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Congenital Fibrosis of the Extraocular Muscles (Autosomal Dominant Congenital External Ophthalmoplegia): Genetic Homogeneity, Linkage Refinement, and Physical Mapping on Chromosome 12

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Summary

Congenital fibrosis of the extraocular muscles (CFEOM) is an autosomal dominant syndrome of congenital external ophthalmoplegia and bilateral ptosis. We previously reported linkage of this disorder in two unrelated families to an 8-cM region near the centromere of human chromosome 12. We now present refinement of linkage in the original two families, linkage analysis of five additional families, and a physical map of the critical region for the CFEOM gene. In each of the seven families the disease gene is linked to the pericentromeric region of chromosome 12. D12S345, D12S59, D12S331, and D12S1048 do not recombine with the disease gene and have combined lod scores of 35.7, 35.6, 16.0, and 31.4, respectively. AFM136xf6 and AFMb320wd9 flank the CFEOM locus, defining a critical region of 3 cM spanning the centromere of chromosome 12. These data support the concept that this may be a genetically homogeneous disorder. We also describe the generation of a YAC contig encompassing the critical region of the CFEOM locus. This interval has been assigned cytogenetically to 12p11.2-q12 and spans the centromere of chromosome 12. These results provide the basis for further molecular analyses of the structure and organization of the CFEOM locus and will help in the identification of candidate genes.

Introduction

Dysfunction of extraocular muscle (EOM) results clinically in a combination of strabismus, ophthalmoplegia, ptosis, and amblyopia. Understanding the etiology of such disorders by conventional electrophysiological and pathological techniques has proved difficult. However, insight into EOM development, function, and susceptibility to disease may be gained by studying genetic disorders of EOM by molecular genetic methods. We are studying congenital fibrosis of EOM (CFEOM; MIM 135700), an autosomal dominant congenital external ophthalmoplegia. This disorder has been referred to in the literature by various names: the pedigrees studied in this paper were originally reported as "congenital familial external ophthalmoplegia with co-contraction"(Cibis 1984; Cibis et al. 1984), "ocular congenital fibrosis syndrome" (Nemet et al. 1985), "hereditary congenital external ophthalmoplegia" (Houtman et al. 1986), and "congenital fibrosis of the extraocular muscles" (Laughlin 1956; Traboulsi et al. 1993; Engle et al. 1994).

Classical CFEOM is characterized by congenital, nonprogressive, bilateral ptosis and external ophthalmoplegia with the eyes fixed in a strabismic and hypotropic position. Thus, affected individuals typically must tilt their heads back to compensate for the ptosis and the fixed downward position of the globes. Residual eye movements are often aberrant, and convergent or divergent movements are typically elicited on attempted upgaze. Forced ductions demonstrate a marked resistance to passive movement. It is unclear if this disorder results from a primary neurogenic or myopathic abnormality. The disorder is autosomal dominant and fully penetrant.

The literature contains reports of individuals and families with slightly variable presentations of congenital ophthalmoplegia with or without ptosis. These include autosomal dominant bilateral external ophthalmoplegia with incomplete penetrance, autosomal dominant unilateral external ophthalmoplegia with and without complete penetrance, autosomal dominant bilateral or unilateral complete (external and internal) ophthalmoplegia, and autosomal recessive external or complete ophthalmoplegia. In the present study, we have limited

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our analysis to families with what we consider "classical CFEOM," or "autosomal dominant congenital external ophthalmoplegia" with complete penetrance.

Elsewhere we have reported linkage mapping of a gene for CFEOM to an 8-cM centromeric region of chromosome 12 in two unrelated families (Engle et al. 1994). In the present report, we demonstrate both linkage in five additional families and refinement of our critical region to 3 cM, and we provide a YAC-based physical map of this region. Our linkage data support the notion that this clinically defined population represents a single, genetically homogeneous disorder.

Material and Methods

Pedigree Collection

The affection status of each family member was determined either by examination by one of us or by another ophthalmologist, by previous examinations documented in the literature, or by telephone discussions with the individual and examination of photographs. The study was approved by the appropriate institutional review board, and all participants were counseled and signed informed-consent forms.

DNA Typing

Blood for DNA extraction was collected from 11 family members of pedigree C, 39 family members of pedigree H, 35 members of pedigree AA, 14 members of pedigree AC, and 13 members of pedigree AD, as indicated by a plus sign (+) in figure 1. To control for possible sample mix-ups, each specimen was split, and the two halves were independently used for direct purification of lymphocyte DNA, as described elsewhere (Kunkel et al. 1977), or by a Puregene kit (Gentra).

