SHORT REPORT

A new syndrome, congenital extraocular muscle fibrosis with ulnar hand anomalies, maps to chromosome 21qter

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Background: Congenital fibrosis of the extraocular muscles (CFEOM) is a heterogeneous group of disorders that may be associated with other anomalies. The association of a CFEOM syndrome with ulnar hand abnormalities (CFEOM/U) has not been reported to date.
Objective: To describe a new autosomal recessive syndrome of CFEOM and ulnar hand abnormalities, and localise the disease causing gene.
Methods: Clinical evaluation of the affected members and positional mapping.
Results: Six affected patients with CFEOM/U (aged 2 to 29 years) from a large consanguineous Turkish family were studied. Ophthalmological involvement was characterised by non-progressive restrictive ophthalmoplegia with blepharoptosis of the right eye. The postaxial oligodactyly/oligosyndactyly of the hands was more severe on the right side. A genome-wide scan established linkage of this new autosomal recessive syndrome to a locus on chromosome 21qter. The multipoint LOD score was 4.53 at microsatellite marker D21S1259, and fine mapping defined a ~1.5 Mb critical region between microsatellite marker D21S1897 and the telomere of the long arm.
Conclusions: CFEOM/U maps to a 1.5 Mb region at chromosome 21qter. Future identification of the disease causing gene may provide insights into the development of the extraocular muscles and brain stem α motor neurones, as well as anteroposterior limb development.

Heuk (1879) was the first to report the combination of congenital blepharoptosis and restricted eye movements.[1] In 1950, Brown described this group of ocular abnormalities in detail and classified the syndromes into five distinct phenotypes: horizontal retraction syndromes; strabismus fixus; vertical retraction syndromes; superior oblique tendon sheath syndromes; and a general fibrosis syndrome.[2] Currently, the horizontal retraction syndromes are referred to as Duane syndrome, the superior oblique tendon sheath syndromes as Brown syndrome, and the remaining syndromes as congenital fibrosis of the extraocular muscles (CFEOM).[3] Recent neuropathological studies have shown that some of the fibrosis syndromes result from developmental defects of particular brain stem α motor neurones and their corresponding axons.[4 5]

Duane syndrome, the most common of the CFEOM syndromes, is characterised by limited abduction, variably limited adduction, and globe retraction on attempted adduction. Most cases are sporadic and only about 10% are familial.[6] Cytogenetic analyses of sporadic cases revealed deletions of 8q13. This locus was designated DURS1 (MIM 126800).[7–9] Linkage analysis in autosomal dominant families mapped the familial disease to the DURS2 locus on 2q31 (MIM 604356).[10] To date, neither the DURS1 nor the DURS2 gene has been identified.

Three other inherited CFEOM syndromes have been mapped to different genetic loci:

- CFEOM1 (MIM 135700), an autosomal dominant disorder, was mapped to 12q12,[11] and the disease causing gene was recently identified as *KIF21A*.[12] Affected individuals have bilateral ptosis and restrictive ophthalmoplegia, and their eyes are fixed below the horizontally neutral position with or without secondary esotropia or exotropia. CFEOM1 is phenotypically variable, with some patients having a milder expression which resembles CFEOM3. However, these families with the milder phenotypes have been linked to the CFEOM1 locus,[13] leading to their designation as CFEOM type 3A (MIM 607034).
- The CFEOM2 locus (MIM 602078), an autosomal recessive disorder, was mapped to chromosome 11q13.3,[14] and subsequently mutations in the *ARIX* gene were described.[15] Affected individuals with CFEOM2 have bilateral ptosis, with both eyes fixed in abduction.
- CFEOM3 (MIM 600638, formerly 604361), an autosomal dominant disorder, was mapped to chromosome 16q24.2.[16] The phenotype of affected individuals in CFEOM3 families was variable and ranged from bilateral ptosis with fixed eyes in an infraducted and exotropic position to normally positioned eyes with minimal limitation of vertical gaze and unilateral or absent ptosis. To date, the gene causing CFEOM3 has not been identified.

Several CFEOM syndromes occur in association with other anomalies including the Duane radial ray syndrome (DRRS) (MIM 607323), the Wildervanck syndrome (MIM 314600), and familial horizontal gaze palsy with progressive scoliosis (MIM 607313).

