

The Cardiac β -Myosin Heavy Chain Gene Is Not the Predominant Gene for Hypertrophic Cardiomyopathy in the Finnish Population

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Objectives. The aim of the study was to screen 36 unrelated patients with hypertrophic cardiomyopathy (HCM; 16 familial and 20 sporadic cases) from a genetically homogeneous area in eastern Finland for variants in the cardiac β -myosin heavy chain (β -MHC) and α -tropomyosin (α -TM) genes.

Background. Mutations in the β -MHC and α -TM genes have been reported to be responsible for 30% to 40% and less than 5% of familial HCM cases, respectively. However, most genetic studies have included patients from tertiary care centers and are subject to referral bias.

Methods. Exons 3-26 and 40 of the β -MHC gene and the nine exons of the α -TM gene were screened with the PCR-SSCP (polymerase chain reaction-single strand conformation polymorphism) method. Linkage analyses between familial HCM locus and two intragenic polymorphic markers (MYO I and MYO II) of the β -MHC gene were performed in 16 familial HCM kindreds.

Results. A previously reported Arg719Trp (arginine converted

to tryptophan in codon 719) mutation of the β -MHC gene was found in one proband and two relatives. In addition, a novel Asn696Ser (asparagine converted to serine in codon 696) substitution was found in one HCM patient. No linkage between familial HCM and the β -MHC gene was observed in 16 familial kindreds. A previously reported Asp175Asn (aspartic acid converted to asparagine in codon 175) mutation of the α -TM gene was found in four probands and 16 relatives. Mutations in the β -MHC and α -TM genes accounted for 6% and 25% familial HCM cases and 3% and 11% of all cases, respectively.

Conclusions. Our results indicate that the β -MHC gene is not the predominant gene for HCM in the Finnish population, whereas HCM caused by the Asp175Asn mutation of the α -TM gene is more common than previously reported.

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Hypertrophic cardiomyopathy (HCM) is characterized by myocardial hypertrophy accompanied with myocyte and myofibrillar disarray (1,2). HCM occurs both as a sporadic form and a familial disorder that is inherited as an autosomal dominant trait (3). Hypertrophic cardiomyopathy is a genetically heterogeneous disease caused by defects in genes encoding the following cardiac sarcomeric proteins: cardiac beta-myosin heavy chain (β -MHC) (4-6), alpha-tropomyosin (α -TM) (7-9), cardiac troponin T (7,8), cardiac myosin binding protein C (10,11), and myosin essential or regulatory light chain (12). Recently, mutations in the cardiac troponin I gene have also been shown to be associated with familial HCM (13).

Missense mutations in the cardiac β -MHC gene have been reported to be responsible for 30% to 40% of familial HCM cases (6,14), whereas mutations in the cardiac troponin T gene account for approximately 15% of cases (8). Mutations in the α -TM and essential or regulatory chains of myosin genes are uncommon causes of HCM, accounting for ~5% and <1% of cases, respectively (8,15,16).

Most of our knowledge of HCM including genetic analyses is derived from studies performed at tertiary care centers, and is profoundly influenced by referral bias (17). Thus, the gene defects accounting for HCM may be distributed differently in the general population. However, so far no genetic studies in unselected populations have been published.

The Finnish population is genetically quite homogeneous, descending mainly from a small number of founders of Baltic and German origin (18). Therefore, the Finnish population offers a unique possibility to investigate the genetic basis of any disease. We used polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) analysis to investigate variants in exons 3-26 and 40 of the cardiac β -MHC gene and in each of the nine exons of the α -TM gene in an unselected population of 36 unrelated Finnish HCM patients.

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Abbreviations and Acronyms

α -TM	= alpha-tropomyosin
Arg719Trp	= arginine converted to tryptophan in codon 719
Asn696Ser	= asparagine converted to serine in codon 696
Asp175Asn	= aspartic acid converted to asparagine in codon 175
β -MHC	= beta-myosin heavy chain
HCM	= hypertrophic cardiomyopathy
PCR	= polymerase chain reaction
SSCP	= single strand conformation polymorphism

Methods

Index patients. The Kuopio University Hospital region in eastern Finland covers a population of 250,000. All patients with suspected or confirmed HCM in this area are sent to the Division of Cardiology of the Department of Medicine of the Kuopio University Hospital for diagnosis and treatment. In this study, all unrelated patients who had suspected or confirmed HCM according to hospital discharge records and previous medical records were evaluated at the Kuopio University Hospital by the same cardiologist (J.K.). Altogether, 36 unrelated patients aged over 16 years and fulfilling the criteria for HCM were included in the study group.

Clinical evaluation. All subjects underwent an interview, physical examination, 12-lead electrocardiogram, M-mode and two-dimensional echocardiography, Doppler echocardiography, and a 24-h Holter electrocardiographic (ECG) registration. Physical examination included measurement of height, weight and blood pressure. Blood pressure was measured twice on the right arm with a standard sphygmomanometer after 10 min of bed rest with a 2-min interval. The latter value was used in statistical analyses. A venous blood sample for genetic analyses was obtained after securing informed consent. Affection status was ascertained without knowledge of the results of genetic analysis.

