

## Identification of a New Autosomal Dominant Limb-Girdle Muscular Dystrophy Locus on Chromosome 7

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### Summary

We report the identification of a new locus for autosomal dominant limb-girdle muscular dystrophy (LGMD1) on 7q. Two of five families (1047 and 1701) demonstrate evidence in favor of linkage to this region. The maximum two-point LOD score for family 1047 was 3.76 for D7S427, and that for family 1701 was 2.63 for D7S3058. Flanking markers place the LGMD1 locus between D7S2423 and D7S427, with multipoint analysis slightly favoring the 9-cM interval spanned by D7S2546 and D7S2423. Three of five families appear to be unlinked to this new locus on chromosome 7, thus establishing further heterogeneity within the LGMD1 diagnostic classification.

### Introduction

Limb-girdle muscular dystrophy (LGMD), which has an incidence of ~1/100,000 (Yates and Emery 1985), encompasses a clinically diverse group of disorders characterized by proximal muscle weakness first affecting the hip and shoulder girdle, elevated creatine kinase values, and absent or reduced deep-tendon reflexes. Both recessive (Jackson and Strehler 1968; Shokeir and Kobrinski 1976) and dominant (Chutkow et al. 1986; Gilchrist et al. 1988) forms have been reported, as have sporadic cases (Morton and Chung 1959).

Extensive locus heterogeneity has been demonstrated in the autosomal recessive LGMDs, with eight loci currently identified, including six cloned genes (Beckmann et al. 1991; Bashir et al. 1994; Bonnemann et al. 1995;

Noguchi et al. 1995; Moreira et al. 1997; Liu et al. 1998). The extent of heterogeneity within the dominant LGMDs mirrors that observed in the recessive form.

Speer et al. (1992) were the first to localize a form of dominant LGMD to 5q (LGMD1A [MIM 159000]) and to demonstrate evidence for locus heterogeneity (Speer et al. 1995) within the autosomal dominant LGMDs. Unique to the 5q-linked family is a dysarthric speech pattern, present in ~50% of affected family members. Three additional autosomal dominant LGMD loci have been localized. LGMD1B (MIM 159001) (van der Kooi et al. 1997), found in pedigrees from the Netherlands, Surinam, and the Caribbean, with a cardiac conduction-system defect affecting the atrial-ventricular node and with congestive heart failure in some patients, has been localized to 1q11-21. LGMD1C (MIM 601253), located at 3p25, is caused by a defect in caveolin 3 (McNally et al. 1998; Minetti et al. 1998). A third LGMD1 locus, in a family with familial dilated cardiomyopathy and a cardiac conduction-system defect, has been located on 6q23 (CDCD3 [MIM 602067]) (Messina et al. 1997). We have continued to characterize our unlinked LGMD1 families and now report identification of a new LGMD1 locus on 7q and establish evidence for further linkage heterogeneity within this diagnostic classification.

### Subjects and Methods

#### *Family Ascertainment and Diagnostic Classification*

Diagnostic classification for LGMD has been established elsewhere (Speer et al. 1992). In brief, individuals were considered affected when they had progressive proximal leg weakness with or without proximal arm weakness, absent ankle deep-tendon reflexes, and elevated creatine kinase values. The diagnostic evaluation of at least one affected member per family documented a myopathic process. Neither electromyography nor muscle biopsy demonstrated any pathognomonic features of other disorders.

Individuals with normal examination results and with

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**Table 1**  
**Family Data and Simulated Z Values for LGMD1 Pedigrees**

FAMILY	NO. OF		SIMULATED Z	
	Samples	Affected Individuals	Average (Standard Error)	Maximum
383	27	9	1.10 (.02)	3.00
1047	32	11	1.82 (.04)	4.88
1701	63	22	3.25 (.05)	9.47
1767	20	9	1.01 (.02)	2.61
1970	11	4	.59 (.01)	1.36

creatine kinase levels within normal limits were assigned gene-carrier risks based on their age at examination. The probability that an individual carries the LGMD1 gene was generated from a normal distribution, by use of the mean age at onset in the families ( $27.1 \pm 8.5$  years). Family members who have signs or symptoms suggestive of LGMD1 but who do not meet the strictly defined diagnostic criteria were considered to be of unknown disease status. All spouses were considered normal with respect to clinical status. Clinical information was scanned optically into the PEDIGENE database (Haynes et al. 1995). Informed consent was obtained from all study participants. This study has been approved by the Duke University Medical Center institutional review board.

