Assignment of the Tibial Muscular Dystrophy Locus to Chromosome 2q31

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Summary

Tibial muscular dystrophy (TMD) is a rare autosomal dominant distal myopathy with late adult onset. The phenotype is relatively mild: muscle weakness manifests in the patient's early 40s and remains confined to the tibial anterior muscles. Histopathological changes in muscle are compatible with muscular dystrophy, with the exception that rimmed vacuoles are a rather common finding. We performed a genomewide scan, with 279 highly polymorphic Cooperative Human Linkage Center microsatellite markers, on 11 affected individuals of one Finnish TMD family. The only evidence for linkage emerged from markers in a 43-cM region on chromosome 2q. In further linkage analyses, which included three other Finnish TMD families and which used a denser set of markers, a maximum two-point LOD score of 10.14 (recombination fraction of .05) was obtained with marker D2S364. Multipoint likelihood calculations, combined with the haplotype and recombination analyses, restricted the TMD locus to an ~1-cM critical chromosomal region without any evidence of heterogeneity. Since all the affecteds share one core haplotype, the dominance of one ancestor mutation is obvious in the Finnish TMD families. The disease locus that was found represents a novel muscular dystrophy locus, providing evidence for the involvement of one additional gene in the distal myopathy group of muscle disorders.

Introduction

Distal myopathies represent a group of relatively rare inherited muscle diseases with unknown etiology and without any identified gene defect, to date. One representative of this group is tibial muscular dystrophy (TMD; MIM 600334), in which the muscle weakness manifests in the patient's early 40s and, to a large extent, remains confined to the tibial anterior muscles, leading to a mild foot drop 10–15 years after the onset of muscle weakness. The overall disability is mild, and patients remain ambulatory throughout their lives (Udd et al. 1991b). The morphological findings are compatible with muscular dystrophy, as anterior tibial muscles are wasted and replaced by adipose and connective tissue (Udd et al. 1992). Rimmed vacuoles are frequently detected in the muscle biopsy (Udd et al. 1993). TMD was originally described in a large Finnish pedigree from an internal isolate (Udd et al. 1991a). Interestingly, in this pedigree with a high degree of remote consanguinity, six family members had a more severe, limb-girdle muscular dystrophy (LGMD). Their clinical features included a progress rate resembling childhood/juvenile-onset LGMD (Udd et al. 1991a).

Additional families with only the classic TMD phenotype have been found subsequently in Finland (Udd et al. 1993; Partanen et al. 1994). The estimated disease prevalence is ⩾5/100,000 (B. Udd, unpublished data). Thus far, TMD has been detected only in Finland, suggesting that it is a new member of the Finnish disease heritage, enrichment of which is due to isolation (Norio 1981; Peltonen et al. 1995).

In some distal myopathies, the locus assignment has been established. Miyoshi myopathy, in which the plantar flexors are initially affected, has been localized to 2p12-14 (Bejaoui et al. 1995). For Nonaka myopathy (in which the anterior tibialis muscles are initially affected) and for hereditary inclusion body myopathy, the loci have been assigned to chromosome 9p1-q1 (Argov et al. 1997; Ikeuchi et al. 1997), and, finally, one autosomal dominant form of distal myopathy has been mapped to chromosome 14 (Laing et al. 1995). The LGMDs are also a genetically heterogeneous group of muscle diseases. Two dominant forms and seven recessive forms have been mapped, thus far, and five defective genes have been identified in the recessive forms (table 1).

Table 1

LGMD Loci and Identified Genes

LGMD Locus	Inheritance[a]	Chromosomal Localization	Gene[b]	Symbol
1A	AD	5q31-33	...	...
1B	AD	1q11-21	...	...
2A	AR	15q15	Calpain3	CAPN3
2B	AR	2p12-13	...	...
2C	AR	13q12	γ-SG	SGCG
2D	AR	17q12-21	α-SG	SGCA
2E	AR	4q12	β-SG	SGCB
2F	AR	5q33	δ-SG	SGCD
2G	AR	17q11-12	...	...

NOTE.—Data from the studies by Beckmann et al. (1991); Ben Othmane et al. (1992); Azibi et al. (1993); Bashir et al. (1994); Roberds et al. (1994); Yamaoka et al. (1994); 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 van der Kooi et al. (1997).

[a] AD = autosomal dominant; AR = autosomal recessive.

[b] SG = sarcoglycan.

