A novel autosomal recessive myopathy with external ophthalmoplegia linked to chromosome 17p13.1-p12

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#Deceased
This work is dedicated to the memory of our dear colleague and friend, the late Dr Lea Averbuch-Heller. Her enthusiasm and wisdom made this work possible.
*These authors contributed equally to this work.

Summary
We describe a new autosomal recessive myopathy of early onset and very slow progression distinguished by the prominent external ophthalmoplegia in 16 subjects of eight families from a large and highly inbred Arab community. Characteristic clinical features include mild facial and skeletal muscle weakness and atrophy more pronounced proximally in the upper limbs, facial dysmorphism and scoliosis associated with conjugate, non-restrictive ocular motility impairment greatest in the upgaze and without ptosis or aberrant eye movements. Orbital MRI in the patients demonstrated atrophy with fatty replacement of the oculorotatory muscles. The major pathological alteration on skeletal muscle biopsy was a marked type 1 fibre predominance with core-like formations. A genome wide search for regions of homozygosity in the affected members from two informative families identified linkage with chromosome 17p13.1-p12 markers. Maximum two-point logarithm of odds scores were obtained at loci D17S1803 and AFMA070WD1 (Zmax = 3.74 at θ = 0). Two independent recombination events at D17S1812 and D17S947 further defined a critical region of 12 cM. Several genes map to this interval, including a cluster of sarcomeric myosin heavy chain genes. One of these genes, MYH2, is involved in inclusion body myopathy 3, but no exonic mutations were found by direct sequencing. The molecular basis for this new myopathy remains to be identified.

Keywords: myopathy; type 1 fibre predominance; external ophthalmoplegia; chromosome 17

Abbreviations: CK = creatine kinase; EO = external ophthalmoplegia; H&E = haematoxylin and eosin; IBM3 = inclusion body myopathy 3; LOD = logarithm of odds; LPS = levator palpebrae superioris; MyHC = myosin heavy chain; T1FP = type 1 muscle fibre predominance


Introduction
External ophthalmoplegia (EO) is a conspicuous feature in a wide range of hereditary neuromuscular disorders manifesting a variable combination of ocular dysmotility, ptosis, strabismus and amblyopia (Rowland, 1992). Multiple congenital myopathies (Goebel and Lenard, 1992), mitochondrial disorders (Hirano and DiMauro, 2001) and congenital myasthenic syndromes (Engel, 1994) can cause EO, as well as myotonic dystrophy (Rowland, 1992), oculopharyngeal muscular dystrophy or related disorders (Rowland et al., 1997), some rimmed vacuolar myopathies (Rose et al., 1997)
and exceptional instances of facioscapulohumeral muscular dystrophy (Krasnianski et al., 2003). In addition, there are congenital cranial dysinnervation disorders (Gutowski et al., 2003), some hereditary neuropathies (Tandan et al., 1990), rare forms of spinal muscular atrophies (Gordon et al., 1996), disorders of the supranuclear ocular motor control that mimic peripheral EO (Leigh and Zee, 1991) and other uncommon syndromes of unclear origin collectively grouped under the title of static or non-progressive EO (Silberberg, 1982).

In view of the multiple possible aetiologies and limited value of the available classical investigation techniques, the traditional classification of EO into myopathic and neurogenic forms is often problematic (Rowland et al., 1997). Clinical and pathological findings help to distinguish these disorders (Jones and North, 1997), but correct diagnosis is sometimes delayed (Gordon et al., 1996) or even impossible without the use of modern genetic analysis (Rowland et al., 1997).

A decade ago, one of us (A.L.) incidentally identified the proband (Patient 1, Family I), who was affected by a peculiar EO with mild facial and skeletal muscle weakness but without ptosis, who attributed her mild functional disabilities to constitutional problems and never sought medical attention. Over the years, we recognized her disease as a very slowly progressive familial myopathy confined to the inhabitants of her village, but its cause remained a diagnostic challenge. With the use of molecular genetic analysis, we now present evidence that this disorder is linked to a locus on chromosome 17p13.1-p12 and represents a new and distinct autosomal recessive myopathy with prominent EO.