Echocardiographic evaluation was performed with a Hewlett-Packard Sonos 1000 scanner with a 2.5-MHz transducer. The M-mode tracings (paper speed 100 mm/s) were obtained with simultaneous electrocardiograms (ECG). All tracings were analyzed with custom-built software by two observers (J.K. and K.S.) without knowledge of the clinical status of the subjects. By using a digitizing table (MM 1201, Summagraphics Co., Fairfield, Connecticut; resolution 0.1 mm), the M-mode measurements were obtained from three cycles and averaged. All measurements were performed according to the standards of the American Society of Echocardiography (19).

The diagnosis of hypertrophic cardiomyopathy (HCM) was based on demonstration of maximal left ventricular (LV) wall thickness of at least 15 mm on two-dimensional echocardiography in the absence of other causes for ventricular hypertrophy, such as hypertension. Subjects were defined as having hypertension if systolic blood pressure was >160 mm Hg or diastolic blood pressure >100 mm Hg, or if the subject was receiving drug treatment for hypertension. Affection status for

HCM in adult relatives of the probands was based on the diagnostic criteria by McKenna et al. (20). Briefly, relatives with LV wall thickness of at least 13 mm in the absence of other causes for ventricular hypertrophy were classified as having HCM. Relatives with LV wall thickness ≥ 13 mm but also with definite or borderline hypertension or a body mass index (BMI) ≥ 35 kg/m², were classified as having suspect HCM. Relatives <16 years of age were diagnosed to have HCM by pediatric cardiologist (T.T.) if the thickness of the interventricular septum on echocardiography exceeded the average values of subjects with similar body surface area by at least 2 SD (21) and if they had ECG signs of LV hypertrophy and/or symptoms supporting the diagnosis. The study protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

Single strand conformation polymorphism analysis (PCR-SSCP). The DNA was prepared from peripheral blood leukocytes by the proteinase K-phenol-chloroform extraction method. Intronic sets of primers flanking each of the exons from 3 to 26 and 40 of the cardiac β -MHC gene were designed according to the reported sequences of the cardiac β -MHC gene (22,23). Primers for the exons 1a, 2b, 3, 4, 5, 6b, 7, 8 and 9a,b of the striated muscle isoform of the α -TM gene were synthesized as previously reported (7). Each of the exons of the cardiac β -MHC gene and of the α -TM gene were amplified with the polymerase chain reaction (PCR). The reaction mixture of 10 μ l consisted of 50 ng genomic DNA, 5 pmol of each primer, 10 mmol/liter Tris-HCl (pH 8.8), 50 mmol/liter KCl, 1.5 mmol/liter of MgCl₂, 0.1% Triton X-100, 200 μ mol/liter dNTP, 0.25 U of DNA polymerase (Dynazyme DNA polymerase, Finnzymes, Finland) and 1.0 μ Ci of alpha ³²P dCTP. The PCR conditions for the β -MHC gene were denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 56–66°C for 30 to 60 s and extension at 72°C for 30 to 60 s with final extension at 72°C for 4 min. The PCR conditions for the α -TM gene were as previously described (7) with an addition of the 3-min denaturation in 94°C and the final extension of 4 min at 72°C. The SSCP analysis was performed essentially according to the method of Orita et al. (24).

To obtain fragments <270 base pairs (bp) long, PCR products were digested with an appropriate restriction enzyme if necessary. For SSCP analysis, PCR products were first diluted 2–20-fold with 0.1% sodium dodecyl sulfate (SDS), 10 mmol/liter EDTA and then diluted (1:1) with loading mix (95% formamide, 20 mmol/liter EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol). After denaturation at 98°C for 3 min, samples were immediately cooled on ice, and 2 μ l of each sample was loaded onto 5% (PCR products >200 bp) or 6% (PCR products ≤ 200 bp) nondenaturing polyacrylamide gel (acrylamide/N,N-methylene-bis-acrylamide ratio 49:1) containing 10% glycerol. Each sample was run at two different gel temperatures: 1) at 38°C for approximately 4 h and 2) at 29°C for approximately 5 h. The gel was autoradiographed overnight at –70°C with intensifying screens.

Direct sequencing. Genomic DNA from individuals with variant single strand conformers was used as a template in the amplification reaction as described above (total volume 50 μ l containing 25 pmol of each primer and 1.25 U of Dynazyme DNA polymerase). Amplified PCR products were purified by electrophoresis on a 1% low-melting-point agarose gel and directly sequenced using Sequenase version 2.0 DNA polymerase (US Biochemicals, Cleveland, Ohio) as previously described (25).