PCR primers to amplify polymorphic CA repeats were purchased from Research Genetics or were synthesized on a model 380B DNA synthesizer. Primer sequences are available from the Genome Data Base (http://gdbwww.gdb.org), Cooperative Human Linkage Center (http://www.chlc.org/), or Généthon (http://www.genethon.fr/genethon_en.html). Amplification of each dinucleotide-repeat polymorphism was performed as reported elsewhere (Engle et al. 1994). D12S1048 and D12S1090 were amplified for 35 cycles of 40 s at 94°C and 30 s at 55°C, followed by 7 min at 72°C. D12S59, D12S61, D12S331, and D12S345 were amplified for 30 cycles of 30 s at 94°C, 75 s at 55°C, and 15 s at 72°C. D12S315 were amplified for 35 cycles of 30 s at 94°C,

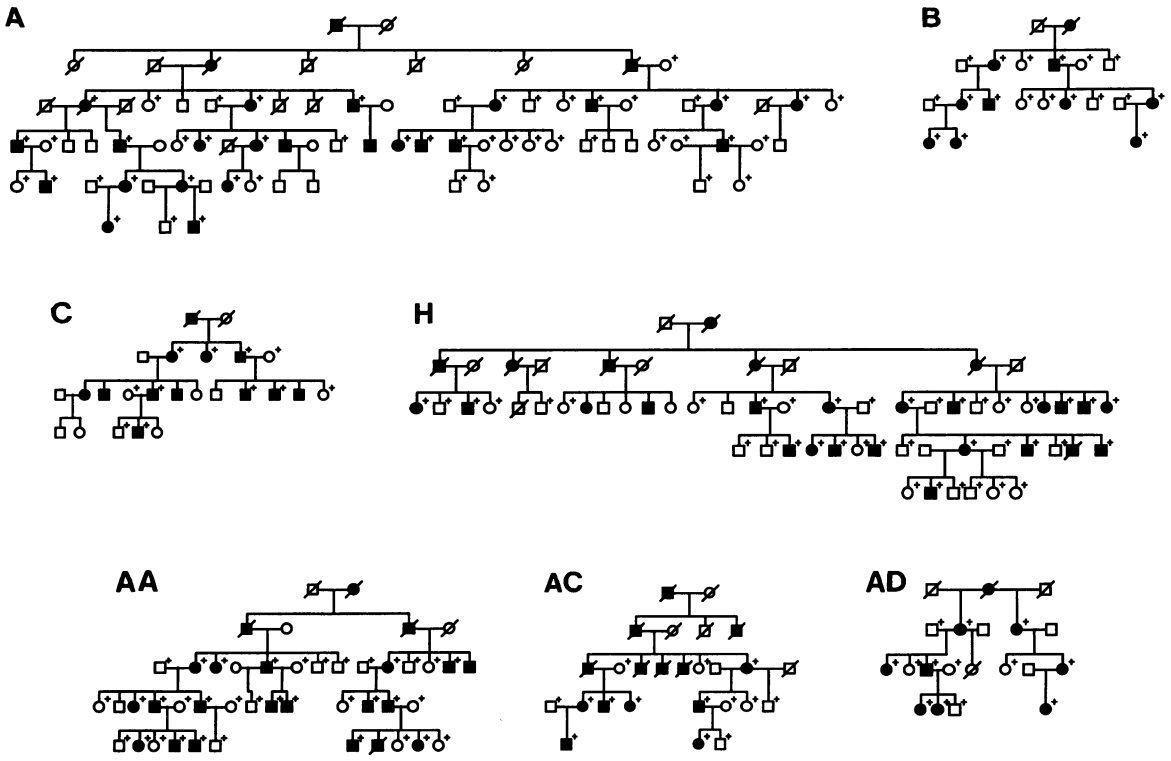

Figure 1 Pedigrees of the seven CFEOM families analyzed. Families are identified by their pedigree code names. Blackened symbols indicate individuals affected with CFEOM. Individuals studied are denoted by a plus sign (+). References to individuals in text refer to pedigree (A, B, C, H, AA, AC, or AD); generation number, from the first down (Roman numeral); and position number within generation, from left to right (Arabic numeral). Offspring of unaffected individuals are not shown, since none were clinically affected.