Methods
Clinical studies
Since 1991, we have personally examined 16 subjects from eight families manifesting EO in association with a variable degree of skeletal muscle weakness, who belong to a large and highly inbred Muslim Israeli-Arab community. Of the 57 living family members, seven parents and 19 siblings were available and agreed to our evaluation. For ethical considerations, we did not examine the patients’ children. All studies were performed after obtaining informed consent of all the participants or the legal guardians of participating minors. The study was approved by the Ethics Committee, Hadassah Medical Organization, Jerusalem.

The following relevant investigations were performed in the affected individuals: thyroid functions; serum creatine kinase (CK, n = 12); lactate (n = 6) and acetylcholine receptor (AchR) antibody level (n = 3); edrophonium test (n = 3); ECGs (n = 7); echocardiogram (ECOH; n = 3); chest X-ray (n = 7); and spirometry (n = 3). Nerve conduction studies (NCS; n = 6), repetitive nerve stimulation at 3 Hz (n = 3), EMG using a concentric needle electrode (n = 6) and single-fibre EMG (SFEMG; n = 2) were also performed. Muscle respiratory chain enzyme function studies and Southern blot analysis for mtDNA deletions were each performed in three patients.

For diagnostic purposes and interpretation of genetic analysis results, we have selected EO as the minimal clinical finding to score an individual as affected. Clinical neuro-ophthalmologic examination was performed by the accepted methods (Leigh and Zee, 1991). For evaluation of the oculomotor functions, the primary position of each globe was determined when looking straight ahead, and duction and version were quantified in four directions of gaze. Ptosis was diagnosed if the upper lid covered ≥2 mm of the iris. Forced duction test was performed with topical anaesthesia in two patients.

Brain and orbital imaging was performed with MRI [MRI unit supplied by 2T unit (Elscint Israel), 1.5T unit (General Electric Milwaukee, WIS, USA)] soon after diagnosis in six patients on a 2T or 1.5T unit using head coils. Images were obtained with thin axial, coronal and sagittal sections using T1, T2 and short tau inversion recovery (STIR) sequences, and T1 sequences after intravenous contrast with fat suppression. For evaluation of keratoconus, photographic documentation of corneal topography was obtained by EyeSys instrumentation. EyeSys Technologies, Houston, TX, USA.

Muscle pathology
Open skeletal muscle biopsy was performed soon after diagnosis in seven patients. Clinically affected proximal limb muscle (deltoid, biceps, quadriceps) was selected in six patients and sternocleidomastoid was sampled in one patient during anterior cervical disectomy for a herniated C5-6 disc.

Frozen sections were stained with haematoxylin and eosin (H&E), modified Gomori trichrome, myofibrillary ATPase after pre-incubation at pH 4.3 and pH 9.4, NADH-tetrazolium reductase (NADH-TR), succinate dehydrogenase and cytochrome C oxidase according to standard methods. Formalin-fixed paraffin-embedded sections were stained with H&E.

Over the years, selected frozen and/or paraffin sections were also stained immunohistochemically: in four cases with monoclonal antibodies for fast myosin (Sigma, Rechovot, Israel, My-32 clone, 1:200) specific for fast myosin heavy chain (MyHC fast); in three cases for slow myosin (Novocastra, Newcastle, UK, NCL-MHCs, 1:50) against MyHC slow; in three cases each for developmental myosin (Novocastra, NCL-MHCd, 1:50) and myogenin (Dako, Carpinteria, CA, USA, F5D, 1:20); and in four cases each for desmin (Dako, DE-R-11, 1:50) and vimentin (Dako, Vim 3B4, 1:500).

Molecular genetic analysis
DNA was isolated using standard methods from peripheral blood samples obtained from seven affected and 13 clinically unaffected individuals from Families I, II (Fig. 1) and III. Families I and II were assigned for linkage analysis. A genome wide search for regions of homozygosity was performed using ~400 fluorescent microsatellite markers of the ABI PRISM™ linkage mapping set version II (Perkin Elmer Cetus/Applied Biosystems, Foster City, CA, USA), which covers the entire human genome with an average spacing of 10 cM. Amplified fragments were electrophoresed and analysed on an automatic sequencer (ABI PRISM 377, Applied Biosystems).

