

A Gene for Autosomal Recessive Limb-Girdle Muscular Dystrophy in Manitoba Hutterites Maps to Chromosome Region 9q31-q33: Evidence for Another Limb-Girdle Muscular Dystrophy Locus

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Summary

Characterized by proximal muscle weakness and wasting, limb-girdle muscular dystrophies (LGMDs) are a heterogeneous group of clinical disorders. Previous reports have documented either autosomal dominant or autosomal recessive modes of inheritance, with genetic linkage studies providing evidence for the existence of at least 12 distinct loci. Gene products have been identified for five genes responsible for autosomal recessive forms of the disorder. We performed a genome scan using pooled DNA from a large Hutterite kindred in which the affected members display a mild form of autosomal recessive LGMD. A total of 200 markers were used to screen pools of DNA from patients and their siblings. Linkage between the LGMD locus and *D9S302* (maximum LOD score 5.99 at recombination fraction .03) was established. Since this marker resides within the chromosomal region known to harbor the gene causing Fukuyama congenital muscular dystrophy (FCMD), we expanded our investigations, to include additional markers in chromosome region 9q31-q34.1. Haplotype analysis revealed five recombinations that place the LGMD locus distal to the FCMD locus. The LGMD locus maps close to *D9S934* (maximum multipoint LOD score 7.61) in a region that is estimated to be ~4.4 Mb (Genetic Location Database composite map). On the basis of an inferred ancestral recombination, the gene may lie in a 300-kb region between *D9S302* and *D9S934*. Our results provide compelling evidence that yet another gene is involved in LGMD; we suggest that it be named “LGMD2H.”

Introduction

The limb-girdle muscular dystrophies (LGMDs) represent a clinically and genetically heterogeneous group of disorders with dominant and recessive inheritance. Seven genes (*LGMD2A–LGMD2G*) encoding autosomal recessive forms of the disorder have now been localized (Bashir et al. 1994; Roberds et al. 1994; Bönnemann et al. 1995; Lim et al. 1995; Noguchi et al. 1995; Richard et al. 1995; Nigro et al. 1996; Passos-Bueno et al. 1996; Moreira et al. 1997), and, on the basis of the exclusion of currently known loci, at least one more is postulated to exist (Moreira et al. 1997; Weiler et al. 1997). Gene products have been identified for five of the autosomal recessive LGMDs; they are calpain 3 (*LGMD2A*) (Richard et al. 1995), and four members of the dystrophin-associated protein complex (Straub and Campbell 1997)— γ -sarcoglycan, α -sarcoglycan, β -sarcoglycan, and δ -sarcoglycan (*LGMD2C–2F*, respectively) (Roberds et al. 1994; Lim et al. 1995; Noguchi et al. 1995; Bönnemann et al. 1996; Nigro et al. 1996; Passos-Bueno et al. 1996). In addition, at least four autosomal dominant LGMDs exist; four have been mapped (Speer et al. 1992; McNally et al. 1997; Messina et al. 1997; van der Kooi et al. 1997; Minetti et al. 1998), and genetic exclusion studies suggest that others may exist (Speer et al. 1995; van der Kooi et al. 1997). LGMDs are characterized clinically by progressive muscle weakness and wasting and, histopathologically, by muscle-cell changes, including variation in fiber size, degeneration, necrosis, regeneration, and fibrosis. Typically, the first muscles affected are those of the shoulder and pelvic girdles, although other muscle groups also can be affected. Facial muscles, however, generally are spared. The different forms of LGMD differ markedly in age at onset, rate of progression, degree of muscle involvement, and clinical severity (Bushby 1995).

We have been studying a relatively mild form of autosomal recessive LGMD (MIM 254110), commonly seen in Manitoba Hutterites, that first was described by

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Shokeir and Kobrinsky in 1976 (Shokeir and Kobrinsky 1976). Recently, we excluded all the known LGMD loci, as well as other candidate genes encoding proteins of the dystrophin-associated protein complex, as causing LGMD in Hutterites and postulated that another gene is responsible for this disorder (Weiler et al. 1997). In the current study, a genome scan was performed on two pools of DNA (patients and sibs) to search for the disease locus. We subsequently mapped the gene for LGMD in Hutterites, a gene that we have designated "LGMD2H," to a small part of chromosome region 9q31-q33, by linkage and haplotype analysis.

