Clinical features of the DOK7 neuromuscular junction synaptopathy

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Mutations in DOK7 have recently been shown to underlie a recessive congenital myasthenic syndrome (CMS) associated with small simplified neuromuscular junctions (‘synaptopathy’) but normal acetylcholine receptor and acetylcholinesterase function. We identified DOK7 mutations in 27 patients from 24 kinships. Mutation 1124_1127dupTGCC was common, present in 20 out of 24 kinships. All patients were found to have at least one allele with a frameshift mutation in DOK7 exon 7, suggesting that loss of function(s) associated with the C-terminal region of Dok-7 underlies this disorder. In 15 patients, we were able to study the clinical features in detail. Clinical onset was usually characterized by difficulty in walking developing after normal motor milestones. Proximal muscles were usually more affected than distal, leading to a ‘limb-girdle’ pattern of weakness; although ptosis was often present from an early age, eye movements were rarely involved. Patients did not show long-term benefit from anticholinesterase medication and sometimes worsened, and where tried responded to ephedrine. The phenotype can be distinguished from ‘limb-girdle’ myasthenia associated with tubular aggregates, where DOK7 mutations were not detected and patients respond to anticholinesterase treatments.

CMS due to DOK7 mutations are common within our UK cohort and is likely to be under-diagnosed; recognition of the phenotype will help clinical diagnosis, targeted genetic screening and appropriate management.

Keywords: congenital myasthenic syndrome; neuromuscular junction; mutations; DOK7; phenotype

Introduction

Congenital myasthenic syndromes (CMS) are disorders of neuromuscular transmission arising from genetic defects in pre-synaptic, synaptic and post-synaptic proteins of the neuromuscular junction (NMJ) (Engel and Sine, 2005). The mutations differentially affect the structure and function of the neuromuscular junction (NMJ), producing weakness that generally is present at birth, though in some cases, particularly the slow channel syndrome, may not be present until adulthood. The weakness may fluctuate with time and is characteristically fatigable, with limb, ocular, bulbar, truncal and respiratory muscles variably affected (Beeson et al., 2005).

The majority of CMS are caused by mutations in genes that encode post-synaptic proteins. Initial studies identified mutations in the genes encoding the AChR subunits that impair ion channel gating or reduce the number of endplate receptors or a combination of the two, giving rise to ‘slow channel’, ‘fast channel’ or AChR deficiency syndromes (Ohno et al., 1995, 1996; Engel et al., 1996). However, CMS may also arise from mutations in proteins involved in the formation and maintenance of the neuromuscular junction such as Rapsyn (Ohno et al., 2002; Burke et al., 2003; Muller et al., 2003; Chevassier et al., 2004).

Many CMS patients do not have an identified genetic mutation of which a major subgroup comprises those with ‘limb-girdle’ weakness (McQuillen, 1996). Some of these CMS patients have tubular aggregates on muscle biopsy (Dobkin and Verity, 1978; Sieb et al., 1996; Furui et al., 1997; Rodolico et al., 2002; Shankar et al., 2002; Chevassier et al., 2005). Other limb-girdle CMS patients lack tubular...
aggregates and in contrast some have abnormally small, simplified neuromuscular junctions that show normal acetylcholine receptor and acetylcholinesterase function (Slater et al., 2006). We recently reported DOK7 mutations in the majority of these (Beeson et al., 2006).

Dok-7 is a newly identified member of the Dok family of cytoplasmic proteins. It interacts with muscle-specific tyrosine kinase (MuSK) and is essential for post-synaptic specialization of the neuromuscular junction. Dok-7 is postulated to have three main functional domains; a pleckstrin homology (PH) domain, essential for membrane association; a phosphotyrosine-binding (PTB) domain involved in the Dok-7 induced activation of MuSK; and a large C-terminal domain containing multiple tyrosine residues (Okada et al., 2006).

