*Proc. Natl. Acad. Sci. USA*
Vol. 90, pp. 6270–6274, July 1993
Genetics

# A familial hypertrophic cardiomyopathy locus maps to chromosome 15q2

LUDWIG THIERFELDER*†, CALUM MACRAE‡§, HUGH WATKINS*§, JAMES TOMFOHRDE¶, MICHELE WILLIAMS¶, WILLIAM MCKENNA§, KATARINA BOHM‖, GERHARD NOESKE**, MARTIN SCHLEPPER**, ANNE BOWCOCK¶, HANS-PETER VOSBERG‖, J. G. SEIDMAN‡, AND CHRISTINE SEIDMAN*††

*Cardiology Division, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115; †Medizinische Universitätsklinik III der Universität Freiburg, D-7800 Freiburg, Germany; ‡Department of Genetics, Harvard Medical School, Boston, MA 02115; §Department of Cardiological Sciences, St. George's Hospital Medical School, London SW17 ORE, United Kingdom; ¶Department of Pediatrics, Southwestern Medical School, Dallas, TX 75235; ‖Max-Planck-Institut fuer Physiologische und Klinische Forschung, D-6350 Bad Nauheim, Germany; and **Kerckhoff Klinik, Abteilung Kardiologie, D-6350 Bad Nauheim, Germany

*Communicated by Eugene Braunwald, April 8, 1993 (received for review January 25, 1993)*

ABSTRACT **We report that a gene responsible for familial hypertrophic cardiomyopathy (FHC) in a kindred with a mild degree of cardiac hypertrophy maps to chromosome 15q2. The gene encoding cardiac actin, located on chromosome 15q, was analyzed and excluded as a candidate for FHC at this locus. Two additional families with typical FHC were studied and the disorder in one also maps to the chromosome 15q2 locus. The maximum combined multipoint logarithm of odds score in the two linked families is 6.02. Although these two kindreds reside in the same country, we believe that their disorder is caused by independent mutations in the 15q2 locus because of the clinical and genotypic differences between affected individuals. Mutations in at least four loci can cause FHC: chromosomes 14q1 (β cardiac myosin heavy chain gene), 1q3, and 15q2 and another unidentified locus, suggesting substantial genetic heterogeneity.**

Cardiac hypertrophy is a complicated pathologic process in which the mass of the heart increases because of cardiac myocyte enlargement. This condition can occur secondary to increased blood pressure or valvular heart diseases or can occur due to a primary genetic defect, termed familial hypertrophic cardiomyopathy (FHC). FHC is an autosomal dominant condition due to mutations in the β cardiac myosin heavy chain (MHC) gene (chromosome 14q1) (1–4), mutations in an unidentified gene on chromosome 1q3 (5), and mutations in at least one other gene. By defining the compendium of mutated genes that cause FHC, we hope to obtain more information about the molecular mechanisms that produce cardiac hypertrophy. To further the process of gene identification, the chromosomal location of all FHC loci must be determined.

Previous linkage analyses of FHC kindreds have involved the study of families where the disorder is ≈100% penetrant and easily diagnosed by echocardiogram and electrocardiogram (1, 4, 6). However, other families exist where the disorder is not highly penetrant and some affected individuals have mild cardiac hypertrophy. Although affected individuals with at least one β cardiac MHC gene mutation (Leu908Val) have this clinically less severe form of the disorder (7), we hypothesized that some of the kindreds with variant clinical presentations of FHC might have mutations in unidentified loci.

Family MZ is a large kindred with FHC. Fifteen members have mild cardiac hypertrophy or are considered affected because they have passed the disorder to at least one offspring. We demonstrate that FHC in family MZ does not map to either chromosome 14q1 or chromosome 1q3. By using short tandem repeat (STR) polymorphisms, a systematic genome search revealed linkage of the disorder to a locus on chromosome 15q2, designated *CMH3*. We have identified a second kindred (MI) with FHC that also maps to *CMH3* in which affected members have profound cardiac hypertrophy. Thus we hypothesize that mutations in the *CMH3* locus can produce a disorder with either mild or severe hypertrophy. Further, since we also have identified an FHC family (D) whose disorder is not linked to chromosome 15q2, chromosome 1q3, or chromosome 14q1, we speculate that there must be at least one other FHC locus.

