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


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 nuclei motor neurones, as well as anteroposterior limb development.

Short Report

H euk (1879) was the first to report the combination of congenital blepharoptosis and restricted eye movements. 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. 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). Recent neuropathological studies have shown that some of the fibrosis syndromes result from developmental defects of particular brain stem nuclei motor neurones and their corresponding axons.

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. Cytogenetic analyses of sporadic cases revealed deletions of 8q13. This locus was designated DURS1 (MIM 126800). Linkage analysis in autosomal dominant families mapped the familial disease to the DURS2 locus on 2q31 (MIM 604356). 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 12q12.1 and the disease causing gene was recently identified as KIF21A. 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.

- The CFEOM2 locus (MIM 602078), an autosomal recessive disorder, was mapped to chromosome 11q13.3 and subsequently mutations in the ARX gene were described. 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. The phenotype of affected individuals in CFEOM3 families was variable and ranged from bilateral ptosis with fixed eyes in an infraocular 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—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.

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
CFEOM with ulnar anomalies maps to 21qter

rs881827
D21S1446
D21S1259
D21S1897
D21S1903
D21S171
D21S1912
D21S1260

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/m² 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 pharmacologic 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

<table>
<thead>
<tr>
<th>Family member</th>
<th>II-9</th>
<th>II-14</th>
<th>III-10</th>
<th>III-11</th>
<th>IV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>20/20</td>
<td>20/20</td>
<td>20/200</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
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<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Ocular motility</td>
<td>Deviation</td>
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<td>12 PD XT</td>
<td>16 PD XT</td>
<td>18 PD XT</td>
</tr>
<tr>
<td>Dysfunctioning muscle(s)</td>
<td>SR,IO</td>
<td>Lev,SR,IO</td>
<td>SR, IO</td>
<td>IO</td>
<td>IO</td>
</tr>
<tr>
<td>Ptosis</td>
<td>None</td>
<td>None</td>
<td>Marked</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Anomalous head posture</td>
<td>Chin elevation</td>
<td>Chin elevation</td>
<td>Slight head tilt</td>
<td>Slight head tilt</td>
<td>Head turn</td>
</tr>
<tr>
<td>Forced duction</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hand abnormalities</td>
<td>Ab 5; Cl Hy 4</td>
<td>Ab 5; small fifth</td>
<td>Ab 5; Cl Hy 4</td>
<td>Ab 5; small fifth</td>
<td>Ab 5; Cl Hy 4</td>
</tr>
</tbody>
</table>
| Numbers following abbreviations represent the relevant finger numbers.

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°C 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.

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).

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. All new markers were on the genome-wide human screening set (version 9) and single chromosome scan autosomal microsatellite markers from the genome-wide human screening set (Invitrogen Life Technologies, Carlsbad, California, USA).

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 MgCl₂, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 200 mM 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°C for 10 minutes, and amplified for 27 cycles with denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension step at
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) under the assumption of autosomal recessive inheritance with full penetrance. The Mega2 program (version 2.5) 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).

**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 °D exotropia and 25 °D 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 hemiparesis and divergent strabismus. He had 12 Δ of exotropia and 25 Δ 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 Δ 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).

**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 Δ exotropia and 20 Δ hypotropia OD. Restricted elevation during adduction was more marked 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 2 ½ 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 enophthalma 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 21q22.11. 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 were 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.

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A generalised fibrosis syndrome. Extraocular muscle involvement with markedly restricted movement was observed in some patients, with atypical CFEOM2 and CFEOM3 having a more generalised and severe pattern of involvement. The fifth cranial nerve (trigeminal) was involved in some cases, and patients had levator palpebralis dysfunction. Phenotypically, three individuals, IV-1 and III-10, respectively, had abnormalities in these muscles.

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 patients. 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. 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 palsy resembling a generalised fibrosis syndrome. The patient had bilateral involvement with secondary esotropia or exotropia. Nerve biopsy findings of patients affected by congenital fibrosis of the extraocular muscles (CFEOM) revealed the absence of the superior division of the oculomotor nerve, which normally innervates the superior rectus and levator palpebrae superioris muscles. In contrast, atypical patients with CFEOM2 and CFEOM3 have a restrictive ophthalmoplegia, caused by deficient function of the muscles innervated by the third or fourth cranial nerves.

