

LETTER TO JMG

Genetic linkage of a novel autosomal dominant restrictive cardiomyopathy locus

J Zhang, A Kumar, L Kaplan, F J Fricker, M R Wallace

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Background: In recent years, non-syndromic idiopathic cardiomyopathies have increasingly been characterised as autosomal dominant conditions caused by single gene mutations. Loci have been identified for hypertrophic and dilated cardiomyopathy, and in some cases the same loci are associated with restrictive cardiomyopathy (RCM). In a kindred with RCM that we previously reported, we ruled out the known cardiomyopathy loci and other candidate genes by linkage analysis and mutation screening.

Methods and Results: Here we report a genome-wide analysis in this family that has resulted in linkage to a region on chromosome 10.

Conclusions: There are no genes in the interval that are known to cause idiopathic cardiomyopathy, and thus this linkage represents localisation of a new RCM locus.

There are three recognised forms of cardiomyopathy, the most common being hypertrophic cardiomyopathy, followed by dilated cardiomyopathy, while the rarest is restrictive cardiomyopathy (RCM). In recent years, Mendelian modes of inheritance in families affected with primary idiopathic cardiomyopathy have been observed and are very commonly autosomal dominant. We now know that these different cardiomyopathy phenotypes may be caused by mutations in the same gene, or even the same mutation within a family, although typically the phenotype holds true within each family.¹ The first such disease gene discovered was a missense mutation in the beta cardiac myosin heavy chain gene in 1990, and since then there have been discoveries of mutations in at least 23 genes (typically those encoding proteins of the sarcomere, Z-disk associated proteins, titin and related proteins, cytoskeletal proteins, and intermediate filaments).^{2–5} There have been a few reports of mutations in RCM, implicating the desmin and cardiac troponin I genes.^{1–6} In the RCM family described in this study, these and other known cardiomyopathy genes were previously ruled out by mutation analysis or linkage analysis.⁷ Thus, we undertook a genome-wide linkage analysis to localise the gene causing the autosomal dominant RCM seen in this family.

METHODS

Clinical data and samples

As previously described, samples from the kindred were obtained with an Institutional Review Board approved protocol, and included 13 from known affected individuals and 13 from at risk or married in individuals.⁷ DNA was extracted from leukocytes using standard methods. Patients had no other features (such as skeletal myopathy) besides cardiomyopathy, although heart biopsies from four individuals showed that this condition was a desminopathy.⁷ Regarding the cardiomyopathy, ages of onset (at first

diagnosis) ranged from 5 to 55 years, with seemingly earlier onset in successive generations. Data from cardiac catheterisation (including ventricular and atrial pressures), electrocardiogram (EKG), echocardiogram, and physical examination were consistent with RCM diagnosis in the 13 individuals designated as affected. Among the clinical findings were dyspnea, anasarca, venous hypertension, atrial flutter/fibrillation, ST changes in EKG, atrial enlargement, and right ventricular conduction delay. Four family members have had heart transplants. A few other family members had possible early symptoms but not sufficient findings to allow a diagnosis of cardiomyopathy, and had phenotype labelled “unknown” for the purposes of linkage analysis, so as to be as stringent as possible.⁷

Genotyping and linkage analysis

As previously described, genotyping of microsatellite markers of all family members was accomplished with PCR using an end labelled primer (P-32), sequencing gel electrophoresis, and analysis of autoradiograms.⁷ We began the genome-wide linkage screen using Research Genetics' Human MapPairs version 8, and added more microsatellite markers as needed to further screen putative positive regions. Two point linkage analysis employed the LINKAGE software package, with autosomal dominant mode of inheritance, equal allele frequencies for markers, mutant allele frequency of 0.0001, full penetrance, and no age of onset curve (simplest model). To avoid false positive results, uncertain phenotypes were scored as unknown to ensure the most conservative analysis of the data. This analysis yielded two point LOD (log of odds) scores, and the MLINK program in this package was used for the three point linkage analysis.

