Oculomotor Nerve and Muscle Abnormalities in Congenital Fibrosis of the Extraocular Muscles

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Congenital fibrosis of the extraocular muscles is an autosomal dominant congenital disorder characterized by bilateral ptosis, restrictive external ophthalmoplegia with the eyes partially or completely fixed in an infraducted (downward) and strabismic position, and markedly limited and aberrant residual eye movements. It has been generally thought that these clinical abnormalities result from myopathic fibrosis of the extraocular muscles. We describe the intracranial and orbital pathology of 1 and the muscle pathology of 2 other affected members of a family with chromosome 12-linked congenital fibrosis of the extraocular muscles. There is an absence of the superior division of the oculomotor nerve and its corresponding alpha motor neurons, and abnormalities of the levator palpebrae superioris and rectus superior (the muscles innervated by the superior division of the oculomotor nerve). In addition, increased numbers of internal nuclei and central mitochondrial clumping are found in other extraocular muscles, suggesting that the muscle pathology extends beyond the muscles innervated by the superior division of cranial nerve III. This report presents evidence that congenital fibrosis of the extraocular muscles results from an abnormality in the development of the extraocular muscle lower motor neuron system.


Congenital fibrosis of the extraocular muscles (CFEOM, MIM 135700) is a relatively static eye movement disorder typically inherited in an autosomal dominant fashion. Affected individuals have congenital, bilateral ptosis and restrictive external ophthalmoplegia, with their eyes partially or completely fixed in an infraducted (downward) and strabismic position. Biopsy specimens of the rectus inferior (IR) from individuals with CFEOM are typically reported to contain fibrous tissue [1–4], or a mixture of myofibers and fibrous tissue [5, 6]. In 1950, Brown categorized CFEOM, horizontal retraction syndrome (Duane’s syndrome), vertical retraction syndrome, superior oblique tendon sheath syndrome (Brown’s syndrome), and strabismus fixus together as the "fibrosis syndromes" [1]. Although the restrictive ophthalmoplegia, "stiff" muscles at surgery, and findings of connective tissue on muscle biopsy examination led many to propose that these disorders resulted from a myopathic process with fibrous changes, it remains unclear whether these disorders result from a primary myopathic or neuropathic process, and whether they are pathophysiologically related to one another.

Although it is known that extraocular muscle (EOM) differs significantly from other skeletal muscle [7, 8], it is not understood why EOM is selectively affected in some disorders and spared in many others that involve the rest of the neuromuscular system. Describing and interpreting pathological changes of EOM has been complicated by its relative inaccessibility to both biopsy and postmortem examination, resulting in the infrequent ascertainment of diseased tissue. Moreover, the normal histology of EOM is unique, and consists of small round fibers of variable size with moderately prominent endomysial connective tissue, features that would be interpreted as signs of myopathy by limb muscle standards [8, 9].

Understanding EOM-specific disorders such as the fibrosis syndromes may lead to a better understanding of the specialized development and biology of the ex-
traocular motor system. Among these syndromes, CFEOM is unique in that it can be approached through a combination of pathologial and molecular genetic investigations. We previously determined that families with autosomal dominant and completely penetrant CFEOM appear to be phenotypically and genetically homogeneous and that their disease gene maps within a 3-cM centrometric region of chromosome 12. We refer to this genetically defined disorder as "classic CFEOM" to distinguish it from sporadic, autosomal recessive, and clinically variant forms [10, 11]. In this report, we present the detailed clinical and pathological findings of several affected members from a family with classic CFEOM whose disease gene maps to the locus on chromosome 12.

Materials and Methods

Participants

Several of the authors (E. C. E., C. A. M., M. S.) examined the living participants to document results of their ophthalmological and neurological examinations. Each participant is a member of a single family with autosomal dominant, fully penetrant CFEOM (Fig 1E). The family’s CFEOM disease gene is linked to chromosome 12, and Patients A, B, C, D, and E all carry the family’s disease haplotype, as previously reported [10, 11]. Living participants were counseled and signed informed consent forms. Consent was obtained for all autopsy material.

Clinical Material

Hematological and chemical assays of blood and cerebrospinal fluid (CSF), electromyography, and magnetic resonance imaging were performed at Children’s Hospital in Boston, using the hospital’s standards and control measurements.