Subjects and Methods

Subjects

The study included four related nuclear families of Hutterite descent (A–D), comprising 40 individuals, all of whom agreed to participate. Individuals were deemed to be affected with LGMD if they (1) showed signs and symptoms of proximal muscle weakness and had creatine kinase (CK) levels more than four times the upper limit of normal, in the absence of any other explanation for CK elevation; (2) showed signs and symptoms of proximal muscle weakness and had either an electromyogram or a muscle biopsy consistent with a myopathic disorder; or (3) showed extremely elevated CK levels (≥ 15 times the upper limit of normal) but were asymptomatic. A muscle biopsy was considered to be consistent with a myopathic disorder if it showed myopathic changes consisting of abnormal variation in fiber size, muscle-fiber degeneration or necrosis with phagocytosis, fiber splitting, internal nuclei, variable degrees of fatty infiltration of the perimysium or endomysium, and endomysial fibrosis. Individuals were considered to be unaffected if they were asymptomatic, had a normal CK level (in females, 28–116 U/liter; in males 52–175 U/liter), and, on the basis of manual muscle testing, had normal muscle bulk, tone, and strength. Individuals were assigned an unknown phenotype if they were asymptomatic and their CK levels were greater than normal but less than four times the upper limit of normal. A total of 18 individuals (11 males and 7 females) were classified as LGMD patients (fig. 1). Six individuals did not meet the criteria as affected or unaffected. Four individuals (I-2, age 60 years; II-17, age 36 years; II-21, age 29 years; and II-28, age 32 years) were asymptomatic, with normal muscle strength, on the basis of manual muscle testing, but had mildly elevated serum CK levels less than four times the upper limit of normal. Two individuals (II-23, age 50 years; and II-30, age 26 years) were asymptomatic on the basis of history and provided DNA samples but were not available for either physical examination or CK testing. The phenotypes of

these six individuals were classified as unknown. Clinical data are summarized in table 1.

DNA Analysis

Genomic DNA was extracted from whole blood, according to the method described by Greenberg et al. (1987) or Zelinski (1991). Oligonucleotide primers designed to amplify microsatellites were obtained from Research Genetics. DNA samples were genotyped by use of protocols reported by Sirugo et al. (1992) and Rodius et al. (1994), with slight amendments.

Genome Scan, DNA Pooling, and Fine Mapping

Genealogical studies of these families (Weiler et al. 1997) suggested that the gene in all patients was identical by descent. We therefore chose to perform a genome scan using a DNA-pooling strategy (Sheffield et al. 1994; Carmi et al. 1995). We pooled 400-ng samples of DNA from each of nine confirmed affected individuals (II-1, II-6, II-16, II-18, II-20, II-24, II-25, II-26, and II-27) into one tube. We also pooled 400-ng samples of DNA from each of the sibs of these patients (II-3, II-4, II-5, II-7, II-17, II-19, II-21, II-22, II-23, II-28, II-29, and II-30) into a second tube, which also contained DNA from two other sibs in family D (not shown in fig. 1). These two individuals were not genotyped for all markers, because of the limited quantities of DNA. The phenotype of individuals II-7, II-17, II-21, II-23, II-28, and II-30 was unknown at the time of DNA pooling, and, therefore, DNA from these individuals was aliquotted into the sib-DNA pool. Each pool was diluted to a concentration of 40 ng/ μ l. Samples of DNA (40-ng) from each of the two DNA pools were genotyped with 200 microsatellite markers (Research Genetics Set 5a, supplemented with 46 additional markers) spaced ~ 20 cM apart. On visual inspection, markers that showed a tendency to fewer bands in the patient pool in comparison with the sib pool were then used to genotype the individual family members indicated in figure 1. Genotypes were obtained for 11 markers in the region of D9S302, the marker, from the genome scan, that showed significant linkage to LGMD2H.