Restriction fragment length polymorphism (RFLP) analysis. A novel amino acid substitution (Asn696Ser) (asparagine converted to serine in codon 696) found in the cardiac β -MHC gene was verified by digestion of the variant PCR product with the restriction endonuclease BsrD1. The AAT696AGT substitution abolishes a BsrD1 restriction site. Therefore, the substitution was confirmed by digestion of the variant PCR fragment by BsrD1 restriction enzyme. Digestion of the 198-bp heterozygous PCR product with BsrD1 yields a 198-bp fragment in case of Asn696Ser substitution in addition to the normal 90-bp and 108-bp products. Digested fragments were then electrophoresed on a 3% agarose gel (NuSieve GTG, FMC Bioproducts, Rockland, Maine).

Detection of polymorphic markers. Primers for intragenic dinucleotide microsatellite markers MYO I and MYO II of the β -MHC gene (26) and $HTM\alpha_{CA}$ of the α -TM gene (7) were synthesized. The primers used for the amplification of the MYO I and MYO II were as follows: MYO I: primer F, 5' ATCTGAGCATATGGGACCAAT 3'; primer R, 5' TCATACACCCACATCTCCCA 3'; MYO II: primer F, 5' CAGCAACTGGATGATGTGAGTAG 3'; primer R, 5' ATCACACCTCTGACTTTTCATT 3'. Primers for the $HTM\alpha_{CA}$ were as previously reported (7). One of the primers for amplification of the microsatellite markers was labeled with fluorescein during synthesis. The polymorphic DNA fragments were amplified with PCR at optimized conditions. The electrophoresis separating dinucleotide alleles of different sizes was carried out with an automated laser fluorescence DNA sequencer (ALFexpress, Pharmacia, Uppsala, Sweden) with 6% Hydrolink gels. Two internal size standards were included in each lane.

Statistical analysis. All calculations were performed with the SPSS/WIN programs (SPSS, Chicago, Illinois). Data are presented as mean \pm SEM. The characteristics of the probands with sporadic and familial HCM were compared with the χ^2 test and Student's two-tailed t test for independent samples, when appropriate. Two-point pairwise linkage analysis was performed using the MLINK program of the LINKAGE 5.2 package (27,28). Dominant inheritance mode was used for HCM with a penetrance of 0.95. Allele frequencies for the MYO I and MYO II markers in population were estimated from the sample of 70 randomly selected healthy men from eastern Finland. Subjects with an uncertain diagnosis were assigned an unknown status in the linkage analysis. Heterogeneity between the families was tested using the HOMOG program (28). Conventional criteria for the evidence of linkage (LOD [logarithm of the odds] score >3) and the exclusion of

Table 1. Clinical Characteristics of the Probands With HCM

Characteristics	n = 36
Men/Women	21/15
Age (yr)	49.3 \pm 2.7
History of cardiac failure	3 (8%)
History of myectomy	3 (8%)
Cardiac symptoms	32 (89%)
Dyspnea	27 (75%)
Chest pain	7 (19%)
Presyncope/syncope	15 (42%)
No symptoms	4 (11%)
Constant atrial fibrillation	4 (11%)
Systolic BP (mm Hg)	131 \pm 2
Diastolic BP (mm Hg)	80 \pm 2
Abnormal auscultation	35 (97%)
S4 without systolic murmur	7 (19%)
Systolic murmur without S4	4 (11%)
S4 + systolic murmur	24 (67%)
Abnormal ECG	36 (100%)
LAH	11 (31%)
LVH	27 (75%)
Pathological Q-waves	17 (47%)
Abnormal T-waves	29 (81%)
Abnormal ST-level	18 (50%)

BP, blood pressure; ECG, electrocardiogram; HCM, hypertrophic cardiomyopathy; LAH, left atrial hypertrophy; LVH, left ventricular hypertrophy. Value for abnormal T-waves includes giant and negative T-waves. Value for abnormal ST-level includes ≥ 1 mm depression or elevation of ST-level. Data are mean \pm SEM or n (%).

linkage (LOD score <-2) between the markers and HCM were applied.

Results

Clinical findings. All but one of the 36 probands fulfilled the aforementioned diagnostic criteria for HCM. This proband (family no. 8), otherwise fulfilling the criteria for HCM, had hypertension but was included in the study because she had one first-degree relative and one second-degree relative with a definite HCM. Fourteen of the 36 probands had a definite family history of HCM (both the proband and at least one first-degree relative fulfilling the diagnostic criteria for definite HCM). In addition, two more probands had at least one first-degree relative with suspected HCM. The remaining 20 probands had no known family history of HCM.

