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Preferential Localization of the Limb-Girdle Muscular Dystrophy Type 2A Gene in the Proximal Part of a 1-cM 15q15.1-q15.3 Interval

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Summary

A gene for a recessive form of limb-girdle muscular dystrophy (LGMD2A) has been localized to chromosome 15. A physical map of the 7-cM candidate 15q15.1-q21.1 region has been constructed by means of a 10–12-Mb continuum of overlapping YAC clones. New microsatellite markers developed from these YACs were genotyped on large, consanguineous LGMD2A pedigrees from different origins. The identification of recombination events in these families allowed the restriction of the LGMD2A region to an estimated 1-cM interval, equivalent to ~3–4 Mb. Linkage disequilibrium data on genetic isolates from the island of Réunion and from the Amish community suggest a preferential location of the LGMD2A gene in the proximal part of this region. Analysis of the interrelated pedigrees from Réunion revealed the existence of at least six different carrier haplotypes. This allelic heterogeneity is incompatible with the presumed existence of a founder effect and suggests that multiple LGMD2A mutations may segregate in this population.

Introduction

Limb-girdle muscular dystrophy (LGMD) is a group of hereditary myopathies usually starting during childhood, characterized by progressive weakness and atrophy predominating in shoulder, pelvic girdle, and trunk muscles, with facial muscles left unaffected. Both dominant and recessive modes of inheritance are known, the latter being more common with a prevalence estimate of 10^{-5} (Emery 1991).

A gene responsible for the recessive form has been localized to 15q (LGMD2A; MIM 253600) by linkage analysis, in a genetically homogeneous group of families from Réunion (Beckmann et al. 1991). This localization has subsequently been confirmed in an Amish population of Indiana (Young et al. 1992) as well as in Brazilian families where genetic heterogeneity has been demonstrated (Passos-Bueno et al. 1993).

Linkage analysis, as well as recombination studies, have allowed the definition of the 15q15.1-q21.1 interval, bracketed by markers D15S129 and D15S143, containing the LGMD2A disease locus. This interval has been assessed as 7 cM, on the basis of linkage analysis on the CEPH reference families (Fougerousse et al. 1994). A physical map of this interval has been established by means of a 10–12-Mb contig of overlapping YAC clones (Fougerousse et al. 1994).

In order to refine genetically this still considerable interval, more meioses, as well as new highly informative markers, were needed. The panel of families has been substantially expanded by the addition of metropolitan French families and Amish kindreds from Indiana and Pennsylvania. The Amish families are particularly suitable for genetic analysis, as they constitute an isolated population composed of large kinships. Yet, the involvement of the LGMD2A gene has been excluded in six LGMD pedigrees from southern Indiana, despite the multiple consanguineous links connecting them to the northern Indiana kindreds who were found to belong to the chromosome 15 group (Allamand et al. 1995). Genetic heterogeneity considerably restricted the number of potentially informative meioses. Since we cannot identify the chromosome 15 families among the small nonconsanguineous pedigrees (especially from metropolitan France), either on a clinical or on a genetic basis, no information on potential crossing-over events can be extracted. We thus limited our studies to two large families from Brazil and to the consanguineous populations from Réunion and the Amish community from northern

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Indiana. The latter two were each thought to represent a single genetic isolate interconnected through multiple consanguineous links and segregating a single unique carrier haplotype.

These characteristics make these families theoretically adequate for homozygosity mapping (Lander and Botstein 1987) and haplotype mapping (i.e., reconstitution of historical recombination events) as well as for linkage disequilibrium studies (Hästbacka et al. 1992). Homozygosity mapping in isolates where a single founder effect is presumed seems a powerful strategy to reduce the region involved by considering that a whole segment of the chromosome flanking the disease locus is likely to be homozygous-by-descent in the affected siblings. Linkage disequilibrium reflects a nonrandom allelic association between two loci, meaning that alleles at these loci occur more frequently together in a population than would be expected on the basis of Hardy-Weinberg equilibrium, therefore suggesting that these loci are both physically and genetically close.

In this paper we present a refined genetic map of the LGMD2A region established by mapping and ordering new microsatellites developed from YAC clones that were part of the LGMD2A contig as well as published markers. Genotyping of polymorphic markers allowed the construction of extended haplotypes and the identification of recombination events in the LGMD2A families, thus permitting the refinement of the region containing the gene involved in this pathology to ~1 cM. Linkage disequilibrium data led us to consider preferentially the proximal part of this interval as the most likely location for the disease locus. Finally, an unexpected high number of carrier haplotypes was identified within the LGMD2A families from Réunion. This unsuspected allelic heterogeneity suggests that several independent mutations are responsible for this myopathy in this population.

Subjects, Material, and Methods

Patient Material

A total of 27 families, including 13 (27 affected individuals) from Réunion (Beckmann et al. 1991), 2 (15 affected individuals) from Brazil (Passos-Bueno et al. 1991), 10 (37 affected individuals) from the Old Order Amish of Adams County, Indiana (Hammond and Jackson 1958; Jackson and Strehler 1968; Young et al. 1992), and 2 (4 affected individuals) from a Pennsylvania Amish population, were examined. Blood from 250 individuals (83 of whom were affected) was obtained for DNA extraction and lymphoblastoid cell line establishment.

Markers and Analysis of Polymorphisms

Eighteen new microsatellite markers were generated from YAC clones 189D1, 296F11, 774G4, 806G4, and 854F9, which are part of the LGMD2A contig (Fougerousse et al. 1994) by subcloning and sequencing of inter-*Alu* products (Pereira de Souza et al. 1994) or subcloning of digested fragments in M13 or Bluescript vectors, using a modified protocol from Vignal et al. (1993). Markers were named after the YAC clone from which they were derived. Markers were tested for polymorphism by PCR amplification of DNA of 64 unrelated individuals from the CEPH reference families. All the newly developed markers were mapped physically by sequence tagged site (STS) screening on YACs, as by Fougerousse et al. (1994), and genetically, on the basis of analysis of recombinant individuals from the CEPH and LGMD2A families. Twenty-one other markers originating from different sources (Weissenbach et al. 1992; Beckmann et al. 1993; Murray et al. 1994) were also used in this study. Genotyping was performed by amplification of 100–200 ng of genomic DNA in a 50-μl reaction mix, as described by Fougerousse et al. (1992).

Statistical Analysis

Two- and multipoint analyses were performed for all these markers, using the LINKAGE software package version 5.10 (Lathrop et al. 1985). Twenty-eight microsatellite markers mapping within the chromosome 15q15.1-q21.1 region were tested for linkage disequilibrium in the families from Réunion, and 20 markers were likewise tested in the Amish population.

Extended haplotypes were constructed manually once phase was determined unequivocally. Parental chromosomes were taken into account, to define two populations: normal and LGMD2A chromosomes. Linkage disequilibrium in the allelic distribution of normal and LGMD2A chromosomes was tested using two methods: the first is referred to as the combined-allele method where the most common allele was considered as one allele, all the others being pooled to form an artificial second allele. A correlation coefficient was calculated according to the method of Hill and Robertson (1968) and Litt and Jorde (1986) and is defined as $r = D/(p_1p_2q_1q_2)^{1/2}$, with p_1, p_2, q_1, and q_2 being the frequencies of alleles 1 and 2 at loci A and B (deduced from the chromosome counts) and $D = P_{11} - p_1q_1$ (P_{11} is the observed frequency of the haplotype A_1B_1). The χ^2 test with 1 df was estimated as $\chi^2 = Nr^2$ with N being the total number of chromosomes studied. In the second method, designated the "multiallelic method," each allele is considered separately. It was, however, sometimes necessary to pool some of the rare alleles so that the expected value of each cell in the contingency table was >5. The χ^2 test was defined as $\chi^2 = N\Sigma D_{ij}^2/p_iq_j$, with N, D, and p_i and q_j defined as above. The number of df's is then equal to $(k - 1) \times (m - 1)$, where k and m are the number of alleles at each locus.

