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Original Article

Identification of the genotypes causing hypertrophic cardiomyopathy in northern Sweden

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Abstract

Hypertrophic cardiomyopathy (HCM) is a heterogenous disease, with variable genotypic and phenotypic expressions, often caused by mutations in sarcomeric protein genes. The aim of this study was to identify the genotypes and associated phenotypes related to HCM in northern Sweden. In 46 unrelated individuals with familial or sporadic HCM, mutation analysis of eight sarcomeric protein genes was performed; the cardiac β-myosin heavy chain, cardiac myosin-binding protein C, cardiac troponin T, α-tropomyosin, cardiac essential and regulatory myosin light chains, cardiac troponin I and cardiac α-actin. A total of 11 mutations, of which six were novel ones, were found in 13 individuals. Seven mutations were located in the myosin-binding protein C gene, two in the β-myosin heavy chain gene and one in the regulatory myosin light chain and troponin I genes, respectively. This is the first Swedish study, where a population with HCM has been genotyped. Mutations in the cardiac myosin-binding protein C gene were the most common ones found in northern Sweden, whereas mutations in the β-myosin heavy chain gene were less frequent than previously described. There are differences in the phenotypes mediated by these genes characterised by a more late-onset disease for the myosin-binding protein C gene mutations. This should be taken into consideration, when evaluating clinical findings in the diagnosis of the disease, especially in young adults in families with HCM, where penetrance can be expected to be incomplete in the presence of a myosin-binding protein C gene mutation.

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1. Introduction

Hypertrophic cardiomyopathy (HCM) is characterised by left and/or right ventricular hypertrophy, with predominant involvement of the interventricular septum in the absence of other causes of hypertrophy, such as hypertension or valvular heart disease [1]. The prevalence in the general population is about 0.2% [2]. The disease is genotypically and phenotypically heterogenous, with a wide variety of clinical manifestations, ranging from asymptomatic individuals to severe symptoms and early death [1]. It is an important cause of sudden cardiac death in children and young adults. Patients with HCM often exhibit symptoms of dyspnoea, chest pain, palpitations and sometimes syncope. Atrial fibrillation and non-sustained ventricular tachycardia (NSVT) are relatively common dysrhythmias. Recognised risk factors for sudden death are family history of sudden death, recurrent syncope, NSVT during ambulatory Holter ECG, abnormal blood pressure response during exercise and severe left ventricular hypertrophy (over 30 mm) [3]. Early experiences from tertiary referral centres indicated a rather adverse prognosis in HCM, with an annual mortality of 2–4% [4–6]. Today, the anatomical and clinical expression of the disease is recognised to encompass a wider range of phenotypes, from a mild

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or focal hypertrophy, limited symptoms and a good prognosis, to massive hypertrophy and a poor outcome. In the adult population, HCM often has a relatively benign prognosis with an estimated annual mortality of <0.7% [7,8]. Typical morphological changes include myocyte hypertrophy and sarcomere disarray surrounding areas of increased loose connective tissue.

In approximately 55% of the cases, HCM has been suggested to be familial, inherited in an autosomal dominant fashion [9]. This conception, however, derives from a time, when molecular genetics was not yet introduced and the actual percentage of familial cases might be much higher. More than 150 different mutations in 10 sarcomeric protein genes have been identified in families with HCM. These protein genes are the cardiac β-myosin heavy chain (*MYH7*) [10], the cardiac myosin-binding protein C (*MYBPC3*) [11–13], the cardiac troponin T (*TNNT2*) [14], the α-tropomyosin (*TPM1*) [14], the cardiac regulatory and essential myosin light chains (*MYL2* and *MYL3*) [15], the cardiac α-actin (*ACTC*) [16], the cardiac troponin I (*TNNI3*) [17], the cardiac troponin C (*TNNC1*) [18] and the titin (*TTN*) [19]. Mutations in a non-sarcomeric protein gene, the γ2 subunit of the protein kinase A (*PRKAG2*), have been described in HCM associated with electrophysiologic abnormalities, particularly the Wolf-Parkinson-White syndrome [20,21]. The role of this gene as a cause of HCM is not yet clear, it has been suggested that mutations in *PRKAG2* cause glycogen storage disease in the heart, mimicking HCM [22], or that an inefficient use of energy, caused by mutations in this gene, plays a central role in the pathogenesis of HCM in these cases [21].

According to available data, the most common disease genes in HCM are *MYH7*, *TNNT2* and *MYBPC3*, accounting for approximately 30–50%, 20% and 20% of familial HCM cases, respectively [23]. However, a recent study has shown that the *MYH7* gene was not the predominant HCM gene in the population of eastern Finland, instead the *MYBPC3* gene was the most common [24]. Genotype-phenotype correlations suggest that mutations in *MYH7* are associated with earlier onset and more severe disease than the *MYBPC3* mutations, which are associated with a milder, late-onset disease. Mutations in *TNNT2* are often associated with a poor prognosis, despite relatively mild cardiac hypertrophy. Genotyping is not yet available in the routine care of HCM patients in Sweden, it is mainly a tool in the research laboratory. However, for some patients it can be expected that the genetic information might play a role in the clinical management in the future.

To our knowledge, there is only one report published, where most of the disease genes have been systematically investigated in the same patient population [24]. No earlier Swedish studies have reported the disease genes associated with HCM, and the aim of this study was to determine the presence and frequency of mutations and the associated phenotypes for eight of the known HCM genes in patients with HCM from northern Sweden.