Clinical characteristics of the probands are shown in Table 1. The study group included 21 men and 15 women with a mean age of 49.3 years. Sex distribution was different between the probands, with sporadic (15 men, 5 women) and familial HCM (6 men, 10 women; $p = 0.023$). Most of the subjects had been diagnosed as having HCM before our study (86%). Three subjects had a history of cardiac failure, and three subjects had undergone a myotomy-myectomy operation. The most common cardiac symptoms were dyspnea and syncope/presyncope. Only 11% of the subjects had a history of constant atrial fibrillation. The probands with sporadic HCM had higher

Table 2. Echocardiographic Findings in the Proband With HCM

Echocardiographic Findings	n = 36
2D IVS (mm)	24.1 ± 0.8
Location of maximal hypertrophy	
Septum	34 (94%)
Free wall	1 (3%)
Concentric	1 (3%)
Left ventricular posterior wall (M-mode, mm)	11.0 ± 0.4
LVEDD (mm)	44.9 ± 1.5
LVESD (mm)	28.9 ± 1.3
FS (%)	36.7 ± 1.5
Base of aorta (mm)	33.2 ± 1.0
Left atrium (mm)	40.5 ± 1.5
Mitral regurgitation	12 (33%)
SAM	4 (11%)
Obstruction at LV outflow tract	3 (8%)
VMAX (m/s)	1.21 ± 0.06

2D IVS, maximal thickness of interventricular septum on two-dimensional echocardiography; FS, fractional shortening; LV, left ventricle; LVEDD, left ventricular end diastolic dimension; LVESD, left ventricular end systolic dimension; SAM, systolic anterior motion of mitral valve; VMAX, maximal flow velocity in the left ventricular outflow tract. Data are mean ± SEM or n (%).

systolic blood pressure than did the probands with familial HCM (135 ± 3 vs. 125 ± 2 mm Hg; $p = 0.017$). Nearly all subjects had both abnormal auscultation and ECG findings. Most subjects (75%) had left ventricular hypertrophy on ECG, and almost half of the subjects had pathological Q-waves on ECG. Eight subjects were diagnosed with short bursts of ventricular tachyarrhythmias on Holter examination.

Echocardiographic data are shown in Table 2. The mean maximal thickness of the interventricular septum (IVS) on two-dimensional echocardiography was 24.1 mm (range 15.4 to 36.1 mm). The maximal hypertrophy was located in the IVS in nearly all of the subjects. Left ventricular end systolic and end diastolic dimensions were within normal limits. Left ventricular end diastolic dimension was greater in the probands with sporadic HCM than in the probands with familial HCM (47.7 ± 1.5 vs. 41.5 ± 2.6 mm; $p = 0.039$). Systolic anterior motion of the mitral valve was present in four patients (11%), and only three (8%) had a LV outflow tract obstruction.

Detection of sequence variants in the β -MHC gene. Exons from 3 to 26 and 40 of the β -MHC gene were screened with the PCR-SSCP method. Sequencing of abnormally migrating DNA fragments revealed a total of eight different variants. A missense mutation previously reported to cause HCM was found in exon 19 (Arg719Trp) (arginine converted to tryptophan in codon 719) in the proband of family no. 25 (Table 3 and Fig. 1a). All available relatives of the proband were both clinically and genetically studied. The mutation was found in two of the proband's young children (individuals IV-2 and IV-3) but in none of the clinically unaffected relatives.

In addition, a novel AAT to AGT substitution in exon 19 of the β -MHC gene resulting in a substitution of asparagine to serine at residue 696 was identified in one HCM patient (heterozygous). This patient was a 12-year-old not belonging to the original study group of 36 probands. He had been clinically

evaluated at the Department of Pediatrics of the Kuopio University Hospital and had a definite HCM with septal and apical hypertrophy.

Other sequence variants (numbered according to reference 22) we found in the cardiac β -MHC gene included four silent nucleotide substitutions (ACC63ACT, TTT244TTC, ACG786ACA and ATT989ATC), an intronic nucleotide substitution (nucleotide 8935 T→C) and a nucleotide substitution at the 3' noncoding region of exon 40 (position 6007 G→A). None of these six variants co-segregated with the disease in families.

