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The Seventh Form of Autosomal Recessive Limb-Girdle Muscular Dystrophy Is Mapped to 17q11-12

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Summary

The group of autosomal recessive (AR) muscular dystrophies includes, among others, two main clinical entities, the limb-girdle muscular dystrophies (LGMDs) and the distal muscular dystrophies. The former are characterized mainly by muscle wasting of the upper and lower limbs, with a wide range of clinical severity. This clinical heterogeneity has been demonstrated at the molecular level, since the genes for six AR forms have been cloned and/or have been mapped to 15q15.1 (LGMD2A), 2p12-16 (LGMD2B), 13q12 (LGMD2C), 17q12-q21.33 (LGMD2D), 4q12 (LGMD2E), and 5q33-34 (LGMD2F). The AR distal muscular dystrophies originally included two subgroups, Miyoshi myopathy, characterized mainly by extremely elevated serum creatine kinase (CK) activity and by a dystrophic muscle pattern, and Nonaka myopathy, which is distinct from the others because of the normal to slightly elevated serum CK levels and a myopathic muscle pattern with rimmed vacuoles. With regard to our unclassified AR LGMD families, analysis of the affected sibs from one of them (family LG61) revealed some clinical and laboratory findings (early involvement of the distal muscles, mildly elevated serum CK levels, and rimmed vacuoles in muscle biopsies) that usually are not observed in the analysis of patients with LGMD2A–LGMD2F. In the present investigation, through a genomewide search in family LG61, we demonstrated linkage of the allele causing this form of muscular dystrophy to a 3-cM region on 17q11-12. We suggest that this form, which, interestingly, clinically resembles AR Kugelberg-Welander disease, should be classified as LGMD2G. In addition, our results indicate the existence of still another locus causing severe LGMD.

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Introduction

Autosomal recessive (AR) limb-girdle muscular dystrophies (LGMDs) represent a heterogeneous group of diseases, characterized by a primary and progressive muscle degeneration of the pelvic and shoulder girdles. A wide spectrum of clinical disability is observed, ranging from very mild to severe forms (Walton and Gardner-Medwin 1988; Bushby and Beckmann 1995).

Within the last few years, six genes that are responsible for the AR LGMDs have been mapped and/or cloned, confirming their clinical heterogeneity at the molecular level. These six different forms have been named chronologically, according to their gene identification, as follows: LGMD2A at 15q15.1 (Beckmann et al. 1991; Richard et al. 1995), LGMD2B at 2p12-16 (Bashir et al. 1994), LGMD2C at 13q12 (Othmane et al. 1992; Noguchi et al. 1995), LGMD2D at 17q12-q21.33 (Roberds et al. 1993, 1994; McNally et al. 1994), LGMD2E at 4q12 (Bönnemann et al. 1995; Lim et al. 1995), and LGMD2F at 5q33-34 (Nigro et al. 1996*a,* 1996*b;* Passos-Bueno et al. 1996*b*). Except for the LGMD2B gene, all the other LGMD genes have been cloned. The gene responsible for LGMD2A encodes calpain 3, or CANP3, a muscle-specific protease (Richard et al. 1995), whereas the genes that cause LGMD2C–LGMD2F encode proteins of the dystrophin-glycoprotein complex (DGC) (Roberds et al. 1993; McNally et al. 1994; Bönnemann et al. 1995; Lim et al. 1995; Noguchi et al. 1995; Richard et al. 1995; Nigro et al. 1996*b*).

The DGC is a large complex formed by dystrophin and its associated proteins (DAPs) and glycoproteins (DAGs) (Campbell 1995; Ozawa et al. 1995; Worton 1995). The DAPs (α-, β1-, and β2-syntrophin and dystrobrevin) are arranged in the syntrophin complex (Adams et al. 1993; Ahn et al. 1994; Peter et al. 1994; Yang et al. 1994; Sadoulet-Puccio et al. 1996). The DAGs are distributed between two subgroups, the dystroglycan complex (α- and β-dystroglycan) (Ibraghimov-Beskrovnaya et al. 1993; Yoshida et al. 1994) and the sarcoglycan complex (α-, β-, γ-, and δ-sarcoglycan and 25DAG) (Campbell 1995; Ozawa et al. 1995). The DGC establishes a connection between the citoskeleton (through actin) and the extracellular matrix (through laminin),