Since a large number of markers were studied (28 and 20 in the families from Réunion and the Amish

Table 1

Characteristics of Newly Developed Microsatellites Derived from Four YAC Clones of the LGMD2A Contig

Name	Origin	Primer Sequences (5′–3′)	Size[a] (bp)	Temperature (°C)	Heterozygosity (*N*)[b]
D15S778	M13	TGCATTCCAGCCACTGACAG GGGATTACAGGTGCATGAGCT	183	50	ND
D15S784	Inter-*Alu*	GAGACCCTGTCTCAAAACAC GAGGAATGACTGAAATGATTT	174	52	.47 (128)
D15S779	M13	TGAGCCCCAGGGATAATAT GCCAGTCAGATCAGGGTAGA	96	59	.84 (86)
D15S780	M13	AAATTTGCAAGTTGGACAGAA CTGATTGGAAGTTCCGTGTG	134	54	.67 (81)
D15S782	BS	ACTGCTCCCAGCCTGTGTGT CACCACTGTCACTCCACCCTC	132	57	.78 (128)
D15S781	BS	AACTTTGCTTTGTCACACTG AGGAAAGAACAGATGAGCAG	186	59	.61 (128)
D15S783	M13	TTGACCACATGGCAACTCTG GACAAATTCTCCCCTGACCA	194	56	.58 (80)

[a] Size of the PCR products.
[b] *N* = number of chromosomes tested; ND = not determined.

population, respectively), the significance level was corrected by using Bonferroni's procedure (Weir 1990) as follows: $\alpha' = 1 - (1 - \alpha)^{1/L}$, with L being the number of individual tests. Considering a type I error, $\alpha = 0.05$, with 28 and 20 comparisons, corrected significance levels became 1.83×10^{-3} and 2.56×10^{-3}, in the Réunion and Amish populations, respectively.

The exact test of Fisher for 2 × 2 contingency tables was also performed using the 2BY2 program (Terwilliger and Ott 1994). Significance level (P_F) for this test was defined as $P_F < .001$ (for compensation to multiple testing, individual P_F values were multiplied by the number of tests carried out at each locus, minus one).

Results

Development of New Markers

Sequencing of $(CA)_n$ containing M13 or Bluescript subclones of selected YACs from the LGMD2A contig (Fougerousse et al. 1994) allowed the development of 18 new STSs. The location of the nonpolymorphic STSs (D15S499, D15S498, D15S500, and D15S496) along the contig map has been described elsewhere (Fougerousse et al. 1994). The remaining 14 markers displayed an observed heterozygosity ranging from .12 to .84 (tables 1 and 2). They were all positioned on the regional physical map. Of these, seven (table 2) had already been described as STSs by Fougerousse et al. (1994).

Restriction of the LGMD2A Interval

Two-point linkage analyses between the disease locus and all the newly developed markers were performed (table 3). Marker D15S782 yielded the highest lodscore ($Z_{max} = 30.54$ at $\theta = .00$) with a one lod support interval of 1.4 cM. These analyses also revealed the existence of recombination events between LGMD2A and markers D15S514 ($Z_{max} = 19.21$ at $\theta = .01$) and D15S222 ($Z_{max} = 16.99$ at $\theta = .01$). The construction of extended haplotypes, with all markers from this region in the LGMD2A families, also allowed visualization of these recombination events. Several recombinants confirmed the previous interval flanked by markers D15S129 and D15S143 (Fougerousse et al. 1994), while others defined a smaller one (fig. 1). The narrowest interval containing the LGMD2A gene was identified by three recombinants: B519-1135 defined the proximal flanking locus as D15S514, whereas R24-62 and A615-147 identified the distal border as D15S222 (figs. 1 and 2). The genetic distance between markers D15S514 and D15S222 was assessed as 1 cM, on the basis of multipoint linkage analysis in the CEPH reference families (data not shown). Considering that this region can be almost en-

Table 2

Characteristics of Newly Developed Microsatellites Markers[a]

Name	Origin	Heterozygosity (*N*)[b]
D15S513	M13	ND
D15S512	M13	.12 (128)
D15S508	M13	.46 (122)
D15S514	M13	.74 (112)
D15S509	M13	ND
D15S516	M13	.24 (127)
D15S517	M13	.19 (124)

[a] Previously described as STSs by Fougerousse et al. (1994).
[b] *N* = number of chromosomes tested; ND = not determined.

Table 3

Pairwise Lod Scores between Chromosomes 15q15.1-q21.1 Newly Developed Markers and the LGMD2A Locus

Markers	Recombination Fraction at θ =							Z_{max} (θ)	One Lod Support Interval
	.00	.01	.05	.10	.20	.30	.40		
D15S514	−∞	19.21	18.00	15.82	11.04	6.31	2.12	19.21 (.01)	<.001–.044
D15S779	26.29	25.65	23.04	19.72	13.10	6.94	2.06	26.29 (.00)	.000–.015
D15S512	6.40	6.27	5.74	5.06	3.61	2.10	.68	6.40 (.00)	.000–.075
D15S782	30.54	29.85	27.01	23.40	16.13	9.08	2.96	30.54 (.00)	.000–.014
D15S780	23.66	23.43	21.63	18.99	13.36	7.70	2.68	23.66 (.00)	.000–.028
D15S508	8.63	8.44	7.68	6.70	4.64	2.56	.77	8.63 (.00)	.000–.053
D15S778	26.38	25.76	23.25	20.08	13.78	7.84	2.74	26.38 (.00)	.000–.016
D15S784	15.89	15.74	14.62	12.89	9.08	5.17	1.70	15.89 (.00)	.000–.041
D15S783	21.34	20.81	18.67	16.00	10.75	5.81	1.74	21.34 (.00)	.000–.018
D15S781	21.48	20.99	19.01	16.52	11.52	6.67	2.35	21.48 (.00)	.000–.020
D15S516	2.55	2.50	2.31	2.03	1.42	.77	.22	2.55 (.00)	.000–.180
D15S509	1.39	1.36	1.24	1.09	.74	.38	.10	1.39 (.00)	.000–.298
D15S517	1.74	1.70	1.55	1.35	.92	.50	.15	1.74 (.00)	.000–.240
D15S222	−∞	16.99	15.73	13.59	9.09	4.80	1.40	16.99 (.01)	.001–.043

