Linkage of Thomsen Disease to the T-Cell-Receptor Beta (TCRB) Locus on Chromosome 7q35

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Summary

The chromosomal localization of the gene for Thomsen disease, an autosomal dominant form of myotonia congenita, is unknown. Electrophysiologic data in Thomsen disease point to defects in muscle-membrane ion-channel function. A mouse model of myotonia congenita appears to result from transposon inactivation of a muscle chloride-channel gene which maps to a region of mouse chromosome 6. The linkage group containing this gene includes several loci which have human homologues on human chromosome 7q31-35 (synteny), and this is a candidate region for the Thomsen disease locus. Linkage analysis of Thomsen disease to the T-cell-receptor beta (TCRB) locus at 7q35 was carried out in four pedigrees (25 affected and 23 unaffected individuals) by using a PCR-based dinucleotide repeat polymorphism in the TCRB gene. Two-point linkage analysis between Thomsen disease and TCRB showed a maximum cumulative lod score of 3.963 at a recombination fraction of .10 (1-lod support interval .048–.275). We conclude that the Thomsen disease locus is linked to the TCRB locus in these families.

Introduction

Thomsen disease is one of several recognized inherited conditions featuring myotonia, a disorder of delayed muscle relaxation (Streib 1987). Transmission is autosomal dominant, and the disease typically presents in the first decade, with painless muscular stiffness that commences with initiation of movement and that slowly resolves with continued activity. Myotonia may be aggravated by prolonged rest and emotional stress. Strength is not impaired, and the only findings on neurological examination are muscle hypertrophy and myotonia. The latter is elicitable on muscle percussion or on action. A recessive variety of myotonia congenita (RGM [recessive generalized myotonia, or Becker-type myotonia]) has also been described, in which the myotonia is generally more severe and may be accompanied by other features (Brooke 1986).

Published family studies in Thomsen disease, for both random (Bender et al. 1989) and candidate loci, have not found linkage. Candidate loci have included the DM locus on 19q13.3, which is the site of mutation in myotonic dystrophy (Koch et al. 1989; Ropers and Pericak-Vance 1991) and SCN4A on 17q23-25, which is the site of an adult skeletal muscle–membrane sodium-channel gene (Abdalla et al., in press). Defects in the latter gene appear to cause hyperkalemic periodic paralaysis and parmyotonia congenita (Fontaine et al. 1990; Ebers et al. 1991; Koch et al. 1991a, 1991b; Ptacek et al. 1991a, 1991b, 1991c; Rojas et al. 1991; McClatchey et al. 1992). Finally, the CFTR (cystic fibrosis transmembrane regulator) gene on 7q31, which exhibits chloride-channel properties in nonexcitable tissues (Riordan et al. 1989), has also been found to be unlinked (Abdalla et al., in press).

Nevertheless, much evidence supports defects in ion-channel function in myotonic patients. Lipicky and Bryant (1973) found a significant reduction in sarcolemmal chloride conductance in intercostal muscle biopsies. Further studies confirmed that myotonic discharges could result from reduced muscle-membrane chloride conductance. It has been possible to

reproduce myotonic behavior in normal skeletal muscle fibers by substituting an impermeable anion for chloride or by using chloride channel–blocking compounds (Furman and Barchi 1978). In addition to having abnormal chloride channel properties, muscle from some myotonic patients exhibits reduced potassium conductance (Lipicky and Bryant 1973). Also, abnormalities of the fast sodium current have been noted in recessive generalized myotonia, in myotonic dystrophy, and in hyperkalemic periodic paralysis (Rudel et al. 1989). Data supporting the abnormal chloride–conductance hypothesis have been more convincing for RGM than for Thomsen disease. Recent studies (Iaizzo et al. 1991) implicate sodium-channel dysfunction as the primary defect in Thomsen disease.

The demonstration of abnormalities in the function of several ion channels has raised some interesting pathophysiologic hypotheses beyond that of a single primary ion-channel defect. These have included the possibilities of genetic heterogeneity or of a genetic defect in a more general factor modifying ion-channel function (Rudel et al. 1989).