protecting muscle fibers from mechanical damage during contractions (Campbell 1995). It recently has been demonstrated that pathogenic mutations in the α-, β-, γ-, and δ-sarcoglycan genes are responsible for LGMD2D, LGMD2E, LGMD2C, and LGMD2F, respectively (Roberds et al. 1994; Bönnemann et al. 1995; Lim et al. 1995; Noguchi et al. 1995; Nigro et al. 1996*a*). LGMD caused by a defect in any of the four components of the sarcoglycan complex has been classified as sarcoglycanopathy.

LGMD patients who have mutations in either the LGMD2A locus or the LGMD2B locus have, in the majority of cases, a relatively mild phenotype—that is, they are able to walk unassisted after the age of 16 years (Beckmann et al. 1991; Bashir et al. 1994; Passos-Bueno et al. 1995*a,* 1996*a;* Richard et al. 1995). On the other hand, patients with any of the four sarcoglycanopathies usually have a severe phenotype associated with total or partial deficiency of α-sarcoglycan in the muscle (Roberds et al. 1994; Bönnemann et al. 1995, 1996; Lim et al. 1995; Noguchi et al. 1995; Passos-Bueno et al. 1995*b;* McNally et al. 1996; Nigro et al. 1996*a;* Vainzof et al. 1996).

AR–muscular dystrophy patients with involvement of both the shoulder and pelvic girdles but with atrophy beginning in the hands or in the feet have been reported in previous studies (Nonaka et al. 1981; Argov and Yarom 1984; Markesbery and Criggs 1986; Miyoshi et al. 1986; Isaacs et al. 1988; Walton and Gardner-Medwin 1988; Sunohara et al. 1989; Yamanouchi et al. 1994). These families originally had been classified as having one of the two forms of distal muscular dystrophy, Miyoshi myopathy (MM) or Nonaka myopathy (also known as "distal myopathy with rimmed-vacuole formation" ["DMRV"]) (Nonaka et al. 1981; Miyoshi et al. 1986).

MM is characterized by (1) very high serum creatine kinase (CK) activity; (2) muscle weakness and atrophy most marked in the legs, with relative sparing of the arms, neck, and small hand muscles; and (3) a dystrophic muscle pattern, with fiber necrosis and regeneration (Miyoshi et al. 1986; Bejaoui et al. 1995). The MM gene recently was mapped to 2p12-14, to the same region as that of the gene for the LGMD2B form (Bejaoui et al. 1995). Weiler et al. (1996) described a large inbred aboriginal Canadian kindred with AR muscular dystrophy also linked to this same chromosome 2p region. Interestingly, seven individuals presented an LGMD phenotype and two an MM phenotype. These authors demonstrated that four LGMD patients and the two MM patients were homozygous for the same haplotype, supporting the hypothesis that LGMD2B and MM may be caused by mutations in the same gene.

DMRV is characterized by (1) onset in the distal muscles of the legs, in the second or third decade of life; (2) a rapid progression, with the proximal muscles of the legs, the small hand muscles, and the arm and neck muscles being involved at an early stage; (3) normal or mildly elevated serum CK levels; and (4) a myopathic muscle pattern (with no apparent fiber necrosis and regeneration) and the presence of rimmed vacuoles as a prominent feature (Nonaka et al. 1981; Isaacs et al. 1988; Sunohara et al. 1989; Yamanouchi et al. 1994; Murakami et al. 1995). The distinction between DMRV and hereditary inclusion body myopathy (HIBM), which are both the result of AR inheritance, is very important (Nonaka et al. 1981; Argov and Yarom 1984). HIBM patients present with progressive muscular weakness, normal or mildly increased serum CK levels, and a myopathic muscle pattern with rimmed vacuoles. However, the distinguishing features of this disorder, as compared with those of other myopathies, are the sparing of the quadriceps and the age at onset, which is usually 20–40 years of age (Argov and Yarom 1984; Mitrani-Rosenbaum et al. 1996). The gene that causes HIBM, which apparently has been reported only among individuals of Persian Jewish descent, recently was mapped to 9p1-q1 (Mitrani-Rosenbaum et al. 1996).

