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PERTTI JÄÄSKELÄINEN

Molecular Genetics of Hypertrophic Cardiomyopathy in Eastern Finland

Doctoral dissertation

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Series editors:

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Professor Martti Hakumäki, M.D., Ph.D.
Department of Physiology

Author's address:

Clinical Research Unit
University of Kuopio
P.O. Box 1627
FIN-70211 KUOPIO
FINLAND
Tel. +358 17 162 738
Fax +358 17 162 445

Supervisors:

Docent Johanna Kuusisto, M.D., Ph.D.
Department of Medicine
University of Kuopio

Professor Markku Laakso, M.D., Ph.D.
Department of Medicine
University of Kuopio

Reviewers:

Professor Markku S. Nieminen, M.D., Ph.D.
Department of Medicine
University of Helsinki

Heikki Swan, M.D., Ph.D.
Department of Medicine
University of Helsinki

Opponent:

Docent Katriina Aalto-Setälä, M.D., Ph.D.
Department of Medicine
University of Tampere

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) is a myocardial disease with an estimated prevalence of 1/500. HCM is caused by mutations in genes encoding sarcomeric proteins. In the Finnish population the genetic background of HCM has not been studied previously. To assess the genetic background and phenotypic expression of HCM in eastern Finland, we clinically characterized and screened 35 unrelated patients with HCM from eastern Finland for variants in eight genes encoding sarcomeric proteins: β-myosin heavy chain (*MYH7*), α-tropomyosin (*TPM1*), cardiac myosin-binding protein C (*MYBPC3*), cardiac troponins I, T and C (*TNNI3*, *TNNT2*, *TNNC1*), and myosin light chains (*MYL3*, *MYL2*). All available relatives of the probands were included in clinical and genetic analyses when applicable. Clinical investigations included electrocardiography, echocardiography, Holter registration and exercise test. Genetic screening was performed using the single-strand conformation polymorphism method.

Six different disease-related mutations in three genes (*MYH7*, *TPM1*, *MYBPC3*) were identified. Previously reported missense mutations Arg719Trp and Asp175Asn were found in *MYH7* and *TPM1*, respectively. Four novel mutations (Gln1061X, IVS5-2A→C, IVS14-13G→A, Ex25ΔLys) expected to result in truncated proteins were found in *MYBPC3*. The *TPM1*-Asp175Asn and *MYBPC3*-Gln1061X mutations were found in four and six different families, respectively, whereas each of the other mutations was present in a single family. Haplotype analysis suggested that the two mutations (*TPM1*-Asp175Asn, *MYBPC3*-Gln1061X) present in multiple families were founder mutations. *MYBPC3* mutations were the most frequent, accounting for ~26% of all cases in the nuclear study group. Mutations in *TPM1* and *MYH7* accounted for ~11% and ~3% of all cases, respectively. Collectively, the six mutations accounted for ~40% of all cases in the nuclear study group. In general, the HCM phenotype in probands and their clinically affected relatives as well as in the carriers of the disease-related mutations was relatively benign.

In conclusion, the genetic profile of HCM in eastern Finland is unique, characterized by a few founder mutations. The phenotypic expression of HCM in patients from eastern Finland, as well as that related to the identified disease-causing mutations, is relatively benign. The genetic findings of the present study have the potential to enhance the accuracy of clinical diagnostics of HCM, allowing the use of molecular diagnosis in up to 40% of eastern Finnish families with HCM.

National Library of Medicine Classification: WG 280, QU 58.5, QH 431, QH 455
Medical subject headings: cardiomyopathy, hypertrophic/genetics; DNA mutational analysis; single-stranded conformational; restriction fragment length; polymorphism (genetics); phenotype; Finland

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Kuopio, May 2002

Pertti Jääskeläinen

SELECTED ABBREVIATIONS

ARVC	arrhythmogenic right ventricular cardiomyopathy
ACTC	cardiac α-actin gene
ATP	adenosine triphosphate
BMI	body mass index
bp	base pair
cDNA	complementary deoxyribonucleic acid
cM	centimorgan
DCM	dilated cardiomyopathy
dCTP	deoxycytidine 5'-triphosphate
dNTP	deoxynucleotide triphosphate
DNA	deoxyribonucleic acid
ECG	electrocardiography, electrocardiographic
HCM	hypertrophic cardiomyopathy
IVS	intervening sequence
LV	left ventricular
LVH	left ventricular hypertrophy
LOD	logarithm of the odds
MYBPC3	cardiac myosin-binding protein C gene
MYH6	α-myosin heavy chain gene
MYH7	β-myosin heavy chain gene
MYL2	myosin regulatory light chain gene
MYL3	myosin essential light chain gene
α-MyHC	α-myosin heavy chain
β-MyHC	β-myosin heavy chain
PCR	polymerase chain reaction
RCM	restrictive cardiomyopathy
RNA	ribonucleic acid
SCD	sudden cardiac death
SSCP	single-strand conformation polymorphism
TNNC1	troponin C gene
TNNI3	cardiac troponin I gene
TNNT2	cardiac troponin T gene
TPM1	α-tropomyosin gene
TTN	titin gene

Three- or one-letter and one-letter abbreviations are used for amino acids and nucleotides, respectively

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which will be referred to by their Roman numerals:

I Jääskeläinen P, Soranta M, Miettinen R, Saarinen L, Pihlajamäki J, Silvennoinen K, Tikanoja T, Laakso M, Kuusisto J. The cardiac β-myosin heavy chain gene is not the predominant gene for hypertrophic cardiomyopathy in the Finnish population. J Am Coll Cardiol 1998; 32: 1709-16.

II Jääskeläinen P, Kuusisto J, Miettinen R, Kärkkäinen P, Kärkkäinen S, Heikkinen S, Peltola P, Pihlajamäki J, Vauhkonen I, Laakso M. Mutations in the cardiac myosin-binding protein C gene are the predominant cause of familial hypertrophic cardiomyopathy in eastern Finland. J Mol Med; In press.

III Jääskeläinen P, Miettinen R, Silvennoinen K, Vauhkonen I, Laakso M, Kuusisto J. The cardiac troponin I gene is not associated with hypertrophic cardiomyopathy in patients from eastern Finland. J Mol Cell Cardiol 1999; 31: 2031-36.

IV Jääskeläinen P, Miettinen R, Kärkkäinen P, Laakso M, Kuusisto J. Genetic background of hypertrophic cardiomyopathy in eastern Finland is characterized by few founder mutations with benign or intermediary phenotypes. Submitted.

CONTENTS

1. INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant primary cardiac disease characterized by myocardial hypertrophy. HCM is both clinically and genetically a heterogeneous disease, ranging from asymptomatic, with mild or absent left ventricular hypertrophy (LVH) and normal life expectancy, to highly symptomatic, with extensive hypertrophy and a high risk of sudden death (*1*). Not only is HCM estimated to be the most common inherited cardiac disease (*2*), it is also the leading cause for sudden cardiac death (SCD) among young (*3*), especially in competitive athletes (*4*).

The first study illustrating the familial occurrence of HCM was reported over 40 years ago (*5*), but only during the past decade has the genetic background of HCM begun to unfold. HCM is caused by mutations in genes encoding cardiomyocyte sarcomeric proteins. The first locus for familial HCM was mapped in 1989 to chromosome 14 (*6*), followed by the identification of the first mutation in 1990 (*7*). Subsequently, an increasing number of new loci and mutations for HCM have been found. To date, approximately 150 mutations in 11 genes encoding sarcomeric proteins have been reported (*8-10*). Each of the genes associated with HCM encode sarcomeric proteins, and thus, HCM has been concluded to be a disease of the sarcomere (*11*). In addition, mutations in genes encoding other than sarcomeric proteins, associated with syndromes having HCM as a feature, have been reported (*8,12*).

The molecular basis of HCM is currently under vigorous investigation. The identification of the disease genes and mutations is important, because it helps to understand the pathogenesis of HCM. Although currently poorly understood, the elucidation of the molecular and cellular pathways triggered by the mutations could contribute to the development of therapy to reduce the cardiac hypertrophy in HCM (*13*). The investigation of the signals that trigger cardiac hypertrophy in HCM may also help to better understand the mechanisms that induce hypertrophy in non-genetic conditions (*13*). Furthermore, the identification of disease-causing mutations may enhance the accuracy of clinical diagnosis in HCM patients (*14*), and facilitate genotype-phenotype comparison between different mutations (*15*). These data may be

beneficial in the risk stratification of patients carrying well-characterized disease-causing mutations (*15*).

Most of our knowledge of HCM, including genetic studies, is derived from studies performed at a few tertiary care centers, and is profoundly influenced by referral bias (*16*). Clinical studies performed in unselected patients have shown HCM to be a more benign disease than previously believed (*17*). Accordingly, most genetic reports on HCM are mainly based on patient populations including patients of various ethnic backgrounds investigated at a few referral centers. Due to both clinical and genetic heterogeneity, the genetic background and phenotypic expression of HCM is likely to be variable between different populations and geographic areas. However, only a few studies systematically evaluating the genetic profiles of HCM have been published (*18,19*).

In this study we investigated the genetic profile of HCM by screening eight previously identified HCM genes for variants in a homogeneous regional patient population from eastern Finland. In addition, the phenotypic expression of HCM and the clinical features associated with the mutations were studied.

2. REVIEW OF THE LITERATURE

2.1 Sarcomere structure and function

The sarcomere is a functional unit of cardiac muscle contraction within myocytes (*20*). It is composed of sarcomeric proteins that are organized into thick and thin filaments. Major thick filament proteins include the myosin heavy chain and essential and regulatory light chains of myosin, whereas the essential proteins of the thin filament are actin, α-tropomyosin and the troponin complex (troponins T, C and I). Other important sarcomeric proteins include myosin-binding protein C and titin (Figure 1) (*21*). Each thick myofilament consists of several hundred myosin molecules bound by myosin-binding protein C. Each myosin molecule is composed of two myosin heavy chains and two pairs of myosin light chains (1 and 2). Thin myofilaments, anchored to the Z-disc, are composed of two helically bound chains of actin, which are regularly incorporated by the troponin complex and α-tropomyosin molecule. Titin is a giant protein representing intermediate filaments, and it binds thick myosin filaments into the Z-disc (*21*). Thick filament proteins generate and modulate contraction force and anchor the sarcomere to the cytoskeleton (*22*), whereas thin filament proteins mainly regulate the cardiac contraction and relaxation (*23*).

Cardiac muscle contraction is achieved by sliding and interdigitating of the thick and thin filaments of sarcomeres (*20*). Contraction is initiated by calcium influx into the sarcomere, which releases troponin I inhibition of actin and enables acto-myosin interaction. Binding of ATP to myosin results in conformational changes at the actin binding sites of myosin that lead to displacement of the head domain of myosin along the thin filament. Force generation is achieved by hydrolysis of ATP, which allows myosin to restore its initial unbound conformation. Several molecules, including cardiac myosin-binding protein C, titin, dystrophin and associated sarcoglycan peptides, transmit force generated by the sarcomere to the myocyte cytoskeleton (*20,22*).

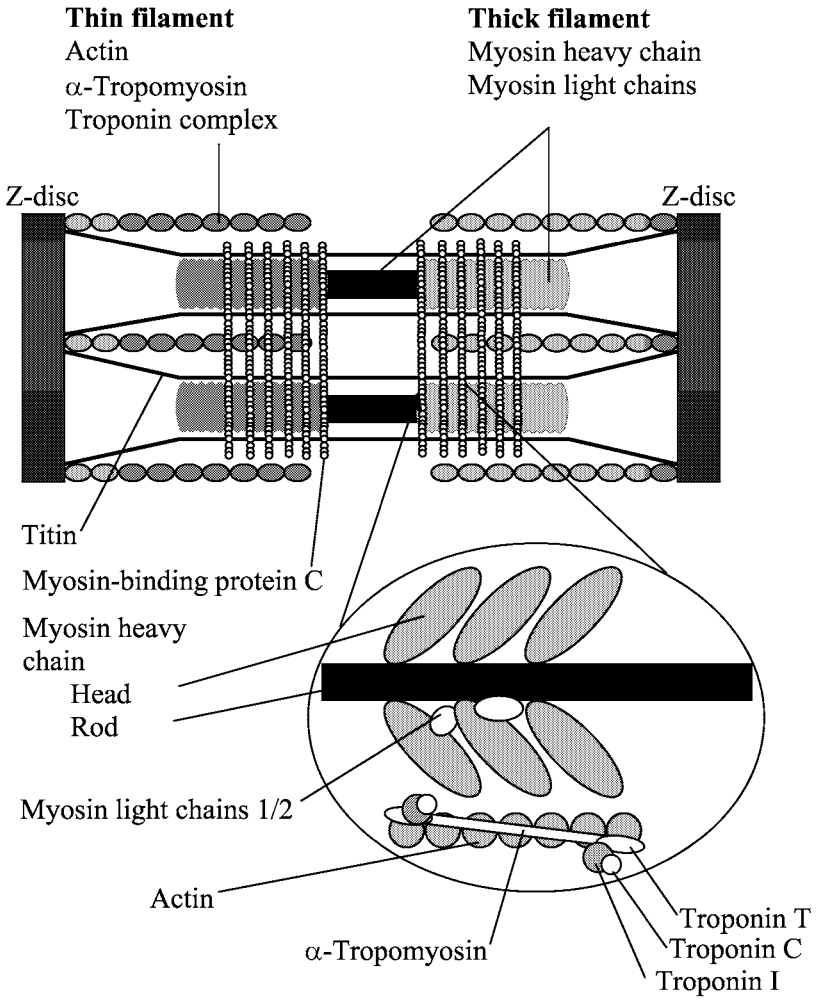

Figure 1. Schematic illustration of the sarcomere and its major constituents (modified from (*24*) and (*25*)).

2.2 Inherited cardiomyopathies

Cardiomyopathies represent a variety of cardiac diseases currently defined as diseases of the myocardium associated with cardiac dysfunction (*26*). Cardiomyopathies are classified by morphologic characteristics as hypertrophic (HCM), dilated (DCM), arrhythmogenic right ventricular (ARVC) and restrictive cardiomyopathy (RCM). The familial inheritance of HCM, DCM and ARVC have been shown, as these

cardiomyopathies are inherited in approximately 50%, 35% and 30% of cases, respectively, but so far no genetic linkage has been shown in RCM (*25*).