DRRS is characterised by the Duane anomaly, radial ray abnormalities, and deafness. The DRRS syndrome—also known as Okihiro syndrome[17]—is inherited as an autosomal dominant trait with variable expressivity. The DRRS locus was mapped to 20q13 and subsequently *SALL4* was identified as the disease causing gene.[18 19]

The features of the Wildervanck syndrome include the Duane anomaly, the Klippel-Feil anomaly (fused cervical vertebrae), and congenital perceptive deafness. This disorder is mostly seen in females, suggesting that the syndrome is

Abbreviations: CFEOM, congenital fibrosis of extraocular muscles; CFEOM/U, congenital fibrosis of extraocular muscles with ulnar hand abnormalities; DRRS, Duane radial ray syndrome; SNP, single nucleotide polymorphism; OD, right eye; OS, left eye

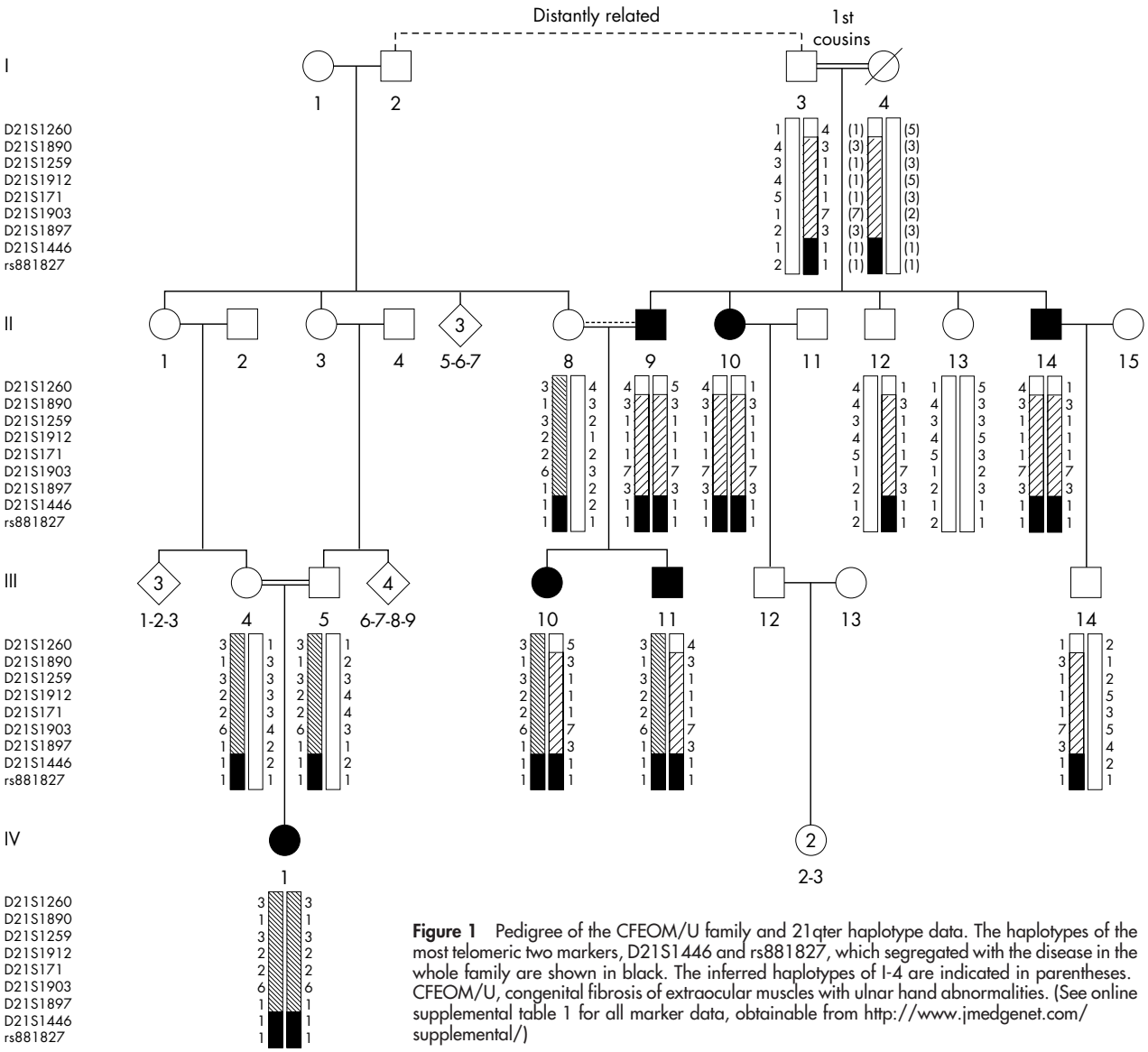

Figure 1 Pedigree of the CFEOM/U family and 21qter haplotype data. The haplotypes of the most telomeric two markers, D21S1446 and rs881827, which segregated with the disease in the whole family are shown in black. The inferred haplotypes of I-4 are indicated in parentheses. CFEOM/U, congenital fibrosis of extraocular muscles with ulnar hand abnormalities. (See online supplemental table 1 for all marker data, obtainable from http://www.jmedgenet.com/supplemental/)

inherited as a sex linked dominant with lethality in affected males.

