Abnormal merosin in adults
A new form of late onset muscular dystrophy not linked to chromosome 6q2

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Summary
We have identified seven patients (including two sib pairs) with a predominantly late onset limb-girdle muscular dystrophy in whom an absence of merosin was noted on immunoblotting. Merosin immunocytochemistry was normal, and no abnormalities were detected on immunostaining for the various proteins known to be involved in the limb-girdle muscular dystrophies (α, β, γ, δ sarcoglycan and calpain 3). Apart from one patient, where muscle problems began in childhood, reported age at onset of muscle weakness involving initially the proximal muscles of the lower limbs ranged from 17 to 40 years. The pattern of muscle involvement was similar from patient to patient, with hypertrophy of at least the calf muscles, absence of scapular winging and predominant involvement of hip flexors and adductors and hamstrings more than quadriceps. Serum creatine kinase in all patients was at least 10 times normal, and muscle biopsies showed non-specific dystrophic features. We believe that the patients described here may represent a genetically distinct subset within the limb-girdle muscular dystrophy group.

Keywords: limb-girdle muscular dystrophy; merosin; muscle proteins

Abbreviation: LGMD = limb-girdle muscular dystrophy

Introduction
As diagnostic techniques improve, it is increasingly possible to characterize patients presenting with a muscular dystrophy by studying the genes and proteins now known to be involved (Bushby, 1996). This has led to an increased understanding of various muscular dystrophies, most notably those which can be defined by the involvement of one of the proteins of the dystrophin-associated complex (Beckmann, 1996). Of the various proteins which form the complex between dystrophin on the outside of the muscle membrane and the extracellular matrix, several have been associated with specific diseases. For example, abnormalities of α, β, δ and γ sarcoglycan are involved in forms of limb-girdle muscular dystrophy (LGMD2D, E, F and C, respectively), and can produce a wide spectrum of disease severity (Bonnemann et al., 1995; Lim et al., 1995; Noguchi et al., 1995; Vainzof et al., 1996). Mutations in calpain 3 are responsible for another type of autosomal recessive LGMD (LGMD2A) (Richard et al., 1995; Spencer et al., 1997), with the gene for a different form (LGMD2B) mapped but not yet cloned (Bashir et al., 1996). The recognition of the involvement of these various proteins in forms of LGMD has contributed to a major reclassification of this group of disorders (Bushby and Beckmann, 1995).

On the outer side of the muscle membrane, α-dystroglycan links the rest of the complex to muscle laminin (merosin) in the extracellular matrix. Merosin is composed of a laminin α2 chain plus a β1 and a γ1 chain (Wewer and Engvall, 1996). Deficiency of the laminin α2 chain has been described in children with congenital muscular dystrophy, an autosomal recessive form of muscular dystrophy, which as the name suggests, has its onset in early childhood and is usually associated with early and severe disability. Merosin deficiency has been demonstrated in approximately half of all children with congenital muscular dystrophy, and children who have total merosin deficiency tend to have a more severe clinical course (Tome et al., 1994; Philpot et al., 1995). Merosin deficiency in these children is associated with a characteristic pattern of white matter changes in the brain detectable by
MRI (Philpot et al., 1995). Mutations in the merosin gene (on chromosome 6q2) (Hillaire et al., 1994) have been demonstrated in some of these cases (Helbling-Leclerc et al., 1995), including some with partial merosin deficiency (Nissinen et al., 1996), though the large size of the gene and the variety of mutations found has meant that the characterization of the primary genetic defect may not be straightforward. On the whole, the phenotype of merosin-negative congenital muscular dystrophy is relatively well defined, though one family with later onset disease and merosin deficiency has been reported. In this family cerebral white matter changes were seen on MRI, and linkage to chromosome 6q was likely (Tan et al., 1997).

All of the proteins of the dystrophin-associated complex which have been implicated in muscular dystrophies can be examined using specific antibodies by the techniques of immunocytochemistry and immunoblotting on muscle biopsy samples (Anderson, 1996). It is therefore possible to characterize patients with a muscular dystrophy with respect to their pattern of muscle protein involvement.