We have identified 26 families with AR LGMD (21 with mild forms and 5 with severe forms), on the basis of clinical evolution, high serum CK levels, and the analysis of muscle biopsies (including the assessment of dystrophin and of the four sarcoglycans). Linkage analysis of these 26 families has demonstrated the existence of at least another locus, since 3 families still were unlinked to any known LGMD loci, as well as to the HIBM locus (Bönnemann et al. 1996; McNally et al. 1996; Passos-Bueno et al. 1996*a,* 1996*b;* E. S. Moreira, M. Zatz, and M. R. Passos-Bueno, unpublished data). Analysis of muscle biopsies from these three kindreds, which were all positive for the sarcoglycan complex, revealed the presence of a great number of rimmed vacuoles in one of them (family LG61). Since family LG61 is large enough for mapping through linkage analysis, we performed a genomewide search in this family. The results suggest the existence of another locus for AR LGMD at 17q11-12.

Subjects and Methods

Subjects

Three AR LGMD families (LG6, LG11, and LG61) reported in previous studies (Passos-Bueno et al. 1996*a,* 1996*b*) were included in the present analysis. Diagnosis was based on clinical examination and course of the disease, family history, serum CK levels, and assessment of dystrophin through immunocytochemistry/western blotting of muscle biopsies. Serum CK analysis was performed by use of Sigma kits; normal levels are considered to be ⩽10 sigma units (SU)/ml for adults and ⩽20

SU/ml for children. Five of the six affected sibs in family LG61, as well as the three patients in family LG11, recently have been clinically and neurologically reexamined by one of us (S.K.M.). Muscle strength was assessed in accordance with the manual muscle test, which is based on the Medical Research Council's recommendations. Muscle biopsies of biceps were performed on at least one individual from each family. Dystrophin and α-sarcoglycan immunofluorescence analyses were performed as reported in previous studies (Vainzof et al. 1991; Passos-Bueno et al. 1995*b*).

Genotyping

DNA was extracted from whole blood, according to standard protocols (Miller et al. 1988). For the genomewide search, DNA samples were analyzed by PCR amplification using microsatellite markers purchased from Research Genetics and from Isogen. For refinement of the candidate area, markers were selected from the study by Dib et al. (1996). Standard protocols were used for PCR reactions. The products were visualized on 6.5% denaturing gels, which were dried and exposed to x-ray films for 2-24 h, as reported in a previous study (Passos-Bueno et al. 1995*b*).

Linkage Analysis

Two-point linkage analysis was performed by use of the computer program MLINK (Lathrop et al. 1984), with an estimated gene frequency of .001 for the disease allele and with equal recombination for both sexes. An equal frequency of the alleles for most of the markers was considered, although we are aware that this could have inflated LOD scores (Terwilliger and Ott 1994). Allele frequencies for the markers mapped to the candidate region were estimated on the basis of the analysis of 86–100 chromosomes from normal Caucasian subjects from our population (table 1). The sizes of the alleles were measured through comparison with the DNA sequence of the bacteriophage M13mp18 (United States Biochemical).

Table 1

Size and Frequency of the Alleles That Are Segregating with the Disease Allele, in Families LG11 and LG61

Marker and Allele(s)[a]	Size (bp)	Frequency
D17S1867:		
2	98	.22
3	96	.54
D17S250:		
7	151	.27
D17S1851:		
2	251	.28
3	249	.27
D17S946:		
2	140	.06
4	136	.31
D17S1818:		
2	147	.05
6	135	.21
D17S1814:		
2	162	.25
8	150	.32

[a] The allele numbers correspond to those shown in the haplotypes of figure 2.

Results

Pedigree and Clinical Description of Family LG61

Family LG61 is a Caucasian nonconsanguineous kindred of Italian ancestry. The parents (66 and 69 years of age) had a total of eight offspring—two of which are unaffected (39 and 44 years of age) and six of which are affected (27–43 years of age). The main clinical findings for all the patients are described in table 2.