Mice with the Dok7 gene ablated die shortly after birth and do not form detectable AChR clusters in diaphragmatic muscle (Okada et al., 2006). In cultured HEK 293 (human) cells or C2C12 (mouse) myoblasts, deletion of the C-terminal region of Dok-7 does not prevent MuSK activation (Okada et al., 2006). Similarly, the common C-terminal frameshift 1124_1127dupTGCC does not disrupt Dok-7-MuSK interaction in HEK 293 cells. However, in mature C2C12 myotubes DOK7-1124_1127dupTGCC impairs MuSK activity and its ability to shape the specialization of post-synaptic structure (Beeson et al., 2006). These observations suggest that this C-terminal truncation of Dok-7 retains partial function. A putative molecular pathway (illustrated in Fig. 1) has DOK7-1124_1127dupTGCC binding MuSK, through interaction of the PTB domain with the crucial MuSK juxtamembrane PTB motif NPXY at amino acids 550–553, but that the loss of the Dok-7 C-terminal region results in partially impaired signalling through kinases downstream of MuSK. The effect on post-synaptic structures may also alter retrograde signalling to the pre-synaptic nerve terminals resulting in the reduced neuromuscular junction size in these patients observed by Slater et al. (2006).

Although the many different CMS share some clinical features, recognition of particular phenotypic characteristics provides clues for targeted genetic screening, predicts the treatment response and may facilitate the identification of new mutations using a candidate gene approach. Here we describes in detail the clinical features of CMS patients with proximal muscle weakness due to DOK7 mutations and contrast our findings with patients with limb-girdle CMS associated with muscle biopsy tubular aggregates.

**Methods**

Patients and DNA samples were recruited through the congenital myasthenia service in Oxford, UK. Approval for this study was received from the Oxford Research Ethics Committee OXREC: 04.OXB.017 and patient consent to publish clinical details was obtained.

**Mutational analysis**

DNA was isolated from peripheral blood using the Nucleon™ II DNA extraction kit (Nucleon Biosciences). The promoter region and the seven coding exons with their immediate flanking regions of DOK7 were amplified by PCR and the resulting products subject to bi-directional DNA sequencing (see www.sciencemag.org/cgi/content/full/1130837/DC1). Details of the oligonucleotides used in the genetic screen are reported in Beeson et al. (2006). Detected mutations were confirmed by analysis of restriction endonuclease digestion of PCR amplicons or allele-specific PCR.
and by the co-segregation of mutant alleles with recessive inheritance of disease within family pedigrees.

**Clinical assessment**

Oxford clinic CMS patients in whom Dok-7 mutations were identified were invited back to clinic as is part of our routine practice for counselling, and for further clinical assessment.

We also identified from our records patients having both a ‘limb-girdle’ pattern of muscle weakness and tubular aggregates on muscle biopsy.

History and examination was performed in a systematic fashion. Assessment was complemented by a quantitative myasthenia gravis (QMG) score, as recommended by the Myasthenia Gravis Foundation of America (Jaretzki et al., 2000). The QMG scoring system consists of measuring 13 objective parameters, with maximum severity score of 39. Forced vital capacity was measured using a Micro Plus Spirometer (Micro Medical, Rochester, UK) using the best of the three trials. An abnormally low value was defined as below 1.43 standard deviations of the mean predicted for sex, age and height (Standardized lung function testing. Official statement of the European Respiratory Society, 1993). Time taken to drink 100 ml of water was included as a supplementary measure of bulbar function. Normal values are available for a 150 ml volume only and we have therefore defined our lower limit of normal as two-third of those published, being 7.3 s for males and 9.3 s for female patients (Wiles et al., 1990).

Electromyography (EMG) was regarded as abnormal if a decrement of >10% occurred at 2–3 Hz stimulation in at least one muscle (Engel, 2001). Single fibre EMG (SFEMG) was regarded as abnormal if more than 10% of fibre potential pairs exceeded normal jitter or had impulse blocking, and/or mean jitter exceeded 38.2 μs (Sarri-giannis et al., 2006). Electrocardiograms were obtained at the time of clinic attendance. Requests were made for echocardiography via the patients’ general practitioners.