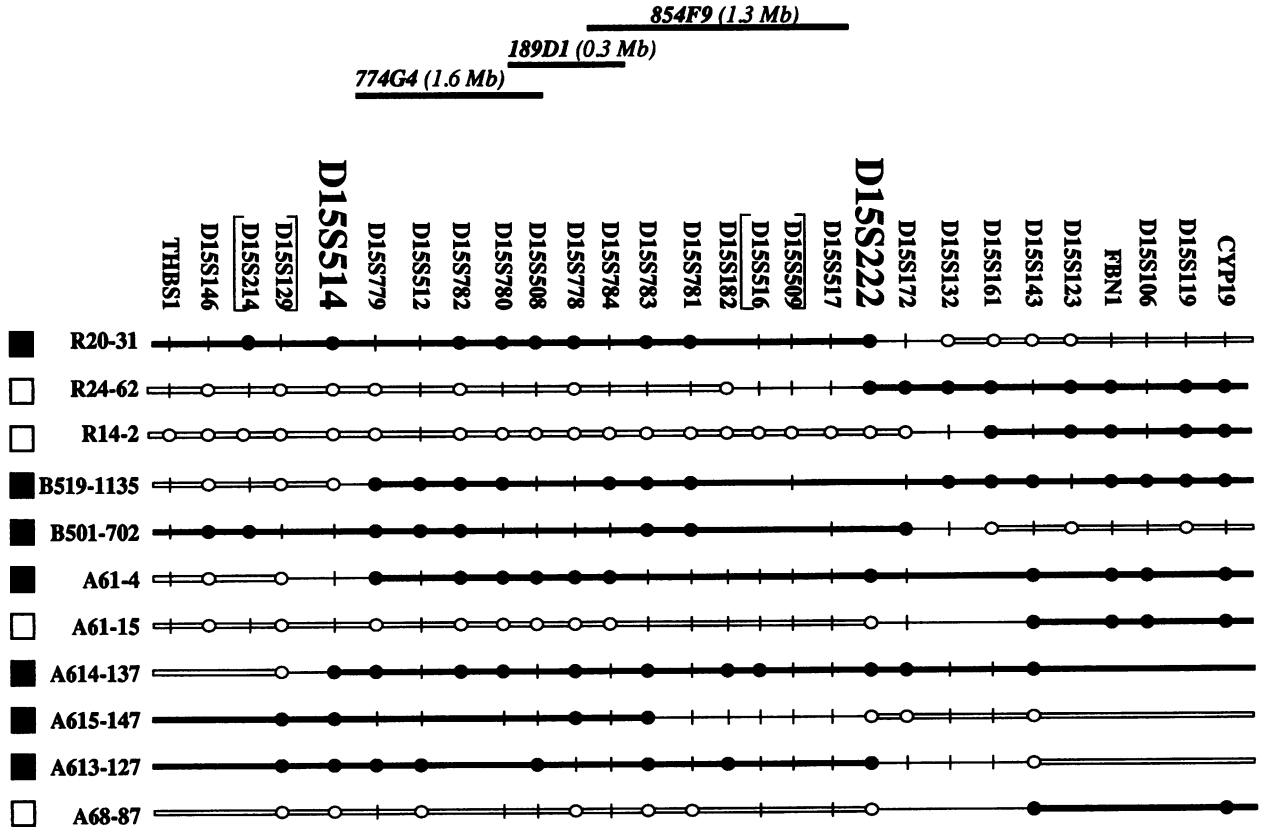

Figure 1 Recombinant haplotypes of chromosome 15q15.1-q21.1 in LGMD2A families. The ordered marker loci have arbitrarily been represented as equidistant. Loci bracketing the smallest interval, defined by recombination events, are noted in larger letters. Bracketed markers could not be ordered. Solid boxes (■) indicate affected individuals, whereas open boxes (□) represent healthy carriers. Recombinant individuals are represented with a letter referring to their origin (R = Réunion; B = Brazil; and A = Amish). The numbers indicate the family and the individual. Parental alleles segregating with the disease allele or the normal allele are coded as solid (●) or open (○) circles, respectively. Uninformative markers are coded by a dash (–) in place of a circle, and ungenotyped markers are left blank. The three nonchimeric YAC clones (and their sizes) covering this interval are represented on top of the figure, reflecting their STS content.

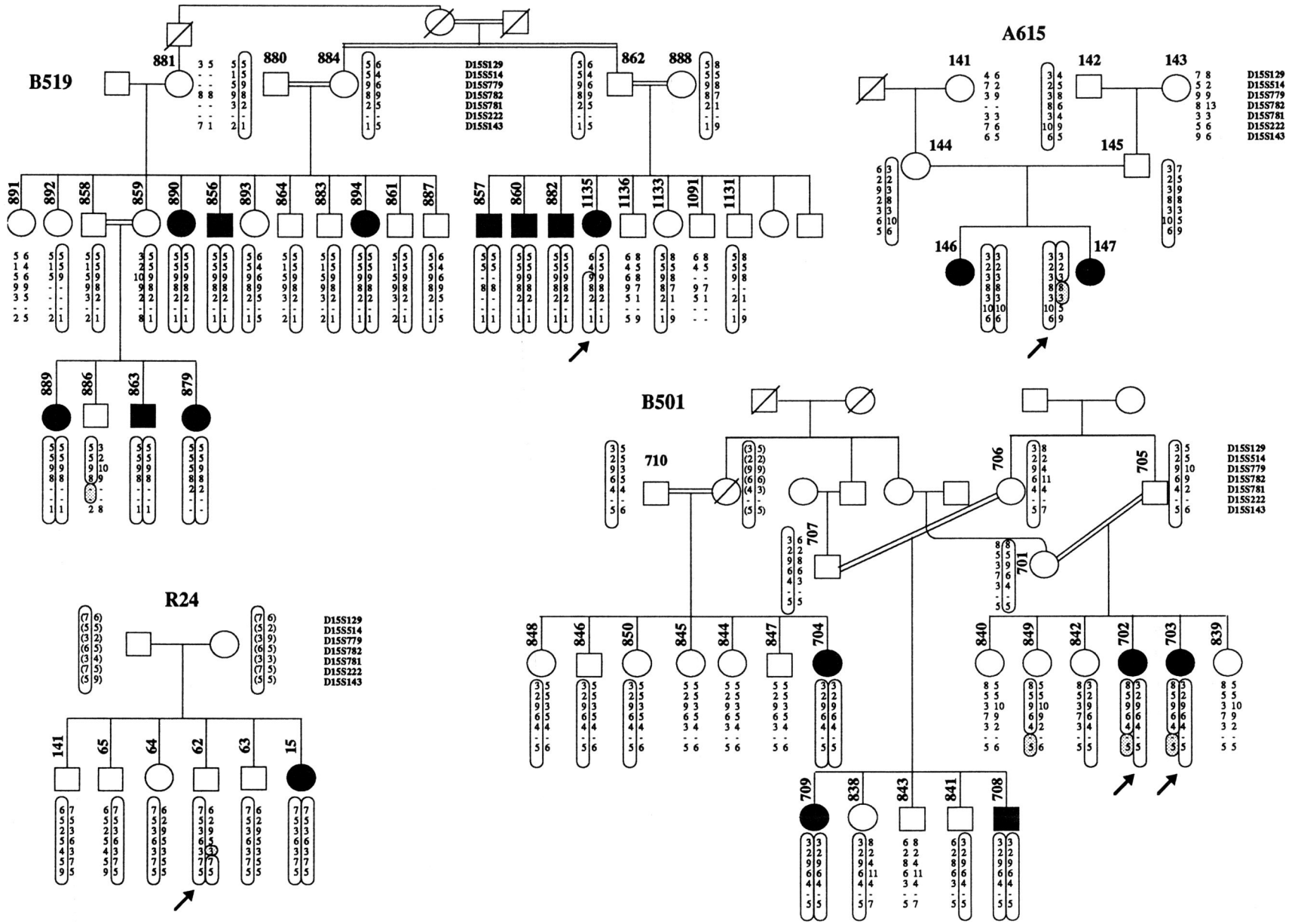

Figure 2 Extended haplotypes of the four families (R24, B501, B519 and A615) presenting recombination events that allow the restriction of the LGMD2A interval between markers D15S514 and D15S222. Solid squares (■) or circles (●) represent affected individuals, and healthy individuals are coded as open squares (□) or circles (○). Marker loci are listed from centromere to telomere. Haplotypes segregating with the disease locus are boxed. Shaded areas indicate that the exact localization of the recombination event could not be determined. Ungenotyped markers are noted with a dash (–). Genotypes in parenthesis were deduced.

tirely covered by three overlapping and nonchimeric YAC clones (774G4, 189D1, and 854F9; fig. 1) of known sizes (1.6 Mb, 0.3 Mb, and 1.3 Mb, respectively), the physical distance between the two flanking markers can be roughly estimated as 3–4 Mb.

However, the analysis of recombination events could not provide further information, and the interval was still too large for the undertaking of a systematic positional cloning strategy. We therefore investigated alternative approaches to refine the LGMD2A region genetically by taking into account the inbreeding characteristics of the populations from Réunion and the Amish community.

Haplotype and Homozygosity Mapping

Haplotypes were analyzed in order to identify historical crossing-over events that would permit narrowing of the LGMD2A interval. Two large Brazilian families (B501 and B519), each with documented consanguinity (Passos-Bueno et al. 1991), were shown to carry different LGMD2A haplotypes (fig. 3). Affected children in pedigree B501 were homozygous for 16 markers scattered between markers D15S779 and D15S106, thus suggesting two hypothetical historical cross-over events that did not allow further restriction of the LGMD2A interval, though consolidating the proximal border, D15S514 (figs. 2 and 3). As for family B519, affected siblings were homozygous-by-descent for all the markers genotyped, within the recombination boundaries. The examination of homozygosity-by-descent in these two kindreds did not permit further restriction of the 1-cM candidate interval.