Linkage Analysis

Data from microsatellite typing of individual family members were analyzed by use of the LINKAGE programs (version 5.2) (Lathrop and Lalouel 1984; Lathrop et al. 1984, 1986) and the FASTLINK version (4.0P) of the LINKAGE programs (Cottingham et al. 1993; Schäffer et al. 1994; Schäffer 1996; Becker et al. 1998). MLINK was used for two-point linkage analysis, and ILINK was used to obtain the maximum-likelihood estimate of the recombination fraction (θ) under the assumption of an autosomal recessive trait with complete

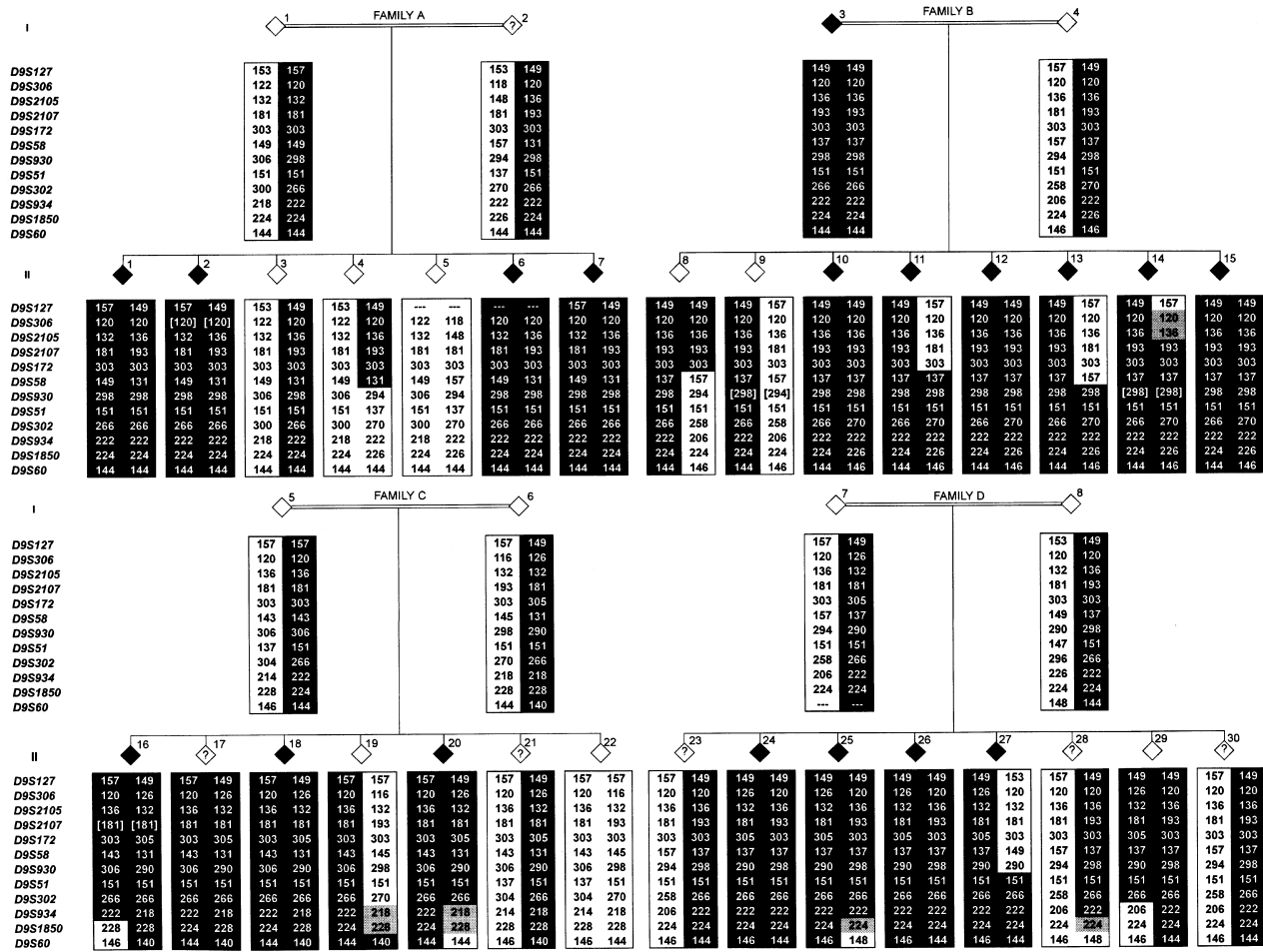


Figure 1 Haplotypes in four Hutterite LGMD families. Affected individuals are represented by blackened diamonds; unaffected individuals are represented by unblackened diamonds; and individuals with an unknown phenotype are denoted by a question mark within a diamond. Inferred genotypes are indicated by square brackets. Blackened haplotypes are those harboring the disease gene; unblackened haplotypes are those that do not carry the disease gene; and gray shading indicates intervals of recombination. The coefficients of kinship between the spouses in family A, family B, family C, and family D are .017, .065, .045, and .059, respectively; and the closest cousin relationships are third cousins once removed in three ways, second cousins in two ways, second cousins, and half-first cousins once removed, respectively. The inbreeding coefficient of parent I-3, who is affected, is .052, and the closest cousin relationship between the parents of I-3 is first cousins once removed (Weiler et al. 1997).

penetrance. Disease-allele frequency was estimated to be .05 (Weiler et al. 1997). Marker-allele frequencies were calculated from the eight parents of the four nuclear families. Multipoint LOD scores were computed at 0.1-cM increments throughout the interval *D9S127*–*D9S60*, by use of the GENEHUNTER program (version 1.1) (Kruglyak et al. 1996). The map order and intermarker distances used were *D9S127*–0.8 cM–*D9S306*–1.2 cM–*D9S2105*–0.2 cM–*D9S2107*–0.3 cM–*D9S172*–5.6 cM–*D9S58*–4.8 cM–*D9S930*–1.3 cM–*D9S51*–2.2 cM–*D9S302*–0.3 cM–*D9S934*–4.1 cM–*D9S1850*–3.1 cM–*D9S60* (fig. 2). Intermarker distances for *D9S127*, *D9S306*, and *D9S2105* were based on physical mapping data (Miyake et al. 1997); those for *D9S2105*,