Autopsy

The neuropathological examination of Patient E was performed following a 20-hour postmortem interval. The brain rostral to the midbrain-thalamic junction was removed from the cranial cavity. The cranial nerves (CNs) were severed at approximately 1 to 2 cm from their point of exit from the
brainstem, and the brainstem and upper cervical spinal cord removed. Small transverse sections of CNS II (optic), III (oculomotor), IV (trochlear), and VI (abducens) were fixed in 2% glutaraldehyde. The right orbital roof was removed to expose the orbital contents, and the rectus lateralis (LR), rectus medialis (MR), rectus inferior (IR), and the obliquus superior (SO) muscles were identified and separated from the globe posterior to their tendinous insertions. The levator palpebrae superioris (LPS) and rectus superior (SR) muscles could not be identified. The optic nerve (ON) was transected near its attachment to the globe. Proximally, the orbital contents were transected at the annulus of Zinn and the entire contents (except the globe) were removed en bloc. The distal portion of the IR was then cut from the block, and the brain, brainstem, orbital contents, and distal IR muscle were fixed in 10% neutral buffered formalin for histological examination.

A standardized survey of forebrain and spinal cord was conducted and sections of the cerebral cortex, thalamus, hypothalamus, amygdala, hippocampus, basal ganglia, and spinal cord were systematically examined. The midbrain and pons were embedded in paraffin and sectioned at 20 μ. Every fifth section was stained with hematoxylin and eosin (H&E) and cresyl violet–Luxol fast blue, to evaluate the neuronal cell populations of CN nuclei. The architecture and morphology of the CNs were examined by light and electron microscopy following embedding of the glutaraldehyde-fixed tissue in epoxy resin (Epon), thick sectioning at 1 μ, and staining with toluidine blue. The right oculomotor nerve was analyzed for total fascicular area, and number, density, and size distribution of myelinated fibers, as reported by Smith and Dyck [12] using BioQuant System IV (R&M Biometrics) image analysis software. The orbital contents, resected en bloc, were embedded in paraffin, sectioned at 15 μ, and every fiftieth section was stained with H&E to evaluate the anatomical relationship among the EOMs and between EOM and nerve.

**Muscle Biopsies**

EOM biopsy specimens were obtained from Patients C and D during corrective strabismus surgery under general anesthesia. For each biopsy, the tendon and muscle were identified and isolated. The width and point of insertion on the globe were measured, and the appearance, texture, and passive stretch noted. We employed a biopsy technique designed to obtain a representative sample of the tendon, transition zone, and muscle. A 3-mm-wide strip along the medial or superior third of the muscle was disconnected from the globe at the point of insertion and longitudinally separated from the remaining muscle fibers, extending as far toward the muscle origin as was safely and anatomically possible. The insertional end of the biopsy specimen was tagged with a suture and the 3-mm strip was cauterized proximally, cut, and removed. Quadriceps muscle biopsy was performed in Patient B at the age of 11 months at Children's Hospital using standard procedures. Quadriceps muscle biopsy was performed in Patient D at the age of 35 months at Dartmouth-Hitchcock Medical Center and was reviewed by the authors.

Each extraocular and quadriceps tissue sample was snap frozen in isopentane and liquid nitrogen, and cut in 10-μ sections. Frozen muscle tissue was evaluated with H&E, Gomori's trichrome, NADH, adenosinetriphosphatase (ATPase) preincubated at varying pHs (4.3, 4.6, and 9.4), periodic acid-Schiff (PAS), and oil red O. To determine the length of the tendinous insertion, the left IR specimen from Patient D and the left IR from a 3-year-old control autopsy subject were serially sectioned at a thickness of 8 and 10 μ, respectively. Every twentieth section was stained with H&E and examined to define the anatomy of the tendon and the transition from tendon to muscle. EOM fiber morphometrics and determination of the frequency of internal nuclei were performed using BioQuant System IV (R&M Biometrics) image analysis software. A nucleus was scored as internal if a minimum of one nuclear width of sarcoplasm was present between the nucleus and the myofiber membrane, and normal if less cytoplasm or no nucleus was visible. All the fibers present in each CFEOM biopsy specimen were scored, and a minimum of 250 fibers from a series of random fields of each autopsy muscle were scored. Total DNA was extracted from an aliquot of biopsied extraocular and skeletal muscle (Patients B, D, and E) and was analyzed for the presence of single or multiple mitochondrial DNA deletions using the widely interspaced primer polymerase chain reaction technique [13].

**Control Tissue**

The caudal midbrains from 6 age-matched autopsied individuals with Alzheimer’s disease (postmortem intervals of 3–26 hours) were sectioned and stained in a similar manner to that described above. The right orbital contents from autopsies of a 3-year-old, 19-year-old, and 70-year-old (postmortem intervals of 5, 4, and 11 hours, respectively) who died from unrelated disorders were removed, processed, sectioned, and stained en bloc as described for Patient E. The left orbital contents were removed and each EOM was dissected from the globe and processed as described for the CFEOM biopsy tissue. Oculomotor nerve morphometric data were compared to previously reported control values [12].