We initially considered the hypothesis of localization of the myotonia congenita gene to the telomeric portion of chromosome 7q, after noting that the gene responsible for a murine myotonia model (ADR mouse) was mapped to mouse chromosome 6 (Rudel 1990) and that, between mouse and human (chromosome 7q31-35) there existed synteny of a large segment (from COLA to TCRB [T-cell-receptor beta]) of this chromosome (fig. 1). Steinmeyer et al. (1991) have recently identified the gene responsible for the ADR phenotype in mice: a muscle-membrane chloride channel which harbors a transposon insertion which is believed to alter chloride-channel function in animals homozygous for this insertion. The candidacy of a similar locus in human Thomsen disease is supported by electrophysiologic data implicating chloride-channel dysfunction in Thomsen disease patients. The ADR locus has been localized to mouse chromosome 6, between the locus for TCRB and the locus for HOX1.

We now demonstrate linkage of Thomsen disease to the human TCRB gene locus on chromosome 7q35 in four families. To do so, we employ a highly polymorphic dinucleotide repeat polymorphism within the TCRB gene (F. C. Cornélis, unpublished data).

Subjects and Methods

Pedigrees and Diagnosis

Four pedigrees were evaluated, (figs. 2–5), including two which were studied by our group for linkage
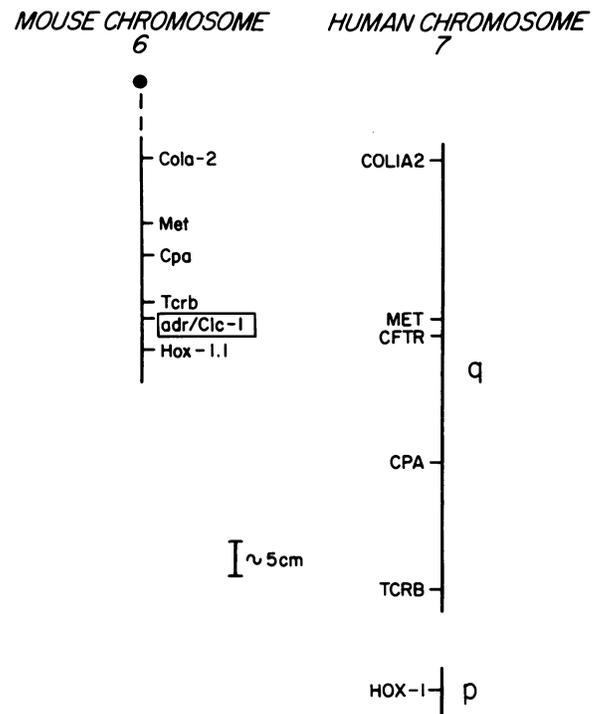

Figure I   Comparison of homologous regions of mouse chromosome 6 and human chromosome 7 (modified after Steinmeyer et al. 1991).