In this article, we present results from a genomewide scan of one large Finnish TMD family and assign the TMD locus to a restricted region on chromosome 2q31 (fig. 1). We also provide initial evidence that, in one inbred family, a more severe, LGMD-type disease is associated with homozygosity of the same disease haplotype.

Subjects, Material, and Methods

Methods used for clinical examination, as well as the diagnostic criteria, have been described elsewhere (Udd et al. 1993). The features of the TMD and LGMD phenotypes are summarized in table 2. The genome scan was performed for 11 affected and 6 healthy individuals from family A (fig. 2). Family B (fig. 3) represents a part of the large inbred pedigree in which TMD was originally described (Udd et al. 1991a). The other two families (C and D, not shown), with four affected individuals in each, are not known to be related to either family A or family B.

DNA for genotyping was extracted from whole-blood samples from consenting individuals (as described in the study by Vandenplas et al. [1984], with modifications for the use of Phase Lock Gel). The study protocol was accepted by the Ethical Board of the University of Helsinki.

The genome scan was performed, by use of the ABI prism system and Genotyper 2.0 software (Perkin-Elmer, Applied Biosystems), with 279 highly polymorphic fluorescent microsatellite markers of Cooperative Human Linkage Center set 6A, which is an improvement of set 5 (Dubovsky et al. 1995). Additional loci were typed by

use of γ-[^{32}P]ATP–labeled markers from the Généthon map (update March 1996). The order of the markers has been obtained mostly from the physical YAC contig map of chromosome 2 (Chumakov et al. 1995), supplemented by the Généthon map. The approximate genetic distances are according to the Généthon map (fig. 1). PCR reactions, electrophoresis, and genotyping were performed with minor modifications to procedures described elsewhere (Aaltonen et al. 1993).

Two-point LOD-score calculations were performed by use of the MLINK option of the LINKAGE package of programs (Lathrop and Lalouel 1984; Cottingham et al. 1993; Schäffer et al. 1994). The SLINK option was utilized to estimate the informativeness of the family material used in the linkage analyses (Ott 1989; Weeks et al. 1990). The VITESSE algorithm was used for multipoint likelihood calculations (O'Connell and Weeks 1995), for which family B had to be subdivided, owing to multiple founders in the family. Autosomal dominant inheritance with complete penetrance was assumed, and the gene frequency was set to .0001; young subjects (<35 years of age) were scored as unknown in the linkage analyses, owing to the late onset of the disease. The patients with the LGMD phenotype were considered as
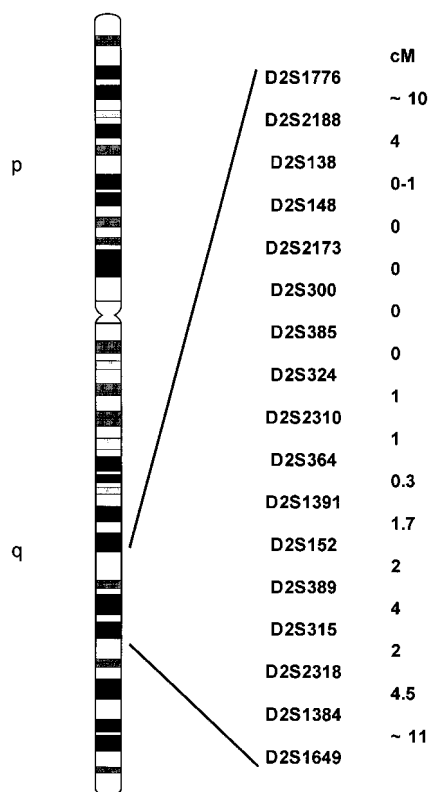

Figure 1 Order of markers and approximate intermarker distances on chromosome 2q31.

Table 2

Comparison of the TMD and LGMD Phenotypes

	TMD Phenotype	LGMD Phenotype
Clinical findings	Weakness in tibial anterior muscles (ankle dorsiflexion affected); patients unable to walk on their heels	Weakness in all proximal muscles; distal muscles less severely affected; facial muscles unaffected
Age at onset	4th–8th decade	1st–3d decade
Progression	Slow progress in anterior compartment muscles; mild proximal leg-muscle weakness in a minority of patients	Loss of ambulation between 3d and 6th decade
Findings from muscle biopsy[a]	Varies from mild myopathic changes to severe muscular dystrophy; rimmed vacuoles may be present	Severe muscular dystrophy in proximal muscles; mild changes in distal muscles
Findings from computed tomography/ magnetic resonance imaging	Fatty degeneration of anterior tibial muscles; patchy lesions in asymptomatic muscles	Fatty replacement and atrophy in proximal muscles

[a] Histopathological changes are variable, depending on the stage of the disease process. All patients in the LGMD group underwent muscle biopsy at a relatively late stage of the disease.

affected in the linkage analyses (fig. 3). The allele frequencies of the markers were obtained from the healthy chromosomes ($n = 20$). Male and female recombination fractions (θ) were assumed to be equal.

