Review

The 10 autosomal recessive limb-girdle muscular dystrophies

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Abstract

Fifteen forms of limb-girdle muscular dystrophies (5 autosomal dominant and 10 autosomal recessive) have already been found. The 10 genes responsible for the autosomal recessive forms, which account for more than 90% of the cases, had their product identified. This review will focus on the most recent data on autosomal recessive-limb-girdle muscular dystrophy and on our own experience of more than 300 patients studied from 120 families who were classified (based on DNA, linkage and muscle protein analysis) in eight different forms of autosomal recessive-limb-girdle muscular dystrophy. Genotype–phenotype correlations in this highly heterogeneous group confirm that patients with mutations in different genes may be clinically indistinguishable. On the other hand, for most forms of autosomal recessive-limb-girdle muscular dystrophy a discordant phenotype, ranging from a relatively severe course to mildly affected or asymptomatic carriers may be seen in patients carrying the same mutation even within the same family. A gender difference in the severity of the phenotype might exist for some forms of autosomal recessive-limb-girdle muscular dystrophy, such as calpainopathy and telethoninopathy but not for others, such as dysferlinopathies or sarcoglycanopathies. Understanding similarities in patients affected by mutations in different genes, differences in patients carrying the same mutations or why some muscles are affected while others are spared remains a major challenge. It will depend on future knowledge of gene expression, gene and protein interactions and on identifying modifying genes and other factors underlying clinical variability.

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1. Introduction

The limb-girdle muscular dystrophies (LGMDs) are a heterogeneous group of genetically determined progressive disorders of skeletal muscle with a primary or predominant involvement of the pelvic or shoulder-girdle musculature. The clinical course in this group is characterized by normal intelligence and great variability, ranging from severe forms with onset in the first decade and rapid progression to milder forms with later onset and a slower course [1,2].

At least 15 genes, 5 autosomal dominant (AD) and 10 autosomal recessive (AR), responsible for LGMD have been mapped. Linkage analysis indicates that there is further genetic heterogeneity both for AD as well as for AR-LGMD [3]. The AD forms are relatively rare and represent less than 10% of all LGMDs. This was confirmed in our population since among a large cohort of more than 100 Brazilian families with multiple affected cases only four showed a typical AD inheritance (unpublished observations). A comprehensive review of all forms of LGMD becomes each time more difficult and therefore this review will focus on the AR forms.

The first AR-LGMD, LGMD2A, was mapped to 15q in 1991 [4] in a group of patients from Réunion Island. Shortly thereafter, linkage analysis in Brazilian families showed evidence of genetic heterogeneity [5]. Indeed, nine additional forms of AR-LGMD were mapped in the last decade, most recently LGMD2J.

The protein products of these 10 genes have been identified (Fig. 1). They are: calpain-3 for LGMD2A [6], dysferlin for LGMD2B [7,8], α-sarcoglycan (SG) for LGMD2D [9,10], β-SG for LGMD2E [11,12], γ-SG for LGMD2C [13,14], δ-SG for LGMD2F [15–17], the sarcomeric protein telethonin for LGMD2G [18,19], TRIM32 for LGMD2H [20], fukutin-related protein (FKRP) for LGMD2I [21] and titin for LGMD2J (Bjarne Udd, personal communication).

Genotype–phenotype correlation studies have been reported for the different forms of LGMD in an attempt to enhance our comprehension on the underlying pathological
mechanisms, to better characterize each of the subgroups and more importantly to try to identify modifier genes or epigenetic factors which might modulate the clinical course in patients who carry the same pathological mutation.

2. LGMD2A—calpainopathy

LGMD2A is caused by mutations in the human CAPN3 gene, which codes for calpain-3, the skeletal muscle-specific member of the calpain family [6]. The demonstration of the involvement of the muscle-specific calcium-activated neutral protease 3 in LGMD2A was the first example of an enzymatic rather than a structural protein defect causing a progressive muscular dystrophy. Calpainopathy is the most frequent form of LGMD in several populations including the Brazilian one, accounting for about 30% of the identified cases. Clinically, LGMD2A is characterized by symmetrical and selective proximal atrophy with no cardiac or facial disturbance and normal intelligence. However, the course is highly variable [2,23]. Calf hypertrophy is apparently rare in European patients but not uncommon in Brazilian LGMD2A families where affected sibs may show a discordant pattern.

Elevated serum creatine kinase (CK), particularly in the active phase of the disorder has been reported for LGMD2A patients by us and others [1,2,6,23,25]. However, most recently we have identified patients, from three unrelated families, who carried mutations in the calpain-3 gene and had normal serum CK (three cases) or a neurogenic pattern on electromyography (two cases) [Starling et al., submitted for publication; Paula et al., in preparation]. These unexpected observations suggest that the spectrum of variability in calpainopathy might be broader than suspected and that a normal CK should not be considered an exclusion criteria for LGMD2A even in ambulant patients.