Familial horizontal gaze palsy with progressive scoliosis is an autosomal recessive disorder characterised by progressive external ophthalmoplegia and scoliosis. The disease causing gene has been mapped to chromosome 11q23–q25.[20]

Here, we describe a new autosomal recessive CFEOM syndrome with prominent ulnar hand abnormalities in a consanguineous Turkish family. The six affected individuals, aged 2 to 29 years, presented with right eye involvement and bilateral postaxial oligodactyly/oligosyndactyly of the hands, with the right more severely affected than the left. A genome scan of DNAs from family members mapped the disease locus to 21q with a multipoint LOD score of 4.525 at microsatellite marker D21S1259. Further interrogation of the locus narrowed the critical region to ~1.5 Mb between D21S1897 and the telomere of the long arm.

METHODS

Subjects and medical evaluation

Six affected individuals from the consanguineous Turkish family (fig 1) were clinically evaluated at the division of medical genetics of the Child Health Institute and the orthoptics clinic of the department of ophthalmology, Istanbul Medical Faculty of Istanbul University, Turkey. The study was approved by the Institutional Review Board of the Child Health Institute of Istanbul University and informed consent was obtained from each participant. Five of the six affected individuals had complete ophthalmological examinations, skeletal *x* rays, abdominal ultrasound, echocardiography, and cranial magnetic resonance imaging (MRI).

Ophthalmological studies

Visual acuity was measured using a Snellen letter chart projector transilluminated at approximately 100 cd/m^2 and line acuity performances at 6 m were recorded. Non-cycloplegic refractive data were obtained using a retinoscope or a Topcon KR-7000P autokeratorefractometer, and binocular status was evaluated with a Clement–Clarke synoptophore. Range of ocular movements was evaluated with Hess screen tests in patients with binocular vision potential. Direct and indirect papillary reactions were recorded, and photographic records of each patient were obtained. Duction

Table 1 Summary of clinical findings in patients with congenital fibrosis of extraocular muscles with ulnar hand abnormalities (CFEOM/U) syndrome

	Family member									
	II-9		II-14		III-10		III-11		IV-1	
Feature	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Visual acuity	20/20	20/20	20/200	20/20	20/20	20/20	20/200	20/20	5/200	20/20
Anterior segment	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Microcornea	Normal
Posterior segment	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Tilted disc	Normal
Ocular motility										
Deviation	14 PD XT 25 PD hT		12 PD XT 25 PD hT		16 PD XT		18 PD XT 20 PD hT			
Dysfunctioning muscle(s)	SR,IO		Lev,SR,IO		SR, IO	IO	SR, IO		All	
Ptosis	None	None	Marked	None	None	None	Mild	None	Marked	None
Anomalous head posture	Chin elevation		Chin elevation		Slight head tilt		Slight head tilt		Head turn	
Forced duction	(–)	(–)	(–)	(–)	(–)	(–)	(+)	(–)		
Hand abnormalities	Ab 5; Cl Hy 4	Ab 5; small fifth finger bud	Ab 5	Ab 5	Ab 5	Hy 4,5; Sy 4–5; Ab nail 5	Ab 5; Cl 4	Ab 5	Ab 3,4,5	Ab 4,5
Hand radiography	Ab MP 5; Hy MP 4; Hy ulnar styloid process; Ab triquetrum and pisiform; fused capitate and hamate	Ab MP 5; biparitie scaphoid	Ab MP 5; Hy ulnar styloid process; fused triquetrum and lunate	Ab MP 5; Hy ulnar styloid process; fused triquetrum and lunate	Ab MP 5	Ab MP 5; Hy MP 4	Ab MP 5	Ab MP 5	Ab MP 3,4,5	Ab MP 4,5

Ab, absent; Cl, clinodactyly; hT, hypotropia; Hy, hypoplastic; IO, inferior oblique muscle; IR, inferior rectus muscle; Lev, *levator palpebrae superioris* muscle; M, metacarpal bone; P, phalangeal bone; PD, prism diopters; SR, superior rectus muscle; Sy, syndactyly; XT, exotropia.
Numbers following abbreviations represent the relevant finger numbers.

and versions in nine diagnostic gaze positions were evaluated with the cover test with special effort to hold the head in a straight position. Deviations in primary position when the head was held straight were measured by the prism cover test and also during the synoptophore examination. Cyclovertical deviations were evaluated with the three step approach, including the Bielschowsky head tilt test. Any aberrant movements, globe retractions, or ptosis were also noted. To assess ptosis, lid openings and levator functions were measured from the upper lid margin while attempted supraduction with frontalis function was controlled by the examiner.