Two-point logarithm of odds (LOD) scores were computed using the LIPEP program version 5.0 (The Rockefeller University, NY, USA) under the assumption of autosomal recessive inheritance with complete penetrance and equal marker allele frequency (Ott, 1974). This was based on clinical examination and pedigree analysis in Families I and II (Fig. 1). The recombination fraction was assumed to be equal for males and females. The marker order used was taken from the Center for Medical Genetics, Marshfield Medical Research Foundation (Marshfield, WI, USA). Recombination events were recognized on the basis of both segregation of haplotypes and examination of LOD score values.
Fig. 1 (A) Pedigree tree of Families I and II and haplotypes of the region 17p13.1-p.12. Patients 1, 2 and 3 are subjects IV:2, IV:7 and IV:9 of Family I, and Patients 4, 5 and 6 are subjects VI:2, VI:3 and VI:4 of Family II. Affected individuals are shown as filled symbols and their shared haplotype by black bars. Grey bars in subjects IV:1 and IV:3 indicate maternal haplotype uninformative for D17S1808 and D17S799. Square = male; circle = female; slashed shape = deceased; proband = arrow. Locus D17S1803 and marker AFMA070WD1 define the homozygosity interval. The genotype of individual III:1 is inferred from his offspring. (B) Ideogram of the short arm of chromosome 17 schematically showing homozygous region defined by the underlined marker AFMA070WD1 and a marker at locus D17S1803. Exhaustive genes of the region assigned according to the Human Genome Project Working Draft (http://genome.cse.ucsc.edu/) and NCBI: MYH 1, 2, 3, 4, 8, 13 (myosin heavy polypeptide 1, 2, 3, 4, 8, 13), SCO1 (SCO cytochrome oxidase deficient homolog 1), MDS006 (similar to ×006 protein), ZNF18 [zinc finger protein 18 (KOX 11)], human gene DNAH9 (axonemal beta heavy chain dynein 9), MAP2K4 (mitogen-activated protein kinase kinase 4), GAS-7 (growth-arrest-specific protein 7) and MYCD (myocardin).
For MYH2 analysis, exons 1 to 38 were PCR-amplified using published primers (Martinsson et al., 2000), and both DNA strands were sequenced using the Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and the ABI PRISM 377 DNA Sequencer.

Two microsatellite DNA markers (D19S421 and D19S422) and a coding single nucleotide polymorphism (SNP) (National Center for Biotechnology Information SNP identity number rs2229144) were studied for haplotype analysis at the RYR1 locus on chromosome 19q (Monnier et al., 2003).

Results
Clinical summary of families I–VIII

The eight families originate from an Arab village near Jerusalem. Most inhabitants in this relatively isolated community belong to a few large kindreds and most can trace their ancestors back to a founder <200 years ago (Zlotogora et al., 1997). Many also follow the tradition of marriages within the kindred (Zlotogora and Chemke, 1995). Since this tradition increases the frequency of autosomal recessive disorders, we interviewed the family members with a particular attention to their genealogical relationships. Parental consanguinity, usually first cousins, was reported in all eight families, while at least one close common ancestor was identified for Families I and II (Fig. 1). All examined parents of the patients were clinically unaffected.

Parents reported uncomplicated early development, normal acquisition of gross and fine motor skills, and healthy childhood with average school achievements. In adulthood, patients did not state specific functional disability, but most preferred not to engage in strenuous physical activity. Table 1 summarizes the clinical characteristics of the 16 patients.

