INVITED REVIEW
Making sense of the limb-girdle muscular dystrophies

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Summary
The clinical heterogeneity which has long been recognized in the limb-girdle muscular dystrophies (LGMD) has been shown to relate to the involvement of a large number of different genes. At least eight forms of autosomal recessive LGMD and three forms of autosomal dominant disease are now recognized and can be defined by the primary gene or protein involved, or by a genetic localization. These advances have combined the approaches of positional cloning and candidate gene analysis to great effect, with the pivotal role of the dystrophin-associated complex confirmed through the involvement of at least four dystrophin-associated proteins in different subtypes of autosomal recessive LGMD (the sarcoglycanopathies). Two novel mechanisms may have to be postulated to explain the involvement of the calpain 3 and dysferlin genes in other forms of LGMD. Using the diagnostic tools which have become available as a result of this increased understanding, the clinical features of the various subtypes are also becoming clearer, with useful diagnostic and prognostic information at last available to the practising clinician.

Keywords: limb-girdle; LGMD; sarcoglycan; calpain; dysferlin

Abbreviations: DGC = dystrophin–glycoprotein complex; FSHD = facioscapulohumeral muscular dystrophy; LGMD = limb-girdle muscular dystrophies

Introduction
The quest for order in the group of diseases designated ‘limb-girdle’ muscular dystrophy (LGMD) in the classifications of the 1950s has led in various directions over the past 40 years (Walton and Nattrass, 1954; Walton, 1955, 1956; Walton and Gardner-Medwin, 1991). For much of this time, LGMD has been an unpopular and poorly defined diagnostic term, carrying with it little in the way of information for either the clinician or the patient. Even in those initial classifications, the description of LGMD emphasized heterogeneity, including the potential for either autosomal recessive or autosomal dominant inheritance, onset of disease in either the pelvic or shoulder girdle musculature, and slow or more rapid progression. The rationale for a separate group was based mainly on the distinction between these patients and those with X-linked disease (now recognized as dystrophinopathy) or facioscapulohumeral muscular dystrophy (FSHD). Exclusion of alternative diagnoses was the only diagnostic tool, and for a while the existence of a separate ‘LGMD’ category at all was questioned (Norman et al., 1989; Walton and Gardner-Medwin, 1991). The situation has now changed dramatically: while the exclusion of alternative diagnoses remains important, there is now a realistic possibility that a precise molecular diagnosis can be achieved in patients presenting with a progressive proximal muscular dystrophy. This ability brings with it increased knowledge about the likely progression and complications of the disease and the opportunity to offer appropriate genetic counselling where required.

This review will describe briefly the progress made to reach this point, and then focus on the disorders that can be discerned within the overall LGMD category. Particular emphasis will be placed on the diagnostic techniques which may now be applied, the clinical features which can help in suggesting a diagnosis and the likely frequency of the different types of LGMD amongst various patient groups.

Linkage analysis—the first step in resolving and revealing heterogeneity
The explosion in our knowledge about the underlying molecular mechanisms involved in producing limb-girdle muscular dystrophy owes much to the techniques of positional
childhood autosomal recessive muscular dystrophy. Heterogeneity in this group, too, with adult-onset cases of proximal muscular dystrophy demonstrated phenotypic variability (Bushby and Beckmann, 1995). The study of families with childhood-onset progressive muscular dystrophy became clear that the level of heterogeneity was likely to be great, with several independent loci identified, a locus-based approach has been obtained through the subsequent proof of cloning. Prior to this molecular era, any reviews of clinical series of LGMD patients demonstrated the huge variability seen within this diagnostic category, meaning that any assumptions of homogeneity for the purposes of linkage analysis would be clearly suspect. The only groups in whom any kind of homogeneity could be assumed were the occasional large family or population isolate in whom a common genetic basis for the disease was likely (Jackson and Carey, 1961; Jackson and Strehler, 1968; Shokeir and Kobrinsky, 1976; Ben Hamida et al., 1983). Careful identification of such large families and linkage analysis in these pedigrees provided the confirmation that a huge amount of genetic heterogeneity underpinned the observed clinical heterogeneity, with at least three loci implicated in autosomal dominant LGMD (Speer et al., 1991; van der Kooi et al., 1992; Minetti et al., 1998) and at least eight in autosomal recessive disease (Beckmann et al., 1991; Azibi et al., 1993; Bashir et al., 1994; Bonnemann et al., 1995; Campbell, 1995; Lim et al., 1995; Nigro et al., 1996; Moreira et al., 1997; Weiler et al., 1998). Validation of this approach has been obtained through the subsequent proof of the widespread geographical distribution of these genetically distinct diseases among those patients identified as having a ‘limb-girdle muscular dystrophy’ phenotype—these rare, genetically homogeneous families have provided molecular answers which can now be widely extrapolated. When it became clear that the level of heterogeneity was likely to be great, with several independent loci identified, a locus-based classification was proposed by a consortium meeting under the auspices of the European Neuromuscular Centre, with the dominant LGMD loci designated LGMD1A, B, C, etc. and the recessive forms as LGMD2A, B, etc. in the order of their identification (see Table 1) (Bushby and Beckmann, 1995). The study of families with childhood-onset progressive proximal muscular dystrophy demonstrated phenotypic heterogeneity in this group too, with adult-onset cases described in the same families. Because of this, the designation ‘severe childhood autosomal recessive muscular dystrophy’ (SCARMD) as a disease and locus description was dropped and the loci already identified for this group were incorporated into the LGMD classification.

### From loci to genes and proteins

The subsequent period of gene identification combined the techniques of positional cloning and candidate gene analysis. The identification of a group of proteins associated with dystrophin (the protein missing or reduced in Duchenne and Becker muscular dystrophy) in the muscle fibre membrane was a milestone in this work. The component members of this protein complex (the dystrophin–glycoprotein complex or DGC) were immediate candidates for involvement in the autosomally inherited muscular dystrophies. Intensive work to characterize these proteins and their genes in various laboratories around the world has led to rapid progress (Campbell and Kahl, 1989; Yoshida and Ozawa, 1990; Yoshida et al., 1994; Matsumura and Campbell, 1994; Madhavan and Jarrett, 1995; Straub and Campbell, 1997; Ozawa et al., 1998). The DGC comprises three groups of proteins: dystroglycan, the sarcoglycans and the syntrophins (Fig. 1). α- and β-Dystroglycan span the sarcolemma, interacting directly with dystrophin inside the sarcolemma and laminin in the extracellular matrix. Total or partial deficiency of laminin A2 is responsible for ~50% of cases of congenital muscular dystrophy (Hillaire et al., 1994; Helbling-Leclerc et al., 1995; Philpot et al., 1995). Dystroglycan is widely expressed, and mice homozygous for dystroglycan mutations are not viable (Williamson et al., 1996). Another protein which appears to be