**Results**

**Clinical Findings**

Patients A (IV-1), B (V-2), C (V-4), and D (VI-1) (see Fig 1) underwent extensive clinical examinations. Both children (Patients B and D) had congenital bilateral ptosis and nearly complete external ophthalmoplegia. Both had mild delay in gross motor milestones during the first 1.5 years of life. When examined at the age of 11 months, Patient B had mild facial diplegia and a mild decrease in both axial and appendicular tone. His hypotonia resolved by approximately 4 years of age and his reflexes were always normal. When examined at 3.5 years, Patient D also had slight facial diplegia, mild hypotonia, slightly tight heel cords, and normal strength and reflexes. Each of their affected parents (Patients A and C) had static congenital ptosis and ophthalmoplegia as well as mild bifacial weakness. Patient C had surgical correction of a cleft palate as a
child (we are aware of no other affected individuals with cleft palate in this or any other classic CFEOM family). None had sensory abnormalities, including within the trigeminal nerve distribution. None experienced fluctuation of symptoms with time of day or fatigue. On general examination, they had no cardiac abnormalities and no additional abnormalities of tone, strength, or reflexes.

All 4 patients maintained a chin-up head posture and had profound bilateral ptosis with almost complete absence of LPS function (see Fig 1). Lid fissures varied eye to eye and ranged from 2 to 5 mm. Both eyes were directed far into downward gaze with a variable degree of both horizontal and vertical strabismus (see Fig 1). There was significant limitation of extraocular movement in both eyes of all 4 patients, always greatest in upgaze, and forced duction tests revealed a marked restriction of globe movement in all directions beyond the limits of voluntary gaze (see Fig 1). In spite of significant ocular misalignment, none of the patients experienced diplopia; Worth four-dot testing in the adults confirmed that this was due to their ability to suppress the retinal image in the deviated eye. The patients demonstrated anomalously directed eye movements. These movements were often dysconjugate and included synergistic convergence and divergence, aberrant downward movement on attempted horizontal gaze, and retraction of the globe into the orbit. No nystagmus was clinically apparent when the eyes were directed in the preferred downward gaze position. However, on attempted movement away from this position there were often unusual, dysconjugate, 2- to 3-Hz beating eye movements that occurred in the direction of attempted gaze in the fixating eye, but not always in this direction in the nonfixating eye. The 2 adults had decreased central acuity in both eyes; the best corrected visual acuity at distance for Patient A was 20/30 in the right and 20/100 in the left eye, and for Patient C was 20/40 in the right and 20/70 in the left eye. This asymmetry in visual acuity was caused by amblyopia. In addition, both children were treated with occlusion therapy for amblyopia. Results of dilated fundus examinations, pupils, and pupillary reactions were normal in all 4 patients.

Affected Patient E died at the age of 71 and the authors did not examine her prior to her death. Her history, medical records, photographs, and DNA studies, however, all confirmed that she had congenital, nonprogressive bilateral infraducted external ophthalmoplegia and ptosis. In 1981 she underwent ptosis surgery and biopsy of the right LPS revealed "fibrous and fibromuscular tissue." She retired from shop work at age 60 years, secondary to ptosis and neck pain. She had hypertension and angina. In the last several years prior to her death she became forgetful and mildly dis-oriented and was diagnosed with probable Alzheimer's disease.

Laboratory Data
Normal laboratory values were found for creatine kinase (CK) and blood lactate levels and electrocardiography (ECG) (Patients A, B, C, D); chemistry panels and blood pyruvate level (Patients B and D); and complete blood cell count, clotting parameters, venous pH, thyroid function tests, urine analysis, urine amino acids, CSF (including levels of protein, glucose, lactate, pyruvate, and amino acids and cell count), and a Tensil test (Patient B). Magnetic resonance imaging studies of the brains of Patients B and D were normal but did not include specific orbital cuts. Brainstem auditory evoked responses were normal in Patient B and revealed a unilateral peripheral auditory defect in Patient A. Results of limb nerve conduction studies and electromyography in Patients A, B, and D were normal.

Postmortem Neuropathological Findings in Patient E
Gross neuropathological examination of the brain revealed bilateral, symmetrical atrophy of the cerebral cortex with decreased brain weight of 990 gm (normal, 1,350 gm), bilateral hippocampal and parahippocampal atrophy, and moderate hydrocephalus ex vacuo, findings consistent with Alzheimer's disease. Microscopic examination revealed neurofibrillar tangles, senile neurofibrillary tangles, senile neuritic plaques, and variable astrocytosis and apparent loss of neurons in the cerebral cortex, hippocampus, subiculum, parahippocampal gyrus, amygdala, substantia innominata, caudate, putamen, thalamus, and the peri-aqueductal gray of the midbrain and raphe nucleus. The optic nerves, chiasm, optic radiations, and calcarine cortex were unremarkable. These postmortem changes of Alzheimer's disease are consistent with Patient E's clinical history of dementia. To our knowledge, Alzheimer's disease has not been reported in association with CFEOM, and dementia is not a feature of the patient's extended family, including affected members with CFEOM. It is quite likely that Alzheimer's disease, a relatively common disease of older individuals, is a coincidental finding in this patient.