**Results**

**Mutational screen**

Initially, DNA was screened for mutations in DOK7 from 26 long-standing CMS patients examined previously in Oxford, that had proved negative in screens for mutations in known CMS genes CHRNA, CHRNB, CHRND, CHRNE, CHAT, COLQ, COLQ and MUSK. Mutations within the DOK7 gene were identified in 12 (included one pair of siblings). Subsequent screening of 175 additional DNA samples from patients referred to the Oxford congenital myasthenia service, who were negative for AChR antibodies, identified mutations within DOK7 in a further 15 patients in 13 kinships. The screen included a cohort of eight ‘limb-girdle’ myasthenia patients confirmed as CMS patients in Newcastle (Slater et al., 2006). Thus DOK7 mutations were detected in 27 patients from 24 kinships. Twenty out of 24 kinships are either homozygous or heterozygous for the mutation 1124_1127dupTGCC. CMS due to DOK7 mutations is a recessive disorder; however, in five kinships harbouring 1124_1127dupTGCC, including two kinships with two affected siblings, we were unable to identify a mutation in the coding sequence of the second allele. 1124_1127dupTGCC has not been detected in DNA from 120 control individuals.

Fifteen patients harbouring DOK7 mutations were assessed in detail in clinic in Oxford (Table 1). One patient, who was assessed in detail previously in Oxford but subsequently died, was included (patient 15). Genetic analysis of patients 1–4, 7 and 11–15 has been reported previously and correspond respectively to index cases 1, 4, 5, 2, 11, 12, 3, 6, 8 and 7 identified in Beeson et al. (2006). Clinical features of patients 7 and 14 were reported as LGM 8 and LGM1 by Slater et al. (2006).

Patient 10 was found to be a compound heterozygote for the frameshift duplication 1124_1127dupTGCC and 473G>A which results in the missense mutation R158Q. R158Q alters a putative site responsible for the interaction of the PTB domain with the phosphotyrosine in the MuSK target motif (NPXY) (Okada et al., 2006). R158Q cosegregates with recessive inheritance of disease and the nucleotide substitution 473G>A was not detected in Bpm 1 restriction endonuclease digests of exon 4 amplicons from 200 control chromosomes suggesting that it is pathogenic (Fig. 2). Patients 8 and 9 are sisters in whom only 1124_1127dupTGCC was detected within the coding sequence of DOK7.

**Clinical features of patients with DOK7 mutations**

In the 15 patients assessed in detail (Table 2), clinical onset was typically after motor milestones had been achieved at normal age, and was characterized by progressive difficulty in walking. Even in the few patients who had earlier symptoms of ptosis, floppy tone and bulbar problems, walking onset was not delayed. No patients required ITU or respiratory support in early life and only one patient had two short-lived apneic episodes. Features typically seen in patients with rapsyn mutations such as congenital joint deformity and strabismus were not present.

Proximal weakness was greater than distal weakness in 11/15 and only one patient had distal greater than proximal weakness (the patient with least weakness). Difficulty walking or running worsened in childhood and was often accompanied by upper limb weakness. Truncal weakness was present in most patients but kyphoscoliosis was significant in only one. Myasthenic fluctuations in symptoms were common. A waddling and lordotic gait was seen where proximal lower limb and truncal weakness occurred.

Ptosis was often noted from an early age, though in some it developed and progressed in childhood. Eye movements were normal in all but one patient, who had a complex ophthalmoplegia. Facial, jaw and neck weakness were common and tongue wasting was seen in about half, bulbar problems typically developing later in the clinical course than limb weakness. Although the forced vital capacity when tested was below the lower limit of normal.
in 8 of 14 patients (one with COAD), respiratory symptoms were only noted by four.

Diagnosis was often delayed and disorders such as muscular dystrophy and congenital myopathy were often suggested. One patient had failed to respond convincingly to immunosuppressive medication following a tentative diagnosis of autoimmune sero-negative myasthenia gravis. She subsequently died from complications due to immunosuppression.

EMG was consistent with a neuromuscular transmission disorder in all patients (showing abnormal decrement in amplitude and/or jitter and blocking on single-fibre studies). Post-tetanic potentiation was assessed in patients with a significant decrement and non-significant (<30%) increases only were seen. There was no repetitive response to a single stimulus.

