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Evidence for Locus Heterogeneity in Autosomal Dominant Limb-Girdle Muscular Dystrophy

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Summary

Limb-girdle muscular dystrophy (LGMD) is a diagnostic classification encompassing a broad group of proximal myopathies. A gene for the dominant form of LGMD (LGMD1A) has recently been localized to a 7-cM region of chromosome 5q between D5S178 and IL9. We studied three additional dominant LGMD families for linkage to these two markers and excluded all from localization to this region, providing evidence for locus heterogeneity within the dominant form of LGMD. Although the patterns of muscle weakness were similar in all families studied, the majority of affected family members in the chromosome 5–linked pedigree have a dysarthric speech pattern, which is not present in any of the five unlinked families. The demonstration of heterogeneity within autosomal dominant LGMD is the first step in attempting to subclassify these families with similar clinical phenotypes on a molecular level.

Introduction

Limb-girdle muscular dystrophy (LGMD), which has an incidence of ~1/10,000 (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 Kobrinsky 1976) and dominant (Chutkow et al. 1986; Gilchrist et al. 1988) forms have been reported, as have sporadic cases (Morton and Chung 1959). The distribution of muscle weakness is similar between the dominant and recessive forms of LGMD; however, while the adult onset form is slowly progressive and leads to loss of ambulation after many years, if at all, the recessive form is generally more severe.

A dominant form of limb-girdle muscular dystrophy (LGMD1A) has been linked to 5q (Speer et al. 1992) with regional localization to a 7-cM interval spanned by D5S178 and IL9 (Yamaoka et al. 1994). Recently, anticipation has been reported in LGMD1A (Speer et al. 1994), which suggests that an unstable trinucleotide repeat may be associated with the disease as has been shown in other neurogenetic disorders demonstrating anticipation (e.g., myotonic dystrophy [Harley et al. 1992; Mahadevan et al. 1992] and Huntington disease [Huntington's Disease Collaborative Research Group 1993]).

In the present study, we demonstrate locus heterogeneity within the dominant form of LGMD by excluding three additional autosomal dominant LGMD families from linkage to the LGMD1A region of 5q. This investigation confirms that the autosomal dominant LGMDs, while clinically similar, are truly a heterogeneous group of disorders.

Material and Methods

Family Studies

Four families (39, 383, 1047, and 1701) are included in the present analysis. Duke family 39 is linked to 5q31 (Yamaoka et al. 1994) and is described in detail elsewhere (Gilchrist et al. 1988; Speer et al. 1992). Families 383 and 1701 have been described elsewhere (Schneiderman et al. 1969; Chutkow et al. 1986). All families are of long-standing American Caucasian ancestry, with the exception of family 383, which originated in Palermo, Sicily. For this study, blood samples were obtained from 336 individuals for DNA extraction and creatine kinase testing, including 229 individuals from family 39 (62 affected), 27 from family 383 (9 affected), 24 from family 1047 (12 affected), and 56 from family 1701 (22 affected).

The diagnostic criteria for LGMD include proximal

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leg weakness with or without proximal arm weakness and elevated creatine kinase values. Affected members of all families demonstrate a similar distribution of proximal muscle weakness ranging from 0 to 4+/5 (MRC scale). There was no evidence of extraocular muscle restriction, facial weakness, calf hypertrophy, or scapular winging in any of the families.

This study was approved by the Institutional Review Board at Duke University Medical Center. Informed consent was obtained from all study participants.

DNA Isolation and Genetic Markers

DNA was isolated from leukocytes by using standard techniques (Yamaoka et al. 1990). Dinucleotide $(CA)_n$ polymorphism genotypings were carried out according to methods described by Speer et al. (1992). The markers IL9 and D5S178 were genotyped in all families, because they are the closest flanking markers to the LGMD1A interval (Yamaoka et al. 1994); in cases where D5S178 was uninformative, D5S210, 3 cM distal to D5S178, was genotyped. Primer sequences for IL9 and D5S210 (Weber et al. 1991) and D5S178 (Szubryt et al. 1993) are as published.

Linkage analysis.—For the linkage analysis, individuals were considered to be affected when they met the diagnostic criteria as described above; asymptomatic, obligate heterozygotes were also considered affected in the linkage analysis. At-risk family members who had signs or symptoms suggestive of LGMD but who did not meet the strictly defined diagnostic criteria and those family members whose only suggestive finding was an elevated creatine kinase value were considered to be of unknown disease status in the linkage analysis. All spouses were considered to be normal with respect to clinical status.