Table 1

Lod Scores of Chromosome 12 Centromeric Markers with CFEOM

Locus and Family	Z at θ = .00	.05	.10	.20	.30	.40	Z_{max}[a]	Maximum θ[a]
D12S61:								
A	−∞	3.8	3.7	3.0	2.2	1.1	3.8	.05
B	−∞	.1	.3	.3	.2	.1	.3	.16
C	.9	.8	.6	.4	.2	.1	.9	.00
H	9.4	8.7	7.9	6.1	4.2	2.1	9.4	.00
AA	6.8	6.3	5.7	4.5	3.1	1.5	6.8	.00
AC	−∞	−.2	.1	.2	.1	.1	.2	.22
AD	.6	.5	.4	.3	.2	.1	.6	.00
Total	−∞	20.0	18.7	14.8	10.2	5.1		
AFM136xf6:								
A	−∞	4.5	4.3	3.4	2.2	.9	4.5	.05
B	3.6	3.3	2.9	2.2	1.4	.6	3.6	.00
C	1.7	1.5	1.3	.9	.4	.1	1.7	.00
H	4.6	4.3	4.0	3.1	2.1	.9	4.6	.00
AA	6.8	6.2	5.6	4.4	3.0	1.5	6.8	.00
AC	−∞	−.5	−.3	−.3	−.3	−.1	−.1	.40
AD	2.3	2.1	1.9	1.5	1.0	.5	2.3	.00
Total	−∞	21.4	19.7	15.2	9.8	4.4		
D12S345:								
A	10.5	9.7	8.8	6.9	4.8	2.5	10.5	.00
B	3.6	3.3	3.0	2.3	1.5	.6	3.6	.00
C	2.0	1.8	1.8	1.1	.9	.3	2.0	.00
H	7.9	7.2	6.5	5.0	3.3	1.5	7.9	.00
AA	7.4	6.8	6.2	4.8	3.3	1.6	7.4	.00
AC	1.7	1.7	1.6	1.3	.9	.4	1.7	.00
AD	2.6	2.4	2.1	1.6	1.0	.5	2.6	.00
Total	35.7	32.9	30.0	23.0	15.7	7.4		
D12S59:								
A	10.0	9.2	8.3	6.5	4.4	2.2	10.0	.00
B	2.1	1.9	1.7	1.4	1.0	.4	2.1	.00
C	1.7	1.5	1.3	1.0	.7	.2	1.7	.00
H	9.7	8.8	8.0	6.1	4.1	1.9	9.7	.00
AA	7.2	6.6	5.9	4.5	3.0	1.3	7.2	.00
AC	2.9	2.6	2.3	1.7	1.1	.4	2.9	.00
AD	2.0	1.8	1.6	1.1	.7	.3	2.0	.00
Total	35.6	32.4	29.1	22.3	15.0	6.7		
D12S331:								
A	5.0	4.6	4.2	3.4	2.4	1.3	5.0	.00
B	.9	.8	.7	.5	.3	.1	.9	.00
C	2.0	1.8	1.6	1.2	.7	.3	2.0	.00
H	3.6	3.3	3.1	2.5	1.7	.9	3.6	.00
AA	3.4	3.2	3.0	2.4	1.6	.7	3.4	.00
AC	.8	.7	.6	.4	.2	.1	.8	.00
AD	.3	.2	.2	.0	−.1	−.1	.3	.00
Total	16.0	14.6	13.4	10.4	6.8	3.3		
D12S1048:								
A	9.4	8.6	7.7	5.9	3.9	1.8	9.4	.00
B	3.9	3.6	3.3	2.6	1.8	.9	3.9	.00
C	1.4	1.2	1.1	.7	.4	.1	1.4	.00
H	7.2	6.6	6.0	4.6	3.1	1.4	7.2	.00
AA	5.3	4.9	4.4	3.5	2.5	1.3	5.3	.00
AC	2.6	2.3	2.0	1.5	.8	.3	2.6	.00
AD	1.6	1.5	1.3	1.0	.7	.3	1.6	.00
Total	31.4	28.7	25.8	19.8	13.2	6.1		

(*continued*)

Table 1 (continued)

Locus and Family	Z at θ = .00	.05	.10	.20	.30	.40	Z_{max}[a]	Maximum θ[a]
AFMb320wd9:								
A	9.1	8.3	7.5	5.7	3.8	1.8	9.1	.00
B	−∞	2.0	2.0	1.7	1.1	.5	2.0	.07
C	2.0	1.8	1.6	1.2	.7	.3	2.0	.00
H	4.3	3.9	3.4	2.5	1.6	.6	4.3	.00
AA	6.8	6.2	5.6	4.3	2.8	1.2	5.8	.00
AC	.6	.6	.6	.6	.4	.2	.6	.08
AD	2.3	2.1	1.9	1.4	.9	.4	2.3	.00
Total	−∞	24.9	22.6	17.4	11.3	5.0		
D12S315:								
A	8.1	7.4	6.6	4.9	3.1	1.2	8.1	.00
B	−∞	.4	.5	.6	.4	.2	.6	.17
C	2.0	1.8	1.6	1.1	.7	.3	2.0	.00
H	6.2	5.7	5.1	3.9	2.5	1.1	6.2	.00
AA	7.4	6.8	6.2	4.9	3.4	1.7	7.4	.00
AC	2.3	2.1	1.8	1.3	.8	.3	2.3	.00
AD	1.7	1.5	1.4	1.0	.7	.3	1.7	.00
Total	−∞	25.7	23.2	17.7	11.6	5.1		
D12S1090:								
A	8.3	7.8	7.2	5.6	3.9	1.9	8.3	.00
B	−∞	2.0	2.0	1.7	1.1	.5	2.0	.07
C	.8	.7	.5	.3	.2	.0	.8	.00
H	−∞	7.5	7.1	5.8	4.1	2.1	7.5	.04
AA	7.7	7.1	6.5	5.1	3.5	1.8	7.7	.00
AC	2.9	2.6	2.3	1.7	1.1	.4	2.9	.00
AD	2.9	2.6	2.4	1.8	1.3	.7	2.9	.00
Total	−∞	30.3	28.0	22.0	15.2	7.4		