### Table 1 Types of LGMD defined by their genetic basis

<table>
<thead>
<tr>
<th>Gene locus</th>
<th>Locus name</th>
<th>Gene product (symbol)</th>
<th>Previous nomenclature</th>
</tr>
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<tbody>
<tr>
<td>5q22-24</td>
<td>LGMD1A</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>1q11-21</td>
<td>LGMD1B</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>p25</td>
<td>LGMD1C</td>
<td>Caveolin 3 (CAV3)</td>
<td>–</td>
</tr>
<tr>
<td>15q15.1-21.2</td>
<td>LGMD2A</td>
<td>Calpain 3 (CAPN3)</td>
<td>–</td>
</tr>
<tr>
<td>2p13</td>
<td>LGMD2B</td>
<td>Dysferlin (DYSF)</td>
<td>–</td>
</tr>
<tr>
<td>17q11-12</td>
<td>LGMD2G</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>9q31-33</td>
<td>LGMD2H</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>17q12-21.33</td>
<td>LGMD2D</td>
<td>α-Sarcoglycan</td>
<td>50 DAG, adhalin, A2, SCARMD2</td>
</tr>
<tr>
<td>4q12</td>
<td>LGMD2E</td>
<td>β-Sarcoglycan (SGCB)</td>
<td>A3b</td>
</tr>
<tr>
<td>13q13</td>
<td>LGMD2C</td>
<td>γ-Sarcoglycan (SGCG)</td>
<td>35 DAG, A4, SCARMD1</td>
</tr>
<tr>
<td>5q33-34</td>
<td>LGMD2F</td>
<td>δ-Sarcoglycan (SGCD)</td>
<td>–</td>
</tr>
</tbody>
</table>

Where the gene product and symbol are known, they are given together with any previously used nomenclature. SCARMD = severe childhood autosomal recessive muscular dystrophy.
The sarcoglycans are transmembrane proteins within the DGC, and are of unknown function (Worton, 1995; Straub and Campbell, 1997; Ozawa et al., 1998). Primary genetic defects in one of α, β, γ and δ sarcoglycan are now established as the cause for four subtypes of autosomal recessive LGMD (Roberds et al., 1994; Noguchi et al., 1995; Lim et al., 1995; Bonnemann et al., 1995; Nigro et al., 1996). A fifth sarcoglycan (ε-sarcoglycan) has recently been identified through its homology to α-sarcoglycan, and its gene has been mapped to chromosome 7q21 (McNally et al., 1998b). It is not yet known to be involved in any muscle disorders. All the known sarcoglycans share a number of features, though sequence homology is highest between γ- and δ-sarcoglycan and between α- and ε-sarcoglycan (McNally et al., 1998b). They all contain a small intracellular domain which may be located at the C-terminus (α-sarcoglycan) or the N-terminus (β-, γ- and δ-sarcoglycan). Each has a single transmembrane domain and a large extracellular domain which contains potential N-glycosylation signals (Ozawa et al., 1998). Expression of α- and γ-sarcoglycan is limited to striated muscle; β-, δ- and ε-sarcoglycan are expressed more widely. Biochemical and ultrastructural analysis and evidence from patients with sarcoglycan deficiency suggest that the sarcoglycans form a distinct subcomplex (Yoshida et al., 1994; Vainzof et al., 1996; Iwata et al., 1996; Inoue et al., 1996; Cullen et al., 1996; McNally et al., 1998b). As loss or deficiency of any of one of the four sarcoglycans causes muscular dystrophy, these proteins must play a critical role in the maintenance of membrane integrity.

While this work has served to emphasize the pivotal role of the dystrophin-associated complex not all forms of LGMD can be accounted for by mutations in these genes. The other forms of autosomal recessive LGMD for which the genes have been identified by a pure positional cloning strategy have suggested the existence of two potentially novel pathways for the production of muscular dystrophy. Following the construction of a meticulous physical and transcript map of the chromosome 15 region to which the LGMD2A gene was localized, a proteolytic enzyme, calpain 3, was shown to be involved in this disorder (Beckmann et al., 1991, 1993; Fougerousse et al., 1994; Allamand et al., 1995; Chiannilkulchai et al., 1995; Richard et al., 1995). Calpain 3 is the muscle-specific member of a family of calcium-dependent proteases. It has four protein domains similar to those found in the ubiquitous calpains, as well as three unique regions (NS, IS1 and IS2) which may confer its muscle specificity (Sorimachi and Suzuki, 1992). Calpain 3 interacts with titin (connectin) via one of these muscle-specific sequences, IS2, which also contains a nucleus translocation signal-like sequence (Sorimachi et al., 1995; Kinbara et al., 1997). The protein appears to exist in both the cytosol and the nucleus, leading to the suggestion that it may have a role in controlling the level of muscle-specific transcription factors and therefore the regulation of muscle cell differentiation (Kinbara et al., 1997). Whether this truly is the role of calpain 3 in muscle and how the absence or deficiency of calpain 3 causes muscular dystrophy remain unknown.
The recently identified protein involved in chromosome 2-linked LGMD (LGMD2B), dysferlin, is the product of a novel mammalian gene with homology detectable only to a nematode protein involved in spermatogenesis (Achanzar and Ward, 1997; Bashir et al., 1998). It localizes to the muscle fibre membrane and is expressed from very early in human development (Anderson et al., 1999). Dysferlin was simultaneously shown to be involved in two forms of muscular dystrophy: LGMD2B and the predominantly distal muscular dystrophy, Miyoshi myopathy (Bashir et al., 1998; Liu et al., 1998). The underlying means by which mutations in this gene are responsible for these different phenotypes is not known. Muscular dystrophies are traditionally classified by their patterns of muscle involvement: the involvement of this single gene in two phenotypically different forms of muscular dystrophy challenges this mode of classification.

As the molecular background of these disorders becomes elucidated and clear groups become discernible, the classification is evolving again to encompass this new and precise information. Even more satisfying to the clinician is that, because categories can be confirmed by gene or protein analysis, clinical correlates are beginning to emerge which help to define the various subgroups. A summary of the nomenclature of the group, including current and previous designations, is shown in Table 1. Where the gene and protein involved in the disorder are known, these provide the preferred designations, is shown in Table 1. Where the gene and protein involved in the disorder are known, these provide the preferred nomenclature. The locus nomenclature remains appropriate where the genes involved have not yet been cloned. For clarity, this review will concentrate on the most recent nomenclature.

The autosomal recessive limb-girdle muscular dystrophies
Sarcoglycanopathies (LGMD2C-2F)
Diagnostic considerations

Analysis of a muscle biopsy sample using specific antibodies to the various sarcoglycans has become the primary diagnostic tool in investigating this group of diseases (Anderson, 1996; Sewry et al., 1996). In some cases immunostaining will suggest which sarcoglycan is primarily involved, e.g. if labelling with one antibody is absent and labelling with the others is normal or reduced. The tight association of the proteins of the DGC may, however, cause problems in establishing a definitive diagnosis. So, for example, abnormal dystrophin has traditionally been regarded as an exclusion criterion for LGMD; however, it is clear that in at least some cases of sarcoglycanopathy, dystrophin analysis is not completely normal (Vainzof et al., 1996; Jones et al., 1998). In our experience, the reduction in dystrophin in these cases is much less than would be expected from the severity of the phenotype, and is accompanied by a much more dramatic deficiency of one or more of the sarcoglycans (R. Pogue, L. V. B. Anderson and K. Bushby, unpublished results). The most worrying diagnostic confusion may arise with isolated females having muscular dystrophy, who, if a full range of antibodies is not used, may be misdiagnosed as manifesting carriers of Duchenne muscular dystrophy, with potentially disastrous results for genetic counselling (Bushby et al., 1997) (Fig. 2). Clearly, however, the issue of misdiagnosis in males (between Becker’s muscular dystrophy and sarcoglycanopathy) can also be important.

