The phenotype of calpainopathy: diagnosis based on a multidisciplinary approach

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Abstract

Calpainopathy (LGMD2A) is the most common type of autosomal recessive limb-girdle muscular dystrophy. We performed a systematic clinical evaluation in 13 calpainopathy patients from 11 families, with particular attention to the pattern of muscle involvement. Eleven patients had a muscle biopsy with deficiency of calpain 3 on western blotting. The other two patients were not biopsied as they were siblings from the same families. Confirmatory \textit{CAPN3} mutations were detected in seven patients. The age at presentation was 2–45 years, wider than previously reported. We confirm the highly characteristic and recognisable phenotype of predominant muscular atrophy with early pelvic girdle involvement, relative sparing of the hip abductors, scapular winging and abdominal laxity. Early primary contractures were also a prominent feature in this group, expanding the breadth of the phenotype. Recognition of the clinical pattern of calpainopathy is of diagnostic significance. It is important, especially in sporadic cases, in targeting and interpreting laboratory investigations in order to provide accurate diagnostic and prognostic information. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

‘La plus ça change la plus c’est la même chose’.

Understanding the phenotype of calpainopathy is a voyage of discovery. Clinically, calpainopathy may be the true successor to the condition, initially described by Erb in 1884, of a predominantly scapulo-humeral, progressive muscular atrophy with juvenile onset.

In 1991, Beckmann mapped the first limb-girdle muscular dystrophy (LGMD) locus to 15q [1], by demonstrating linkage to D15S25, in an inbred population of Réunion Island. Subsequently a proteolytic enzyme, calpain 3, was implicated in the disease [2]. The pathologic mechanism remains uncertain though it has been suggested that apoptosis may be involved. By 1999, 97 different \textit{CAPN3} mutations had been identified, including nonsense, missense and splice-site mutations, small deletions and insertions, distributed throughout the gene [3]. Fardeau provided a detailed account of the clinical correlates of the disease in communities on Réunion Island [4] and in mainland France [5]. He described a predominantly atrophic and symmetrical disease with a marked selectivity of muscle involvement that included scapular winging. Onset was usually in the second decade and associated with a relatively slow progression.

In 1998, Anderson developed monoclonal antibodies to calpain 3 that can be used for immunoblotting [6]. In practice, this provides a useful guide to diagnosis and can help to target mutation detection.

In Newcastle, we adopt a multidisciplinary approach to diagnosis and care. This facilitates close liaison between the clinical service, the muscle biopsy service for immunohistochemistry and immunoblotting and also molecular genetics. It enables us to offer not only a strategic approach to diagnosis but also the possibility to test the diagnostic hypothesis. This is particularly important in sporadic cases and where the clinical presentation is atypical.

Most of the clinical descriptions of calpainopathy so far described relate to the isolated communities of the Amish [7], Basque [8] and Turkey [9]. This raises the question of whether this phenotype is representative of other communities such as that of the UK. We have addressed this issue in our population.
2. Patients and methods

The first three patients (1a, 1b, 2) in whom we were able to diagnose calpainopathy had previously had a muscle biopsy many years ago, from which frozen muscle tissue was still available. Although, in each case, muscle histology had shown evidence of a muscular dystrophy, dystrophin and the sarcoglycans were normal and a definitive diagnosis had not been possible. With the development of monoclonal antibodies to calpain 3 the biopsies were re-examined and a marked reduction of calpain 3 demonstrated. It was readily apparent that, although the patients’ clinical features were in keeping with the correlates of the disease as previously described, there were also important new features, including primary contractures, which were present in all 3 patients. Mutation screening provided confirmation that the patients did indeed have calpainopathy and not a secondary decrease in calpain 3.

This permitted us to use the clinical findings as a template for calpainopathy against which we could measure other undiagnosed limb-girdle muscular dystrophies. This was useful for establishing the gestalt of the condition but also provided a starting point for a more detailed examination of the pattern of muscle involvement. By building upon this clinical foundation it became apparent that it is possible to suggest the diagnosis from the clinical findings with subsequent confirmation from protein and molecular techniques. Each diagnostic modality may thus offer an important test of the diagnostic hypothesis.