Results

Family A was utilized in the initial genomewide scan for the TMD locus, since, in the simulation analysis, this family alone was predicted to provide significant evidence for linkage, with an expected average LOD score of 4.34. The primary genomewide scan, using 279 markers, revealed only one interesting area, with a maximum two-point LOD score >3 in the pairwise analyses. Positive LOD scores were observed with markers D2S1776, D2S1391, D2S1384, and D2S1649, which span a 43-cM region (fig. 1). The maximum two-point LOD score of 6.31 was obtained with marker D2S364 ($\theta = .00$). When three additional TMD families were analyzed with the markers for this region, significant LOD scores of 3.12 ($\theta = .00$) and 3.47 ($\theta = .00$) were obtained in family B, with markers D2S364 and D2S385, respectively. Families C and D did not provide significant evidence for linkage to any of the markers in the pairwise analyses. However, when information from all four families was combined, the evidence for linkage increased. The maximum two-point LOD score of 10.14 ($\theta = .05$) was obtained with marker D2S364, in the pooled data set, and no evidence for genetic heterogeneity emerged from the statistical analyses.

Multipoint likelihood calculations assigned the TMD locus to be within close proximity of marker D2S324, which gave a maximum multipoint LOD score of 10.05

($\theta = .00$) (fig. 4). Haplotype analysis was performed on the basis of the haplotypes formed when a minimum number of recombination events was allowed (figs. 2 and 3). In families A, B, and D the analysis revealed a common core haplotype, since all affected individuals shared identical alleles on markers D2S138, D2S148, D2S2173, D2S300, D2S385, D2S324, D2S2310, and D2S364. Family C shared this haplotype identity with markers D2S138, D2S148, D2S2173, D2S300, D2S385, and D2S324, suggesting one restricted core haplotype in all the families analyzed. Furthermore, one healthy individual in family B shared the chromosomal haplotype of TMD, up to marker D2S148. This set the proximal boundary for the TMD region (fig. 3). The core haplotype and the recombinations thus restrict the critical area to an ~1-cM region between markers D2S148 and D2S2310.

Six patients in family B had a more severe, LGMD-type of disease. Three individuals, with childhood onset of LGMD and loss of ambulation already in the 3d decade, were homozygous for the disease allele (fig. 3). Although the other three individuals had LGMD, onset of the disease had been in adolescence, and they had lost ambulation in the 6th decade. They shared only one TMD chromosome, like that found in the TMD-affected members of the family (data not shown).

Discussion

Our findings provide evidence for a novel muscular dystrophy locus, via the assignment of the TMD locus to chromosome 2q31, between markers D2S148 and D2S2310. The number of disease chromosomes in our
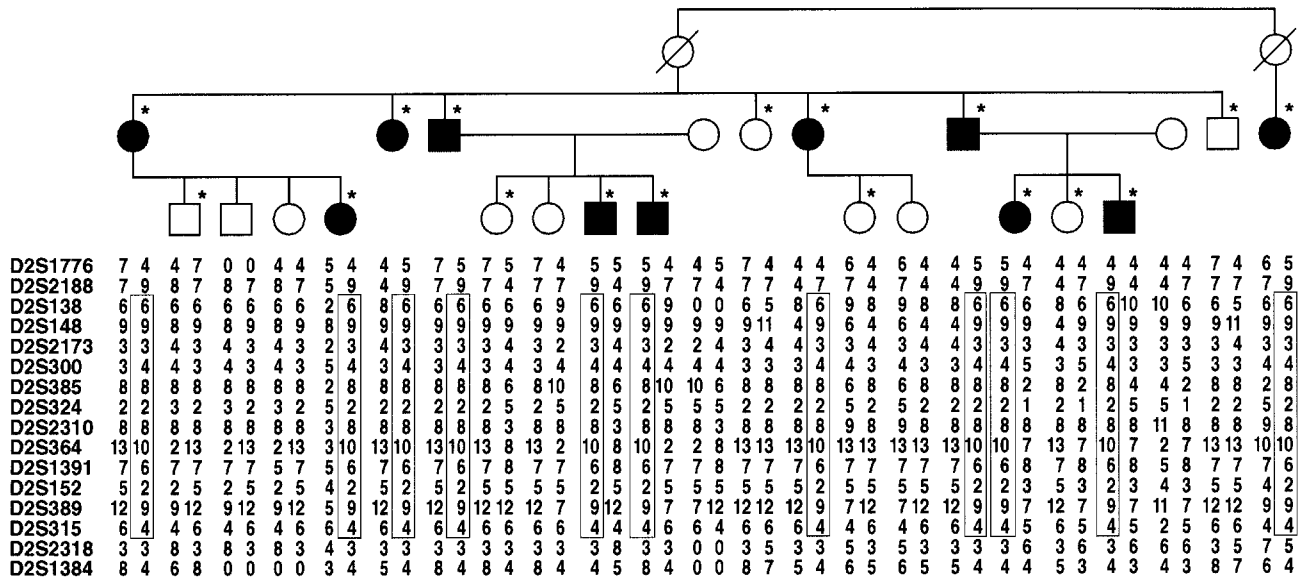
Figure 2 Pedigree of family A, showing chromosome 2q haplotypes. Individuals marked with an asterisk (*) were included in the genome scan. The haplotype cosegregating with the disease is boxed.