A second potentially important pitfall is that a single antibody (most commonly to α-sarcoglycan alone) is often used to screen for sarcoglycan deficiency, on the premise that a primary loss of any one component of the sarcoglycan complex will lead to a secondary reduction of all of the others. This is not necessarily a universal finding, especially where the primary deficiency is of α- or γ-sarcoglycan (Vainzof et al., 1996; Jones et al., 1998; Ozawa et al., 1998). Deficiencies of β- and δ-sarcoglycan do seem to be more uniformly associated with a total deficiency of the complex (Bonnemann et al., 1996; Vainzof et al., 1996). Use of all four sarcoglycan antibodies is therefore most likely to give a clear answer. Multiplex Western blotting may also be very helpful in this situation (Anderson and Davison, 1999).

Where a sarcoglycan deficiency has been suggested by an abnormality of immunostaining, in families where the structure is suitable, haplotype or linkage analysis using polymorphic markers linked to the various sarcoglycan genes may also help. However, where the primary abnormality needs to be established with certainty (e.g. to be able to provide carrier testing or prenatal diagnosis), mutation analysis will continue to be necessary. In all of the sarcoglycan genes a wide range of mutations have been described (Table 2), with recurrent mutations common only in α-sarcoglycan (Carrie et al., 1997). In α-sarcoglycan it is logical to start by testing for the common mutations; in most situations, however, an exon-by-exon approach to mutation detection cannot be avoided, except in populations where a founder mutation is known to be present (Lim et al., 1995; Noguchi et al., 1995; Piccolo et al., 1996). Multiplexing techniques help to reduce the time taken for this sort of analysis, at least to some degree (Pogue et al., 1998). It is important to note that not all patients ascertained with absent or reduced sarcoglycan staining on a muscle biopsy can be shown to have a mutation in one of the sarcoglycan genes by standard techniques. One study, which used α-sarcoglycan labelling as the tool to ascertain cases of all of the different types of sarcoglycanopathy, found mutations in only 39% of partial deficiency cases, compared with 90% of the cases where α-sarcoglycan was completely absent (Duggan et al., 1997a, b). This may have been due to a high frequency of types of mutation missed by these techniques, mutations involving parts of the gene not studied in this analysis, or the involvement of a still unidentified gene, primary abnormalities of which lead to the secondary sarcoglycan deficiency.

It is not yet known whether subtle differences in the pattern of muscle involvement may distinguish the various sarcoglycanopathies (Noguchi et al., 1995; McNally et al.,
Table 2 Types and distribution of mutations in the known autosomal recessive LGMD genes

<table>
<thead>
<tr>
<th>Gene product (symbol)</th>
<th>Number of exons</th>
<th>Type of mutation</th>
<th>Distribution of mutations, common mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Sarcoglycan (SGCA)</td>
<td>8</td>
<td>Nonsense mutations, small splice site duplications</td>
<td>Exon 3 most common site for mutations. Otherwise mostly in extracellular domain. Several recurrent mutations described. R77C mutations especially frequent (~40% of mutations).</td>
</tr>
<tr>
<td>β-Sarcoglycan (SGCB)</td>
<td>6</td>
<td>Missense and truncating mutations seen</td>
<td>Missense mutations clustered in immediate extracellular domain. Founder mutation in the Amish population (T151R).</td>
</tr>
<tr>
<td>γ-Sarcoglycan (SGCG)</td>
<td>8</td>
<td>Often small deletions, relatively few missense mutations</td>
<td>Founder mutations described in North African (del 521T) and European gypsy population (C283Y).</td>
</tr>
<tr>
<td>δ-Sarcoglycan (SGCD)</td>
<td>9</td>
<td>Single nucleotide substitution or deletions</td>
<td>Not yet reported.</td>
</tr>
<tr>
<td>Calpain 3 (CAPN3)</td>
<td>24</td>
<td>Predominantly missense mutations, small deletions and splice site mutations</td>
<td>Mutations distributed throughout gene. Founder mutations in Amish, Reunion, Basque and Turkish populations. Occasional recurrent mutations seen on different genetic backgrounds but not at usefully high frequency.</td>
</tr>
<tr>
<td>Dysferlin (DYSF)</td>
<td>~55</td>
<td>Few mutations described to date: missense, small deletions, duplication.</td>
<td>Mutations appear to be found across gene. Apparently founder mutation in Libyan Jewish population.</td>
</tr>
</tbody>
</table>

Note the variability of mutation seen in most of these genes and the large number of exons to be tested, which can make it a laborious and time-consuming task to detect mutations in any given patient. The distinction between recurrent mutations (where the same mutation is seen on a different genetic background) and founder mutations (where the same mutation is seen at an unusually high frequency in a particular population) is important. References to these mutations are as in the text.

1996). No very clear correlations have yet been shown between the type of mutation seen and the clinical course, although this is, of course, complicated by the fact that, except within inbred populations, most affected individuals are compound heterozygotes for two different mutations. It is striking that so many mutations in these genes are missense mutations and that most mutations are found in the portion of the genes encoding the large extracellular domains. It has been suggested that missense mutations in these membrane proteins may lead to aberrant protein processing and degradation. From this model, missense mutations in a member of the complex might cause improper processing or assembly of the whole group, leading to rapid turnover of the mutated protein (Campbell, 1995).

Clinical characteristics of the sarcoglycanopathies  
α-Sarcoglycanopathy (LGMD2D). A number of reports contain variably detailed information about the clinical features of patients with genetically proven cases of α-sarcoglycanopathy (Romero et al., 1994; Ljunggren et al., 1995; Piccolo et al., 1995; Kawai et al., 1995; Carrie et al., 1997; Passos-Bueno et al., 1999), and the paper of Eymard and colleagues is a very comprehensive review (Eymard et al., 1997) from which a number of useful clinical guidelines can be established. α-Sarcoglycanopathy occurs world-wide. Patients most commonly present with difficulty running and climbing stairs, but, as in dystrophinopathy, presentation with muscle cramps or exercise intolerance has been reported. Toe-walking was an early feature in fewer than half of the patients (Fig. 2). Presymptomatic massive elevation of creatine kinase and elevation of creatine kinase to around the upper limit of normal in carriers has been reported, but the predictive value of creatine kinase levels in determining carrier status is not known. The age at onset among the 20 patients reported by Eymard and colleagues (Eymard et al., 1997) ranged from 3 to 15 years, with a mean of 8.5 years. Other reports highlighted delayed walking in some patients (Kawai et al., 1995), while adult-onset cases have also been reported, including an asymptomatic patient aged 36 years (Fanin et al., 1997). These adult cases are seen less commonly than those with childhood onset. While patients rarely have symptomatic upper limb weakness at presentation, most have scapular winging, often to a more pronounced degree than is seen at a similar stage in patients with dystrophinopathy. Early involvement of the deltoid was also noted, and weakness of the biceps with relative preservation of the triceps. In the lower limbs at presentation, femoral muscles are less involved than the pelvic group. The quadriceps and hamstrings are usually equally affected, as distinct from dystrophinopathy, in which quadriceps weakness predominates. Any early distal lower limb involvement tends to be in the anterior tibial group. Calf hypertrophy, occasionally also involving other muscle groups, is seen in almost all patients at some stage. As the disease progresses the selectivity of muscle weakness
to describe a genetically pure sarcoglycan genes reported, these reports cannot be assumed to exist in this population, with mutations in different sarcoglycan genes. The clinical reviews of the North African autosomal recessive muscular dystrophy population predate this analysis available. The clinical descriptions of LGMD2C patients with genetically proven γ-sarcoglycanopathy are few in number so far, but reports of patients with proven δ-sarcoglycanopathy are rare. Reports of patients with proven γ-sarcoglycanopathy are rare. The clinical reviews of the North African autosomal recessive muscular dystrophy population predate this analysis (Ben Hamida et al., 1983), and as genetic heterogeneity is known to exist in this population, with mutations in different sarcoglycan genes reported, these reports cannot be assumed to describe a genetically pure γ-sarcoglycanopathy population. On the whole, the clinical descriptions of γ-sarcoglycanopathy which do exist, however, tend to make the same points as are made in the α-sarcoglycanopathy literature—presentation with proximal lower limb weakness, frequent calf hypertrophy, variability in severity which may be intrafamilial, a predominance of childhood onset, usually a lack of cardiac complications and normal intelligence.