2.1. Patients

Thirteen patients from 11 different families were examined by Newcastle clinicians. The patients all had in common the finding of reduced or absent calpain 3 on immunoblotting of frozen muscle biopsy samples (see below). In two patients (4 and 11) examination findings were based on notes made by Newcastle neurologists. All patients had presented with a limb-girdle distribution of muscle weakness and had a raised serum creatine kinase. Eleven of the patients were revisited by one of the authors (KB and/or CP) and examined using a standard protocol. This included a detailed examination of muscle power using the Medical Research Council (MRC) grading system [10]. Specifically, a comparison of the relative strength of the flexors and extensors, adductors and abductors of both the proximal and distal upper and lower limbs was made in each of the patients. The presence of spinal rigidity and of contractures at the wrist, elbow, hip, knee and ankle were documented. Spirometry was performed in seven of the patients and the forced vital capacity was recorded.

2.2. Samples

A blood sample in EDTA was taken from each patient for DNA analysis. For 11 of the patients (including one of each sibling pair) a muscle biopsy had been taken as part of the routine diagnostic procedure and was stored in liquid nitrogen until required.

2.3. Immunoblotting

The antibodies to calpain 3 work on western blots only [6]. A biphasic system of electrophoresis and blotting was used, as described previously [11]. This had been optimized to resolve all the known muscular dystrophy proteins on a single pair of gels/blots. Thus the lower half of the gel contained 7% acrylamide (for resolving calpain 3, merosin and the sarcoglycans, in the molecular mass range of 30–00 kDa) while the upper half contained a gradient of 5.5–4% (for resolving myosin heavy chain, dysferlin and dystrophin in the range 200–400 kDa). A 3% stacking gel was used. The frozen tissue samples were weighed and kept frozen until homogenized with 19 volumes of electrophoresis treatment buffer containing 4% sodium dodecyl sulphate and 4 M urea (with no additional protease inhibitors). Lanes of control muscle (with no fat or fibrous connective tissue) typically contained 200 μg protein [12]. After electrophoresis the gels were blotted, labelled with antibody multiplexes which were followed by a peroxidase conjugated secondary antibody and visualized with hydrogen peroxide and diaminobenzidine (DAB). The antibody combinations currently in use are (1) 1/10 Dy8/6C5 to the C-terminus of dystrophin (400 kDa band), 1/300 NCL-hamlet to exon 52 of dysferlin (230 kDa), 1/10 Calp3d/2C4 to exon 1 of calpain 3 (94 and 30 kDa), 1/2 Ad1/20A6 to α-sarcoglycan (50 kDa) and 1/30 43DAG/8D5 to β-dystroglycan (43 kDa); and (2) 1/10 Dy4/6D3 to the rod domain of dystrophin (400 kDa doublet of bands plus smaller metabolites), 1/10 Calp3c/12A2 to exon 8 of calpain 3 (94 and ~60 kDa), 1/1000 commercial Chemicon antibody 1922 to the laminin α2 chain of merosin (80 kDa), 1/30 43DAG/8D5 to β-dystroglycan (43 kDa) and 1/2 35DAG/21B5 to γ-sarcoglycan (35 kDa, plus an unknown immunoreactive band or doublet at 120 kDa). The antibody to β-dystroglycan was used on both blots as an internal control. The amount of muscle protein loaded (as opposed to fat and fibrous connective tissue) was assessed from the corresponding myosin heavy chain bands on the postblotted gel, which was stained with coomassie blue.