Photographs of the affected individuals were taken with their or their parents' permission and blood samples were obtained by venepuncture for DNA isolation and gene mapping studies.

Histochemical and ultrastructural studies

Samples of the right superior rectus (SR) and inferior oblique (IO) muscles, obtained from patient III-11 at surgical correction for strabismus, were frozen immediately in liquid nitrogen and kept at −80℃ until use. Fresh frozen muscle tissue was processed for routine histology, histochemical staining (including haematoxylin and eosin, Gomori's modified trichrome, NADH, alkaline phosphatase, acid phosphatase, ATPase at pH 9.4 and 4.2, and esterase), and electron microscopy using standard techniques.[21]

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood collected in EDTA from patients and family members using the DNA isolation kit for mammalian blood (Roche, Istanbul, Turkey). For the initial genome scan, DNAs were analysed using 422 autosomal microsatellite markers from the genome-wide human screening set (version 9) and single chromosome scan set (Invitrogen Life Technologies, Carlsbad, California, USA). As needed, additional microsatellite markers were obtained from public databases (NCBI, Marshfield Institute, deCode), or new microsatellite markers were designed using the tandem repeat finder program.[22] All new markers were on the genomic contig NT_011515 and were named by their positions on the contig in kb (Human May 2004[hg17] assembly[NCBI Build 35] on the UCSC human genome browser). The new markers and their positions on chromosome 21 were as follows: 1305K (44 748 500 base pairs (bp)), 2044K (45 585 432 bp), 2849K (46 292 500 bp), 3086K (46 529 500 bp), 3258K (46 701 500 bp) (for primer information, see online supplemental table 2, obtainable from http://www.jmedgenet.com/supplemental/). The primers were designed using Primer3 software,[23] and the fluorescent dye labelled forward primers were synthesised by Invitrogen Life Technologies. When no informative microsatellite markers were found in a particular region, single nucleotide polymorphisms (SNPs) were used for linkage analysis. SNP information was obtained from the UCSC Genome Browser and NCBI web sites, and primers were designed as above.

Genomic DNAs were polymerase chain reaction (PCR) amplified in 96-well microtitre plates in an oil-free system using a DNA Engine PTC-200 thermal cycler (MJ Research, Waltham, Massachusetts, USA). Reaction mixtures (10 µl) contained 10 ng of genomic DNA, 2 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 200 nM of each primer, 0.2 mM dNTPs, and 0.5 U of *Taq* DNA polymerase (AmpliTaq Gold, Applied Biosystems, Foster City, California, USA). For PCR, the reaction mixtures were initially incubated at 95℃ for 10 minutes, and amplified for 27 cycles with denaturation at 94℃ for 30 seconds, annealing at 56℃ for 30 seconds, extension at 72℃ for 30 seconds, and a final extension step at