## MATERIALS AND METHODS

**Clinical Evaluations.** All studies were performed in accordance with the Brigham and Women's Hospital Human Subjects Committee. Family members from three FHC families (MZ, MI, and D) who were age >16 were clinically evaluated by history, physical exam, 12-lead electrocardiogram, and two-dimensional echocardiogram. FHC was diagnosed if left, right, or biventricular cardiac hypertrophy was demonstrable by two-dimensional echocardiography or if the offspring of an affected individual had electrocardiogram abnormalities in the absence of other cardiovascular disease (8–10). Four of five individuals in family MZ (III-6, III-7, IV-4, and IV-10) and one in family MI (II-8) with arterial hypertension were included in the genotypic analysis but clinically scored as individuals with uncertain phenotype. Deceased family members were scored affected if clinical antemortem or autopsy data demonstrated FHC.

**Genotype Analyses.** DNA was extracted from a peripheral blood sample obtained from each family member as described (11). Southern blot analysis of a restriction fragment length polymorphism located within the β cardiac MHC gene (pSC14) was performed as described (11). Polymorphic STR sequences located throughout the genome were amplified using the polymerase chain reaction (PCR) and analyzed as described (11). A dinucleotide-STR polymorphism (*D15S169*) (M.W., J.T., and A.B., unpublished data) from a chromosome 15 library was also studied. Primer sequences of *D15S169* are as follows: forward primer, CAG GAG AGA GCC TTG GAT; reverse primer, GAG ACA TCT CTT CTG AAA GCT C.

**Linkage Analysis.** Logarithm of odds (LOD) scores were calculated assuming a disease penetrance of 0.95 (except where indicated). Allele frequencies were estimated from the

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Abbreviations: FHC, familial hypertrophic cardiomyopathy; LOD, logarithm of odds; MHC, myosin heavy chain; STR, short tandem repeat; LVWT, left ventricular wall thickness; cM, centimorgan(s).

††To whom reprint requests should be addressed.

study population. The MLINK program was used to calculate two-point LOD scores (12). Multipoint linkage analyses were performed using LINKMAP with STR polymorphisms at loci *D15S98, D15S108*, and *D15S125*, as these contributed the maximum information in the study families. The 90% confidence interval containing the *CMH3* locus was defined as the region where the LOD score was within 1 unit of the maximum multipoint LOD score. The HOMOG program was used to test for heterogeneity (13).

**Other Statistical Tests.** Mean maximal left ventricular wall thicknesses (LVWTs) were compared with an unpaired Student's *t* test.

## RESULTS

**Clinical Findings.** Family MZ (Fig. 1) is of Northern European origin. Five surviving affected members (III-11, III-15, III-17, III-20, and IV-7) had cardiac hypertrophy (maximal LVWT >13 mm; see Table 1). Necropsy studies from two deceased members (IV-15 and V-1) reported typical findings of FHC including cardiac hypertrophy with myocyte disarray.

Eight individuals in this family were also scored affected despite the absence of typical echocardiographic signs of the disorder. Three individuals (III-2, III-12, and IV-1) had clinical and/or electrocardiogram abnormalities and transmitted typical FHC to offspring. Individual III-2 had arterial hypertension, a dilated left ventricle, and affected offspring. She was considered affected and died of intractable heart failure during the course of this study. Five individuals (IV-9, IV-18, IV-23, IV-27, and V-3) aged between 18 and 43 years were also considered to be affected, although cardiac hypertrophy was not detected by echocardiography. They presented with clinical and electrocardiogram features of FHC

