Localization of Gene for Familial Hypertrophic Cardiomyopathy to Chromosome 14q1 in a Diverse US Population

J. Fielding Hejtmancik, PhD, MD; Paul A. Brink, MD; Jeffrey Towbin, MD; Rita Hill, BSN; Lucy Brink, MS; Terry Tapscott, BS; Anatole Trakhtenbroit, MD; and Robert Roberts, MD

Background. Familial hypertrophic cardiomyopathy, an inherited primary cardiac abnormality characterized by ventricular hypertrophy, is the leading cause of sudden death in the young. Recent application of restriction fragment length polymorphism markers has provided provocative results, with localization to chromosome 18 (Japanese studies), 16 (Italian studies), 14 (US and French-Canadian studies), and two (National Institutes of Health studies) indicating genetic heterogeneity. Interpretation remains speculative until at least one of these loci is confirmed in unrelated pedigrees by independent investigators.

Methods and Results. We studied eight unrelated families of varied ethnic origins across the United States. DNA from each individual was digested with restriction enzymes TaqI or BamHI and analyzed by Southern blots followed by hybridization with probes T cell receptor α (TCRA), myosin heavy chain β, D14S25, and D14S26. Multipoint linkage analysis showed a maximum lod score of 4.3, placing the locus 10 cm from D14S26 between D14S26 and TCRA, with an odds ratio of 20,000:1 and 90% confidence limits of 12 cM proximal to D14S25 to 4 cM distal to TCRA. The probability of linkage to 14q1 was more than 99%.

Conclusions. These results indicate that the loci for familial hypertrophic cardiomyopathy in our families is primarily 14q1 but does not exclude other loci in a small proportion of the families. Thus, 14q1 appears to be the locus for familial hypertrophic cardiomyopathy in a significant proportion of the US population. (Circulation 1991;83:1592–1597)

Elucidation of the molecular basis for normal and abnormal organ function is being accelerated as a result of the application of the techniques of molecular genetics and linkage analysis. Application of these techniques to cardiac disorders has been sparse until recent investigation of familial hypertrophic cardiomyopathy (FHCM). FHCM, an inherited primary cardiac abnormality characterized by ventricular hypertrophy, is the leading cause of sudden death in the young.1,2 This disorder has undergone exhaustive clinical, hemodynamic, and pathological study that has contributed significantly to our understanding of ventricular function and cardiac failure. Compared with most instances of cardiac hypertrophy, hypertrophy of the cardiac muscle in hypertrophic cardiomyopathy (HCM) appears to be primary rather than secondary to increased workload. It is also characterized by impaired diastolic relaxation, a feature of cardiac failure from other causes.3 This suggests that the underlying genetic defect is fundamental to cardiac growth and sarcomere assembly; thus, FHCM may serve as an excellent paradigm for the study of molecular mechanisms underlying cardiac repair, growth, and hypertrophy. Clinical studies4 confirm autosomal dominant inheritance, but the presence and severity of signs and symptoms vary markedly, tending to increase with age.

The development of DNA markers recognizing restriction fragment length polymorphisms (RFLPs) spanning the human genome has provided the geneticist with a method of mapping the location of genes previously unavailable. Random changes in the DNA
sequence, which occur every 100–500 base pairs in the human genome, can result in an altered pattern produced on a Southern blot by hybridizing with a cloned probe homologous to adjacent DNA sequences. Each of these patterns is inherited in an autosomal codominant fashion so that the presence or absence of a recognition site (i.e., each allele of the RFLP) can be unambiguously determined, and its inheritance from parent to child can be followed in families. If particular alleles of an RFLP tend to be coinherited with a disease with a more-than-random frequency within a family, the disease is said to be linked to the marker. It is important to note that this does not mean that a particular allele of the RFLP will be associated with the disease in the general population. Genetic linkage of two loci reflects close physical proximity on the same chromosome, with coinheritance varying from 100% for contiguous DNA segments to 50% for loci that are distant or on separate chromosomes (i.e., unlinked). The percentage of meioses demonstrating lack of coinheritance (i.e., showing recombination between the marker and disease) is called the recombination fraction and designated by \( \theta \).