Among the Amish population, a unique carrier haplotype (fig. 3, family A61) segregated within the 10 northern Indiana kindreds, with the exception of a hypothetical historical crossing-over event detected in one family, in agreement with D15S222 being the distal flanking marker. The observation of a common carrier haplotype was expected, in light of the high level of consanguinity in this population (Hammond and Jackson 1958; Young et al. 1992). A subset of markers was also analyzed in two Pennsylvania Amish families (A617 and A618; fig. 3). The carrier haplotype in these Amish kindreds was identical to that presented by family A61, except for locus D15S514 (allele 5 instead of allele 2; fig. 3). It should be noted that there are no records showing exactly how far back these two Amish populations separated, although the records available suggest that it was before 1700. The variation at marker D15S514 could be due to mutation(s) of this microsatellite that would be posterior to the geographical separation of these kindreds or else could reflect a historical crossing-over. In both groups of families, nonrecombinant affected siblings were homozygous for all the markers in the 10–12-Mb region. Therefore, this study of homozygosity-by-descent did not provide any valuable information for further restriction of the interval.

In contrast with the homogeneous situation encountered among the Amish LGMD2A chromosomes, an unexpected diversity of carrier haplotypes segregating within the 13 pedigrees from Réunion was revealed (fig. 3). At least six different haplotypes were identified, none of which was identical to those seen in the Brazilian or Amish pedigrees. These included two major haplotypes (I and II; fig. 3) that were encountered in ≥ 9 (R11, R14, R16, R17, R19, R21, R23, R26, and R27) and 5 (R15, R16, R17, R19, and R24) families, respectively. The total was greater than the number of examined families (13), because in three pedigrees (R16, R17 and R19) affected children carried both haplotypes in a heterozygous state. The finding of these multiple carrier haplotypes was thus suggestive of a previously unsuspected allelic heterogeneity at the LGMD2A locus in this population.

Although homozygosity mapping did not permit further restriction of the LGMD2A region in this population, because of the haplotype heterogeneity, it nevertheless could be used to ascertain the involvement of the chromosome 15 locus in family R12, a pedigree with only one affected child who displayed a unique minor haplotype (V; fig. 3) in a homozygous state. The cosegregation of minor and major haplotypes as compound heterozygotes in affected individuals permitted the identification of "at least" three minor LGMD2A haplotypes: haplotype III, present once in families R17 and R14 (and possibly also in R15), and haplotypes IV and VI, seen once only (in families R14 and R27, respectively). Although the haplotypes of family R15 could belong to group II or III, they were nevertheless classified into group II, as they shared alleles at two biallelic markers within this interval for which they differed from haplotype III (data not shown). The only family that could not be definitively ascertained to belong to the chromosome 15–linked group was family R20, in which affected children were compound heterozygotes carrying two haplotypes (VII and VIII; fig. 3) that were not seen in any other family.

Being able to subdivide the Réunion LGMD2A haplotypes into several subgroups allowed the identification of two hypothetical historical crossing-over events by comparison of the shared alleles between families displaying haplotype II. The haplotypes seen in family R15 (fig. 3) did confirm D15S222 as the distal flanking marker, while suggesting D15S779 as a new proximal boundary.

Gametic Linkage Disequilibrium Between Markers and the Disease Locus

Although the smallest LGMD2A interval region had been restricted to ~1 cM, the boundaries were essentially defined only by a small number of recombinants. There was thus a risk that the latter might result from errors rather than recombination events. We therefore

Families / Markers	THBS1	D15S146	D15S214	D15S129	D15S514	D15S779	D15S512	D15S782	D15S780	D15S508	D15S778	D15S784	D15S783	D15S781	D15S182	D15S516	D15S509	D15S517	D15S222	D15S172	D15S132	D15S161	D15S143	D15S123	FBN1	D15S106	D15S119	CYP19	Haplotypes
Réunion Island																													
R11 *	2	5	5	6	2	3	3	6	3	2	5	5	2	3	-	2	-	4	7	2	3	6	-	8	4	5	4	3	I
R16	2	5	5	6	2	3	3	6	3	1;2	5	5	2	3	5;7	2	2	4	7	2	3	6	9	8	4	5	4	3	I
R17	2	5	5	6	2	3	3	7	3	2	5	5	2	4	-	2	2	4	7	2	3	6	9	8	4	5	4	3	I
R19	2	5	5	6	2	3	3	6	2	2	5	5	2	3	5	2	2	4	7	2	3	6	9	8	4	5	4	3	I
R21	2	5	5	6	2	3	3	6	3	2	5	5	2	3	5	2	2	4	7	2	3	6	9	8	4	5	4	3	I
R26	2	5	5	6	2	3	3	6	3	2	5	5	2	3	5	2	2	4	7	2	3	6	9	8	4	5	4	3	I
R27	2	5	5	6	2	3	3	6	3	2	5	5	2	3	5;6	2	2	4	7	2	3	6	9	8	4	5	4	3	I
R21	2	5	5	6	2	3	3	6	3	2	5	5	2	3	5	2	2	2;4	7	2	2	6	9	8	2	5	4	7	I
R14	2	5	5	6	2	3	3	6	3	2	5	5	2	3	-	2	2	4	7	2	2	6	9	8	2	5	4	7	I
R23	2	5	5	6	2	3	3	6	3	2	5	5	2	3	5	2	2	4	7	2	3	6	9	8	2	5	4	7	I
R23	2	5	5	6	2	3	3	6	3	2	5	5	2	3	4;5	2	2	4	7	2	3	6	9	8	2	3	5	6	I
R26	2	5	5	6	2	3	3	6	3	2	5	5	2	3	5;6	2	2	4	7	2	3	6	9	8	2	4	4	3	I
R16	2	4	6	7	5	3	3	6	5	1	2	5	3	3	5	2	2	4	7	6	8	3	5	8	4	5	5	7	II
R17	2	4	6	7	5	3	3	6	5	1	2	5	3	3	-	2	2	4	7	6	8	3	5	8	4	5	5	7	II
R19	2	4	6	7	5	3	3	6	5	1	2	5	3	3	5	2	2	4	7	6	8	3	5	8	4	5	5	7	II
R24 *	2	4	6	7	5	3	3	6	5	1	2	5	3	3	5	2	2	4	7	6	8	3	5	8	4	5	5	7	II
R15	2	4	6	5	8	10	3	6	5	1	5	5	3	4	5	2	2	4	4	4	4	5	5	3	2	4	4	6	II
R15	2	3	6	7	8	10	3	6	5	1	5	5	3	4	5	2	2	4	4	4	4	5	5	3	2	4	4	6	II
R14	1	4	6	3	5	8	3	7	5	1	5	5	3	4	-	2	2	4	4	3	3	6	5	6	2	4	6	6	III
R17	2	5	6	3	5	8	3	6	5	1	5	5	3;2	3	-	2	2	4	4	3	3	7	5	6	2	4	6	6	III
R14	1	4	6	3	2	3	3	4	3	2	4	5	2	4	4;5	2	2	2	6	6	3	2	9	4	2	5	4	5	IV
R20	2	5	6	6	5	9	3	8	3	2	4	5	2	3	5;6	2	2	4	10	6	8	3	9	8	2	5	5	7	V
R20	1	3	6	7	5	9	3	7	3	2	7	5	3	4	5	2	2	4	9	6	6	8	2	6	4	5	3	6	VI
R12 *	2	5	2	8	7	5	3	4	5	1	4	4	3	4	5	3	3	4	5	3	4	2	8	4	2	4	5	7	VII
R27	2	3	3	6	5	8	3	6	5	2	5	5	2	4	5	2	2	2	6	6	6	6	5	4	2	5	4	3	VIII
Amish																													
A61 *	2	5	-	3	2	3	3	8	4	2	4	5	2	3	5	2	-	4	10	6	-	-	6	-	4	5	-	5	
A617, A618	-	-	-	-	5	3	3	8	4	2	4	5	2	3	-	2	-	4	10	-	-	-	-	-	-	-	-	-	
Brazil																													
B501	1	4	7	3	2	9	3	6	4	2	4	7	3	4	-	-	-	4	-	2	8	7	5	4	2	4	4	7	
B501	2	2	4	8	5	9	3	6	4	2	4	7	3	4	-	-	-	4	-	2	8	7	5	-	2	4	5	7	
B519 *	2	3	7	5	5	9	4	8	3	2	4	2	4	2	-	-	-	-	-	6	9	7	1	7	4	5	4	6	

Figure 3 Carrier haplotypes segregating within the families from Réunion (R), the Amish population (A), and Brazil (B). Ordered marker loci and examined LGMD2A families are listed in the first row and column, respectively. Markers flanking the 1-cM interval defined by recombination events are indicated in larger letters. When phase could not be determined, both alleles are shown, separated by a semicolon. Ungenotyped markers are indicated by a dash (-). Microsatellite mutations could explain the occasional shifts within a conserved haplotype (underlined alleles). Families showing a single carrier haplotype in a homozygous state are indicated by an asterisk (*).

chose to investigate linkage disequilibrium for markers scattered along an ~10-cM interval (between THSB1 and CYP19). Twenty-eight markers were individually tested for allelic association with the disease locus in the families from Réunion and 20 in the Amish population. Different estimates were used to investigate linkage disequilibrium between multiallelic microsatellite markers and LGMD2A (for details, see Subjects, Material, and Methods).