DCM is characterized by ventricular dilation and impaired contractile function associated with heart failure, serious arrhythmias and thromboembolic events (*20*). DCM is most often inherited in an autosomal dominant fashion but also autosomal recessive, X-chromosomal and mitochondrial DNA linked DCM exists. More than 15 genetic loci with 12 identified genes associated with isolated DCM or DCM accompanied by additional phenotypes (e.g. conduction abnormalities, skeletal myopathy) are presently known (*25,27-29*). Mutations involve mainly proteins of the cytoskeleton or sarcomere that result in deficits of force generation, force transmission or energy production, depending on the mutated protein (*30*).

ARVC, formerly called arrhythmogenic right ventricular dysplasia (ARVD), involves primarily the right ventricle with gradual replacement of myocardial tissue by fibrosis and fat and is clinically characterized by ventricular arrhythmias, heart failure and sudden death (*25,31*). Most ARVC families show an autosomal dominant pattern of inheritance. Six autosomal dominant and two autosomal recessive loci with two identified disease genes have so far been reported. The two autosomal recessive loci are associated with Naxos disease, characterized by ARVC, palmoplantar keratoderma and woolly hair (*25*). The mutations found in genes encoding plakoglobin and desmoplakin suggest myocellular apoptosis due to defective desmosomal cell junctions as the pathogenic mechanism of autosomal recessive ARVC (*32,33*). Features of ARVC have also been described in some cases with cardiac ryanodine receptor gene (*RYR2*) mutations (*34*).

2.3 Hypertrophic cardiomyopathy

2.3.1 Classification and prevalence

HCM is a primary myocardial disease characterized by myocardial hypertrophy in the absence of a co-existing condition, such as aortic valvular stenosis or hypertension (*35*). Since the first unequivocal recognition of its pathology in the late 1950s (*36*), multiple descriptive names of HCM, including idiopathic subaortic stenosis, asymmetric septal hypertrophy and hypertrophic obstructive cardiomyopathy, have been used (*13*).

Current estimates indicate that HCM is a relatively common disorder, with a prevalence of 0.2% in the general population (*37*) and 1% in primary cardiologic practice (*38*).

2.3.2 Major morphological, pathophysiological and clinical features

The classical morphological characteristics of HCM include asymmetric LVH usually located in the interventricular septum. Less frequently, hypertrophy may be located in the apex, free wall of the left ventricle, or papillary muscles, or present as concentric LVH. Histopathological findings include myocyte hypertrophy and disarray, and interstitial fibrosis. Additionally, malformations of the mitral valve, thickened intramural coronary arteries and increased ventricular chamber stiffness may appear in HCM (*2*).

The major pathophysiological findings attributable to HCM include left ventricular (LV) diastolic dysfunction, LV outflow tract obstruction (present in ~25% of cases), impaired coronary vasodilator reserve and myocardial ischemia, and supraventricular and ventricular arrhythmias (especially atrial fibrillation and ventricular tachycardia). The aforementioned features of HCM are related to the severity of symptoms and clinical course of the disease but have variable occurrence in individual patients (*2,17*).

The most common symptoms of HCM are dyspnea on exertion, chest pain, pre-syncope/ syncope and palpitations. Symptoms are often mild or absent and SCD upon exertion may be the first sign of HCM (*2,17*). In ~25% of HCM cases the hypertrophied septum causes obstruction at the subaortic or midventricular level, or both, often showing clinical signs such as biphasic carotid artery pulsation, prominent A wave in the jugular venous pulse, palpable double systolic impulse at the LV apex, fourth heart sound and a systolic ejection murmur on the left sternal border on auscultation. These clinical signs are often mild or absent in the non-obstructive form of HCM (*39*). Electrocardiographic (ECG) findings associated with HCM include LVH with repolarization changes, abnormal Q- and T-waves and ST-changes. ECG signs may be absent in mild forms of hypertrophy, but in general ECG and echocardiography have similar diagnostic values for HCM in adults (*39,40*). On echocardiography, LVH, most often involving the interventricular septum, and normal or reduced LV dimensions can usually be detected. LV ejection fraction (EF) is frequently preserved or enhanced in

HCM. Other echocardiographic signs of HCM include enlarged left atrium, mild mitral regurgitation and diastolic dysfunction, which is often detected regardless of the extent of LVH. Systolic anterior movement of the mitral valve and gradient at the LV outflow tract are typical findings in obstructive HCM (*1,13*).

The clinical course of HCM is more favorable than previously believed (*17*). The annual mortality rate of HCM in unselected populations is reported to be 1% or less (*16,41-43*), compared to 3-4% in referral center populations (*44*), often including patients with pronounced clinical phenotypes (*17*). HCM causes mortality most often by SCD. Progressive heart failure and stroke associated with atrial fibrillation are more unusual causes of death in HCM (*1*). SCD is usually caused by ventricular arrhythmias (*45*). Major risk factors for SCD in HCM include previous cardiac arrest or sustained ventricular tachycardia, repetitive or prolonged bursts of non-sustained ventricular tachycardia on Holter ECG, recurrent syncope, massive LVH (≥30 mm), impaired blood-pressure response in clinical exercise test, family history of HCM-related SCDs and adverse disease-causing mutation (*1,2,17,46*). Although no single test or risk factor can reliably predict the risk of SCD in HCM, patients with multiple risk factors of SCD have been shown to have a markedly increased risk of SCD (*47,48*), justifying consideration for primary prevention with an implantable cardioverter-defibrillator (ICD)(*3*). Importantly, the absence of the acknowledged risk factors of SCD predicts favorable prognosis in HCM, and generally more than half of HCM patients are considered to possess a low risk of SCD (*3,47*). Therefore, a risk stratification assessment of the presently known risk factors using non-invasive methods, including two-dimensional echocardiography, ambulatory Holter ECG and clinical exercise test, is currently recommended for most patients with HCM (*3*).

2.3.3 Diagnostics

The conventional criterion for the diagnosis of HCM used in index patients is the demonstration of LVH of 13-15 mm or more on two-dimensional echocardiography in the absence of secondary reasons for LVH, such as hypertension (*13*). For adult members of affected HCM families more sensitive criteria, including not only LVH detected in echocardiography but also other echocardiographic findings characteristic of

HCM, ECG abnormalities and cardiac symptoms, have been proposed (*49*) (see Table 2). The clinical signs of HCM usually do not develop until late adolescence (*50*), and mutations in certain genes (e.g. *TNNT2* or *MYBPC3*) have been shown to be associated with only mild or subclinical LVH, non-diagnostic findings on ECG and mild symptoms, averting clinical detection of some patients (*40*). On the other hand, conditions other than HCM either simulating or resulting in LVH (e.g. sigmoid septum of the elderly, LV false tendons, hypertension, physical training) may lead to a false positive echocardiographic diagnosis of HCM (*51*). Therefore, genetic testing of disease-related mutations improves diagnostic accuracy and allows preclinical diagnosis of HCM (*14*). However, due to the wide genetic heterogeneity of HCM, molecular diagnostics is currently restricted to those families with an identified disease-causing mutation.

2.3.4 Molecular genetics

2.3.4.1 General aspects

HCM is inherited in an autosomal dominant way, and a familial pattern of inheritance is seen in >50% of cases (*8*). All of the genes responsible for HCM encode myocardial contractile proteins, and therefore HCM is considered a disease of the sarcomere (*11*). In addition, mutations in genes encoding other than sarcomeric proteins are also known to result in cardiac phenotypes similar to HCM in patients with syndromes in which HCM is only one manifestation (*52*). The genetic background of HCM shows wide locus and allelic heterogeneity. To date, approximately 150 mutations in 11 genes encoding sarcomeric proteins have been reported (*8-10*) (Table 1). Both familial inheritance, and rarely, de-novo occurrence (*53,54*) of mutations in which neither parent of an affected patient carry the disease-related mutation, occur. De-novo mutations usually become familial, as they are inherited by the offspring (*8*). Most mutations causing HCM in distinct families have arisen independently, with rare occasions of founder effects (*55,56*). Causative mutations of HCM include mainly single-base pair (bp) substitutions resulting in amino acid substitutions in the encoded proteins. Nonsense mutations, splice site defects, deletions and insertions, all of which mostly result in truncated proteins, can also cause HCM. Truncation mutations are common

especially in the cardiac myosin-binding protein C gene (*MYBPC3*) (*57*). Mutations in the β-myosin heavy chain (*MYH7*), *MYBPC3* and cardiac troponin T (*TNNT2*) genes are estimated to most frequently cause HCM, whereas mutations in the other genes are uncommon. Collectively, mutations in the genes identified so far account for more than 2/3 of all cases of HCM (*8*).

Table 1. Sarcomere protein genes associated with HCM.

Gene	Symbol	Locus	Mutation type	%[1]	First report
β-myosin heavy chain	*MYH7*	14q11.2-q12	M, N, D	~30	(*7*)
Cardiac myosin-binding protein C	*MYBPC3*	11p11.2	M, N, S, D, I	~20	(*58,59*)
Cardiac Troponin T	*TNNT2*	1q32	M, S, D	~20	(*11*)
α-tropomyosin	*TPM1*	15q22.1	M	~5	(*11*)
Cardiac troponin I	*TNNI3*	19q13.4	M, D	~5	(*60*)
Myosin essential light chain	*MYL3*	3p21.3-p21.2	M	<5	(*61*)
Myosin regulatory light chain	*MYL2*	12q23-q24.3	M, S	<5	(*61*)
Cardiac α-actin	*ACTC*	15q14	M	<5	(*62*)
Titin	*TTN*	2q24.3	M	<5	(*63*)
Troponin C	*TNNC1*	3p21.3-p14.3	M	?	(*9*)
α-myosin heavy chain	*MYH6*	14q12	M	?	(*10*)

[1] Estimated percentage of HCM attributable to this locus according to Marian et al. (*8*). D, deletion; I, insertion; M, missense; N, nonsense; S, splice defect.

2.3.4.2 Disease-causing genes

β-myosin heavy chain gene (*MYH7*)

MYH7 is the first mapped gene found to be responsible for HCM and is located on chromosome 14q12 (*6,64*). It is composed of 38 coding exons encompassing ~23kb of DNA (*65,66*). *MYH7* encodes β-myosin heavy chain (β-MyHC), which is the major isoform of the human ventricle and of slow-twitch skeletal muscle (*22*). Mutations of *MYH7* are globally the most common cause of HCM, accounting for approximately 30% of all cases (*8*). More than 60 mutations, mostly resulting in amino acid

substitutions, have been reported (reviewed in (*13,24*)). With few exceptions (*67*), the mutations are located in the region encoding the globular head and head-rod junction of the protein, with codons 403 (*68*) and 719 (*69*) considered as mutational hot spots (*8*). Recently, *MYH7* mutations resulting in DCM have also been reported (*70*).

Cardiac myosin-binding protein C gene (*MYBPC3*)

MYBPC3 is located on chromosome 11p11 (*71,72*). It consists of 34 coding exons with ~21kb of DNA (*57*). The encoded protein (MyBPC) is specifically expressed in the human myocardium (*73,74*). *MYBPC3* is estimated to be the second most common gene for HCM, accounting for ~20% of all cases of HCM (*8*). So far, more than 30 mutations, most of which result in truncated proteins, have been described (reviewed in (*13*)).

Cardiac troponin T gene (*TNNT2*)

TNNT2, located on chromosome 1q32 (*75,76*), is composed of 16 coding exons spread over 17 kb and encodes several isoforms in the heart through alternative splicing of the single gene (*76,77*). *TNNT2* is considered the third most common gene for HCM, accounting for ~20% of cases (*8*). More than 10 missense mutations, one splice site defect and one deletion have been reported in *TNNT2* (*8,13*). Three of the missense mutations are located at codon 102, which is considered a mutational hot spot (*78*). A deletion in *TNNT2* has also been reported to cause DCM (*70*).

α-tropomyosin gene (*TPM1*)

TPM1 is located on chromosome 15q22 (*79,80*) but its complete genomic organization has not been reported in humans (*24*). Several isoforms of *TPM1* generated by alternative splicing of the 15 exons exist, and the cardiac isoform, expressed in the ventricular myocardium and in fast-twitch skeletal muscles, is encoded by 9 exons (*81*). Mutations of *TPM1* are estimated to be relatively infrequent causes of HCM, six reported missense mutations (*11,82-85*) accounting for ~5% of HCM (*8*). Recently, two novel missense mutations of *TPM1* were reported to cause DCM (*27*).

Cardiac troponin I gene (*TNNI3*)

TNNI3, located on chromome 19q13, consists of eight coding exons encompassing 6.2 kb of genomic DNA (*86,87*). The isoform encoded by *TNNI3* is specifically expressed in cardiac muscle (*88*). To date, nine mutations, seven of them missense mutations and two deletions, have been reported for *TNNI3* (*10,60,89*), accounting for ~5% of HCM (*8*).

Myosin essential light chain (*MYL3*) and regulatory light chain (*MYL2*) genes

MYL3 is composed of six coding exons, located on chromosome 3p21 (*90*). *MYL3* encodes ventricular myosin essential light chain (MLC-1), expressed both in the myocardium and in the slow skeletal muscles (*91*). Three missense mutations in *MYL3* have been reported (*61,92*). *MYL2*, located on chromosome 12q23-q24, includes seven coding exons encompassing ~12 kb of genomic DNA (*93*). The encoded protein myosin regulatory light chain (MLC-2) is expressed in both ventricular heart muscle and in the slow skeletal muscles (*24*). Thus far, one splice site defect and seven missense mutations have been identified in *MYL2* (*61,94,95*). Mutations in the myosin light chain genes (*MYL3* and *MYL2*) are estimated to be rare, accounting for <5% of HCM (*8*).

Cardiac α-actin gene (*ACTC*)

ACTC is comprised of six exons located on chromosome 15q14 (*96,97*). The encoded protein cardiac α-actin is expressed mostly in the myocardium and less extensively in a variety of tissues (*13*). Recently, four missense mutations were reported in *ACTC* (*62,98*). Mutations of *ACTC* are estimated to be a rare cause of HCM, accounting for less than 5% of all cases (*8*). However, *ACTC* mutations have also been identified as a cause of DCM (*99*).

Titin (*TTN*), troponin C (*TNNC1*) and α-myosin heavy chain (*MYH6*) genes

TTN, located on chromosome 2q24, is the largest of the HCM-associated genes, with 363 exons. *TTN* encodes a giant protein expressed in both cardiac and skeletal muscles (*100-102*). *TNNC1*, located on chromosome 3p21-14, consists of six coding exons and encodes a protein expressed in heart and slow-twitch skeletal muscles (*103,104*). *MYH6*,

which is located only ~4 kb downstream of *MYH7* on chromosome 14q12, encodes the α-myosin heavy chain (α-MyHC). α-MyHC has over 90% homology with β-MyHC, which is encoded by *MYH7* (*65,105*). α-MyHC is the predominant isoform expressed in the human cardiac atria (*22*).