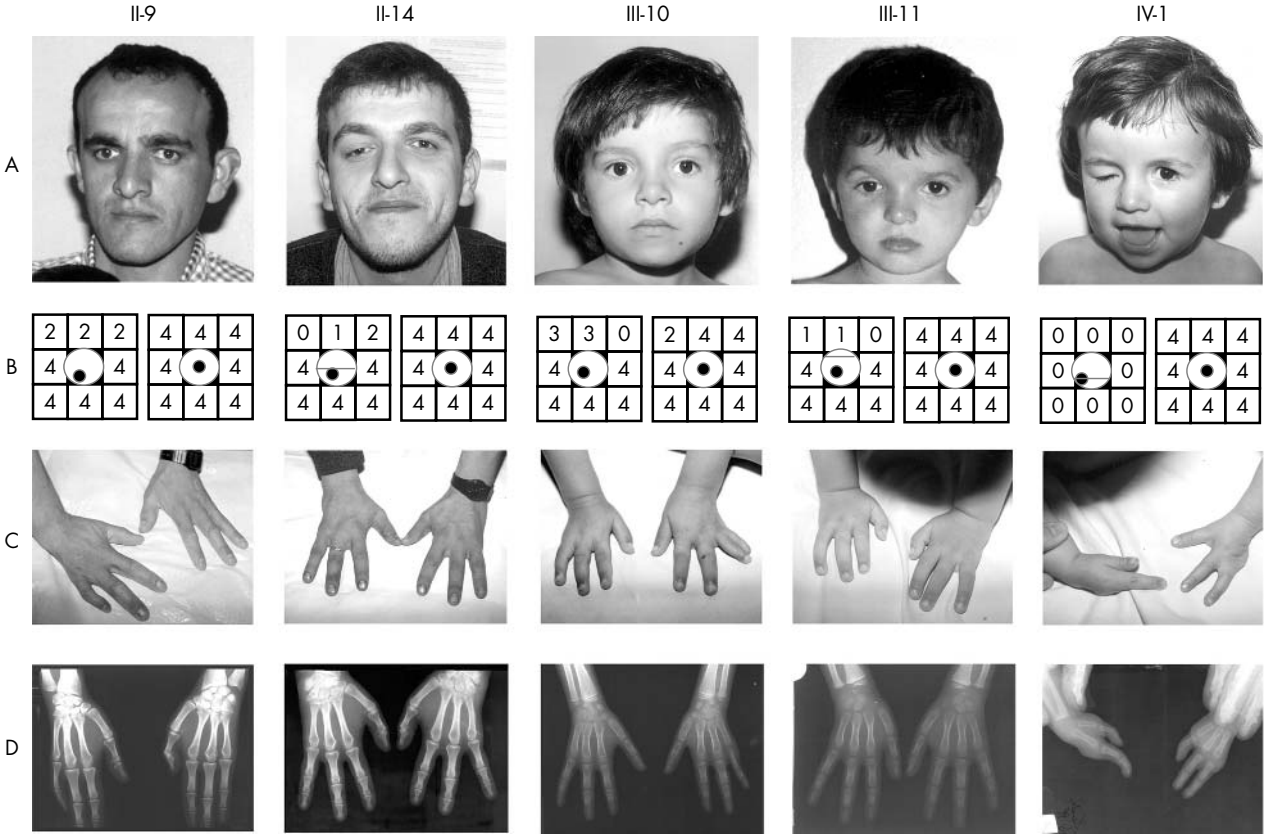

Figure 2 Clinical findings of the patients with congenital fibrosis of extraocular muscles with ulnar hand abnormalities (CFEOM/U). (A) facial photographs. (B) Centre position of the eyes and eyelids (bar) if ptosis was present, and range of eye movement; a score of 4 indicates normal unrestricted movement; 3, 2, and 1 denote decreasing movement in the indicated direction. (C) and (D) Hand photographs and *x* rays. See text and table 1 for details. Permission was obtained from the subjects or their parents for the photographs to be reproduced here.

72°C for seven minutes. PCR products were analysed with either an ABI Prism 3100 genetic analyser or on an ABI Prism 377 DNA sequencer using GeneScan analysis software (version 3.1.2) and Genotyper software (version 2.5) (Perkin-Elmer-Cetus, Norwalk, Connecticut, USA).

PCRs for SNPs were undertaken in a final volume of 50 µl and at specific annealing temperatures for each fragment for 35 cycles. Amplicons were sequenced on an ABI Prism 3700 capillary array sequencer using the ABI Prism BigDye Terminator ready reaction mix (Perkin-Elmer-Cetus). Electropherograms were inspected using ABI Prism sequencing analysis software (version 3.4.1).

Linkage analysis

The multipoint LOD score calculations for each chromosome were individually carried out with the SimWalk2 program (version 2.86)[24] under the assumption of autosomal recessive inheritance with full penetrance. The Mega2 program (version 2.5)[25] was used to create input files for the SimWalk2 program. As data on the population incidence of this unique disorder were unavailable, we used 0.001 as the disease allele frequency. Loci with suggestive LOD scores were genotyped with a denser marker set. The highest LOD score obtained with SimWalk2 was confirmed with the Linkmap program (Fastlink package version 4.1).[26]

RESULTS

Clinical evaluations

Figure 1 shows the pedigree of the consanguineous Turkish family with six affected members in three sibships, of which four (II-9, II-10, II-14, and IV-1) were offspring of first cousin marriages, suggesting autosomal recessive inheritance. Of note, two affected siblings (III-10 and III-11) were the offspring of an affected father (II-9) and a healthy mother (II-8). Although consanguinity between the parents could not clearly be documented, both originated from the same small village and thought they were distantly related. The physical and neurological examinations of all affected members were normal with the exception of the ophthalmological and hand abnormalities. The feet were normal and there were no abdominal ultrasound or echocardiographic abnormalities in the affected individuals. The relevant findings in each patient are described below and summarised in table 1.

Patient 1 (II-9)

This was a 29 year old man who had a right divergent strabismus with hypotropia and 20/20 visual acuity bilaterally, with no refractive error. He had 14 ΔD exotropia and 25 ΔD hypotropia OD, with no torsional component and a slight ''chin up'' anomalous head posture. He had a markedly restricted elevation OD, as well as secondary overaction of the inferior oblique muscle OS. Hess screen test results showed restriction of the entire right superior gaze field. Cranial MRI showed volume loss of the right superior rectus muscle and hypertrophy of the inferior rectus muscle. A forced duction test done under general anaesthesia showed no passive restriction of eye movements, and the patient was diagnosed as having right double elevator palsy. He had absent fifth fingers bilaterally, and the proximal part of his right hand

was markedly hypoplastic compared with the left. While the fourth finger on the right hand was incurved and hypoplastic (clinodactyly), the fourth finger of the left hand was normally developed and had a very small (~3–4 mm) fifth finger bud emerging from the ulnar side at the metacarpophalangeal level. On *x* ray, the metacarpal and phalangeal bones of the fifth fingers were absent bilaterally, and were hypoplastic for the right fourth finger. The right ulnar styloid process was also hypoplastic. In addition, the carpal bones of the right hand were abnormal, the triquetrum and pisiform bones were absent, and the capitate and hamate bones were fused. The scaphoid bone of the left hand was bipartite (fig 2).