Using a combination of immunocytochemistry and immunoblotting to screen our patients, we have identified a group with predominantly adult onset muscular dystrophy who show a normal immunocytochemical pattern for dystrophin, the sarcoglycans and merosin, but who have a consistent deficiency of merosin on immunoblotting.

Patients and methods

Patients

As new antibodies to muscle proteins are identified, we use these to screen muscle biopsies from the archive at the Muscular Dystrophy Group Research Laboratories at Newcastle General Hospital. The patients reported here had presented with proximal muscle weakness with elevation of serum creatine kinase (see below) and had been shown to have a muscular dystrophy on histological grounds. No definitive diagnosis of the type of muscular dystrophy had been possible because these patients had normal dystrophin, normal sarcoglycans and normal calpain 3 (thereby excluding a diagnosis of Duchenne or Becker muscular dystrophy, a sarcoglycanopathy or LGMD2A). Each patient was revisited by one of the authors (K.B. and/or C.P.) and a full muscle examination was performed blind of the results of examination in the other patients (Anon, 1986).

Immunocytochemistry and immunoblotting

Immunocytochemical analysis of unfixed frozen tissue sections was performed by the indirect peroxidase method. Electrophoresis and Western blotting techniques were performed as previously described (Nicholson et al., 1989, 1990). Commercial antibodies to laminin α1 chain (Chemicon MAB 1924; Chemicon International Inc., Temecula, Calif., USA), α2 chain or merosin (Chemicon MAB 1922), β1 chain (Chemicon MAB 1921) and γ1 chain (Chemicon MAB 1920) were obtained from Chemicon. As defined by the data sheet, the Chemicon antibody MAB 1922 reacts with the 80 kDa fragment of merosin. The monoclonal antibodies to dystrophin and the associated proteins used have been described previously (Nicholson et al., 1993; Sewry et al., 1996). An additional monoclonal antibody to merosin, Mer3/22B2, was isolated from the fusion of X63.Ag8.653 myeloma cells with splenocytes from BALB/C mice immunized with purified merosin from human placenta (Gibco). The antibody does not work on blots, but shows the same pattern of immunoreactivity on sections as the 300 kDa antibody used in Tan et al. (1997) (C. Sewry, personal communication).

Genetic analysis

DNA samples were collected from all patients in whom a reduction in merosin was detected and also, where possible, from their families. Haplotypes around the merosin gene on chromosome 6q2 were constructed in the families, where sufficient family members were available, using the microsatellite markers D6S407, D6S1620 and D6S1705, which are closely linked to the merosin gene (Naom et al., 1997).

Results

Clinical history

Clinical details are summarized in Table 1. No patients were from families with a previous history of muscle disease and there was no consanguinity. Patients 1a and 1b are sisters as are Patients 5a and 5b: the others have no affected relatives. There was a striking female predominance (6/7). Only in one patient (Patient 4) was there any problem with mobility in early childhood—she reported markedly earlier onset and a more rapidly progressive clinical course than the other patients in the group. She had walked at a normal age, but complained of calf cramps from age 2 years and was always noted to have big calves. Her performance at school sport was poor. Her mother commented on her unusual gait from around age 11 years, and as a teenager she remembers avoiding stairs wherever possible. Deterioration in Patient 4 appeared to accelerate in her late teens following a road traffic accident. In the other patients, detailed questioning about their early physical prowess failed to reveal any specific problems, though all described themselves as slow runners. Progression of muscle weakness in these patients was gradual but steady. Patient 4 required full-time use of a wheelchair from the age of 26 years but all other patients remain independently mobile. All patients noticed that upper limb involvement followed lower limb involvement by several years. Intelligence in all patients appeared normal, and all had attended mainstream schools.