A wide intra and interfamilial clinical variability ranging from severe to milder forms was reported in a multicenter study of 163 European patients [23]. The mean age at onset was 13.7 (ranging from 2 to 40 years old) and the mean age at loss of walking ability was 17.3 years (range 5–39 years) after onset with no sex difference in age at onset or progression. In the British population, a slightly younger age at onset in female than in male patients was observed [24], although the sample was relatively small (seven males and six females). We have analysed 93 (47 females and 46 males, from 38 families) Brazilian patients who were characterized at the molecular and most of them at the protein level as well [25]. Eighteen patients (~19%) were confined to a wheelchair (mean age 21.7 years, range 12–45). When we compared both sexes, it was observed that, the mean age at onset and ascertainment did not differ significantly between males and females. However, a statistically significant difference was seen for loss of ambulation (observed for 12 males but only 6 females) which occurred on an average earlier in males (mean age 21.7 years, range 12–45) than in females (29.7 years, ranging from 18 to 45 years old).
This observation suggests a more rapid progression in LGMD2A affected males than in females Brazilian patients. Interestingly, in a recent screening of calpain-3 deficiency through muscle protein analysis in Italian patients [26] there was a statistically greater proportion of affected males (43) than females (23). This gender disproportion caught our attention. One possible explanation is that there were more severely affected males than females who were therefore more likely to be ascertained in accordance to our data.

The CAPN-3 gene comprises 24 exons and covers a genomic region of 50 kb. It is expressed as a 3.5 kb transcript, and a 94 kDa translated protein. Screening of mutations in a large set of European, Brazilian and Japanese patients has revealed more than 100 distinct pathogenic CAPN3 mutations distributed along the entire length of the gene ([23,25,27], Leiden database at http://www.dmd.nl/capn3_home.html).

Most of the mutations represent private variants, although some mutations were found more frequently in isolated communities, with a high degree of inbreeding [23,28,29]. In a recent study of 21 Japanese LGMD2A patients, 10 of 13 identified mutations were not found in other populations (including six novel ones). Three of them, accounting for 71% of the cases, were recurrent mutations [27]. In the Brazilian population, we identified two recurrent null mutations (R110X and 2362-2363AG > TCATCT) and seven novel pathogenic mutations. Interestingly, 41 of the identified mutations (~80%) were concentrated in only six exons (1, 2, 4, 5, 11 and 22), which have important implications for diagnostic purposes. This observation may be particularly helpful for patients who are severely affected and in whom getting a muscle biopsy may be difficult or for patients who show normal results for all known LGMD proteins including calpain-3 amount [25,30].

3. Protein analysis

Calpain-3, a muscle-specific 94 kDa calcium-activated neutral protease 3, binds to titin. It is a cysteine protease, which plays a role in the disassembly of sarcomeric proteins, but it may also have a regulatory role in modulation of transcription factors. Calpainopathy seems to result from the loss of proper substrate processing activity of calpain-3 [31].

Western blot (WB) analysis of muscle calpain-3 in LGMD2A patients can show a total, partial, or more rarely, no apparent deficiency at all, with no direct correlation between the amount of calpain and the severity of the phenotype. Very low levels or no expression of calpain-3 were seen in European and Brazilian patients with a clinical course varying from mild to severe [30,32]. Normal or almost normal 94 kDa calpain-3 bands were found in some patients with missense mutations [25,30] or more recently in a Brazilian patient compound heterozygote for a missense/null mutation (unpublished observation).

The analysis of calpain-3 in other forms of muscular dystrophy revealed no apparent alteration in sarcoglycanopathy [32,33] or telethoninopathy patients [33]. However, a secondary reduction of calpain-3 was first reported in LGMD2B patients, suggesting a possible association between calpain-3 and dysferlin [32,34,35]. Subsequently, other studies have shown a secondary reduction of calpain-3 in other forms of LGMD such as LGMD2I [22], and 2q-linked muscular dystrophy [36–38], which requires further studies.

The functional role of calpain-3 is still under discussion but several pathogenic hypotheses have been proposed to explain why mutations in this gene cause muscular dystrophy: calpain-3 could have a protective effect and be involved in muscle detoxification preventing a degradation of the muscle fibers. Alternatively it could have a role in the proper processing and assembly of the structural scaffold of the muscle cell. It has been suggested also that it may play a significant role in intracellular signal transduction systems [39–42]. According to Bagdiguian et al. [43], calpain-3 deficiency would be associated with myonuclear apoptosis and a profound perturbation of the IkBα/NF-κB pathway.

4. Genotype–phenotype correlations

Although there is a marked inter and intrafamilial heterogeneity in the severity of the clinical course in LGMD2A muscular dystrophy [2,23,44] it has been suggested that on an average missense mutations are usually associated to a milder phenotype than null mutations [23,27,30].