Studies to determine the power of the available pedigree material were performed by SIMLINK (Boehnke 1986; Ploughman and Boehnke 1989). For these simulations, we used a model similar to that for the linkage analysis. We further assumed the availability of a tetraallelic marker with heterozygosity of .70, linked at 5% recombination with the disease locus. One thousand replicates were performed.

*DNA Collection and Genotyping for Genomic Screening*

For DNA extraction, a minimum of 24 ml of blood was collected in either EDTA or acid citrate-dextrose tubes, by venipuncture of participating individuals. Each sample was assigned a consecutive sample number, was entered into PEDIGENE, and was identified by that number subsequently, for all genotyping analyses. DNA was extracted from the lymphocytes in the DNA bank of the Center for Human Genetics (CHG), by use of standard protocols (Vance 1998).

One hundred thirty-five pre-labeled multiplex primer sets (comprising 430 primers) were used for the initial genomic screening set and provided an ~7-cM screen of the genome. Plates were amplified on either MJ DNA engines or Hybaid Omnigenes and then were loaded onto a 110-lane dual-gel C.B.S. electrophoretic unit, for size separation, by means of a Hamilton syringe.

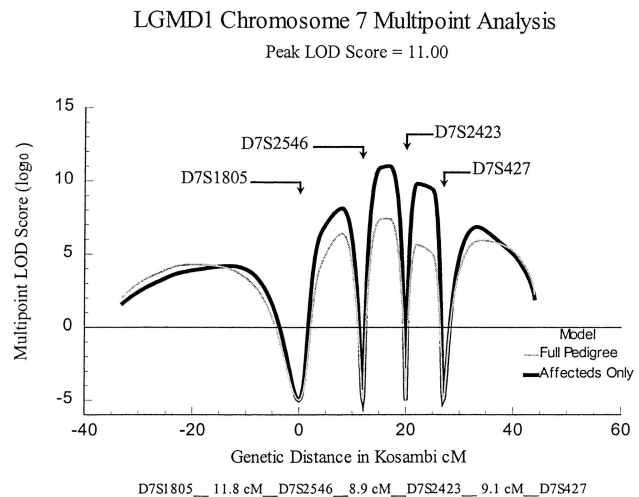
The gels were then scanned on an Hitachi FMBIO II, and the image was downloaded to the CHG network. Allele size was determined semiautomatically by the whole-band analysis program of BIOIMAGE. The genotypes were then downloaded into PEDIGENE. The integrity of the genotyping data was assessed routinely, via standard quality-control checks.

*Haplotype Analysis and Identification of the Minimum Candidate Region (MCR)*

Haplotype analysis, in which the ordered genetic markers surrounding the region of interest were assigned the most likely chromosomal configurations (haplotypes), was performed to identify critical recombination events between affected individuals. This haplotype analysis was both performed by hand and confirmed numerically by SimWalk (Weeks et al. 1995). Genetic marker genotypes for critical recombination events were confirmed on an independent DNA sample (blood spots, buccal swabs, or an additional blood sample), when possible.

*Statistical Analysis*

Two-point and multipoint LOD score (Z) values were calculated by the VITESSE computer program (Weeks et al. 1995). Both a full-pedigree analysis and an “affecteds-only” analysis were performed. The full-pedigree analysis requires specification of a penetrance model and incorporates all the available phenotypic and genotypic data, allowing the pedigree material to be maximally informative. The affecteds-only analysis utilizes the phenotypic data *only* on those individuals whose diagnostic status is most certain—that is, the affected individuals.