to SCN4A (17q23-25) and to CFTR (7q31). Diagnosis was established by history and examination, with electromyography (EMG) used only in rare instances. Diagnostic criteria for Thomsen disease included a history of episodic stiffness which is precipitated by sudden movement, particularly after a period of rest, and which subsides with continued activity; lack of significant effect of cold on the presence of stiffness; lack of relation to ingestion of potassium-rich foods; absence of periodic paralysis or, indeed, of any weakness; presence of some degree of muscle hypertrophy; presence of percussion or action myotonia on exam and/or EMG evidence of myotonia; and autosomal dominant inheritance pattern (Brooke 1986; Streib 1987). All affected individuals from the four Thomsen disease families met these criteria. For the purpose of linkage analysis, the status of deceased members in pedigrees 1 and 4 was inferred from historical data from family members, and no individual under the age of 5 years was included in the analysis. Presumed recombinant individuals underwent EMG testing. The pedigree diagrams in figures 2–5 are consistent with an autosomal dominant pattern of inheritance with complete penetrance.
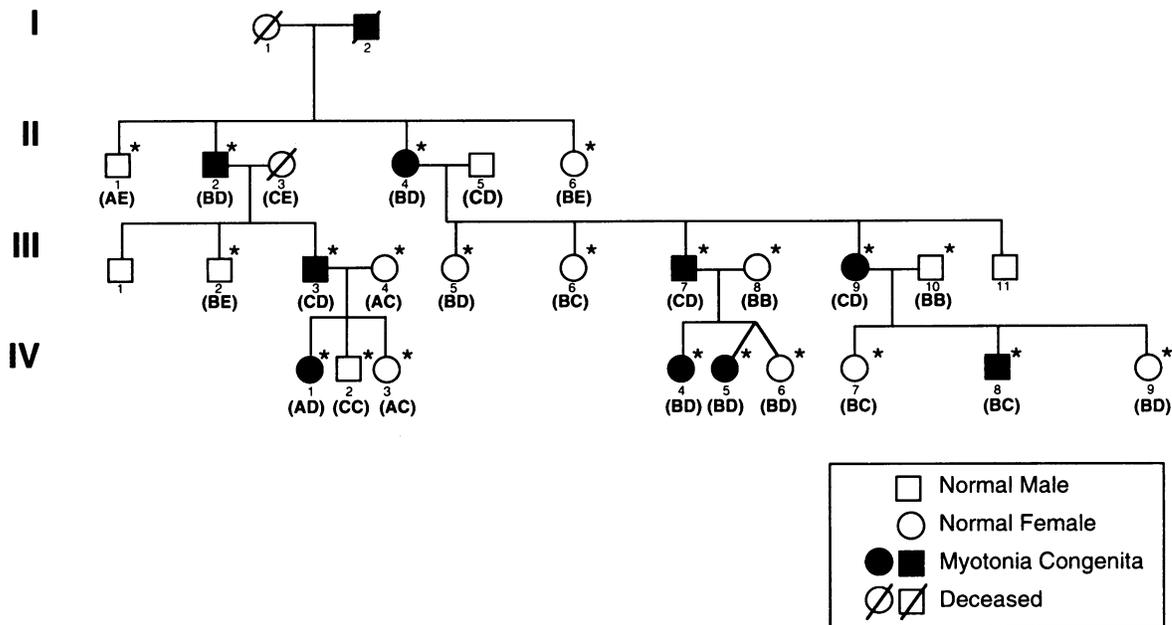
Figure 2   Pedigree 1. An asterisk (*) indicates an individual who was examined by one of the authors and whose blood was collected for DNA analysis. TCRB alleles are listed under each individual. Genotypes of individuals I-1, I-2, and II-3 are derived. Allele A = 240 bp; allele B = 238 bp; allele C = 236 bp; allele D = 234 bp; and allele E = 224 bp.

DNA Analysis

To detect the dinucleotide-repeat sequence polymorphism in the TCRB locus on 7q35 (F. C. Cornélis, unpublished data), genomic DNA was extracted from EDTA-anticoagulated blood of affected and unaffected family members according to a standard protocol (Madisen et al. 1987). Primers were chosen from
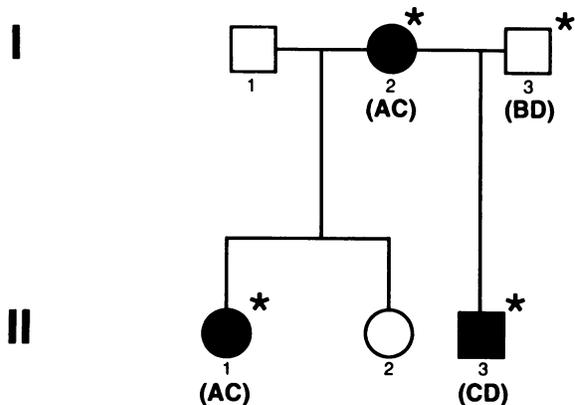

Figure 4   Pedigree 3. An asterisk (*) indicates that the individual was studied. TCRB alleles are listed under each individual. Allele A = 240 bp; allele B = 238 bp; allele C = 236 bp; allele D = 228 bp; allele E = 224 bp; and allele F = 222 bp. Symbols are as in fig. 2.