For the remaining individuals who were at risk but asymptomatic and had creatine kinase values within normal limits, penetrance was considered to be dependent on age at examination. These individuals were assigned a risk to carry the LGMD gene on the basis of age at examination as follows: since the age-at-onset distributions were similar for the families, age-at-onset data from these families were pooled. Liability classes for the linkage analysis were assigned by calculating the probability of carrying the LGMD gene, under the assumption that age at onset was normally distributed with mean and standard deviation as obtained directly from the pooled pedigree data.

Linkage analysis was performed under the assumption that LGMD was an autosomal dominant trait with a disease allele frequency of .0001; penetrance was dependent on age at examination, as described above. Two-point and multipoint linkage analyses were performed using the MLINK and LINKMAP modules, respectively, of the LINKAGE package (version 5.1; Lathrop et al. 1984) as implemented in the FASTLINK program (Cottingham et al. 1993; Schaffer et al. 1994). The genetic distance between D5S178 and IL9 has been estimated previously as 7 cM, without evidence for sex-specific differences in recombination in this interval (Yamaoka et al. 1994); the genetic distance between IL9 and D5S210 has been estimated as 10 cM (Jabs et al. 1993). Map distances were converted to recombination fractions for use in the multipoint analysis allowing for a Kosambi level of interference. Allele frequencies were calculated directly from the available pedigree data on at least 100 unrelated individuals. Frequencies for these markers and others are available via anonymous file transfer protocol (dnadoc.mc.duke.edu in the /pub/ALLELE_FREQ directory).

Simulation studies were performed to determine the maximum attainable LOD score for the family material by using the SIMLINK program (Boehnke 1986; Ploughman and Boehnke 1989) and assuming a genetic model identical to that utilized for the linkage analysis. This analysis of power further assumed the availability of a tetra-allelic marker with equally frequent alleles and that the marker demonstrated no recombination with the disease locus.

In addition, "low-penetrance" analyses were performed by using only affected family members to ensure that results were not due to asymptomatic individuals who were nonpenetrant gene carriers. For this analysis, disease phenotype information was removed for all at-risk family members while marker data on all individuals were retained (regardless of their affection status), thereby maximizing the available data for inferences of unavailable parental marker genotypes.

Investigations of heterogeneity.—Testing for linkage homogeneity was performed using the results of the two point and multipoint linkage analyses for the full pedigree and the affecteds-only analysis separately. In cases where D5S178 was uninformative, results for D5S210 were incorporated into the analyses. This procedure allows for a conservative test, since D5S210 is located further from the LGMD1A locus than is D5S178. The homogeneity testing was performed by using the admixture test as implemented in the HOMOG program (Ott 1991). Because multipoint LOD scores can be multimodal, exact interpretation of the homogeneity testing using the multipoint LOD scores is not possible (with respect to degrees of freedom), and the likelihood ratio in support of linkage heterogeneity consequently is reported for the multipoint cases.

Haplotype analysis was performed by visual inspection of the pedigree data. An interval between two markers (either IL9–D5S178 or IL9–D5S210) was considered to be excluded when two affected relatives within a family inherited different haplotypes from an affected parent; double recombination events would be expected to occur with frequency $<1\%$, under the conservative assumption of no interference.

Table 1

Two-Point LOD Scores for LGMD Families for D5S178 and IL9

	D5S178 LOD Score at θ =			IL9 LOD Score at θ =			Maximum Attainable LOD Score[a]
	.00	.05	.10	.00	.05	.10	
(a) Full Pedigree Analysis:							
39	−99.99	14.79	14.22	−99.99	19.36	18.47	NA
383[b]	−99.99	−1.49	−.97	−99.99	−3.02	−1.93	3.75
1047	−99.99	−1.51	−.80	−99.99	−1.29	−.47	4.10
1701	−99.99	−4.50	−2.47	−99.99	−3.39	−1.64	7.72
(b) Affecteds only:							
39	−99.99	9.40	8.97	−99.99	13.14	12.13	...
383[b]	−99.99	−1.54	−1.04	−99.99	−3.23	−2.14	...
1047	−99.99	−.62	−.19	−99.99	1.04	1.08	...
1701	−99.99	−1.84	−.68	−99.99	−.48	.29	...

NOTE.—NA = not applicable.

[a] See text for details of genetic model used to generate LOD scores.

[b] Results are for D5S210; see text for details.