[a] Maximum-likelihood estimate.

60 s at 56°C, and 3 min at 72°C. AFMb320wd9 and AFM136xf6 were amplified for 35 cycles of 30 s at 94°C, 60 s at 54°C, and 3 min at 72°C. Radiolabeled PCR products were separated on denaturing polyacrylamide sequencing gels and were subjected to autoradiography.

Linkage Analysis

Lod score (Z) values were calculated by the Linkage 5.1 package of programs (Lathrop et al. 1984), under the assumption of autosomal dominant inheritance with complete penetrance and a disease-gene frequency of .000005 (i.e., disease incidence of 1/100,000 births). Because of the small sample sizes and diverse ethnic backgrounds of the CFEOM families, each polymorphism was assumed to have 10 alleles of equal frequency. Changing allele frequencies does not alter the recombination analysis used to determine the CFEOM gene location. Nevertheless, observed allele frequencies among unrelated members of all seven pedigree were calculated and used for a repeat analysis of the four nonrecombinant markers (D12S345, D12S59, D12S331, and D12S1048), resulting in only minimal effects (i.e., a change of <10%) on the Z values. In particular, the maximum Z (Z_{max}) values for families C, AC, and AD stayed the same or increased by 0.1 or 0.2 units.

Physical Map Construction

The Quickmap database (Cohen et al. 1993) of the CEPH mega YAC library (Chumakov et al. 1992; Gyapay et al. 1994) was searched for YACs containing D12S345 and D12S331. Nearest-neighbor analysis of 15 resultant clones identified a total of 126 YACs associated with the CFEOM critical region. DNA from these YACs was prepared and screened with a number of chromosome 12–specific markers.

To increase the density of markers in this interval, several YAC ends were isolated by the vectorette-PCR method (Riley et al. 1990; LeBlanc-Straceski et al. 1994). Ends thus isolated were labeled and used to screen filters containing *Eco*RI-digested DNA from YACs in the collection. Each of the ends was expected to hybridize to the YAC from which it was derived. If an end did not hybridize to any other YAC, it was presumed to have resulted from a non–chromosome 12

chimeric fragment and was discarded. If the end hybridized to a number of other YACs, it was considered to be derived from chromosome 12. These latter YACs were used to generate an additional 13 STS markers by subjecting the ends to sequencing and generating primers from these sequences. Construction of a near-complete, second-generation physical map of chromosome 12 is described in more detail by Krauter et al. (1995).

FISH

Selected YAC clones were FISH mapped on metaphase chromosome spreads as described by Lichter et al. (1990), with 350–500 ng of biotin-labeled total yeast (YAC + genomic) DNA used as the probe. An R-banding pattern was generated by cohybridization of a digoxigenin-labeled oligonucleotide corresponding to positions 52–129 of the Alu consensus sequence (Matera and Ward 1992). Biotinylated probes were detected with fluorescein-labeled avidin (5 μg/ml), while the Alu oligonucleotide was detected with anti-digoxigenin Fab (2 μg/ml) conjugated to rhodamine. Digital images were captured by a computer-controlled Zeiss axioskop epifluorescence microscope coupled to a cooled CCD camera (Photometrics).