D9S2107, and *D9S172* were estimates based on linkage disequilibrium (Toda et al. 1996) and an arbitrary assumption that *FCMD* is ~20 kb centromeric to *D9S2107*; and those for *D9S172*–*D9S60* were obtained from the chromosome 9 summary map of the Genetic Location Database (Collins et al. 1996). One centimorgan was assumed to be equivalent to 1 Mb. Both multipoint analysis of three markers (*D9S302*, *D9S934*, and *D9S1850*) in the region of the highest LOD scores, on the basis of the LINKMAP program in the FASTLINK version of the LINKAGE programs, and multipoint analysis of eight markers (*D9S172*–*D9S60*), on the basis of the VITESSE program (O’Connell and Weeks 1995), gave similar results to those produced by the GENE-

HUNTER program (data not shown). Haplotypes were constructed with a minimal number of recombinations, under the assumption that no marker mutations had occurred. A small number of missing genotypes were inferred, where possible.

Results

Genome Scan Using a DNA-Pooling Strategy

Previous genealogical analysis of Hutterite families with LGMD showed that this autosomal recessive disease could be traced back to ≥ 10 ancestors born in the 1700s, which suggested that all affected individuals might be homozygous by descent, at the disease locus (Weiler et al. 1997). We therefore initiated a genome scan, using a DNA-pooling strategy with two pools: a patient pool containing DNA from nine affected individuals, and a control pool comprising DNA from 14 sibs of patients. Of the 200 markers that were tested, 5 (*D1S236*, *D6S259*, *D6S1003*, *D9S302*, and *D17S849*) gave a banding pattern suggestive of excess homozygosity in the patients' DNA pool compared with the sibs' DNA pool (fig. 3). These markers were then used to genotype individual family members, as shown in figure 1. Two-point linkage analysis was done. *D9S302*, located in chromosome region 9q31-q33, showed significant linkage to *LGMD2H*, with a maximum LOD score (Z_{max}) of 5.99 at a maximum θ ($\hat{\theta}$) of .03.