β-Sarcoglycanopathy (LGMD2E). An extremely broad range of clinical severity has been reported in the few patients with genetically proven β-sarcoglycanopathy. Amongst the Amish families shown to be carrying the same homozygous missense mutation (Lim et al., 1995b), intrafamilial variability was marked: age at onset was at a mean age of 7.6 years, and age at loss of ambulation ranged from 12 to 38 years (mean, 26 years). An even milder phenotype has been reported in an Amish family heterozygous for two other missense mutations. The onset of disease in these patients was in adult life with proximal weakness and enlarged calves, and independent ambulation was still possible in the sixth decade (Duclos et al., 1998). It is clear, however, that a much more severe disease may also occur in β-sarcoglycanopathy (Bonnemann et al., 1995, 1996). These patients presented in early childhood with similar symptoms, but were confined to a wheelchair by their early teens. While one of the mutations described in these patients was a frameshifting deletion, the others were missense. The contrast between the severity of the phenotype in these patients compared with the mild phenotype in the Amish patients with missense mutations suggests that the part of the gene in which these mutations were found (immediately beyond the transmembrane domain) may be particularly sensitive to the effect of mutations which might affect the secondary structure of the protein.

δ-Sarcoglycanopathy (LGMD2F). Reports of patients with proven δ-sarcoglycanopathy are few in number so far, but have shown a very severe clinical course (Nigro et al., 1996b; Duggan et al., 1997b). Age at onset ranged from 4 to 10 years, with confinement to a wheelchair between 9 and 16 years and death between 9 and 19 years. These patients may potentially be at particular risk of cardiac complications—the Syrian cardiomyopathic hamster has a mutation in δ-sarcoglycan and may manifest either a dilated or a hypertrophic cardiomyopathy, though the skeletal muscles are not prominently involved (Sakamoto et al., 1997; Nigro et al., 1997). Genetically uncharacterized cases of sarcoglycan deficiency and cardiomyopathy may represent involvement of this gene (Fadic et al., 1996).

Calpain-deficient LGMD (calpainopathy) (LGMD2A)

Diagnostic considerations

The gold standard in confirming the diagnosis of calpainopathy is the demonstration of mutations in the CAPN3 gene, though this may be guided by linkage to chromosome 15 in families where the structure is suitable. As with the sarcoglycan genes, the mutations may be missense...
or nonsense substitutions, small deletions and small insertions, spread widely over the gene (Table 2) (though missense mutations are relatively infrequent in the first protein domain). Even in the original population on the island of La Reunion, in whom the gene was first localized to chromosome 15 and where mutations in CAPN3 were subsequently identified, a surprising heterogeneity of mutations was discovered, known as the ‘Reunion paradox’ (van Ommen, 1995; Richard et al., 1995). In this highly isolated population with LGMD, which is apparently descended from a small founder population, it was possible to identify six distinct disease-associated haplotypes (and subsequently mutations). This unexpected finding remains unexplained. The original suggestion that the disease might be more properly regarded as a digenic rather than a monogenic disorder has not been proven (Beckmann, 1996; Zlotogora et al., 1996; Richard et al., 1997). In other populations where calpainopathy is seen, it behaves exactly as predicted from a straightforward autosomal recessive model. Only a few recurrent mutations (Table 2) have been reported—one does appear to be conserved with a founder haplotype in families from such diverse geographical origins as America, Brazil and Reunion (Richard et al., 1997). A common mutation in Turkey and another in the Basque region of Spain have also been described (Topaloglu et al., 1997; Dincer et al., 1997; Urtasun et al., 1998).

Calpainopathy patients have normal dystrophin and sarcoglycan labelling on muscle biopsies. Initial studies had shown that calpain 3 was undetectable in normal muscle, leading to the suggestion that the protein was very rapidly subjected to autoproteolysis and consequently had a very short half-life, as RNA levels were high but protein levels were not (Sorimachi et al., 1993). Subsequent studies have shown that calpain 3 is detectable in skeletal muscle (Spencer et al., 1997) and a recently generated monoclonal antibody to calpain 3 can now be used for immunoblotting of muscle biopsy samples, though the antibody does not work on muscle sections (Anderson et al., 1998). Given the heterogeneity of the mutations seen in this very large gene, examination of a muscle biopsy will probably still prove a more straightforward starting point for diagnosis than mutation detection, though this will remain necessary for carrier detection and prenatal diagnosis where this is required (Restagno et al., 1996).

Clinical characteristics of calpainopathy

A distinct and clear phenotype is emerging from the literature about patients with genetically proven calpainopathy (Fardeau et al., 1996; Topaloglu et al., 1997; Richard et al., 1997; Dincer et al., 1997; Urtasun et al., 1998; Passos-Bueno et al., 1999). The disease is predominantly symmetrical and atrophic, with prominent calves seen in only a minority of cases in most series. Achilles tendon contractures may be an early sign, and contractures elsewhere, including the spine, may also be prominent (C. Pollitt et al., unpublished observations). Pelvic girdle weakness is present and symptomatic from the onset, but often with very striking sparing of the hip abductors, even relatively late in the course of the disease. These features, combined with a marked laxity of the abdominal muscles, lead to the development of a particularly characteristic stance in these patients (Fig. 3) (Fardeau et al., 1996). Scapular winging is usually present from the early stages, though it may be asymptomatic. In some patients, the scapular winging has been so pronounced that a diagnosis of scapulohumeral muscular dystrophy was initially considered, and in our experience this is a fairly commonly suggested differential diagnosis. Facial muscles are spared, and the neck muscles are often also well preserved. While the majority of patients present with difficulty running or climbing stairs or have frequent falls, a small minority have had muscle pain and have been misdiagnosed as having polymyositis or metabolic myopathy (Penisson-Besnier et al., 1998).