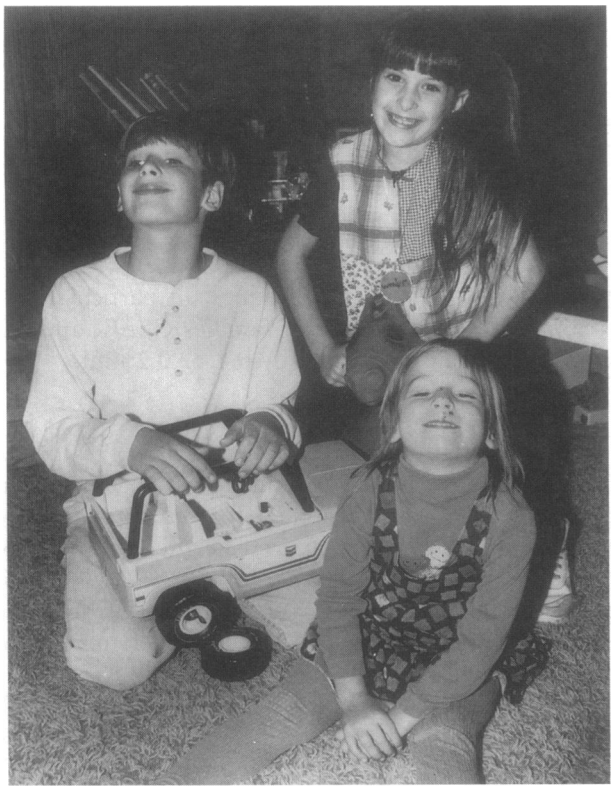

Figure 2 Three siblings of Pedigree AA. Left is affected individual V-7, back right is unaffected individual V-9, and front right is affected individual V-10. The two affected children have ptosis and hypotropic strabismus with a compensatory backward tilt to the head. Note also their horizontal smile in comparison with that of their unaffected sister.

Results

Clinical Description

The present study is based on the two CFEOM pedigrees—A and B—reported elsewhere (Engle et al. 1994) and on five additional CFEOM pedigrees—C, H, AA, AC, and AD—as illustrated in figure 1. Pedigrees C (Nemet et al. 1985), H (Flieringa 1924; Houtman et al. 1986; Jonkers 1950), AA (Laughlin 1956), AC (Traboulsi et al. 1993), and AD (Cibis 1984; Cibis et al. 1984) have been described in earlier reports.

The disorder observed in these seven families is clinically homogeneous. As was also noted in our previous description of pedigrees A and B (Engle et al. 1994), all affected members of each family have bilateral congenital ptosis and hypotropic external ophthalmoplegia (fig. 2). Within each of the families, we find that there is variation in the degree and symmetry of the ptosis and strabismus, in the extent of residual horizontal eye movements, and in the character and degree of aberrant convergent or divergent horizontal movements on attempted gaze. Interestingly, many affected members of each pedigree appear to have mild facial weakness (fig. 2). The families are ethnically diverse. Pedigree C lives in Israel and is of Sephardic Jewish descent, pedigree H lives in the Netherlands and is of Dutch descent, and pedigrees AA, AC, and AD live in the United States and are of Anglo-Saxon, French-Canadian, and mixed European ancestry, respectively. Chromosome studies with a focused analysis on chromosome 12 were performed on at least one affected member each of pedigrees A, B, H, AA, AC, and AD, and all were normal.

Linkage of CFEOM to Chromosome 12

The CFEOM locus has been previously localized to an 8-cM pericentromeric region of chromosome 12, flanked by D12S87 on the p arm and by D12S85 on the q arm (Engle et al. 1994). Subsequent recombination data have placed D12S61 centromeric to D12S87, changing the original flanking markers to D12S61 and D12S85, and this order has been confirmed by physical mapping on a YAC contig (Krauter et al. 1995). We tested the five new CFEOM families with PCR primers that amplify polymorphic repeats in the centromeric region of chromosome 12, and we included the previously reported two families when testing newly identified markers in the region (AFM136xf6, D12S345, D12S331, D12S1048, AFMb320wd9, D12S315, and D12S1090). All of these new markers are within the interval defined by D12S61 and D12S85, with AFM136xf6 <1 cM centromeric to D12S61 on the p arm and with D12S1090 ~5 cM centromeric to D12S85 on the q arm.

Table 2

Key Recombination Events Involving CFEOM Locus and Selected Markers

Distance (cM)	Marker	Gametes Transmitted[b]							CFEOM Critical Region[c]
		Affected Offspring				Unaffected Offspring			
		Total (n = 88)	B, IV-1 (n = 1)	H, III-28 (n = 1)	B, III-2 (n = 1)	Total (n = 60)	A, IV-19 (n = 1)	AC, IV-7 (n = 1)	
53	D12S61	−/ni	+	−	ni	−/ni	+	+	+
	AFM136xf6	−/ni	−	−	−	−/ni	+	+	+
54	D12S345	−/ni	−	−	−	−/ni	−	−	−
	D12S59	−/ni	−	−	−	−/ni	−	−	−
	centromere								
55	D12S331	−/ni	ni	ni	ni	−/ni	−	−	−
	D12S1048	−/ni	−	−	−	−/ni	−	−	−
56	AFMb320wd9	−/ni	−	−	+	−/ni	−	ni	+
	D12S315	−/ni	−	−	+	−/ni	−	ni	+
57	D12S1090	−/ni	−	+	+	−/ni	−	−	+

NOTE.—A plus sign (+) denotes a recombinant event; a minus sign (−) denotes no recombinant event; and "ni" denotes that the marker is not informative for individual.

