Clinical phenotype and linkage analysis of the congenital fibrosis of the extraocular muscles in an Indian family

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Purpose: To describe the clinical phenotype and linkage analysis of the congenital fibrosis of the extraocular muscles (CFEOM) in an Indian family.

Methods: Individuals were examined and their peripheral blood samples were withdrawn for genetic analysis. The disorder was tested for linkage to two known autosomal dominant CFEOM loci on chromosome 12p11.2-q12 (CFEOM1) and chromosome 16q24 (CFEOM3) using microsatellite markers.

Results: Nine individuals including seven affecteds participated in the study. All seven affecteds had a classic form of CFEOM which included congenital bilateral ptosis, hypotropia, and chin elevation. The disorder segregated as an autosomal dominant trait in this family. The maximum simulated lod score in this family was 2.02. Linkage to CFEOM3 was excluded ($Z<-2.00$), whereas analysis of chromosome 12 markers was positive. The maximum observed two-point lod score was 1.8 (given the size and structure of the family) at theta=0 with marker D12S345. Markers D12S61, D12S1631, D12S87, D12S345, D12S59, D12S1048, and D12S1668 cosegregated with the disease locus in all affecteds. Haplotype analysis showed that the candidate region spanned the centromere.

Conclusions: The present data showed a classic CFEOM phenotype in an Indian family. The family's phenotype is consistent with linkage to CFEOM1 locus on chromosome 12p11.2-q12.

In humans, the eyeballs are suspended in the orbits by extraocular muscles (EOMs), connective tissue fascia, and fat [1]. There are six EOMs (four recti and two oblique) that control the movement of the eyeball, and one muscle (levator palpebrae superioris) that raises the eyelid [1]. Each EOM muscle fibre has a long, tendonous origin and insertion consisting of fibrous connective tissue [2]. Dysfunction of these muscles leads to a combination of strabismus, ophthalmoplegia, ptosis, and amblyopia [3]. Congenital fibrosis of the extraocular muscles (CFEOM) is a syndrome of congenital nonprogressive bilateral ophthalmoplegia and ptosis, an infraducted primary position of each eye with the inability to raise either eye above the midline, and forced duction testing positive for restriction [4]. A typical CFEOM patient has congenital non-progressive bilateral ptosis and external ophthalmoplegia with eyes fixed in a hypotropic position [3]. Affected individuals typically must tilt their heads backward with their chin elevated to compensate for the ptosis and fixed downward position of the globes in order to see below their eyelids. In affected individuals, any residual eye movements are typically in horizontal plane and it may vary between the two eyes. Affected individuals frequently have significant refractive errors with high astigmatism, lack binocular vision and can develop amblyopia [5]. At surgery, the EOMs are stiff and tight,

and biopsies frequently consist of fibrous connective tissues. Several attempts have been made to correct this disorder surgically which include the recession of appropriate EOMs, adjustment of the globe, and correction of ptosis [1].

CFEOM is genetically heterogeneous disorder. In some families, CFEOM is inherited as an autosomal recessive trait. In three consanguineous families from Saudi Arabia, Wang et al. [6] mapped a CFEOM locus to chromosome 11q13 (CFEOM2). CFEOM can also be inherited as an autosomal dominant trait with loci mapped to chromosome 12p11.2-q12 (CFEOM1, MIM 135700) in 17 families from the USA, Canada, Israel, Italy, Ireland, Japan, the Netherlands and Turkey [3,4,7,8] and to chromosome 16q24.2-q24.3 (CFEOM3) in three families from the USA and Canada [4,9]. We report here the clinical phenotype and linkage analysis of CFEOM in an Indian family in which the disease segregates as an autosomal dominant trait. The results demonstrate that the family's phenotype is consistent with linkage to CFEOM1 locus on chromosome 12p11.2-q12.

METHODS

Subjects: The four-generation Hindu CFEOM family IISC-1 lives in the state of Karnataka, India. The pedigree consists of 11 members, and 9 members participated in the study and went through clinical examination. All affecteds had the classic form of CFEOM. No other abnormalities were noticed in affecteds and their intelligence and development appeared to be normal. Informed consent was obtained for research following the guidelines of the Indian Council of Medical Research, New

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Delhi. Informed and signed consent was obtained from each subject for publication of a full-face photograph (Figure 1).