We have compared Brazilian patients in whom both mutations were identified [25], who were classified in three groups according to the nature of the mutation: (1) patients with missense mutations on both alleles or one missense and one in frame deletion; (2) patients who were compound heterozygous for missense/null mutations and (3) patients carrying null mutations (frameshift or stop codon mutations) or splicing site changes on both alleles. Comparison among the three groups showed that on an average the ages at onset and ascertainment were significantly higher in group (1) than for both groups (2) and (3) in accordance with Richard et al. [23]. However, the mean ages of onset and ascertainment did not differ between groups (2) and (3) which suggests that one null mutation is enough to determine a more severe phenotype.

No direct correlation has been observed between the protein amount and the severity of the phenotype or type of mutation although null mutations were more often associated with total absence of muscle calpain-3 while missense mutation with partial deficiency. Almost normal 94 kDa calpain-3 amount was found in about 10% of LGMD2A Brazilian patients (3 among 31 unrelated patients in whom muscle biopsies were available for protein studies) [25] and unpublished observation). However, their clinical course was not milder which suggests that although present...
the protein is non-functional. In addition, the observation that patients carrying two pathogenic mutations may have an apparently normal calpain amount also suggests that the relative proportion of calpainopathy screened through protein analysis might be underestimated.

5. LGMD2B—dysferlinopathies

This form of LGMD includes Miyoshi myopathy (MM), a distal muscle disorder that preferentially affects the gastrocnemius muscle or LGMD type 2B with characteristic proximal weakness at onset. Although the initial presentation may be different the distinction between patients with distal or proximal onset is very difficult after many years of progression. This form of LGMD arises from mutations in the dysferlin gene, which contains over 55 exons and spans a region of 150 kb [7,8,45]. The clinical picture is on an average less severe than other AR-LGMD forms although there is a wide intra and interfamilial variability [46,47]. We also observed this in 81 Brazilian patients (40 males and 41 females) from 26 families. Calf hypertrophy is rare but is also observed in some cases. Interestingly, patients often lose their ability to walk on tiptoes before the ability to walk on their heels, a feature which might be helpful for differential diagnosis. Confinement to wheelchair may occur, on an average 10–20 years after onset of the disease but on an average it is less frequent and occurs later than among calpainopathy patients [2,44]. Among Brazilian patients, about 10% (five males and three females) were confined to a wheelchair (mean age 37.8 ± 9.02, ranging from 27 to 50 years). Cardiac and respiratory muscles are not involved in either MM or LGMD2B and patients have normal intelligence. Serum CK is always elevated among these patients even in the pre-clinical stages. LGMD2B may be the most prevalent form (35–45%) in some populations such as in the Cajun/Arcadian population of North America [35]. In the Brazilian population it is the second more prevalent form of AR-LGMD accounting for ~22% of the classified forms.

6. Protein analysis

Dysferlin is a ubiquitously expressed 230 kDa molecule that is localized in the periphery of skeletal muscle fibers, linked to the sarcolemmal membrane [48]. It is expressed in early stages of development [22,35,48] and can be detected also in blood, skin and in chorionic villus biopsy [35,49].

Given the homology of dysferlin to a nematode spermatogenesis factor that is required for successful membrane fusion, it has been suggested that the lack of dysferlin might cause faulty myotube fusion and impair muscle regeneration [7,48].

Protein analyses in LGMD2B have shown a total deficiency of dysferlin, both through IF and WB. Although a partial deficiency has been reported in LGMD2B patients from UK [48], dysferlin deficiency seems to be specific to LGMD2B in our patients [35].

A reduced sarcolemmal expression along with accumulation of intracellular staining has been described in skeletal muscle of patients with disruption in the dystrophin–glycoprotein complex or DGC [50]. In our experience, however, the analysis of dysferlin has shown a normal localization and molecular weight (MW) in Duchenne (DMD) and sarcoglycanopathy patients, suggesting no interaction between dysferlin and the DGC [35]. A possible association between dysferlin and caveolin3 has recently been described [51].

Following the cloning of dysferlin, two additional human genes, with strong homology to dysferlin, coding for myoferlin and cardioferlin, were identified indicating the existence of a family of ferlin-related proteins [50]. It has been suggested that myoferlin, a plasma membrane protein highly expressed in cardiac and skeletal muscle, could act as a possible modifier of the muscular dystrophy phenotype [52]. However, our studies in LGMD2B patients showed no correlation between myoferlin expression and the severity of the phenotype, suggesting that this protein does not seem to modify the clinical course of dysferlin deficient patients [35].

7. Genotype–phenotype correlations

Due to the large size of the dysferlin gene, and the absence of any apparent mutation hotspot region, few mutations have been identified to date [49]. Therefore the diagnosis is mainly performed through linkage (in large pedigrees) or muscle dysferlin deficiency in patients’ muscle biopsies. Only six mutations were identified to date in seven Brazilian families (two homozygous and five in heterozygous state) [53]. A novel splicing mutation was reported in a LGMD2B family associated with inflammation [54]. A striking feature associated with dysferlinopathy which remains unexplained is why patients carrying the same mutation, even within the same family, differ not only in severity but also in the distribution of muscle weakness [55].