**Figure 1** Multipoint linkage analysis for families 1701 and 1047, for the full-pedigree and low-penetrance analyses.

**Table 2**  
Age-Dependent Data for Chromosome 7 Markers

MARKER AND FAMILY	FULL-PEDIGREE ANALYSIS					AFFECTEDS- ONLY ANALYSIS		
	Two-Point Z Value at $\theta =$				$Z_{\max}$	$\hat{\theta}$ (Support Interval)	$Z_{\max}$	$\hat{\theta}$
	.00	.05	.10	.15				
D7S1815:								
383	-6.568	-1.694	-1.127	-.804	NA	NA	NA	NA
1047	1.277	1.164	1.046	.925	1.277	.00 (.00-.40)	.573	.00
1701	-99.999	.561	.667	.646	.667	.10 (NA)	.751	.10
1767	-3.944	-.234	-.020	.068	NA	NA	NA	NA
1970	-99.999	-1.726	-1.147	-.819	NA	NA	NA	NA
D7S3058:								
383	.232	.200	.170	.142	NA	NA	NA	NA
1047	1.599	1.398	1.196	.995	1.599	.00 (.00-.26)	1.512	.00
1701	-1.619	2.080	2.568	2.627	2.627	.15 (.03-.33)	5.236	.00
1767	-99.999	-.614	-.088	.158	NA	NA	NA	NA
1970	-99.999	-3.387	-2.262	-1.618	NA	NA	NA	NA
D7S427:								
383	-2.388	-1.538	-.946	-.600	NA	NA	NA	NA
1047	3.762	3.351	2.931	2.504	3.762	.00 (.00-.12)	2.037	.00
1701	-99.999	-1.696	-.347	.217	.438	.20 (NA)	1.955	.10
1767	-99.999	-.463	-.231	-.119	NA	NA	NA	NA
1970	-99.999	-1.721	-1.143	-.815	NA	NA	NA	NA

NOTE.—NA = not applicable.

The genotypic marker data on all family members was retained in the analysis, to maximize the ability to infer genotypes from deceased relatives. Significant evidence in favor of linkage was declared when the  $Z$  at any value of the recombination fraction ( $\theta$ ) was greater than or equal to the standard  $Z$  score of 3.0. 1- $Z$ -unit support intervals for the maximum-likelihood estimate of  $\theta$  were calculated from two-point  $Z$  values, by means of the 1- $Z$ -down method (Ott 1991).

Marker alleles were estimated from a series of  $\geq 25$  unrelated individuals from an ethnically and geographically matched control population. These allele frequencies and relevant CEPH standards are available from the Duke Center for Human Genetics (Duke University Medical Center). Assessment of heterogeneity of the  $Z$  values was performed by means of the admixture test, as implemented in the HOMOG package (Ott 1991).