Figure 3   Pedigree 2. An asterisk (*) indicates that the individual was studied. TCRB alleles are listed under each individual. Allele A = 240 bp; allele B = 236 bp; allele C = 234 bp; and allele D = 224 bp. Symbols are as in fig. 2.
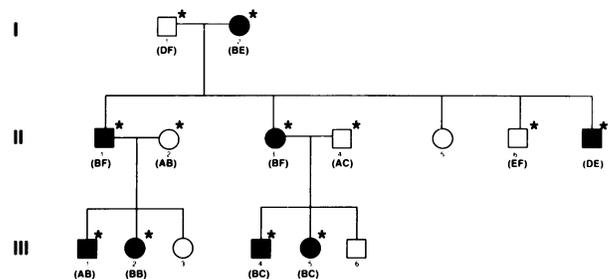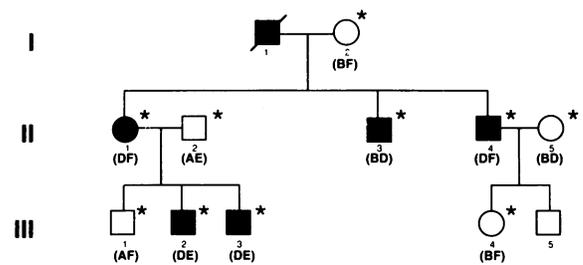

Figure 5   Pedigree 4. An asterisk (*) indicates that the individual was studied. TCRB alleles are listed under each individual. Allele A = 240 bp; allele B = 238 bp; allele C = 236 bp; allele D = 234 bp; allele E = 224 bp; and allele F = 220 bp. Symbols are as in fig. 2.

a previously published sequence for the TCRB gene (Li et al. 1991), in the V beta 6.7 region of the gene. Primer sequences are as follows: Vb 6.7L, TCTTCT-GGGTGGCCTTCTGTCTCC; and Vb 6.7R, GGA-TCACACCTGAGCTCTACATCC. PCR assays were set up according to a standard protocol (Weber and May 1989) with some modifications. Each 50-μl PCR reaction tube contained 300 ng genomic DNA, 5 μl 10 × reaction buffer (100 mM Tris HCl, 500 mM KCl, 15 mM MgCl₂, pH 8.3 [Boehringer-Mannheim] at 20°C), 200 μM of each dNTP, 0.5 μM of each primer, and 2 units of *Taq* polymerase (Boehringer-Mannheim). One primer was 5′-end labeled with ^{32}P by using polynucleotide kinase (Pharmacia) (Sambrook et al. 1989, pp. 11.31–11.32). One microliter of tetramethylammonium chloride (TMAC) was added to enhance specificity of amplification (Hung et al. 1990). Thirty-one cycles of PCR were performed on a Hybaid thermal cycler as follows: denaturation at 94°C for 60 s (except for the first cycle, for which denaturation was extended to 5 min), annealing at 60°C for 60 s, and extension at 72°C for 60 s. PCR products were electrophoresed through a 6% denaturing polyacrylamide gel, transferred to Whatman 3MM paper, and exposed to X-ray film for 16–24 h.

The presence of a polymorphism in the size of PCR products had been previously determined in normal controls. It showed at least 10 alleles of size 254–277 bp, with a heterozygosity index of 90% (F. C. Cornélis, unpublished data; H. K. Cousin and L. Hashimoto, unpublished data) (fig. 6).

Linkage Analysis

Two blinded investigators independently scored the autoradiographs, with complete concordance of allele
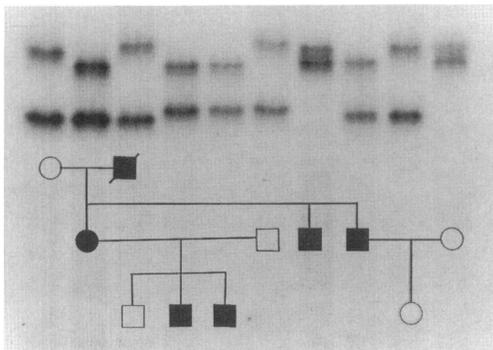

Figure 6 Autoradiograph showing the TCRB polymorphism in pedigree 4. Individuals shown are I-2 (lane 1), II-1 (lane 2), III-1 (lane 3), III-2 (lane 4), III-3 (lane 5), II-2 (lane 6), II-3 (lane 7), II-4 (lane 8), III-4 (lane 9), and II-5 (lane 10).