Because an RFLP allele and a disease could be coinherited by chance even if they are not linked, powerful statistical tests have been developed to determine whether apparent genetic linkage is real. In general, this is carried out by calculating the likelihood that the observed coinheritance would occur if the marker and disease were linked with a particular recombination frequency (\( \theta \)) as well as the likelihood that this degree of coinheritance would occur if they were in reality unlinked (i.e., the recombination frequency is 0.5 so that random assortment holds). The ratio of these likelihoods is called the odds of linkage, and the logarithm of this value is called the lod score, frequently abbreviated as \( Z \). The lod score can be calculated at a variety of recombination fractions. The recombination fraction with the highest lod score represents the most likely true value of \( \theta \) and is called the maximum likelihood estimate of \( \theta \), designated as \( \hat{\theta} \). The lod score at this \( \hat{\theta} \) is abbreviated \( Z \). In general, a lod score of 3 (representing a likelihood ratio of 1,000:1 in favor of linkage) is considered significant. Because of the initially high odds against linkage of two random markers in the human genome, this actually represents about a 95% probability of linkage. Maximum likelihood estimation can be applied simultaneously to several markers that are known to be linked to each other, a process called multipoint linkage analysis. The amount of information obtained from multipoint analysis can be severalfold that obtained from two-point analysis.

When mutations at two or more different genes (possibly at widely separated genetic locations) can cause a clinically identical disease, genetic heterogeneity is said to exist. As might be expected, this complicates linkage analysis. The major difficulty is that the presence of families with an unlinked form of a disease can obscure linkage in a subset of families in whom the disease is caused by mutations in a linked gene. Multipoint analysis is especially sensitive to this problem. Genetic heterogeneity can, however, be taken into account by using maximum likelihood estimation to determine the fraction of families linked, referred to as \( \alpha \). These results are necessarily given in probabilistic terms, reflecting the statistical nature of the analysis.

The molecular genetics of FHCM are beginning to be elucidated. Initial suggestions of linkage of HCM to the human leukocyte (HLA) region have not been confirmed by subsequent studies. In the past year, application of linkage analysis using RFLP markers to determine the locus of the gene for FHCM has provided exciting and provocative results. Summarizing recent results, linkage to prealbumin on chromosome 18 has been suggested by Nishi et al. In an Italian study in which more indirect methods were used, the investigators suggested that the gene for FHCM was located on chromosome 16. In a recent study of one large French-Canadian family, Jarcho et al. documented linkage in 14q1 with a lod score of 9.37, whereas Solomon et al. documented that other families are not linked to this locus. Investigators at the National Institutes of Health studied a large number of individuals from several families and reported the locus in at least one of these families to be on chromosome 2q. These results suggest genetic heterogeneity. Because of the varied phenotypic expression of the clinical features of this disease, clinicians have for some time expected the disease to be heterogeneous in its etiology and pathogenesis. However, until there is confirmation of at least one of these loci in unrelated pedigrees by independent investigations, definitive interpretation remains speculative. In the present study, we studied eight unrelated families of varied ethnic origins from across the United States with autosomal dominant inheritance of the gene for FHCM. The families under study showed linkage to markers in 14q1 with no statistical evidence for genetic heterogeneity, although this possibility could not be excluded. This confirms the linkage of FHCM to chromosome 14q1 and suggests that a gene in this region might be a frequent cause of FHCM in the US population.

**Methods**

**Patient Studies**

Eight families identified through an affected family member underwent detailed cardiovascular examination and two-dimensional echocardiography. Some family members were deceased, and clinical status was determined by medical records. The echocardiographic criterion for being affected was the presence of septal or ventricular hypertrophy as determined by wall thickness of 13 mm or more in the absence of other potential causes such as hypertension. In pediatric patients, the criterion was adjusted for age and weight. We did not include anyone less than 10
years old or more than 60 years old or individuals in whom hypertension or other potential causes of the hypertrophy existed.

**Genetic Marker Studies**

EDTA and ACD or heparinized blood samples were collected from all affected individuals, their spouses, and, where available, all first-degree relatives. The EDTA samples were analyzed for polymorphisms in 16 red cell antigen, enzyme, and plasma proteins by the Genetic Marker Laboratory, University of Texas Health Science Center, Houston. Lymphoblastoid cell lines were established from ACD and heparinized blood samples, and DNA was prepared from cell lines and any excess ACD and heparinized blood samples using a nucleic acid extractor (model 340A, Applied Biosystems, Foster City, Calif.). Restriction digests of genomic DNA were essentially performed as previously described. DNA samples (5 μg) were digested with a threefold excess of restriction enzyme for at least 4 hours, following the recommendations of the supplier. DNA fragments were separated by agarose gel electrophoresis in TEA buffer, alkali denatured, and transferred to nylon membranes (Zeta Probe, Bio-Rad, Cambridge, Mass.) using capillary transfer. DNA probes were labeled to more than 10⁶ cpm/μg with [³²P]dCTP (du Pont, Wilmington, Del.) by random oligonucleotide priming and hybridized to the membrane-bound genomic DNA using a modified Church-Gilbert method. After 12–24 hours, the filters were washed with 40 mM NaPi and 1% SDS at 65°C for 30–60 minutes with at least two changes of buffer and exposed to radiographic film (Kodak XAR-5) with a du Pont Cronex intensifying screen at –80°C for 2–4 days. Membranes were stripped by incubating in 0.1 N NaOH at 42°C for 30 minutes. RFLP alleles were analyzed by three individuals independently and without reference to phenotype or pedigree position, and only results in uniform agreement were accepted.