TTN, *TNNC1* and *MYH6* are the most recently reported HCM genes. However, there is only limited evidence that these genes are associated with HCM, as only one missense mutation in each gene in patients with sporadic HCM have been reported (*9,10,63*). Satoh et al. (*63*) reported a missense mutation of *TTN* (found in one of 82 HCM patients screened) that was associated with increased binding affinity of titin to α-actinin in a yeast two-hybrid assay. More recently, a missense mutation was reported in *TNNC1* (found in a single patient) (*9*) and *MYH6* (found in 1 of 31 patients screened) (*10*). However, previous studies by Kimura et al. (*60*) and Regitz-Zagrosek et al. (*106*) did not identify any disease-related mutations in *TNNC1* among 184 and 120 probands with HCM, respectively. No other studies regarding mutation screening in *TTN*, *TNNC1* or *MYH6* have been published, and thus, HCM-related mutations in these genes are probably uncommon (*8*). However, a deletion in *TTN* was recently reported to cause familial DCM (*28*).

Non-sarcomeric genes

Mutations in genes encoding other than sarcomeric proteins resulting in cardiac phenotypes similar to HCM have been identified in patients with syndromes in which HCM is only one manifestation. Several mutations in mitochondrial DNA have been associated with maternally inherited HCM accompanied by multi-organ disorders (e.g. neurologic and endocrine disorders) (*107-109*). Co-existence of mitochondrial DNA and *MYH7* mutations in HCM patients with late congestive heart failure has also been reported (*110*). In addition, HCM-like cardiac manifestations have been reported as one feature in two triplet repeat syndromes, myotonic dystrophy and Friedreich ataxia, caused by trinucleotide repeat expansions in genes encoding dystrophia myotonica protein kinase (*DMPK*) and frataxin (*FRDA*), respectively (*52,111,112*). Furthermore, two mutations in the gene encoding γ2-subunit of protein kinase A (*PRKAG2*) located on chromosome 7q36 were recently identified in two families with HCM accompanied

by Wolff-Parkinson-White (WPW) syndrome (*12,113*), previously linked to chromosome 7q3 (*114*). Shortly afterwards, another two mutations of the same gene were reported to cause plain WPW syndrome (*115,116*). However, just recently, Arad et al. reported that mutations of *PRKAG2* do not cause HCM, but rather lead to a novel glycogen storage disease mimicking HCM (*117*).

2.3.5 Molecular pathogenesis

Mutations in sarcomeric proteins lead to abnormalities in the sarcomere structure and/or function that eventually lead to the development of the clinical manifestations of HCM. *In vitro* functional studies using animal and human tissues have demonstrated various defects caused by mutated proteins, and the clinical manifestations of HCM have been recapitulated in several transgenic, knock-out and knock-in animal models of HCM (reviewed in (*8,24,118,119*)). The first and currently the best-characterized *in vivo* animal model of HCM is a mouse line harboring the Arg403Gln mutation in the murine α-MyHC gene (which is the predominant ventricular myosin isoform in mice, in contrast to β-MyHC in humans (*22*)), and corresponds to the identical *MYH7* mutation causing HCM in humans (*120*). The α-MyHC knock-in mice showed myocyte disarray, interstitial fibrosis and cardiac dysfunction and also mild cardiac hypertrophy. Heterozygous mice survived for one year but those homozygous for the mutation died after a few days. Subsequently, transgenic animal models expressing human HCM-related mutations in *TNNT2*, *MYBPC3*, *MYL3*, *TPM1* and *TNNI3*, with variable recapitulation of the human HCM phenotype, have been generated (*8*). The first animal models to express mutant β-MyHC (Arg403Gln) were transgenic rabbits that showed full resemblance of human disease, including marked LVH (*121*).

As several types of mutations (i.e. missense mutations or mutations resulting in truncated proteins) can cause HCM, there are at least two different mechanisms by which mutations could result in HCM. According to the "poison polypeptide" hypothesis, mutant sarcomeric proteins incorporate into the sarcomere and act as dominant negative proteins (*8*). Alternatively, the "haplo-insufficiency" hypothesis, postulated for mutations resulting in truncated proteins (*122*), suggests that HCM is

caused by insufficient incorporation of mutated protein into myofibrils, and thus, insufficient amount of sarcomeric proteins (*8*). Current data from experimental studies indicate that the mutated proteins usually incorporate into the sarcomere and cause HCM by dominant negative action (*20*).

The morphological manifestations of HCM (i.e. myocardial hypertrophy, sarcomeric disarray and interstitial fibrosis) are believed to be secondary to functional defects triggered by mutations. In experimental studies, mutant sarcomeric proteins have been shown to cause a variety of mechanical (e.g. reduced myocyte performance and acto-myosin interaction), biochemical (e.g. impaired ATPase activity, altered Ca^{2+} sensitivity) and structural (e.g. sarcomere dysgenesis, altered stoichiometry of the sarcomeric proteins) initial defects in the sarcomere, depending on the mutated protein and the mutation-induced structural defect in it (*8*). Mutations of *MYH7* as well as some other genes frequently show mechanical dysfunction between the thick and thin filaments and reduced actin-activated ATPase activity (*8*). Increased Ca^{2+} sensitivity of force development is observed in most mutations affecting thin filament proteins (*23*). Recently, Morimoto et al. reported that in rabbit cardiac muscle fibers a *TNNT2* mutation responsible for HCM results in Ca^{2+} sensitization of force generation, whereas another *TNNT2* mutation causing DCM leads to Ca^{2+} desensitization, suggesting altered Ca^{2+} sensitivity as the primary mechanism of both HCM and DCM involving *TNNT2* mutations (*123*). In some experimental studies mutated proteins have been shown to cause impaired sarcomere assembly and dysgenesis. (*124,125*).

The cascade of events, triggered by distinct mutations, at cellular and molecular level leading to HCM is unknown. Some theories for the mechanism of HCM common to all mutations exist. Recently, Marian (*126*) proposed that mutated sarcomeric proteins impair cardiac myocyte contractility, resulting in increased myocyte stress which contributes to activation of stress-responsive trophic and mitotic factors common to all forms of cardiac hypertrophy in the myocardium, which in turn leads to the phenotypic manifestations of HCM. Activation of Ca^{2+} sensitive signaling molecules due to altered Ca^{2+} sensitivity may also have a critical role in myocyte hypertrophy (*8,20*). Inability to maintain normal ATP levels in the myocyte, leading to a failure of

energy-dependent homeostatic mechanisms and myocyte hypertrophy, has also been suggested as a common pathogenic mechanism for HCM (*12*).

2.3.6 Genotype-phenotype correlations

The clinical phenotype of HCM is influenced by the primary gene defect, modifying genetic factors and environmental factors (*8*). Identification of the causal mutations of HCM has allowed the characterization of phenotypes associated with distinct mutations. Genotype-phenotype studies are important in risk stratification of HCM, particularly with respect to SCD (*15,127*). The phenotype of HCM and the risk of SCD associated with distinct primary defects is variable, depending on the affected gene, and the type and location of the mutation in it (*15*). In a study by Watkins et al. missense mutations of *MYH7* resulting in a charge change were associated with shorter life expectancy compared to neutral substitutions (*128*). Furthermore, homozygous (*129-131*) and compound/double heterozygous (*132,133*) mutations result in a more pronounced phenotype of HCM and increased risk of SCD. However, the clinical phenotype of HCM is often highly variable even in patients carrying identical disease-causing mutations (*8*). Therefore, prediction of clinical outcome of HCM based on the disease-related mutation only is of limited value.

Mutations of *MYH7* are usually associated with more extensive hypertrophy and an earlier onset of the disease compared to mutations of *MYBPC3* (*134,135*). However, distinct *MYH7* mutations present variable clinical phenotypes and prognosis (*69,128,136-140*). *MYH7* mutations Arg403Gln (*128,141,142*) and Arg719Trp (*69,143*) are associated with high penetrance and a marked risk of SCD, whereas mutations Val606Met (*128,137*) and Leu908Val (*141*) have near-normal life expectancy. Mutations of *MYBPC3* (*135,144,145*) and *TPM1* (*83,146*) are generally associated with mild to moderate LVH, low penetrance and a relatively low risk of disease-related death. *MYBPC3* defects frequently show age-related penetrance, characterized by late onset of the disease and favorable prognosis, but with increasing risk of SCD with age (*135,144,147*). Some *TPM1* mutations with a poor prognosis have also been reported (*83,85*). Although most mutations of *TNNT2* present only mild or subclinical LVH, they are frequently associated with a high risk of SCD (*148-151*). Due to recent identification

and relatively infrequent occurrence of mutations in the other HCM genes, the genotype-phenotype correlations of these genes are largely unknown (*15*). Some mutations of *MYL2* and *MYL3* may be associated with papillary muscle hypertrophy and LV mid-cavity obstruction (*61*), whereas defects of *TNNI3* have been reported to be associated with apical hypertrophy in Asian HCM patients (*60*). Furthermore, mutations of *TNNI3* and *PRKAG2* have been reported to result in HCM accompanied by WPW syndrome (*12,60*).

The variable clinical expression of HCM is partly explained by modifying genetic factors, referred to as modifier genes (*8*). The most widely studied genetic modifier of LVH is an insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme-1 gene (*ACE-1*) (*152*). In ungenotyped patient groups with HCM, the I/D-polymorphism of *ACE-1* has been shown to be associated with the risk of SCD (*153*) and the magnitude of LVH (*154*). Variants in genes encoding endothelin-1, tumor necrosis factor-α and angiotensin II receptor-1 have also been shown to modify the phenotypic expression of HCM (*155-157*). In genotyped patient groups, the influence of the *ACE-1* I/D-polymorphism on HCM phenotype seems to be dependent on the disease-related mutation (*158*).

2.4 Population history of Finland

Archeological evidence of human activity in Finland date back more than 9000 years (*159,160*). The single-origin theory of the inhabitation of Finland suggests the founding of the country ~2000 years ago by a single group of founders from the south (*161,162*). However, the dual theory, supported by common ancestral Y chromosome haplotypes, assumes an occurrence of an earlier immigration wave from the east some 4000 years ago (*163*), followed by another group of settlers from the south. The present population of Finland is descended mainly from a small number of founders immigrated from the south over the Gulf of Finland approximately 2000 years ago (*161-165*). The coastal parts of southern and western Finland (early settlement area) were first inhabited, and for centuries, the population was concentrated on these areas with little internal population movement. Internal migration from a small southeastern area toward the middle, western and finally northern and eastern parts of the country (late settlement

area) began in the 16th century, resulting in isolated rural populations. Famine and disease epidemics resulted in a one-third reduction in population size (from 400,000 to ~250,000), but since the 18th century population growth was fast, leading to a ~20-fold increase in population size (from 250 000 to ~5 million) in few hundred years (*165*).

The population history of Finns, characterized by a small number of founders, isolation, population bottlenecks and rapid population expansion, is reflected in the genetics of hereditary diseases (*165*). The term Finnish disease heritage refers to a group of inherited disorders that are enriched or only encountered in Finland and, on the other hand, the lack or relatively low frequency of some diseases more frequent in other populations (*166*). This group currently includes more than 30 diseases, most of which are rare, have autosomal recessive pattern of inheritance, and mostly single major causative mutation with a common ancestral origin (*160,165*). Furthermore, more prevalent and usually dominantly inherited diseases, such as familial hypercholesterolemia (FH), are mainly caused by only a few founder mutations in the Finnish population (*160,167*). A single FH mutation accounts for almost 90% of cases in an eastern subpopulation of North Karelia (*168*). Recently, two Finnish founder mutations causing long QT syndrome (LQTS) were reported (*169,170*). The more common mutation, which clustered in families originated from eastern Finland, accounted for 30% of Finnish cases with LQTS (*170*).

In previous studies HCM has been reported to be caused mostly by numerous distinct mutations with rare occurrence of founder events (*55*). Given the special population history of Finns and the genetic findings of many other hereditary diseases in Finland, the genetic background of HCM in the Finnish population is likely to be characterized by a few major mutations, reflecting a founder effect.

3. AIMS OF THE STUDY

The main purpose of this study was to investigate the genetic background of HCM in patients from eastern Finland. Also, the clinical features of HCM and the clinical characteristics of the identified disease-causing mutations were studied. Specifically, the following questions were addressed:

Do mutations of the β-myosin heavy chain gene (*MYH7*) and the α-tropomyosin gene (*TPM1*) cause HCM in patients from eastern Finland? (Study I)

Do mutations of the cardiac myosin-binding protein C gene (*MYBPC3*) cause HCM in patients from eastern Finland? (Study II)

Do mutations of the cardiac troponin I gene (*TNNI3*) cause HCM in patients from eastern Finland? (Study III)

Do mutations of the genes encoding cardiac troponin T (*TNNT2*), myosin light chains (*MYL3* and *MYL2*) and troponin C (*TNNC1*) cause HCM in patients from eastern Finland? (Study IV)

What is the genetic profile of HCM and what are the clinical characteristics of HCM in patients from eastern Finland as well as the clinical characteristics attributable to the mutations identified? (Study IV)

4. SUBJECTS AND METHODS

4.1. Subjects

4.1.1 Index patients with HCM (Studies I-IV)

All patients aged ≥16 years with suspected or confirmed HCM from the Kuopio University Hospital region, covering a population of approximately 250,000, are sent to the Division of Cardiology for diagnosis and treatment. 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. Of 48 subjects evaluated initially, 12 were excluded because they did not meet the diagnostic criteria for definite HCM. The original study group included 36 probands, of which 35 resided in eastern Finland and one proband in the adjacent county in central Finland. All but one of the probands fulfilled the diagnostic criteria for definite HCM. One proband with concomitant hypertension, but with a definite family history of HCM, was included in the original study group (n=36; Study I). Subsequently, two of the 36 probands were found to be related (first cousins), and therefore, these two families were united as a single family. These 35 probands and two newly identified probands with definite HCM were included in the subsequent genetic screenings (n=37; Studies II-III). Finally, these 37 probands and four additional newly identified probands with definite HCM were included in Study IV (n=41). Thus, 35 of the 41 probands were included in the genetic screening in all studies I-IV, and subsequently these probands (n=35) are referred to as the nuclear study group.