Twelve markers gave significant results on the Amish population, even after correction for multiple testing, 10 of which mapped inside the 7-cM interval containing the disease locus. Eight of them were located within the 1-cM LGMD2A interval. Three markers located in the proximal part of the interval showed the most significant linkage disequilibrium with the disease locus: D15S779, D15S780, and D15S782 (data not shown).

Among the 28 markers tested in the Réunion island families, only 7 showed significant linkage disequilibrium (table 4). Of these, six mapped within the previously identified 7-cM interval, and four of them (D15S779, D15S782, D15S783, and D15S781) were located within the 1-cM interval determined in this study. Marker D15S779 was clearly the marker displaying the strongest association with the disease locus, with allele 3 present in 69% of the LGMD2A chromosome and in none of the normal chromosomes, resulting in a P_F value of 8×10^{-8}. Significant χ^2s were obtained for marker D15S783 with the multiallelic method ($\chi^2 = 22.20$; $P = 1.5 \times 10^{-5}$), whereas the combined allele method failed to detect significant linkage disequilibrium ($\chi^2 = 0.62$; $P > 1.83 \times 10^{-3}$). This is explained by the fact that allele 2, which displays linkage disequilibrium, is not the most common allele, and therefore, when the combined-allele method was used, it was not allele 2, but allele 3, that was tested for association. The relatively smaller number of markers manifesting significant linkage disequilibrium in the families from Réunion, as compared with those in the Amish kindreds, is likely to be a consequence of the multiplicity of carrier haplotypes observed in the Réunion island population.

In both the Réunion and Amish populations, the χ^2 values obtained by using the combined-allele method are presented in figure 4. Information was lost using the combined-allele method, since it reduced multiallelic markers to biallelic systems. It had, however, the advantage that all χ^2 values had 1 df and could therefore be compared directly with one another (this would not be the case with the multiallelic method, as various markers may have different numbers of alleles, and, consequently, χ^2 values would have different df's). In any event, results were in agreement using both methods (table 4). Figure 4 clearly shows that markers displaying significant linkage disequilibrium were scattered along the entire LGMD2A region, with the "highest peak" observed for marker D15S779 in both the Réunion and the Amish populations. Two other markers displayed very significant association, namely D15S222, which mapped outside the smallest interval defined by recombination events, and D15S782. The latter was derived from the same YAC clone 774G4 as D15S779, from which it is separated by one microsatellite (D15S512) and cannot be farther apart than 1.6 Mb, the size of the original YAC clone.

Discussion

A gene for the recessive form of LGMD, LGMD2A, has been localized within a 7-cM interval on chromosome 15q15.1-q21.1, and a physical map of the region has been established by means of a 10–12-Mb contig of YAC clones (Fougerousse et al. 1994). In the absence of any cytogenetic or biochemical indication or a suitable candidate gene, positional cloning appeared as the only valid option to identify the LGMD2A locus. However, it was difficult to consider launching such a daunting task without further refinement of the region containing the disease locus.

We therefore undertook a genetic investigation of LGMD2A, an analysis which was complicated by the genetic heterogeneity of the autosomal recessive LGMDs (Passos-Bueno et al. 1993; Bashir et al. 1994; Allamand et al. 1995) and the difficulty of ascertaining the involvement of the chromosome 15 locus in small pedigrees. This study was thus restricted to families with three different origins (2 large pedigrees from Brazil, 12 from an Amish community in the United States, and 13 families from Réunion).

Restriction of the LGMD2A Interval by Recombination Mapping

The genotyping of published and newly developed markers of the region allowed the construction of extended haplotypes. The study of the segregation of these haplotypes within the LGMD2A families led to the delineation of a 1-cM interval containing the disease gene that was identified by three recombination events. The physical distance could be assessed as 3–4 Mb, considering the sizes of the three YAC clones that roughly cover this region. This relation between genetic and physical distances differs from the generally accepted rule (i.e., 1 cM corresponding to 1 Mb), possibly a reflection of the fact that this region may be a "cold spot" for recombination.

There were no other large LGMD2A kinships available. Small LGMD2A pedigrees could have contributed to a more precise mapping, had we been able to identify them. This, unfortunately, was not the case, and this still large region of ~3–4 Mb was the smallest interval that could be delineated on the basis of recombination events in the available informative families.

Haplotype Analysis in Two Consanguineous Genetic Isolates

There was only weak evidence for the assignment of proximal and distal borders of the interval, since they were defined by, respectively, one and two authentic recombinants (as opposed to historical ones). We therefore tried to extract additional genetic information from the families from Réunion and the Amish kindreds.

Extended LGMD2A haplotypes and homozygosity-by-descent among the affected siblings were investigated. Homozygosity mapping in highly consanguineous populations has been used successfully as an approach to map disease genes such as ataxia with selective vitamin E deficiency (Ben Hamida et al. 1993), alkaptonuria (Pollak et al. 1993), and Hirschprung disease (Puffenberger et al. 1994).

A high proportion of consanguinity had been reported among the northern Indiana Amish population (Hammond and Jackson 1958), and genealogical reports

Table 4

Significant Allelic Association between Chromosome 15q15.1-q21.1 Markers and LGMD2A in the Population from Réunion