Linkage and Haplotype Analysis

LGMD2H was linked to *D9S302*, which is estimated to be 11 Mb from *FCMD* (Collins et al. 1996). It is possible, therefore, that the loci for *LGMD2H* and *FCMD* are the same. To obtain evidence that these disease loci are distinguishable, family members were genotyped for six markers known to be linked to *FCMD* (*D9S127*, *D9S306*, *D9S2105*, *D9S2107*, *D9S172*, and

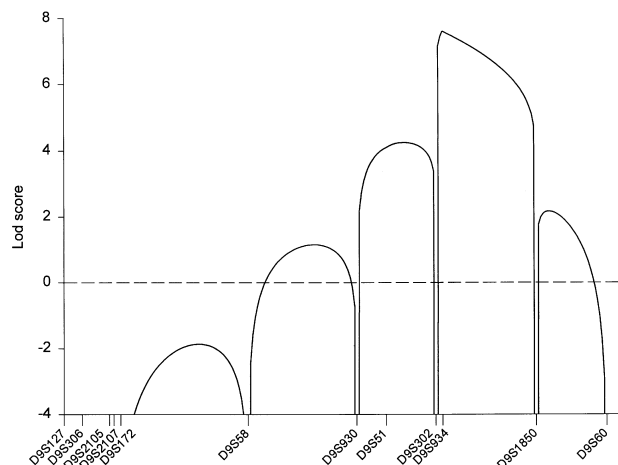


Figure 2 Multipoint LOD score of location of *LGMD2H*, for the four pedigrees shown in fig. 1; LOD scores < -4 are not shown. Marker positions are indicated by vertical tick marks on the X-axis. The total distance between *D9S127* and *D9S60* was estimated to be ~ 23.9 cM. *FCMD* is estimated to be ~ 20 kb from *D9S2107* (Toda et al. 1996).

D9S58) (Toda et al. 1993, 1994, 1996). Two-point linkage analysis of *LGMD2H* versus markers *D9S127* and *D9S2107* (located, respectively, ~ 2 Mb centromeric and ~ 20 kb telomeric of *FCMD*) showed significantly negative LOD scores, of -5.17 and -4.03 , respectively, at $\theta = .01$ (table 2). One of the markers linked to *FCMD* (*D9S306*) showed significant linkage to the disease gene ($Z_{max} = 3.15$ at $\theta = 0$). However, recombinants between *D9S2107*, the marker closest to *FCMD*, and *LGMD2H* were apparent in individuals II-8, II-11, II-13, II-27, and II-29.

Five additional markers surrounding *D9S302* and telomeric to *FCMD* (*D9S930*, *D9S51*, *D9S934*, *D9S1850*, and *D9S60*) were also used to genotype all

Table 1

Summary of Clinical Data of Subjects with LGMD

	Symptomatic (N)	Asymptomatic (N)
Subjects (from fig. 1)	I-3, II-1, II-2, II-6, II-10, II-11, II-12, II-15, II-16, II-18, II-20, II-24, II-25, II-26, II-27 (15)	II-7, II-13, II-14 (3)
Age at onset (years)	8-27 (15)	N/A
Age at presentation (years)	21-53 (15)	N/A
Presenting symptoms	Proximal weakness (9), back or neck pain (6), fatigue (4), waddling gait (2), muscle wasting and weakness (1), wasting of shoulder girdle (1), difficulty climbing stairs (1), weak legs (1)	N/A
CK (U/liter) ^a	250-3,130 U/liter (15)	2,740-4,280 U/liter (3)
Muscle biopsy	Dystrophic (5)	Dystrophic (1)
Electromyogram	Myopathic (7), myopathic/neurogenic (1)	None tested
Status in 1997	Ambulatory at age 23-39 years (11), ambulatory with difficulty at age 37-46 years (3), wheelchair bound at age 61 years (1)	Asymptomatic at age 23-26 years (3)

SOURCE.—Weiler et al. (1997); authors' unpublished data.

^a Highest recorded values; normal range for females is 28-116 U/liter, and that for males is 52-175 U/liter.