Patient 2 (II-14)

This was a 26 year old man who had right hemiptosis and divergent strabismus. He had 12 ΔD of exotropia and 25 ΔD of hypotropia OD without measurable torsional deviation. Elevation of the right eye was restricted, and this was more significant in abduction. The Bielschowsky head tilt test was slightly positive on the right and he had a chin up head posture. His visual acuity was 20/200 (OD) and 20/20 (OS). Cranial MRI was similar to that of patient II-9, with volume loss of the superior rectus muscle and hypertrophy of the inferior rectus muscle of the right eye. Under general anaesthesia, the forced duction test showed no passive restriction in ocular movements. The fifth fingers of both hands were absent, and the fourth finger of the right hand was slightly thinner than on the left, although the patient was right handed. On *x* ray, both hands were symmetrical, and the metacarpal and phalangeal bones of the fifth fingers were absent. The ulnar styloid processes were hypoplastic, and the triquetrum and lunate bones were fused bilaterally (fig 2).

Patient 3 (III-10)

This was a four year old girl who had 20/20 visual acuity bilaterally, 16 ΔD exotropia OD, and restricted elevation on adduction of both eyes, which was more marked on the right. Head posture appeared normal, but a slight head tilt to the right shoulder occurred occasionally. Cranial MRI was normal. The forced duction test was normal, and the patient was diagnosed as having pseudo-Brown syndrome because of bilateral inferior oblique muscle dysfunction. The fifth finger of the right hand was absent, and she had syndactyly of the fourth and fifth fingers on the left, both of which were hypoplastic, and the fifth finger had no nail. On *x* ray, the metacarpal and phalangeal bones of the fifth fingers were absent bilaterally, and the metacarpal and phalangeal bones of the left fourth finger were hypoplastic (fig 2).

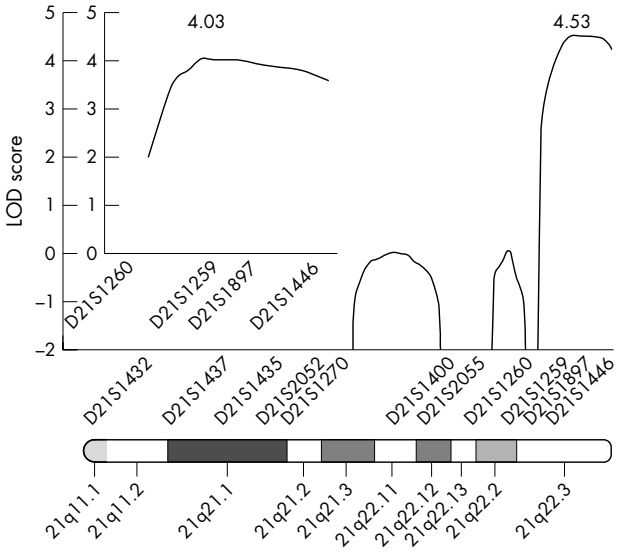

Figure 3 Graphical presentation of multipoint LOD scores. Plot of the scores obtained by the SimWalk2 program for chromosome 21. Insert: Plot of the score obtained by Linkmap program in the critical region only.

Patient 4 (III-11)

This was a three year old boy who had a visual acuity of 20/200 (OD) and 20/20 (OS) with no significant refractive error. He had 18 ΔD exotropia and 20 ΔD hypotropia OD. Restricted elevation during adduction was more marked than on abduction. He had a normal head posture, but a slight head tilt toward the right occurred intermittently. He had a positive forced duction test, showing a markedly restricted elevation during adduction resembling that found in Brown syndrome. Cranial MRI was normal. His fifth fingers were absent bilaterally, and the right fourth finger was slightly incurved. On *x* ray, the metacarpal and phalangeal bones of the fifth fingers were absent bilaterally (fig 2).