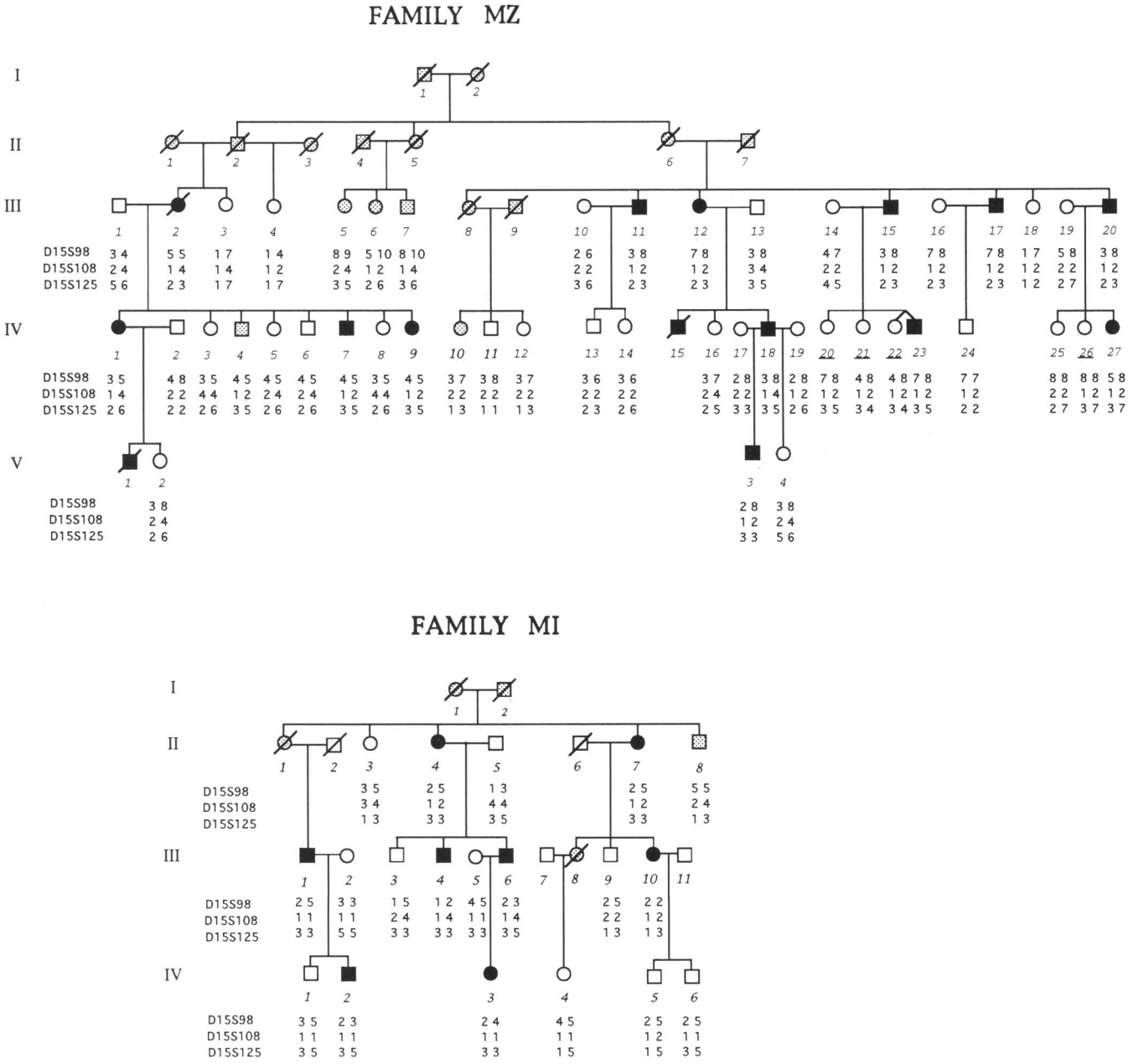


FIG. 1. Pedigrees of families MZ and MI. Pedigree numbers for each individual are given in italics; circles indicate women and squares indicate men. Clinical status is denoted: open symbols, unaffected; solid symbols, affected; stippled symbols, undetermined; slashed, deceased. Underlined numbers denote recombinants at the chromosome 15q2 locus (see text for details). Individual alleles of three STR polymorphisms at *CMH3* (*D15S98, D15S108*, and *D15S125*) are shown for family MZ and MI.