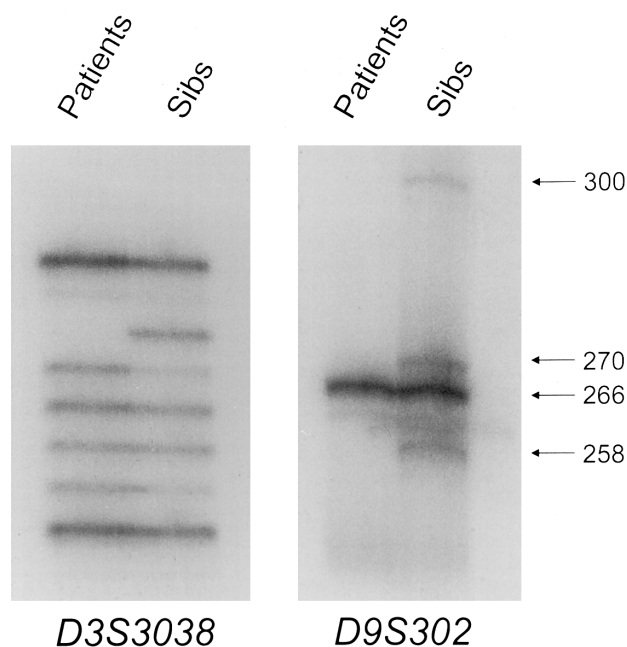


Figure 3 Autoradiogram of two pools of DNA genotyped with either *D3S3038* or *D9S302*. Banding patterns are indicative of an unlinked marker (*D3S3038*) and a linked marker (*D9S302*).

individuals in figure 1. Two-point analysis of *LGMD2H* versus *D9S930*, *D9S302*, and *D9S934* yielded LOD scores >3.0 (table 2). *D9S60*, located ~ 7.2 Mb telomeric to *D9S934* (Collins et al. 1996), showed a significantly negative LOD score, -5.25 , at $\theta = .01$. A distal recombination is evident between *D9S934* and *D9S1850* in individual II-16. Taken together, the proximal and distal recombinations narrow the candidate region, which is flanked by *D9S302* and *D9S1850* and contains *D9S934*, to ~ 4.4 Mb (3.9 cM in males, 6.2 cM in females) (Collins et al. 1996). An estimate of the minimum candidate interval defined by recombinant haplotypes in affected individuals (II-16 and II-27) is 7.9 Mb (5.9 cM in males, 10.0 cM in females), flanked by *D9S930* and *D9S1850* (Collins et al. 1996).

Figure 2 shows the multipoint LOD scores computed, at 0.1-cM increments, by use of the GENEHUNTER program. The multipoint analysis excludes *LGMD2H* from being linked to a 5-cM region encompassing *FCMD* (LOD scores <-2) and indicates that the most likely location of *LGMD2H* is close to *D9S934*. The multipoint Z_{\max} was 7.61 at *D9S934*; the 3-unit-LOD-score support interval (Terwilliger and Ott 1994) extends from slightly distal to *D9S302* to slightly proximal to *D9S1850*, a region estimated to be ~ 4.4 Mb.

Discussion

This study of a large extended Hutterite kindred has led to the localization of yet another gene causing

LGMD. Because the Hutterite population is a genetic isolate and because the parents of the four families in our study are related (ranging from first cousins once removed to half-third cousins once removed), we assumed that the disease alleles were identical by descent (Weiler et al. 1997). Under this assumption, we were able to use a DNA-pooling strategy to perform an efficient genome scan. This resulted in linkage being detected between *LGMD2H* and *D9S302*.

The cytogenetic location of *D9S302* in chromosome region 9q31-q33 agrees with that reported for *FCMD* (Toda et al. 1993). *FCMD* is a severe congenital muscular dystrophy associated with mental retardation, although considerable heterogeneity has been reported (Kondo-Iida et al. 1997). The form of *LGMD* in the Hutterite kindred described in the present report also exhibits clinical heterogeneity (Weiler et al. 1997). However, we have strong evidence that excludes *FCMD* as a candidate gene for *LGMD2H*, on the basis of a significantly negative LOD score with *D9S2107*, a marker estimated to be ~ 20 kb from *FCMD* (Toda et al. 1996). Furthermore, five recombinations were observed between this marker and *LGMD2H*. Multipoint analysis excludes *LGMD2H* from being linked to a 5-cM region encompassing *FCMD*. Two-point LOD scores >3.0 were obtained for *D9S930*, *D9S302*, and *D9S934*, all located telomeric to *FCMD* (Collins et al. 1996). Proximal and distal recombination events are evident in individuals II-29 and II-16, which narrows the candidate region within the ~ 4.4 -Mb region bounded by *D9S302* and *D9S1850* (Collins et al. 1996). The multipoint analysis indicates that the most likely location of *LGMD2H* is close to *D9S934* (multipoint $Z_{\max} = 7.61$).