study material was too low to provide statistical evidence for linkage disequilibrium in this dominant disorder. However, the haplotype analyses suggested that all affected individuals share one common core haplotype and, thus, predict one major ancestral TMD mutation

in the Finnish population. Analysis of the core haplotype, defined by use of a relatively dense set of markers, actually provided information on ancient recombination events and restricted the critical chromosomal region.

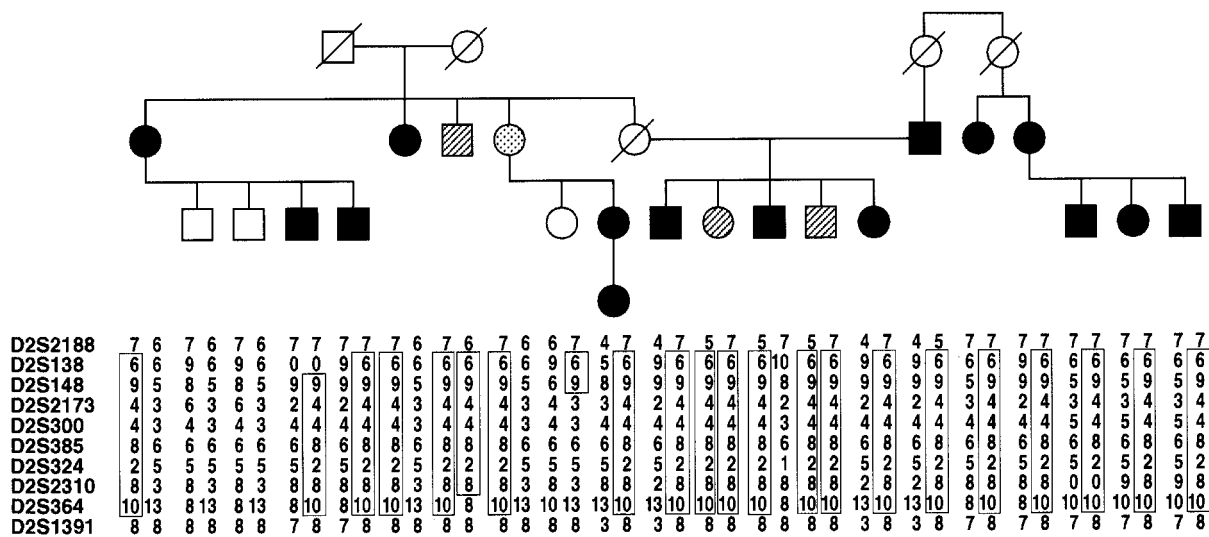In the highly inbred family B, the occurrence of the