Table 1. Clinical characteristics of family MZ and MI

| Pedigree number | Age/ sex | Clinical symptoms | EKG abnormalities | Max. LVWT, mm |
|---|---|---|---|---|
| Family MZ | | | | |
| III-2 | 65/F | A, D, H | AF, ST | 8 |
| III-11 | 67/M | A, D, S | LVH | 18 |
| III-12 | 63/F | D, A | RVH | 11 |
| III-15 | 60/M | None | AF, ST | 14 |
| III-17 | 56/M | A | QRS | 14 |
| III-20 | 41/M | D | AF, QRS | 16 |
| IV-1 | 43/F | None | QRS | 8 |
| IV-7 | 31/M | None | LVH | 17 |
| IV-9 | 23/F | D | ST | 8 |
| IV-15 | 8/M | SD | NA | 23 |
| IV-18 | 38/M | None | Q | 12 |
| IV-20 | 36/F | None | None | 8 |
| IV-21 | 32/F | None | None | 9 |
| IV-22 | 25/F | None | None | 8 |
| IV-23 | 25/M | None | ST | 10 |
| IV-26 | 30/F | None | None | 8 |
| IV-27 | 24/F | S | LVH, ST | 8 |
| V-1 | 6 mo/M | SD | NA | 9 |
| V-3 | 18/M | None | QRS, ST | 8 |
| Family MI | | | | |
| II-4 | 76/F | D, A | ST | 15 |
| II-7 | 75/F | D | RBBB | 22 |
| III-1 | 52/M | None | LVH, ST | 22 |
| III-4 | 41/M | D | None | 20 |
| III-6 | 40/M | None | QRS, ST | 17 |
| III-10 | 38*/F | D* | None* | 23* |
| IV-2 | 18/M | SD | LVH, ST | 23 |
| IV-3 | 12*/F | D, A* | LBBB* | NA* |

Age is in years unless otherwise indicated. F, female; M, male; A, angina pectoris; D, dyspnea; H, arterial hypertension; S, syncope; SD, sudden cardiac death; AF, atrial fibrillation; ST, ST-segment abnormalities; LVH/RVH, left/right ventricular hypertrophy; QRS, intraventricular conduction defects; Q, abnormal Q waves; RBBB/LBBB, right/left bundle branch block; NA, not available; mo, months.
*Before myectomy.

(Table 1), did not suffer from any other cardiac or circulatory disease, and were direct descendants of affected members. Individual III-5 was not available for clinical evaluation.

The extent of cardiac hypertrophy detected by echocardiography in affected members of family MZ was significantly milder than that of other FHC families. Solomon *et al.* (14) reported that the mean maximal LVWT for 39 affected individuals from six FHC families with different $\beta$ cardiac MHC mutations was 23.7 ± 7.7 mm. The mean maximal LVWT for affected adults in family MZ was only 12.5 ± 4.7 mm ($P < 0.0001$).

FHC family MI is of the same ethnic origins as family MZ but is not known to share common ancestors. Eight affected members in family MI were diagnosed with FHC. These individuals exhibited typical clinical symptoms of the disorder and both echocardiography and electrocardiography demonstrated cardiac hypertrophy (Table 1). Premyectomy echocardiographic data of individual IV-3 were not available. Cardiac hypertrophy in affected members of family MI was significantly greater (mean maximal LVWT = 20.3 ± 3.1 mm) than in those of family MZ ($P < 0.0004$) but was not different from that found in families with $\beta$ cardiac MHC mutations (23.7 ± 7.7 mm).