Seven of the nine disease chromosomes among the parents of the four sibships carry the same *D9S51-D9S302-D9S934-D9S1850* haplotype (151-266-222-224 bp). If we assume that the haplotype (151-266-218-

Table 2

LOD Scores for Linkage between *LGMD2H* and Markers in Chromosome Region 9q31-q34.1

MARKER	LOD SCORE AT $\theta =$							$\hat{\theta}$	Z_{\max}
	.00	.01	.05	.10	.20	.30			
<i>D9S127</i>	$-\infty$	-5.17	-1.39	-1.0	.60	.51	.23	.62	
<i>D9S306</i>	3.15	3.09	2.82	2.48	1.74	.98	.00	3.15	
<i>D9S2105</i>	$-\infty$	-.37	.20	.34	.33	.20	.14	.36	
<i>D9S2107</i>	$-\infty$	-4.03	-1.46	-.54	.06	.14	.28	.14	
<i>D9S172</i>	1.58	1.55	1.42	1.26	.89	.51	.00	1.58	
<i>D9S58</i>	$-\infty$	-.26	1.47	1.89	1.75	1.14	.13	1.93	
<i>D9S930</i>	$-\infty$	2.63	3.56	3.56	2.85	1.78	.07	3.62	
<i>D9S51</i>	2.21	2.17	2.00	1.77	1.26	.72	.00	2.21	
<i>D9S302</i>	$-\infty$	5.80	5.92	5.46	4.12	2.52	.03	5.99	
<i>D9S934</i>	5.97	5.86	5.40	4.81	3.53	2.14	.00	5.97	
<i>D9S1850</i>	$-\infty$	1.54	2.01	2.01	1.66	1.10	.07	2.04	
<i>D9S60</i>	$-\infty$	-5.25	-2.57	-1.51	-.61	-.22	.50	.00	

228) carried by parent I-6 resulted from an ancestral recombination, then *LGMD2H* must be proximal to *D9S934*. Furthermore, if we assume complete penetrance, the recombinant event in individual II-29 places *LGMD2H* distal to *D9S302*. Thus, *LGMD2H* may lie within a 0.3-Mb region between *D9S302* and *D9S934* (Collins et al. 1996). Finally, the 270-bp allele of *D9S302* on the disease haplotype carried by parent I-4 differs from that of the common disease-associated allele (266 bp) at this locus. However, the alleles of the two flanking markers are the same as those on the disease-associated haplotype. This suggests that a mutation of the tetranucleotide repeat, *D9S302*, has occurred (Weber and Wong 1993). It is therefore likely that all of the parents share the same *LGMD2H* mutation identical by descent.

More than 40 genes have been mapped to chromosome region 9q31-q33, the region harboring *LGMD2H*. After inspection of the map location, expression pattern, and function of these genes, none appears to be a convincing candidate gene. Hexabrachion, although located centromeric to our candidate region (Collins et al. 1996), has been detected in the muscles of Duchenne muscular dystrophy patients (Mayer et al. 1997). Since it may be implicated in the disorder described here, we will be examining this possibility. Additionally, two mouse phenotypes—muscular dystrophy with myositis (Muller-Seitz et al. 1993) and vacillans (Sirlin 1956, 1957; Marbois et al. 1994)—that map to mouse chromosome regions that are homologous to the *LGMD2H* candidate region have been described. Further analysis of new patients, especially in the other two subdivisions of the Hutterite population (Fujiwara et al. 1989), should allow us to narrow the region sufficiently for positional cloning or identification of the gene on the basis of mapped expressed sequences and known genes (Schuler et al. 1996).

We have provided evidence for the location of a gene for autosomal recessive LGMD in chromosome region 9q31-q33 in the Hutterites. There was no recombination with *D9S934*, a marker in a region flanked by *D9S302* and *D9S1850* and estimated to be ~4.4 Mb. Haplotype analysis identified a putative ancestral recombination that would further narrow the candidate region, to ~300 kb.

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Electronic-Database Information

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

Genetic Location Database, <http://cedar.genetics.soton.ac.uk/public.html/> (for distances for *D9S172–D9S60*)

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for autosomal recessive LGMD [MIM 254110])

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