Interestingly, in the animal model for dysferlinopathy [56] it was observed that males have on an average a more severe course than females (Reginald Bittner, personal communication). However, in opposition to calpainopathy, no gender difference was seen among our Brazilian patients (unpublished observation) for age of onset, ascertainment or wheelchair confinement. The identification of new mutations in the dysferlin gene is of great interest to enhance our comprehension on the underlying mechanism associated with such a variable phenotype.
8. Sarcoglycanopathies

Among AR-LGMD, the sarcoglycanopathies (LGMD2C–2F) represent a subgroup caused by mutations in one of the genes that encode γ-, α-, β- and δ-SGs, respectively [9,11–13,16]. Severe clinical Duchenne-like presentations tend to be more common among SG patients. Serum CK is always greatly elevated, in particular when patients are still ambulant. Onset usually occurs early in the childhood and confinement to a wheelchair before the age of 16 [2,44,57–60]. Nevertheless, milder courses in LGMD2C–2E patients that harbor missense or even nonsense mutations have also been found by us and others [11,14,61,62]. The SGs are transmembrane glycoproteins which, together with sarcospan, dystrophin, dystroglycans, syntrophins and α-dystrobrevin, constitute the DGC [63–66]. In addition to the four SGs that comprise the SG–sarcospan subcomplex of the DGC in the striated muscle [67], a fifth SG, named ε-SG, is expressed in a wide variety of tissues [68,69]. In the smooth muscle, ε-SG replaces α-SG as an integral component of a unique SG–sarcospan complex composed of ε-, β-, γ- and δ-SGs and sarcospan [70]. Recently, it has been shown that mutations in ε-SG cause myoclonus-dystonia syndrome, an AD non-degenerative central nervous system disorder [71,72].

Until now, 77 distinct pathogenic mutations have been found in SG patients: 41 in the α-SG gene, 20 in the β-SG gene, 10 in the γ-SG gene and 6 in the δ-SG gene (Leiden Database at http://www.dmd.nl/capn3_home.htm). We have been able to confirm at the molecular level a diagnosis of sarcoglycanopathy in 39 Brazilian families with the following relative frequency: 23% for LGMD2C, 40% for LGMD2D, 23% for LGMD2E and 14% for LGMD2F. As a group, sarcoglycanopathies account for about 1/3 of the classified forms of AR-LGMD in our population. Interestingly the four subtypes are well represented in Brazil, in contrast to what has been observed in other countries. While in Europe and North America, the great majority of the patients deficient for the SG proteins are affected by LGMD2D [57,73,74], LGMD2C corresponds to almost 100% of the sarcoglycanopathies in Northern Africa [75]. In addition, LGMD2F seems to be very rare all over the world [44,57,60]. Probably, the relative balance among the different forms in Brazil may be attributed to the high degree of miscegenation of our population and increased consanguinity particularly for some groups. Indeed, the rate of consanguinity in LGMD2F is 100%, compared to values between 43 and 75% in the other three forms. Therefore, the higher prevalence of LGMD2F among Brazilian sarcoglycanopathies may reflect both a founder effect as well as the inbred nature of this sample.

Out of the 18 distinct SG mutations that are segregating in the Brazilian LGMD2C–2F families, seven were found to be recurrent [76] and three of them account for more than 60% of the disease alleles. These are c.521delT in the γ-SG gene, c.656delC in the δ-SG gene and R77C in α-SG, which were detected in 100, 80 and 64% of the LGMD2C, 2F and 2D alleles, respectively. The alterations γ-SG/c.521delT and δ-SG/c.656delC have probably been introduced in our population by African-Brazilians, since all patients in each of these two subgroups share a common haplotype [14,77]. On the other hand, the R77C mutation is associated with at least three distinct haplotypes in Brazil [78]. Actually, this mutation is the most frequent alteration in LGMD2D alleles in other populations [60,73,79–81], and this cytosine, in a CpG island, has been considered a mutational hotspot [81]. Nevertheless, the percentage of the R77C mutation among the LGMD2D alleles in our country is apparently the highest reported so far.

Among the remaining 15 mutations identified by us, the great majority lie either in the α- or in the β-SG genes, while only one lies in δ-SG. As most of the reports described in the literature [57,60,73,79–81], we found only missense mutations associated to LGMD2D. They are spread across five exons and all of them result in amino acid substitutions localized in the extracellular domain of the protein, which corresponds to its largest portion. With regard to β-SG, although missense, frameshift and splicing mutations were detected, 75% of them lie in exons 3 and 4.