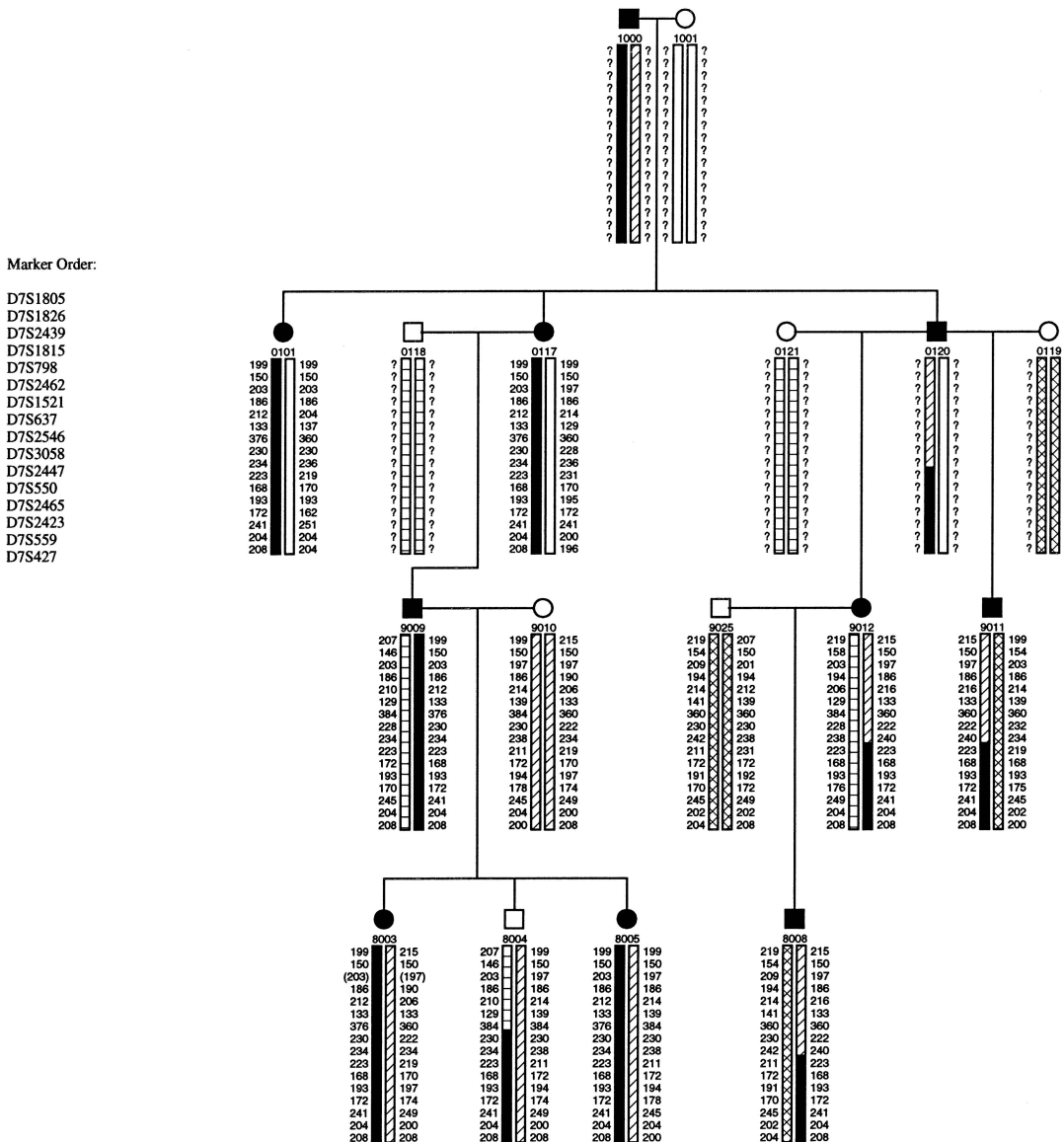
## Results

Power-study results for the available family material are presented in table 1. Of the five families, three (383, 1047, and 1701) have the capability of generating a  $Z \geq 3.0$  whereas the remaining two (1767 and 1970) can yield  $Z$  values of 2.52 and 1.36.

Genomic screening identified an area of interest on chromosome 7. Two point  $Z$  values for our five families, for markers D7S1815, D7S3058, and D7S427, are shown in table 2. For the full-pedigree model, the peak  $Z$  ( $Z_{\max}$ ) of 3.76 for family 1047 occurs for marker D7S427 at  $\theta = .00$ . For family 1701, the  $Z_{\max}$  of 2.63

occurred for D7S3058 at  $\theta = .15$ . The three remaining families (383, 1767, and 1970) yielded negative or uninformative  $Z$  values for markers in this region. Analyses performed under the low penetrance or "affecteds-only" model were consistent with the above interpretation, and in addition provided significant evidence for linkage of family 1701 to this region of chromosome 7 ( $Z_{\max} = 5.24$ ; maximum  $\theta$  [ $\hat{\theta}$ ] = .00). Evidence for linkage and for heterogeneity was significant when D7S3058, the most informative marker, was used in this low-penetrance model ( $\chi^2_1 = 5.85$ ;  $P = .008$ ). A multipoint analysis performed in all five families, using the map D7S1815-D7S3058-D7S427 with the low-penetrance model, identified two families (1047 and 1701) to be linked with  $>95\%$  probability. The remaining three families yielded posterior probabilities of linkage  $<10\%$ , as well as negative  $Z$  values, throughout the bulk of the interval. Thus, additional genotyping to identify the minimum candidate interval for the disease gene was limited to families 1047 and 1701.

Families 1047 and 1701 were subsequently genotyped for polymorphic markers between D7S1815 and D7S427, to refine the disease-gene interval. Multipoint linkage analysis was performed with the map D7S1805-11.8 cM-D7S2546-8.9 cM-D7S2423-9.1 cM-D7S427. In the full-penetrance analysis, the overall  $Z_{\max}$  was 7.40 when the disease gene was placed between D7S2546 and D7S2423; family 1047 has a  $Z_{\max}$  of 4.93, and family 1701 has a  $Z_{\max}$  of 3.68. This location is supported over the next most likely location, between D7S2423 and D7S427, with odds of 10:1. Under the