The antibodies used produce three bands for calpain 3. These correspond to the full-size protein at 94 kDa, a set of rather diffuse breakdown bands that commence at ~60 kDa and a clear ‘fragment’ at 30 kDa (Fig. 1). For the purposes of comparison between patients, the labelling intensity of each size band was assessed separately and scored by eye as ++++ (strong labelling), +++ (moderate), + + (clearly visible), + (faint), +/− (very faint) and − (not visible). This is the same method as used previously [6] and permitted comparison of the bands at ~60 kDa, which are not amenable to densitometry. Scoring the intensity of band labelling is also more useful to general diagnostic laboratories, where densitometry is not routinely undertaken. The
Fig. 1. Multiplex western blot analysis in a group of patients with limb-girdle muscular dystrophy. (A) is labelled with monoclonal antibodies to the C-terminus of dystrophin (400 kD), exon 1 of calpain 3 (94 and 30 kD), α-sarcoglycan (50 kD) and β-dystroglycan (43 kD). (B) is labelled with monoclonal antibodies to the rod domain of dystrophin (400 kD, with metabolite bands down to below 200 kD), exon 8 of calpain 3 (94 and 60 kD), the laminin α-2 chain of merosin (80 kD) and β-dystroglycan (43 kD). (C) is the coomassie blue stained myosin heavy chain band from one of the corresponding post-blotted gels. This is used to indicate how much muscle protein, as opposed to fat and fibrous connective tissue, is loaded in each lane. Dys, dystrophin; αSG, α-sarcoglycan; β-dys, β-dystroglycan. Lanes 1 and 4, normal controls; lane 2 = patient 8; lane 3 = patient 1a; lane 6 = patient 6; lane 7 = patient 4; lane 5 = patient later identified as having LGMD2B; lane 8 = patient with unresolved LGMD.
use of multiple antibodies was considered essential in the study of calpain 3 because, in a few patients, abnormalities are observed in more than one protein.

2.4. Mutation detection

Genomic DNA was amplified by PCR (50–200 ng genomic template, 0.25 μM primer, 0.2 mM dNTP, 0.5 unit Taq polymerase/reaction). Mutation detection analysis was then performed using the single-strand conformational polymorphism/heteroduplex analysis (SSCP/HA) technique [19]. Seven microlitres of each PCR product was added to an equal volume of denaturing loading buffer (95% formamide, 20 mM EDTA, 20 mM NaOH, 0.5% xylene cyanol, 0.5% bromophenol blue). This was then denatured for 3 min at 95°C, and plunged into ice to minimise re-annealing of the single strands. Ten microlitres of the mixture was loaded on a mutation detection enhancement (MDE, Flowgen) polyacrylamide gel containing 5% glycerol. Electrophoresis was carried out under two different sets of conditions: 300 V for 24 h at 10°C and 300 V for 16–18 h at room temperature. Results were visualised by silver staining and band shifts were sequenced using the Thermosequenase sequencing kit (Amersham).

3. Results

Results of immunoblotting and mutation searching are presented in Table 1. None of these patients had any abnormalities of any of the other muscle proteins examined. Mutations were detected in seven of the eleven families (see Table 1). All had a clinical profile and pattern of muscle involvement that fitted with previous descriptions of patients with LGMD2A (Tables 2 and 3), though additional features were noted in some patients.

3.1. Clinical history

Patients 1a and 1b are siblings, as are patients 7a and 7b. None of the patients had a family history of muscle disease and there was no consanguinity. There were seven affected males and six females. All patients reported that lower limb weakness preceded upper limb involvement and all had intellectual function within the normal range.

The range of age of onset was 2–45 years. This is broader than that described in other studies though the average age is comparable. In our population, the age of onset in females tended to be slightly younger than in males. One female patient reported an exacerbation of muscle weakness after pregnancy and at least two patients complained of difficulty in picking up their children as a relatively early feature of the disease. In all cases children were born to affected women by normal vaginal delivery and there was no evidence to suggest that Caesarean section was more common.

Three patients were diagnosed in the first decade of life. Although, in all cases, early motor milestones were said to be normal, four of the patients toe-walked in childhood and all patients reported toe-walking at some stage. The most striking of these was patient 1a who toe-walked at the age of 2 years. This was initially attributed to ‘habit’. He subsequently presented with muscle weakness and was diagnosed by a paediatric neurologist as having severe childhood auto-