9. Analysis of the SG proteins

The DGC acts as a linker between the cytoskeleton of the muscle cell and the extracellular matrix, providing mechanical support to the plasma membrane during myofiber contraction [65,82]. Besides this structural function, there is now increasing evidence that the DGC might play a role in cellular communication, as highlighted by its interaction with signaling molecules such as calmodulin, nitric oxide synthase and Grb2 [66,83].

In the majority of muscle biopsies from patients with a mutation in one of the SG genes, the primary loss or deficiency of any one of the four SGs, in particular of β- and δ-SG, leads to a secondary deficiency of the whole subcomplex [2,44,84–86]. However, exceptions may occur, such as minor deficiencies of γ-SG with partial preservation of the other three SG in LGMD2C [67] or the partial deficiency of only α-SG with the retention of the other three in LGMD2D [85,87]. The observation of a complete deficiency of one SG with partial deficiency of the others may help to indicate which gene should be first screened for mutations.

Patients with SG mutations may also have a secondary reduction in dystrophin, particularly those with primary γ-SG deficiency, suggesting that γ-SG might interact more directly with dystrophin [86]. In addition, this observation should be taken into account for differential diagnosis with X-linked Becker dystrophy in patients with no identified mutation.
Animal models lacking α-, β-, γ- and δ-SG have been described and have provided new insights into the pathogenesis of muscular dystrophy as well as in the function of the SG complex. Based on these studies there are now evidences that this complex may be an independent signaling module [84].

10. Genotype–phenotype correlations

Some frequent alterations, like R77C in the α-SG [44, 79,80,86,88] and c.521delT in the γ-SG genes [14], have been associated with both severe and mild forms with discordant phenotypes among affected sibs. More recently, this was observed by us in two brothers homozygous for a novel mutation c.2T > C in the β-SG gene both showing an identical immunohistochemical profile for the SG complex [76].

Brazilian patients harboring null mutations in both alleles of one of the SG genes as well as a drastic decrease of the entire SG complex usually but not always show a severe phenotype. In one of our patients in whom muscle analysis revealed deficiency of the four SGs, no mutation was found suggesting that the pathogenic mutation(s) may be located in unexplored non-coding regions in one of the SG genes or in another known or unknown gene encoding or interfering with a protein of the DGC [89].

Most patients with missense changes in both alleles also show a dramatic reduction of the primary protein together with variable deficiency of the secondary SGs. This pattern has been associated usually with a severe clinical course in LGMD2E and 2F but with both mild and severe presentations in LGMD2D. Within this last subgroup, clinical variability among patients homozygous for the same mutation seems to correlate with the residual amount of γ-SG in the muscle, despite the absence of the other three SGs in all of them [86]. However, normal or almost normal levels of the primarily involved SG in the muscle have been found in some rare LGMD2C and LGMD2D patients [67,81,87].

Interestingly there were significantly more females (61) than males (40) in our samples of 101 sarcoglycanopathy patients (from 39 families). One possible explanation is that affected females are already ascertained with a probable diagnosis of LGMD while severely affected males in whom a muscle biopsy could not be obtained may be erroneously classified as Xp21 muscular dystrophy. Indeed when the probands were excluded the proportion of affected females: males (34F:28M) did not differ significantly from the expected. Comparison between genders showed no statistically significant difference for age of onset (8.0 ± 3.0 for females and 8.4 ± 3.6 for males) or wheelchair confinement (13.0 ± 2.8 for females and 14.0 ± 7.6 for males).

11. LGMD2G—telethoninopathy

LGMD2G is a rare form of AR-LGMD, which was mapped in Brazilian patients in 1997 [18]. Subsequently it was shown that this form of AR-LGMD is caused by mutations in the gene encoding the sarcomeric protein telethonin. Patients from four unrelated Brazilian genealogies had null mutations in the telethonin gene and deficiency of this protein in muscle [19]. Screening of mutations in the telethonin gene in other populations did not allow so far the identification of any other case of telethoninopathy. This is surprising since the genealogy, which led to the mapping of the LGMD2G gene, is of Italian origin and affected patients in this family are compound heterozygotes for two different null mutations. In addition, mutations in heterozygous state were identified among Italian patients [90]. On the other hand, recently, a partial deficiency of muscle telethonin was identified in patients from two unrelated families from United States but no mutation was identified in the telethonin gene suggesting that this probably represents a secondary effect [91].

12. Protein analysis

Telethonin is a sarcomeric protein of 19 kDa, present in the Z disk of the sarcomere of the striated and cardiac muscle [92]. It was found to be one of the substrates of the serine kinase domain of titin. The specific function of telethonin and its interaction with the other proteins from the muscle is still unknown.