On gross inspection, the midbrain and pons were well formed, and all of the CNs were identified bilaterally and exited the skull foramina appropriately. Microscopic examination of the serially sectioned midbrain revealed a group of large multipolar neurons with prominent chunky Nissl substance and central nuclei located on either side of the central gray matter, ventral to the cerebral aqueduct (Fig 2d, e). These cells corresponded to a similar group of neurons in the Alzheimer control brains (Fig 2a, b) and were identified as alpha motor neurons belonging to the complex of ventral, dorsal, intermedi-
Fig 2. Cross section of the oculomotor nuclei in control and affected individuals. (a) The caudal aspect of the oculomotor nucleus within the midbrain of an age-matched control individual with Alzheimer's disease. (× 5 before 84% reduction.) (b) Normal alpha motor neurons with blocks of Nissl substance within the lateral subnuclei indicated by box b in (a). (× 63 before 70% reduction.) (c) Normal alpha motor neurons with blocks of Nissl substance within the caudal central subnuclear area indicated by box c in (a). (× 63 before 70% reduction.) (d) The caudal aspect of the oculomotor nucleus within the midbrain of Patient E. (× 5 before 84% reduction.) (e) Normal-appearing alpha motor neurons with blocks of Nissl substance within the lateral subnuclei indicated by box e in (d). (× 63 before 70% reduction.) (f) Round-to-oval neurons with eccentric nuclei and fine granular Nissl substance within the area of the central caudal nuclear area indicated by box f in (d) are morphologically distinct from normal alpha motor neurons. (× 63 before 70% reduction.) (Cresyl violet–Luxol fast blue.)
ate, and medial oculomotor subnuclei, which innervate the MR, IR, IO, and SR muscles, respectively [14–16]. Because the subnuclei within this complex overlap, the presence or absence of a specific subnucleus cannot be determined without tracer studies. There appeared, however, to be fewer alpha motor neurons within this entire complex of Patient E’s oculomotor nucleus than within control midbrains (compare Fig 2a and d). In contrast to these overlapping subnuclei, the alpha motor neurons whose axons innervate the LPS muscle are located in the unpaired caudal oculomotor subnucleus. This subnucleus lies in the midline and is distinct and nonoverlapping with the other oculomotor subnuclei [14, 16, 17]. These centrally located alpha motor neurons are morphologically similar but slightly smaller than the lateral alpha motor neurons, and were easily identified in the appropriate location at multiple levels in sections of normal and Alzheimer control midbrains (Fig 2c). In contrast, these cells could not be identified at any level in Patient E’s brainstem. We did identify occasional neuronal cell bodies in the region of the caudal central subnucleus of Patient E (Fig 2f); however, these cells were round to oval with eccentric nuclei and fine granular Nissl substance. In addition, unlike the alpha motor neurons of the normal caudal central subnucleus that are restricted to within the confines of the subnucleus (see Fig 2c), the round-to-oval neurons identified in the caudal central subnuclear region were also found more diffusely in the central midbrain. Therefore, these neurons are morphologically distinct from and have a different distribution from that of normal caudal central alpha motor neurons, and are likely a different neuronal population. Histological examination of the caudal midbrain and the rostral pons revealed bilateral trochlear and abducens nuclei containing alpha motor neurons. Similar to the lateral oculomotor subnuclei, however, there appeared to be a qualitative decrease in the neuronal content of the abducens nuclei bilaterally. The paramedian pontine reticular formation, a putative region involved in coordinated eye movements, was unremarkable. There was no additional evidence of dysgenesis or gliosis of the CN nuclei or brainstem.

The proximal portions of CNs III and IV were multifascicular, and that of CN VI was multifascicular. The endoneurial areas were made up of transverse profiles of large and small myelinated fibers with unremarkable structure as shown by light and electron microscopy, and no areas of increased connective tissue (data not shown). The proximal portion of CN III had a total fascicular area of 0.6 mm² and contained 5,229 myelinated fibers (8,715 myelinated fibers/mm²). This cross section, located within 2 cm of the brainstem, is proximal to CN III’s normal division into superior and inferior branches. Similarly obtained and processed oculomotor nerve data from 15 control individuals (mean age, 52 years; range, 28–81 years) were published by Smith and Dyck [12]. They found an average total fascicular area of 2.7 mm² (standard deviation [SD] = 0.6), an average of 23,000 myelinated fibers/nerve (SD = 2,400), and 8,900 myelinated fibers/mm² (SD = 1,800). Thus, Patient E’s CN III fascicular area and fiber number were significantly reduced compared to control values, but the fiber density was within normal limits. In addition, although Patient E’s oculomotor fiber diameter peaks (Fig 3, bar graph) generally corresponded in size to previously published control data (Fig 3, dashed line [12, 18]), the ratio of large to small fibers was reversed, as the CFEOM nerve had a larger population of small fibers than large fibers.