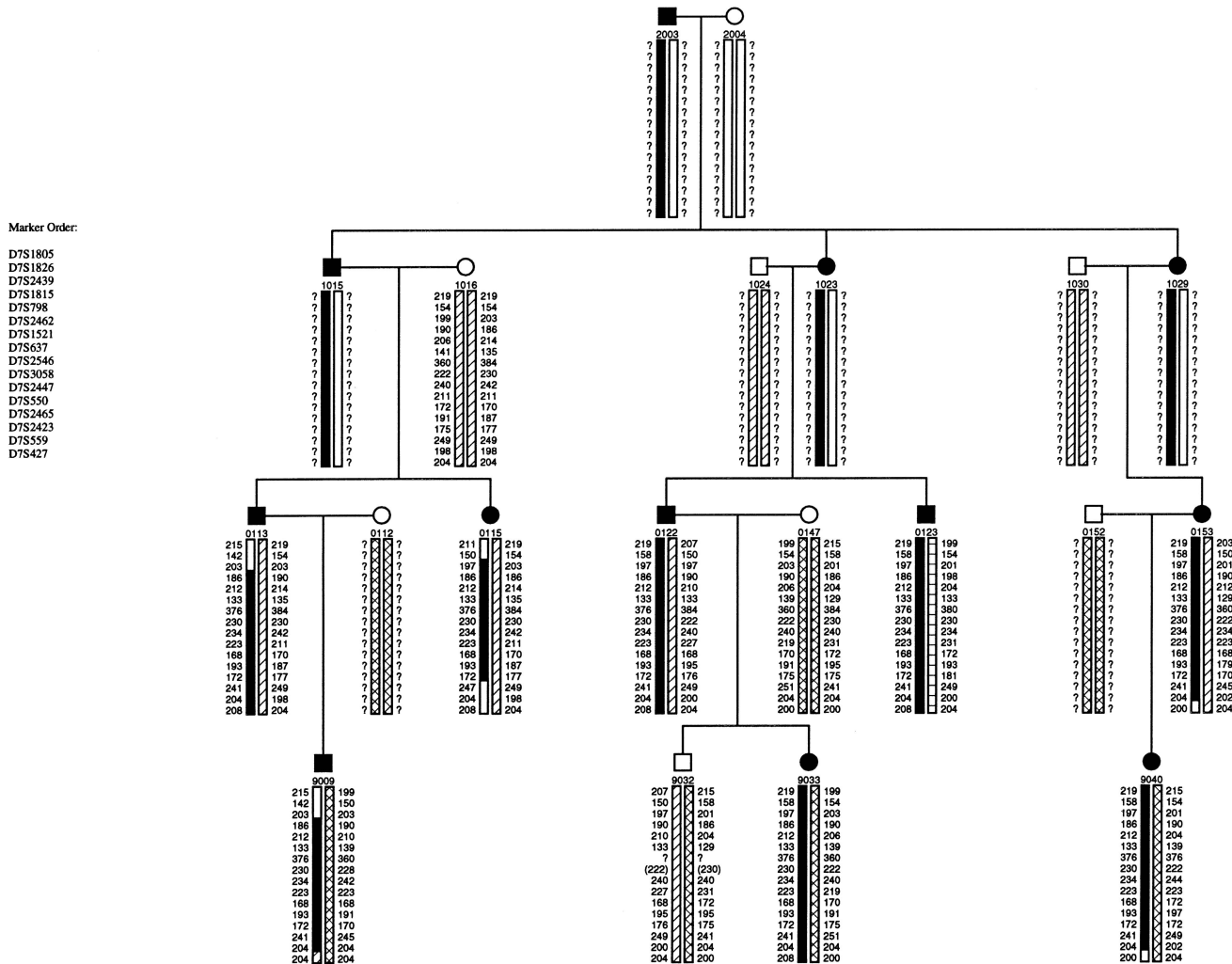


**Figure 2** Selected subset of pedigree 1047, demonstrating the proximal breakpoint of D7S2546. This recombination event likely occurred in transmission from affected individual 1000 to affected son 0120. The blackened bars represent areas of chromosome 7 that harbor the LGMD1 gene in this family. Establishment of linkage phase was performed visually and was confirmed with SimWalk (Weeks et al. 1995). Inferred genotypes are in parentheses. Note that markers D7S637 and D7S2546 are linked at 0% recombination.

low-penetrance model, the  $Z_{max}$  obtained for family 1047 was 3.85, and that for family 1701 was 7.57, with an overall  $Z_{max}$  of 11.00 in the same interval as in the full-pedigree analysis (fig. 1).