The age at presentation is extremely variable. The majority probably present between the ages of 8 and 15 years, though a range from 2 to 40 years has been reported (Richard et al., 1997). The rate of deterioration varies between families, but intrafamilial variation is less marked than is frequently reported for the sarcoglycanopathies. The disease is not usually as severe as that usually seen, for example, with Duchenne muscular dystrophy, and may be extremely mild. Confinement to a wheelchair occurs at the earliest typically 11–28 years after the onset of symptoms. Respiratory, but not cardiac complications have been reported. Genotype–phenotype correlations are difficult except in patients who are homozygous for a mutation: as a general rule patients with homozygous null mutations tend to have the most severe...
clinical course. While there are occasional reports of elevated creatine kinase in carriers [especially, it has been suggested, in carriers of a particularly deleterious mutation (Topaloglu et al., 1997)], the general applicability of this has yet to be determined.

**Dysferlin-deficient LGMD (dysferlinopathy)** (LGMD2B)

**Diagnosis**

Proof of the diagnosis of dysferlinopathy is the demonstration of mutations in the dysferlin gene, though linkage to chromosome 2p13 in large families may be indicative of the diagnosis. Dystrophin, sarcoglycan and calpain 3 analysis in these patients is normal. Only a few mutations have yet been described in this large gene, but even from this preliminary analysis mutations appear to be variable and spread widely over the coding sequence of the gene (Table 2) (Liu et al., 1998; Bashir et al., 1998). Antibodies to dysferlin have now been developed, and they are likely to become part of the diagnostic process based on the examination of muscle biopsies (Anderson et al., 1999). As with the other forms of LGMD, muscle biopsies show non-specific dystrophic changes. Creatine kinase is usually very markedly elevated at presentation (often 20–150 times or more above the normal range) (Mahjneh et al., 1996).

**Clinical features**

The LGMD2B locus was first identified in families with a predominantly proximal muscular dystrophy with onset in the late teens (Mahjneh et al., 1992, 1996; Bashir et al., 1994). These patients had normal mobility in childhood and usually a very slowly progressive muscle disease. Calf hypertrophy as a transient feature early in the course of the disease was reported in a minority of patients (Bashir et al., 1998). Early contractures were rare. Even at the later stages of the disease the anterior distal leg muscles and the distal arm muscles were relatively spared. In contrast to calpain deficiency, scapular involvement was minor and was not present at the onset. While the first localization of detectable muscle weakness was in the pelvisfemoral muscles, muscle CT scanning in some patients showed early involvement of the gastrocnemius muscle which was usually asymptomatic, though early inability to walk on tiptoe is an important clinical clue (Passos-Bueno et al., 1999). Calf wasting is also seen (Fig. 4).

These observations became particularly pertinent when the gene for Miyoshi myopathy was shown to be localized to the same genetic interval on chromosome 2 as LGMD2B (Bejaoui et al., 1995). Miyoshi myopathy is a predominantly distal muscular dystrophy with early involvement of the posterior compartment of the lower limb. It has been increasingly recognized in the West over the last few years, having previously been described mainly in Japan (Linssen et al., 1997). The initial muscle involvement, usually in early adult life, is of the gastrocnemius, so that in this disorder the inability to stand on tiptoes is a very common early symptom. Proximal lower and upper limb weakness often develops as the disease progresses. While Miyoshi myopathy most commonly causes a slowly progressive muscular dystrophy, it is now recognized that progression does occur, with one-third of a Dutch series needing a wheelchair for mobility outside from ~10 years after the onset of the disease (Linssen et al., 1997). It appears that not all cases of Miyoshi myopathy map to the dysferlin gene and a second locus has tentatively been assigned to chromosome 10 (Linssen et al., 1998).

Families linked to chromosome 2p13 with different members affected by variable phenotypes have been reported (Weiler et al., 1996; Illarioshkin et al., 1996). In a large aboriginal Canadian kindred with nine affected family members, seven presented with an LGMD phenotype and two with a distal myopathy; haplotypes were shared between the patients with different presentations, suggesting a shared genetic basis for their diseases. Illarioshkin and colleagues described a family of ethnic Avars from the northern Caucasus where either a very clearly distinct LGMD or distal myopathy phenotype could be recognized (Illarioshkin et al., 1996). The phenotype was constant in affected sibs and a common homozygous phenotype was shared between all affected family members, whatever the phenotype. Mutations in the dysferlin gene can now be identified, and in some families it has now been confirmed that patients from the same family who are homozygous for the same mutation may present with either a limb-girdle or a Miyoshi muscular dystrophy (Liu et al., 1998; Bashir et al., 1998). To complicate matters further, some of the patients in whom dysferlin mutations have been described presented with a predominantly anterior distal involvement rather than the posterior distal involvement.
that is characteristically seen in Miyoshi myopathy (Liu et al., 1998). This suggests that additional factors, genetic or non-genetic, may contribute to the pattern of muscle involvement observed.

**LGMD2G**

This form of autosomal recessive LGMD is defined by its gene localization only, as the gene itself has not yet been cloned (Moreira et al., 1997). Using the resource of their many large LGMD families as a starting point, Moriera and colleagues were able to identify a family which failed to show linkage to any of the other LGMD loci. Examination of muscle biopsies from affected family members showed normal sarcoglycan staining; however, on histopathological analysis the biopsies contained a large number of rimmed vacuoles, a feature not commonly reported in autosomal recessive LGMD. The creatine kinase concentration was elevated between 3 and 17 times normal. Clinically, patients typically presented early in the second decade with difficulty climbing stairs and running, though foot-drop was also an early feature. Proximal and distal lower limb weakness was therefore present from the onset while in the upper limbs the proximal musculature was more severely affected. Confinement to a wheelchair took place ~18 years after onset. Linkage to chromosome 17q11-12 was identified in this family; analysis of a further family, not large enough to generate a significant lod score but with a clinically similar pattern, was also suggestive of linkage to this locus.

**LGMD2H**

As with LGMD2G, a chromosomal localization only has been reported for the eighth autosomal recessive LGMD locus. The disease, now linked to chromosome 9q31-33, was first described in 1976, and affects a population of Hutterites in Manitoba (Shokeir and Kobrinsky, 1976; Weiler et al., 1998). Affected individuals are described as presenting usually with proximal lower limb weakness between 8 and 27 years of age, with an elevated serum creatine kinase concentration (2–30 times normal). Facial muscles were involved as the disease progressed, as were the proximal upper limb muscles, predominantly the trapezius and deltoid. Mild distal limb involvement was also seen, involving the brachioradialis and anterior peroneal muscles. Most patients have remained ambulant late into adult life.

**Epidemiology of autosomal recessive LGMD**

No studies have yet revisited the population frequency of LGMD as a whole since precise molecular diagnosis has become possible. However, various estimates of the different types of autosomal recessive LGMD are beginning to emerge. A single population-based study of sarcoglycanopathy has been reported (Fanin et al., 1997), suggesting a prevalence of sarcoglycanopathy in north-east Italy of $5.6 \times 10^{-6}$. This compares with a prevalence in the same population for spinal muscular atrophy types 2 and 3 of $6.8 \times 10^{-6}$ and $6.8 \times 10^{-6}$, respectively, for congenital muscular dystrophy. Of the sarcoglycanopathy patients ascertained by immunological analysis of muscle biopsies, 39% had $\alpha$-sarcoglycanopathy, 22% had $\gamma$-sarcoglycanopathy, 11% had $\beta$-sarcoglycan deficiency and no patients had $\delta$-sarcoglycanopathy. In 28% of the patients no mutations were found.