4.1.2 Relatives of the index patients (Studies I-IV)

All available first-degree relatives (aged 16 years or more) of the index patients were recruited to the study based on family information provided in a questionnaire by the probands. The probands included in the nuclear study group (n=35) had 155 living first-degree relatives, of which 131 were evaluated. In addition, 105 second-degree or more distant relatives (from 18 families) of the probands were included in the study. Altogether, 236 relatives of the probands of the nuclear study group were evaluated. All relatives were evaluated clinically and were included in the screening of variants as well

as in linkage and haplotype analyses, when appropriate. In study IV, 53 clinically affected relatives of the probands and all identified genetic carriers of disease-causing mutations were included in the analysis of clinical features of HCM. Furthermore, two Finnish HCM families from other areas of Finland and not belonging to the original study group, one carrying a *MYBPC3* mutation (Gln1061X; 4 subjects) and another carrying a *TPM1* mutation (Asp175Asn; 4 subjects), respectively, were included in the analysis of clinical characteristics and haplotype studies of these specific mutations.

4.1.3 Control subjects (Studies II-IV)

Controls were healthy unrelated subjects from our previous population studies (*171-173*). In studies II-III, the frequency of identified genetic variants was tested in 111 control subjects (83 men, 28 women). Study IV included 37 controls (37 men). Control subjects did not have any chronic disease, including diabetes, hypertension, obesity or coronary heart disease. Echocardiography excluding HCM had been performed previously on 82 of these subjects.

4.2 Methods

4.2.1 Clinical evaluation of the index patients and relatives

All subjects underwent an interview, physical examination, 12-lead ECG and M-mode, two-dimensional and Doppler echocardiography. 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 minutes of bed rest with a 2-minute interval. The latter value was used in statistical analyses. All echocardiograms were performed by the same cardiologist with a Hewlett-Packard Sonos 1000 or 5500 scanner (Palo Alto, CA, USA) with a 2.5 MHz transducer. All measurements were performed according to the standards of the American Society of Echocardiography (*174*). In Studies I and III M-mode tracings of the index patients were measured by using a digitizing table (MM 1201, Summagraphics Co., Fairfield, CT, USA; resolution 0.1 mm).

Table 2. Proposed diagnostic criteria for HCM in adult members of affected families according to McKenna et al. (*49*) used in the clinical diagnosis of relatives.

Major criteria	Minor criteria
Echocardiography	
Left ventricular wall thickness ≥ 13 mm in the anterior septum or posterior wall or ≥ 15 mm in the posterior septum or free wall	Left ventricular wall thickness of 12 mm in the anterior septum or posterior wall or of 14 mm in the posterior septum or free wall
Severe systolic anterior motion of the mitral valve (septal-leaflet contact)	Moderate systolic anterior motion of the mitral valve (no leaflet-septal contact)
	Redundant mitral valve leaflets
Electrocardiography	
Left ventricular hypertrophy + repolarization changes	Complete bundle branch block or (minor) interventricular conduction defect
T-wave inversion in leads I and aVL (≥ 3 mm) (with QRS-T wave axis difference ≥ 30°), V3-V6 (≥ 3 mm) or II and III and aVF (≥ 5 mm)	Minor repolarization changes in LV leads
Abnormal Q (> 40 ms or > 25% R wave) in at least 2 leads from II, III, aVF (in absence of left anterior hemiblock), V1-V4; or I, aVL, V5-V6	Deep S V2 (> 25 mm)
	Unexplained chest pain, dyspnea or syncope

The diagnosis of HCM is fulfilled by the presence of one major criterion (echocardiographic or electrocardiographic), two minor echocardiographic criteria, or one minor echocardiographic criterion with two minor electrocardiographic criteria

The diagnosis of HCM in the index patients was based on demonstration of maximal LV wall thickness of at least 15 mm on two-dimensional echocardiography without other causes for LV hypertrophy, such as hypertension or valvular heart disease. 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. For adult relatives (≥16 years of age) of the index patients, the diagnosis of HCM was based on the diagnostic criteria by McKenna et al. (*49*) (Table 2). In general, relatives were diagnosed to have HCM mostly based on LV wall thickness of at least 13 mm on two-dimensional echocardiography, or pathological Q-waves on ECG. Relatives with LV thickness of at least 13 mm but also with definite or borderline hypertension, body mass index ≥35 kg/m^2, or other secondary causes for

LVH were classified as having suspected HCM. Relatives <16 years of age (in Study I) were diagnosed to have HCM by a pediatric cardiologist if the thickness of the interventricular septum on echocardiography exceeded the average values of subjects with similar body surface area by at least 2 SDs (*175*) and if they had ECG signs of LV hypertrophy or symptoms supporting the diagnosis.

All probands, 38 of 53 clinically affected relatives and 17 other subjects harboring disease-related mutations underwent a 24-h Holter ECG registration. In addition, 15 of 35 probands, 25 of 53 clinically affected relatives and 9 other subjects with disease-related mutations underwent a standard clinical exercise test (Study IV). Blood samples for genetic analyses were collected from all subjects evaluated.

4.2.2 Genetic analyses

4.2.2.1 DNA extraction

DNA was prepared from peripheral blood leucocytes by using previously described proteinase K-phenol-chloroform (*176*) and salt precipitation (*177*) methods, with some modifications.

4.2.2.2 Polymerase chain reaction

Polymerase chain reactions (PCR) were performed with thermo cyclers (PTC-100 Programmable Thermal controller, MJ-research Inc., Watertown, MA, USA or Uno-Thermoblock, Biometra, Göttingen, Germany). Intronic sets of primers for the genes screened were designed according to the published genomic sequences available at Genbank (*178*), or alternatively, previously published primers were used (Table 3). Exons and exon-intron junctions of the genes were amplified. PCR amplifications for the subsequent mutation screening were done in a volume of 5.6-6μl (10 μl in Study I) containing 30-50 ng of genomic DNA, 3 pmol (5 pmol in Study I) of each primer, 10 mmol/l Tris/HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l of $MgCl_2$, 0.1% Triton X-100, 200 μmol/l dNTP and 0.15 units (0.25 U in Study I) of DNA polymerase (Dynazyme DNA polymerase, Finnzymes, Espoo, Finland). PCRs were radioactively labeled by adding 1.0 μCi of α-^{32}P dCTP (Study I) or 0.25-0.7 μCi of α-^{33}P dCTP (Studies II-IV)

(NEN Life Science Products, Boston, MA, USA) into the mix. PCR conditions were optimized by modifying the annealing temperatures and extension times. The PCR program included a denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 55-66°C for 30-60 seconds and extension at 72°C for 30-60 seconds with final extension at 72°C for 4 minutes.

Table 3. List of the genes screened in Studies I-IV and accession numbers of the GenBank sequences used in primer design.

Study	Gene	Number of coding exons	Exons studied	GenBank sequence(s) used in primer design
I	*MYH7*	38	3-26, 40	X52889, M57965
I	*TPM1*	9	1a-9a,b	See ref. (*11*)
II	*MYBPC3*	34	1-35	Y10129, U91629
III	*TNNI3*	8	1-8	X90780
IV	*TNNT2*	16	2-17	AY044273
IV	*MYL2*	7	1-7	Z15030
IV	*MYL3*	6	1-6	M24242-47
IV	*TNNC1*	6	1-6	M37984

4.2.2.3 Single-strand conformation polymorphism analysis

Single-strand conformation polymorphism analysis (SSCP) (*179*) was used to screen the genes of interest for sequence variants. To obtain fragments <270 bp long, radiolabeled PCR products were digested with an appropriate restriction enzyme if necessary. PCR products were first diluted 2-20 fold with 0.1% SDS and 10 mmol/l EDTA and then diluted (1:1) with loading mix (95% formamide, 20 mmol/l EDTA, 0.05% bromphenolblue, 0.05% xylene cyanol). After denaturation at 98°C for 3 minutes, samples were immediately cooled on ice and 2-3 μl of each sample were loaded onto a 5-6% nondenaturing polyacrylamide gel (acrylamide/ N,N -methylene-bis-acrylamide ratio 49:1) containing 10% glycerol. Samples were run at two different gel temperatures: 1) at 38°C for ~4 hours and 2) at 29°C for ~5 hours. The gel was dried on filter paper and autoradiographed at -20°C or -70°C with intensifying screens.

4.2.2.4 Sequencing

DNA fragments with variant SSCP conformers were reamplified with PCR using the same primers and purified by electrophoresis on a 1-2% low-melting-point agarose gel. Sequencing reactions were performed by using a radiolabeled (^{35}S or ^{33}P) Sanger dideoxy chain termination reaction as a non-cyclic reaction (Sequenase 2.0 Sequencing Kit, US Biochemicals, Cleveland, OH, USA) (Study I) or as a cyclic reaction (Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit, US Biochemicals, Cleveland, OH, USA) (Studies II-III). The sequencing reactions were visualized by polyacrylamide gel electrophoresis, followed by autoradiography. In Studies II and IV, fluorescein labeled cyclic reaction (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit) was used, followed by analysis with an automated laser fluorescence sequencer (ABI Prism 310 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA).

4.2.2.5 Specific DNA and RNA analyses

To confirm the presence of identified sequence variants and to allow simple and fast genotyping of relatives of affected probands or control subjects, PCR samples containing the variant of interest were digested with specific restriction enzymes, when available, and electrophoresed on a 2-3% agarose gel (NuSieve GTG, FMC Bioproducts, Rockland, ME, USA) and visualized by ethidiumbromide staining (*MYH7*-Arg719Trp, Asn696Ser, *MYBPC3*-IVS5-2A→C, IVS14-13G→A, Gln1061X). When suitable restriction enzyme was not available, direct sequencing with both forward and reverse primers was used (*TPM1*-Asp175Asn). The presence of the deletion Ex25ΔLys in *MYBPC3* was confirmed by sequencing the PCR fragments cloned into pBluescript SK +/- (Stratagene Inc., La Jolla, CA, USA) using an automated laser fluorescence DNA sequencer (ALFexpress, Amersham Pharmacia Biotech, Uppsala, Sweden). To investigate the possible aberrant mRNA splicing of *MYBPC3* due to *MYBPC3* mutation IVS14-13G→A total RNA from a patient carrying this defect was isolated from peripheral blood leucocytes by using TRIzol Reagent (Gibco BRL, Life Technologies, Grand Island, NY, USA). The cDNA synthesis and first round of PCR using outer

primers was performed with the Titan One Tube RT-PCR system (Roche Diagnostics, Indianapolis, IN, USA). The PCR products were diluted 1:1000 and used as a template in a second round of PCR, which was done using nested primers. cDNA-PCR fragments amplified from a patient with the *MYBPC3*-IVS14-13G→A mutation and from a genetically unaffected control were purified and sequenced with ABI Prism 310 Genetic Analyzer (PE Applied Biosystems).

4.2.2.6 Detection of polymorphic markers and haplotype analysis

Primers for extragenic or intragenic microsatellite markers (dinucleotide repeats) located at the region of interest were synthesized. One of the primers was labeled with fluorescein during synthesis. The polymorphic DNA fragments were amplified with PCR and analyzed with an automated laser fluorescence DNA sequencer (ALFexpress, Amersham Pharmacia Biotech). To investigate the possible common ancestral origin between families with identical mutations, haplotype analysis was carried out by using intragenic or extragenic microsatellite markers situated in, or near, the gene of interest. Haplotypes were constructed manually.

4.2.2.7 Linkage analysis

Linkage analysis was performed by using intragenic polymorphic microsatellites (Study I) or identified gene defects (Study II) as markers. Two-point pairwise linkage analysis was performed by using the MLINK module of the LINKAGE 5.2. package (*180,181*). In linkage analysis, autosomal dominant inheritance pattern with a disease-penetrance of 95% (Study I) or 90% (Study II) was used. Allele frequencies for the markers were estimated from control subjects. Subjects with an uncertain diagnosis of HCM were assigned an unknown diagnosis in the analyses. A LOD score ≥ 3 or ≤ -2 was considered significant.

4.2.3 Statistical analyses

All statistical analyses were performed with the SPSS/Win statistical program (SPSS Inc, Chicago, IL, USA). Differences between two groups were compared with the chi-

square test or the Fisher's test for categorical variables and with the Student's *t*-test for continuous variables. All data are presented as means ± SEM (Study I-III) or means ± SD (Study IV). P-value <0.05 was considered statistically significant.

4.3 Approval of ethics committee

Informed consent was obtained from all subjects. The study protocol was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital and was in accordance with the Helsinki Declaration.

5. RESULTS

5.1 Genes with disease-related mutations (Studies I, II)

5.1.1 *MYH7* and *TPM1* (Study I)

In Study I, 36 unrelated probands with HCM were screened for variants in exons 3-26 and 40 of *MYH7* and in the nine exons of *TPM1* by using the PCR-SSCP method. Two-point linkage analysis between *MYH7* and HCM was performed using two intragenic polymorphic microsatellite markers MYO I and MYO II in 16 families with familial HCM.

A previously reported Arg719Trp missense mutation (C to G substitution) in exon 19 of *MYH7* (*69*) was found in one proband and two relatives, accounting for ~3% (1/36) of all cases in the study group of Study I. A clinically affected mother (in whom a sigmoid septum with a false tendon structure was revealed in a later echocardiographic study) of the proband with the Arg719Trp mutation did not carry the defect, whereas two of the proband's young children (age 1 and 2 years, respectively), of whom the older one had a clinical diagnosis of HCM, inherited the mutation. Because the proband's father did not have a history of HCM (he was not clinically or genetically evaluated in our study), the Arg719Trp may have been a de-novo mutation, or alternatively the mutation was inherited from the father. Furthermore, a novel Asn696Ser amino acid substitution (A to G substitution), located in a conserved region of *MYH7* encoding residues critical for ATP and actin binding, was found in one child with HCM from the Kuopio University Hospital area not belonging to the study group of 36 adult probands. No evidence of definite linkage was found between the *MYH7* markers MYO I and MYO II and HCM in any of the 16 families studied. In six families individual LOD scores for either MYO I or MYO II indicated the exlusion of linkage between *MYH7* and HCM but the LOD scores for the rest of the families provided little or no evidence supporting or refuting linkage to the *MYH7* locus. In addition, some silent polymorphisms were found in *MYH7*.