Analysis of the recombination events in affected family members confirmed that current flanking markers are D7S2546 (fig. 2) proximally and D72423 (fig. 3) distally. Specifically, a recombination event distal to D7S2546 is evident in individuals 9012 and 9011 in family 1047; minimization of the recombination events in this family implies that the event occurred in the transmission from individual 1000 to individual 0120. In family 1701, a

recombination event occurred proximal to D7S2423 in individual 0115. Interpretation of this distal breakpoint in family 1701, however, is complicated by the fact that the only difference between the disease-associated haplotype and the “recombinant” interval occurs at D7S2423, suggesting that the 247 allele may possibly represent a new mutation. If that is the case, the distal breakpoint for this gene may be D7S427 (fig. 3), thereby increasing the size of the interval to 18 cM. Individual 8004 in family 1047 was asymptomatic and was 25 years old at the time of examination, well below the average age at onset in this family, and thus the recom-



**Figure 3** Subset of family 1701, demonstrating a recombination event in O115 that confirms the distal breakpoint of D7S2423. Establishment of linkage phase was performed visually and was confirmed with SimWalk (Weeks et al. 1995). The most likely linkage phases are in parentheses. Note that markers D7S637 and D7S2546 are linked at 0% recombination.

bination event in this individual cannot currently be evaluated. Interestingly, families 1701 and 1047 share a common haplotype for the disease-associated region, suggesting that a common ancestor links the two pedigrees. However, family histories through the preceding three generations fail to identify a common ancestor.

**Discussion**

We have established evidence for linkage of two large LGMD1 pedigrees to 7q, in the 9-cM interval spanned by D7S2546 and D7S2423. This new LGMD1 gene lies in the terminal region of chromosome 7. Odds for support of this localization, however, are weak (10:1 over next most likely interval), because of the pedigree structure (earlier generations of the pedigrees are unavail-

able), limiting the ability to accurately assign linkage phase. Recruitment of additional family members is currently underway to fully refine the interval. Interestingly, several members of family 1701 are asymptomatic and likely are gene carriers, on the basis of haplotype analysis. These individuals include four (ages 27, 33, 34, and 34 years ) who are either at or above the average age at onset and two individuals (ages 6 and 21 years) who are below the average age at onset. Their inclusion in the linkage analysis utilizing an age-at-onset curve serves to lower the Z values in the full-pedigree analysis, compared with that in the affecteds-only analysis.

In addition, we have established additional underlying linkage heterogeneity within the LGMD1 classification. Clinically, all five pedigrees demonstrate identical patterns of muscle weakness in affected individuals; the only

discernible difference among the pedigrees is that family 1767 demonstrates earlier age at onset (mean  $\pm$  SD =  $6 \pm 2.3$  years) than is seen in the other pedigrees.

Clinical data on linked families 1047 and 1701 has been published previously (Schneiderman et al. 1969; Speer et al. 1995). Clinically, these families differ from other linked LGMD1 families, in their lack of other associated findings. For instance, in addition to the characteristic patterns of muscle weakness, the chromosome 5-linked LGMD1A family has a unique dysarthric speech pattern, and the chromosome 6-linked (van der Kooi et al. 1997) and chromosome 1-linked families (Messina et al. 1997) have associated cardiac abnormalities. Age at onset is similar (i.e., onset occurs in adulthood) in all linked families—with the exception of those in which the disease is due to mutations in caveolin-3, which have onset during childhood (Minetti et al. 1998).

Interestingly, the extensive heterogeneity evident within the autosomal dominant LGMDs closely mirrors that characterized in the autosomal recessive LGMDs. In the autosomal recessive LGMDs, six genes have been cloned that are involved in disease development, and four of them are known to be small transmembrane proteins, components of the sarcoglycan complex. This finding has allowed elucidation of an entire family of proteins that serve a previously unknown function in the dystrophin-glycoprotein complex. Thus, the autosomal recessive LGMDs have transformed this group of diseases from a diffuse clinical “collection of illnesses” into a group providing seminal data about the pathophysiology of the dystrophin-glycoprotein complex. The further characterization of the autosomal dominant LGMDs promises similar insights, and the clarification of the phenotypic similarities between these genetically different entities will undoubtedly shed light on important muscle pathologies.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Duke Center for Human Genetics, Duke University Medical

Center, <http://www2.mc.duke.edu/depts/medicine/medgen> (for allele frequencies and CEPH standards)  
Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omin>(for LGMD1A [MIM 159000], LGMD1B [MIM 159001], LGMD1C [MIM 601253], and CDCD3 [MIM 602067])

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