Other studies address the frequency of sarcoglycanopathy in different ways. As the denominator in all of these studies is different, they are difficult to compare. Most only used a single sarcoglycan antibody to confirm patients ($\alpha$-sarcoglycan). These studies do agree that sarcoglycan abnormalities are found only in a true ‘muscular dystrophy’ population and not among patients with myopathy (Hayashi 1995; Duggan et al., 1997a; van der Kooi et al., 1998a). Duggan and colleagues (Duggan et al., 1997a) showed $\alpha$-sarcoglycan deficiency in 22% of patients with a progressive muscular dystrophy beginning in childhood. Among patients with an adult-onset LGMD phenotype, 6% were found to be $\alpha$-sarcoglycan-deficient. In only 58% of the patients with sarcoglycan deficiency was it possible to define the primary genetic abnormality. The frequency of the mutations found in the various sarcoglycan genes was as follows: $\alpha$-sarcoglycan, 34%; $\beta$-sarcoglycan, 16%; $\gamma$-sarcoglycan, 8%. The molecular frequency of $\delta$-sarcoglycan mutations in biopsies ascertained through $\alpha$-sarcoglycan deficiency was ~4% (Duggan et al., 1997b).

In the Netherlands, an exhaustive clinical review identified 37 families with a strict diagnosis of autosomal recessive or sporadic LGMD and found sarcoglycan deficiency in 25% of them (van der Kooi et al., 1998a). Hiyashi and colleagues (Hiyashi et al., 1995) found that 8.8% of biopsies from patients with either an uncharacterized progressive muscular dystrophy or LGMD showed sarcoglycan deficiency. When only families large enough for linkage analysis were studied, 55% of LGMD families in Brazil had a sarcoglycanopathy, again with $\alpha$-sarcoglycanopathy the most frequent and $\gamma$-, $\beta$- and $\beta$-sarcoglycanopathies having approximately similar frequencies (Passos-Bueno et al., 1999).

So although it is now clear that these disorders exist in all populations, the population frequency of the various sarcoglycanopathies is extremely variable. However, part of the variability described may be due to differences in the method of ascertainment.

Various lines of evidence suggest that calpainopathy may be a relatively common cause of LGMD. Richard and colleagues found that 39% of an unselected LGMD population had calpain-related LGMD, rising to 45% when patients known to be sarcoglycan positive were included (Richard et al., 1997). In a Turkish LGMD population, calpainopathy accounted for half of all cases, though a founder effect appears to be important in this population, in which there is a single predominant mutation (Dincer et al., 1997). There is a similar effect in the Basque population of Spain, where
the prevalence of LGMD has been estimated to be 69 per million persons; in the vast majority of cases (79%) the calpainopathy is accounted for by a single founder mutation (Urtasun et al., 1998). In La Reunion, the prevalence of calpainopathy is estimated to be 48 per million persons (Fardeau et al., 1996b).

Only one estimate of the frequency of dysferlinopathy exists, from the linkage studies in large Brazilian families, in which there was linkage to chromosome 2p13 in ~25% of the families compared with 17.5% for calpainopathy (Passos-Bueno et al., 1999). A founder mutation appears to be present in the Libyan Jewish population (Bashir et al., 1998; Z. Argov et al., unpublished observations). Further studies are necessary in order to pursue these issues.

The autosomal dominant limb-girdle muscular dystrophies

Only a relatively small proportion of LGMD (possibly ~10%) is autosomal dominant in inheritance (Bushby, 1992). Van der Kooi and colleagues (van der Kooi et al., 1994) point out that, on careful reappraisal, ~8% of patients with a diagnosis of LGMD may in fact have FSHD. In our experience, as would be expected, this misdiagnosis is especially common in families with a dominant family history. The clinical spectrum of FSHD can include prominent pelvic girdle weakness and, in some individuals, only minimal facial muscle involvement, leading to even more confusion in diagnosis. Now that it is possible to diagnose 95% of FSHD cases by direct DNA analysis, it is probably worth excluding this diagnosis in families with a positive family history (Upadhyaya et al., 1997).

Three genetically determined types of autosomal dominant LGMD have been delineated. An important distinguishing feature in all of these families compared with those with autosomal recessive LGMD is the much lower serum creatine kinase concentration usually seen in autosomal dominant disease.

LGMD1A

The LGMD1A gene maps to chromosome 5q, based on the study of a single large North American pedigree with a predominantly proximal muscular dystrophy associated in some cases with dysarthria and tight Achilles tendons. No patients had arm weakness without leg weakness, and distal weakness was a late feature (Gilchrist et al., 1988; Speer et al., 1992). Age at onset ranged from 18 to 35 years, with some suggestion of anticipation (Speer et al., 1994). Progression of the disease was very slow, and very few patients were confined to a wheelchair. The creatine kinase concentration was usually only mildly elevated. No other LGMD families have yet been identified which map to this locus.

LGMD1B

The LGMD1B gene has been localized to chromosome 1q11-1q21 (van der Kooi et al., 1997). The families whose disease linked to this locus had a dominant limb-girdle muscular dystrophy associated with cardiac involvement (van der Kooi et al., 1996). The age at onset of problems in these families ranged from 4 to 38 years, with proximal lower limb symptoms such as difficulty running and climbing stairs. About half of all patients presented in childhood. Muscle weakness was slowly progressive. Calf hypertrophy was variable, and contractures (of the Achilles tendon or elbows) were minimal or late. The creatine kinase concentration was normal or mildly elevated. Atrophicventricular conduction defects increased with age in these patients, so that before the age of 25 years most had normal atrophicventricular conduction, progressing to first-degree heart block in about one-third of patients between 25 and 35 years and second-degree heart block, often requiring pacemaker insertion, between 35 and 45 years of age. Sudden death was common among untreated patients. A minority of patients also had a dilated cardiomyopathy. While cardiac problems very rarely preceded muscle symptoms, in some patients their muscle symptoms were recognized only in retrospect. A similar phenotype has been described by Fang and colleagues (Fang et al., 1997).

The phenotype in these patients is apparently different from autosomal dominant Emery–Dreifuss muscular dystrophy, in which early spinal rigidity, Achilles tendon and elbow contractures are usually seen in association with a predominantly humeroperoneal muscular dystrophy, though the cardiac involvement may be similar (Emery, 1989; Rudenskaya et al., 1994). This phenotype overlaps significantly with that described in association with secondary β1 laminin deficiency (Taylor et al., 1997). Linkage of the autosomal dominant Emery–Dreifuss muscular dystrophy gene to the same region of chromosome 1q as the LGMD1B locus suggests that there may be a variable phenotype (with or without prominent contractures) in association with this locus (Bonne et al., 1998). On the other hand (Messina et al., 1997), the disease in a family with dilated cardiomyopathy, cardiac conduction defects and an adult-onset, slowly progressive proximal muscular dystrophy maps to chromosome 6q23, showing that the combination of an autosomal dominant muscular dystrophy and cardiac disease is itself genetically heterogeneous.