[a] Cumulative genetic distance from the teleomere of the short arm of chromosome 12 (Krauter et al. 1995).

[b] Family and individual designations are as in fig. 1. n = total no. of gametes analyzed with the indicated pattern of recombination.

[c] Boxed area denotes nonrecombinant markers.

D12S345, D12S59, D12S331, and D12S1048 cosegregate with the disease in all seven families, giving combined Z_{max} values of 35.7, 35.6, 16.0, and 31.4, respectively, at a recombination fraction (θ) of .00 (table 1). The nearest flanking marker on the p arm, AFM136xf6, has recombined twice, once in an unaffected female member of pedigree A (A IV-19) and once in an unaffected female member of pedigree AC (AC IV-7). The nearest flanking marker on the q arm, AFMb320wd9, has recombined in an affected female member of pedigree B (B III-2) (table 2).

Haplotype analysis of the four nonrecombinant markers in each of the seven families revealed no common haplotype associated with the CFEOM mutation, and for each marker there was no single allele associated with the disorder (data not shown). Formal analysis for linkage disequilibrium was not performed, because of the diverse ethnic background of these families. At least one affected member of each family had two distinct alleles for each of the nonrecombinant markers, suggesting that there are no large deletions of this region.

Development of a Physical Map of 12 cen

To construct a physical map of the CFEOM critical region between AFM136xf6 and AFMb320wd9, we assembled a set of YACs that contain sequences from this region and developed and identified a series of sequence-tagged sites (STS) markers with which to generate a contig map based on STS content. The resulting map, shown in figure 3, contains two contigs of 31 YACs each, separated by a gap that probably includes the centromere (see below).

The region contains 24 markers, including 13 monomorphic STS markers generated from YAC ends and 11 highly polymorphic markers derived previously by three separate groups (Marshfield, Généthon, and Cooperative Human Linkage Center). Since the critical interval spans 3 cM, the polymorphic markers provide an average genetic resolution of 0.3 cM. The average density of YACs that are positive for each marker throughout the map is 10.6, with a range of 4–18, and, by comparing STS content of partially overlapping YACs, we have been able to unambiguously order 21 of the 24 markers.

To integrate the physical map with the cytogenetic map and to determine the location of the centromere, several YACs in the contig were used for FISH. YAC 813h9 maps to 12p11.2, and YAC 958e2 maps to 12q12 (fig. 4). Additional FISH studies revealed that YACs 832f4 and 946d5, in the same contig as 813h9, hybridized to the p arm of chromosome 12, whereas YACs 956a5, 933e11, and 798b12, in the other contig, give a hybridization signal on the q arm of chromosome 12 (fig. 3; data not shown). Therefore, we conclude that the region covered by the contigs—and, thus, the critical region for the CFEOM gene—spans the centromere of chromosome 12 and includes 12p11.2-q12.

Although the size of the gap between the two contigs could not be determined, it is possible to estimate the size of the CFEOM critical region covered by the two YAC contigs by adding the sizes of nonchimeric YACs