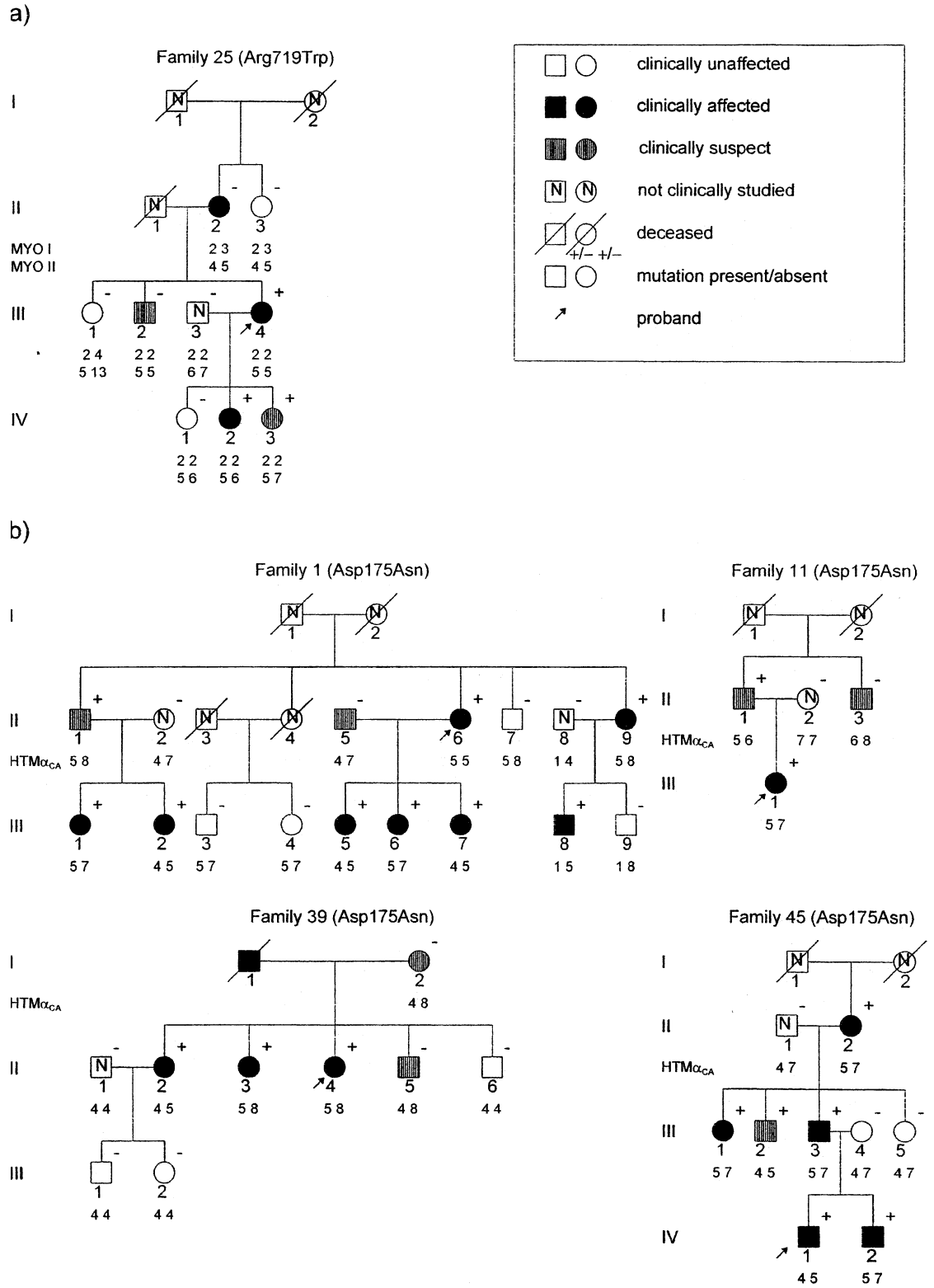
Identification of the Asp175Asn substitution in the α -TM gene. A previously reported missense mutation (Asp175Asn) (aspartic acid converted to asparagine in codon 175) in exon 5 of the α -TM gene was found in four probands (families no. 1, 11, 39 and 45; Table 3 and Fig. 1b). Most first-degree relatives and some of the second-degree relatives of these four probands were both clinically and genetically studied. Altogether 20 probands and relatives from the four families carried the mutation. No other sequence variants were found in the α -TM gene.

Linkage analysis. Linkage analysis was performed in 16 families with confirmed or suspected familial HCM using the intragenic polymorphic markers MYO I and MYO II of the cardiac β -MHC gene (Table 4). No evidence of linkage was found between the HCM locus and markers MYO I ($Z = -11.43$, $\theta = 0$) and MYO II ($Z = -18.78$, $\theta = 0$). Allowing heterogeneity between families did not change the results. In six families (1, 8, 12, 17, 39 and 45) individual LOD (logarithm of the odds) scores for either MYO I or MYO II indicated the exclusion of linkage between HCM and the β -MHC gene. The LOD scores for the rest of the families provided little or no evidence supporting or refuting linkage to the β -MHC locus. To investigate the possible founder effect of the Asp175Asn substitution of the α -TM gene in families 1, 11, 39 and 45, DNA samples from the members of these families were genotyped for the intragenic microsatellite marker HTM α_{CA} . All family members with the Asp175Asn substitution carried the same allele (no. 5) of the marker (Fig. 1b).

Table 3. Mutations in the Cardiac Beta-Myosin Heavy Chain (β -MHC) and Alpha-Tropomyosin (α -TM) Genes Causing Hypertrophic Cardiomyopathy

	β -MHC Arg719Trp	β -MHC Asn696Ser	α -TM Asp175Asn
Nucleotide change	CGG→TGG	AAT→AGT	GAC→AAC
No. of probands (n = 36) with mutation	1	0*	4
No. of relatives with mutation	2	not screened	16
Percentage of probands with familial HCM with mutation	6%	0%*	25%

*The patient with HCM carrying the Asn696Ser substitution did not belong to the original study group of 36 probands.



Discussion

Study population. Finland is known for its “Finnish disease heritage,” meaning that uncommon genetic, mostly recessive,

Figure 1a. Pedigrees of the families carrying (a) cardiac β -myosin heavy chain gene Arg719Trp and (b) α -tropomyosin gene Asp175Asn substitutions. Alleles for intragenic microsatellite markers (a) MYO I and MYO II and (b) $HTM\alpha_{CA}$ are depicted under each family member.