LGMD1C (caveolin 3 deficiency)

The demonstration of caveolin 3 deficiency in the group of patients now designated LGMD1C illustrates again the power of approaching disease characterization by the analysis of a candidate protein. Caveolin 3 is a muscle-specific component of the caveolin membrane which is probably involved in signal transduction. Caveolin 3 localizes to the sarcolemma, coinciding with the distribution of dystrophin, with which it
can also be shown to associate by immunoprecipitation experiments (Song et al., 1996). The caveolin 3 gene has been localized to chromosome 3p25. In two families with autosomal dominant LGMD, caveolin 3 labelling on muscle biopsies was shown to be reduced, and mutations in the gene were demonstrated in these families. These patients had normal motor milestones, onset of disease at ~5 years of age, with cramping muscle pains after exercise, calf hypertrophy and mild to moderate proximal muscle weakness. Disease progression was variable: it was slow in one family but more rapid in another. The creatine kinase concentration was elevated to between 4 and 25 times normal. In two other families, mutations of uncertain significance have been shown in the caveolin gene (McNally et al., 1998a).

**LGMD with secondary protein deficiencies.**

With the wide range of diagnostic investigations now available for the study of patients with muscular dystrophy, it is not surprising that a number of apparently secondary protein abnormalities have been observed in which the primary defect remains unknown. Two groups of patients can be fairly consistently recognized in this way. A secondary deficiency of laminin A2 (merosin) detectable only on immunoblotting has been described in a group of predominantly adult-onset patients with a limb-girdle weakness, mild facial weakness and calf hypertrophy (Bushby et al., 1998). This group appears to have an autosomal recessive form of LGMD, as two sibling pairs have been described in whom the primary defect may reside in a protein affecting the stability of merosin. It is also important to note that partial primary laminin A2 deficiency can result in an LGMD phenotype.

In another group of patients, an apparently autosomal dominant phenotype characterized by spinal rigidity and contractures of the Achilles tendons and elbows can be shown to have a secondary deficiency of laminin β-1 on biopsy, detectable particularly when the biopsy is taken in adult life (Taylor et al., 1997). This phenotype overlaps with both Bethlem myopathy and the autosomal dominant Emery–Dreifuss muscular dystrophy phenotype (Bethlem and Wijngaarden, 1976; Malandrini et al., 1995). Molecular analysis should help to resolve these issues of secondary protein involvement in time, and may well provide insights into the relationships of the various proteins involved and their function in muscle. Meantime, observation and careful delineation of these phenotypes remains important.

**Conclusions**

If clinical information, protein analysis and genetic information is available, a primary diagnosis may be achievable in the majority of patients with a progressive predominantly proximal muscular dystrophy. A summary of the diagnostic criteria for the different groups and their clinical correlates is shown in Table 3 and a practical approach to diagnosis is outlined in Table 4. All of the different types of autosomal recessive LGMD appear to be seen world-wide, though the relative frequencies of the different types is variable. A definition based on the primary gene or protein defect should now be the preferred disease designation, especially as the disorders encompassed within the traditionally defined LGMD sphere have been recognized to include ones where distal onset may be apparent (dysferlinopathy and LGMD2G). This disease nomenclature, based on molecular pathology, can accommodate our increasing understanding of the potential phenotypic variation seen in these types of muscular dystrophy.

Nonetheless, some broad clinical guidelines are possible. A sarcoglycanopathy is most likely to be diagnosed in patients presenting with proximal lower limb weakness associated with calf hypertrophy and often asymptomatic scapular winging. Muscle biopsy shows a dystrophic pattern. Patients with sarcoglycanopathy account for between 8 and 25% of all patients with a progressive proximal muscular dystrophy. This figure is higher among childhood-onset cases than among cases with onset in adulthood. Use of antibodies to two or more sarcoglycans will be most likely to identify all cases and may point to the primary genetic abnormality, the use of all four sarcoglycan antibodies giving the best chance of defining which sarcoglycan gene harbours the primary mutation. A proportion of patients with sarcoglycan deficiency on muscle biopsy will not have a detectable mutation in one of the known sarcoglycan genes. This proportion is highest in patients with partial sarcoglycan deficiency. Some cases of sarcoglycanopathy may have mildly abnormal dystrophin, and, as for dystrophinopathy, the spectrum of clinical severity in sarcoglycanopathy is very broad. It is not yet possible to say whether the different sarcoglycanopathies can be distinguished clinically one from the other, but differences may be discernible between sarcoglycanopathy and dystrophinopathy. These include the mode of inheritance (by definition), a more marked early predilection for scapular involvement, equal involvement of quadriceps and hamstrings, less frequent cardiac involvement and lack of intellectual impairment. While the use of the terms ‘Duchenne’ and ‘Becker-like’ to describe the group may be evocative in terms of the rate of progression and the frequent presence of calf hypertrophy, too widespread usage of these terms may serve to devalue these potentially informative clinical clues.

Calpainopathy may account for ~40% of patients with ‘LGMD’. The presentation may be in childhood or adult life. Patients with calpainopathy typically have a symmetrical, predominantly proximal and atrophic pattern of muscle weakness at onset, often with asymptomatic scapular involvement. Achilles tendon contractures are commonly seen early in the course of the disease. It may be possible to distinguish this phenotype from sarcoglycanopathy and dystrophinopathy. The muscle biopsy is dystrophic and the serum creatine kinase concentration at presentation is usually
Table 3 Summary of the diagnostic tools available and the clinical correlates relevant to the genetically defined groups of LGMD