The patients described here can be classified as atypical CFEOM because 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, is frequently accompanied by dysfunction of other cranial nerves, and may also be associated with arthrogryposis or hand abnormalities but is quite distinct from the syndrome we present here. It is estimated that approximately 1/600 newborn infants have a congenital abnormality of the upper limb. 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). 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 have been reported. 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 from the new microsatellite marker, 2044K, to qter. The homozygous region between 2044K and the telomer was critical, spanning about 2 Mb to 1.5 Mb from the new microsatellite marker, 2044K, to qter. The homozygous region between 2044K and the telomer was critical, spanning about 2 Mb to 1.5 Mb from the new microsatellite marker, 2044K, to qter.

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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.

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Further evidence for LBP-1c/CP2/LSF association in Alzheimer’s disease families

L Bertram, M Parkinson, M B McQueen, K Mullin, M Hsiao, R Menon, T J Moscarillo, D Blacker, R E Tanzi

Objectives: Several studies suggested chromosome 12 harbours an Alzheimer’s disease (AD) risk factor gene. Significant association of a single nucleotide polymorphism (SNP) in the 3’ UTR of transcription factor CP2 (LBP-1c/CP2/LSF or TFCP2) at 12q13 was reported in three independent case-control studies, but no family based analyses have been performed to date.

Methods: Genotypes for three SNPs were generated in two independent AD family samples. A meta-analysis on all published case-control studies was also performed.

Results: The A allele of the 3’ UTR SNP was associated with increased risk for AD in one sample (odds ratio (OR) 2.1, 95% confidence interval (95% CI) 1.1 to 4.3), but not in the other, possibly due to low power. Haplotype analyses showed that this allele is part of a putative risk-haplotype overtransmitted to affected individuals in one sample and in both samples combined. Meta-analysis of the previously associated 3’ UTR SNP showed a trend towards a protective effect of the A allele in AD (OR 0.73, 95% CI 0.5 to 1.1).

Conclusions: This is the first study to examine LBP-1c/CP2/LSF in AD families, and the fifth to independently show significant association. While our results support a role of this gene in AD pathogenesis, the direction of the effect remains uncertain, possibly indicating linkage disequilibrium with another variant nearby.

Abbreviations: AD, Alzheimer’s disease; CAG, Consortium on Alzheimer’s Genetics; CLR, conditional logistic regression; LD, linkage disequilibrium; OR, odds ratio; PDT, pedigree disequilibrium test; SNP, single nucleotide polymorphism; 95% CI, 95% confidence interval

A lzheimer’s disease (AD) is a neurodegenerative disorder with a complex genetic background. The rare, early onset autosomal dominant forms of AD are caused by mutations in three genes (APP, PSEN1, and PSEN2), all of which lead to an increase in β amyloid protein (Aβ) levels in brain.1 Disease onset is typically before the sixth decade of life, and pathogenic mutations display virtually 100% penetrance. The more common late onset form of AD (that is, with disease onset usually between 60 and 80 years of age) is likely governed by genetic susceptibility factors of smaller effect and greatly reduced penetrance, which are transmitted in a non-Mendelian fashion.2 The only established risk factor to date is the ε4 allele of apolipoprotein E (APOE) on chromosome 19q13, which is involved in the accumulation and/or clearance of Aβ in the brain of AD patients.3 While several dozen papers are published each year claiming or refuting association with additional candidate genes on just about every chromosome, none of these has been unequivocally confirmed.4

Since the discovery of APOE ε4, numerous laboratories worldwide have performed either linkage, or association based, full genome screens in search of other AD predisposing variants. Linkage to chromosome 12 was one of the first major signals to emerge from these efforts. However, while some studies predominantly observed the strongest findings on the short arm of this chromosome (on 12p13, near 10 Mb),5,6 other groups detected more pronounced linkage approximately 40 Mb distal (on 12q13, near 50 Mb).4,7 To date, it remains unclear whether these signals are caused by the same underlying locus, or are actually the result of two distinct genes. The latter hypothesis is supported by the fact that there is at least one candidate gene in each region that has received independent confirmation—albeit not unequivocally—in at least ten studies: α2-macroglobulin (A2M) on 12p13, and the lipoprotein receptor related protein-1 (LRP1) on 12q13 (reviewed in Bertram and Tanzi8 and in Saunders et al.9). Other candidates on chromosome 12 reported to be associated with AD include: on 12p13, oxidised lipoprotein receptor-1 (OLR1); and on 12q13, transcription factor CP2 (LBP-1c/CP2/LSF) and neurotrophin-3 (NTF3). Of these, LBP-1c/CP2/LSF (also known as TFCP2) has received the most consistent support from independent groups with four papers reporting significant association, and thus far no published negative study.10–13