At the time of removal of the orbital contents, the SR and LPS muscles could not be identified in Patient E. The remaining EOMs were well demarcated from the surrounding orbital fat, and were brown-pink and of normal consistency. On microscopic examination, in the normal location of the SR and LPS muscles there was an abnormal, small oval-shaped structure surrounded by connective tissue and containing fat, fibrous tissue and sparse myofibers (Fig 4b–d). No nerve twigs were identified within this structure. This structure was not identified until after the orbital contents were separated from the globe and orbital wall and, therefore, its insertion is not known. Its course, however, is most consistent with an SR muscle. The IR, MR, LR, and SO muscles (see Fig 4b) were easily identified, although the IR muscle extended a shorter distance because its distal aspect had been removed and fixed separately (Fig 4e–f). On microscopic examination, the myofibers were normally round and variable in size, and were well innervated. There was no evidence of degeneration or regeneration, inflammatory

![Fig 3. Bar graph histogram of the size distribution of fiber diameters of all myelinated axons (number on left axis) within proximal cranial nerve III of Patient E. For comparison, the superimposed dashed line summarizes key data points (number of myelinated fibers per mm²; right axis) from control individuals published by Smith and Dyck [12].](image-url)
cells, or increased perimysial or endomysial connective tissue. The muscles did, however, contain an increase in the percent of myofibers with internal nuclei when compared with aged-matched control EOMs (Table).

The superior division of CN III could not be identified within the microscopic sections of the posterior orbital contents. The optic nerve, trochlear nerve, abducens nerve, inferior division of CN III, superior and nasociliary branches of the trigeminal nerve, ciliary ganglion, and ophthalmic artery were identified in their appropriate locations (see Fig 4b). The courses of the trochlear and abducens nerves were followed to their normal sites of innervation of the SO and LR muscles, respectively. The diameter of the inferior division of CN III (see Fig 4b) was smaller than that in control tissue (Fig 4a), but followed the normal course and bifurcated just proximal to the level of the ciliary ganglion, sending branches to innervate the MR, IR, and obliquus inferior. The frontal branch of the ophthalmic division of the trigeminal nerve was present above the abnormal SR/LPS complex. The nasociliary branch of the ophthalmic division of the trigeminal nerve followed the appropriate course; in the posterior orbit it was located inferior to the lateral aspect of the abnormal SR/LPS complex, and several of its small branches coursed down toward the ciliary ganglion. Further into the orbit, the nasociliary nerve coursed from the lateral to medial orbit rostral to the optic
Frequency of Internal Nuclei in Extraocular Muscles of Congenital Fibrosis of the Extraocular Muscles (CFEOM) Patients and Control Individuals

<table>
<thead>
<tr>
<th>% Myofibers with Internal Nuclei</th>
<th>IR</th>
<th>LR</th>
<th>MR</th>
<th>SR</th>
<th>LPS</th>
<th>SO</th>
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<td>CFEOM</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Patient C</td>
<td>7.6</td>
<td>8.0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Patient D</td>
<td>5.3</td>
<td>8.6</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Patient E</td>
<td>5.0</td>
<td>4.6</td>
<td>3.9</td>
<td>7.1*</td>
<td>6.8</td>
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<td>19 yr</td>
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<td>2.9</td>
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<tr>
<td>70 yr</td>
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<td>1.8</td>
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*SR/LPS complex.

IR = rectus inferior; LR = rectus lateralis; MR = rectus medialis; SR = rectus superior; LPS = levator palpebrae superioris; SO = obliquus superior.

nerve, to then travel in the superior-medial quadrant of the orbit.

In contrast to these findings, the orbital contents of each of the 3 control autopsied subjects revealed normal SR and LPS muscles, and the superior branch of the oculomotor nerve was present inferolateral to the SR, which it subsequently entered and innervated (see Fig 4a). The nasociliary nerve was present and, similar to Patient E, passed over the optic nerve to subsequently lie along the superior-medial quadrant of the orbit.

