (1993) Congenital fibrosis of the extraocular muscles: report of 24 cases illustrating the clinical spectrum and surgical management. *Am Orthopt J* 43:45–53
- Wurst W, Auerbach A, Joyner A (1994) Multiple developmental defects in *Engrailed-1* mutant mice: an early mid- and hindbrain deletion and patterning defects in forelimbs and sternum. *Development* 120:2065–2075