Figure 3 Pedigree of family B, showing chromosome 2q haplotypes. The common core haplotype shared with family A is boxed. Hatched symbols indicate individuals with the more severe, LGMD phenotype. The dotted symbol indicates an individual with an unknown phenotype. In marker D2S2173, allele 4 probably represents an old mutated version of allele 3. The major alleles of the markers are as follows: D2S2188 allele 7; D2S138 allele 6; D2S148 allele 9; D2S2173 allele 3; D2S300 allele 4; D2S385 allele 6; D2S324 alleles 1 and 5; D2S2310 alleles 3 and 8; D2S364 alleles 2 and 13; and D2S1391 alleles 5, 7, and 8. Each of these alleles was present in $\geqslant 25\%$ of healthy chromosomes.
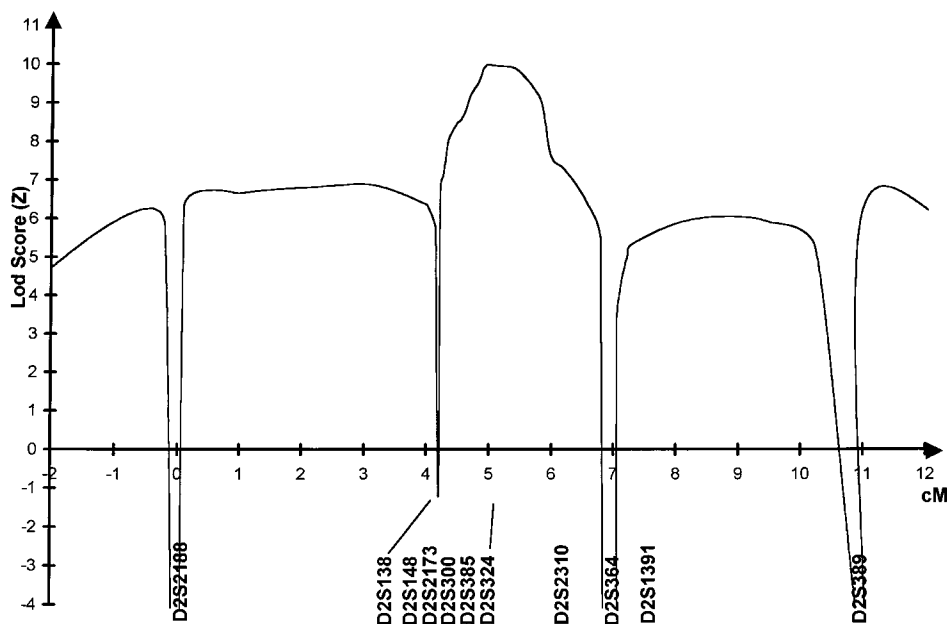
Figure 4 Multipoint linkage analysis of the TMD locus, against a fixed set of markers. The markers used are shown below the horizontal axis. The intermarker distances and the order between the markers indicated as a cluster are not well established. The order indicated here is most compatible with the haplotypes analyzed. Distances are indicated in centimorgans (cM).

more severe, LGMD phenotype together with the milder TMD phenotype is not a unique event in families with muscular dystrophy. Two families have been described with a distal myopathy and LGMD phenotypes, which segregate in an autosomal recessive fashion. Linkage analyses of these families revealed a chromosome 2p locus that harbors both the autosomal recessive LGMD locus (LGMD2B) and the Miyoshi myopathy locus. It was suggested that the different disease phenotypes resulted from the same disease-causing mutation but that the phenotype was additionally influenced by other genetic or environmental factors (Illarioshkin et al. 1996; Weiler et al. 1996). In family B, the two phenotypes also are linked to the same locus, and the most severe LGMD phenotype seems to be associated with homozygosity of the disease allele. This could be caused by inbreeding, and the LGMD phenotype could result from the presence of another recessive muscular dystrophy locus cosegregating in the family. Alternatively, a secondary gene may directly influence disease severity, as was described recently for a mouse model of cystic fibrosis (Rozmahel et al. 1996).

Our findings provide the basis for further attempts to identify the TMD gene. The relatively well restricted critical chromosomal region on 2q31 contains good candidate genes, including a gene for titin, a giant structural protein of muscle (Labeit and Kolmerer 1995). In ad-

dition, two genes encoding collagen chains (COL3A1 and COL5A2), expressed in the endo- and perimysium of the muscle, were initially assigned to 2q32 (Collins et al. 1996). The titin is claimed to contribute to the myofibrillar assembly and elasticity of striated muscle (Labeit and Kolmerer 1995). Furthermore, titin is composed of slightly different domains in different types of skeletal and heart muscle, owing to alternative splicing (Labeit and Kolmerer 1995; Kolmerer et al. 1996; Sorimachi et al. 1997). The tissue-specific expression of the differentially spliced titin transcripts could explain the selective muscular affection pattern of TMD. Consequently, the titin gene could be an excellent candidate gene for TMD.

Our further studies are focused on the structural analyses of this gigantic (100-kb cDNA) gene, as well as on other candidate genes in the critical chromosomal region. Also, novel positional candidate genes will be identified via the construction of the complete transcript map. These efforts eventually should result in the characterization of the gene defect causing TMD.

Acknowledgments

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