Protein analysis in six Brazilian patients, from four unrelated families, carrying the same frameshift mutation in the telethonin gene showed deficiency of the protein telethonin in muscle. Additional protein studies in these patients showed normal expression for dystrophin, the four SGs, dysferlin, calpain-3 and titin. Immunofluorescence analysis for α-actinin-2 and myotilin showed a normal cross striation pattern, suggesting that at least part of the Z-line of the sarcomere is preserved. Ultrastructural analysis confirmed the maintenance of the integrity of the sarcomeric architecture. Thus, as mutations in the telethonin gene do not seem to alter the sarcomere integrity, the underlying pathogenic mechanism is still under investigation [33]. However, since this form of LGMD has been confirmed exclusively in Brazilian patients carrying the same mutation, the existence and effect of other mutations in the telethonin gene are still unknown.

13. Genotype–phenotype correlations

A wide spectrum of inter and intrafamilial clinical variability was observed in 14 patients (six males and eight
new gene, FKRP gene that mapped at the same locus of the LGMD2I. This gene was identified through homology to the Fukuyama congenital muscular dystrophy (FCMD) gene (a severe progressive muscular weakness from early infancy and marked central nervous system involvement [101]).

The FKRP gene consists of four exons, but the entire coding sequence is located within exon 4. Mutations in this gene were shown to cause LGMD2I as well as the congenital muscular dystrophy 1C (CMD1C) [21,100]. CMD1C is a severe condition (age at onset in the first few weeks of life and inability to walk) while LGMD2I has a milder and variable course (age at onset in the second or third decade of life and slower progression), both with preserved intelligence and elevated serum CK. In both disorders, weakness and wasting of the shoulder-girdle muscles and primary restrictive respiratory and cardiac involvement have been reported [21,100,102].

Although first identified in Tunisia, LGMD2I was subsequently found to be rare in this country but the commonest form in several European countries. Among German patients, LGMD2I has been shown to represent the most frequent differential diagnosis between DMD and BMD [122; Thomas Voit, personal communication].

Interestingly, some LGMD Hutterite families did not appear to be linked to the chromosome 9 loci. Indeed when the putative disease-causing mutation (D487N in TRIM32) for LGMD2H was identified, none of the affected individuals in these families carried this mutation, indicating genetic heterogeneity. More recently, DNA sequence analysis of the FKRP gene in these patients revealed that they carry the missense C826A mutation [20]. Surprisingly this mutation has been shown to be the most common mutation among LGMD2I patients. The presence of both LGMD2H and LGMD2I in the Hutterite population further emphasizes the importance of taking into account the possibility of genetic heterogeneity in gene mapping studies even in genetically isolated population.

The function of the FKRP is still unknown but it has sequence similarities to a family of proteins involved in the glycosylation of cell surface molecules [100]. Direct protein quantification of FKRP was not reported yet, since there is no available antibody to date. However, some interesting secondary protein alterations have been reported in LGMD2I and CMD1C patients. These include a secondary laminin α2 reduction, mainly on immunoblots, reduced WB labeling for laminin β1 and secondary reduction of calpain-3. A variable reduction of α-DG expression was also observed in the skeletal muscle biopsy of affected individuals, with a reduction in the MW on immunoblotting, which could indicate a glycosylation defect associated to this disease, a novel pathogenic mechanism in muscular dystrophy [100,102]. Until a proper antibody against FKRP is developed, muscle proteins analysis showing a relative secondary deficiency of α-DG and laminin α2 might suggest a diagnosis of LGMD2I, which should be confirmed through molecular analysis.
16. Genotype–phenotype correlations

Similar to severe DMD and the milder allelic form of BMD, both caused by mutations in the dystrophin gene, it was shown that mutations in the FKRP gene could cause two clinically distinct disorders with overlapping symptoms resulting in an intermediate phenotype. At the most severe end of the spectrum, children never achieve independent ambulation or may even be associated to severe mental retardation as recently shown in two unrelated Turkish patients [103]. At the other end of the spectrum patients may be asymptomatic until their fifth or sixth decades. The common C826A mutation was found in the majority of the mildly affected patients. Intermediate cases often are compound heterozygotes for this mutation and another missense or stop mutation. The most severe cases were found to be compound heterozygotes for two other missense mutations or one missense and one stop codon mutation. No patients have yet been reported with two null type mutation [22]. However, similar to most forms of LGMD there is intrafamilial variability in the presence of other complications such as respiratory or cardiac failure or in the severity of the phenotype [104].

The common prevalent C826A missense mutation was found in most European LGMD2I families associated frequently within two distinct haplotypes, suggesting few independent mutation events [21]. Our research group has identified 16 clinically affected patients from 13 Brazilian LGMD2I genealogies who carry mutations in the FKRP gene (de Paula et al., manuscript in preparation). Five families share the recurrent C826A change (four in homozygous and one in heterozygous state). Eight missense pathogenic changes (not found in at least 100 normal controls) were identified in the other patients. With exception of one affected female, deceased at age 33, the patients from these 11 genealogies (seven females and four males), aged 25–51, are still ambulant. In one additional isolated affected male, with an intermediate clinical course, a FKRP mutation was identified in just one allele. The most severe course was observed in three affected sisters who are compound heterozygous for one missense and one stop codon mutation. The two older sisters had a Duchenne-like course, deceased at age 14 and 15 but the younger sister can still walk short distances at age 24. On the other end of the spectrum four individuals from two unrelated families, carrying novel homozygous missense mutations were completely asymptomatic [105]. It is important to point out that these cases would remain undetected if they did not have clinically affected relatives, which suggests that the frequency of mutations in the FKRP gene may be underestimated.