Table 1 Clinical findings in 16 patients with autosomal recessive myopathy and external ophthalmoplegia linked to chromosome 17p13.1-p12

<table>
<thead>
<tr>
<th>Male to female ratio</th>
<th>11:5</th>
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<tbody>
<tr>
<td>Mean age at diagnosis (years, range)</td>
<td>27 (7–56)</td>
</tr>
<tr>
<td>Mean duration of follow up (years, range)</td>
<td>4 (0–12)</td>
</tr>
<tr>
<td>Muscle weakness at last follow up*</td>
<td>Mild-50%, moderate-50%</td>
</tr>
<tr>
<td>Facial</td>
<td>4–100%</td>
</tr>
<tr>
<td>Neck flexors</td>
<td>4–81%, grade 5–19%</td>
</tr>
<tr>
<td>Shoulder girdle</td>
<td>4–69%, grade 5–31%</td>
</tr>
<tr>
<td>Distal upper limbs</td>
<td>4–44%, grade 5–56%</td>
</tr>
<tr>
<td>Pelvic girdle</td>
<td>4–63%, grade 5–37%</td>
</tr>
<tr>
<td>Proximal lower limbs</td>
<td>4–31%, grade 5–69%</td>
</tr>
<tr>
<td>Distal lower limbs</td>
<td>5–100%</td>
</tr>
<tr>
<td>Scoliosis (n = 11)</td>
<td>73%</td>
</tr>
<tr>
<td>Mean CK (normal &lt;200 U/L, range, n = 12)</td>
<td>112 (23–265)</td>
</tr>
<tr>
<td>Myopathic EMG pattern (n = 6)</td>
<td>50%</td>
</tr>
</tbody>
</table>

*Skeletal muscle weakness Medical Research Council grading: 4 = movement against variable resistance; 5 = movement against full resistance.

Since none specifically reported symptoms attributable to extraocular, facial or skeletal muscles, the age of onset could not be established with certainty except for Patient 15. This girl started to walk at the age of 18 months with physiotherapy for ‘weak legs’, but then had no medical problems until the age of 5 years, when ‘hypomobile’ eyes were noted on a routine visual childhood screening. At the age of 7 years, she had reduced visual acuity due to astigmatism later corrected with glasses, limited eye movements, eye closure weakness without ptosis, and mild limb girdle weakness. Muscle biopsy performed for a suspected mitochondrial disorder was interpreted as showing no specific changes (see Muscle pathology section). Additional information regarding the onset was available for Patient 7, who was treated for corneal trauma at the age of 7 years when normal eye movements were documented. Since then, he was lost for follow-up until the diagnosis 19 years later.

The ocular motility abnormalities were similar in character in all the patients, and none complained of diplopia or maintained abnormal head posture. The eyes were well aligned in a neutral primary position with orthotropia on the cover test. A minimal degree of exophoria was noted on alternating cover in five patients. The extraocular movements appeared slow and limited in both eyes, with a variable range limitation in the horizontal and vertical plane, always greatest in the upgaze (Table 2). In Families I, II and VI, the elder patients tended to be more affected. Saccades were slow and limited, pursuit was smooth in the unlimited range of movement, and convergence was impaired. A forced duction test showed no resistance to passive eye manipulation in the two patients tested. Passive rapid head rotation produced slow movement in the opposite direction, but did not succeed in driving the eyes into paretic fields, vestibulo-ocular reflex cancellation was preserved, and optokinetic nystagmus was of low amplitude. Head movements in the direction of attempted large gaze shift were often noted in six patients, but no aberrant eye movements or snykinesia phenomena were observed. There was no ptosis, but eight patients used forehead contraction on attempted upgaze and 10 had minimal eyelid retraction when looking straight-ahead. Pupillary reactions were normal.

The distribution of muscle weakness was symmetric and relatively homogeneous, but the clinical severity somewhat varied in different patients (Table 1). Facial and nuchal muscles were always affected giving the appearance of a long, thin face accompanied by nasal quality voice with high-arched and low mobility palate in the more affected individuals. The gag reflex was well preserved. There was atrophy of the masseters and, to a lesser extent, of the temporalis. Weakness of the orbicularis oculi and neck flexors was more pronounced than of the orbicularis oris and neck extensors. Mild limb and limb girdle weakness was present in 13 patients. Arms were more commonly affected than legs and proximal muscles more than distal. When affected, biceps and deltoid were weaker than triceps, and iliopsoas and quadriceps weaker than glutei and hamstrings. Atrophy of the trapezius and supraspinatus (n = 12), the intrinsic hand muscle weakness (n = 7), scapular...
Table 2 Ocular findings in 16 patients with autosomal recessive myopathy and external ophthalmoplegia linked to chromosome 17p13.1-p12