Results

Linkage and heterogeneity analysis.—The maximum LOD score attainable, under the assumptions for age-dependent penetrance as described earlier, for each of the families is shown in table 1*a*. Results of the two-point linkage analysis for D5S178 and IL9 in the full pedigree analysis, which allows for age-dependent penetrance, are shown in table 1*a*. There was no evidence for any family to be linked to the 5q markers, except for family 39. Because D5S178 was uninformative in family 383, the more distal marker D5S210 was genotyped and replaces D5S178 in all subsequent analyses of this family. Multipoint linkage analysis (data not shown) conclusively excluded the LGMD1A interval in all three families (i.e., LOD scores ≤ -2.0 at all tested values of the recombination fraction). Evidence in favor of linkage heterogeneity was significant for both D5S178 ($\chi^2_1 = 15.85$; $P < .001$) and IL9 ($\chi^2_1 = 6.09$; $P = .007$) in the two-point linkage analysis. A test of homogeneity using the multipoint LOD scores yielded a likelihood ratio of 4.60×10^5 in favor of heterogeneity. All families yield posterior probabilities to be linked to the LGMDIA interval of <1%.

Results from the "low-penetrance" (affecteds-only) linkage analysis (two- point and multipoint) showed results consistent with those obtained in the full pedigree analysis. Two-point LOD scores for the low-penetrance analysis are shown in table 1*b*.

Haplotype analysis.—Haplotype analysis of the D5S178–IL9 interval for families 1047 and 1701 and the D5S210–IL9 interval in family 383 showed clear evidence that affected siblings within a family inherited entirely different LGMD1A intervals (fig. 1), thus confirming the exclusion of the LGMD1A interval as the gene locus in these families. On the basis of this haplotype analysis, there is no evidence for any of these three families to be linked to the LGMD1A locus.

Clinical comparisons.—While the distribution of extremity muscle weakness is consistent among all families, comparison of the clinical features of the four LGMD families (table 2) shows notable phenotypic differences between some of the families. In family 39, >50% of affected family members have an unusual, nasal quality to their speech. This characteristic speech pattern is the only consistent phenotypic difference that differentiates this chromosome 5–linked family from the remaining, unlinked families. Three of 15 affected family members of family 1701 have dysphagia, which is not observed in any affected family members in the remaining families. Onset of symptoms is reported to be adult in all families, although considerable intrafamilial differences are noted.

Discussion

Clinicians (e.g., Brooke 1986) have long been suspicious that LGMD is a diagnosis of broad scope, a "catch-all" diagnosis for proximal myopathies not otherwise classifiable. Other than LGMD, the differential diagnosis of proximal upper- and lower-extremity weakness without ocular or facial involvement with a normal or mildly elevated creatine kinase includes the progressive spinal muscular atrophies, the congenital myopathies, and metabolic myopathies. Emery-Dreifuss muscular dystrophy or Bethlem myopathy would also be included, if X-linked inheritance and/or contractures had been present. Electrophysiological studies and muscle biopsies from representative individuals within each