assignments (fig. 6). Linkage of Thomsen disease to the TCRB polymorphism was assessed with the LIPED computer program (Ott 1974). To permit analysis, the multiallelic DNR was coded as a tetraallelic locus. This was done by arbitrarily designating alleles by letter name and then converting all but the three most informative alleles to a fourth letter (these were in most cases alleles introduced into the families by unrelated spouses). We attributed frequencies of .1 to each of the first three alleles and .7 to the last. The frequency of the Thomsen disease allele was specified at .0001, the lowest allowed in this program. The model of inheritance used for linkage was autosomal dominant with complete penetrance. Phenotypes for the TCRB locus of deceased individuals were easily inferred by genotyping their children. Two-point lod scores were obtained at recombination fractions (RF) values of 0–.5, and the 1-lod support interval was determined (Conneally et al. 1985; Ott 1991).

Results

Between the TCRB locus and the disease phenotype, we found only 3 recombination events (individuals IV-6, IV-8, and IV-9) among 18 informative meioses in pedigree 1, 1 (individual II-7) among 7 in pedigree 3, and 0 among 6 in pedigree 4. Pedigree 2 proved to be uninformative for this marker. Of the four recombinant individuals, two had unambiguous clinical signs of myotonia, and two were unaffected. EMGs in the recombinant individuals were concordant with the clinical impression of the examiner. The lod scores obtained are shown in table 1. The highest cumulative lod score was obtained at an RF of .10. The sum of the lod scores in these families at this RF is 3.963. The 1-lod support interval is 0.048–0.275.

Discussion

The finding of a lod score of 3.963 at RF .1 (range .048–.275) indicates that the Thomsen locus likely lies within an interval of approximately 4.8–27.5 cM from TCRB, either telomeric or centromeric. Studies are underway in our laboratory to clarify this by using markers which flank TCRB.

Certain assumptions were made in this study: the true frequency of the disease allele is unknown but, in our population approaches, .0001. In our experience with this condition, penetrance is complete, and there are in the literature no reports of incomplete penetrance or late-onset cases. Previous linkage studies in Thomsen disease have also assumed complete pene-

Table I

Two-Point Lod Scores between Thomsen Disease Locus and TCRB Locus

	LOD SCORES			
	Pedigree			
RF[a]	1	3	4	Total
.450	.39	.17	.18	.73
.400	.75	.32	.37	1.44
.350	1.09	.45	.56	2.10
.300	1.39	.57	.75	2.71
.250	1.62	.68	.95	3.24
.200	1.77	.76	1.13	3.66
.150	1.82	.80	1.31	3.93
.100	1.69	.79	1.49	3.96
.050	1.16	.65	1.65	3.46
.001	− 3.59	− .90	1.80	− 2.68
.000	∞	∞	1.81	∞

[a] Peak RF .10 (range .048–.275).

trance (Koch et al. 1989). The frequencies of the TCRB alleles have been determined elsewhere (F. C. Cornélis, unpublished data; H. K. Cousin and L. Hashimoto, unpublished data). The process of conversion of a multiallelic locus to a tetraallelic locus, if anything, might lead to underestimation of the degree of linkage but would not overestimate linkage. Finally, mistaken paternity is very unlikely, in light of the segregation of the TCRB marker and other highly polymorphic markers tested on these same pedigrees in previous studies (Abdalla et al., in press).

It was the finding of homology between mouse and human chromosomal regions which led us initially to consider 7q31-35 as the site of the Thomsen disease gene, although there were no known candidate genes in this region. It is tempting to postulate at this point that a human muscle-membrane chloride-channel gene lies approximately 10 cM from TCRB and that a mutation(s) therein is (are) responsible for Thomsen disease and perhaps other forms of myotonia. This has also been suggested by Steinmeyer et al. (1991). Further studies will be required to pinpoint the responsible gene(s) and their product(s). The possibility of heterogeneity in autosomal dominant myotonia congenita remains a consideration, since the number of families studied thus far is relatively small.

Acknowledgments

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