<p>| Table 1 | Protein and gene expression in patients with LGMD2A* |
|-----------------------------------------------|
| <strong>Calpain 3 protein expression</strong> | <strong>Calpain 3 gene expression</strong> |</p>
<table>
<thead>
<tr>
<th>94 kDa full size</th>
<th>30 kDa fragment</th>
<th>60 kDa products</th>
<th>Mutations</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>Del 1373C</td>
</tr>
<tr>
<td>Patients 1a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Del 1983A</td>
</tr>
<tr>
<td>2</td>
<td>+/−</td>
<td>-</td>
<td>-</td>
<td>A1435G 2nd not identified</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
<td>+/−</td>
<td>+/−</td>
<td>G649A</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>G2093C</td>
</tr>
<tr>
<td>5</td>
<td>+/−</td>
<td>-</td>
<td>-</td>
<td>Del 550A 2nd not identified</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+/−</td>
<td>+/−</td>
<td>Del 550A</td>
</tr>
<tr>
<td>7b</td>
<td>-</td>
<td>-</td>
<td>+/−</td>
<td>A2114G</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>G → T exon 18-1</td>
</tr>
<tr>
<td>9</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>C328A, 2nd not identified</td>
</tr>
<tr>
<td>10</td>
<td>+/−</td>
<td>-</td>
<td>-</td>
<td>none identified</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+/−</td>
<td>+/−</td>
<td>none identified</td>
</tr>
</tbody>
</table>

*Key to band labelling: ++++, strong; +++, moderate, ++, clearly visible; +, faint; +/-, very faint; −, not visible.
Table 2
Clinical details of the patients

<table>
<thead>
<tr>
<th>Patient (gender)</th>
<th>Age presented (years)</th>
<th>Symptoms at presentation</th>
<th>CK [age in years] (Normal &lt;160 Iu/l)</th>
<th>Age last seen (years)</th>
<th>Functional ability when last seen</th>
<th>Contractures</th>
<th>Current FVC (litres) (% predicted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a (M)</td>
<td>2</td>
<td>Presented with toe walking 2 years.</td>
<td>&gt; 9000 [7]</td>
<td>14</td>
<td>Severe toe-walking. Wheelchair part-time since age 13 years. Climbs stairs with railing.</td>
<td>Paraspinal, finger and ankle. Lordosis. Scoiosis. Broad feet with pes cavus.</td>
<td>3.25 (80%)</td>
</tr>
<tr>
<td>1b (M)</td>
<td>6</td>
<td>Diagnosed because of affected younger brother. Asymptomatic until 11 years when difficulty climbing stairs. Toe walking 12 years.</td>
<td>1181 [38]</td>
<td>38</td>
<td>Severe toe-walking. Climbs stairs both hands on banister.</td>
<td>Ankles. Severe spinal rigidity.</td>
<td>3.33 (90%)</td>
</tr>
<tr>
<td>4 (F)</td>
<td>11</td>
<td>Toe-walking 11 years. Calf pain on exercise. Frequent falls. Difficulty climbing stairs.</td>
<td>555 [16]</td>
<td>34</td>
<td>Walks unaided around house. Wheelchair part-time since age 30 years. Unable to climb stairs since age 29 years.</td>
<td>Severe lordosis. Wrist, fingers, ankles, toes.</td>
<td>3.72 (81%)</td>
</tr>
<tr>
<td>6 (M)</td>
<td>24</td>
<td>Age 23 years passed medical exam for RAF but then difficulty rising from chair + running.</td>
<td>385 [20]</td>
<td>30</td>
<td>Waddling, toe-walking gait unaided. Unable to climb stairs since age 26 years.</td>
<td>Severe lordosis, mild scoliosis. Ankle. contractures.</td>
<td>5.07 (123%)</td>
</tr>
<tr>
<td>7a (F)</td>
<td>12</td>
<td>Diagnosed because of affected older brother. Sport: slower than peers. 12 years difficulty rising from chair.</td>
<td>6625 [24]</td>
<td>30</td>
<td>Stands but unable to walk. Wheelchair part-time since age 29 years. Unable to climb stairs since age 29 years.</td>
<td>Severe lordosis. Mild scoliosis. Neck, fingers.</td>
<td>1.59 (44%)</td>
</tr>
<tr>
<td>7b (M)</td>
<td>16</td>
<td>Presented with walking difficulty associated with clawing of toes.</td>
<td>3330 [34]</td>
<td>41</td>
<td>Walks unaided. Climbs stairs both hands on banister.</td>
<td>Severe lordosis. Mild contractures shoulder, wrist and hip. Broad feet.</td>
<td>3.58 (75%)</td>
</tr>
<tr>
<td>8 (M)</td>
<td>45</td>
<td>Good at sport: football coach. Presented with difficulty climbing kerb and 3 years history difficulty throwing a ball.</td>
<td>1540 [18]</td>
<td>18</td>
<td>Climbs stairs both hands on banister.</td>
<td>Not recorded</td>
<td>Not done</td>
</tr>
<tr>
<td>9 (F)</td>
<td>24</td>
<td>Good at sport. Difficulty climbing stairs and frequent falls.</td>
<td>3330 [34]</td>
<td>41</td>
<td>Walks unaided. Climbs stairs both hands on banister.</td>
<td>Severe lordosis. Mild contractures shoulder, wrist and hip. Broad feet.</td>
<td>Not done</td>
</tr>
<tr>
<td>10 (M)</td>
<td>29</td>
<td>Always poor at sport. Difficulty carrying his children upstairs.</td>
<td>3330 [34]</td>
<td>41</td>
<td>Walks unaided. Climbs stairs both hands on banister.</td>
<td>Severe lordosis. Mild contractures shoulder, wrist and hip. Broad feet.</td>
<td>Not done</td>
</tr>
</tbody>
</table>
### Table 3
Pattern of muscle involvement in patients with LGMD 2A