<table>
<thead>
<tr>
<th>Patient</th>
<th>Visual acuity</th>
<th>Range of movements</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Ptosis</td>
</tr>
<tr>
<td>1</td>
<td>6/60</td>
<td>6/60</td>
<td>0 2</td>
<td>0 2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6/12</td>
<td>6/12</td>
<td>0 2</td>
<td>0 2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>6/9</td>
<td>6/9-</td>
<td>0 2</td>
<td>0 2</td>
<td>2 3</td>
</tr>
<tr>
<td>4</td>
<td>6/7.5+</td>
<td>6/6-</td>
<td>1 3</td>
<td>1 3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>6/6-</td>
<td>6/7.5</td>
<td>2 2</td>
<td>1 2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>6/6-</td>
<td>6/6-</td>
<td>2 3</td>
<td>3 2</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>6/12</td>
<td>6/6-</td>
<td>0 2</td>
<td>0 2</td>
<td>2 1</td>
</tr>
<tr>
<td>8</td>
<td>6/15</td>
<td>6/30</td>
<td>2 2</td>
<td>2 2</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>6/6</td>
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<td>10</td>
<td>6/7.5</td>
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<td>0 1</td>
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<tr>
<td>11</td>
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<tr>
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<td>1 4</td>
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<tr>
<td>15</td>
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<td>6/6</td>
<td>3 4</td>
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<td>0</td>
</tr>
<tr>
<td>16</td>
<td>6/8</td>
<td>6/7.5</td>
<td>2 3</td>
<td>2 3</td>
<td>0</td>
</tr>
</tbody>
</table>

Visual acuity on a Snellen optotype chart at 6 m with the existing optical correction. Range of movements at last follow-up: 0 = no movement; 1 = marked restriction to <25% of full range; 2 = moderate restriction to 25–50% of full range; 3 = mild restriction to 50–75% of full range; 4 = movements >75% of full range. Movements are measured from the primary resting position of each eye. ND = not done.
wringing ($n = 4$) and scoliosis ($n = 8$) were present in the more affected individuals, but none complained of dysphagia. Fluctuations, fatigability, percussion myotonia or joint contractures were not observed. Otherwise, there were no abnormal neurological findings, except for Patient 10 who had spastic paraparesis related to multiple sclerosis, Patient 9 who manifested simple motor tic disorder, and Patient 16 with cervical myelopathy who had anterior discectomy for a herniated C5-6 disc at the age of 53 years.

After the initial examination, 10 patients were followed for $>3$ years and four patients for $>10$ years. However, the results of sequential quantitation of motor functions were available for seven patients; four of these showed very slowly progressive limitation of ocular motility always greatest in the upgaze. This was evident only 5 years or more after the diagnosis. In the other three patients, maximal upgaze range limitation was already present at the initial examination and no further deterioration could be definitely confirmed. The course of skeletal muscle weakness was relatively static, except for Patients 1, 3 and 7 who had a slow progression most evident in the distal arms and hands.

Routine blood tests, lactate and assay for AchR antibody were normal or negative except for a tendency for slightly elevated CK in the younger patients (Table 1). Edrophonium challenge did not affect the ophthalmoplegia. Motor and sensory NCS including late response was normal, and no double response to single nerve stimulation was recorded. Repetitive nerve stimulation for decrement at 3 Hz and SFEMG for jitter or blocking in the clinically affected proximal limb and facial muscles was normal or negative. EMG of the clinically affected facial (two patients) and skeletal (six patients) muscles showed no abnormal insertional or spontaneous activity. Myopathic changes consisting of brief, small polyphasic units with full recruitment on mild effort were recorded from facial muscles in the proband and in Patient 7, and from the trapezius in Patient 16. Early recruitment on mild effort with increased polyphasia was also present in the deltoid and biceps of Patient 7. Muscle respiratory chain enzyme function in Patients 1, 3 and 16 was normal, and Southern blot analysis in Patients 1, 2 and 3 detected no mtDNA deletions. Patient 3 was also tested for the C3256T tRNA Leu, A3243G tRNA Leu and T4285C tRNA Ile mutations and was negative. There were no signs of myocardial dysfunction on ECG or ECHO, and forced vital capacity measurements were 78–99% of the predicted standing values with 2–4% decrement on lying down. Screening with corneal topography was performed following the diagnosis of keratoconus in Patient 2 (Table 2). In five of the eight studied patients, this demonstrated keratoconic features or keratoconus. However, three of the six studied neurologically unaffected relatives had keratoconus as well, and we assume that it represents an associated but genetically separate disorder in this kindred.