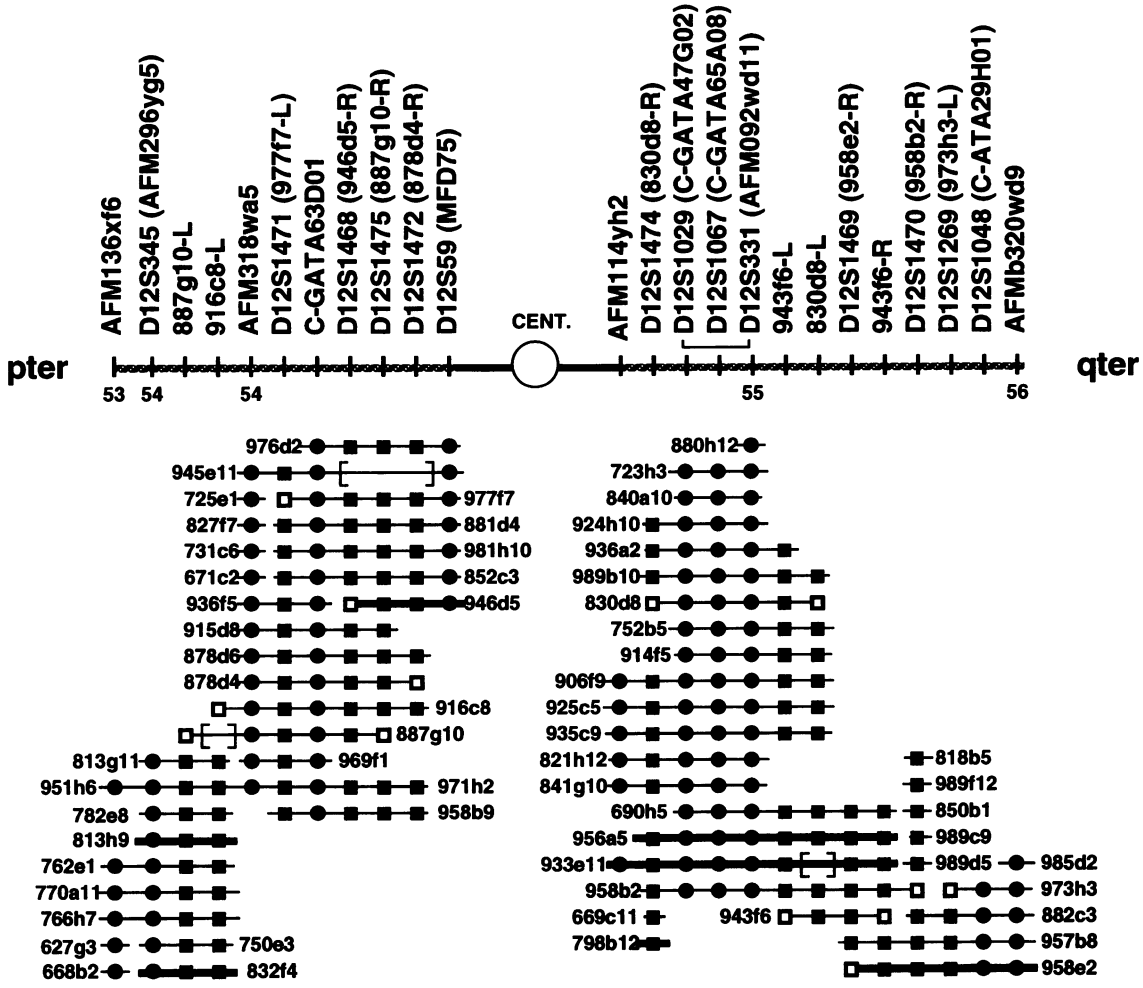

Figure 3 Physical map of the CFEOM critical region. The horizontal line at the top of the figure represents the pericentromeric region of chromosome 12, with pter to the left of the centromere and with qter to the right. Markers are indicated above the line; those whose relative order could not be established unambiguously are enclosed within a square bracket. The genetic distance (in cM), as derived from the Généthon linkage map, is indicated for the appropriate markers below the horizontal line. The 62 YACs are represented below the line, each with its corresponding address shown. FISH-mapped YACs are indicated by a thicker line. Markers present on individual YACs are shown by corresponding symbols: highly polymorphic markers are denoted by black dots; monomorphic markers are denoted by blackened squares; and YAC ends from which markers were derived are denoted by unblackened squares. Square brackets on YACs indicate deletions of markers at that position.

that constitute a minimal tiling path between the flanking markers. These YACs are 762e1 (220 kb), 916c8 (1,590 kb), 933e11 (1,320 kb), and 957b8 (1,410 kb), and they constitute a combined length of 4.5 Mb (fig. 3). The size of the centromeric alpha-satellite–containing region has been estimated at 2 Mb (Looijenga et al. 1992). However, the entire CFEOM critical region is almost certainly <6.5 Mb, since there is significant overlap between the YACs included in this calculation.

Discussion

Refining the map location of CFEOM and developing a YAC contig across the region are important steps toward identifying the mutated gene. Our data confirm the linkage of CFEOM to the pericentromeric region of chromosome 12 in five additional families and refine the disease-gene location, in all seven families, to an ~3-cM interval between markers AFM136xf6 and AFMb320wd9. Z_{max} is >3 in families A, B, H, and AA (10.5, 3.9, 9.7, and 7.7, respectively). Families C, AC, and AD are small and provide <10 informative meioses. Despite there being no recombination events, their small size results in a Z_{max} for each of these families that is close to but less than 3 (2.0, 2.9, and 2.9, respectively) for the most informative markers. Given the clinical homogeneity among our families, these data provide strong evidence for genetic homogeneity of CFEOM.