Patient 5 (IV-1)

This 2½ year old girl was the most severely affected. She had total ptosis and enophthalmia OD, and the right cornea was smaller (radius, R = 11 mm) than the left (R = 12 mm). She could not fixate and follow objects with her right eye owing to profound amblyopia. Ocular movements of the right eye were restricted in all directions, while they were normal on the left. Examination of the fundus revealed a tilted disc OD. The MRI confirmed enophthalmia but did not reveal any additional abnormalities. The right third, fourth, and fifth fingers and the left fourth and fifth fingers were absent. On *x* ray, the relevant metacarpal and phalangeal bones were absent, while the other bony structures were normal (fig 2).

Histopathological studies

Histological examination of the right superior rectus from patient III-11 showed primarily fibroadipose tissue and no muscle cells. In contrast, the skeletal muscle from the right inferior oblique biopsy had mild non-specific variation in fibre size on light microscopy, but no abnormalities were observed on histochemical analysis or electron microscopy (data not shown).

Genome scan and linkage to 21qter

A genome scan with 422 autosomal microsatellite markers was carried out using genomic DNA from 13 family members, and multipoint linkage analysis was used to map the disease locus. A location score of 3.13 was obtained at marker D21S1259 on chromosome 21q22.3. The addition of the polymorphic markers D21S1260 and D21S1897—which were centromeric and telomeric to D21S1259, respectively—increased the location score to 4.53. The Linkmap program gave a LOD score of 4.03 at D21S1259 (fig 3). Initial calculations were made assuming that II-8 and II-9 were not consanguineous. This assumption, and the limited informativeness of the most telomeric marker, D21S1446, resulted in the highest location score being more centromeric, located among more informative markers.

Haplotype analysis assuming non-consanguinity between II-8 and II-9 confined the critical region between D21S1260 and the telomere, a critical region of ~5.2 Mb (fig 1). Only the most telomeric marker, D21S1446, was homozygous in all affected individuals. Thus the critical region was interrogated using four additional microsatellite markers (D21S1890, D21S1912, D21S171, and D21S1903), and four SNPs

(rs234781, rs2839627, rs170916, and rs234728) which spanned the region. Only the SNP data further narrowed the centromeric boundary of the critical region by 2 Mb to ~3.5 Mb from rs234728 to the telomere (online supplemental table 1, obtainable from http://www.jmedgenet.com/supplemental/).

As additional microsatellite markers in the region (available in public databases) were not informative, several new markers were identified and tested (online supplemental tables 1 and 2). Of these, 2044K was the most telomeric heterozygous marker in patients III-10 and III-11, thereby further narrowing the critical region to ~1.5 Mb. All microsatellite markers and SNPs telomeric to 2044K (rs2838917, rs725358, 2849K, rs2839168, rs1060609, 3086K, rs2839235, 3258K, rs2839281, D2151446, rs9722, and rs881827) were homozygous for the same allele in all patients. Although these markers were not very informative in II-9 to II-14, SNP rs881827 clearly showed that affected and unaffected individuals inherited different alleles. Thus the *CFEOM/U1* gene locus was localised to a critical region of ~1.5 Mb from the new microsatellite marker, 2044K, to qter. Despite the fact that the new markers in the homozygous region were not very informative, the location and LOD scores were still significant when calculations were repeated assuming consanguinity between II-8 and II-9, and with the inclusion of the new microsatellite markers and SNPs. Using the Simwalk2 program, a multipoint location score of 3.28 was obtained at microsatellite D21S1446 through SNP rs881827. This result was confirmed with the Linkmap program, which gave a multipoint LOD score of 3.12 at D21S1446.

DISCUSSION

The consanguineous Turkish patients described here define a new autosomal recessive syndrome of CFEOM with ulnar hand abnormalities (designated CFEOM/U). The affected individuals all had CFEOM of varying severity, which was mainly confined to the right eye, and bilateral postaxial oligodactyly/oligosyndactyly of the hands, which was more severe on the right. Although the clinical findings were variable between affected individuals, penetrance was complete. Interestingly, the severity of the eye and hand involvement was consistent in the same individual. This was clearly observed in the most and least severely affected individuals, IV-1 and III-10, respectively.

All five patients had superior rectus and inferior oblique muscle involvement, and three (II-14, III-11, and IV-1) also had levator palpebralis dysfunction. Phenotypically, three patients (II-9, II-14, and III-11) had a double elevator palsy, perhaps caused by a superior rectus muscle paresis.[27] Patient III-11 was distinctive in having a more marked mechanical restriction of elevation during adduction, resulting in a positive forced duction test, as observed in patients with Brown syndrome; and patient III-10 had bilateral restricted elevation during adduction with a negative forced duction test, which was interpreted as a pseudo-Brown syndrome because of bilateral inferior oblique dysfunction. The fifth patient had a more generalised and severe pattern of extraocular muscle involvement with markedly restricted eye movements in all directions and a total ptosis resembling a generalised fibrosis syndrome.