FHC family D is of Eastern European descent and has been described in detail elsewhere (6). Echocardiographic assessment of cardiac hypertrophy in family D was different from family MZ (maximal LVWT = 22.2 ± 7.1 mm versus 12.5 ± 4.7 mm; $P < 0.0001$) but was not different from family MI (20.3 ± 3.1 mm) or FHC families with $\beta$ cardiac MHC mutations (23.7 ± 7.7 mm).

**Genetic Findings.** Genetic analyses were performed to determine whether the mutated locus responsible for FHC in family MZ was linked to either *CMH1* (the $\beta$ cardiac MHC gene on chromosome 14q1) or *CMH2* (an unidentified gene on chromosome 1q3). Two polymorphic sequences within the $\beta$ cardiac MHC gene (identified by probes pSC14 and Int24) excluded linkage to *CMH1* (LOD score = − 7.7; $\theta = 0.0$; Table 2). The disease locus in family MZ was also not linked to the *CMH2* locus (Table 2 and ref. 5). LOD scores calculated at a reduced penetrance (50%) similarly excluded linkage to *CMH1* (LOD score = −4.15 at $\theta = 0.00$) and *CMH2* [LOD score < −2 for the 10-centimorgan (cM) interval flanking *CMH2*].

A genome-wide search was then performed by analyzing the inheritance of highly polymorphic, randomly distributed, STR polymorphisms in family MZ. One-hundred twenty loci were analyzed and found to be unlinked to FHC in this family, thereby excluding ≈50% of the genome. Linkage analysis with an anonymous STR locus, *D15S108*, located on chromosome 15q2, gave a maximum LOD score of 2.15 at $\theta = 0.1$ (Table 2). Subsequent evaluation with several loci on chromosome 15q2 confirmed linkage of FHC in family MZ to this region. The two-point LOD scores of FHC in family MZ at four loci on chromosome 15q2 are listed in Table 2. Multipoint linkage analyses using *D15S98*, *D15S108*, and

Table 2. Two-point LOD score analyses of family MZ and MI

| Locus | Chromosome | Recombination fraction ($\theta$) 0.00 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 |
|---|---|---|---|---|---|---|---|
| Family MZ | | | | | | | |
| INT24 | 14q1 | −7.71 | −1.36 | −0.54 | 0.00 | 0.10 | 0.04 |
| *F13B* | 1q3 | −17.36 | −5.36 | −3.25 | −1.35 | −0.54 | −3.75 |
| *ACTC* | 15q | −∞ | −6.39 | −4.22 | −2.14 | −1.03 | −0.37 |
| *D15S98* | 15q2 | −2.75 | 0.30 | 1.03 | 1.47 | 1.27 | 0.68 |
| *D15S108* | 15q2 | 1.06 | 1.86 | 2.15 | 2.13 | 1.68 | 0.94 |
| *D15S125* | 15q2 | −5.84 | 2.88 | 3.23 | 3.02 | 2.24 | 1.09 |
| *D15S169* | 15q2 | −13.8 | −3.50 | −1.74 | −0.24 | 0.25 | 0.24 |
| Family MI | | | | | | | |
| INT24 | 14q1 | −9.24 | −3.02 | −2.00 | −1.00 | −0.47 | −0.17 |
| *F13B* | 1q3 | −3.59 | −1.24 | −0.88 | −0.46 | −0.22 | −0.08 |
| *ACTC* | 15q | −3.53 | −0.38 | −0.04 | 0.20 | 0.21 | 0.13 |
| *D15S98* | 15q2 | 2.14 | 1.88 | 1.62 | 1.12 | 0.62 | 0.18 |
| *D15S108* | 15q2 | 2.88 | 2.72 | 2.48 | 1.89 | 1.18 | 0.44 |
| *D15S125* | 15q2 | 1.64 | 1.47 | 1.30 | 0.95 | 0.60 | 0.26 |
| *D15S169* | 15q2 | −2.92 | 0.87 | 1.18 | 1.15 | 0.81 | 0.34 |

*D15S125* yielded a maximum multipoint LOD score of 4.16 at *D15S108*. Assessment of linkage using a reduced penetrance (50%) increases the maximum multipoint LOD score to 5.19 (data not shown). We denote the FHC locus on chromosome 15q2 as *CMH3*.