Pattern of muscle involvement

The main features of the muscle involvement seen in the seven patients is shown in Table 2. Five patients had mild
Table 1 Clinical details of the seven patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Early mobility</th>
<th>Age (years)</th>
<th>First symptoms</th>
<th>Age last seen (years)</th>
<th>Functional ability when last seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>F</td>
<td>Normal</td>
<td>40</td>
<td>Difficulty climbing stairs and running</td>
<td>43</td>
<td>Mild waddle; uses both hands on railing to climb stairs; raises arms in full circle</td>
</tr>
<tr>
<td>1b</td>
<td>F</td>
<td>Normal</td>
<td>35</td>
<td>Difficulty climbing stairs and rising from floor</td>
<td>45</td>
<td>Mild waddle; uses one banister to climb stairs; raises arms in full circle</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Normal</td>
<td>27</td>
<td>Legs giving way; increasing effort to climb stairs</td>
<td>34</td>
<td>Needs two rails for stairs; uses wheelchair for long distances; able to walk on the flat</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>Normal</td>
<td>29</td>
<td>Problems with steps and rising from sitting</td>
<td>34</td>
<td>Uses both hands on railing to climb stairs; waddling gait; difficulty rising from chair; raises arms in full circle</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Muscle cramps from age 2</td>
<td>11–14</td>
<td>Calf cramps in early childhood; abnormal gait from age 11; difficulty running from age 14</td>
<td>27</td>
<td>Wheelchair full time from age 26; unable to stretch out arms forward</td>
</tr>
<tr>
<td>5a</td>
<td>F</td>
<td>Normal</td>
<td>18</td>
<td>Falls; slow walking; unable to run; never good at school sport</td>
<td>38</td>
<td>Climbs stairs on all fours; requires assistance to walk long distances; unable to lift arms above head</td>
</tr>
<tr>
<td>5b</td>
<td>F</td>
<td>Normal</td>
<td>17</td>
<td>Falls; has difficulty rising from floor; previously a slow runner</td>
<td>34</td>
<td>Uses both hands on railing to climb stairs; uses a stick for support for long distances; unable to lift arms above head</td>
</tr>
</tbody>
</table>

weakness of eye closure (Fig. 1) but no other facial muscle involvement was seen. Neck flexion was weaker than extension in all patients. Hypertrophy of at least the calf muscles was a feature in all patients (Fig. 1) and brachioradialis hypertrophy was also a common feature (5/7). In the lower limbs, there was a predominant and consistent pattern of muscle weakness with usually greater involvement of the hip adductors and flexors compared with abductors and extensors, and of the hamstrings more than the quadriceps, though with increasing weakness the selectivity was less noticeable. The selectivity of muscle involvement in the upper limbs was less uniform; however, scapular winging was not seen in any patient. In both upper and lower limbs distal muscles were involved less than proximal, though the peronei were more involved than the anterior tibial group in the lower limbs. Some asymmetry of muscle involvement was seen. Reflexes were normal early in the disease but were later lost. There were no contractures except for a mild reduction in lateral flexion and rotation of the neck, no scoliosis and no spinal rigidity in any of the patients. All had a marked lumbar lordosis.

Investigations
Table 3 shows the results of the investigations performed. Creatine kinase was significantly elevated in all patients (normal range in our laboratory is up to 140 IU/l). Both EMG and muscle biopsy changes were indicative of chronic muscle disease. Forced vital capacity was reduced to between 30 and 93% of the predicted value. Brain MRI scans were carried out in three patients and all were entirely normal with no sign of the type of abnormalities characteristically seen in patients with merosin-negative congenital muscular dystrophy. Cardiac investigations (ECG and echocardiogram where available) showed a coarctation of the aorta in one patient, but were essentially normal in the rest.

Immunoanalysis
Immunoblotting of the 80 kDa merosin fragment detected by the Chemicon antibody MAB (monoclonal antibody) 1922 revealed an absence or near-absence of labelling in all the patients, although labelling for dystrophin, α sarcoglycan
Table 2 Muscle involvement in patients with absent merosin immunoblotting