MRI scanning of the orbits identified extraocular muscle changes in all six patients studied (Fig. 2). The primary alteration on the T1-weighted images consisted of reduced size of variable degree and distribution. In three patients, all oculorotatory muscles were severely involved, whereas in two patients, size reduction was mild to moderate and was more pronounced in the superior rectus (arrow) and in the elder brother. The fatty replacement manifests increased signal intensity best seen centrally in the muscles with a relatively preserved tissue bulk. Levator palpebrae superioris could not be separately identified.

Fig. 2 Representative T1-weighted MRI orbital images in Patient 10 (A, B) and Patient 12 (C, D) at the age of 30 and of 28 years, respectively. Coronal sections at the level of optic nerve (A, C) and globe (B, D) showing atrophy with fatty replacement of the oculorotatory muscles. The atrophy is more pronounced in the superior rectus (arrow) and in the elder brother. The fatty replacement manifests increased signal intensity best seen centrally in the muscles with a relatively preserved tissue bulk. Levator palpebrae superioris could not be separately identified.
**Muscle pathology**

Significant findings on microscopic examination included mild variation in muscle fibre size in three patients, slightly increased number of internal nuclei in four patients, and the presence of a few angulated fibres in six patients. There were no signs of necrosis, regeneration or endomysial fibrosis. Rimmed vacuoles and ragged-red fibres were not identified. Enzyme histochemistry revealed irregular staining of the intermyofibrillar network in five patients with patchy, focal loss of oxidative enzyme activity in a core-like pattern (Fig. 3). There was a marked type 1 muscle fibre predominance (T1FP) best noted in sections stained immunohistochemically for fast and/or slow MyHC in Patients 2, 7, 10 and 15 (Fig. 3). This was sometimes difficult to ascertain on enzyme histochemistry, which gave the impression of loss of the normal muscle mosaic, since very few myofibres—and as few as a single fibre in two cases—were immunoreactive for fast MyHC as type 2 fibres in the entire biopsy specimen. In the other two cases, ~5–10% of the fibres stained for fast MyHC. There was no size difference between the two myofibre groups. No tissue was available for immunohistochemistry in Patients 1, 3 and 16. One of these had marked T1FP, while in the other two patients repeated attempts failed to differentiate type 1 or type 2 fibres, with no apparent mosaic on ATPase or NADH-TR stains.

Immunostaining for developmental myosin demonstrated a single myofibre in the specimen from two patients and none in one; stains for desmin, vimentin and myogenin were normal.

Ultrastructural examination did not reveal any significant alterations in the myofibres. However, fibres with core-like disorganization were not identified in the tissue sampled for electron microscopy.

**Linkage analysis**

Initial genome wide search for regions of homozygosity in Families I and II identified three loci including D17S799 in all six affected individuals. Further genotyping with additional markers confirmed homozygosity only at AFMA070WD1, D17S1852, D17S954, D17S1875 and D17S1803 loci on chromosome 17 (Fig. 1). Maximal LOD score values were obtained for markers AFMa219ye9 at locus D17S1803 and AFMA070WD1 (Zmax = 3.74 at θ = 0). Two independent recombination events (Fig. 1) were observed at loci D17S1812 (Patient 3) and D17S947 (Patient 6). Therefore, we concluded that the disease gene maps to a 12 cM interval defined by these markers, including a region of homozygosity of at least 8 cM (markers AFMA070WD1, D17S954, D17S1852, D17S1875 and D17S1803).