The inheritance pattern within all seven families is consistent with autosomal dominant inheritance and

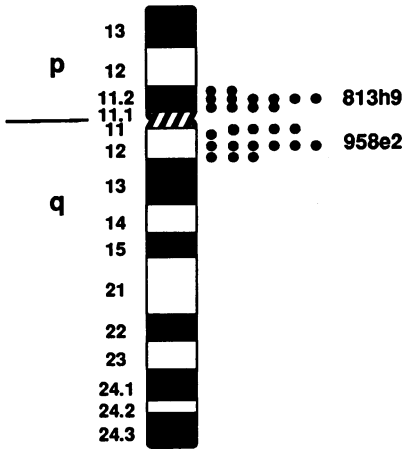

Figure 4 Signals from FISH of YACs 813h9 and 958e2. Cytogenetic positions are listed to the left of the representation of chromosome 12. Each dot denotes data from a single chromosome and indicates the location of the YAC probe on chromosome 12.

complete penetrance; 113 (56%) of 202 offspring of affected individuals are affected, no offspring of an unaffected individual is affected, and no unaffected individual inherited the entire CFEOM-containing critical region from an affected parent. Therefore, we have defined the p-arm boundary of the critical region on the basis of two recombination events for marker AFM136xf6 in unaffected individuals A IV-19 and AC IV-7 (table 2). Although unlikely, it remains possible that "classical" CFEOM is not a fully penetrant disorder and that these two individuals are nonpenetrant carriers of the mutated gene. If this were true, the distal p-arm boundary would then be defined by the recombinant event at D12S61 in affected offspring B IV-1 (table 2).

On the basis of the physical map and the Généthon linkage map, the flanking markers, AFM136xf6 and AFMb320wd9, span the centromere and are located at 53 and 56 cM cumulative distance, respectively, from the telomere of 12p. Therefore, we do not yet know if the gene resides on the long or short arm of chromosome 12. Recombination events allow the ordering of some markers, as indicated in table 2, and this is consistent with the STS content–based YAC contig map of the chromosome (fig. 3). The nearest physically mapped genes are KRAS2 at 12p12.1 (Kucherlapati et al. 1994) and contactin (CNTN1) at 12q12 (Krauter et al. 1995), but our FISH data place YAC 813h9, which is located immediately proximal to the flanking polymorphic marker AFM136xf6, at 12p11.2, making 12p11.2-q12 the most likely current cytogenetic location for the CFEOM gene.

These results place the CFEOM gene in the immediate vicinity of the centromere of chromosome 12, a location that likely hinders further meiotic mapping. Previous studies have demonstrated a significant reduction of recombination events in the pericentromeric region of other chromosomes, particularly in male meioses (Wu et al. 1990; Bamshad et al. 1994). All five gametes recombinant for the markers shown in table 2 were products of female meioses. In our data set, 3 of 153 meioses are recombinant for the flanking markers (table 2), giving a genetic distance of 2.0 cM, similar to the 3-cM distance reported for the chromosome 12 collaborative map (Krauter et al. 1995). Because of recombination suppression, however, this genetic distance likely corresponds to a physical distance significantly >2 Mb and, in fact, adding the sizes of overlapping YACs in a minimal tiling path suggests that this region may be 4–5 Mb in size. Although linkage disequilibrium can provide additional mapping data for some disorders (Jorde 1995), there is no evidence for it at the CFEOM locus. This is not surprising, given that the families are of diverse ethnic background and that several of the families provided oral histories suggesting that the disorder appeared for the first time in recent generations. Together, these facts suggest that there have been multiple disease-causing mutational events at the CFEOM locus. In addition, each family contained affected members who were heterozygous for the nonrecombinant markers, indicating the absence of microdeletions that might assist in mapping the gene.

Currently, there are no known candidate genes within the CFEOM critical region. The YAC-contig map places HoxC outside our region, at 72–75 cM, and places contactin (CNTN1), a neuronal cell adhesion molecule, between AFMb320wd9 and D12S315, ruling these out as potential candidate genes (Krauter et al. 1995). The development of our physical map for this region will now facilitate the identification and mapping of further candidate genes.

The seven CFEOM families included in this analysis share the clinical phenotype of congenital, bilateral ptosis and external ophthalmoplegia, with the eyes partially or completely fixed in the orbit, in a hypotropic and strabismic position. Thus far, we have found that families with autosomal dominant, completely penetrant inheritance of these symptoms are genetically homogeneous. This finding will now permit us to pool clinical and laboratory investigative data and to begin to address the pathological and pathophysiological basis of this genetically defined disorder. In addition, we are in the process of collecting and analyzing families with slight phenotypic or genetic variation, to determine if these disorders are allelic or are separate disease entities. Classifying the various inherited strabismic disorders and determining their genetic etiology is a first step toward a better understanding of EOM function and its selective role in muscle-disease processes.

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