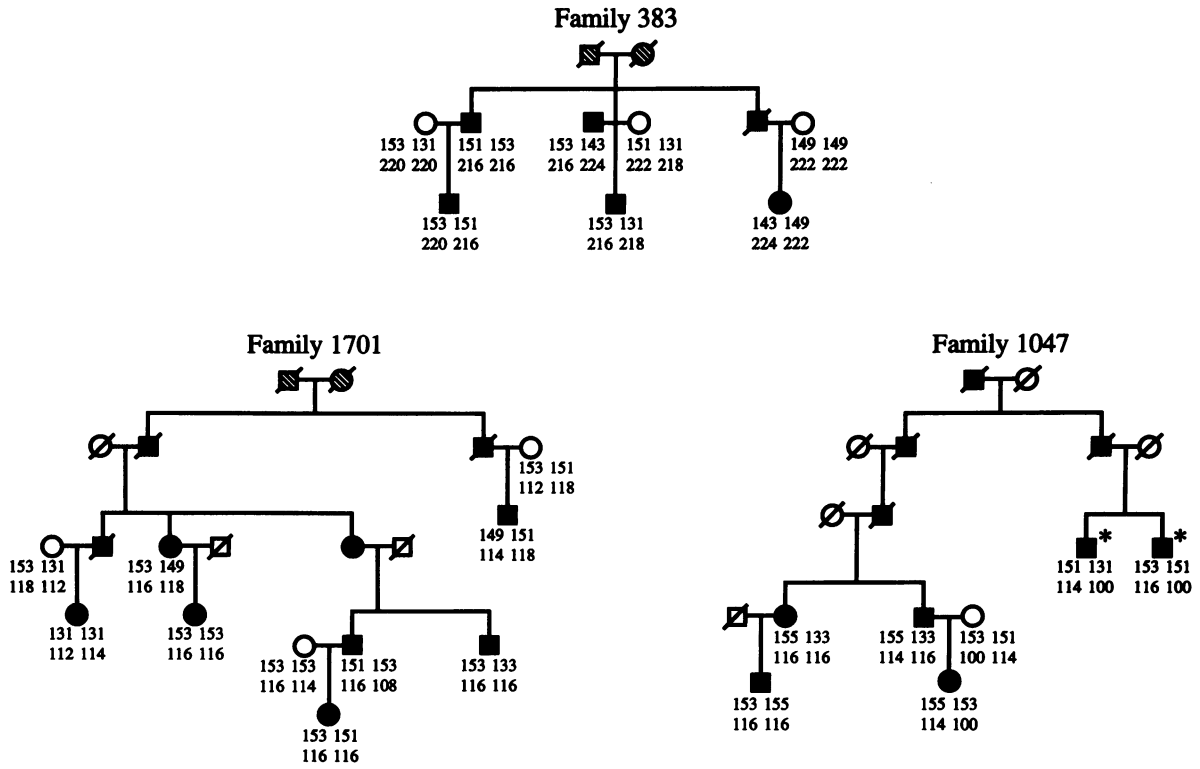

Figure 1 Partial pedigrees, showing crossover events excluding the LGMD1A interval. Alleles are shown in base pairs in the order IL9, D5S178 (D5S210 when D5S178 is uninformative). Shaded individuals are affected with LGMD, unshaded individuals are unaffected. Asterisks (*) by individuals in family 1047 indicate that additional marker genotypic data is available on unaffected siblings (who are not shown on the pedigree), to provide support for these inferred haplotypes.

of our families were consistently myopathic, and these results thereby rule out the progressive spinal muscular atrophies or other neuropathic considerations. Likewise, muscle biopsy results revealed no abnormalities of histochemical staining, accumulated vacuolated material, or abnormal ultrastructure to suggest metabolic disease. Congenital myopathies are not a consideration given the adult onset of dystrophy in these families.

A measure of the uncertainty associated with this diagnosis was demonstrated by Norman et al. (1989), who found 4/15 males diagnosed with LGMD to have a deletion within the dystrophin gene at Xp21. This report was the first attempt to subclassify individuals diagnosed with LGMD on the basis of molecular studies. The demonstration herein of locus heterogeneity within these dominant LGMD families confirms the utility of molecular means in improving their diagnostic classification, a goal already partially achieved with the dominant familial spastic paraplegias (Hazan et al. 1993, 1994; Fink et al. 1995) with at least three known loci and some families remaining unlinked. The familial spastic paraplegias are a paradigm for the extensive heterogeneity found in neurogenetic disorders with clinical similarity.

Locus heterogeneity has already been demonstrated within the recessive LGMDs. A series of recessive families from La Réunion Island (LGMD2A) was the first to be linked (Beckmann et al. 1991), with evidence for linkage to markers on chromosome 15. Subsequently, loci on chromosomes 2 (LGMD2B; Bashir et al. 1994), 13 (LGMD2C; Ben Othmane et al. 1993), and 17 (LGMD2D; Roberds et al. 1994) have been detected, with a subset of these families failing to demonstrate linkage to any known locus.

Attempts at localizing the genes responsible for the muscular dystrophy in these families are in progress and should help to illuminate the genetic bases for this heterogeneous group of disorders. The possibility of homology (Donlon and Malcolm 1991) between a large region of chromosome 5 (where LGMD1A is localized) and 15q (where LGMD2A is localized) lends credence to hypotheses postulating evolutionary and/or pathogenetic commonalities.

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Table 2

Clinical Characterization of the Four LGMD1 Families

Family	Age at Onset $\mu \pm \sigma$ (N)[a]	Creatine Kinase	Electromyography	Muscle Biopsy	Other Associated Findings
39	27.1 ± 8.5 (58)	1.5–9 × normal	Myopathic features with spontaneous activity	Increased internal nuclei, fiber splitting, myofiber degeneration, variation in fiber size, normal histochemical stains	Nasal quality in the speech of >50% of affected family members
383	35.6 ± 11.5 (6)	1.5–2 × normal	Sensory and motor velocities normal; increased numbers of small amplitude, brief duration, polyphasics	Nonspecific atrophic, hypertrophic, and fiber splitting of type 1, 2, or both; internal nuclei; myofiber necrosis and regeneration is present	None evident
1047	23.3 ± 14.3 (8)	1.5–10 × normal	Mildly abnormal, most consistent with myositis	Nonspecific targetoid changes, variation in fiber size, internal nuclei, fibrosis, normal histochemical stains, no inflammatory cells	None evident
1701	37.9 ± 11.6 (15)	1.5–3 × normal	Normal motor and sensory responses; increased polyphasic units and early recruitment, most evident in iliopsoas and gluteus medius	Small, angulated and rounded fibers, fiber splitting, fibrosis, with normal histochemical stains	3/15 affecteds with moderately severe dysphagia

[a] N = no. of individuals with age-at-onset information; μ = mean; σ = SD.

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