Homozygosity at the same region was further demonstrated in Patient 7 from Family III, who shared common alleles at each of the contiguous polymorphic markers of the homozygous interval (data not shown). The presence of the common ancestral mutant haplotype between the AFMA070WD1 and D17S1803 suggests linkage disequilibrium and founder effect, as assumed for the whole kindred.

![Fig. 3 Main pathological findings in muscle biopsy from (A) Patient 7, (B) Patient 2, (C) Patient 10 and (D) Patient 15. (A) Increased number of internal nuclei (H&E ×200). (B) Disorganization of the intermyofibrillar network with core-like formations (NADH-TR, ×200). (C) Several angulated myofibres (*semithin section stained with toluidine blue, ×400). (D) Type 1 fibre predominance (slow myosin immunostain, ×100).]
Since MYH2 at 17p13.1 is mutated in inclusion body myopathy 3 (IBM3) (Martinsson et al., 2000), this gene was regarded as a candidate by position and function. However, direct sequence analysis of the protein-coding exons identified no deleterious changes.

Although the clinical and pathological findings suggested that the RYR1 locus might be involved (Muntoni and Sewry, 2003), it was excluded by haplotype analysis showing neither a homozygous region nor a linked haplotype formed by alleles of the microsatellite and SNP markers.

Discussion

We report a new familial myopathy in 16 members of a large Arab kindred. The high rate of consanguinity, the involvement of both genders, and the absence of clinical abnormality in the parents suggest autosomal recessive inheritance. The clinical phenotype is relatively stereotyped and is marked by the following: (i) conjugate, non-restrictive ocular motility impairment greatest in the upward direction but without ptosis; (ii) mild to moderate facial and neck muscle weakness and atrophy; (iii) mild skeletal muscle weakness and atrophy involving proximal limbs more than distal, and upper limbs more than lower; (iv) mild scoliosis and the appearance of a long, thin face and nasal quality voice suggestive of a long-standing disease process; (v) slightly variable clinical severity not strictly dependent on age; and (vi) documented onset in childhood with a very slowly progressive course and little functional impairment.

The most distinctive feature in these patients is EO and the results of our clinical investigation, together with the oculorotatory muscle atrophy on MRI, favour its neuromuscular origin (Leigh and Zee, 1991). Comparable type of EO, selective muscle weakness and atrophy, facial dysmorphism, and scoliosis with normal or slightly elevated CK and myopathic EMG occur in non-aggressive forms of hereditary and congenital myopathies with delayed symptomatic onset (Case records of the Massachusetts General Hospital, 1985; Scelsa et al., 1996; Miro et al., 2000), but the diagnosis is based on specific morphological alterations in the muscle. The outstanding pathological finding in our patients was a marked T1FP, best demonstrated on myosin immunohistochemistry and accompanied by a core-like oxidative staining pattern. Although these findings are not specific (Goebel and Lenard, 1992), they may suggest a possibility of RYR1-related myopathy, such as central core or multifillicore disease (Muntoni and Sewry, 2003). Moreover, instances of EO have been documented in association with recessive mutations at this locus (Monnier et al., 2003; Romero et al., 2003), which was excluded in our study.

Similar pathological changes also characterize uniform type 1 fibre myopathy described in patients from North America, Europe and Japan (Oh and Danon, 1983; Vallat et al., 1983; Riggs et al., 1989; Ohtaki et al., 1990; Jung et al., 1997; Muranaka et al., 1997; Pavlovsky et al., 2001). Typical manifestations include congenital or early onset hypotonia, delayed motor development, EO, mild facial, nuchal and skeletal muscle weakness, hyporeflexia, and a non-progressive course with normal CK and myopathic EMG. Familial occurrence is sometimes mentioned in twins (Oh and Danon, 1983), in siblings (Vallat et al., 1983; Pavlovsky et al., 2001) and, possibly, in parents (Muranaka et al., 1997). The clinical and pathological phenotype in our patients is very similar yet distinguished by the dissociation of EO and ptosis, normal tendon reflexes, a very slow progression, a clear autosomal recessive inheritance and demographic clustering.