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Diagnostic criteria</th>
<th>Clinical correlates</th>
</tr>
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<tbody>
<tr>
<td>AD LGMD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD1A</td>
<td>Genetic: Linkage to chromosome 5q22–24 only available tool</td>
<td>(i) Described only in one North American pedigree.</td>
</tr>
<tr>
<td></td>
<td>Biochemical: Serum CK normal or mildly elevated</td>
<td>(ii) Onset aged 18–35 years; slow progression.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(iii) Affected individuals may have dysarthria and Achilles tendon contractures.</td>
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<tr>
<td></td>
<td></td>
<td>(iv) May show anticipation.</td>
</tr>
<tr>
<td>LGMD1B</td>
<td>Genetic: Linkage to chromosome 1q11–21 only available tool</td>
<td>(i) Cardiac conduction defects a prominent complication.</td>
</tr>
<tr>
<td></td>
<td>Biochemical: Serum CK normal or mildly elevated</td>
<td>(ii) Onset at age 4–38 years; slow progression.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(iii) May be allelic with autosomal dominant Emery–Dreifuss muscular dystrophy where contractures (especially of spine, elbows, Achilles tendons) are a major feature.</td>
</tr>
<tr>
<td>LGMD1C (caveolin deficiency)</td>
<td>Genetic: Linkage to chromosome 3p25 or mutations in caveolin 3 gene</td>
<td>(i) Few cases as yet described: calf hypertrophy and muscle pains on exertion noted as features.</td>
</tr>
<tr>
<td></td>
<td>Protein: Absence of caveolin 3 on immunocytochemistry of muscle biopsy samples</td>
<td>(ii) Onset around age 5 years; variable progression.</td>
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<tr>
<td>AR LGMD</td>
<td></td>
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<tr>
<td>Sarcoglycanopathy</td>
<td>Genetic: In suitable families, linkage to a sarcoglycan locus; where possible, confirmation of the primary mutation</td>
<td>(i) Calf hypertrophy a common feature. Involvement of scapular muscle and deltoid in upper limb at onset; pelvic girdle involvement in lower limb greater than femoral.</td>
</tr>
<tr>
<td></td>
<td>Protein: Major tool is the use of at least two sarcoglycan antibodies on immunocytochemistry or immunoblotting of muscle biopsy</td>
<td>(ii) Majority present in childhood but adult onset is also reported. Progression ranges from very severe to very mild.</td>
</tr>
<tr>
<td></td>
<td>Biochemical: Serum CK &gt;10 times normal</td>
<td>(iii) Cardiac involvement has been reported in association with various primary sarcoglycan deficiencies. Respiratory complications are important.</td>
</tr>
<tr>
<td>Calpainopathy</td>
<td>Genetic: In suitable families, linkage to CAPN3 gene; where possible, confirmation of the primary mutation</td>
<td>(i) Calf hypertrophy reported but rare: predominantly atrophic pattern of muscle involvement. Achilles tendon contractures common at presentation. Muscle involvement may be ‘scapulohumeral’ at presentation. Sparing of hip abductors a consistent sign in the lower limbs.</td>
</tr>
<tr>
<td></td>
<td>Protein: Absence or deficiency of calpain 3 on immunoblotting</td>
<td>(ii) Majority present between 8 and 15 years but adult onset fairly common. Progression variable.</td>
</tr>
<tr>
<td></td>
<td>Biochemical: Serum CK &gt;10 times normal</td>
<td>(iii) Respiratory complications may be severe. Cardiac involvement has not been reported.</td>
</tr>
<tr>
<td>Dysferlinopathy</td>
<td>Genetic: In suitable families, linkage to chromosome 2p13; where possible, confirmation of primary mutation</td>
<td>(i) Predominant pattern at presentation may be with proximal or distal lower limb involvement. Early inability to stand on tiptoes is a common symptom. Little scapular or upper limb involvement at onset.</td>
</tr>
<tr>
<td></td>
<td>Protein: Absence or reduction of dysferlin on immunocytochemistry</td>
<td>(ii) Onset is most commonly in the late teens with usually slow progression.</td>
</tr>
<tr>
<td></td>
<td>Biochemical: Serum CK often massively elevated (may be &gt;100 times normal)</td>
<td>(iii) Other complications have not been reported.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variable phenotype (e.g. distal vs proximal presentation) may be present in the same family.</td>
</tr>
</tbody>
</table>

AD = autosomal dominant; AR = autosomal recessive; CK = creatine kinase.

at least 10 times the normal level. Dystrophin and sarcoglycan staining is normal in calpainopathy. Mutation detection involves screening the whole gene, except in defined populations, but antibody analysis of a muscle biopsy (currently only possible by Western blotting) may identify those patients in whom mutation detection is likely to be worthwhile. The progression of the disease is variable: respiratory complications may be important but cardiac
Table 4 The practical approach to LGMD: guidelines to the approach to diagnosis in individual patients with a suspected LGMD

(1) History and examination
Clinical clues (see text and Table 3) may suggest most logical route to investigations
- Calf hypertrophy and childhood onset: suspect sarcoglycans.
- Atrophic muscles, scapular winging and sparing of hip abductors: suspect calpain 3.
- Distal involvement with onset in late teens: suspect dysferlin.
- Contractures a very prominent feature: suspect EDMD/laminin β1 deficiency.
(In many populations, calpainopathy and dysferlinopathy may be more common than sarcoglycan deficiency.)

(2) General investigations
Exclude alternative causes of proximal muscle weakness.
- Serum creatine kinase: if normal or mild elevation in active disease suspect dominant condition.
- EMG: myopathic, no specific features.
- Muscle biopsy: dystrophic pattern.

(3) Family history
Dominant family history
- Exclude FSHD, EDMD.
- Check caveolin 3 on biopsy.
- Check laminin β1 on biopsy.
- Linkage analysis to dominant LGMD loci if family structure suitable.

Large family with recessive disease
- Haplotype analysis at ARLGMD loci may be informative.
- This will be useful only in a small minority of families and should be used as an adjunct to protein analysis of a muscle biopsy.

(4) Specific investigations
Look at biopsy first.
- Immunocytochemistry: use at least two sarcoglycan antibodies as well as dystrophin antibodies.
- Immunoblotting: blotting necessary to examine calpain 3.
- Multiplex blotting may also show which sarcoglycan is primarily involved.

Genetic analysis
- Direct detection of the mutation is the final proof of diagnosis and allows carrier detection/prenatal diagnosis. However, given the complexity of the genes and the heterogeneity of the mutations, this is best directed by the results of protein analysis.

Notes on the interpretation of biopsy findings
- Dystrophin may be abnormal in sarcoglycanopathy.
- If abnormalities are detected on immunocytochemistry with dystrophin or sarcoglycan antibodies, then the full range of sarcoglycan antibodies should be used to attempt to pinpoint the primary deficiency.
- With α- and γ-sarcoglycan deficiency, secondary changes detected with the other sarcoglycan antibodies may not be present or may be minor.
- With β- and δ-sarcoglycan deficiency especially, total loss of all sarcoglycan components may be seen.
- Multiplex immunoblotting offers an alternative approach to defining the primary sarcoglycan involvement. We have rarely seen sarcoglycan deficiency missed on immunocytochemistry but detectable only on blotting.
- Sarcoglycans are normal in calpainopathy and dysferlinopathy.
- Dysferlin can be studied by immunocytochemistry, but calpain 3 examination currently requires immunoblotting.

EDMD = Emery–Dreifuss muscular dystrophy.

involvement and intellectual impairment have not been described in these patients.

Dysferlinopathy should be suspected in muscular dystrophy patients with presentation in their late teens, especially if posterior distal lower limb involvement can be demonstrated early on. This may be asymptomatic, or manifest as early inability to stand on the toes. The clinical spectrum of dysferlinopathy encompasses different modes of presentation in terms of the pattern of muscle involvement. Shared features appear to be a tendency for the age at onset to be most commonly in the late teens or early twenties and an elevated creatine kinase concentration (often >100 times normal in the early stages). The disease is progressive, but rarely rapidly so. Diagnosis is confirmed by protein analysis and mutation detection.

In all of the various types of autosomal recessive LGMD, there are anecdotal reports of mild elevation of serum creatine kinase in obligate carriers (e.g. Passos-Bueno et al., 1999). This information is not secure enough at present to be able to offer testing on this basis. In families with a known mutation or in populations with a common genetic basis for a particular form of LGMD, carrier detection through identification of the mutation may be possible. For unrelated individuals in other populations, however, it remains problematic because of the large number of distinct mutations described in all of these genes.
Autosomal dominant LGMD is relatively rare, and exclusion of FSHD in this group is important. Experience with the genetically defined types of dominant LGMD remains limited; dominant disease should be suspected in patients with a relatively low serum creatine kinase concentration at presentation or where contractures are a particular feature of the disease.

Limb-girdle muscular dystrophy need no longer be an ‘orphan’ diagnosis but should be the starting point for the identification of the precise molecular defect through the use of the new techniques available (Table 4). As experience with the various subtypes continues to accumulate, this will translate into a greater understanding of prognostic and treatment implications, as well as the potential for accurate genetic counselling.

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