Table 4. LOD Scores of the Two-Point Linkage Analysis Between Familial HCM Locus (Dominant Inheritance Mode With a Penetrance of 95%) and Two Polymorphic Markers MYO I and MYO II of the Cardiac β -Myosin Heavy Chain Gene With Recombination Fractions (θ) 0 and 0.01 in 16 Families With Confirmed or Suspected Familial HCM

Family	n	MYO I		MYO II	
		$\theta = 0$	$\theta = 0.01$	$\theta = 0$	$\theta = 0.01$
All	133	-11.43	-9.45	-18.78	-12.24
1	16	-2.33	-2.25	-2.22	-1.03
7	4	-0.16	-0.15	-0.22	-0.21
8	15	-1.96	-1.71	-3.10	-2.21
11	4	0.00	0.00	0.00	0.00
12	11	0.32	0.32	-2.20	-1.34
15	10	0.10	0.15	0.72	0.71
17	4	0.00	0.00	-3.24	-1.40
18	6	-1.02	-0.88	-0.90	-0.80
19	8	-0.20	-0.20	-0.22	-0.22
25	9	-1.22	-1.06	-1.37	-1.19
26	10	-0.65	-0.54	-0.68	-0.58
28	6	-0.76	-0.68	0.07	0.07
37	5	-0.46	-0.43	-0.46	-0.39
39	9	-0.39	-0.35	-2.38	-1.11
40	7	-0.32	-0.31	-0.49	-0.47
45	9	-2.38	-1.36	-2.09	-2.07

LOD, logarithm of the odds.

disorders are enriched or only encountered in Finland (18,29). Because of genetic isolation, the Finnish population, particularly in eastern Finland, is an ideal population to investigate distribution of different variants responsible for HCM. In the present study all known and suspected HCM patients of the Kuopio University Hospital region, encompassing a population of about 250,000, were included. Therefore, our study population is probably representative of all symptomatic HCM cases in the study area and very likely reflects the distribution of gene defects causing HCM in the Finnish population, at least in the eastern part of the country.

Screening of variants in the β -MHC and α -TM genes. We screened the exons encoding the head and hinge regions (and exon 40 encoding the 3'-end of the rod region) of the cardiac β -MHC gene and the nine exons of the α -TM gene in 36 unselected Finnish patients with HCM. Only two amino acid substitutions (Arg719Trp and Asn696Ser) in exon 19 of the β -MHC gene were found. A previously reported mutation (Asp175Asn) in exon 5 of the α -TM gene was found in four probands. These data indicate that, unlike in other European HCM kindreds (6), the cardiac β -MHC gene is not the predominant gene for HCM in the Finnish population because it accounted for only ~3% of all cases and less than 10% of familial cases. In contrast, the Asp175Asn substitution of the α -TM gene was a substantially more common cause for HCM than previously reported (8). This substitution was found in 11% of all cases and in approximately 25% of familial cases.

Accordingly, a recent study of Japanese HCM patients indicated that mutations in the β -MHC gene are not the predominant cause for HCM in Japanese patients (30). These

results imply genetic heterogeneity of HCM among different populations.

Interestingly, in our study all patients with the Asp175Asn substitution from four unrelated kindreds carried the same repeat sequence allele of the H_{TM} α_{CA} marker. Hence, the Asp175Asn mutation may have arisen from a common ancestor in these families. Recently, Coviello et al. (31) described three European HCM kindreds with the Asp175Asn substitution arising from independent origins, suggesting that the codon 579 of the α -TM gene is a mutational hot spot.

Patients with the variants in the β -MHC gene. The proband of family 25 (Fig. 1a) with the Arg719Trp substitution of the β -MHC gene was 28 years old and had been diagnosed with HCM at the age of 18. The maximal thickness of the interventricular septum (IVS) was 25.0 mm. The oldest of the proband's children (individual IV-1, age 4 years) was normal by echocardiography and did not carry the mutation. The older of the proband's two children carrying the mutation (individual IV-2, age 2 years) had definite HCM (IVS thickness of 8 mm [+2 SD]). The proband's youngest child (individual IV-3, age 1 year) had the mutation but did not fulfill the echocardiographic criteria for HCM (IVS thickness of 5.5 mm [+0.5 SD]). The proband's mother (individual II-2) did not carry the Arg719Trp substitution although her IVS was 17.0 mm thick and there were no other obvious causes for the hypertrophy. The proband's father (individual II-1) died of cancer at the age of 45 and did not have medical history indicating HCM. The proband's brother (individual III-2) also showed left ventricular (LV) hypertrophy with IVS thickness of 14.2 mm but did not carry the Arg719Trp substitution. He had been a professional athlete and was still exercising intensively, which was the probable cause for mild LV hypertrophy.