After excluding the known possible aetiologies, we performed a genome wide search for homozygosity. The only consistent region of homozygosity was flanked by two recombination events at D17S1812 and D17S947 loci defining a critical interval of 12 cM on chromosome 17p13.1-p12. Several genes map to the same genetic interval (Fig. 1), including an ordered cluster of six genes that encode a group of sarcromeric MyHC isoforms (Weiss et al., 1999). Each of these genes is developmentally regulated, exhibits a specific pattern of expression and, therefore, represents a serious candidate by both position and function. So far, only MYH2 has been associated with human myopathy (Martinsson et al., 2000). It is involved in IBM3, which despite major differences, also shares some features in common with the present disorder, including EO that is only rarely accompanied by ptosis (Darun et al., 1998). MYH2 codes for MyHC IIA, which is the main isoform in adult type 2A fibres (Martinsson et al., 2000) and is expressed in oculorotatory muscles (Kjellgren et al., 2003). This explains the co-occurrence of skeletal myopathy with EO. Since MyHC IIA is also present in levator palpebrae superioris (LPS) (Kjellgren et al., 2003), the infrequency of ptosis is unclear but may be related to the distinct composition of fibres in this muscle (Porter et al., 1995). Instances of T1FP and of irregular intermyofibrillar network on oxidative stains are also reported in IBM3, but represent only minor alterations overshadowed by rimmed vacuolar changes (Tajsharghi et al., 2002). We have excluded exonic mutations in MYH2, but intronic mutations leading to aberrant splicing are still a possibility.

Of the other MyHC genes in this region, MYH1 codes for MyHC Ix, which is the main isoform in adult type 2B fibres (Tajsharghi et al., 2002) and is present in oculorotatory muscles and in LPS, at least in rodents (Asmussen et al., 1993). In this respect, it is similar to MYH2 and may be regarded a plausible candidate. Furthermore, targeted disruption of a corresponding gene in mice results in muscle phenotype with disorganization of the intermyofibrillar network in a moth-eaten pattern and in kyphosis (Acakpo-Satchivi et al., 1997). MYH4 for MyHC Iib is not expressed in human muscle fibres (Wingmore and Evans, 2002), whereas MYH13 for extraocular MyHC is restricted to oculorotatory muscles, some fibres in LPS and several head muscles (Winters et al., 1998). It is not expressed in skeletal muscles and cannot be expected to explain skeletal myopathy. MYH3 and MYH8 encode embryonic and perinatal MyHCs that
predominate during early skeletal muscle development (Wingmore and Evans, 2002). Both are repressed postnatally, except in some extraocular muscles, and are re-expressed in regenerating fibres (Weiss et al., 1999). During the initial phases of fibre type diversification, perinatal MyHC marks some of the future fast fibres (Wingmore and Evans, 2002) and may be an interesting option.

The non-MyHC genes in this region and of a known function (Fig. 1) seem less likely candidates; they include SCO1, which encodes a putative mitochondrial copper transport protein (Valnet et al., 2000). This is associated with autosomal recessive neonatal-onset hepatopathy and encephalopathy with hyperlactataemia and muscle lipid accumulation due to cytochrome c oxidase deficiency, which was not detected in our patients.

In conclusion, we have mapped a locus on chromosome 17p13.1-p12 responsible for a new autosomal recessive myopathy of early onset and a very slow progression distinguished by the prominent EO without ptosis and characterized histologically by T1FP and core-like oxidative pattern. Several sarcomeric MyHC genes localized to the same genetic interval (Fig. 1) seem less likely candidates; they include SCO1, which encodes a putative mitochondrial copper transport protein (Valnet et al., 2000). This is associated with autosomal recessive neonatal-onset hepatopathy and encephalopathy with hyperlactataemia and muscle lipid accumulation due to cytochrome c oxidase deficiency, which was not detected in our patients.

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References


Hirano M, DiMauro S. ANT1, Twinkle, POLG, and TP. New genes open our eyes to ophthalmoplegia. [Review]. Neurology 2001; 57: 2163–5.