Marker and Alleles	No. (%) of Chromosomes		χ^2		Fisher P_F[b]
	N	LGMD	Combined Allele (P)[a]	Multiallele (P)[a]	
D15S779:					
2	3 (10.34)	0 (0)	...		...
3	0 (0)	20 (68.96)	30.5 ($<10^{-6}$)	30.76 (10^{-6})	8×10^{-8}
4	1 (3.45)	0 (0)	...	...	...
5	2 (6.90)	2 (6.90)	...	...	...
6	2 (6.90)	0 (0)	...	...	...
7	1 (3.45)	0 (0)	...	...	...
8	6 (20.69)	3 (10.34)	...	...	...
9	8 (27.58)	2 (6.90)	...	...	...
10	6 (20.69)	2 (6.90)	...	...	...
D15S782:					
3	4 (13.80)	0 (0)	...	...	...
4	0 (0)	3 (10.34)	...	...	...
5	9 (31.02)	0 (0)	...	...	...
6	6 (20.69)	22 (75.87)	17.68 (2.6×10^{-5})	21.14 (9.9×10^{-5})	8.26×10^{-5}
7	6 (20.69)	3 (10.34)	...	...	...
8	2 (6.90)	1 (3.45)	...	...	...
9	1 (3.45)	0 (0)	...	...	...
10	1 (3.45)	0 (0)	...	...	...
D15S783:					
2	2 (6.9)	16 (55.17)	0.62 (NS)	22.20 (1.5×10^{-5})	2.58×10^{-4}
3	16 (55.17)	13 (44.83)	...	...	...
4	5 (17.24)	0 (0)	...	...	...
7	1 (3.45)	0 (0)	...	...	...
8	5 (17.24)	0 (0)	...	...	...
D15S781:					
1	2 (6.9)	0 (0)	...	...	...
2	4 (13.80)	0 (0)	...	...	...
3	8 (27.60)	20 (69.0)	9.94 (1.6×10^{-3})	9.94 (1.6×10^{-3})	NS[c]
4	15 (51.70)	9 (31.00)	...	...	...
D15S222:					
1	1 (3.45)	0 (0)	...	...	...
2	1 (3.45)	0 (0)	...	...	...
4	3 (10.34)	4 (13.80)	...	...	...
5	14 (48.27)	2 (6.90)	...	...	...
6	2 (6.90)	2 (6.90)	...	...	...
7	3 (10.34)	19 (65.50)	18.75 (1.5×10^{-5})	21.44 (8.5×10^{-5})	1.1×10^{-4}
8	1 (3.45)	0 (0)	...	...	...
9	0 (0)	1 (3.45)	...	...	...
10	4 (13.80)	1 (3.45)	...	...	...
D15S132:					
2	0 (0)	2 (6.90)	...	...	...
3	1 (3.45)	14 (48.27)	2.75 (NS)	18.49 (3.5×10^{-4})	4.69×10^{-4}
4	3 (10.34)	4 (13.80)	...	...	...
5	1 (3.45)	0 (0)	...	...	...
6	10 (34.50)	2 (6.90)	...	...	...
8	13 (44.81)	7 (24.13)	...	...	...
10	1 (3.45)	0 (0)	...	...	...
D15S123:					
1	1 (3.45)	0 (0)	...	...	...
3	4 (13.80)	2 (6.90)	...	...	...
4	5 (17.24)	4 (13.80)	...	...	...
5	9 (31.03)	0 (0)	...	...	...
6	2 (6.90)	3 (10.34)	...	...	...
8	5 (17.24)	20 (68.96)	15.82 (7.10^{-5})	21.49 (8.3×10^{-5})	4.46×10^{-4}
9	3 (10.34)	0 (0)	...	...	...

[a] Corrected significant level (α') was estimated from the Bonferroni's procedure to account for multiple testing. A total number of 28 loci were examined for linkage disequilibrium, therefore leading to $\alpha' = 1.83 \times 10^{-3}$.

[b] The one-sided Fisher P_F value was corrected for multiple testing by multiplying by the number of tests carried out for each locus minus one.

[c] NS = not significant ($P > 1.83 \times 10^{-3}$ for the χ^2 test, and $P_F > .001$ for the Fisher test).

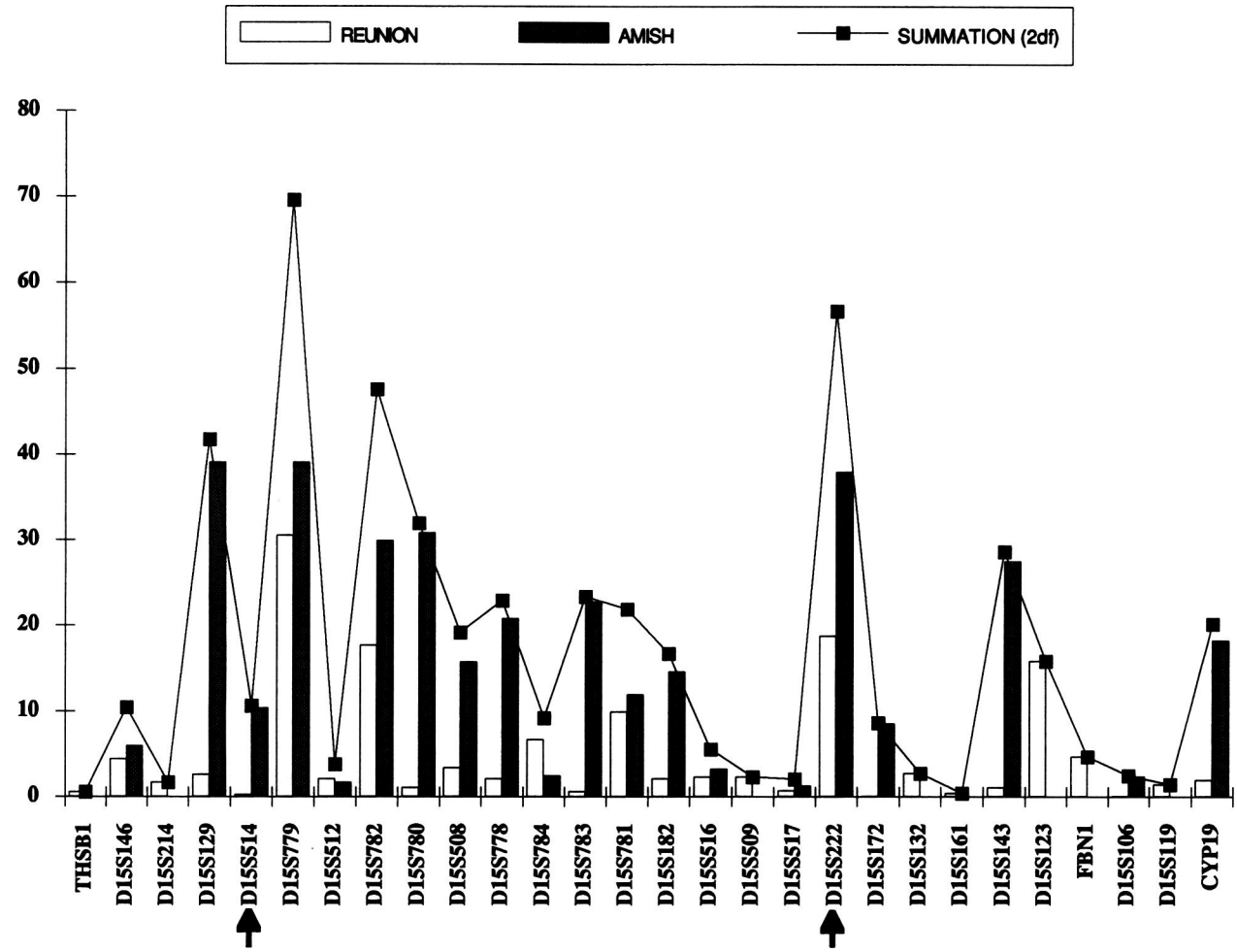

Figure 4 χ^2 values of linkage disequilibrium obtained on the Réunion and Amish populations using the combined-allele method. Marker loci are listed from centromere (*left*) to telomere (*right*). Marker loci flanking the 1-cM interval defined by recombination events are indicated by *arrows*.

traced these families back to a single ancestral couple (Jackson and Strehler 1968; Young et al. 1992; Allamand et al. 1995). As expected, because of a presumed unique founder effect, a single LGMD2A carrier haplotype segregating within this population was revealed (fig. 3). In light of the sharing of such an extensive haplotype (>7 cM), no historical crossing-over event could be identified. Moreover, the homozygosity-by-descent approach did not permit restriction of the LGMD2A interval, since all affected children in both the Indiana and Pennsylvania Amish populations were homozygous for all markers genotyped along the LGMD2A interval.

This is to be contrasted with the situation seen among the Réunion island patients, where, despite the multiple consanguineous links and the genealogically reported common ancestor who landed on this island 13 generations ago (Beckmann et al. 1991), at least six different carrier haplotypes could be identified in this LGMD2A population: two haplotypes were present in a majority of families, but at least four minor haplotypes could also be observed (fig. 3). Affected children carrying the same haplotype on both their chromosomes were homozygous for almost all the markers genotyped (spanning a 10-cM region). Affected offspring carrying different haplotypes were homozygous for a reduced number of markers scattered along the LGMD2A region, but this homozygosity was most probably a reflection of arbitrary concordance of the associated alleles rather than an expression of homozygosity-by-descent. This unexpected diversity of carrier haplotypes complicated and obscured the homozygosity mapping. Therefore, this analysis did not permit refinement of the location of the disease gene. In contrast, the identification of historical crossing-over events in these pedigrees confirmed D15S222 as the distal flanking marker while suggesting D15S779 as a new proximal boundary (fig. 3).