The origin of the Arg719Trp substitution of the cardiac β -MHC gene in family 25 is uncertain. The proband's mother did not carry the mutation, although she fulfilled the echocardiographic criteria for HCM. Therefore, it is possible that the mutation has arisen de novo in the proband, who has transmitted it to two of her three children. On the basis of haplotype analysis of the markers MYO I and MYO II, this seems possible because the proband and her genetically unaffected brother (individual III-2 of the family 25) share the same alleles of both markers (Fig. 1a). Alternatively, the proband has inherited the mutation from her father, but her brother (individual III-2) has inherited a recombination of alleles MYO I and MYO II.

The patient with the Asn696Ser substitution of the β -MHC gene was 9 years old when diagnosed with HCM. Echocardiography showed asymmetrical hypertrophy of the apical and IVS with a maximal IVS thickness of 17.0 mm. He had no symptoms until he died suddenly during exertion. The presence of the Asn696Ser substitution among relatives could not be studied because the patient had been adopted and samples were not available from the biological parents or other relatives. The Asn696Ser substitution does not change the charge of the cardiac β -MHC protein. However, because the mutation was located in the region critical for adenosine triphosphate

(ATP) and actin binding sites, which is highly conserved among different species (22), the Asn696Ser substitution was likely to be the cause for HCM in this patient.

Patients with the Asp175Asn mutation. In patients with the Asp175Asn substitution of the α -TM gene (4 probands and 16 relatives), mean maximal thickness of the IVS was 18.9 mm (range 11.2 to 30.0 mm). Three individuals (family no. 1, individual II-1; family no. 11, individual II-1; family no. 45, individual III-2; Fig. 1b) carrying the Asp175Asn substitution had hypertension; therefore, they were clinically classified to have suspect HCM. Individual II-5 of family no. 1 and individuals I-2 and II-5 of family no. 39 (Fig. 1b) were classified to have suspect HCM because they had left ventricular hypertrophy and also high blood pressure values. None of them carried the mutation. In family no. 39, the Asp175Asn mutation was inherited from the proband's father (individual I-1), who had died suddenly at the age of 48, and cardiomyopathy (most likely HCM) was diagnosed at autopsy.

Screening of variants. The SSCP generally detects ~80% of all mutations. To increase the probability of finding sequence variants, we screened all exons at two different temperatures. Furthermore, all the PCR fragments were <270 bp in length to maximize the probability of finding mutations in the β -MHC and α -TM genes. The method we used has been shown to detect all sequence variants of the lipoprotein lipase gene (32,33), and it has also been successfully applied in the screening of the green and red opsin genes (34,35) and the insulin receptor substrate-1 gene (36). Therefore, it is not likely that we have missed a significant number of sequence variants in the β -MHC and α -TM genes.

Conclusions. We conclude that the cardiac β -myosin heavy chain gene is not the predominant disease-causing gene for HCM in the Finnish population because it accounted for less than 10% of familial and 3% of all cases of HCM. In contrast, the Asp175Asn mutation in the α -tropomyosin gene was more common than previously reported (8), accounting for approximately 25% of Finnish familial HCM cases. Therefore, this substitution could be the predominant gene defect leading to familial HCM in the Finnish population. Because the Finnish population has descended from a small number of founders it is possible that many probands in our patient population may have a common ancestor. Thus, only a few mutations might account for the majority of HCM cases. Interestingly, none of the sporadic HCM cases were caused by mutations in the β -MHC gene or α -TM gene, and over half of the familial HCM cases were not caused by defects in the aforementioned genes either. Consequently, the major genes responsible for HCM in the Finnish population are yet to be identified.