Linkage analyses demonstrated that FHC in family MI was not due to mutations in either *CMH1* or *CMH2* (Table 2). We have shown (5, 6) that family D is not linked to the β cardiac MHC gene or to chromosome 1q3. To determine whether mutations at the *CMH3* locus account for FHC in other families, we asked whether the disorder in families D and MI was linked to chromosome 15q2. Two-point linkage analysis with four loci (Table 2) suggested that the disease gene in family MI was linked to *CMH3*. Multipoint linkage analyses gave a peak LOD score of 2.88 at *D15S98* for FHC in family MI (Fig. 2). By using HOMOG, the calculated probability that the disorder in families MI and MZ are due to mutations at the same locus is >0.99.

Two-point linkage analyses (data not shown) in family D and multipoint linkage analyses (Fig. 2) using chromosome 15q2 loci did not suggest linkage between the FHC gene in this family and *CMH3*. Calculations using the HOMOG program indicate a probability <5% that the disease gene in family D is located at *CMH3*.

**Candidate Gene Assessment.** Because β cardiac MHC gene mutations cause FHC, genes encoding other sarcomeric proteins including cardiac actin have been suggested as candidate genes for FHC that is unlinked to *CMH1*. The cardiac actin gene maps to chromosome 15q, ≈40 cM centromeric of *D15S108*. Mutations within the cardiac actin gene as cause for FHC in families MZ and MI were excluded by linkage analyses of a STR polymorphism within this gene (*ACTC*; Table 2 and ref. 15).

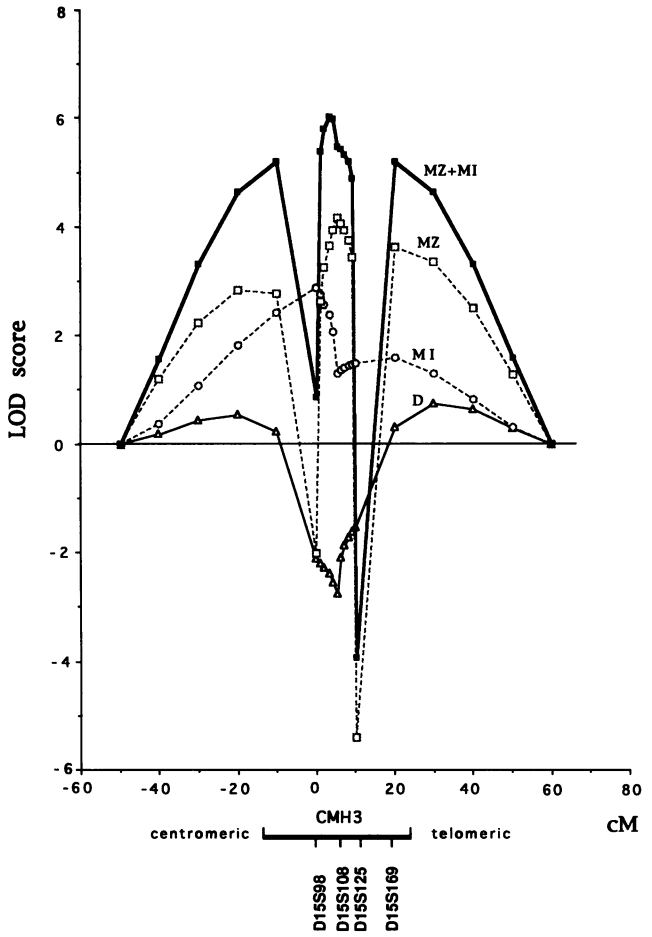


FIG. 2. Graphic representation of multipoint LOD scores at *CMH3* on chromosome 15q2 in three families (MZ, MI, D, and MZ+MI). Locus *D15S98* is set arbitrarily as 0. The order and distance of four STR polymorphisms at *CMH3* (*D15S98*, *D15S108*, *D15S125*, and *D15S169*) were determined by two of us (A.B. and J.T.). The thick bar represents the interval that contains *CMH3* (90% confidence).