In short, genotype–phenotype correlation studies for the FKRP gene are still emerging. However, the identification of pathogenic mutations in patients from different populations suggests that the spectrum of clinical variability associated with mutations in the FKRP gene seems to be wider than for other forms of LGMD [106].

17. LGMD2J

A new form of LGMD has been recently classified as LGMD2J based on the description of a Finnish family with patients homozygous for a titin mutation, which in heterozygous states causes AD tibial muscular dystrophy ([38,107], Bjarne Udd, personal communication). The coding region of the titin gene has 363 exons, which encodes a giant structural protein of the sarcomere [108]. The titin molecule has several ligand-binding sites along its structure. Two of the titin ligands, the muscle-specific protease calpain and the telethonin are of special interest because they are mutated in LGMD2A [6] and LGMD2G [19].

An interesting question is whether we should classify patients carrying two mutated titin alleles as affected by an AR form of LGMD, that is LGMD2J, or as homozygous for AD tibial muscular dystrophy. The observation that homozygous patients have a different phenotype, a more severe-childhood-onset-LGMD ([38] Bjarne Udd, personal communication) when compared with the dominant form (where clinical symptoms occur at age 35–45 or much later) suggests that the first alternative is probably more appropriate.

18. Relative proportions of the different forms

The relative proportion of the different AR-LGMD forms varies in different populations. For example in North Africa and Turkey, sarcoglycanopathies seem to be the most prevalent forms while in Europe, LGMD2A is more common in some countries such as Italy or Spain and LGMD2I in others such as England [88,109]. Apparently LGMD2H has been found so far only among Hutterites [20] while the incidence of LGMD2J around the world is still unknown.

In Brazil the relative proportion of the different forms among classified patients (through DNA and/or muscle protein analysis) from 120 families was found to be: 32% for LGMD2A, 22% for LGMD2B, 32% for the sarcoglycanopathy group, 3% for LGMD2G and 11% for LGMD2I. Therefore, the most prevalent form is LGMD2A and the less common are telethoninopathy and 8-SG although both forms have been identified in our population (Table 1).

19. Main remarks and conclusions

For differential diagnosis, the following observations should be considered. Among patients with a milder course, those with distal atrophy, inability to walk on tip toes
and very high serum CK levels are more likely to have LGMD2B. However, the clinical presentation may be very similar in patients with the different forms of AR-LGMD. Those with a severe DMD-like course and proximal weakness are more likely to belong to the sarcoglycanopathy, LGMD2I or calpainopathy subgroups [22,23,44]. Testing for defective protein expression is a very useful tool for directing the search for gene mutations. For