In *TPM1*, a previously reported Asp175Asn missense mutation (G to A substitution) in exon 5 (*11*) was identified in four probands and 16 relatives, accounting for ~11% (4/36) of HCM cases in the study group of Study I. No other variants were found in *TPM1*.

5.1.2 *MYBPC3* (Study II)

The entire coding region of *MYBPC3* encompassing 35 exons was screened with the PCR-SSCP method in 37 unrelated patients with HCM. Two-point LOD scores for each identified variant were calculated.

In addition to numerous silent variants, seven novel (Gln1061X, IVS5-2A→C, IVS14-13G→A, Ex25ΔLys, Pro147Leu, Ser236Gly, Arg1138His) and two previously reported (Arg326Gln, Val896Met) variants, all of which are predicted to affect the structure of the encoded protein, were identified (Table 4). Four of the nine variants, a nonsense mutation Gln1061X, a splice acceptor mutation (IVS5-2A→C), a substitution in intron 14 (IVS14-13G→A), and a 3-bp deletion in exon 25 (Ex25ΔLys) were concluded to be disease-causing mutations because they co-segregated with the HCM phenotype or were absent in more than 200 normal chromosomes, or both. Furthermore, all of these four mutations were located at evolutionary conserved regions of *MYBPC3*. Altogether, the four HCM-associated mutations accounted for ~24% (9/37) of all cases in the study group of Study II (Table 4).

A novel C to T substitution at codon 1061 in exon 29 of *MYBPC* was expected to exchange glutamine for a premature stop codon (Gln1061X) that would lead to expression of truncated protein lacking the important binding sites for myosin and possibly titin. Gln1061X was the most frequently found mutation in the study group, being present in six separate families (6 probands and 17 relatives; Figure 2) and accounting for ~16% (6/37) of all cases studied. The mutation co-segregated with HCM in families, was found in a few healthy carriers and was absent from 111 control subjects. However, because of small family sizes, healthy carriers and clinically suspected cases, two-point LOD scores calculated for the Gln1061X mutation in each of the affected families (+0.30, +1.45, -0.44, -0.21, +0.00, -1.70) showed no evident linkage between the mutation and HCM.

Three additional novel mutations (IVS5-2A→C, IVS14-13G→A, Ex25ΔLys) were identified. The defect IVS5-2A→C was located at the splice acceptor site of intron 5, and was expected to impair the function of the splice site, resulting in a truncated protein lacking the binding sites for myosin and titin. The defect IVS14-13G→A

created a cryptic splice acceptor consensus sequence (cag) 11 bp upstream of the consensus splice acceptor site of intron 14. The aberrant splicing of *MYBPC3* mRNA caused by the IVS14-13G→A mutation was ascertained by mRNA/cDNA analysis of an affected patient. The 3-bp deletion in exon 25 was located at evolutionary conserved region of *MYBPC3*, and was expected to result in deletion of a lysine from the encoded protein without disruption of the reading frame. Each of the three mutations were present in single families, each defect accounting for ~3% (1/37) of all cases in the study group. The IVS5-2A→C mutation showed clear co-segregation with HCM (LOD score +1.17). Due to small family sizes and the presence of healthy carriers and clinically suspected patients, however, limited evidence of co-segregation existed with the IVS14-13G→A and Ex25ΔLys mutations (LOD scores +0.15 and -0.70, respectively).

Two defects previously reported as disease-causing mutations (Arg326Gln, Val896Met) and three novel (Pro147Leu, Ser236Gly, Arg1138His) missense variants were also found, but none of these variants clearly co-segregated with HCM and all of them were present in controls. Results of the two-point linkage analysis of these variants showed no evidence supporting or refuting linkage to the *MYBPC3* locus. Only one of the five variants (Arg1138His) was located at a highly evolutionary conserved residue of *MYBPC3*. In addition, one clinically unaffected individual homozygous with respect to the Val896Met substitution, and two clinically unaffected individuals homozygous with respect to the Arg1138His substitution were found. Therefore, these variants were suspected to be neutral polymorphisms. Furthermore, each of the three novel missense variants (Pro147Leu, Ser236Gly, Arg1138His) were present in families in which another *MYBPC3* defect (Gln1061X, IVS5-2A→C, IVS14-13G→A) co-segregated with the HCM phenotype, whereas any of the three missense variants did not, further suggesting these missense variants as polymorphisms (Figure 2). Compound heterozygous individuals with respect to the defects Gln1061X and Ser236Gly (2 patients) or Arg1138His (2 patients) (Figure 2), as well with respect to IVS14-13G→A and Pro147Leu (1 patient), were identified.

Table 4. Variants predicted to affect the protein structure of myosin-binding protein C found in *MYBPC3*.

Variant	Previous report as HCM-related mutation	No. of probands/ relatives with variant	Co-segregation with HCM	Two-point LOD score (θ=0) [1]	No. of controls (n=111) with variant
Mutations associated with HCM					
CAG1061TAG (Gln→X)	-	6 / 17	+	-0.60	0
IVS5-2A→C	-	1 / 9	+	+1.17	0
IVS14-13G→A	-	1 / 4	+	+0.15	0
Ex25Δ3bp (ΔLys) codon 811-815 [2]	-	1 / 2	?	-0.70	0
Variants with equivocal significance					
CGG326CAG (Arg→Gln)	(*10,147*)	1 / 1	?	-0.21	7
GTG896ATG (Val→Met)	(*56*)	1 / 2	? [3]	+0.00	5
CCT147CTT (Pro→Leu)	-	2 / 4	-	-0.41	2
AGC236GGC (Ser→Gly)	-	6 / 13	-	-0.45	22
CGC1138CAC (Arg→His)	-	3 / 12	-	-0.28	5

[1] The LOD scores for the variants present in multiple families are shown as combined values
[2] The exact location of the deletion could not be indicated due to a lysine repeat
[3] Clinically unaffected mother of the proband was homozygous for the substitution

5.1.3 Founder effect of the mutations present in multiple families (Studies I-II, IV)

To investigate the possible common ancestral origin of the *TPM1*-Asp175Asn mutation, members of the four families with this mutation were genotyped for an intragenic microsatellite marker (HTMα_{CA}). The Asp175Asn mutation co-segregated with an identical allele of the marker in all four families, indicating a possible founder effect (Study I). In study IV, extended haplotype analysis using not only the intragenic marker HTMα_{CA}, but also two extragenic microsatellite markers (D15S1036, D15S108) flanking the *TPM1* locus, was performed in the four families, and in an additional

family carrying the mutation. A conserved haplotype between the three markers, spanning a region of ~3 cM (Marshfield Map) and co-segregating with the mutation, was observed in all five families.

The possible founder effect of the *MYBPC3*-Gln1061X mutation found in six separate families was studied with haplotype analysis using four highly polymorphic microsatellite markers (D11S4133, D11S1344, D11S1350, D11S1326) flanking the *MYBPC3* locus (Study II). A common haplotype 2-9-13-1 co-segregating with the mutation in five of the six families was observed, indicating a likely founder effect (Figure 2). An apparent recombination event with the uppermost marker had occurred in one of the families (family 28). Thus, a common haplotype in all six families was observed between the markers D11S1344 and D11S1326 spanning a region of less than 2 cM (Genethon Map) or ~6 Mb (Sequence Map) (Figure 2). The identical four-marker haplotype was also observed in an additional family with the Gln1061X mutation (Study IV).

Geographical clustering of the birthplaces of the oldest carriers of the mutations *TPM1*-Asp175Asn and Gln1061X was observed, further suggesting these defects to be founder mutations (Study IV). The two mutations affected families descending mainly from eastern (Gln1061X) and central (Asp175Asn) parts of the country.

Haplotype analysis of *MYBPC3* variants Arg326Gln, Val896Met, Pro147Leu and Arg1138His, found both in HCM patients and controls, suggested common ancestral origins of the defects among the HCM families and affected controls. On the other hand, multiple haplotypes co-segregating with the Ser236Gln variant were observed, suggesting multiple origins (Study II).

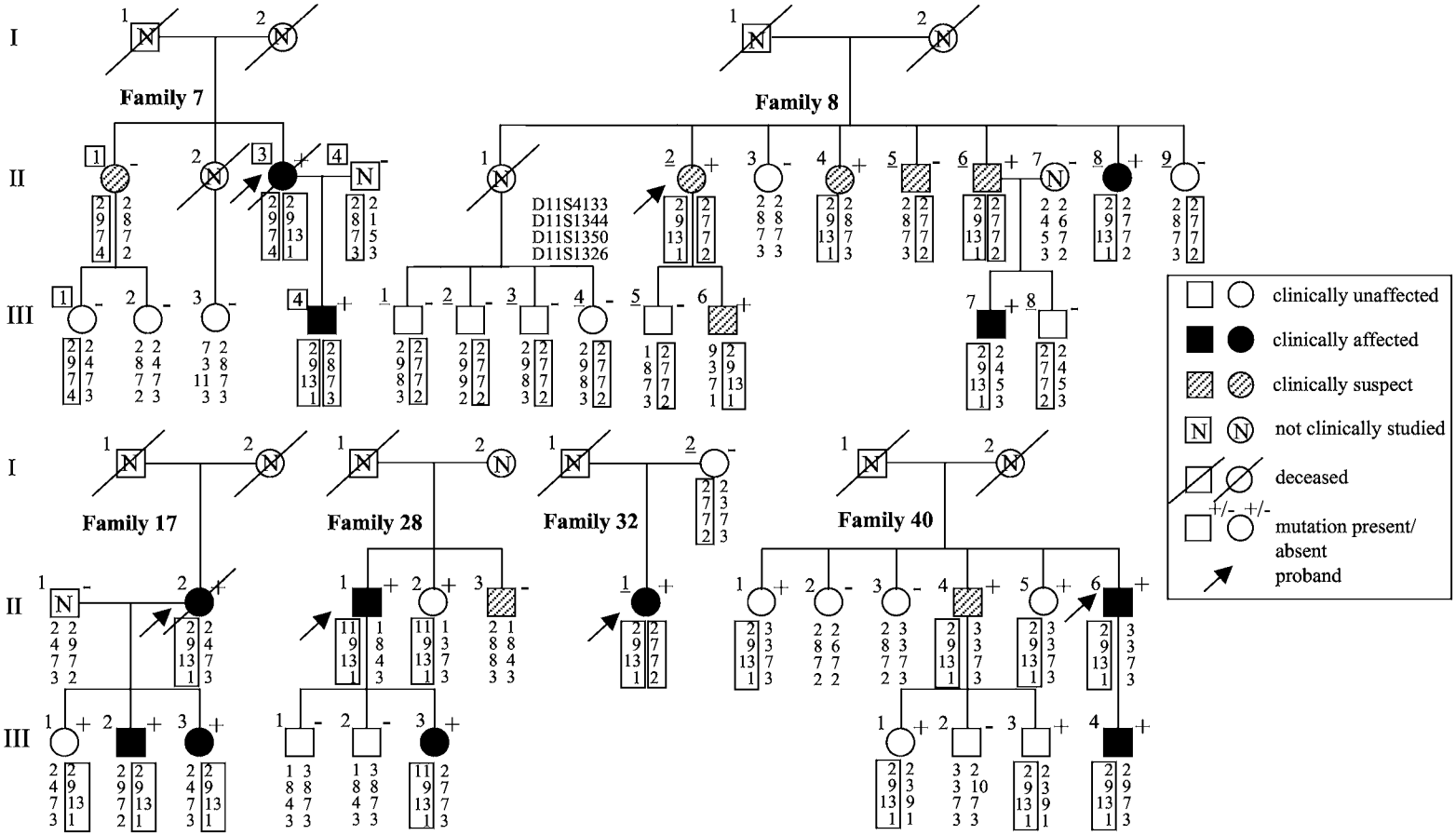

Figure 2. Pedigrees of the families carrying the *MYBPC3*-Gln1061X founder mutation. Compound heterozygous individuals with respect to the Gln1061X mutation and either the Ser236Gly or Arg1138His substitution were identified in families 7, 8, and 32, respectively. The individuals carrying the Ser236Gly (family 7) and Arg1138His (families 8 and 32) substitutions are indicated by boxed and underlined identification numbers, respectively. Haplotypes constructed from the markers D11S4133, D11S1344, D11S1350 and D11S1326 are depicted under each individual. The haplotypes co-segregating with the corresponding variants are boxed.

5.2 Genes without disease-causing mutations (Studies III-IV)

5.2.1 *TNNI3* (Study III)

In this study the entire coding region of *TNNI3* (8 exons) was screened in 37 probands with HCM by using the PCR-SSCP method. A total of five variants (IVS1+100C→G, IVS2-8T→A, IVS3+21G→A, IVS3-26T→G, CGG68CGT), three of which were novel and two reported previously as polymorphisms, were identified. None of the variants co-segregated with HCM, and four of the five variants were present in a panel of 111 controls. Furthermore, none of the identified variants were located at functional splice sites of *TNNI3*.

5.2.2 *TNNT2*, *MYL2*, *MYL3*, *TNNC1* (Study IV) and *ACTC*

In study IV, the entire coding regions of four sarcomeric genes, *TNNT2* (16 exons), *MYL2* (7 exons), *MYL3* (6 exons) and *TNNC1* (6 exons), previously shown to be associated with HCM, were screened in 41 unrelated probands with HCM by using the PCR-SSCP method. Altogether, 14 different variants in *TNNT2*, three in *MYL2* and one in *MYL3* were identified (Table 5). No variants were found in *TNNC1*. One of the variants found in *TNNT2* results in an amino acid substitution (Lys263Arg), four were silent nucleotide substitutions located in exons, and 10 variants were located in the non-coding regions of *TNNT2* and *MYL2*. None of the intronic variants were located at functional splice sites of *TNNT2* or *MYL2*. Seven of the 14 variants showed homozygosity for both normal and variant alleles among the study group, and all of the variants were also found in a panel of 37 control subjects. Seven of the 14 variants, including the Lys263Arg amino acid substitution in *TNNT2*, have been reported previously as neutral polymorphisms. Accordingly, the Lys263Arg substitution did not co-segregate with HCM in family studies.

Furthermore, in a previous study not included in the thesis, 40 of the 41 probands have been screened for variants in exons 1-6 of *ACTC* without detection of any variants (*182*).

Table 5. Variants found in *TNNT2*, *MYL2*, *MYL3* and *TNNC1*.