Gametic Linkage Disequilibrium Analysis Supports a Proximal Location of the LGMD2A Gene

We next tested for nonrandom association between chromosome 15 marker loci and the disease locus among the families from the Réunion and the Amish population. In isolated populations, such a preferential association is expected to occur between loci that are tightly linked (Hästbacka et al. 1992; Sirugo et al. 1992) and has been used to identify candidate regions and/or to refine the location of disease genes, as reported for progressive myoclonus epilepsy (Lehesjoki et al. 1993), Wilson disease (Bowcock et al. 1994), and autosomal dominant polycystic kidney disease (Snarey et al. 1994). The detection of linkage disequilibrium has also contributed to the identification of the cystic fibrosis gene (Kerem et al. 1989), the Huntington disease gene (The Huntington's Disease Collaborative Research Group 1993), and, recently, the diastrophic dysplasia gene (Hästbacka et al. 1994).

Linkage disequilibrium data based on the analysis of small populations like those from the Réunion island and the Amish community must, however, be interpreted with caution. Because of the rarity and genetic heterogeneity of the disease (and therefore the difficulty in obtaining a large number of informative pedigrees), the sample size could not be increased. And, in light of the failure to further restrict the 3–4-Mb candidate region by haplotype and homozygosity mapping, it was worth attempting to see whether linkage disequilibrium studies could permit the reaching of this goal.

A number of loci along the LGMD2A interval displayed significant associations with the disease allele in both the Réunion island and Amish pedigrees. Furthermore, in both populations, the proximal region of the 1-cM LGMD2A interval appeared to show a "higher density" of such markers (D15S779, D15S782, and D15S780) than the distal part (fig. 4).

Problems Associated with Fine Mapping Using Linkage Disequilibrium Analysis

The adequacy of gametic association studies to narrow a genetic interval on such relatively young populations as the two genetic isolates studied here needs to be addressed. The impact of allele frequencies on linkage disequilibrium estimates also poses problems. The latter were well illustrated when considering marker D15S784, which, although displaying eight alleles, never gave significant association, because allele 5, which was present in 100% of the LGMD2A chromosomes, was also the most frequent allele on control chromosomes. This was also the case for marker D15S512, which, although located between two markers showing highly significant linkage disequilibrium (D15S779 and D15S782), failed to reveal significant association with the disease locus.

Yet, however tenuous the linkage disequilibrium analysis in our sample, the markers that exhibited the most significant linkage disequilibrium with the disease locus were preferentially clustered in the proximal part of the interval in both the Réunion island and Amish populations (fig. 4). In this context, it is of interest to note that one metropolitan French family shares a four-marker haplotype (D15S779, D15S512, D15S782, and D15S780 are all assigned to the proximal part of the interval) with the Amish families (data not shown). The chance occurrence of such a haplotype can be estimated on the basis of the allele frequencies taken from the CEPH reference families as $<8.4 \times 10^{-4}$. This observation is thus suggestive of a common ancestral origin and further supports the preferential location. It remains to be demonstrated whether or not both LGMD2A chromosomes carry the same disease-causing mutation.

The candidate region might, thus, accordingly have been reduced from 3–4 Mb to 1.6 Mb, which corresponds to the size of YAC 774G4 carrying markers D15S779, D15S780, and D15S782. This hypothesis served as the starting point for further investigations. A cosmid contig has been established from this YAC clone (I. Richard, C. Roudaut, F. Fougerousse, N. Chiannilkulchai, and J. S. Beckman, unpublished data), and a search for expressed sequences in this region has been undertaken (Chiannilkulchai et al. 1995). This work eventually led to the identification of an attractive positional and functional candidate gene. The latter encodes for the large subunit of the muscle-specific intracellular calpain 3. Finally, 15 distinct pathological mutations were identified in this locus, thereby demonstrating the role of this protein in the etiology of LGMD2A (Richard et al. 1995).

Extent of Linkage Disequilibrium Estimate

Thus, despite the many consanguineous links connecting the families within the Réunion island and Amish populations, links which, at first sight, made them adequate for homozygosity mapping, this analysis did not permit further restriction of the 1-cM interval containing the LGMD2A gene. Indeed, the conserved haplotypes spanned a region of >7 cM. One explanation for the extended common haplotype could be the "linkage drag" (Stam and Zeven 1981). The latter refers to the observation that the proportion of chromosomal DNA retained on a series of successive crosses (e.g., backcrosses) around a trait under selection is substantially larger than would be theoretically expected for a random chromosomal segment after the successive recombination-mediated series of dilution of the introgressed genome. As a matter of fact, the investigated LGMD2A families appear to be equivalent to a population under selection, since we are essentially interested in the carrier haplotypes. Therefore, the results obtained (conservation of haplotypes and homozygosity along the 7–10-cM interval) are the consequence and reflection of this selection bias.

Moreover, both the Amish and Réunion island populations can be considered as "young," displaying a large area of linkage disequilibrium so that linkage disequilibrium estimates can be very powerful to demonstrate or confirm linkage even with distant markers. However, the adequacy of these estimates for the precise localization of a disease locus is questionable, as they are too sensitive to fluctuations in the frequencies of the associated marker alleles. It is likely thus that "older" populations would be better adapted for such studies, since enough generations would separate the founder event from present-day patients so that only genetically and physically tightly linked markers would display linkage disequilibrium. Eventually, however, a densely saturated map of equifrequent biallelic markers (e.g., one every 100 kb, on the average) would provide more power to assess linkage disequilibrium than the highly polymorphic microsatellites with potentially as much information (e.g., as highly informative haplotypes). Using such equifrequent markers, it should then be possible to tend toward the resolution shown by Jorde et al. (1994).

The observation of multiple haplotypes in the genetic isolate of Réunion still needs explanation. A number of hypotheses can be put forward. The first one would be that the different haplotypes result from multiple, historical recombination events that would be expressed in present-day generations as apparent double recombinants. This, however, is highly unlikely, considering the 1-cM interval separating the flanking markers defined by recombination events, and could not explain as many as six or more distinct haplotypes. The second hypothesis would be that the common ancestor (13 generations before contemporary patients) was already a compound heterozygote and contributed two different LGMD2A alleles. Even then it is hard to explain all of the haplotypes. A combination of these two hypotheses would by no means lead to a diversity of haplotypes as seen within the Réunion population. The instability of microsatellite markers may have contributed to the haplotype divergence (fig. 3). Yet, even the combined effects of recombination events, microsatellite mutations, and compound heterozygote are insufficient to account for the number of carrier chromosomes. We therefore infer that a number of independent mutations do cosegregate in this population and that the haplotype heterogeneity reflects this phenomenon. This hypothesis was subsequently demonstrated by the identification of at least six distinct calpain mutations within the Réunion island pedigrees (Richard et al. 1995; I. Richard and J. S. Beckmann, unpublished data).

Thus, one needs to reconcile the high number of suspected mutations in this small isolated population with the reported low prevalence of the disease, the so-called Réunion paradox discussed by Richard et al. (in press), who propose that LGMD2A is an example of a digenic or more complex inheritance and that the phenotypic expression of the calpain mutations might be controlled by a second unlinked nuclear, or even a mitochondrial locus (e.g., a modifier gene), which would be fixed in the Reunion families. This model could thus account for the multiple mutations in other small populations reported by others (Bach et al. 1994; Rodius et al. 1994; Heinisch et al. 1995) and might thus reflect a new general genetic mechanism.

To conclude, this extensive genetic analysis of a recessive form of LGMD has allowed a further restriction of the candidate interval, which eventually led to the identification of the disease locus (Richard et al. 1995), thereby validating the experimental approach followed to narrow the incriminated genetic interval. The recognition of LGMD2A carrier haplotypes among the two populations examined could be valuable in the future for presymptomatic, prenatal, or prenuptial diagnosis of heterozygous and homozygous carriers and also to recognize the involvement of the LGMD2A locus in other LGMD families that share a common partial haplotype with one of those already identified. Moreover, this study also raised issues that are likely to apply to other diseases.