The T cell receptor α (TCRA) and myosin heavy chain β (MYHβ) probes were kindly provided by Mary Ann Robinson and H.P. Vosberg, respectively. The probes CRI-C436 and CRI-C329 were purchased from Collaborative Research Inc., Bedford, Mass. Allele characteristics and frequencies were taken from the human gene mapping 10⁶ and, for the D14S25 and D14S26 allele frequencies, personal communication from Collaborative Research.

**Data Analysis**

Two-point and multipoint linkage analyses were performed using version 5.03 of the LINKAGE program package on a UTT 386 computer with an 80387 math coprocessor (CH Systems, Houston). Genetic distances separating markers were taken from Mitchell et al., Donis-Keller et al., and Jarcho et al. Because markers used in the present study were separated by several centimorgans, linkage equilibrium was assumed; hence, haplotype frequencies were not used. Equal male and female recombination rates were used as a conservative approach to this analysis. Recombination fractions were converted to genetic distances in centimorgans according to the Kosambi formulation and tested for homogeneity using the HOMOG program of Ott. Ninety percent probability limits for were estimated as recommended by Conneally et al.

Because of the clinical variability of HCM, two types of penetrance (partial penetrance and full penetrance) were assumed in the linkage analysis. The first was an age-related analysis based on our clinical experience and that of other investigators applied uniformly across the different pedigrees. Between 10 and 20 years of age, 16% penetrance was assumed, with a false-positive diagnosis rate of 1%. Between 20 and 40 years of age, 80% penetrance was assumed, with a false-positive diagnosis rate of 2%. Between 40 and 60 years of age, 80% penetrance was assumed, with a false-positive diagnosis rate of 4%. Individuals with marginal diagnoses were coded as unknown. Because of the arbitrary nature of the penetrance curve and the difficulty in deriving an accurate age-related penetrance curve for these families, linkage was also analyzed assuming full penetrance to show that evidence of linkage persists under even the most stringent assumption.

**Results**

Pedigrees of the families studied are shown in Figure 1. All individuals marked as affected met the stringent diagnostic criterion detailed in "Methods.” Individuals not examined are marked as unknown, as are individuals who marginally met or missed the diagnostic criterion. In addition, individuals with diseases that might confound the diagnosis or who belonged in age groups that were considered to obscure the diagnosis are also marked as unknown.

Two-point linkage analysis was performed with a variety of protein and blood group markers and with RFLP markers on chromosomes 6, 17, and 19 and gave no evidence for linkage (data not shown). Analysis with probes of the HLA region (pCH6, 21-hydroxylase, HLA-A2, HLA-B7, DRα, DRβ, DQα, DQβ, and factor 13) excluded linkage to this region. Two-point linkage analysis using markers in the chromosome 14q1 region was suggestive of linkage. The resulting summed lod scores ranged from 2.63 with TCRA to 1.34 with D14S26 (Table 2). Using the age-dependent penetrance curve described in “Methods,” D14S25 and D14S26 showed maximum lod scores at recombination frequencies of 0.03 and 0.05, respectively. Under the arbitrary assumption of complete penetrance and no false-positive diagnoses, single obligate recombinants occurred between HCM and D14S25 in families 101, 112, and 120 and between D14S26 and HCM in family 112. A single obligate recombinant occurred between HCM and MYHβ in family 112 and between TCRA and HCM in family 112. The single obligate recombinant with MYHβ in family 112 is of particular interest.
because MYHβ has been demonstrated to be the cause of HCM in at least two families.22,23 This recombination occurred between individuals III-32 (phenotypically normal female) and several others, most directly 3 (her affected teenage brother); both of these individuals inherited the paternal 3.5 kb allele for the MYHβ RFLP. This individual (III-32) is a normal 10-year-old girl with no symptoms or signs of HCM, and her echocardiographic heart examination is normal. Therefore, this represents inheritance of the same MYHβ allele by an affected as well as an unaffected individual. The possibility of mistaken paternity was excluded on the basis of the analysis of 67 polymorphic markers including 16 serum markers spread across the genome, 45 standard two-allele RFLPs, and six variable number of tandem repeat markers from chromosomes 6, 17, 18, and 19 in our earlier efforts to map HCM. Although the results obtained with MYHβ are somewhat suggestive of heterogeneity, the incomplete and age-related penetrance of familial HCM implies that these results cannot be taken as strong evidence of