Gene	Variant	Previous report
TNNT2	Intron 2 +41 c→t	-
	Intron 3 del ctctt *	(*183*)
	Intron 4 –71 c→t	-
	Intron 4 –70 g→a *	-
	Intron 6 –50 g→a *	-
	Exon 9 TCG79TCA (Ser→Ser)	(*148,183*)
	Exon 10 ATC116ATT (Ile→Ile) *	(*11,148,183*)
	Intron 10 –98 del c	-
	Intron 12 –32 c→a	(*183*)
	Intron 13 –81 c→t	-
	Exon 15 AAG263AGG (Lys→Arg)	(*148*)
	Intron 15 –33 c→t	-
	Intron 16 +46 c→t	-
	3' untranslated region +6 c→t	-
MYL2	Exon 3 ATT44ATC (Ile→Ile) *	-
	Intron 4 +17 g→c *	-
	Intron 5 +20 del g *	-
MYL3	Exon 1 CCC23CCT (Pro→Pro)	(*95*)
TNNC1	-	

Del, deletion. * Homozygosity for both normal and variant alleles were observed in the study group. Exons and codons have been numbered according to Farza et al. (*183*)

5.3 Genetic profile of HCM in eastern Finland (Study IV)

In studies I-IV, 35 of the 41 probands with HCM in our study group were screened for variants in all eight sarcomeric genes (*MYH7*, *TPM1*, *MYBPC3*, *TNNI3*, *TNNT2*, *MYL2*, *MYL3*, *TNNC1*). Furthermore, *ACTC* was screened in 40 of the 41 probands in a previous study (*182*). Mutations of *MYBPC3* were the most common cause of HCM, four novel mutations (Gln1061X, IVS5-2A→C, IVS14-13G→A, Ex25ΔLys) accounting for ~39% familial and ~26% of all cases in the nuclear study group (Table 6). *TPM1* was the second most common gene, a single mutation (Asp175Asn) accounting for ~17% of familial and ~11% of all cases. Only one mutation in *MYH7* (Arg719Trp), accounting for ~4% of familial and ~3% of all cases, was found. No disease-causing mutations in the other genes were found. Collectively, the six different mutations found in three genes (*MYH7*, *TPM1*, *MYBPC3*) accounted for approximately 61% of familial and 40% of all cases of HCM in the nuclear study group of 35 probands. In addition, these six disease-causing mutations identified among the nuclear study group were not found in the six of 41 probands not belonging to the nuclear study

group. Furthermore, none of these six mutations have been found in the probands who were excluded from the initial study group due to an equivocal diagnosis of HCM (n=12, mean age 54±14 years, mean maximal LV wall thickness 20±4 mm). One excluded proband had maximal LV wall thickness <15 mm while the rest of the 12 probands having LVH of at least 15 mm were excluded due to concomitant hypertension.

Table 6. Genetic profile of HCM in the nuclear study group of 35 probands that underwent genetic screening of nine genes encoding sarcomeric proteins.

Gene	No. of mutations	No. of affected families	No. of mutation carriers	% of familial cases (n=23)	% of all cases (n=35)
MYBPC3	4	9	52	~39%	~26%
TPM1	1	4	28	~17%	~11%
MYH7	1	1	3	~4%	~3%
TNNI3	0	0	0	0%	0%
TNNT2	0	0	0	0%	0%
MYL2	0	0	0	0%	0%
MYL3	0	0	0	0%	0%
TNNC1	0	0	0	0%	0%
ACTC	0	0	0	0%	0%
ALL GENES	**6**	**14**	**83**	**61%**	**40%**

5.4 Clinical characteristics

5.4.1 Probands and adult relatives with HCM (Studies I-IV)

In studies I-IV, a total of 41 unrelated probands were clinically evaluated. The nuclear study group included 35 probands who underwent the genetic screening of all eight genes included in Studies I-IV. Fifty-three (22%) of the 236 adult relatives of 35 probands fulfilled the diagnostic criteria for definite HCM, and 32/236 relatives (14%) were classified as having suspected HCM. The rest of the relatives did not meet the diagnostic criteria for HCM. Twenty-three of the 35 probands (66%) were considered to have familial HCM (definite or suspected family history of HCM or a presence of a documented disease-causing mutation in proband and in at least one first-degree relative).

Table 7 shows the clinical characteristics, and the findings in Holter registration and exercise test, of the nuclear study group of 35 probands and their clinically affected relatives with definite HCM (n=53).

Clinical findings: Most of the probands (97%) and about half of the clinically affected (47%) relatives had either a confirmed diagnosis of HCM, or HCM had been suspected prior to our study. History of cardiac failure (9% in probands, 2% in relatives) and myotomy-myectomy operation (9% in probands, 0% in relatives) was rare. More than half of the patients had cardiac symptoms, most often dyspnea and palpitations. However, symptoms were usually mild, most subjects having a NYHA functional class of I-II. Most of the probands (97%) and clinically affected relatives (70%) had abnormal auscultation findings. Probands had most often S4 combined with systolic murmur (69%) or isolated S4 (17%), whereas among the relatives isolated systolic murmur (28%) and that combined with S4 (23%) were most frequently observed.

Electrocardiogram: All of the probands (100%) and most of the relatives (74%) had abnormal ECG findings. On ECG, LVH with repolarization changes was observed in 29% and 15% of probands and relatives, respectively. Half of the probands and one third of the relatives had pathological Q-waves on ECG.

24-h ambulatory electrocardiogram and exercise test: On Holter examination, 11% of probands and none of the relatives (0/38) had constant atrial fibrillation. Short bursts of ventricular tachycardia were observed in 11% of probands and in 8% of relatives who underwent Holter examination. Most of the probands and relatives had normal functional capacity in the exercise test. Inadequate increase (<20 mm Hg) or fall (≥20 mm Hg) in systolic blood pressure during exercise was seen in 20% of probands and in 16% of relatives evaluated.

Echocardiography: Table 8 shows the echocardiographic findings among the probands and relatives. The mean maximal thickness of the LV wall on two-dimensional echocardiography was 24 mm (range 15-36 mm) in the probands and 18 mm (range 11-30 mm) in the relatives. The interventricular septum was thickened virtually in all patients. No patients with apical or papillary muscle hypertrophy were observed. LV end diastolic and end systolic dimensions were within normal limits, and only two probands and three relatives had abnormal fractional shortening (below 25%).

Mild to moderate mitral regurgitation was present in 34% of probands and 15% of relatives. Systolic anterior motion of the mitral valve was present in 11% of probands and in 4% of relatives, and only 9% of probands and 8% of relatives had LV outflow tract obstruction.

During the follow-up of 1-6 years, five of the 35 probands and three relatives with definite or suspected HCM have died. No SCDs definitely related to HCM, and only two non-sudden HCM-related deaths, have occurred among the probands and relatives included in our study. The two HCM-related deaths occurred in two of 23 subjects carrying the *MYBPC3*-Gln1061X mutation (individuals II-3 of family 7 and II-2 of family 17; Figure 2; discussed in Study IV in detail). In four patients HCM may have contributed to death. In addition, one proband now aged 29 years was successfully resuscitated from HCM-related ventricular fibrillation at the age of 14 years, and subsequently underwent a myotomy-myectomy operation.

Upon clinical evaluation, 18 of 35 probands (51%) reported an occurrence of a sudden (possibly cardiac) death in at least one first-degree relative (altogether 25 suddenly deceased first-degree relatives; mean age 61 years, range 23-81 years). Death certificates were available in 8 of 25 cases. In two cases from two of 35 families (6%) HCM-related SCD could be verified (sudden death judged to be caused by HCM documented in autopsy), three deaths were verified not to be HCM-related, and in three of eight cases the possibility of HCM-related SCD could not be excluded (i.e. no autopsy performed). In addition, HCM-related SCD was verified in a second cousin of one proband of the nuclear study group, and in a member of a HCM family from western Finland not belonging to the nuclear study group. Three of the four kindreds with a verified family history of HCM-related SCD carried the *TPM1*-Asp175Asn mutation (one individual died while riding a bicycle at age 19 years; another two individuals died at age 9 and 49 years, respectively, with no specific data of the incidents available). One family had the *MYBPC3*-Gln1061X mutation (one individual died suddenly at age 45 years, but no specific data of the incident was available). Furthermore, one sporadic child patient carrying the *MYH7*-Asn696Ser mutation, and not belonging to the nuclear study group of 35 adult probands, suffered a documented HCM-related SCD (while running at school yard) at the age of 12 years.

Table 7. Clinical, Holter and exercise test findings in the nuclear study group, clinically affected relatives and genetic carriers of disease-causing mutations.

	Phenotype +		Genotype +						
			MYH7	*TPM1*	*MYBPC3*				
	Probands (n=35)	Clinically affected relatives (n=53)	Arg719Trp (n=1)	Asp175Asn (n=32)	Gln1061X (n=27)	IVS5 -2A→C (n=21)	IVS14 -13G→A (n=5)	Ex25 ΔLys (n=3)	All *MYBPC3* mutations (n=56)
Men/Women	21/14	27/26	0/1	13/19	14/13	12/9	3/2	2/1	31/25
Age at study (y)	49±16	43±14	28	40±14	44±14	45±15	46±19	37±13	44±14
Diagnosis of definite or suspected HCM [1]	35 (100%)	53 (100%)	+	29 (91%)	18 (67%)	18 (86%)	4 (80%)	1 (33%)	41 (73%)
History of CF	3 (9%)	1 (2%)	-	1 (3%)	1 (4%)	1 (5%)	1 (20%)	0	3 (5%)
History of myectomy	3 (9%)	0	-	1 (3%)	1 (4%)	0	0	0	1 (2%)
Cardiac symptoms	31 (89%)	37 (70%)	+	22 (69%)	18 (67%)	15 (71%)	5 (100%)	2 (67%)	40 (71%)
Chest pain	6 (17%)	8 (15%)	-	5 (16%)	7 (26%)	3 (14%)	1 (20%)	0	11 (20%)
Dyspnea	26 (74%)	25 (47%)	+	16 (50%)	14 (52%)	13 (62%)	3 (60%)	1 (33%)	31 (55%)
Palpitations	24 (69%)	30 (57%)	+	19 (59%)	13 (48%)	10 (48%)	4 (80%)	2 (67%)	29 (52%)
Presyncope/syncope	14 (40%)	16 (30%)	+	12 (38%)	8 (30%)	5 (24%)	2 (40%)	0	15 (27%)
NYHA functional class									
I-II [2]	31 (89%)	50 (94%)	+ (II)	31 (97%)	22 (81%)	19 (90%)	4 (80%)	3 (100%)	48 (86%)
III-IV	4 (11%)	3 (6%)	-	1 (3%)	5 (19%)	2 (10%)	1 (20%)	0	8 (14%)

Systolic BP (mm Hg)	131±14	131±14	100	129±16	138±17	137±14	153±28	128±7	139±17
Diastolic BP (mm Hg)	80±10	79±9	68	81±13	82±9	80±8	94±14	80±10	82±10
Abnormal ECG	35 (100%)	39 (74%)	+	24 (75%)	19 (70%)	11 (52%)	4 (80%)	2 (67%)	36 (64%)
LAH	11 (31%)	4 (8%)	-	4 (13%)	6 (22%)	1 (5%)	3 (60%)	1 (33%)	11 (20%)
LVH + repolarization Changes	10 (29%)	8 (15%)	-	5 (16%)	4 (15%)	1 (5%)	2 (40%)	1 (33%)	8 (14%)
Abnormal Q-waves	17 (49%)	15 (28%)	-	16 (50%)	7 (26%)	2 (10%)	3 (60%)	1 (33%)	13 (23%)
Abnormal T-waves [3]	28 (80%)	26 (49%	+	15 (47%)	15 (56%)	6 (29%)	2 (40%)	1 (33%)	24 (43%)
Abnormal ST-level [4]	18 (51%)	12 (22%)	-	10 (31%)	6 (22%)	2 (10%)	2 (40%)	1 (33%)	11 (20%)
Holter	(n=35)	(n=38)	+	(n=30)	(n=15)	(n=11)	(n=4)	(n=1)	(n=31)
Constant AF	4 (11%)	0	-	1 (3%)	2 (13%)	0	0	-	2 (6%)
VT	4 (11%)	3 (8%)	+	0	3 (20%)	2 (18%)	0	-	6 (19%)
Exercise test	(n=15)	(n=25)	No data	(n=26)	(n=3)	(n=7)	(n=2)	No data	(n=12)
4 minute moderate workload adjusted to age and gender (%)	80±29	90±21	No data	82±23	77±76	93±16	42±10	No data	81±34
Abnormal systolic BP response during exercise [5]	3 (20%)	4 (16%)	No data	4 (15%)	1 (50%)	0	0	No data	1 (8%)

CF=Cardiac failure; NYHA=New York Heart Association; BP=Blood pressure; LAH=Left atrial hypertrophy; LVH=Left ventricular hypertrophy; AF=Atrial fibrillation; VT=Ventricular tachycardia. [1] Probands: LVH ≥15 mm on two-dimensional echocardiography; relatives fulfilling the diagnostic criteria by McKenna et al. (*49*) in the absence of secondary causes. Relatives having LVH ≥13 mm with concomitant secondary causes were classified as having clinically suspected HCM. [2] Includes asymptomatic subjects with normal exercise capacity; [3] Includes giant and negative T-waves; [4] Includes ≥1 mm depression or elevation of ST-level. [5] Increase of <20mmHg, any decrease below baseline level without initial increase, or decrease of ≥20 mmHg after initial increase. Data are means ± SD or n (%).

Table 8. Echocardiographic findings in the nuclear study group, clinically affected relatives and genetic carriers of disease-causing mutations.