This family was first seen in our center 20 years ago. The mean age at onset was 12.5 years, which is when the patients showed difficulty with walking, running, and climbing stairs. They report that at the same time or soon after the first signs were noted, they were unable to perform ankle dorsiflexion. Difficulty with walking on the heels appeared prior to difficulty with walking on the toes. Extraocular and facial muscles were spared in all the patients. In five sibs, who recently have been clinically and neurologically reassessed (table 3), the neck muscles are only very mildly affected or not affected. In the upper limbs, proximal muscle atrophy is marked, whereas, in the lower limbs, proximal and distal muscle atrophy is evident, which is in accordance with the pattern of muscle weakness observed in both limb girdles (table 3). It is interesting that in patient II-3, who shows the mildest clinical evolution, the proximal muscles of the upper and lower limbs are less affected than the distal muscles (table 3). The tendon reflexes are abolished, without involvement of sensory and cranial nerves or of coordination. In addition, foot-drop is a common feature in all of these five patients. Four of the six affected sibs are already in a wheelchair. No evidence of cardiac disease was detected. CK levels were elevated 3–17-fold in the first stages of the disease, but they have decreased, as the patients have aged, to almost normal levels in those patients already in a wheelchair (table 2).

Muscle Pathology

The analysis of a muscle biopsy, from patient II-8 of family LG61, showed round-shaped fibers, with a marked variation in fiber size. Necrotic and regenerating fibers were found, with an increased number of central myonuclei. Infiltration of connective tissue also was ob-

Table 2

Main Clinical Findings in the LG61 Patients

Patient	Current Age (years)	Age at Onset (years)	CK Level (× Normal)			Wheelchair Confinement (Age [years])
			1976	1990	1997	
II-2	43	15	5.4	1.6	1.3	Yes (39)
II-3	41	13	4.5	3.8	4.2	No
II-5	37	13	10.4	2.5	1.2	Yes (31)
II-6	36	9	17.5	4.0	2.0	Yes (34)
II-7	33	13	3.5	3.5	2.0	Yes (31)
II-8	27	12	4.0	3.1	4.7	No

served. One rimmed vacuole per fiber was detected in a great proportion of the muscle cells (fig. 1*A*). There was no predominance of type 1 or type 2 fibers and no type grouping (fig. 1*B*). Dystrophin and α-sarcoglycan showed a positive sarcolemmal staining through immunohistochemical studies.

Linkage Analysis

In a previous study, family LG61 had been excluded for the six known AR LGMD loci, as well as for eight additional candidate regions, where the syntrophin, dystroglycan, and HIBM genes have been mapped (Passos-Bueno et al. 1996*a,* 1996*b;* E. S. Moreira, M. Zatz, and M. R. Passos-Bueno, unpublished data). Therefore, a genomewide search was performed for this genealogy.

A total of 402 markers (spaced 5–10 cM) were tested in this kindred. Evidence of linkage was observed with the microsatellite marker D17S250 (Z_{max} = 3.26; θ = .00), which is located at 17q11-12. The following additional marker sequences near this locus were genotyped: D17S1824–3 cM–D17S1800–2 cM–D17S1880–3 cM–D17S1850; D17S1833–2 cM–D17S1872–1 cM–D17S933–1 cM–D17S1867–1 cM–D17S1851;

Table 3

Manual Muscle-Strength Evaluation of the Patients from Family LG61

Movement Tested	Strength Rating, for Each Patient[a]				
	II-2	II-3	II-5	II-6	II-8
Neck flexion	5	4	4	5	4
Neck extension	5	5	5	5	5
Shoulder abduction	1	3	1	1	2
Shoulder external rotation	1	5	1	1	2
Elbow flexion	0	5	0	0	2
Elbow extension	2	5	2	2	4
Wrist flexion	4	4	4	4	5
Wrist extension	2	3	2	2	4
Thumb abduction	5	4	2	3	5
Hip flexion	1	2	1	1	2
Hip extension	1	2	1	1	2
Knee flexion	0	3	0	0	2
Knee extension	2	3	2	2	3
Ankle dorsiflexion	0	0	0	0	0
Ankle plantarflexion	0	1	0	0	0
Toe flexion	0	0	0	0	0
Toe extension	0	0	0	0	0

[a] 0 = no movement; 1 = flicker of movement; 2 = movement of the joint when the effect of gravity is eliminated; 3 = movement through full range of the joint, against gravity but not against resistance; 4 = movement of the joint, against gravity and against added resistance; and 5 = full strength.