Surgical Observations and Biopsy Results
Patient C underwent strabismus surgery with recession of the right MR and both IR muscles at the age of 30 years. Traction testing under anesthesia verified the limitations to globe movement (see Fig 1). The right MR was abnormally tight but had relatively good elastic properties; the right IR felt more tight and was inelastic; and the left IR was extremely tight, inelastic, and difficult to expose. Biopsy specimens measuring 17 mm, 17 mm, and 11 mm respectively, were obtained (all measurements reflect lengths prior to contraction of the muscle sample, which occurred on removal). Fibers of the SO tendon were identified but, despite extensive exploration, neither a SR tendon nor muscle was found. Patient D underwent bilateral recession of the IR and exploration of the left LR at the age of 5 years, at which time all of the muscles felt abnormally tight and inelastic. Traction testing showed significant restriction to globe movement which lessened slightly with globe retropulsion and was relieved by disinsertion of the muscles from the globe. Biopsy samples of the left LR and the left and right IR measured 16 mm, 15 mm, and 10 mm, respectively. At age 6, Patient D underwent a second procedure with recession of the bilateral LR and exploration of the bilateral SR. A right LR biopsy specimen measured 17 mm and a right SR biopsy specimen measured 10 mm. In both Patients C and D, the conjunctiva and Tenon's capsule appeared normal and, for the muscles identified, the location of their insertions on the globe were appropriate.

Microscopic examination of the bilateral IR and left LR specimens from Patient D revealed dense connective tissue within the portion of each sample adjacent to the insertion on the globe (Fig 5a), and myofibers in the portion of each specimen closest to the muscle belly (Fig 5c). Pathologically long tendinous insertions of EOMs in CFEOM patients would result in reduced contractile length and could account for some degree of restrictive ophthalmoplegia and the tight, inelastic, and noncontractile properties of the muscle. To test this hypothesis we examined the serial sections of Patient D's IR biopsy specimen and the age-matched control subject's IR sample obtained at autopsy, and

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Fig 5. Cross sections of a surgical biopsy specimen of the rectus inferior from Patient D showing the normal transition of tendon to muscle at distances of (a) 3.5 mm, (b) 5.8 mm, and (c) 7 mm from the point of the insertion of the muscle onto the globe. The inset is of a myofiber with abnormal mitochondrial clumping. ct = connective tissue; m = myofiber; mc = mitochondrial clumping; in = internal nucleus. (Hematoxylin and eosin.)
calculated (1) the length of the tendons from the insertion to the appearance of the first muscle fiber, and (2) the length of the transition from the appearance of the first myofiber to the point at which the cross section contained myofibers and no excess connective tissue. These measurements reflected lengths following contraction of the muscles with removal and freezing. The first myofibers appeared at a distance of 2.8 mm from the insertion in the control subject and 4.1 mm in Patient D. With greater distance from the insertion, the percentage of cross-sectional area occupied by myofibers increased, and the percentage occupied by connective tissue decreased (Fig 5b). The transition ended at a distance of 5.2 mm in the control subject and 5.5 mm in the patient (see Fig 5c). The small difference in the measured distance from the tendon insertion to the beginning of the transition may result from our comparison of biopsy and autopsy specimens. In cross sections of the autopsy muscle, the myofibers first appear near the center of the muscle. These myofibers would not be included in the small peripheral biopsy sample from the CFEOM patient, resulting in an artifactually long measurement. This artifact would be less likely to affect the measurement of the end of the transition, as the last connective tissue to disappear is around the muscle periphery.

Microscopic sections taken from the noninsertional end of the right MR and bilateral IR biopsy specimens from Patient C and the right LR from Patient D contained a mixture of myofibers and connective tissue indistinguishable from sections of control tissue taken from within the transition zone from tendon to muscle. In contrast, Patient D’s SR specimens contained only a few clumps of myofibers that were surrounded by loose connective tissue and fat (Fig 6) and did not resemble normal control muscle, tendon, or transition zone.

The myofibers present in all of the biopsy specimens from both patients, including the clumps of myofibers found in the SR, were histologically similar to control myofibers in being round and variable in fiber size; the IR fibers of Patient D had an average diameter of 21.4 ± 5.3 µm, and the LR fibers had an average diameter of 17.3 ± 4.0 µm. There was normal vasculature, no inflammatory infiltrate, and no degenerating or regenerating fibers. As found in the autopsy subject, the muscles contained an increase in the percent of myofibers with internal nuclei when compared to control tissue (see Table). Several areas of the left IR and LR biopsy specimens from Patient D contained myofibers with central accumulations of clumped material that appeared dark by H&E, trichrome, and NADH staining, and did not stain with ATPase (see Fig 5c, inset), most consistent with abnormal central aggregates of mitochondria.

Patient B’s quadriceps muscle biopsy specimen revealed fiber-type disproportion (type I-II ratio was 1:0.6, normal is 1:2) with a preponderance of hypotrophic type I fibers with some tendency of grouping. Ultrastructural examination revealed no structural abnormalities. Patient D’s quadriceps muscle biopsy specimen also revealed slight type I fiber predominance (type I-II ratio was 1:1.3) and a mild increase in fiber size variation. None of the extraocular and skeletal muscle specimens (Patients B, D, and E) harbored single or multiple mitochondrial DNA deletions.