	Phenotype +		Genotype +						
			MYH7	*TPM1*	*MYBPC3*				
	Probands (n=35)	Clinically affected relatives (n=53)	Arg719Trp (n=1)	Asp175Asn (n=32)	Gln1061X (n=27)	IVS5 -2A→C (n=21)	IVS14 -13G→A (n=5)	Ex25 ΔLys (n=3)	All *MYBPC3* mutations (n=56)
2D IVS (mm)	24.2±5.1 (15-36)	18.2±5.0 (11-30)	25	17.9±5.6 (7-30)	18.6±7.6 (10-36)	17.2±5.6 (11-30)	18.6±4.5 (11-22)	14.4±7.5 (9-23)	17.8±6.6 (9-36)
LV posterior wall (mm)	11.6±2.2	10.6±1.8	8	10.5±1.8	11.0±2.0	10.8±2.4	11.8±2.4	10.2±1.3	11.0±2.2
LVEDD (mm)	43.4±8.1	43.5±7.0	39	41.5±7.2	45.8±6.9	43.8±5.4	48.8±6.2	44.9±8.6	45.3±6.4
LVESD (mm)	27.8±6.7	27.1±6.2	27	26.4±7.1	29.3±5.7	28.0±5.2	30.4±8.3	24.4±4.9	28.6±5.7
FS (%)	36.7±9.9	37.8±8.9	32	38.1±11.7	36.2±6.2	36.1±8.5	38.6±11.6	45.9±2.1	36.9±7.7
Left atrium (mm)	41.4±9.0	39.4±8.1	46	38.9±6.9	41.0±8.3	38.9±11.1	40.5±3.9	37.1±3.8	39.9±9.0
Mitral regurgitation	12 (34%)	8 (15%)	-	5 (16%)	3 (11%)	4 (19%)	1 (20%)	0	8 (14%)
SAM	4 (11%)	2 (4%)	-	2 (6%)	1 (4%)	1 (5%)	0	0	2 (4%)
Obstruction at LV outflow tract	3 (9%)	4 (8%)	-	3 (9%)	0	2 (10%)	0	0	2 (4%)
VMAX (m/s)	1.21±0.37	1.30±0.47	1.2	1.32±0.35	1.16±0.27	1.45±0.63	1.37±0.50	1.09±0.11	1.28±0.46

2D IVS=Maximal thickness of the interventricular septum on two-dimensional echocardiography; LV=Left ventricular; LVEDD=Left ventricular end-diastolic dimension; LVESD=Left ventricular end-systolic dimension; FS=Fractional shortening; SAM=Systolic anterior motion of mitral valve; VMAX=Maximal flow velocity in the left ventricular outflow tract. Data are means ± SD or n (%).

5.4.2 Disease-related mutations (Study IV)

In study IV, the clinical features of the patients carrying HCM-related mutations of *MYH7*, *TPM1* and *MYPBC3* were reported, and the features between the larger mutation groups were compared (Tables 7 and 8). Collectively, differences in the clinical features between different mutations groups were small, and the overall clinical phenotype associated with each documented mutation was relatively mild. Moreover, the clinical phenotype of HCM in individual patients with identical mutations was variable. Among the families (n=14) with an identified disease-causing mutation, 5% (2/41) of relatives with clinically definite HCM did not carry the mutation of the pedigree (false positive clinical diagnosis).

The disease-penetrance between the mutation groups varied between 33%-91% (Table 7). Molecular diagnosis of families revealed three *TPM1*-Asp175Asn mutation carriers (9%), nine *MYBPC3*-Gln1061X mutation carriers (33%), and three *MYBPC3*-IVS5-2A→C mutation carriers (14%), who were clinically void of the disease, thus indicating incomplete penetrance in all of the largest mutation groups. The *TPM1*-Asp175Asn mutation was associated with higher penetrance (91%) compared to the *MYBPC3*-Gln1061X mutation (67%; $P<0.05$).

Systolic blood pressure was lower in patients with the *TPM1*-Asp175Asn mutation (129±16 mm Hg) compared to *MYBPC3*-Gln1061X (138±17 mm Hg; $P<0.05$), and all *MYBPC3* mutations (139±17 mm Hg; $P<0.05$). Pathological Q-waves were more common in patients with *TPM1*-Asp175Asn (50%) compared to *MYBPC3*-IVS5-2A→C (10%; $P<0.01$) and all *MYBPC3* mutations (23%; $P<0.05$). In addition, short bursts of ventricular tachycardia occurred more often in the *MYBPC3*-Gln1061X group (20%) than in the *TPM1*-Asp175Asn group (0%; $P<0.05$).

On echocardiography, the mean maximal LV wall thickness was moderate in every group (range 14-25 mm) (Table 8). The LV end-diastolic dimension (LVEDD) was smaller in patients with *TPM1*-Asp175Asn (41.5±7.1 mm) compared to *MYBPC3*-Gln1061X (45.8±5.4 mm; $P<0.05$) and all *MYBPC3* mutations (45.2±6.4 mm; $P<0.05$).

6. DISCUSSION

6.1 Representativeness of the study subjects

A few studies with a systematic evaluation of regional HCM patient populations have been published previously (*41-43*). Our study group was a representative group of HCM patients from eastern Finland without a significant referral bias. However, it is possible that a number of patients with HCM in the Kuopio University Hospital area have not been recognized, and thus, were not included in our study. Most patients without an established diagnosis of HCM may have mild clinical phenotypes escaping clinical detection. On the other hand, patients with the most severe clinical HCM phenotypes associated with a high risk of SCD at a young age may also be underrepresented in our study group. Therefore, no reliable estimation of e.g. the prevalence of HCM in the general population can be made on the basis of our study.

The establishment of a solid diagnosis of HCM excluding patients with secondary causes of LVH is important. In a South African panel including patients with an equivocal diagnosis of HCM disease-related molecular defect was identified only in 6% of cases compared to a non-equivocal group with a mutation detection rate of 57% (*18*). Accordingly, none of the probands (n=12) excluded from our initial study group due to an equivocal diagnosis of HCM carried any of the disease-causing mutations identified among the nuclear study group of 35 probands.

6.2 Evaluation of the clinical and molecular methods

6.2.1 Echocardiography and ECG

In our study the diagnosis of HCM in index patients was based on the demonstration of LVH on two-dimensional echocardiography, which is an internationally accepted practice in the diagnosis of HCM (*1,3*). Echocardiography is a relatively cheap, rapid and reproducible method in the diagnostics and evaluation of patients with HCM (*184*). Furthermore, in our study all echocardiograms were performed by the same experienced cardiologist, assuring consistent results. Compared to magnetic resonance imaging (MRI), the sensitivity of echocardiography in detecting LVH is limited by its small acoustic window. Therefore, some patients with localized myocardial hypertrophy may

have been missed (*184,185*). However, the interventricular septum, which is the thickest LV segment in the majority of HCM patients (*1*), is virtually always visualized well on echocardiography. Therefore, the failure to detect existing HCM-related myocardial hypertrophy by echocardiography is probably uncommon. The echocardiographic criterion of HCM used among the probands (LVH ≥15 mm) excludes patients with mild hypertrophy frequently associated with mutations of *MYBPC3* (*144*), and also with *TNNT2* mutations reported to confer a high risk of SCD (*148,150*). However, application of strict echocardiographic criteria based on evident hypertrophy provides better specificity, which is essential for successful genetic screening of probands. Because the risk of HCM in first-degree relatives of HCM patients is very high, more sensitive diagnostic criteria (*49*), including milder LVH (≥13 mm) on echocardiography and minor echocardiographic and ECG abnormalities, were used for the relatives. Echocardiography and ECG have been reported to be of similar diagnostic value for HCM in genotyped adults (*40*), but in carriers of certain mutations ECG has been reported to be a more sensitive indicator of HCM than echocardiography (*186,187*). Nevertheless, only two relatives in our study group were diagnosed to have HCM based on abnormal ECG (pathological Q-waves) only, without detectable LVH on echocardiography.

6.2.2 Single-strand conformation polymorphism (SSCP) analysis

SSCP analysis, used in the screening of variants in Studies I-IV, is probably the most frequently used method for screening of unknown sequence variants. This technically simple method enables the screening of a large number of subjects with a relatively high sensitivity (~80-90%) and specificity (*188-190*). However, the sensitivity of the SSCP method depends on various factors, such as type, position and number variants on the fragment of interest, length and G+C content (%) of a fragment, and electrophoresis conditions (*190*). A weakness of the SSCP method is its inability to detect large deletions or insertions in genomic DNA, but large deletions or insertions have only rarely been reported to cause HCM (*191*). We used standardized running conditions with two different temperatures and a gel matrix that has performed well in previous

studies (*190*). Our SSCP conditions have been validated against the known variants of the lipoprotein lipase gene (*192,193*), and the same method we used has been successfully applied in several previous studies (*194-196*). Therefore, it is not likely that we have missed a significant number of variants in the genes studied.

6.3 Molecular genetics of HCM in eastern Finland

6.3.1 A unique genetic profile of HCM in eastern Finland

We identified a total of six definite or probable disease-causing mutations (*MYH7*-Arg719Trp, *TPM1*-Asp175Asn, *MYBPC3*-Gln1061X, IVS5-2A→C, IVS14-13G→A, Ex25ΔLys) in three genes, accounting for approximately 61% of familial and 40% of all cases of HCM in the nuclear study group of 35 probands. In addition, a novel Asn696Ser missense mutation of *MYH7* in a child patient not belonging to the nuclear study group of adults with HCM was found.

Our genetic findings differed from several recent studies including HCM patients of European, Asian and South African origin (Table 9). First, the total number of mutations accounting for HCM was small in our study group compared to the other European and Asian studies, even when taking into account that these studies included larger patient groups than ours. Secondly, there were differences in the distribution of mutations among the disease genes. In our study group *MYBPC3* was the most common cause of HCM, accounting for 26% of all cases. Accordingly, mutations of *MYBPC3* were most frequently observed in Danish, German and French HCM populations (17-53% of cases), suggesting that *MYBPC3* is the predominant gene for HCM in European populations. However, in the Asian and South African study groups *MYBPC3* mutations were relatively uncommon (prevalence 12.5% and 5%, respectively) In the current literature *MYBPC3* is estimated to account for 15-20% of the cases of HCM (*8,13*). In contrast to the studies presented in Table 9, and in the previous literature (*8,148*), *TPM1* was a relatively common disease gene in our study group, accounting for 11% of HCM cases. However, mutations in two globally common genes, *MYH7* and *TNNT2*, were rare or absent in our patients. In the recent European, Asian and South African studies

Table 9. Comparison of the distribution of causative mutations of HCM in different populations.

Reference	Our study	Havndrup et al. (*197*)	Regitz-Zagrosek et al. (*106*)	Richard et al. (*198*)	Kimura et al. (*19*)	Moolman et al. (*18*)
Nationality	Finnish	Danish	German	French	Asian	South African
No. of probands	35	48	120	102	184 [2]	37
No. of genes with mutations	3	5	5	5	8	3
No. of mutations	6	15	45	76	61	9
MYBPC3 (%)	26	17	20	53	12.5	5
TPM1 (%)	11	4	1	0	0.5	0
MYH7 (%)	3	14	15	32	18	38
TNNI3 (%)	0	6	1	7	3	0
TNNT2 (%)	0	0	Not specified	2	11	14
MYL2 (%)	0	6	No data	6	1	0
MYL3 (%	0	0	No data	No data	1	0
ACTC (%)	0	0	No data	0	0	No data
TTN (%)	No data	No data	No data	No data	0.5	No data
TNNC1 (%)	0	No data	0	No data	No data	No data
MYH6 (%)	No data	No data	No data	No data	No data	No data
All genes (%)	**40**	**47**	**~ 40** [1]	**100** [1]	**~ 47**	**57**

[1] Some patients were reported to have double mutations either in the same or different gene.
[2] Included only familial cases

(Table 9) *MYH7* was reported as the most common or second most common cause for HCM. In accordance with our results *TNNT2* mutations were also rare in other European studies. We did not find any mutations in *TNNI3*, *MYL2*, *MYL3*, *ACTC* and *TNNC1*. Mutations in these genes appear to be infrequent causes of HCM also in other populations (Table 9)(*8*). Only a few studies have screened *TTN, TNNC1* and *MYH6*. Since no familial HCM cases carrying mutations in these genes have been reported (*9,10,63*), it is somewhat unclear whether these genes are associated with HCM. The total percentage of cases explained by mutations in the genes screened in our study (40%) resembles that of other studies (Table 9). The full coverage of HCM cases by mutations in the study by Richard et al. (*198*) is explained by presentation of data from

a selected genotyped population. However, neither in our study nor in the studies presented in Table 9 were all known genes responsible for HCM screened. Therefore, the actual percentage covered by the known sarcomeric genes may be higher than that presented in our and other studies.

Why is the genetic profile of HCM in our study group different compared to the available literature and the recent reports from other populations? Several factors, such as study design, including patient selection and diagnostic criteria, and the peculiar population history of Finland are likely to have an influence in our unique results.

First, most of the available literature on the genetics of HCM is derived from referral center studies including highly selected HCM families of various ethnic or racial origin with penetrant disease. The estimated prevalence of mutations in the disease genes has been largely based on the results of linkage analysis of these selected pedigrees (*13*), which does not necessarily reflect the mutation distribution in the general population. In contrast, less selected regional HCM patient populations free of significant referral center bias are more likely to reflect the overall genetic profile of HCM. Mutations of *MYBPC3* and *TPM1*, previously reported to be associated with benign or intermediary phenotypes (*135,144,146*), were common in our study group whereas mutations of the more malignant genes, such as *MYH7* (*69*), were rare. These results support the hypothesis that in regionally collected HCM patients the genetic profile of HCM is characterized by disease-genes and mutations associated with more favorable phenotypes compared to referral center populations.

Second, the diagnostic criteria in our study included only the probands alive with evident LVH, which may have caused the underrepresentation of mutations of genes (e.g. *TNNT2*) associated with mild hypertrophy or high mortality (no cases of SCD were included in the study group). However, similar diagnostic criteria in probands (LVH ≥13-15mm on echocardiography) have been used in most other studies (*13*), and in accordance with our study mutations of *TNNT2* are reported to be uncommon in most European HCM patients (Table 9).

Third, the genetic background of HCM and other hereditary diseases is likely to vary between different populations and geographic areas. A likely founder effect, supported by common disease haplotypes and geographical clustering of the affected

families, was observed in the case of the two most common mutations (*MYBPC3*-Gln1061X, *TPM1*-Asp175Asn) in our study group. Thus, the genetic isolation of the Finnish population, reflected by a strong founder effect and the enrichment of few disease alleles, is likely to be a major factor influencing the genetic profile of HCM and other genetic diseases in Finland (*165*). However, founder events (mostly incidental) have also been reported in previous studies including European and South African HCM patients (*55,56,58,131,145,199*). As all of the families carrying the two founder mutations and most of the families with the four other mutations identified in our study group have originated from the late settlement area (central, eastern and northern parts of the country), it can be speculated that the mutations identified in our study appear mostly in families descended from inhabitants of this area. However, due to the presence of numerous regional population isolates in the late settlement area and different population history of the early settlement area (southern and western coastal parts of the country), no reliable extrapolation of the genetic profile of HCM to the entire Finnish population can be made.

Finally, our genetic findings are limited by the modest number of patients with HCM in our study group. A larger number of patients are therefore needed to confirm the unique genetic profile of HCM in the Finnish population.