RESULTS

We genotyped 430 microsatellite markers across the genome in this family. Regions that could not be eliminated by negative linkage were further studied with a denser selection of markers to obtain a conclusive answer. No two point LOD scores reached a score of 3.0 (considered accepted positive linkage) due to lack of informativeness in parts of the pedigree, although the highest score was 2.436 at $\theta = 0$ for marker D10S1242 (table 1). $\theta = 0$ indicates no recombination between the segregation of the marker and the disease allele. Table 1 shows the maximum two point LOD scores obtained from this area, and the corresponding maximum θ (estimated genetic distance between marker and disease gene).

Only three other markers in the genome yielded a LOD score above 1.2 (on different chromosomes), but linkage was ruled out via analysis of closer, flanking markers in those areas. Pursuing the chromosome 10 lead from the two point LOD scores, we developed additional markers from bacterial

Abbreviations: EKG, electrocardiogram; RCM, restrictive cardiomyopathy

Table 1 Two point LOD scores for RCM

Marker (D10S)	Max LOD score	Max θ
541	0.967	0.089
1739	0.829	0.083
1242	2.436	0
2542	2.052	0
1753	1.474	0
2540	2.397	0
2539	0.971	0
2541	1.085	0
564	1.745	0
1173	0.031	0.411
583	0.869	0.145
185	0.694	0.193

Table 2 Three point LOD scores ($\theta=0$)

Marker combination v RCM	Max LOD
1242+2542	4.01
2542+1753	3.00
1753+2539	3.37
2539+2541	3.21
2541+2540	2.03
2540+564	2.82

10 000:1. This region spans 3.2 Mb on chromosome 10, and is considered to be at the cytogenetic band 10q23.3.

artificial chromosome derived human genome sequence in the non-recombinant interval (spanning D10S1242), using Repeat Masker software (see <http://repeatmasker.genome.washington.edu>). We characterised three new CA-repeat polymorphisms and a TATG tetranucleotide repeat (D numbers D10S2539, D10S2540, D10S2541, and D10S2542, respectively) (primers listed in the Genome Database, www.gdb.org) within 200 kb of D10S1242. Unfortunately, these new markers were also uninformative in some key individuals and thus did not yield any larger two point LOD scores. However, multipoint analysis of adjacent pairs of markers, including these, yielded more useful information. Using three point analysis, a maximum LOD score of 4.01 at $\theta=0$ (no recombination) was obtained for marker combination D10S1242+D10S2542, indicating linkage to this region in an interval between nearest flanking recombinant markers D10S1739 and D10S1173 (table 2, fig 1) at odds greater than

DISCUSSION

The linkage data indicate that a gene in the non-recombinant region contains a mutation leading to RCM in this family. Cardiac related genes originally known to be in the general vicinity (ACTA2, FER1L3, LBX1, VCL, and LDB3) were initially interesting candidates before the region was fully mapped and sequenced, which subsequently showed that they lie outside the interval. The closest of these were ACTA2 (200 kb centromeric of the interval) and FER1L3 (60 kb telomeric). Despite the small non-recombinant interval (given that it is a single family analysis), current genome analysis (NCBI build 35.1) indicates that 25 transcripts lie in this interval, 21 of which are named genes. Thus, this is a gene-rich region and future investigation and mutation screening will be needed to prioritise and identify the disease causing gene. The region does not contain any genes known to be associated with dominant cardiomyopathy, but has some possible interesting candidates at first glance. One is