Discussion
Our postmortem examination of 1 individual and muscle biopsy examination of 2 other individuals revealed pathological abnormalities in the brainstem, CNs, and EOMs in patients with classic (chromosome 12-linked) CFEOM. The postmortem examination revealed an absence or marked abnormality of the caudal central alpha motor neurons of the oculomotor nucleus (which normally send their axons through the superior division of CN III and innervate the LPS muscle), a loss of large motor axons in the proximal portion of CN III, complete absence of the superior division of CN III (which normally innervates the SR and LPS muscles), and abnormalities of the SR and LPS muscles that consisted of a single structure containing fat, connective tissue, and a few myofibers. Without tracer studies, we could not determine the presence or absence of the alpha motor neurons of the medial subnucleus, which also send their axons through the superior division of CN III to innervate the SR muscle. Consistent with the autopsy findings, a surgical biopsy specimen from another patient’s SR contained a disorganized mixture of connective tissue, fat, and a small clump of myofibers, and despite extensive exploration, an SR muscle could not be identified during a third patient’s surgery. Our findings are consistent with the

Fig 6. Light microscopy of a biopsy specimen of the rectus superior from Patient D showing residual muscle fibers, fat, and connective tissue. m = myofiber; f = fat; ct = connective tissue. (Epoxy resin embedded, toluidine blue.)
only previously reported postmortem examination of a patient with CFEOM, by Heuck in 1879 [19]. His investigation was limited to visual inspection of the orbital contents of an affected member of an autosomal dominant pedigree. He could not identify a left LPS, the right LPS was “delicate and ill-developed,” and the SR muscles were very thin and membranous.

Because our findings are limited to one autopsy and several biopsies, it will be important to confirm them by additional postmortem examinations of individuals from classic CFEOM families. Additional cases will also provide the statistical power to quantify the neuronal populations of the CN nuclei. The anatomical abnormalities identified, however, do provide an explanation for the ptosis, lack of upgaze, globe infraduction, and pupillary sparing in individuals with CFEOM. Ptosis results from dysfunction of the abnormal or absent LPS and the axons and motor neurons that innervate it. Similarly, the lack of upgaze and globe infraduction results from lack of function of the abnormal SR in the presence of a normal or partially functional IR muscle. Presumably, the resulting imbalance of antagonists leads to secondary infraduction and immobility of the globe. Consistent with pupillary sparing in CFEOM, the ciliary ganglion is present and CN III exhibits a greater depletion of large axons (which innervate muscle fibers) than small axons (which innervate both muscle fibers and the parasympathetic pupillary sphincter and ciliary muscles [18]). The lack of axonal degeneration or excessive connective tissue within the nerve may suggest that these axons were never present.

Several of our findings suggest that CFEOM muscle pathology extends beyond the muscles innervated by the superior division of CN III. First, when individuals with CFEOM are compared to age-matched control subjects, the proportion of myofibers with internal nuclei are increased in muscles innervated by CNs III, IV, and VI. The significance of increased internal nuclei in EOM is not known, although in limb skeletal muscle it is found in both myopathies and chronic peripheral neuropathies [20]. Second, abnormal central aggregations of mitochondria are present in some IR and LR myofibers of individuals with CFEOM. Central mitochondrial aggregates have been found in the obliquus inferior muscle from individuals with primary or secondary “overacting inferior oblique syndrome” [21, 22], in muscle biopsy specimens from individuals with strabismus [23] or congenital nystagmus [24], and with low frequency in normal EOM [23]. Mitochondrial abnormalities are also found in autosomal dominant progressive external ophthamoplegia, a disorder associated with multiple mitochondrial DNA deletions caused by defective nuclear genes at least three different loci (chromosomes 10q, 3p, and unknown) [25]. None of the CFEOM patients studied harbored a pathologically significant quantity of these characteristic multiple mitochondrial DNA deletions. The increased internal nuclei and the central mitochondrial clumping present in these additional muscles may reflect a lower susceptibility to the same pathological process that affects the SR/LPS complex, may be the result of aberrant innervation, or may be secondary changes from overactivity and lack of antagonistic movements during development. We do not know if these findings are the pathological correlates of the muscle stiffness and secondary globe restriction found in individuals affected by CFEOM.