<table>
<thead>
<tr>
<th>LGMD</th>
<th>Protein</th>
<th>Age at onset</th>
<th>Loss of ambulation in most cases</th>
<th>Serum CK fold increased</th>
<th>Gender distribution</th>
<th>Relative proportions</th>
<th>Cardiac involvement</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD2A</td>
<td>Calpain-3</td>
<td>First to second decade</td>
<td>Second to fifth decade</td>
<td>Normal to &gt;60</td>
<td>May be more severe in males than females</td>
<td>Relatively common in many countries; 32% in Brazil</td>
<td>Rare</td>
<td>DNA and muscle protein</td>
</tr>
<tr>
<td>LGMD2B</td>
<td>Dysferlin</td>
<td>Second to sixth decade</td>
<td>Third to sixth–seventh decade</td>
<td>6 to &gt;150</td>
<td>No gender difference</td>
<td>Relatively common in many countries; 22% in Brazil</td>
<td>Rare</td>
<td>Muscle protein</td>
</tr>
<tr>
<td>LGMD2C</td>
<td>γ-SG</td>
<td>First to third decade</td>
<td>Second to third decade</td>
<td>10 to &gt;100</td>
<td>No gender difference</td>
<td>Reported in many countries; very common in North Africa; 23% of Brazilian SG</td>
<td>Common</td>
<td>DNA and muscle protein</td>
</tr>
<tr>
<td>LGMD2D</td>
<td>α-SG</td>
<td>First to third decade</td>
<td>Second to fourth or fifth decade</td>
<td>10 to &gt;100</td>
<td>No gender difference</td>
<td>The most frequent SG-glycanopathy in many countries; 40% of Brazilian SG</td>
<td>Common</td>
<td>DNA and muscle protein</td>
</tr>
<tr>
<td>LGMD2E</td>
<td>β-SG</td>
<td>First to third decade</td>
<td>Second to fourth decade</td>
<td>10 to &gt;100</td>
<td>No gender difference</td>
<td>Reported in many countries; 23% of Brazilian SG</td>
<td>Common</td>
<td>DNA and muscle protein</td>
</tr>
<tr>
<td>LGMD2F</td>
<td>δ-SG</td>
<td>First decade</td>
<td>Second decade</td>
<td>10 to &gt;100</td>
<td>No gender difference</td>
<td>Relatively rare 14% of Brazilian SG</td>
<td>Common</td>
<td>DNA and muscle protein</td>
</tr>
<tr>
<td>LGMD2G</td>
<td>Telethonin</td>
<td>Second to third decade</td>
<td>Third to fifth decade</td>
<td>1.5–25</td>
<td>Males more severely affected</td>
<td>Described only in Brazil—3% of the cases</td>
<td>Common</td>
<td>DNA and muscle protein</td>
</tr>
<tr>
<td>LGMD2H</td>
<td>TRIM32</td>
<td>Second to third decade</td>
<td>Most ambulant in the sixth decade</td>
<td>On average fourfold above normal</td>
<td>No reported gender difference</td>
<td>Described only in Hutterites</td>
<td>?</td>
<td>DNA</td>
</tr>
<tr>
<td>LGMD2I</td>
<td>FKRP CMD1C</td>
<td>From birth to fourth or fifth decade</td>
<td>Extremely variable from early childhood to absence of weakness in fourth decade</td>
<td>20 to &gt;100</td>
<td>No reported gender difference</td>
<td>The commonest AR-LGMD in many countries; 13% in Brazil</td>
<td>Common</td>
<td>DNA</td>
</tr>
<tr>
<td>LGMD2J</td>
<td>Titin</td>
<td>First to third decade</td>
<td>Normal to two-threefold</td>
<td>No reported gender difference</td>
<td>Described in Finland; still unknown</td>
<td>?</td>
<td>DNA</td>
<td></td>
</tr>
</tbody>
</table>
example, we start mutation screening in the α-SG gene in patients with deficiency of any component of the SG complex since this is the most prevalent sarcoglycanopathy in our population. Among the non-sarcoglycanopathies, the absence of dysferlin or telethonin on muscle biopsy strongly suggests a diagnosis of LGMD2B or LGMD2G, respectively. However, results on calpain analysis should be interpreted with caution since normal or only slight reductions may be found in LGMD2A patients while secondary deficiencies may occur in other forms of MD. When no muscle is available, the choice of what gene should be screened first for mutations will depend on the relative frequency of each form in different populations.

Among the 10 AR-LGMD forms already identified, with exception of LGMD2H and 2J, all others are well represented in the Brazilian population. This allows some interesting comparative observations for the eight AR-LGMD subtypes present in Brazil, such as:

(a) Inter and intrafamilial clinical variability was observed among patients from all groups with exception of LGMD2F where all patients reported so far present a severe phenotype.
(b) Calf hypertrophy was observed in patients from all groups, but is rare among LGMD2B patients.
(c) Heart involvement was observed in Brazilian patients from all groups in particular in sarcoglycanopathies and LGMD2I patients. It is rare among calpainopathy and dysferlinopathy patients.
(d) On an average, LGMD2B (dysferlin) patients were the least affected but some missense mutations in the FKRP gene may be associated with a very mild phenotype or even non-penetrance in the fourth decade.
(e) In addition to sarcoglycanopathies, a severe Duchenne-like phenotype may be seen among LGMD2I and LGMD2A patients.
(f) Serum CK activities showed on an average the highest values in sarcoglycanopathies and dysferlinopathy patients and are elevated in all ambulant patients. In the telethonin group serum CK may range from borderline to 30-fold increase.
(g) Although most ambulant LGMD2A patients have elevated serum CK, some may have normal serum levels even in the active phase of the disease.
(h) Some LGMD2G (telethonin) patients had distal atrophy with a phenotype resembling MM while the others had calf hypertrophy with a typical LGMD presentation.
(i) A gender difference in the severity of the clinical course, affecting more males than females, may exist for calpainopathy and telethoninopathy but apparently not for dysferlinopathy and sarcoglycanopathy.
(j) The spectrum of clinical variability associated with mutations in the FKRP gene seems to be wider than for other forms of LGMD ranging from severe congenital forms to absence of symptoms in the fourth decade.

Understanding how clinically indistinguishable phenotypes may be observed among patients with different forms of LGMD, while a discordant phenotype may be seen in unrelated patients carrying the same mutation and even among affected sibs remains a major challenge. It will depend on future knowledge on gene expression, gene and protein interactions, protein processing and on identifying modifying genes or other mechanisms underlying clinical variability.

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