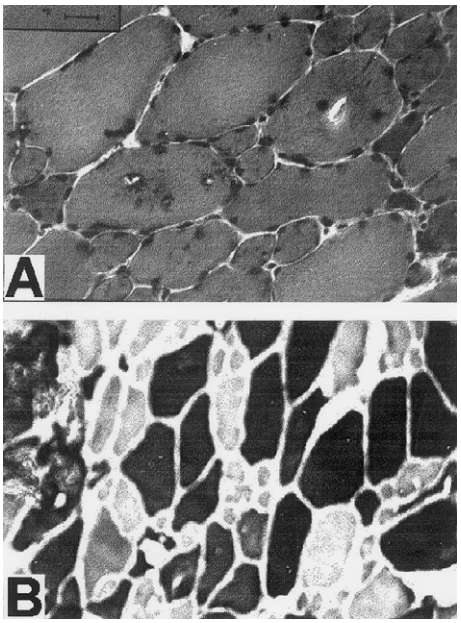

Figure 1 *A,* Muscle biopsy, from patient II-8 of family LG61, showing evidence of rimmed vacuoles in affected fibers (haematoxylin and eosin). *B,* Muscle biopsy, from patient II-8 of family LG61, showing no predominance of type 1 or type 2 fibers (ATPase stain at pH 9.4). Bar = 25 μm.