DNA typing: Peripheral blood for DNA extraction was collected from 9 family members of the CFEOM family IISC-1. DNA was extracted from the blood using a Wizard ™ Genomic DNA Purification kits (Promega Inc., Madison, WI, USA). The family was genotyped for nine chromosome 12 markers (viz., D12S61, D12S1631, D12S87, D12S345, D12S59, D12S1048, D12S1668, D12S1090, and D12S85) as well as five chromosome 16 markers (viz., D16S498, D16S476, D16S413, D16S3121 and D16S671) [3,6-10]. PCR primers for microsatellite markers were purchased from Research Genetics Inc. (Huntsville, AL, USA). Amplification of each microsatellite marker was performed as reported in Kumar et al. [11,12]. Radiolabelled PCR products were separated on 6% denaturing polyacrylamide sequencing gels and were subjected to Phosphor Image analysis or exposed to X-ray films.

Linkage analysis: The program SIMLINK [13] was used in order to determine the maximum potential lod score possible in the family IISC-1 assuming a tetra-allelic marker with a heterozygosity = 0.75 and performing 10,000 replicates. Lod score (Z) were calculated using VITESSE [14], under assumption of autosomal dominant mode of inheritance with complete penetrance and a disease-gene frequency of 0.00001 [3]. Population specific marker allele frequencies are not available for the Indian population. However in the pedigree in Figure 2, the majority of individuals have DNA available for genotyping. Thus equal marker allele frequencies were assumed for the analysis found in Table 1 and Table 2. Varying the allele frequencies did not substantially change the linkage results. Two-point (MLINK program) analysis was executed in order to maximize the linkage information [15].

RESULTS

All the affected individuals in the CFEOM family IISC-1 ascertained from the state of Karnataka, India had congenital, non-progressive bilateral ptosis and external ophthalmople-

gia with the eyes fixed in a strabismic and hypotropic position (Figure 1). The clinical phenotype and diagrammatic representation of right and left eye extraocular muscle range of movement are given in Figure 3. All the affected individuals tilted their heads backward to compensate for the ptosis and fixed downward position of the globe. In addition, individual III-5 had perverted convergence on upgaze; individual IV-5 had exotropia, perverted convergence on upgaze, perverted divergence on down gaze; individual IV-6 had esotropia, perverted convergence on upgaze, perverted divergence on down gaze; individual IV-7 had exotropia, amblyopia of the left eye and tilted disc in the left eye; III-1 had exotropia and perverted convergence on upgaze; IV-1 had esotropia, nystagmoid movements and perverted convergence on upgaze; and, individual IV-2 had exotropia, excyclotropia (excycloversion) on attempted convergence and nystagmoid movements. These data suggest a classic CFEOM phenotype in affected individuals in the Indian CFEOM family IISC-1.

The inheritance pattern of CFEOM in this family was consistent with an autosomal dominant mode of inheritance with complete penetrance (Figure 2). The maximum expected simulated lod score in this family (4 allele locus; HET=0.75; 10,000 replicates) is 2.02. To assess linkage of CFEOM in this family to autosomal dominant CFEOM1 and CFEOM3 loci, microsatellite markers from both within and flanking the candidate regions were genotyped. Linkage to CFEOM3 candidate region was excluded (Table 1), whereas the result of eight

TABLE 1. TWO-POINT LOD SCORES OF CFEOM3 MARKERS IN THE CFEOM FAMILY IISC-1

Markers	lod scores at theta						
	0.000	0.050	0.100	0.150	0.200	0.300	0.400
D16S498	-3.301	-0.644	-0.385	-0.250	-0.165	-0.068	-0.021
D16S476	-2.875	-0.613	-0.355	-0.221	-0.139	-0.048	-0.010
D16S413	-inf	-0.819	-0.369	-0.167	-0.065	0.005	0.006
D16S3121	-inf	-0.944	-0.493	-0.290	-0.185	-0.100	-0.062
D16S671	-inf	-1.345	-0.813	-0.531	-0.350	-0.136	-0.032

TABLE 2. TWO-POINT LOD SCORES OF CFEOM1 MARKERS IN THE CFEOM FAMILY IISC-1

Markers	lod scores at theta						
	0.000	0.050	0.100	0.150	0.200	0.300	0.400
D12S61	-0.000	-0.016	-0.023	-0.024	-0.019	-0.006	0.003
D12S1631	1.408	1.234	1.061	0.889	0.721	0.398	0.124
D12S87	0.359	0.295	0.235	0.180	0.131	0.055	0.013
D12S345	1.806	1.609	1.408	1.206	1.003	0.605	0.242
D12S59	1.575	1.397	1.218	1.039	0.863	0.523	0.214
D12S1048	0.329	0.270	0.215	0.166	0.123	0.055	0.014
D12S1668	-2.070	0.327	0.474	0.493	0.457	0.307	0.122
D12S1090	-1.671	0.458	0.580	0.573	0.512	0.314	0.101
D12S85	0.204	0.163	0.125	0.093	0.066	0.027	0.006

Figure 1. Photographs of individuals from the Indian CFEOM family IISC-1. **A**, **B**, **C**, and **D** represent individuals III-5, IV-5, IV-6 and IV-7, respectively in the pedigree shown in Figure 2. Note, chin elevation, bilateral ptosis and hypotropia in all affected individuals.

chromosome 12 markers was positive (Table 2, Figure 2). The maximum two-point lod score was 1.8 at theta=0 with marker D12S345 (Table 2). Markers D12S61, D12S1631, D12S87, D12S345, D12S59, D12S1048, and D12S1668 cosegregated with the disease locus in all affecteds (Figure 2). Haplotype analysis showed that the candidate region spanned the centromere (Figure 2).