## DISCUSSION

We provide evidence for an FHC disease locus, *CMH3*, located on chromosome 15q2 in the region of loci *D15S98*, *D15S108*, *D15S125*, and *D15S169*. FHC in two independent families maps to *CMH3* (combined maximum multipoint LOD score = 6.02; Fig. 2). However, FHC in one other family (D) does not map to *CMH1*, *CMH2*, or *CMH3*. We conclude that mutations in at least four loci can cause FHC: chromosome 14q1 (β cardiac MHC gene), 1q3, 15q2, and another unidentified locus.

The extent of cardiac hypertrophy in FHC has been clinically recognized to vary extensively within and between families. Identification of the genetic basis of FHC has permitted correlation between genotype and phenotype. In one study (14), a similar extent of cardiac hypertrophy was found in affected individuals with different β cardiac MHC gene mutations. Their hypertrophy was significantly greater than that found in unaffected relatives. Recently, Epstein *et al.* (7) described a family with FHC due to a β cardiac MHC gene mutation (Leu908Val). Only 61% of affected individuals had a maximal LVWT measured ≥12 mm. Clinical analyses in two families (MZ and MI) with FHC that maps to *CMH3* similarly suggest that a range of phenotypes can be caused by mutations at this locus. The cardiac hypertrophy found in affected individuals from family MI is much more severe than that found in patients from family MZ ($P < 0.0004$). By analogy to the phenotype–genotype correlation associated with mutations in the β cardiac MHC gene, we suggest that families MZ and MI have independent mutations at the *CMH3* locus. Alternatively, these diverse phenotypes may reflect mutations of two closely linked genes on chromosome 15q2.

Clinical assessment of family MZ was complicated by the absence of echocardiographic manifestations of cardiac hypertrophy in 50% of affected individuals. This difficulty was reflected in information obtained from genotype analyses. Four adults in family MZ (IV-20, IV-21, IV-22, and IV-26) were diagnosed as unaffected but have the same haplotype as affected relatives. Either four double crossover events have occurred between *D15S98* and *D15S125* in these individuals or these individuals carry the FHC mutation but lack demonstrable disease. Given that the region between these loci is ≈10 cM, the probability of four double crossovers is low. Calculation of multipoint LOD scores in family MZ using a reduced penetrance (50%) increases the maximum multipoint LOD score at *CMH3* without altering the location for the disease gene.

Assessment of candidate genes at the *CMH1* locus led to the identification of FHC-causing mutations within the β cardiac MHC gene (2–4). The only known sarcomeric protein encoded on chromosome 15q is cardiac actin. Linkage analyses reported here demonstrate that cardiac actin is not located at *CMH3*; mutations in this gene cannot account for FHC in families MZ or MI. We anticipate that the FHC genes at *CMH2* and *CMH3* will soon be identified. Characterization of these genes will give clues to the search for additional FHC loci and will broaden our understanding of the molecular basis for inheritable cardiac hypertrophy.

These studies would not have been possible without the generous assistance of family members. We are grateful to Yah-Huei Chou for advice on selection of DNA polymorphisms and to Hiromi Seo,

Laszlo Levai, and Stavros Constantinides for assembling data on family members. This work was supported by grants from the Deutsche Forschungsgemeinschaft (L.T. and H.-P.V.), British Heart Foundation (H.W. and W.M.), Fritz-Thyssen Stiftung (H.-P.V.), National Institutes of Health (HG00461 to A.B., HL46320 to J.G.S., and HL41474 and HL42467 to C.S.), Howard Hughes Medical Foundation (J.G.S. and C.M.), the American Heart Association (C.S.), and Bristol–Myers Squibb.

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