![Schematic showing inheritance of familial hypertrophic cardiomyopathy in eight pedigrees (PED) that have been truncated for analysis purposes. Generation number is indicated at left (IV), subject number is above subject, and disease status of each family member is indicated by symbol. Squares denote male members, and circles denote female members. Solid symbols indicate family members affected with hypertrophic cardiomyopathy; open symbols denote unaffected individuals; and hatched symbols indicate members in whom the diagnosis is marginal or who could not be examined. There were eight individuals who could not be examined: three were spouses (101-167, 101-271, and 112-038), and five were blood-related (101-228, 101-264, 112-7, 120-9, and 120-17). Slashes indicate deceased individuals, and arrows denote probands.]

---

**Table 1. Chromosome 14 Markers Used in Study**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Probe</th>
<th>Polymorphism</th>
<th>Size (kb)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCRA</td>
<td>pY1.4</td>
<td>Taq1</td>
<td>7.6/2.1/2.0</td>
<td>0.45/0.54/0.01</td>
</tr>
<tr>
<td>MYHβ</td>
<td>pSC14</td>
<td>BamHI</td>
<td>3.5/1.6</td>
<td>0.68/0.32</td>
</tr>
<tr>
<td>D14S25</td>
<td>CRI-C329</td>
<td>Taq1</td>
<td>12.0/9.5/8.5</td>
<td>0.63/0.01/0.36</td>
</tr>
<tr>
<td>D14S26</td>
<td>CRI-C436</td>
<td>Taq1</td>
<td>4.2/2.2</td>
<td>0.52/0.48</td>
</tr>
</tbody>
</table>

TCRA, T cell receptor α; MYHβ, myosin heavy chain β.

**Table 2. Two-Point Lod Scores for Hypertrophic Cardiomyopathy With Chromosome 14 Markers**

<table>
<thead>
<tr>
<th>Marker</th>
<th>0</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>( \hat{Z} )</th>
<th>( \hat{\theta} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCRA</td>
<td>2.63</td>
<td>2.59</td>
<td>2.41</td>
<td>2.11</td>
<td>1.47</td>
<td>0.86</td>
<td>0.35</td>
<td>2.63</td>
<td>0</td>
</tr>
<tr>
<td>MYHβ</td>
<td>1.46</td>
<td>1.46</td>
<td>1.44</td>
<td>1.34</td>
<td>1.01</td>
<td>0.62</td>
<td>0.28</td>
<td>1.46</td>
<td>0.01</td>
</tr>
<tr>
<td>D14S25</td>
<td>2.15</td>
<td>2.17</td>
<td>2.16</td>
<td>2.01</td>
<td>1.52</td>
<td>0.94</td>
<td>0.40</td>
<td>2.19</td>
<td>0.03</td>
</tr>
<tr>
<td>D14S26</td>
<td>1.15</td>
<td>1.24</td>
<td>1.34</td>
<td>1.31</td>
<td>1.11</td>
<td>0.81</td>
<td>0.44</td>
<td>1.34</td>
<td>0.05</td>
</tr>
</tbody>
</table>

TCRA, T cell receptor α; MYHβ, myosin heavy chain β.
heterogeneity in these families. Although these two-point lod scores do not reach the value of 3 generally accepted as statistically significant, taken with the demonstration by Jarcho et al of linkage of HCM to these markers in a large Canadian family, they encouraged us to carry out multipoint analysis using these markers.