Dok-7 is expressed in cardiac as well as skeletal muscle (Okada et al., 2006), raising the possibility of

### Table 1 Clinical features of patients with Dok Mutations

<table>
<thead>
<tr>
<th>Dok-7 mutation</th>
<th>Age/sex</th>
<th>Age at onset</th>
<th>Manifestation(s) at onset</th>
<th>Current course</th>
<th>Response to treatmenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 c.1124.1127dupTGCC c.1339.1342dupCTGG</td>
<td>34/M</td>
<td>Birth</td>
<td>Feeding problems and SOB</td>
<td>Improving</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>2 c.1124.1127dupTGCC c.1263insC</td>
<td>63/F</td>
<td>1–2 yr</td>
<td>Difficulty walking</td>
<td>Worsening</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>3 c.1124.1127dupTGCC c.1263insC</td>
<td>49/F</td>
<td>9mnth</td>
<td>Ptosis</td>
<td>Worsening</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>4 c.1124.1127dupTGCC c.1124.1127dupTGCC</td>
<td>55/M</td>
<td>2 yr</td>
<td>Difficulty walking</td>
<td>Worsening**</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>5 c.1124.1127dupTGCC-</td>
<td>19/M</td>
<td>3 yr</td>
<td>Difficulty walking</td>
<td>Improving</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>6 c.593G&gt;C c.1124.1127dupTGCC</td>
<td>55/M</td>
<td>5–6 yr</td>
<td>Difficulty walking/running</td>
<td>Stable</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>7 c.593G&gt;C c.1124.1127dupTGCC</td>
<td>50/F</td>
<td>1.5–2 yr</td>
<td>Difficulty walking</td>
<td>Stable</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>8 c.1124.1127dupTGCC-</td>
<td>38/F</td>
<td>Birth</td>
<td>Floppy infant</td>
<td>Worsening</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>9 c.1124.1127dupTGCC-</td>
<td>45/F</td>
<td>Birth</td>
<td>Floppy infant</td>
<td>Worsening</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>10 c.473G&gt;A c.1124.1127dupTGCC</td>
<td>26/F</td>
<td>3 yr</td>
<td>Difficulty walking</td>
<td>Worsening</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>11 c.1124.1127dupTGCC c.1124.1127dupTGCC</td>
<td>41/F</td>
<td>3–4 yr</td>
<td>Difficulty walking</td>
<td>Worsening</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>12 c.1124.1127dupTGCC c.1263insC</td>
<td>63/F</td>
<td>1 yr</td>
<td>Difficulty walking</td>
<td>Worsening</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>13 c.1124.1127dupTGCC c.1124.1127dupTGCC</td>
<td>49/F</td>
<td>Birth</td>
<td>Floppy infant; feeding problems</td>
<td>Stable</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>14 c.1143insC c.1143insC</td>
<td>28/F</td>
<td>1.5 yr</td>
<td>Difficulty walking</td>
<td>Improving</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
<tr>
<td>15 c.548551delTCCT c.1124.1127dupTGCC</td>
<td>26/F</td>
<td>2–3 yr</td>
<td>Ptosis and lordosis</td>
<td>Stable</td>
<td>Pyridostigmine, 3,4 DAP, Ephedrine</td>
</tr>
</tbody>
</table>

*Not all patients received all treatments, ↓ deteriorated, → unchanged, ↑ improved, — treatment not tried; **severe chronic obstructive airways disease causing deterioration in respiratory function and mobility; SOB, shortness of breath.
cardiac involvement. However, no patients had symptoms of arrhythmia or cardiac failure. Electrocardiograms were normal in all patients as was echocardiography in the six patients. To date seven patients have had echocardiography and this was normal in six patients. Patient 5 had a reduced left ventricular ejection fraction of 49% which was asymptomatic. One patient had unrelated coronary and pulmonary problems.

**Limb-girdle CMS patients with tubular aggregates**

Four CMS patients with a limb-girdle pattern of muscle weakness and tubular aggregates on muscle biopsy were assessed (Table 3). Mutations in DOK7 were not identified in these patients. They were notable for a more exclusively ‘limb-girdle’ picture, with relative sparing of ocular and distal muscles. Onset was characterized by difficulty in walking and in one patient motor milestones were delayed. Mobility tended to decrease during childhood and then to stabilize in adolescence. Muscle biopsy in all four patients in this group showed subsarcolemmal inclusions visible on electronmicroscopy, similar to those previously described (Dobkin and Verity, 1978; Seib et al., 1996; Furui et al., 1997; Rodolico et al., 2002; Chevassier et al., 2005).