<table>
<thead>
<tr>
<th>Patient</th>
<th>Facial weakness</th>
<th>Neck flexion weaker than extension</th>
<th>Shoulder adduction weaker than abduction</th>
<th>Elbow flexion weaker than extension</th>
<th>Wrist flexion weaker than extension</th>
<th>Finger extension weaker than flexion</th>
<th>Hip flexion weaker than extension</th>
<th>Hip adduction weaker than abduction</th>
<th>Knee flexion weaker than extension</th>
<th>Ankle dorsiflexion weaker than plantar flexion</th>
<th>Ankle eversion weaker than inversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>NO</td>
<td>YES (4+/5)</td>
<td>YES (4+/5)</td>
<td>YES (3+/4+)</td>
<td>NO (5/4+)</td>
<td>YES (4+/5)</td>
<td>YES (4+/5)</td>
<td>NO (5/4+)</td>
<td>YES (4+/5)</td>
<td>YES (4+/5)</td>
<td>YES (4/5)</td>
</tr>
<tr>
<td>2</td>
<td>NO</td>
<td>= (5/5)</td>
<td>YES (3+/4+)</td>
<td>NO (5/4+)</td>
<td>?</td>
<td>NO (3/2)</td>
<td>YES (2/4)</td>
<td>YES (3+/4-)</td>
<td>= (4+/4+)</td>
<td>YES (4/5)</td>
<td>= (5/5)</td>
</tr>
<tr>
<td>5</td>
<td>NO</td>
<td>YES (4+/5)</td>
<td>YES (2+/4+)</td>
<td>YES (3/4)</td>
<td>NO (5/4+)</td>
<td>?</td>
<td>YES (1/1)</td>
<td>YES (1/4+)</td>
<td>YES (4+/5)</td>
<td>YES (4/5)</td>
<td>= (5/5)</td>
</tr>
<tr>
<td>6</td>
<td>NO</td>
<td>YES (4+/5)</td>
<td>= (2/2)</td>
<td>YES (1/2+)</td>
<td>NO (5/3)</td>
<td>YES (3/)</td>
<td>YES (1/2)</td>
<td>YES (3/5)</td>
<td>YES (4/5)</td>
<td>YES (4/5)</td>
<td>= (5/5)</td>
</tr>
<tr>
<td>7a</td>
<td>NO</td>
<td>YES (4/5)</td>
<td>YES (1/)</td>
<td>YES (2/3)</td>
<td>NO (4/3)</td>
<td>YES (3/5)</td>
<td>YES (4/)</td>
<td>YES (1/4)</td>
<td>YES (1/2)</td>
<td>YES (3/5)</td>
<td>YES (3/5)</td>
</tr>
<tr>
<td>7b</td>
<td>YES</td>
<td>YES (4+/5)</td>
<td>YES (1/2)</td>
<td>YES (2/4-)</td>
<td>NO (4/4-)</td>
<td>YES (3/5)</td>
<td>YES (0/1)</td>
<td>YES (1/4-)</td>
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<td>YES (3/5)</td>
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<td>8</td>
<td>NO</td>
<td>YES (4+/5)</td>
<td>YES (4/4+)</td>
<td>NO (5/4+)</td>
<td>NO (5/4+)</td>
<td>?</td>
<td>YES (4/4-)</td>
<td>NO (4/4+)</td>
<td>= (4/)</td>
<td>YES (4/5)</td>
<td>= (5/5)</td>
</tr>
<tr>
<td>9</td>
<td>YES</td>
<td>YES (3/4-)</td>
<td>YES (2/3)</td>
<td>= (4/4-)</td>
<td>YES (4/4+)</td>
<td>YES (1/2)</td>
<td>YES (2/4-)</td>
<td>= (1/1)</td>
<td>YES (4/4+)</td>
<td>YES (4/5)</td>
<td>= (5/5)</td>
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<tr>
<td>10</td>
<td>NO</td>
<td>YES (5-/5)</td>
<td>= (4+/4+)</td>
<td>YES (3/5)</td>
<td>NO (5/5-)</td>
<td>YES (5-/5)</td>
<td>YES (2/3)</td>
<td>YES (2/4-)</td>
<td>NO (4/4-)</td>
<td>YES (5-/5)</td>
<td>= (5/5)</td>
</tr>
</tbody>
</table>