<table>
<thead>
<tr>
<th>Patient</th>
<th>Muscle hypertrophy</th>
<th>Facial muscle involvement</th>
<th>Hip flexion weaker than extension</th>
<th>Hip adduction weaker than abduction</th>
<th>Hamstrings weaker than extension</th>
<th>Shoulder adduction weaker than abduction</th>
<th>Biceps weaker than triceps</th>
<th>Wrist flexion weaker than extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a Calves, brachioradialis</td>
<td>Mild weakness, eye closure</td>
<td>Yes 4 vs 5</td>
<td>Yes 4 vs 4 vs 5</td>
<td>Yes 2 vs 4</td>
<td>Yes 4 vs 5</td>
<td>Equal 4 vs 4 vs 5</td>
<td>Yes 4 vs 5</td>
<td>Equal 5 vs 5</td>
</tr>
<tr>
<td>1b Calves, brachioradialis</td>
<td>Mild weakness, eye closure</td>
<td>Yes 4 vs 5</td>
<td>Yes 4 vs 4 vs 5</td>
<td>Yes 3 vs 4</td>
<td>Yes 4 vs 5</td>
<td>Equal 4 vs 4 vs 5</td>
<td>Yes 4 vs 4</td>
<td>Equal 4 vs 4 vs 5</td>
</tr>
<tr>
<td>2 Calves</td>
<td>No</td>
<td>Yes 4 vs 5</td>
<td>No 4 vs 4 vs 5</td>
<td>Yes 2 vs 3</td>
<td>No 4 vs 4 vs 4</td>
<td>Equal 4 vs 4 vs 5</td>
<td>Yes 4 vs 4</td>
<td>Equal 4 vs 4 vs 5</td>
</tr>
<tr>
<td>3 Calves, brachioradialis</td>
<td>No</td>
<td>Yes 4 vs 5</td>
<td>Yes 1 vs 4</td>
<td>Equal 1 vs 1</td>
<td>Yes 3 vs 4</td>
<td>Yes 4 vs 4</td>
<td>Equal 4 vs 4</td>
<td>Yes 4 vs 4</td>
</tr>
<tr>
<td>4 Calves, brachioradialis, anterior tibial muscles, triceps</td>
<td>Mild weakness, eye closure</td>
<td>Yes 4 vs 4</td>
<td>Yes 0 vs 2</td>
<td>Equal 1 vs 1</td>
<td>Equal 3 vs 3</td>
<td>Yes 2 vs 3</td>
<td>Yes 3 vs 4</td>
<td>Equal 4 vs 4</td>
</tr>
<tr>
<td>5a Calves</td>
<td>Mild weakness, eye closure</td>
<td>Yes 4 vs 5</td>
<td>Yes 2 vs 4 vs 4</td>
<td>Yes 2 vs 4</td>
<td>Yes 4 vs 4</td>
<td>Equal 4 vs 4</td>
<td>Equal 4 vs 4</td>
<td>Yes 3 vs 4</td>
</tr>
<tr>
<td>5b Calves, brachioradialis, triceps</td>
<td>Mild weakness, eye closure</td>
<td>Yes 4 vs 5</td>
<td>Yes 2 vs 4 vs 4</td>
<td>Yes 2 vs 4</td>
<td>Yes 4 vs 4</td>
<td>Equal 4 vs 4</td>
<td>No 4 vs 4</td>
<td>Yes 4 vs 4</td>
</tr>
</tbody>
</table>

Muscle scores are given according to the MRC scale.

and β sarcoglycan on the same Western blot was normal (Fig. 2). In contrast, sections labelled with the same antibody failed to demonstrate a comparable reduction. Immunocytochemical analysis with the 300 kDa-type merosin antibody Mer3/22B2 was also normal. Biopsies were obtained from both sisters, Patients 1a and 1b. They showed identical results.

Genetic studies

Haplotypes around the merosin locus were constructed for Patients 1a and 1b and their parents and the families of Patients 2 and 3. For Patients 1a and 1b, haplotyping showed that the two sisters had inherited the same paternal haplotype but opposite maternal haplotypes around the merosin locus. Patient 3 and his unaffected sister and brother shared the same haplotype around the merosin locus. The analysis for Patient 2 and her family was not informative. These results are consistent with absence of linkage to the merosin gene.

Discussion

We describe a group of patients with a ‘limb-girdle’ pattern of muscular dystrophy who have the unusual finding of a reduction of merosin on immunoblotting but not immunocytochemical analysis of muscle biopsy samples. Genetic linkage analysis and the results of the MRI scans performed in three affected patients indicated that the merosin gene itself is not involved in this group.