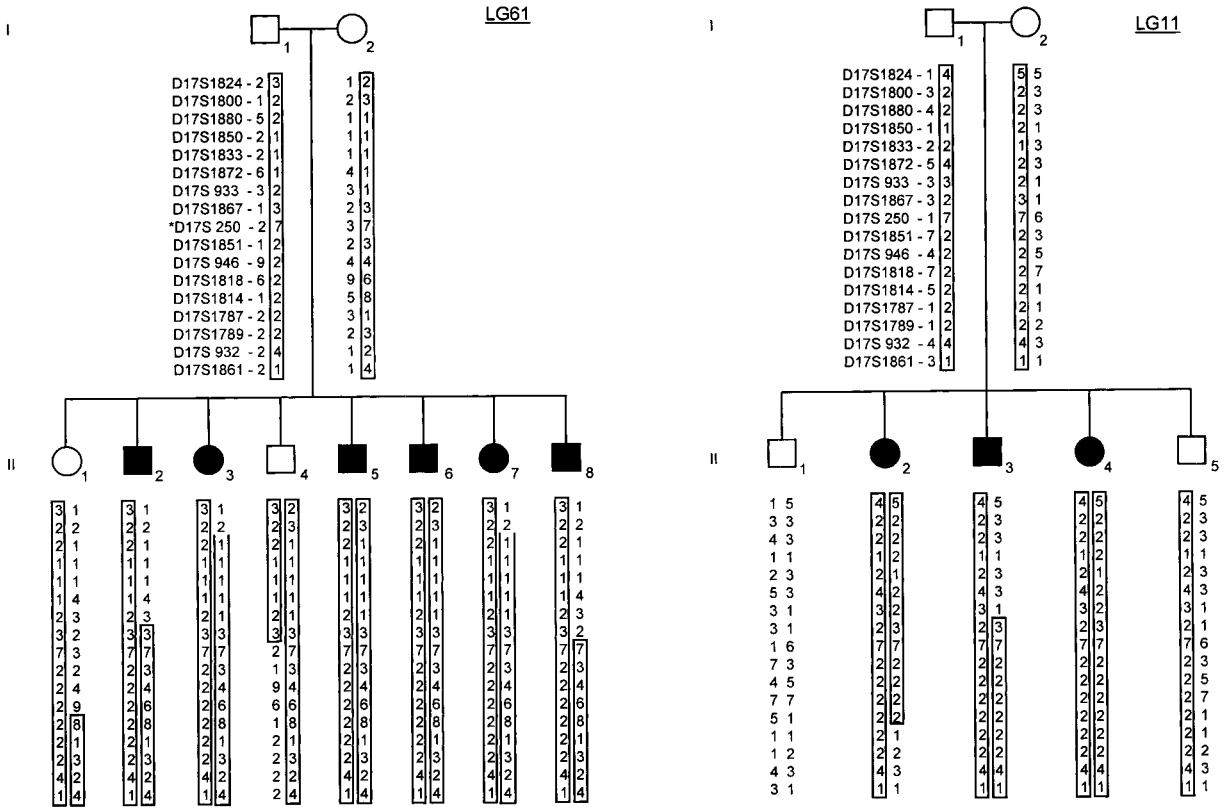

Figure 2 Haplotypes with markers from the 17q11-12 region in the two LGMD2G families. The haplotype of individual I-1 from family LG11 was deduced from the family's descendants. The haplotype or the portions of it presumed to be ancestrally associated with the disease in the families are boxed; a partially boxed haplotype indicates that it was not possible to establish with certainty the haplotype at risk (owing to lack of informativeness of the parents). An asterisk (*) indicates a marker for which the exact position is unknown; the position estimate was based on the analysis of recombinants found in this genealogy (see text).

D17S946–1 cM–D17S1818–1 cM–D17S1814–1 cM–D17S1787–1 cM–D17S1789; and D17S932–1 cM–D17S1861. Although the exact position of D17S250 is unknown, we have suggested that this marker may lie between D17S1867 and D17S1814, on the basis of analysis of recombinants observed in family LG61 (fig. 2).

Results of two-point linkage analysis between these microsatellites and the disease gene in family LG61 are described in table 4. Confirmation of linkage (LOD score >3.00) was obtained by use of two other markers, D17S1851 and D17S1818.

Two important recombinations were observed in this genealogy, involving affected individual II-8 and one of his normal sibs, individual II-1. In individual II-8, a recombination between D17S1867 and D17S1851 placed the gene distal to D17S1867, whereas, in the normal sib (individual II-1), the recombination between D17S1814 and D17S1818 positioned the gene proximal to D17S1814. Therefore, the gene must lie in the interval between D17S1867 and D17S1814. Homozygosity was found only for the 151-bp allele of marker D17S250; however, this allele is the most common in our population (fig. 2; table 1).

The markers from the candidate region also were tested in two other families (LG6 and LG11), which are unlinked to any known LGMD or candidate loci (Passos-Bueno et al., 1996*a*, 1996*b*; E. S. Moreira, M. Zatz, and M. R. Passos-Bueno, unpublished data). Positive LOD scores were detected in only one family (LG11), which was genotyped further for the other 16 markers from the 17q11-12 region (table 4; fig. 2).

In family LG11, although the LOD score was <3.00, owing to the small family size, no recombinants were found within an interval of 5 cM. In addition, homozygosity was observed between the markers D17S250 and D17S1814. Interestingly, this haplotype, which was not found in our control population (data not shown), was observed in heterozygosity, in all the affected sibs from family LG61 (fig. 2).

Discussion

In the present report, we were able to demonstrate linkage to 17q11-12 of another subtype of AR muscular dystrophy. Interestingly, this new locus has been mapped near the α-sarcoglycan gene (Roberds et al.