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References

Allamand V, Broux O, Bourg N, Richard I, Tischfield J, Hodes ME, Conneally PM, et al (1995) Genetic heterogeneity of autosomal recessive limb-girdle muscular dystrophy in a genetic isolate (Amish) and evidence for a new locus. Hum Mol Genet 4:459–464

Bach G, Moskowitz SM, Tieu PT, Matynia A, Neufeld EF (1994) Molecular analysis of Hurler syndrome in Druze and Muslim Arab patients in Israel: multiple allelic mutations of the *IDUA* gene in a small geographic area. Am J Hum Genet 53:330–338

Bashir R, Strachan T, Keers S, Stephenson A, Mahjneh I, Marconi G, Nashef L, et al (1994) A gene for autosomal recessive limb-girdle muscular dystrophy maps to chromosome 2p. Hum Mol Genet 3:455–457

Beckmann JS, Richard I, Hillaire D, Broux O, Antignac C, Bois E, Cann H, et al (1991) A gene for limb-girdle muscular

dystrophy maps to chromosome 15 by linkage. C R Acad Sci III 312:141–148

Beckmann JS, Tomfohrde J, Barnes RI, Williams M, Broux O, Richard I, Weissenbach J, et al (1993) A linkage map of human chromosome 15 with an average resolution of 2 cM and containing 55 polymorphic microsatellites. Hum Mol Genet 2:2019–2030

Ben Hamida C, Doerflinger N, Belal S, Linder C, Reutenauer L, Dib C, Gyapay G, et al (1993) Localization of Friedreich ataxia phenotype with selective vitamin E deficiency to chromosome 8q by homozygosity mapping. Nat Genet 5:195–200

Bowcock AM, Tomfohrde J, Weissenbach J, Bonne-Tamir B, St George-Hyslop P, Giagheddu M, Cavalli-Sforza LL, et al (1994) Refining the position of Wilson disease by linkage disequilibrium with polymorphic microsatellites. Am J Hum Genet 54:79–87

Chiannilkulchai N, Pasturaud P, Richard I, Auffray C, Beckmann JS (1995) A primary expression map of the chromosome 15q15 region containing the LGMD2A gene. Hum Mol Genet 4:717–726

Emery AEH (1991) Population frequencies of inherited neuromuscular diseases: a world survey. Neuromuscul Disord 1:19–29

Fougerousse F, Broux O, Richard I, Allamand V, Pereira de Souza A, Bourg N, Brenguier L, et al (1994) Mapping of a chromosme 15 region involved in Limb-Girdle Muscular Dystrophy. Hum Mol Genet 3:285–293

Fougerousse F, Richard I, Broux O, Cohen D, Beckmann JS (1992) Mapping of two chromosome 15 microsatellites. Genomics 13:903–904

Hammond, DT, Jackson, CE (1958) Consanguinity in a midwestern United States isolate. Am J Hum Genet 10:61–63

Hästbacka J, de la Chapelle A, Kaitila I, Sistonen P, Weaver A, Lander E (1992) Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. Nat Genet 2:204–211

Hästbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, et al (1994) The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 78:1073–1087

Heinisch U, Zlotogora J, Kafert S, Gieselmann V (1995) Multiple mutations are responsible for the high frequency of metachromatic leukodystrophy in a small geographic area. Am J Hum Genet 56:51–57

Hill WG, Robertson A (1968) Linkage disequilibrium in finite populations. Theor Appl Genet 38:226–231

Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983

Jackson CE, Strehler DA (1968) Limb-girdle muscular dystrophy: clinical manifestations and detection of preclinical disease. Pediatrics 41:495–502

Jorde LB, Watkins WS, Carlson M, Groden J, Albertsen H, Thliveris A, Leppert M (1994) Linkage disequilibrium predicts physical distance in the adenomatous polyposis coli region. Am J Hum Genet 54:884–898

Kerem B, Rommens JM, Buchanan JA, Markiewizc D, Cox TK, Chakravarti A, Buchwald M, et al (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245:1073–1080

Lander ES, Botstein D (1987) Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. Science 236:1567–1570

Lathrop GM, Lalouel JM, Julier C, Ott J (1985) Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

Lehesjoki AE, Koskiniemi M, Norio R, Tirrito S, Sistonen P, Lander E, de la Chapelle A (1993) Localization of the EPM1 gene for progressive myoclonus epilepsy on chromosome 21: linkage disequilibrium allows high resolution mapping. Hum Mol Genet 2:1229–1234

Litt M, Jorde LB (1986) Linkage disequilibria between pairs of loci within a highly polymorphic region of chromosome 2q. Am J Hum Genet 39:166–178

Murray JC, Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Manion F, Quillen J, et al (1994) A comprehensive human linkage map with centimorgan density. Science 265:2049–2054

Passos-Bueno M-R, Richard I, Vainzof M, Fougerousse F, Weissenbach J, Broux O, Cohen D, et al (1993) Evidence of genetic heterogeneity in the autosomal recessive adult forms of limb-girdle muscular dystrophy following linkage analysis with 15q probes in Brazilian families. J Med Genet 30:385–387

Passos-Bueno M-R, Vainzof M, de Cassia M. Pavanello R, Pavanello-Filho I, Lima MABO, Zatz M (1991) Limb-girdle syndrome: a genetic study of 22 large Brazilian families—comparison with X-linked Duchenne and Becker dystrophies. J Neurol Sci 103:65–75

Pereira de Souza A, Allamand V, Richard I, Brenguier L, Chumakov I, Cohen D, Beckmann JS (1994) Targeted development of microsatellite markers from inter-Alu amplification of YAC clones. Genomics 19:391–393

Pollak MR, Wu Chou Y-H, Cerda JJ, Steinmann B, La Du BN, Seidman JG, Seidman CE (1993) Homozygosity mapping of the gene for alkaptonuria to chromosome 3q2. Nat Genet 5:201–204

Puffenberger EG, Kauffman ER, Bolk S, Matise TC, Washington SS, Angrist M, Weissenbach J, et al (1994) Identity-by-descent and association mapping of a recessive gene for Hirschsprung disease on human chromosome 13q22. Hum Mol Genet 3:1217–1225

Richard I, Broux O, Allamand V, Fougerousse F, Chiannilkulchai N, Bourg N, Brenguier L, et al (1995) Mutations in the proteolytic enzyme, calpain 3, cause limb girdle muscular dystrophy type 2A. Cell 81:27–40

Rodius F, Duclos F, Wrogemann K, Le Paslier D, Ougen P, Billault A, Belal S, et al (1994) Recombinations in individuals homozygous by descent localize the Friedrich Ataxia locus in a cloned 450-kb interval. Am J Hum Genet 54:1050–1059

Sirugo G, Keats B, Fujita R, Duclos F, Purohit K, Koenig M, Mandel JL (1992) Friedreich ataxia in Louisiana Acadians: Demonstration of a founder effect by analysis of microsatellite-generated extended haplotypes. Am J Hum Genet 50:559–566

Snarey A, Thomas S, Schneider MC, Pound SE, Barton N, Wright AF, Somlo S, et al (1994) Linkage disequilibrium

in the region of the autosomal dominant polycystic kidney disease gene (PKD1). Am J Hum Genet 55:365–371

Stam P, Zeven AC (1981) The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica 30:227–238

Terwilliger JD, Ott J (1994) Handbook of human genetic linkage. The John Hopkins University Press, Baltimore

Vignal A, Gyappay G, Hazan J, Nguyen S, Dupraz S, Cheron N, Becuwe N, et al (1993) Non-radioactive multiplex procedure for genotyping of microsatellite markers. In: Adolph KW (ed) Methods in Molecular Genetics, 1st ed. Academic Press, San Diego, pp 211–221

Weir BS (1990) Genetic data analysis. Sinauer, Sunderland, MA

Weissenbach J, Gyapay G, Dib C, Vignal A, Morisette J, Millasseau P, Vaysseix G, et al (1992) A second-generation linkage map of the human genome. Nature 359:794–801

Young K, Foroud T, Williams P, Jackson CE, Beckmann JS, Cohen D, Conneally PM, et al (1992) Confirmation of linkage of limb-girdle muscular dystrophy, type-2, to chromosome 15. Genomics 13:1370–1371