**Histological changes in DOK7 muscle**

Reports from previously performed muscle biopsies were obtained on 8 of 15 patients all of whom had standard stains. Tubular aggregates were not seen in patients with DOK7 mutations. Mild myopathic changes were described on conventional biopsies with increased fibre size variability, type II fibre atrophy and occasional internalized nuclei. Fibre necrosis and inflammation was described in one of three biopsies taken from patient 8 and was not present in other patients. Two patients had ‘slight’ type I fibre clustering, and demonstrated only mild myopathic changes, with increased fibre size variability, type II fibre atrophy and occasional internalized nuclei. Motor point biopsies from patients 1, 2, 4 and 12 were described in detail in Beeson et al. (2006), and patients 7 and 14 in Slater et al. (2006).
Response to treatment

The previous response to treatment was documented from the patient history and notes. Two patients had been formally assessed with pre- and post-treatment QMG scores (patients 5 and 10). Pyridostigmine had either no effect or had made the weakness worse although a short-lived initial response or positive tensilon test (two of eight documented) was occasionally reported. In one patient a combination of pyridostigmine and 3,4-DAP was documented as beneficial (case 5, QMG score pre and post: 13 and 11). 3,4-DAP was occasionally reported to improve strength (e.g. patient 10 QMG scores: pre; post-pyridostigmine; post 3,4-DAP: 12, 12, 11). Ephedrine, when tried, was usually felt to be helpful. At the time of this study eight patients had stopped all treatment, three were taking 3,4-DAP alone, one was on 3,4-DAP and ephedrine, two were on ephedrine alone, and one was on pyridostigmine and 3,4-DAP.

In contrast with patients having DOK7 mutations, limb-girdle CMS patients with tubular aggregates all reported a sustained response to pyridostigmine and have thus continued to date on this medication and one additionally finds 3,4-DAP useful.

Discussion

Mutations in the DOK7 gene underlie the first identified CMS synaptopathy and are associated with a characteristic phenotype. Many patients with DOK7 mutations were previously classified as ‘limb-girdle CMS’. However, it is now clear that the DOK7 mutation phenotype and the ‘limb girdle with tubular aggregate’ group are separate entities. Patients with DOK7 mutations have a generalized pattern of weakness although proximal weakness is usually greater than distal weakness and eye movements are usually spared. Their response to treatment is different to CMS with severely reduced AChR numbers: pyridostigmine can make the weakness worse; there is a variable response to 3,4-DAP; and a response to ephedrine may be seen. Both pyridostigmine and 3,4-DAP increase the available ACh; the former by decreasing its breakdown, and the latter by increasing its release by prolonging the nerve action potential. The novel characteristic of a reduced post-synaptic end plate area (Slater et al., 2006) in patients with DOK7 mutations could limit the effectiveness of increasing endplate ACh with pyridostigmine and increase the risk of depolarization blockade. The mechanism of action of ephedrine at the neuromuscular junction is still unclear, but it may increase quantal release (Sieb and Engel, 1993) and may block the AChR when in the open state (Milone et al., 1996). Ephedrine has also recently been reported helpful in some cases of endplate acetylcholinesterase deficiency (Bestue-Cardiel et al., 2005).

We did not identify DOK7 mutations in the tubular aggregate group who contrasted in their response to pyridostigmine and their more classical limb-girdle pattern of weakness. Both groups were notable for achieving motor milestones such as standing and walking at a normal age and for the subsequent development of increasing loss of mobility as the presenting symptom in early childhood. Only one patient with tubular aggregates had delayed walking age and even in the few patients with DOK7 mutations who

<table>
<thead>
<tr>
<th>Muscle weakness</th>
<th>Tongue wasting</th>
<th>Reflexes</th>
<th>VC</th>
<th>Swallow</th>
<th>QMGS</th>
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<tr>
<td>Ptosisa</td>
<td>Face/Jjawa</td>
<td>Necka</td>
<td>Prox. limbsa</td>
<td>Distal limbsa</td>
<td>Trunk</td>
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<td>1</td>
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</table>

Note: Muscle weakness, N, normal no weakness; Y, present.
aWeakness: +, mild; ++, moderate; ++++, severe (QMGS and MRC grade).
bFasiculations present
c↓, reduced.
dReduced respiratory function generally asymptomatic.
eSwallow affected when time to drink 100 ml water was increased above normal value.
fHigher QMGS score with increased weakness.
had earlier symptoms of ptosis, floppy tone and bulbar problems, walking onset was not delayed.