Table 4

Results of Two-Point Linkage Analysis between the Disease Gene and 17q11-12 Markers

	LOD Score for Family LG11, at θ =[a]					LOD Score for Family LG61, at θ =				
Marker	.00	.01	.05	.10	.20	.00	.01	.05	.10	.20
D17S1824	.55	.54	.49	.43	.30	−∞	−2.49	−.60	.05	.40
D17S1800	−∞	−.57	.03	.21	.25	−∞	−2.49	−.60	.05	.40
D17S1880	−∞	−.57	.02	.19	.22	1.15	1.14	1.06	.96	.73
D17S1850	−∞	−1.00	−.39	−.19	−.06	1.15	1.14	1.06	.96	.73
D17S1833	−∞	−.96	−.35	−.15	−.03	1.15	1.14	1.06	.96	.73
D17S1872	−∞	−.57	.02	.19	.22	−∞	−2.49	−.60	.05	.40
D17S933	−∞	−1.00	−.38	−.17	−.04	−∞	−2.49	−.60	.05	.40
D17S1867	1.45	1.42	1.29	1.13	.78	−∞	−.49	.68	.99	.98
D17S250	1.45	1.42	1.30	1.13	.79	3.26	3.20	2.95	2.63	1.94
D17S1851	1.45	1.42	1.30	1.13	.79	3.26	3.20	2.95	2.63	1.94
D17S946	1.45	1.42	1.30	1.13	.79	1.45	1.43	1.33	1.20	.91
D17S1818	1.45	1.42	1.30	1.13	.79	3.26	3.20	2.95	2.63	1.94
D17S1814	1.45	1.42	1.30	1.13	.79	−∞	1.50	1.96	1.95	1.58
D17S1787	−∞	−.57	.03	.21	.25	1.15	1.14	1.06	.96	.73
D17S1789	.55	.54	.49	.43	.30	1.15	1.14	1.06	.96	.73
D17S932	−∞	−.84	−.24	−.05	.04	−∞	1.50	1.96	1.95	1.58
D17S1861	.55	.54	.49	.43	.30	−∞	1.50	1.96	1.95	1.58

[a] Homogeneity test (Terwilliger and Ott 1994): $\chi^2 = 1.385$; $P > .05$. Heterogeneity test (Terwilliger and Ott 1994): $\chi^2 = 22.87$; $P < .001$.

1994); however, it is located at least 9 cM apart from the α-sarcoglycan gene.

Although family LG61 is not consanguineous, we have found homozygosity with the 151-bp allele of marker D17S250. Since this allele is the most common in our population, this data does not support evidence for linkage disequilibrium. Therefore, on the basis of the analysis of recombinants observed in this kindred, we have estimated that the candidate region for this new locus spans ~3 cM. Several genes lie within this area; however, their products are not specifically expressed in the muscle; therefore, they were not considered to be good functional candidate genes for this disease (OMIM 1996).

The phenotypes of the affected patients from this 17q-linked family were relatively mild until the third decade of life. However, four of the patients were confined to a wheelchair before 40 years of age, and the youngest brother, who is currently 27 years of age, walks with great difficulty and only with support, suggesting that he will not be able to walk much longer. On the other hand, patient II-3 (tables 2 and 3), who is currently 41 years of age, is showing a milder course, illustrating the intrafamilial variability in the expression of this disease gene. Although there is severe involvement of the proximal muscles of the upper and lower limbs, the marked weakness in the distal muscles of the legs, the moderately increased serum CK levels, and the presence of rimmed vacuoles in the muscle biopsy distinguish this form of AR muscular dystrophy from LGMD2A, LGMD2B, and the sarcoglycanopathies. It is important to point out that the patients noted difficulty with walking on the heels prior to difficulty with walking on the toes. This observation suggests that the tibial anterior muscle was affected before the gastrocnemius, which is just the opposite of what is described for LGMD2B or MM patients. On the other hand, the observation that the neck muscles, as well as the small hand muscles, were only slightly or not involved and the finding that the muscle pathology was typically dystrophic differs from DMRV. In addition, the patients reported here were confined to wheelchairs at least 18 years after the first signs, whereas DMRV leads to the loss of ambulation within 10 years after its onset (Murakami et al. 1995). These observations suggest that the family reported here has a different muscle disease.