In contrast to the two-point analysis, multipoint analysis of HCM in our pedigrees using the genetic map of D14S25–4 cM–D14S26–24 cM–TCRA demonstrated linkage to chromosome 14 that was statistically significant. The results of this analysis are shown in Figure 2. According to these results, the most likely location for the HCM locus is 10 cM from D14S26 between D14S26 and TCRA with a maximum lod score of 4.3 and a 90% confidence interval ranging from 12 cM proximal to D14S25 to 4 cM distal to TCRA, assuming the age-related penetrance curve described in “Methods.” When the analysis was repeated under the assumption of complete penetrance, the maximum lod score decreased but was still statistically significant. Assuming full penetrance, the maximum lod score, 3.48, occurred 19 cM from D14S26 between D14S26 and TCRA. Because the published marker-to-marker distances were not in precise agreement, the analysis was repeated for D14S26–TCRA map distances ranging from 20 to 28 cM, with statistical significance (lod of more than 3) maintained for all values tested. In addition, the analysis was repeated with the D14S25–D14S26 order inverted, which gave a lod score of 3.99, 22 cM from D14S26 between D14S26 and TCRA.

Because of concern regarding genetic heterogeneity of linkage in HCM, the linkage data were examined on a family-by-family basis using data assuming both age-related penetrance and full penetrance. No evidence of heterogeneity was demonstrated by simple examination of the individual family multipoint lod scores across the D14S25–TCRA region. The lod scores for individual families under the assumption of age-related penetrance at the suggested location for HCM 10 cM distal to D14S26 were 1.3 for family 101, 0.3 for family 103, 0.5 for family 105, 0.01 for family 110, 0.3 for family 112, 0.4 for family 120, 0.034 for family 122, and 1.5 for family 134. As can be seen, not all the families were highly informative in this region. However, no family was excluded from this region (exclusion usually requires lod scores of −2.0). In addition, linkage results were analyzed using the HOMOG program. No evidence for heterogeneity was found with this program, nor was heterogeneity excluded. The fraction of families showing linkage to this region was estimated to be 0.85 with 95% confidence limits of 0.15 and 1.0, the results being weakened by the relatively small size of the families analyzed.

**Figure 2.** Curves of multipoint lod scores of eight pedigrees calculated to define location of gene for familial hypertrophic cardiomyopathy in relation to three linked loci on chromosome 14. Fixed distances between loci recognized by CRI-L329 (D14S25), CRI-L436 (D14S26), and T cell receptor α (TCRA) and their order have been previously described. Using CRI-L436 as the origin, CRI-L329 is 4 cM from CRI-L436 and TCRA is 24 cM from CRI-L436. The lod scores were calculated at 1-cM intervals. Graphs were generated for assumptions of partial penetrance (panel B) and full penetrance (panel A).
Discussion

We have demonstrated linkage of HCM to genetic markers in the chromosome 14q1 region using a group of eight unrelated US families. The results obtained with these families gave no evidence of heterogeneity, although this possibility cannot be excluded. The summed multipoint lod score of 4.3 obtained in the present study corresponds to an odds ratio of almost 20,000:1 favoring linkage, giving a posterior probability of linkage of more than 99%. This research supports the results of the study by Jarch et al, who reported the initial linkage between chromosome 14 markers and HCM in a large Canadian family. Solomon and coworkers analyzed three additional families, finding evidence for linkage to markers on chromosome 14 and identifying causative mutations in MYH 2,22,23. Like Jarch and coworkers, we excluded linkage to the HLA region in our set of families. This has been suggested to be the location of HCM in a group of Japanese families. Additional data suggesting linkage to chromosome 18 and 16 have been reported. We are in the process of examining our families with markers in each of these regions, with special attention to family 112. Although it is not possible to reliably estimate what portion of HCM mutations map to chromosome 14q1 with the present information, our data suggest that a locus in this region might be responsible for a significant fraction of HCM seen by cardiologists in the US population. The multiple loci reported as linked to HCM by other researchers might reflect different genetic loci causing HCM in the specific populations studied, although this should become clearer with time.

Acknowledgments

We wish to acknowledge Dr. Terry Burton of the University of Texas Health Science Center, Houston, for analyzing the serum markers and Dr. Steve Daiger for his assistance with and advice on linkage analysis and preparation of this manuscript. The excellent secretarial assistance of Debra Weaver and Karen Robins is gratefully acknowledged.

References


KEY WORDS • familial hypertrophic cardiomyopathy • DNA • ventricular hypertrophy
Localization of gene for familial hypertrophic cardiomyopathy to chromosome 14q1 in a diverse US population.

J F Hejtmancik, P A Brink, J Towbin, R Hill, L Brink, T Tapscott, A Trakhtenbroit and R Roberts

_Circulation_. 1991;83:1592-1597
doi: 10.1161/01.CIR.83.5.1592

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/83/5/1592

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/