* Muscle scores are given according to the MRC scale. EMG, electromyography; % of predicted value was based on the patient's gender, age and height; ECG, electrocardiogram.
somal recessive muscular dystrophy (SCARMD). Creatine kinase was raised (>9000) and muscle biopsy at that time showed a dystrophic pattern. His elder brother was asymptomatic but was diagnosed at age six years. Both brothers, but especially the elder who had a slightly milder phenotype, had primary contractures, not only of the tendo-achilles but also of the fingers and elbows. The elder brother remains ambulant and is studying at university. His younger brother has used a wheelchair part-time since the age of 13 years (Fig. 2).

This was the first of our patients who was noted to have a reduction in calpain on western blot. He subsequently proved to have two frameshift mutations. The finding of early primary contractures in this sibship thus signalled that they might well be an integral part of the disease and alerted us to their presence in other patients. At the opposite end of the spectrum, patient 6 (now 80 years old) was asymptomatic until the age of 24 years. Early motor milestones were completely normal. He presented with difficulty running and rising from a chair. Muscle biopsy revealed a wide variation in fibre size and some vacuolated fibres. He remained ambulant with the use of a wheelchair on a part-time basis until the age of 70. The diagnosis of calpainopathy was finally made on a repeat muscle biopsy sample at the age of 76. He has recently developed a significant degree of restrictive respiratory impairment so that he requires nocturnal home ventilation with additional ventilatory support for acute chest infections.

Age of onset was not necessarily a predictor of rate of progression or age of wheelchair use. Thus one woman with onset at nine years of age was still ambulant at 38 years whereas another with onset at age 24 years was using a wheelchair part-time at the age of 29 years. She had presented with mild weakness whilst travelling. She was initially diagnosed as having polymyositis and was treated with steroids. She had significant hand contractures and, interestingly, pictures from early childhood show that in several photographs her fingers were curled. The diagnosis of calpainopathy was made independently on the basis of immunoblotting and clinical findings. No mutation has been detected.

Eight patients were still ambulant ten years after onset of the disease. Moreover, even when using a wheelchair to aid mobility, most patients retained the ability to stand with only minimal support. This usually consisted of fingertip pressure to maintain balance. The stance is almost characteristic: broad-based (owing to the relative preservation of the hip abductors) with locked knees, a lumbar lordosis and a degree of abdominal laxity.