It is intriguing that the abnormality was picked up only on Western blots and not immunocytochemistry. Unlike fixed frozen sections, where the protein is in a native state, electrophoresis and blotting involves extensive processing: homogenization in the presence of detergents and reducing agents, boiling and centrifugation. It is known that the large merosin molecule is prone to fracture since the 80 kDa polypeptide detected with this antibody is a natural fragment and not a genetically distinct subunit.

It is possible that these patients share a predisposition to merosin fragility which is exposed by the tissue processing required to perform the immunoblot analysis, or that the deficit is in a protein which binds merosin and would normally stabilize it. That this is a genetic disorder is suggested by the presence of two sibling pairs with identical clinical features and identical finding of absent merosin on blots in muscle biopsies taken and analysed at different times. We believe therefore that the finding is unlikely to be artefactual. Merosin is usually an extremely stable protein; the extracellular matrix proteins tend to persist a long time after other proteins have degraded. For example, if samples have thawed merosin is still detectable at normal levels when dystrophin and the sarcoglycans have been lost. All of the
biopsies reported here were in good condition, and in >100 other patients with a diagnosis of limb-girdle muscular dystrophy with normal dystrophin and sarcoglycans this merosin abnormality was not detected.

Until recently the merosin-negative muscular dystrophies have been regarded as a fairly homogeneous group. The diagnostic criteria for congenital muscular dystrophy are clear (Dubowitz, 1997): hypotonia from birth or noticed soon after, with severe muscle weakness and contractures. Patients with partial merosin deficiency tend to have milder disease, but with the exception of the family reported by Tan et al. (1997), still tend to present in early childhood. Consistent amongst all of the merosin-negative families reported is the finding of cerebral white matter changes on MRI scanning. Families with merosin deficiency unlinked to chromosome 6q2 have not yet been described (though families are often small) and mutations in the merosin gene have been demonstrated in cases of both total and partial merosin deficiency (Helbling-Leclerc et al., 1995; Nissinen et al., 1996). There is as yet no pathological or histochemical marker for the cases of congenital muscular dystrophies with normal merosin. These cases are often milder again than the patients with merosin reduction. MRI scans are normal and families are rarely big enough to be tested for linkage. In a small group of merosin-positive patients a deficiency of α-actinin 3 has been described (North and Beggs, 1996). These patients quite clearly fulfil the criteria for congenital muscular dystrophy, unlike the patients reported here. It is likely that the merosin-positive cases of congenital muscular dystrophy represent a genetically heterogeneous group.

The patients we report here do not have congenital muscular dystrophy (Dubowitz, 1997). Their presentation and clinical features fit with the phenotype of ‘limb-girdle’ muscular dystrophy in its broadest definition (Bushby, 1997). They share many characteristics—normal early mobility in all but one patient with reported onset of muscle problems most often in adult life, normal IQ, steady progression of weakness and, strikingly, a pattern of muscle involvement, especially in the lower limbs, which is relatively constant from patient to patient. Investigations in all patients were consistent with the diagnosis of a muscular dystrophy, with progressive involvement of the respiratory muscles but no related cardiac problems. Although the group is small, the female predominance is striking and cannot easily be explained. There is no gender bias in the biopsies we investigate by immunoblotting, and in fact more men have been investigated in this way than women.

The limb-girdle muscular dystrophies can now be studied in much greater detail than was previously possible: forms of recessive LGMD can be defined by mutations in calpain 3 (Richard et al., 1995), α, β and δ, and γ sarcoglycan (Vainzof et al., 1996) or by linkage to the LGMD2B locus on chromosome 2p13 (Bashir et al., 1996). Other forms of LGMD undoubtedly remain to be characterized, and even where a deficiency of a sarcoglycan can be demonstrated it may not always be possible to define the causative mutation (Duggan et al., 1997), suggesting the interaction of yet other genes. Clinical correlates for at least some of the genetically defined subgroups of LGMD are beginning to emerge, and it would appear that our patients have some features which...
Table 3 A summary of the investigations performed on the group