DISCUSSION

Here we report the clinical phenotype and linkage analysis of an autosomal dominant form of CFEOM in an Indian family. Since the linkage to chromosome 16 locus is excluded and the maximum two-point lod score in the family is 1.806, despite not obtaining a lod score of 3.00 due to small size and structure of the family, these results are suggestive of linkage of this family to chromosome 12 locus. D12S61, D12S1631, D12S87, D12S345, D12S59, D12S1048, and D12S1668 cosegregated with the disease locus. This family is clinically identical to 17 families reported earlier [3,4,7,8]. Engle et al. [3,7] have reported the linkage of CFEOM to chromosome 12 (12p11.2-q12) in eight families from the USA, Canada, Israel, the Netherlands, and Turkey. The minimum critical region (MCR) reported by Engle et al. [3] is flanked by AFM136xf6 and D12S1668 (AFMb320wd9), defining a critical region of 3 cM spanning the centromere of chromosome 12. Markers D12S345, D12S59, and D12S1048 did not recombine with the disease locus [3]. Our results corroborate their findings. However, they do not narrow the MCR further as reported by Engle et al. [3].

Neuropathological studies indicate that CFEOM may result from the maldevelopment of the oculomotor (nIII) and trochlear (nIV) cranial nerve nuclei [16]. Recently, Nakano et al. [16] showed that homozygous mutations in a homeodomain transcription factor protein, ARIX (PHOX2A), causes autosomal recessive CFEOM that maps to chromosome 11 (CFEOM2). ARIX protein was shown to be required for the development of oculomotor and trochlear cranial nerve nuclei in mouse and zebrafish. They suggested that CFEOM2 results from the abnormal development of nIII/nIV and that the CFEOM2 gene product (ARIX protein) plays a critical role in the development of these midbrain nuclei [16]. A number of ESTs and candidate genes have been reported in the MCR. However, none of these seem to have homology with the ARIX gene (GeneMap'99, [17]). One of the genes in the MCR, *BICD1*, is near D12S345 and encodes a human homologue of the *Drosophila Bicaudal-D* gene (GeneMap'99, [17]). BICD1 protein is expressed in brain, heart, and skeletal muscles [18]. The *BICD1* gene encodes a cytoskeleton-like coiled-coil polypeptide, with a leucine zipper and five alpha-helix domains or heptad repeats showing sequence similarity to the tail domains of myosin heavy chains. It is possible that the mutations in the *BICD1* gene result in CFEOM1.
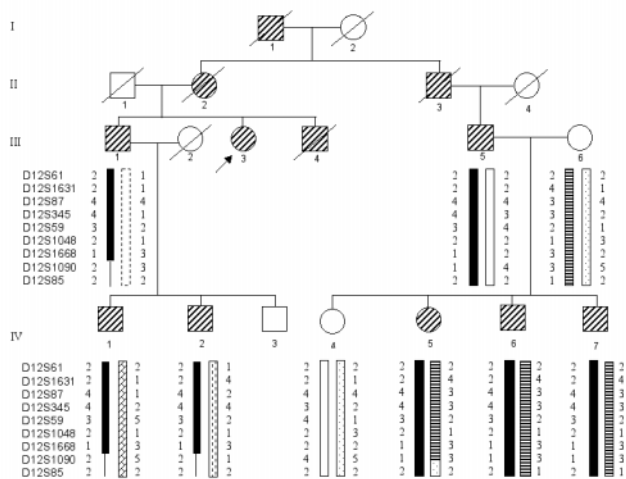
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Figure 2. Pedigree of the CFEOM family IISC-1. Pedigree diagram and haplotype analysis of the CFEOM family IISC-1. Hatched symbols denote affecteds with CFEOM.

Figure 3. The phenotype and diagrammatic representation of extraocular muscle range of movement. The clinical phenotype and diagrammatic representation of right and left eye extraocular muscle range of movement. Black dot indicates the pupillary position with the head held straight. Movements are measured from each eye's primary resting position. IO, inferior oblique; IR, inferior rectus; SR, superior rectus; LR, lateral rectus; MR, medial rectus; SO, superior oblique; 3, full range of movement; 2, moderate restriction; 1, marked restriction; o, no movement.

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