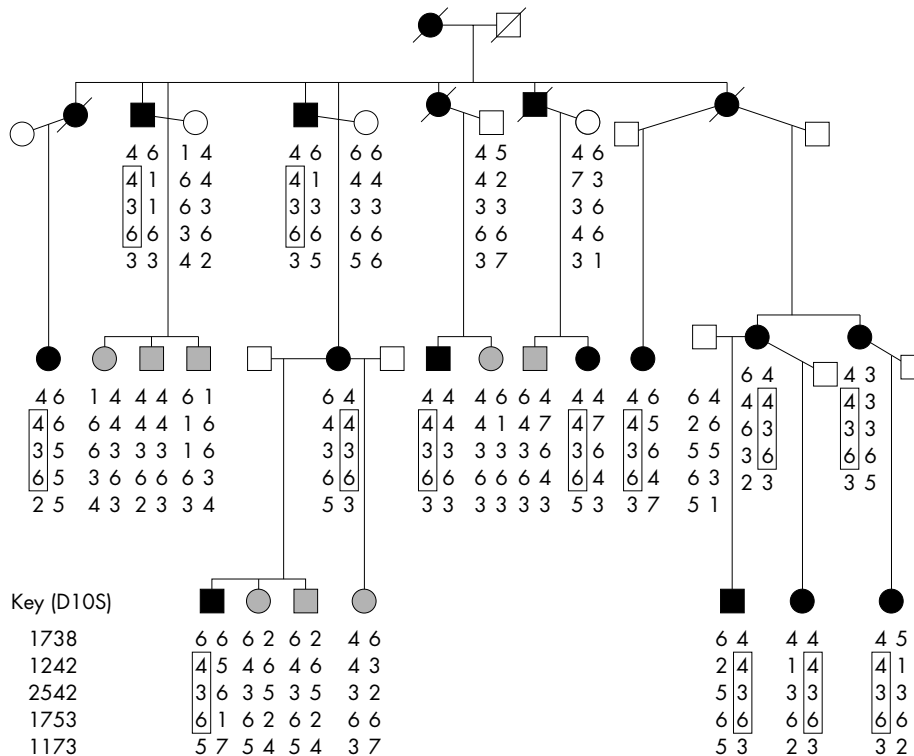


Figure 1 Pedigree of four generation family with restrictive cardiomyopathy. Black filled symbols represent individuals confirmed to have the condition. Shaded symbols indicate individuals at risk for the condition, but with insufficient data or symptoms to be definitive about diagnosis. Open symbols are unaffected individuals married into the family. Genotypes at each marker are listed below each individual according to the key. Haplotypes were inferred to minimise recombination events, and are suggested by alleles shown vertically for each individual. The haplotype that is segregating with the condition is boxed.

the ankyrin repeat domain gene, encoding a protein thought to aid in signalling between the sarcomere and nucleus.⁸ In addition, several metabolic enzymes (for example, lipase A, cholesterol 25-hydroxylase), a family of interferon induced proteins, and a number of putative transcription factors and phosphatases (or regulatory units of phosphatases) also lie in this region. The latter group may be very interesting in light of the fact that desminopathies represent accumulation of the phosphorylated form of desmin.⁹ Furthermore, other phosphorylation abnormalities have been implicated in cardiomyopathy in several pathways.^{10–13} The identification of this new RCM gene will ultimately shed light on inherited restrictive cardiomyopathy and possibly other heart conditions.

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ELECTRONIC-DATABASE INFORMATION



The GDB Human Genome Database is at www.gdb.org. Repeat Masker can be found at <http://repeatmasker.genome.washington.edu>.

Authors' affiliations

J Zhang, Department of Neurosurgery, Johns Hopkins University, Baltimore, MD, USA

A Kumar, Children's Heart Center, Indianapolis, IN, USA

L Kaplan, M R Wallace, Department of Molecular Genetics and Microbiology, IDP Graduate Program in Genetics, University of Florida, Gainesville, FL, USA

F J Fricker, Department of Pediatrics, Division of Cardiology, University of Florida, Gainesville, FL, USA

M R Wallace, Departments of Molecular Genetics and Microbiology, and Pediatric Genetics, University of Florida, Gainesville, FL, USA

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Correspondence to: Dr Margaret Wallace, Molecular Genetics, Box 100266, University of Florida, Gainesville, FL 32610–0266, USA; peggyw@ufl.edu

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