3.2. Clinical examination

The overall pattern of muscle involvement showed interfamilial consistency. Ten out of 12 patients had scapular winging. One patient had calf pseudohypertrophy early in the course of the disease but the rest had quite marked atrophy. Two patients had mild facial weakness. In ten out of 13 cases neck flexion was weaker than extension. Primary contractures, especially of the tendo-achilles but also of the hands and elbows, were a notable feature. They were most striking in the patients of younger onset. The boy who was toe-walking at the age of two years, for example, exhibited primary elbow and finger contractures in adolescence. Three patients had evidence of spinal rigidity. (All have known mutations.)

In the upper limb, in 11 out of 12 patients, shoulder adduction was equal to, or weaker than, abduction and in ten out of 13, elbow flexion was weaker than extension. Conversely, in ten out of 12 patients, wrist extension was weaker than flexion. In the lower limb, 11 out of 12 patients were markedly weaker in hip adduction than abduction. Hip flexion was weaker than extension in nine out of 12 patients. In ten out of 13 patients, knee flexion was weaker than extension. In 12 out of 12 patients, ankle dorsiflexion was equal to, or weaker than, plantar flexion and ankle eversion weaker than inversion.

Five of the seven patients tested had evidence of mild respiratory impairment when last examined. In one other patient this was sufficient to require nocturnal ventilatory support when he was in his late 70s. Respiratory impairment did not correlate with spinal rigidity. None of our patients had clinically significant cardiac abnormalities though one had an arrhythmia.

4. Discussion

In 1996 an archetypal form of limb-girdle muscular dystrophy was observed by Fardeau and colleagues in the population of Reunion Island [4]. The gene causing this disease had been identified by Beckmann and colleagues [2] and was confirmed in another isolated population, the Old Order Amish [7]. The exclusive nature of these clinical groups meant that very few clinicians had actually seen LGMD2A for themselves, and the differential diagnosis of LGMD in more cosmopolitan populations was uncertain. However, the generation of diagnostic monoclonal antibodies (via synthetic peptides from the gene sequence and biopsies from some of the original Reunion Island patients) has helped to change that perspective. Immunoanalysis identified individuals who potentially had LGMD2A from among those patients with indeterminate limb-girdle muscular dystrophy. Genetic analysis confirmed the diagnosis in the defined patients, permitting the clinical characteristics of the disease to be observed by a wider group of clinicians. As more patients were identified, a characteristic clinical profile emerged, and now patients can be identified primarily from these clinical patterns, with secondary confirmation from gene and protein analysis.

Clinical features fall into two categories. Firstly there are the typical features that are almost always present, and which may be considered as the main ‘theme’ of calpaino-
Fig. 2. Montage of clinical photographs. (A) Generalized muscle wasting and mild scapular winging; (B) Spinal rigidity; (C) abdominal laxity (D) contractures of tendo achilles; (E) characteristic stance and calf wasting (F) calf wasting and ankle contractures; (G) scapular winging (H) generalized muscle wasting; (I) weakness of finger extensors (J) finger contractures.
pathy. Secondly there are the ‘variations’; the features that we observed in our group of patients that add to the previously published clinical descriptions [4,5,8,9,13].

The range of age of onset and rate of progression is wider in this group of patients than has been previously described. The main difference in the phenotype is the finding of early primary contractures in our group of patients. In the Réunion Island population Fardeau [4] reported that, in the early stages of the disease, contractures were limited to the calf muscles but that they progressed rapidly when ambulation was lost. Two cases in the French series [5] also had early contractures which were more widespread and affected the hip and elbow in addition to the Achilles tendon. Early finger or hand contractures have not however been previously reported. In our population the presence of relatively early distal involvement, though not severe, is noteworthy and well conserved throughout the group. It is not as marked as is seen in LGMD 2B (dysferlinopathy) and does not appear to constitute a separate form of the disease. Moreover, scapular winging, which is a well-recognised feature of calpainopathy and which was present in all our patients, would be considered extremely unusual in the proximal form of dysferlinopathy.