LBP-1c/CP/LSF encodes a nuclear transcription factor that regulates the expression of A2M and glycogen synthase kinase-3β (GSK3β),14,15 and also interacts with Fe65,16 which serves as an adapter molecule for the cytoplasmic domain of Aβ precursor protein (APP), and may also be involved in the regulation of gene expression via interaction with the APP intracellular domain (AICD).17 The original paper associating LBP-1c/CP/LSF with AD examined three independent case-control studies from France, the US, and the UK (table 1).13 While two of these (France and UK) showed a significant protective effect in carriers of the A allele at a single nucleotide polymorphism (SNP) in the 3’ UTR of the gene, the authors detected no significant association in the US sample. Combining all three populations revealed a significantly decreased risk of developing AD in carriers of the A allele v the G/G genotype (odds ratio (OR) 0.58, 95% confidence intervals (95% CI) 0.44 to 0.75; table 1). Two subsequent independent case-control studies by Taylor et al.13 and Luedeking-Zimmer et al.14 replicated the protective effect of the A allele with similar effect sizes (table 1). Finally, a fourth case-control study from Italy also detected a significant association between this SNP and AD.15 In contrast to the other papers, however, the data of this report suggested an over-representation of the A allele in AD cases v controls. To date, there are no published reports investigating the potential role of the LBP-1c/CP2/LSF gene in family based AD datasets, which have the advantage of being unbiased in the presence of population admixture. In this study we have examined a total of three SNPs in LBP-1c/CP2/LSF.
LSF in two independent and carefully ascertained and evaluated AD family samples, and provide further support for a significant role of this gene in contributing to overall AD risk.

**METHODS**

Samples

The NIMH AD genetics initiative study sample

Subjects were collected following a standardised protocol applying NINCDS/ADRDA criteria for the diagnosis of AD. Over the 10 years that the participating families have been followed, a clinical diagnosis of AD has been confirmed at autopsy in 94% of the cases. The NIMH sample includes 1439 individuals (69% female) from 437 families with at least two affected individuals (994 affected individuals (mean SD) age of onset 72.4 (7.7) years, range 50–97 years), 411 unaffected individuals, and 34 with unknown phenotype.

Consortium on Alzheimer’s Genetics (CAG) study sample

Subjects for this second, independently ascertained, AD family sample were collected under the auspices of the Consortium on Alzheimer’s Genetics, a collaborative effort of the Massachusetts AD Research Center, the University of California, Los Angeles, the University of California, San Diego, and the University of Rochester Medical Center. NINCDS/ADRDA criteria were used for a clinical diagnosis of AD, and probands were included only if they had at least one unaffected living sibling willing to participate in this study. Unlike the NIMH sample, no affected individual beyond the proband was required; thus, the vast majority of families are not multiplex. Currently, data and specimen collection is completed for 489 individuals (62.6% female) from 217 sibships in which all affected individuals displayed an onset age ≥50 years (n = 224 affected individuals (mean SD) age of onset 71.2 (9.1) years, range 50–89 years), n = 265 unaffected individuals. Most sibships consisted of just one discordant sibpair, but in 41 families there were more than two siblings available.

Genotyping

Genotypes for a total of three polymorphisms (that is, the original 3’ UTR SNP, rs4438107 (~10 kb proximal), and rs10876135 (773 bp distal)) in LBP-1c/CP/LSF were generated using fluorescent polarization detection single base extension (FP-SBE) on a Criterion Analyst AD (Molecular Devices, Sunnyvale, CA). PCR primers were designed to yield a product of approximately 250 bp in length and added to ~10 ng of genomic DNA using individually optimised PCR conditions (sequences available on request). PCR primers and unincorporated dNTPs were degraded by the direct addition of exonuclease 1 (0.1–0.15 U/rxn) and shrimp alkaline phosphatase (1 U/rxn). The single base extension step was carried out using Thermosequenase (0.4 U/rxn) and the appropriate mix of R110-ddNTP, TAMRA-ddNTP (3 mM), and all four unlabeled ddNTPs (22 or 25 μM) to the Exo1/SAP treated PCR product. To assess genotyping quality and ensure consistency of the genotyping calls, ~10% of the samples were randomly duplicated and genotyped twice. For all three SNPs combined, average genotyping efficiency was 97.8%, and the discrepancy rate (based on comparison to blinded duplicated samples) was below 0.3% in both samples.