Individuals affected with classical CFEOM (that is, CFEOM1) usually have bilateral ptosis and restrictive ophthalmoplegia, with their eyes fixed below the horizontal neutral position with or without secondary esotropia or exotropia. Necropsy examinations of individuals affected by classical CFEOM revealed the absence of the superior division of the oculomotor nerve, which normally innervates the superior rectus and levator palpebrae superioris muscles.[5] In contrast, atypical patients with CFEOM2 and CFEOM3 have a restrictive ophthalmoplegia, caused by deficient function of the muscles innerved by the third or fourth cranial nerves. Unlike the classical type, they can raise their eyes above the horizontal line or have unilateral involvement. For atypical CFEOM, marked phenotypic variability has been reported.[4]

The patients described here can be classified as atypical CFEOM as they have involvement of the superior and inferior divisions of the third cranial nerve, although patient IV-1 may also have involvement of the fourth and sixth cranial nerves, indicating the variability in the ocular phenotype. In addition to the ocular abnormalities, these patients had oligodactyly/oligosyndactyly of the hands. The only ocular motility disorder with upper limb defects is the Duane-radial ray syndrome with radial ray abnormalities ranging from hypoplasia of the thenar eminence to absence of the radial bone or forearm. In the family presented here, neither the ocular nor the skeletal findings resembled the clinical findings of DRRS. Moebius syndrome, which is characterised by congenital paresis or paralysis of the seventh (facial) cranial nerve frequently accompanied by dysfunction of other cranial nerves, may also be associated with arthrogryposis and hand abnormalities but is quite distinctive from the syndrome we present here.

It is estimated that approximately 1/600 newborn infants have a congenital abnormality of the upper limb.[28] Postaxial limb deficiencies are most often unilateral and sporadic. They also occur as a feature of various syndromes. An autosomal dominant, non-syndromic postaxial oligodactyly which affects all four extremities has also been described (MIM 176240).[29] However, the association of a congenital fibrosis syndrome with postaxial oligodactyly/oligosyndactyly is novel and has not been reported to date. Of note, only a few causative genes for this group of disorders have been mapped or identified.[30]

By multipoint linkage analysis, the disease locus for CFEOM/U1 was mapped to chromosome 21 between the new microsatellite marker 2044K and the chromosome 21 telomer, a critical region spanning ~1.5 Mb. Initial calculations were made assuming that II-8 and II-9 were not consanguineous, in order to prevent the lower location scores that would have been obtained because of the limited density and informability of the marker grid in the genome-wide marker sets. In fact, the markers initially available lacked the ability to detect the very small homozygous region shared by siblings III-10 and III-11 and all other affected individuals. The homozygous region between 2044K and the telomer was further refined by identifying several additional microsatellites and SNPs in the region, which were less than 500 kb apart. Although the telomeric markers were not very informative, recalculating the location score assuming consanguinity gave a score of 3.28 at microsatellite marker D21S1446 through SNP rs881827. This result was confirmed with the Linkmap program which gave a LOD score of 3.12 at marker D21S1446.

Based on the current Human May 2004 (hg17) Assembly (NCBI Build 35) on the UCSC human genome browser, this region of ~1.5 Mb contains 17 genes (C21orf123, COL18A1, SLC19A1, PCBP3, COL6A1, COL6A2, FTCD, C21orf56, LSS, MCM3APAS, AF426262, C21ofr57, C21orf58, PCNT2, C21orf106, S100B, and HRMT1L1), which have corresponding entries in PDB or SWISS-PROT, or are NCBI reference sequence mRNAs with a ''reviewed'' status. As there may be unrecognised genes in this ~1.5 Mb region, it may contain about 20 genes. However, there were no obvious candidate genes, and no obvious motifs. Efforts are under way to further refine the region of homozygosity and to identify the disease causing gene.

Conclusions

A new autosomal recessive ocular motility syndrome with postaxial oligodactyly and syndactyly (designated CFEOM/U1) was identified and its locus mapped to the most telomeric 1.5 Mb of chromosome 21. Future identification and functional studies of the gene causing this new syndrome may provide insights into the development of the extraocular muscles and their cranial motor nuclei, as well as antero-posterior limb development.

ELECTRONIC DATABASE INFORMATION

- Center for Medical Genetics, Marshfield Clinic Research Foundation, http://research.marshfieldclinic.org/genetics/Map_Markers/maps/IndexMapFrames.html
- National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/
- Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
- Primer3 primer design program, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi
- Tandem Repeat Finder Program, http://c3.biomath.mssm.edu/trf.html
- University of California Santa Cruz (UCSC), Human Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway

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Supplementary tables 1 and 2 can be found on our web site, www.jmedgenet.com/supplemental

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