Mutations of the currently known sarcomeric genes are estimated to account for more than two-thirds of all cases of HCM (*8*). However, in our study the screening of eight (nine) genes revealed the disease-causing mutation in approximately 40% of the patients, whereas no mutation was found in 39% (9/23) of familial and in 60% of all cases in the nuclear study group. Therefore, additional mutations responsible for HCM in our study group are yet to be found. Some mutations might exist in the other known HCM genes not included in the present study (i.e. *TTN* and *MYH6*). In the case of *MYH7* only the exons encoding the head and hinge regions (3-26) and exon 40 of β-MyHC were screened. Hence, some unidentified mutations in *MYH7* encoding the rod domain might exist. Just recently, two novel *MYH7* mutations located in exons 30 and 37, respectively, were reported to cause HCM (*67*). Some mutations might also be found in yet unidentified genes. Novel sarcomeric proteins such as myotilin (*200*), calsarcins (*201*) and obscurin (*202*) have recently been found, and the genes encoding

them are good candidates for HCM. Furthermore, since the PCR-SSCP method is not 100% sensitive in mutation detection (*188*), some variants may have been missed. Finally, 34% (12/35) of the probands in our study group did not have a family history of HCM, and no disease-related mutations in these patients were found either. This raises the possibility that some of these individuals may not have genetically determined HCM.

6.3.2 Variants with equivocal significance

In *MYBPC3*, five missense variants (Pro147Leu, Ser236Gly, Arg326Gln, Val896Met, Arg1138His) were considered as suspected polymorphisms due to non-co-segregation with HCM in families having another defect more likely responsible for the disease, presence in controls or homozygous occurrence of the variants in healthy subjects. However, two of these variants (Arg326Gln and Val896Met) have previously been reported as potential disease-causing mutations (*10,56,147*). Results of the haplotype analysis suggested that four of the five amino acid substitutions (Arg326Gln, Val896Met, Pro147Leu, Arg1138His) have common ancestral origins. It is possible that these four variants with a common ancestor are disease-causing mutations exhibiting mild HCM phenotypes, but a founder effect does not solely prove these variants as disease-related mutations. On the other hand, the novel Ser236Gly substitution co-segregated with multiple distinct haplotypes among the HCM families and control subjects, suggesting the Ser236Gly variant as a likely polymorphism. Accordingly, some neutral amino acid polymorphisms not causing HCM have been reported previously in *TNNI3* (*60*) and *TNNT2* (*148*). Furthermore, as homozygosity with respect to a disease-causing mutation usually results in severe HCM (*129-131*), the homozygous occurrence of the variants Val896Met and Arg1138His in healthy subjects suggests that these variants are polymorphisms.

A few studies reporting HCM patients with compound or double heterozygous mutations involving two separate defects located in either the same or different genes have been published (*110,132,133,203*). Most of these reports have described a co-existence of two independently disease-causing mutations in a single family, resulting in a more severe HCM phenotype when carried simultaneously (*110,133*). In a study by

Jeschke et al. (*132*) only one of the two *MYH7* defects carried by a HCM family was concluded to be a disease-causing mutation, but the co-existence of these defects was suggested to be associated with a high-risk phenotype. Accordingly, although not causative mutations of HCM, some of the amino acid substitutions found in our study group might act as modifying gene defects contributing to the phenotype in the presence of a causative HCM mutation. However, due to the small number of compound heterozygous patients in our study group, the effect of the missense variants on HCM phenotype could not be studied.

In conclusion, functional studies are required to determine the effect of the five *MYBPC3* missense variants on sarcomere structure and function.

6.4 Phenotypic expression of HCM

6.4.1 Index patients and relatives with HCM

Based on the data obtained from the clinical evaluation of the probands and clinically affected relatives, the clinical phenotype of HCM in eastern Finland seems to be relatively favorable, which is in accordance with the results of the studies performed on regional populations free of significant referral center bias (*41,43,204*). During a follow-up of 1-6 years, only 3% of the probands and clinically affected relatives in our study group have died from confirmed HCM-related causes, and 9% of the probands had a family history of documented HCM-related SCD. Maron et al. (*204*) reported an occurrence of HCM-related death in 12% of patients during a follow-up of 8±7 years in a large non-referral-based HCM patient population. Due to the cross-sectional setting of our study, a longer follow-up of the patients is needed to make definite prognostic assumptions of HCM in our study group. However, the overall clinical phenotype among the probands and relatives, characterized by mild symptoms, mild to moderate LVH without LV outflow tract obstruction, rare occurrence of arrhythmias and normal blood-pressure response in an exercise test, suggests a good prognosis in most patients of our study group (*3*).

6.4.2 Disease-related mutations

The small number of carriers of the *MYH7*-Arg719Trp mutation in our study group did not allow reliable genotype-phenotype assessment of this defect. This mutation seems to result in rather pronounced LVH with an early onset of the disease, since one of the proband's two affected children had a clinical diagnosis of HCM at the age of two years. In previous studies the Arg719Trp mutation has been reported to be associated with significantly reduced life expectancy (*69,139,143*). Accordingly, the novel *MYH7*-Asn696Ser mutation, identified in a child with HCM (not belonging to the study group) who suffered a premature HCM-related SCD at the age of 12 years, might be associated with reduced life expectancy.

The observation of a mild to moderate HCM phenotype associated with the two founder mutations (*TPM1*-Asp175Asn, *MYBPC3*-Gln1061X), as well as with the other three *MYBPC3* mutations in our study group agrees with the findings of earlier studies regarding the phenotype attributable to mutations in these genes (*135,144,146*). Coviello et al. (*146*) reported the association of the *TPM1*-Asp175Asn mutation with variable LVH and near normal life expectancy among three HCM families, whereas Niimura et al. (*144*) have shown an association of *MYBPC3* mutations with delayed clinical expression and favorable clinical course among 212 genotyped subjects. The comparison of clinical features between the carriers of the *TPM1*-Asp175Asn and the *MYBPC3* mutations revealed no dramatic phenotypic differences between the mutation groups, although some statistical differences were found. In accordance with the results of Charron et al. (*135*) the phenotypic differences between the two largest *MYBPC3* mutation groups (Gln1061X, IVS5-2A→C) were small. The relatively small genotyped patient groups carrying individual *TPM1* or *MYBPC3* mutations and the small number of documented HCM-related deaths among them and their family members did not allow reliable prognostic assessment of these mutations. However, the results of previous studies regarding the *TPM1*-Asp175Asn and *MYBPC3* mutations, as well as the relatively mild clinical picture associated with these mutations in our study group suggest that the identified mutations in *TPM1* and *MYBPC3* are associated with a benign or intermediary clinical course of HCM.

6.5 Clinical implications

A disease-causing mutation was found in 40% of the probands and 77% of the clinically affected relatives in our study group. In addition, 38% of the relatives in the entire study group classified as having suspected HCM carried a disease-related mutation, confirming the clinical diagnosis in these patients. On the other hand, genetic analysis provided exclusion of HCM in 25% of the relatives with clinically suspected HCM. Among the families (n=14) with an identified HCM-related mutation, genotyping suggested that 5% of relatives with clinically definite HCM had a false positive clinical diagnosis of HCM. In addition, the genotyping of family members revealed 15 carriers of disease-causing mutations (mostly *MYBPC3*-Gln1061X) who did not fulfill the clinical criteria of HCM. Thus, the genetic analyses provided accuracy to the clinical diagnosis of affected patients, but also showed the presence of several 'healthy' mutations carriers in the families. This implies that clinical methods do not recognize all subjects potentially at risk of HCM (*147*).

Although identification of disease-causing mutations is a definite way to establish whether an individual has HCM or is at risk of developing the disease in the future, genetic testing is not routinely used in clinical practice (*3*). The reasons for this include the wide genetic heterogeneity of HCM and the lack of identification of all mutations causing the disease, which makes the testing expensive and time-consuming. In addition, because the genotype-phenotype correlations of most mutations have not been thoroughly characterized, the benefit of the knowledge of the genotype in patient care is currently limited (*3*). Furthermore, due to the variable clinical expression of HCM even among individuals carrying identical defects (*8*), prognostic counseling of individual family members based on DNA testing would be difficult (*205*).

Genetic diagnosis may be used to confirm the clinical diagnosis of HCM especially in patients having other conditions (e.g. concomitant hypertension) that prevent reliable clinical diagnosis of HCM (*14*). Also, the identification of disease-causing mutations in probands enables genetic screening of family members. As the clinical expression of HCM is age-dependent, and in the case of *MYBPC3* mutations often delayed until middle-age (*144*), family members of HCM patients cannot be reassured not to be at risk of developing HCM later in life based on clinical methods. Therefore, the use of

genetic diagnosis in the family members of genotyped HCM patients would provide focused clinical management so that only those family members carrying a disease-related mutation would need regular follow-up (i.e. periodic echocardiographic evaluations). Since the phenotype of HCM and particularly the risk of SCD is dependent on the disease-causing mutation (*206*), a careful phenotypic characterization of the mutations, based on large patient groups carrying identical defects, could benefit the clinical management of affected patients (*15*). Unfortunately, for the time being there are no studies to suggest that any pharmacological treatment could improve the prognosis of HCM patients. The only measure to prevent SCD is an implantable cardiac defibrillator (*45*).

The mutations found in our study lead to the identification of a disease-causing mutation in up to 40% of HCM patients in eastern Finland, the two most frequently observed founder mutations (*MYBPC3*-Gln1061X and *TPM1*-Asp175Asn) accounting for ~30% of all cases. The screening of these two potentially widespread founder mutations, by using simple PCR-based tests, in all clinically diagnosed probands with HCM in Finland could be useful, because the identification of a large number of clinically characterized patients carrying these gene defects would allow reliable phenotypic assessment of these mutations. Large genetically homogeneous patient groups would also provide the opportunity to conduct pharmacological trials in these patients, and to investigate the factors (e.g. modifying gene defects) influencing the phenotypic expression of HCM.

6.6 Concluding remarks

The screening of eight genes encoding sarcomeric proteins revealed a total of six different mutations in three genes responsible for HCM in 14 different families. Two of these mutations, *MYBPC3*-Gln1061X and *TPM1*-Asp175Asn, were detected in multiple families, whereas the rest of the mutations were found in single kindreds. The two most common mutations found in *MYBPC3* and *TPM1* accounted for 17% and 11%, respectively, of all cases of HCM in the nuclear study group of 35 probands. Collectively the six mutations found in *MYBPC3*, *TPM1* and *MYH7* accounted for 40%

of all HCM cases in the present study group. Because no mutations were found in *TNNI3*, *TNNT2*, *MYL2*, *MYL3* and *TNNC1*, these genes appear not to have a major role in the genetics of HCM in patients from eastern Finland.

According to our results, the genetic profile of HCM in eastern Finland is different compared to that in other populations. A small number of mutations accounted for the disease, and defects in globally common genes, such as *MYH7* and *TNNT2* were rare or absent. In contrast, mutations in *TPM1* were more common than previously reported due to a single founder mutation effect and its enrichment in the eastern Finland population. In accordance with recent European studies, *MYBPC3* appears to be the predominant gene for HCM in eastern Finland. Our results suggest that the genetic background of HCM is variable in different populations. Therefore, the extrapolation of the genetic profile from previous referral center studies may not be appropriate. Studies including patients from regional populations are needed to establish the genetic background of HCM in different populations.

As in the case of many other hereditary diseases in Finland, a likely founder effect involving the two most frequently found mutations (*MYBPC3*-Gln1061X, *TPM1*-Asp175Asn) was observed. The *MYBPC3*-Gln1061X mutation appears to be enriched in eastern Finland, whereas the *TPM1*-Asp175Asn mutation affects mainly HCM families descended from central and western parts of the country. The founder effect partly explains the small number and the unique distribution of mutations in our study population.

The phenotype of HCM in the probands and in their clinically affected relatives was relatively benign, characterized by mild symptoms, mild to moderate myocardial wall thickening without obstruction, rare occurrence of arrhythmias, good exercise tolerance and few confirmed disease-related deaths. Although further follow-up studies are needed to confirm our findings, our results are in accordance with previous studies indicating that in regional patient populations the clinical picture of HCM is often more favorable compared to referral center-based populations. As reported in previous studies, the phenotype associated with the identified disease-causing mutations in *MYBPC3* and *TPM1*, especially *MYBPC3*-Gln1061X and *TPM1*-Asp175Asn, was

relatively mild with small phenotypic differences between mutations, but with marked phenotypic variability between the carriers of identical mutations.

The molecular findings of our study have the potential to improve the accuracy of clinical diagnosis of HCM in eastern Finland and to allow the identification of family members at risk of developing HCM in genotyped families. The identification of two potentially widespread founder mutations can be applied in the screening of patients with clinically confirmed or suspected HCM in Finland. Further genotype-phenotype characterization of the identified mutations may enhance the overall risk stratification of HCM patients.

7. SUMMARY

Study I: The previously reported missense mutations Arg719Trp (one family) in *MYH7* and Asp175Asn in *TPM1* (four families) were found among 36 probands with HCM. Additionally, a novel Asn696Ser missense mutation was identified in one child with HCM. Haplotype analysis with an intragenic microsatellite marker suggested a founder effect of the Asp175Asn mutation. In contrast to previous studies, mutations of *MYH7* were uncommon in our study group, whereas the *TPM1*-Asp175Asn mutation was more common than previously reported.

Study II: Four novel mutations (Gln1061X, IVS5-2A→C, IVS14-13G→A, Ex25ΔLys) associated with HCM were found among 37 probands. Gln1061X, indicated as a likely founder mutation in haplotype analysis, was found in six separate families, whereas the other three were found in single families. In addition, five missense variants with equivocal significance with respect to HCM were found. The four mutations found in *MYBPC3* accounted for ~24% (9/37) of all cases in the study group. *MYBPC3* was thus concluded to be the predominant gene for HCM in eastern Finland.

Study III: Five silent polymorphisms, but no disease-causing mutations, in *TNNI3* were found in 37 probands with HCM.

Study IV: No disease-causing mutations were found in *TNNT2*, *MYL2*, *MYL3* and *TNNC1* in 41 probands. Collectively, mutations in the nine sarcomeric genes screened in the present and previous studies accounted for 61% of familial and 40% of all cases in the nuclear study group of 35 probands. The clinical phenotype of HCM among probands and clinically affected relatives was relatively benign. The previously identitified mutations in *MYBPC3* and *TPM1* were associated with benign or intermediary phenotypes with small phenotypic differences between mutations.

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