**Statistical analyses**

Single locus and haplotype based tests of association were done in FBAT (v.1.5.3). FBAT uses a generalised score statistic to perform a variety of TDT type tests and despite the true underlying genetic model, FBAT performs best assuming an additive genetic model, which was used here. We used the empirical variance function of the program to account for the presence of linkage in the area (as was suggested by previous studies), and an equal weight offset correction to incorporate genotypes from both affected and unaffected individuals. All analyses were performed on the full NIMH sample, the CAG sample, and on the two samples combined. All single locus analyses were repeated using the pedigree disequilibrium test (PDT) to confirm results obtained with FBAT (note that the PDT currently does not accommodate haplotype tests). While both tests compute valid p values under the null hypothesis of linkage but no association, the PDT statistic can be less efficient under some circumstances as it only includes discordant sibships and ignores families where only affected siblings are available. Further, to assess the magnitude of any potential effect on disease risk for the 3’ UTR SNP, we performed conditional logistic regression (CLR) stratified on family, comparing carriers of the A allele to carriers of the GG genotype. All OR are adjusted for age, gender, and APOE ε4 allele status. Note that confidence intervals may be too narrow because CLR may slightly underestimate the standard errors when multiple affected and unaffected subjects are included in each family. However, the magnitude of this effect is expected to be small unless genetic effects are very large. Finally, to assess whether families showing association with the 3’ UTR SNP in LBP-1c/CP/LSF overlap with families associated with the intron 18 deletion in A2M, we determined for both polymorphisms which of the NIMH pedigrees showed
transmission of at least two risk alleles to affected individuals (using the Viewstat option in FBAT).

**Haplotype block predictions**

Haplotype blocks were estimated using the program Haploview based on the four gamete rule (fourth gamete at 0.02 frequency; see Haploview website for details at http://www.broad.mit.edu/personal/jcbarret/haploview/index.php). Haploview was also used for the calculation of pairwise linkage disequilibrium (LD) measures across all three SNPs.

**Meta analysis**

Study specific crude ORs and 95% CIs were calculated from the raw data for each of the case-control studies investigating the association between the LBP-1c/CP/LSF 3′ UTR SNP and AD. The Q statistic, a test for heterogeneity among the study specific ORs, that is distributed approximately as χ² with k−1 degrees of freedom (k = number of studies), resulted in a p value <0.1, suggesting significant between-study heterogeneity. Therefore, to calculate a summary OR for all studies, we used the DerSimonian and Laird random effects model, which utilises weights that incorporate both the within study and between study variance. Note the ORs estimated from the CLR in our family based analyses are adjusted for a number of co-variables (see above), which is why we elected not to combine them with the crude or differently adjusted ORs from the case-control studies. Statistical Analysis System (SAS) was used for the statistical analyses and resulting graphs.

**RESULTS**

The results of the single locus and haplotype association analyses are shown in table 2. Allele frequencies, as estimated by FBAT, were very similar for all three SNPs in both samples, and for the 3′ UTR SNP are comparable to the previous reports (see legend to table 2). Genotype frequencies for all SNPs were in Hardy-Weinberg equilibrium (p > 0.90). Testing the 3′ UTR SNP in FBAT revealed significant overtransmission of the A allele to affected individuals in the NIMH families (p = 0.05). This was confirmed using the PDT (p = 0.04), and resulted in a significant risk increase in carriers of the A allele vs non-carriers (OR 2.1, 95% CI 1.1 to 4.3) using CLR stratified on family. Due to the low numbers, heterozygous and homozygous A allele carriers could not be examined separately, but all three subjects carrying the A/A heterozygous and homozygous A allele carriers could not be examined separately, but all three subjects carrying the A/A heterozygous and homozygous A allele carriers could not be examined separately, but all three subjects carrying the A allele of the previous studies demonstrating a protective role for the A allele of the 3′ UTR SNP were comprised of late onset AD families. In this study, however, effect size estimates are quite comparable in families of late (OR 1.8 (1.0–3.2)) and early/mixed onset (OR 2.2 (0.7–7.2); combined sample).

Combining all four previously published case-control studies into one meta-analysis revealed an overall protective effect of the A allele, although this did not reach statistical significance when all six independent case-control series were considered (0.73, 95% CI 0.5 to 1.1; fig 1). Interestingly, allele-frequency estimates across studies were quite similar for the AD cases (ranging from 0.04 to 0.06), but were considerably more variable in the control populations (ranging from 0.02 to 0.09; table 1). In an attempt to reduce this variability, we removed the two populations with the most extreme allele frequency estimates in healthy controls (that is, Panza et al. and the UK sample from Lambert et al.) and repeated the analyses. As expected, the resulting summary OR proved somewhat more stable, indicating a significant protective effect across the remaining samples (OR 0.62, 95% CI 0.5 to 0.8; fig 1).