<table>
<thead>
<tr>
<th>Patient</th>
<th>Creatine kinase (IU/l) (age in years)</th>
<th>EMG</th>
<th>Muscle histology</th>
<th>Brain MRI scan</th>
<th>FVC (litres) (% predicted)</th>
<th>Cardiac investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>2230 (40)</td>
<td>Mild chronic patchy necrotizing myopathy</td>
<td>Increased range of fibre diameter; foci of necrosis and phagocytosis; increased internal nuclei; occasional hyaline fibres</td>
<td>Normal</td>
<td>2.32 (86%)</td>
<td>Normal ECG</td>
</tr>
<tr>
<td>1b</td>
<td>1123 (45)</td>
<td>Not done</td>
<td>Increased range of fibre diameter; some necrotic fibres</td>
<td>Not done</td>
<td>0.36 (85%)</td>
<td>Coarctation of aorta</td>
</tr>
<tr>
<td>2</td>
<td>2870 (29)</td>
<td>Chronic active necrotizing myopathy</td>
<td>Wide range of fibre diameter; many foci of necrosis; Some flocular fibres</td>
<td>Normal</td>
<td>2.82 (78%)</td>
<td>Normal ECG</td>
</tr>
<tr>
<td>3</td>
<td>3696 (19)</td>
<td>Chronic mildly active necrotizing myopathy</td>
<td>Increased range of fibre diameter; occasional hyaline fibres; some fibres necrotic</td>
<td>Normal</td>
<td>1.53 (30%)</td>
<td>Normal ECG</td>
</tr>
<tr>
<td>4</td>
<td>3270 (19)</td>
<td>Chronic rather inactive necrotizing myopathy</td>
<td>Considerable variation in fibre size, some fibre splitting and internal nuclei</td>
<td>Not done</td>
<td>1.37 (35%)</td>
<td>ECG showed relatively poor R wave progression</td>
</tr>
<tr>
<td>5a</td>
<td>&gt;1500 (37)</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>3.14 (93%)</td>
<td>Normal echo and ECG</td>
</tr>
<tr>
<td>5b</td>
<td>1545 (34)</td>
<td>Chronic myopathy</td>
<td>Variation in muscle fibre diameter; some necrosis</td>
<td>Not done</td>
<td>2.20 (71%)</td>
<td>Normal echo and ECG</td>
</tr>
</tbody>
</table>

EMG and muscle biopsy were not repeated in Patient 5a after the diagnosis was established in her sister. FVC = forced vital capacity. Percentage of predicted value was based on the patient’s sex, age and height.

distinguish them clinically from the other groups. For example, in LGMD2A, scapular winging and early Achilles tendon contractures, both absent in our patients, are described (Fardeau et al., 1996). In LGMD2B, which may well prove to be allelic to the distal muscular dystrophy Miyoshi myopathy (Bejaoui et al., 1995), careful clinical studies can often detect presymptomatic distal weakness (Mahjneh et al., 1996), which again was not present in the patients reported here.

It is possible to define the genetic fault, protein phenotype and clinical pattern of an increasing number of muscular dystrophies. The tools are now available to study molecular or protein abnormalities amongst the broader spectrum of as yet uncharacterized muscular dystrophies. The families we describe here represent another subset amongst the ‘LGMD’ group which may be genetically distinct. Candidate genes or proteins potentially responsible for the disease in these patients would include any proteins which interact with merosin and affect its stability or which might be responsible for its increased breakdown.

Fig. 2 Immunoblot showing Patient (1–4) and control muscle (C) labelled with antibodies to dystrophin (D), merosin (M), α sarcoglycan (αS) and β dystroglycan (βD) as described in the text.
Acknowledgements
We thank Dr Margaret Johnson for immunocytochemical analysis and Dr Peter Hudgson for referring one of the patients. Our work is supported by the Muscular Dystrophy Group of Great Britain, the Medical Research Council of Great Britain and Action Research.

References

Anon. Aids to the examination of the peripheral nervous system. 443–6.


Late onset muscular dystrophy with cerebral white matter changes due to partial merosin deficiency. Neuromuscul Disord 1997; 7: 85–9.


Received August 8, 1997. Revised September 30, 1997
Accepted November 12, 1997