**Frequency of DOK7 mutations in CMS**

We found DOK7 mutations in a large proportion (12/26, 46%) of our longstanding CMS patients who had undergone full investigation in Oxford and in whom screening had failed to detect a mutation in other CMS genes. To date, of approximately 200 kinships where genetic analysis in Oxford has confirmed a CMS, 97 have mutations in the AChR ε-subunit (CHRNE), 44 in RAPSN and 24 in DOK7. Thus, DOK7 mutations are present in around 12% of CMS kinships confirmed by genetic diagnosis, and DOK7 is the third most commonly affected gene in our cohort of samples. Twenty-two out of the 24 kinships were of Caucasian descent and two originally from the Indian subcontinent. As these patients were identified by screening DNA from genetically undiagnosed CMS adults already stored in Oxford, the true proportion of patients with DOK7 mutations may be greater. The four-nucleotide frameshift mutation 1124_1127dupTGCC was found to be a common mutation (present in 20/24 kinships).

All patients were found to have at least one allele with a frameshift mutation in DOK7 exon 7. It is probable that these C-terminal region frameshift mutations, 1143insC, 1263insC and 1339_1342dupCTGG, in a similar fashion to 1124_1127dupTGCC, generate truncated Dok-7 that can interact with the juxtamembrane PTB motif of MuSK, and is partially functional. Targeted genetic screen of this exon should facilitate rapid genetic diagnosis.

There is only one reported case of a CMS due to mutations in MUSK (Chevessier et al., 2004). The patient harboured a null allele in combination with the missense mutation V790M, which may reduce MuSK stability (Chevessier et al., 2004) and possibly impair Dok-7-MuSK interaction (Okada et al., 2006). It is not yet possible to determine if there are distinct phenotypic differences between CMS due to mutations in DOK7 and MUSK.

**Limb-girdle myasthenia with associated tubular aggregates**

The presence of tubular aggregates in muscle is thought to be secondary to non-specific phenomena probably linked to disturbance of intra-sarcoplasmic calcium homoeostasis (Chevessier et al., 2005). It likely reflects a molecular mechanism that differs from the DOK7 group in these ‘limb-girdle’ CMS patients, which in some other reported cases may be autoimmune (Rodolico et al., 2002, 2005). Within the group of patients described by Slater et al. (2006), one (LGM2) had typical tubular aggregates and did not harbour DOK7 mutations. In contrast with the patients with DOK7 mutations that had normal postsynaptic AChR density, the motor-point biopsy from LGM2 showed reduced AChR density, a more striking reduction of
post-synaptic folds, an increased area of cholinesterase staining and α-bungarotoxin binding. Additionally LGM2 was the only patient who showed a dramatic improvement with pyridostigmine and 3,4-DAP (Table 4).

Tubular aggregates have been observed in many reports of ‘limb-girdle’ CMS (Dobkin and Verity, 1978; Seib et al., 1996; Furui et al., 1997; Rodolico et al., 2002; Shankar et al., 2002; Chevassier et al., 2005), and it is not clear how many previously described ‘limb-girdle’ CMS are due to DOK7 mutations. In our CMS cohort, ‘limb-girdle’ patients with tubular aggregates were a minority. This may be due to the lack of a clear definition of the limb-girdle pattern. The DOK7 mutation phenotype has a less well-defined limb-girdle distribution, distal weakness also being present, and the previous studies may have used stricter criteria for limb-girdle pattern weakness (Table 4).

Summary

Our study has identified DOK7 mutations as the likely causal factor in a sizeable proportion of the well-characterized CMS patients previously without a genetic diagnosis. Moreover, we have identified phenotypic differences between these two forms of ‘limb-girdle’ CMS: those with DOK7 mutations, and those with tubular aggregates in whom the genetic mutations have not been identified. Our findings make it highly likely that these disorders are also genetically distinct. These conditions are probably under-diagnosed owing to their similarities to other non-CMS muscle disorders and our phenotypic characterization should help direct genetic testing. The presence of the common mutation 1124_1127dupTGCC facilitates screening. Because patients with DOK7 mutations may often worsen with conventional CMS treatments but respond to ephedrine (an unconventional treatment), early genetic screening is important to establish the diagnosis as soon as possible. Patients with suspected DOK7 mutations and significant weakness should have supervised assessment of their 3,4-DAP response and not routinely be prescribed pyridostigmine.

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