References

1. Maron BJ, Bonow RO, Cannon RO, Leon MB, Epstein SE. Hypertrophic cardiomyopathy: Interrelations of clinical manifestations, pathophysiology, and therapy I. *N Engl J Med* 1987;316:780–9.
2. Maron BJ, Bonow RO, Cannon RO, Leon MB, Epstein SE. Hypertrophic cardiomyopathy: Interrelations of clinical manifestations, pathophysiology, and therapy II. *N Engl J Med* 1987;316:844–52.
3. Watkins H, Thierfelder L, Hwang DS, McKenna W, Seidman JG, Seidman CE. Sporadic hypertrophic cardiomyopathy due to de novo myosin mutations. *J Clin Invest* 1992;90:1666–71.
4. Geisterfer Lowrance AA, Kass S, Tanigawa G, et al. A molecular basis for familial hypertrophic cardiomyopathy: a beta-cardiac myosin heavy chain gene missense mutation. *Cell* 1990;62:999–1006.
5. Marian AJ, Yu QT, Mares A Jr, Hill R, Roberts R, Perryman MB. Detection of a new mutation in the beta-myosin heavy chain gene in an individual with hypertrophic cardiomyopathy. *J Clin Invest* 1992;90:2156–65.
6. Watkins H, Rosenzweig A, Hwang DS, et al. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. *N Engl J Med* 1992;326:1108–14.
7. Thierfelder L, Watkins H, MacRae C, et al. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell* 1994;77:701–12.
8. Watkins H, McKenna WJ, Thierfelder L, et al. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. *N Engl J Med* 1995;332:1058–64.
9. Nakajima-Taniguchi C, Matsui H, Nagata S, Kishimoto T, Yamauchi Takihara K. Novel missense mutation in alpha-tropomyosin gene found in Japanese patients with hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 1995;27:2053–8.
10. Watkins H, Conner D, Thierfelder L, et al. Mutations in the cardiac myosin binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. *Nat Genet* 1995;11:434–7.
11. Bonne G, Carrier L, Bercovici J, et al. Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. *Nat Genet* 1995;11:438–40.
12. Poetter K, Jiang H, Hassanzadeh S, et al. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat Genet* 1996;13:63–69.
13. Kimura A, Harada H, Park JE, et al. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. *Nat Genet* 1997;16:379–82.
14. Marian AJ, Roberts R. Recent advances in the molecular genetics of hypertrophic cardiomyopathy. *Circulation* 1995;92:1336–47.
15. Yamauchi-Takihara K, Nakajima-Taniguchi C, Matsui H, et al. Clinical implications of hypertrophic cardiomyopathy associated with mutations in the alpha-tropomyosin gene. *Heart* 1996;76:63–5.
16. Spirito P, Seidman CE, McKenna WJ, Maron BJ. The management of hypertrophic cardiomyopathy. *N Engl J Med* 1997;336:775–85.
17. Spirito P, Chiarella F, Carratino L, Berisso MZ, Bellotti P, Vecchio C. Clinical course and prognosis of hypertrophic cardiomyopathy in an outpatient population. *N Engl J Med* 1989;320:749–55.
18. de la Chapelle A. Disease gene mapping in isolated human populations: the example of Finland. *J Med Genet* 1993;30:857–65.
19. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978;58:1072–83.
20. McKenna WJ, Spirito P, Desnos M, Dubourg O, Komajda M. Experience from clinical genetics in hypertrophic cardiomyopathy: proposal for new diagnostic criteria in adult members of affected families. *Heart* 1997;77:130–2.
21. Huwez FU, Houston AB, Watson J, McLaughlin S, Macfarlane PW. Age and body surface area related normal upper and lower limits of M mode echocardiographic measurements and left ventricular volume and mass from infancy to early adulthood. *Br Heart J* 1994;72:276–80.
22. Jaenicke T, Diederich KW, Haas W, et al. The complete sequence of the human beta-myosin heavy chain gene and a comparative analysis of its product. *Genomics* 1990;8:194–206.
23. Liew CC, Sole MJ, Yamauchi-Takihara K, et al. Complete sequence and organization of the human cardiac beta-myosin heavy chain gene. *Nucleic Acids Res* 1990;18:3647–51.
24. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci USA* 1989;86:2766–70.
25. Kretz KA, Carson GS, O'Brien JS. Direct sequencing from low-melt agarose with Sequenase. *Nucleic Acids Res* 1989;17:5864.
26. Schwartz K, Beckmann J, Dufour C, et al. Exclusion of cardiac myosin heavy

- chain and actin gene involvement in hypertrophic cardiomyopathy of several French families. *Circ Res* 1992;71:3-8.
27. Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 1984;36:460-5.
 28. Ott J. *Analysis of Human Genetic Linkage*. 2nd ed. Baltimore: The Johns Hopkins University Press, 1992.
 29. Peltonen L, Pekkarinen P, Aaltonen J. Messages from an isolate: lessons from the Finnish gene pool. *Biol Chem Hoppe Seyler* 1995;376:697-704.
 30. Machida M. Genetic heterogeneity of hypertrophic cardiomyopathy in Japanese. *Hokkaido Igaku Zasshi* 1994;69:1024-34.
 31. Coviello DA, Maron BJ, Spirito P, et al. Clinical features of hypertrophic cardiomyopathy caused by mutation of a "hot spot" in the alpha-tropomyosin gene. *J Am Coll Cardiol* 1997;29:635-40.
 32. Reina M, Brunzell JD, Deeb SS. Molecular basis of familial chylomicronemia: mutations in the lipoprotein lipase and apolipoprotein C-II genes. *J Lipid Res* 1992;33:1823-32.
 33. Nevin DN, Brunzell JD, Deeb SS. The LPL gene in individuals with familial combined hyperlipidemia and decreased LPL activity. *Arterioscler Thromb* 1994;14:869-73.
 34. Winderickx J, Lindsey DT, Sanocki E, Teller DY, Motulsky AG, Deeb SS. Polymorphism in red photopigment underlies variation in colour matching. *Nature* 1992;356:431-3.
 35. Winderickx J, Battisti L, Motulsky AG, Deeb SS. Selective expression of human X chromosome-linked green opsin genes. *Proc Natl Acad Sci USA* 1992;89:9710-4.
 36. Laakso M, Malkki M, Kekäläinen P, Kuusisto J, Deeb SS. Insulin receptor substrate 1 variants in non-insulin-dependent diabetes. *J Clin Invest* 1994; 94:1141-6.