With the exception of the SR/LPS complex, we found that the CFEOM EOM did not contain a conspicuous increase in the amount of endomysial or perimysial connective tissue compared to control EOM. This contrasts with several previous reports [1–6]. One possible explanation for this discrepancy may be that the previous studies included patients with diseases other than chromosome 12–linked CFEOM. Alternatively, the discrepancy may result from different biopsy techniques. Normal EOMs have long tendinous insertions of fibrous connective tissue [9], and a large series of biopsy samples from patients with nonrestrictive strabismus showed that occasionally the normal tendon rather than the muscle is sampled [26]. CFEOM biopsies typically sample the IR muscle, and the fixed infraducted position of the globe and the taut and inelastic properties of this muscle markedly increase the risk of sampling the connective tissue tendon or the transition zone. Therefore it may be that in some cases of CFEOM, reports of fibrotic EOM may actually result from the inadvertent biopsy of normal fibrous tendon. Consistent with this hypothesis, we found that near the muscle insertion and through much of each sample’s length, our control and CFEOM biopsy tissue consisted of tendon that is not distinguishable from the “pathological fibrosis” described in the CFEOM literature [1–4]. Similarly, in the transition from tendon to muscle, our control and biopsy tissue contained a mixture of myofibers and connective tissue that is similar to previous findings of CFEOM biopsy specimens containing connective tissue with “islands of normal muscle” [5, 6]. In addition, the CFEOM and the control IR tendon and transition zone are of equivalent length. Therefore, employing our longitudinal biopsy technique, we demonstrated the presence of apparently normal tendon, tendon to muscle transition, and nonfibrotic muscle in individuals with chromosome 12–linked CFEOM.

In normal human and mouse development, the EOM primordia segment into six distinct EOMs, are innervated [27], and then migrate out to the appropriate locations within the orbit and become associated with the developing globe [28]. The SR and LPS muscles remain intimately associated during this period;
they share a common anlage, migrate into the orbit as a single structure, and only after arrival in the orbit do the two begin to slowly separate from one another [28]. Thus, the residual abnormal SR/LPS structure identified at autopsy could result from absence of a subgroup of precursor cells, early degeneration of the myofibers from lack of innervation, or failure of appropriate separation of the two muscles.

The Wnt-1 null mutant mouse lacks midbrain structures, including the oculomotor and trochlear alpha motor neurons and CNs [29]. Despite the absence of appropriate innervation, the EOMs migrate to their correct locations within the orbit prior to the degeneration of uninnervated myofibers [8]. Some myofibers within the oculomotor and trochlear innervated muscles survive, however, and these fibers become abnormally innervated by axons of the abducens nerve [8]. This model suggests that in CFEOM, the SR/LPS muscle precursor could migrate to the appropriate position in the orbit despite a lack of innervation. Furthermore, we must consider the possibility that in CFEOM, the EOM CNs may innervate the wrong muscles in the absence of their proper innervation.

The pathology of CFEOM has interesting parallels to Duane's syndrome, in which aberrant innervation occurs. Autopsies of one bilateral [30] and one unilateral [31] case of Duane's syndrome found absence of the abducens nucleus and nerve on the affected side(s). The oculomotor and trochlear nuclei and nerves were normal, and the LR muscles were partially innervated by branches from the oculomotor nerve [30, 31]. Thus, CFEOM may be a partial oculomotor nerve analogue of Duane's syndrome, which primarily affects the abducens nerve and results in aberrant innervation of the LR.

The pathology of Duane's syndrome and the Wnt-1 mouse mutant combined with the clinical, electrophysiological, and pathological data of CFEOM suggest that aberrant innervation of the EOMs may account for the abnormal residual eye movements found in individuals with CFEOM. Clinically, aberrant innervation is suggested by reports of the co-occurrence of sporadic CFEOM and Marcus Gunn's jaw winking phenomenon [6, 32]. Electrophysiologically, it is suggested by studies performed in members of two CFEOM families [33, 34] whose disease genes were subsequently mapped to the chromosome 12 locus [11]. In the first family, electromyographic potentials recorded from the LR of an affected individual increased with attempted upgaze. Clinically, convergence-like movements and globe retraction occurred, suggesting inappropriate co-contraction of the MR and LR. In the second family, aberrantly increased MR firing was recorded in an affected individual on attempted upgaze, and no motor unit activity of the SR muscles could be detected in 2 other affected family members. If aberrant innervation occurs either within the brainstem, between the residual fibers of CN III, or by extremely small branches between CNs III, IV, and VI within the orbit of individuals with CFEOM, we might have failed to identify it at postmortem examination.

It remains to be determined whether the etiology of CFEOM results from a primary brainstem or a primary EOM process, and whether it is an abnormality of the development or of the early maintenance of any of these structures. Our pathological studies provide evidence that classic (chromosome 12-linked) CFEOM results from an anatomical abnormality of the motor neurons, axons, and muscles of the superior division of the oculomotor nerve. In addition, the autopsy findings of a qualitative decrease in the number of alpha motor neurons in all oculomotor subnuclei and the abducens nucleus, decreased diameter of the inferior division of the oculomotor nerve, and an increase in internal nuclei within myofibers of all EOMs examined suggest that the pathology of CFEOM extends beyond the SR/LPS complex to all of the EOMs. Mild facial weakness, hypotonia gross motor delay, and the nonspecific abnormalities of the quadriceps muscle biopsy specimens from affected family members suggest that the normal CFEOM gene product may play at least a transient role in normal skeletal muscle development or function.

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