These new variations on the phenotype have particular importance in sporadic cases where it is clinically important to elucidate the inheritance pattern of the disease and establish the diagnosis. In view of the presence of contractures, various autosomal dominant disorders should be considered in the differential diagnosis. These would include Bethlem myopathy [14] and autosomal dominant Emery–Dreifuss muscular dystrophy [15,16], both of which may be associated with a secondary beta 1 laminin deficiency [17]. In Bethlem myopathy the main discriminating features are the extremely early onset and severity of contractures (which may include congenital talipes or torticollis) coupled with an often almost imperceptible rate of progression. The pattern of muscle involvement is also different, with relative weakness of the flexors, including the wrist flexors but marked weakness of the finger extensors. Facial weakness is also more common and scapular winging is absent. The relative strength and weakness of the hip abductors and adductors is very variable and this contrasts with calpain deficiency. LGMD 1B is also characterized by a relatively slow progression of muscle weakness and atrophy in a humeroperoneal distribution but in association with a cardiac conduction disturbance. Indeed the latter may occur in the absence of muscle involvement though, at present, the converse does not appear to be true.

The importance of the pattern of muscle involvement in LGMD 2A should not be underestimated. Not only was the basic pattern conserved interfamiliarly in our patients but it was also conserved through time. Thus the hip abductors were spared even late in the disease. Moreover our findings appear to confirm the findings of other groups and especially the early reports of Fardeau from the Réunion Island population [4]. In Newcastle, we do not have ready access to diagnostic muscle CT. This is an invaluable adjunct to characterisation of the selectivity of muscle involvement not only in calpainopathy but in all forms of LGMD. It would also facilitate comparison of the patterning of muscle involvement in patients in different centres. In the absence of this we have presented the results of muscle examination in terms of a balance of forces around a particular joint. We find this useful on several counts, and our patients have themselves found it helpful. It enables them to set a limit to their perceived weakness and to discriminate between the muscles involved.

The pattern of muscle involvement or balance of forces may explain some of the typical features of the disease. It underpins the characteristic stance with hips abducted, knees hyperextended and with the weight on the lateral border of the feet. It also explains why most patients were able to stand with minimal support (often with only finger tip pressure for balance) until the late stages of the disease. Similarly the ankle plantar flexion contractures and the finger flexion contractures may be regarded as in keeping with the pattern of distal weakness. However the early elbow contractures in ambulant patients are a little surprising give the weakness of triceps relative to biceps. The pathological mechanism of the contractures is not known. A genotype–phenotype correlation is beyond the scope of this paper. It would, however, be interesting to look in a larger population, to see if there is a relationship between certain mutations and a higher prevalence of contractures.

The two techniques used for mutation detection, SSCP and heteroduplex analysis, each yield a mutation detection efficiency of 60–70%. We were unable to find caprin 3 mutations in four of the eleven families reported here. The search for mutations may not always be as straightforward as SSCP analysis of the genomic structure, or even sequencing of the entire gene. Mutations such as large scale deletions, pathological changes deeper within the introns, or changes in promoter or enhancer elements will not necessarily be detected using these methods. Richard & Beckmann [2] have suggested that synonymous codon changes may, in some cases, cause faults in the function of a gene leading to a pathological phenotype. Furthermore, Chou et al. [18] reported two patients that were heterozygous for a single mutation at the DNA level whereas only the mutant allele was observed at the RNA level. This supports the view that there are undetectable, nondeletion mutations that can alter or reduce expression of the CAPN3 gene. None of the patients reported here had any detectable abnormality in any of the other muscle proteins examined. This, together with the utter consistency of the phenotype, leads us to believe that they do represent true cases of calpainopathy, and not cases with a secondary reduction of calpain 3, even though CAPN3 mutations have not been detected by the methods used.

In the patients detailed here, therefore, the diagnosis of calpainopathy has been achieved on the evidence of the muscle biopsy, clinical profile and, in some cases, mutation screening. The feedback offered by this approach has
enabled us to extend our appreciation of the phenotype. Several of our patients have significant primary contrac-
tures, including spinal rigidity and distal weakness which
have not been described previously. Just as importantly, it
has enabled us to see the features that are conserved and
which may be considered the core of the phenotype. This is
the main clinical theme of calpainopathy against which we
measure local variation.

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