<table>
<thead>
<tr>
<th>FBAT</th>
<th>Single locus analyses*</th>
<th>3′ UTR</th>
<th>Rs10876135</th>
<th>Haplotype analysis†</th>
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<tr>
<td></td>
<td>rs4438107</td>
<td></td>
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<tr>
<td></td>
<td>z score (p)</td>
<td>z score (p)</td>
<td>z score (p)</td>
<td>Allele (p)</td>
</tr>
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<td>1.9 (0.05)</td>
<td>−2.0 (0.04)</td>
<td>H4 (p) = 0.03</td>
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<td>CAG (n = 217)</td>
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<td>1.1 (0.03)</td>
<td>1.4 (0.2)</td>
<td>H4 (p = 0.02)</td>
</tr>
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<td>Combined (n = 654)</td>
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<td>2.2 (0.03)</td>
<td>−0.9 (0.4)</td>
<td>H4 (p = 0.01)</td>
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<td>z² (p)</td>
<td>z² (p)</td>
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<tr>
<td>NIMH (n = 437)</td>
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<td>5.1 (0.02)</td>
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<tr>
<td>CAG (n = 217)</td>
<td>1.2 (0.3)</td>
<td>0.5 (0.5)</td>
<td>1.5 (0.2)</td>
<td>−</td>
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<tr>
<td>Combined (n = 654)</td>
<td>0 (0.9)</td>
<td>5.5 (0.02)</td>
<td>2.1 (0.15)</td>
<td>−</td>
</tr>
</tbody>
</table>

*Minor allele frequencies in the NIMH (CAG) sample are: rs4438107 (T) = 0.46 (0.47), 3′ UTR (A) = 0.06 (0.07), rs10876135 (T) = 0.08 (0.06). z score for minor allele or denoted haplotype allele (positive values indicate overtransmission to affected individuals).
†Haplotype frequencies in the NIMH (CAG) sample are: H1 [C-G] = 0.5 (0.48), H2 [T-G-C] = 0.39 (0.43), H3 [T-G-T] = 0.06 (0.04), H4 [C-A-C] = 0.04 (0.04). Association statistics are presented for risk associated haplotype (H4) and 1 df.

**Table 2** Association analyses of three SNPs in LBP-1c/CP/LSF in two independent family samples
**DISCUSSION**

This is the first study to assess the previously reported association between LBP-1c/CP2/LSF and AD using family based methods. Examining two independently ascertained and carefully characterised AD family samples, we observed a significant risk effect of the A allele of the 3’ UTR SNP in the NIMH families, and a similar but insignificant effect in the smaller CAG dataset. Combining both samples resulted in the same effect size estimate as for the NIMH families alone, with slightly narrower confidence intervals. This could indicate that the A allele is also a risk factor in the CAG families, which possibly remained undetected due to insufficent power. Interestingly, only two of the NIMH families found to be associated with LBP-1c/CP2/LSF here also show association with the intron 18 deletion in A2M published earlier by our group using the same NIMH dataset, if association is assumed when at least two risk alleles are transmitted (as indicated by the Viewstat function in FBAT; see Methods) to affected individuals for each polymorphism. This suggests that these two genes, which are located ~40 Mb apart on chromosome 12, likely function as independent risk factors.

In addition to the findings with the previously reported 3’ UTR SNP, we also detected evidence of association with a nearby second SNP (rs10876135) in the NIMH families. It is interesting that the minor allele (that is, T) of this SNP was undertransmitted to affected individuals, reminiscent of the transmission pattern observed in all but one previous case-control study for the 3’ UTR SNP. Overtransmitted alleles for both SNPs were located on a rare haplotype (H4), which was associated with risk for AD in the NIMH sample. A similar overtransmission was found in the CAG sample, although this did not reach statistical significance, possibly due to low power (that is, there were only 17 informative transmissions in 30 in the NIMH sample). Taken together, our findings clearly support and extend the previously suggested role of the LBP-1c/CP2/LSF gene in AD pathogenesis.