In addition, one of our still-unlinked LGMD genealogies (LG11) also may be linked to this locus. The three affected sibs from this family also show a relatively mild phenotype, with onset of the disease at the beginning of the second decade of life. Two of them, who are 19 and 24 years of age, are still walking without difficulties; however, the third one, who is 23 years of age, is still walking without support but is unable to climb stairs. Although in an earlier stage, the pattern of muscle involvement in this family seems to be very similar to that presented by family LG61, in particular with regard to the initial weakness of the tibial anterior muscle (table 5). The patients' serum CK levels are only slightly elevated (31 SU). Rimmed vacuoles were observed in their

Table 5

Manual Muscle-Strength Evaluation of the Patients from Family LG11

Movement Tested	Strength Rating, for Each Patient[a]		
	II-2	II-3	II-4
Neck flexion	5	5	5
Neck extension	5	5	5
Shoulder abduction	5	1	5
Shoulder external rotation	5	1	5
Elbow flexion	5	0	5
Elbow extension	5	1	5
Wrist flexion	4	4	5
Wrist extension	4	4	4
Thumb abduction	5	4	4
Hip flexion	4	1	3
Hip extension	4	0	3
Knee flexion	4	0	3
Knee extension	4	1	3
Ankle dorsiflexion	0	0	2
Ankle plantarflexion	4	3	4
Toe flexion	4	4	4
Toe extension	4	0	0

[a] 0 = no movement; 1 = flicker of movement; 2 = movement of the joint when the effect of gravity is eliminated; 3 = movement through full range of the joint, against gravity but not against resistance; 4 = movement of the joint, against gravity and against added resistance; and 5 = full strength.

muscle biopsies; however, these vacuoles were very rare. The clinical evolution and the laboratory findings are compatible with those of the 17q-linked kindred (LG61) reported here. In addition, it is important to point out that these patients share the same haplotype as those of family LG61, which further supports the hypothesis that the disease in this family also may have been caused by mutations in this 17q locus. However, definite proof will only be possible after the identification of this gene.

The fact that one of our LGMD kindreds (LG6) was excluded from this locus was not surprising, since the patients' phenotypes are quite different from those observed in families LG11 and LG61 (Passos-Bueno et al. 1996*b*). Although also positive for α-sarcoglycan, the three affected sibs from the LG6 genealogy have very high serum CK levels and a severe clinical course typical of Duchenne-like muscular dystrophy. Since this family is not linked to any of the candidate genes already known, this result confirms the existence of at least another locus causing severe LGMD.

The classification of the muscular dystrophies, based on only clinical and histopathological aspects, has become more and more difficult, mainly for the AR subgroup, and a new classification based on molecular findings seems necessary. This is illustrated clearly by the occurrence of both LGMD and MM phenotypes in one family and by the demonstration that these two phenotypes might have been caused by mutations in the same gene (Weiler et al. 1996). Another example is the large inbred Finnish family reported by Udd et al. (1991). In this kindred, two different muscular manifestations, one compatible with LGMD and the other with distal myopathy, are segregating (Udd et al. 1991; Udd 1992).

On the basis of our findings and of the above-described observations, we propose to classify this new form of AR muscular dystrophy as LGMD2G. The fact that this 17q-linked family (LG61), which is of Italian ancestry, is nonconsanguineous suggests that mutations in this locus may be relatively common. Therefore, we think that it will be extremely important to test this new locus in AR–muscular dystrophy families who are positive for the sarcoglycan complex independently of early distal muscle involvement. Interestingly, there are some patients diagnosed as having Kugelberg-Welander disease (KW; also known as "spinal muscular atrophy type III") who have slightly elevated serum CK levels and rimmed vacuoles in their muscle biopsies, thus presenting with a phenotype resembling that of the LGMD family (LG61) described here (Fukahara et al. 1980; Aubry et al. 1995; Lefebvre et al. 1995; Rodrigues et al. 1996). In most of the KW patients, the disease is due to mutations in the survival motor-neuron (SMN) gene; however, in 5%–10% of these patients, KW seems to be caused by mutations at another locus (Aubry et al. 1995; Lefebvre et al. 1995; Rodrigues et al. 1996; Wang et al. 1996). The possibility that the pathology in these cases also is caused by mutations in the LGMD2G gene should be considered.

The identification of the genetic defects responsible for the different types of LGMD has been extremely important for the enhancement of our comprehension of the underlying pathological mechanisms. Therefore, the cloning of the gene that, when mutated, causes LGMD2G will be of fundamental importance.

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