Thus far, only one study has investigated the potential consequences of the 3’ UTR SNP on LBP-1c/CP2/LSF expression and protein function. These experiments were somewhat preliminary and revealed that the A allele—which the same authors found to be underrepresented (that is, protective) in their AD cases as compared to controls—had a lower affinity to nuclear proteins in neuroblastoma cells using electrophoresis mobility shift assays, potentially suggesting a decreased function of this nuclear transcription factor. However, when LBP-1c/CP2/LSF mRNA expression was compared in lymphocytes from AD cases and controls, the A allele carriers showed no detectable differences. In fact, only the affected G/G carriers showed an overall reduced expression of this gene in cases v controls. However, the numbers in these latter experiments were very low (only three A allele carriers in each group) and were only performed in peripheral blood cells. Thus, no inference can be currently drawn from these data regarding any pathophysiological consequences in the brain.

Despite these promising findings, there remains discrepancy with respect to the direction of the potential effect uncovered by the genetic analyses, where two studies now show a risk effect, while three studies favour a protective role for the A allele. There are at least three conceivable scenarios to explain the observed discrepancies across studies. The first possibility is that LBP-1c/CP2/LSF is not an AD susceptibility gene, and the previously published reports constitute false-positive findings published on the basis of publication bias. While currently the possibility of publication bias cannot be assessed reliably due to the limited number of studies, this scenario appears unlikely as it is typically only an issue for the first, and usually positive, publication. In AD and other genetically complex diseases, the majority of promising initial findings are followed by a number of non-confirmative studies, which—at least in the past—have mostly outweighed the positive results. However and as mentioned earlier, LBP-1c/CP2/LSF has thus far been quite remarkable in that only positive studies have been published following the initial report, our study being no exception. A second explanation for the discrepant findings is that the families driving the association in our analyses (that is, NIMH) were
ascertained based on the presence of at least two AD cases in first degree relatives of the same pedigree, while all previous samples did not specifically consider family history. This could potentially lead to the sampling of genetically distinct populations, that is, samples that are governed by different genetic risk factors and risk alleles. However, the observation that at least one other investigation (by Panza et al) also described an over-representation of the A allele in their AD samples as compared to controls, suggests that differences in ascertainment are probably not responsible for the observed differences in allele and genotype distributions across studies. Finally, it is possible that the 3’ UTR SNP is not actually pathogenic, but that the observed associations merely reflect LD with another genetic variant nearby. In this case, the—still elusive—true disease predisposing variant would have independently occurred on the haplotype background of the major allele in four of the examined case-control populations, while in the sample by Panza et al and our study it has arisen coupled with the minor allele. In the former samples the A allele would thus appear as protective (since the actual risk allele is actually in LD with the G allele at the 3’ UTR SNP), while in the latter cases it would appear as a risk factor. On the other hand, there could be several independent and rare disease modifying variants within the LBP-1c/CP2/LSF gene, which would have also arisen on different haplotype backgrounds (for example, similar to the A allele which is observed for PSEN1). Both alternatives are consistent with the analyses provided in this study, which—at least in the families analysed here—favour the existence of risk increasing variant(s) on the H4 background. It is noteworthy that similar observations, that is, significant associations with opposite alleles across different samples and populations, have actually been reported with several other AD candidate genes in the past (for example, A2M (recently reviewed in Saunders et al9), LRP1,20,21 tumour necrosis factor α (TNFA),32,33 and butyrylcholinesterase K (BChE-K)36,37). If they do not merely represent a collection of varying false-positive findings, these differences could be attributed to the different patterns of LD across populations of different origin and/or differing degrees of population heterogeneity. While we favour this last alternative as the most likely explanation for the observed differences with the 3’ UTR SNP, clearly more studies need to be performed on the potential association of this and possibly other polymorphisms in LBP-1c/CP2/LSF and AD.

In conclusion, we provide additional and independent evidence suggesting that genetic variants in LBP-1c/CP2/LSF significantly alter the risk for developing AD. More studies will need to be performed to further establish this association, and to more definitively assess which variant(s) are actually responsible for the observed effects, and how they affect disease pathogenesis.

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ELECTRONIC-DATABASE INFORMATION

The Haploview website can be found at http://www.broad.mit.edu/personal/cabernet/haploview/index.php and International HapMap Project can be found at http://www.hapmap.org/.

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REFERENCES


www.jmedgenet.com
In the paper titled, A new syndrome, congenital extraocular muscle fibrosis with ulnar hand anomalies, maps to chromosome 21qter (J Med Genet 2004;41:408-15) there are a number of errors. The affiliations and correspondence details were incorrect, the correct details have been listed below:

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In figure 3 the marker D21S1400 should be D21S1403.

On page 413 in the second paragraph, the marker D21S1446 should be D21S1440.