2. Materials and methods

2.1. Patients and clinical evaluation

The region of northern Sweden has a population of 883 000 inhabitants. Specialised cardiac care is provided by the Heart Center at Umeå University Hospital. The Hospital Discharge Register of the National Board of Health and Welfare in Stockholm was used to identify patients that had been hospitalised with HCM and 50 possible cases were identified. Moreover, the physicians in charge of cardiology at the 12 other hospitals in the region were contacted to obtain information about known patients with HCM and another 60 possible cases were found. Criterium for the diagnosis of HCM was left ventricular hypertrophy demonstrated at echocardiography, with a wall thickness of ≥15 mm [25]. For the family members of an affected proband, the diagnostic criteria suggested by McKenna et al. [26] were used. Exclusion criteria were arterial hypertension, defined as blood pressure >160/90 mmHg or ongoing antihypertensive treatment, significant valvular disease or known systemic disease capable of producing cardiac hypertrophy. All patients >18 years old of Swedish ethnic origin were considered for the study. When medical records were scrutinised, 64 cases were excluded; 25 hypertensive individuals and two with aortic stenosis. In eight cases with other conditions, the HCM diagnosis was incorrect by documentation error in the medical records (wrong ICD10 diagnosis code given) and 29 cases were not included for various reasons (not giving consent to the study, age <18 years, non-Swedish ethnic background, previous cardiac transplant, other illnesses, e.g. psychiatric disease).

Thus, 46 unrelated individuals with HCM were included in the study, 25 men and 21 women with a mean age of 61.6 years (range 26–80 years). Each individual had at least one living first-degree relative >18 years old (maximum 11 relatives). In 12 cases, all first-degree relatives were studied and in another 28 cases, as many as possible, but not all were studied. In six cases, no relatives were studied, mainly because they were asymptomatic and did not wish to participate. When family members had moved outside the region of northern Sweden, information about the phenotype was sometimes obtained through medical records from other hospitals, but no genotyping was performed. In 11 cases, the disease was definitely familial, defined as the finding of at least two affected subjects in the family, including deceased individuals, where a positive diagnosis could be established from medical records. In the remaining 35 cases, the first-degree relatives studied in 29 cases did not fulfil the major diagnostic criteria. Two relatives had a borderline phenotype with a septal thickness of 13 mm in the presence of arterial hypertension, and were not considered to be affected. No family history of HCM was known and the disease was considered likely to be sporadic in these cases. The patients underwent physical examination, including patient history, echocardiography (M-mode, two-dimensional and Doppler), 12-lead electrocardiogram (ECG), 24-h Holter ECG and a

venous blood sample was taken for the genotyping. Echocardiographic evaluation was performed with an Acuson xp/10 or Acuson Sequoia ultrasound system (Acuson, Mountain View, CA, USA). Views of the heart were obtained from the parasternal, apical and subcostal positions. All measurements were done according to the standards of the American Society of Echocardiography [27]. DNA from at least 200 chromosomes from healthy individuals was used as normal control. Informed consent was obtained from each individual and the protocol was approved by the Ethics Committee of Umeå University.

2.2. Genetic analysis

DNA was extracted from peripheral blood leucocytes by standard protocol. The *MYH7*, *MYBPC3*, *MYL2* and the *TNNI3* genes were screened for mutations using single-stranded conformation polymorphism (SSCP) as previously described [28,29]. The *TNNT2*, *TPM1*, *MYL3* and the *ACTC* genes were analysed by denaturing high performance liquid chromatography (DHPLC) [30]. Intronic sets of oligonucleotide primers were designed according to the published genomic sequence of the genes. In the analysis of the *MYH7*, only the first 24 exons were examined, whereas each coding exon of the seven other genes was amplified by "touch down" polymerase chain reaction (PCR) and then subjected to an SSCP- or a DHPLC analysis. For SSCP analysis of *MYH7*, *MYL2* and *TNNI3*, the PCR products were heat denatured at 94 °C for 5 min in a saccharose buffer, and resolved on a 10% polyacrylamide gel (acrylamide/bisacrylamide ratio of 37.5:1) at 8 mA per gel (10 cm) in 0.8× Tris-borate-EDTA (TBE) buffer, run at 7 and 25 °C. After migration, DNA was visualised by silver staining (Pharmacia, Uppsala, Sweden) of the gels [31]. SSCP analysis of *MYBPC3* was carried out using an automated laser fluorescence system (ALF, Pharmacia, Uppsala, Sweden). Composition of the gels and running conditions were the same as described above, except that the PCR fragments were denatured in formamide buffer and the gels were run at 38 mA. Both forward and reverse primers were fluoresceine-labelled and sequence variations were identified as abnormal fluorescence peaks. Sometimes, running at 7 °C yielded technically unsatisfactory results and those exons were reanalysed on standard gels.

For the DHPLC analysis, the PCR was followed by a heteroduplex formation step, where the PCR products were slowly cooled down from 95 °C to room temperature at 1.5 °C/min. Heteroduplexes were resolved from the corresponding homoduplexes using the WAVE system (Transgenomic, San Jose, CA, USA), an automated HPLC with a DNA separation column. The Wavemaker™ software was used to calculate specific melting curves for each PCR fragment, and to determine the optimal temperature for heteroduplex separation. Most of the fragments required more than one temperature for mutation analysis, due to differences of the melting temperatures in some domains of the PCR fragment (the DHPLC running conditions and sequences of the oligonucleotide primers are available from the authors upon request). The nature of the mutation was then determined by direct sequencing of the PCR product using both forward and reverse primers on an automated fluorescence DNA sequencer, ABI 377 (PE Applied Biosystems, Foster City, CA, USA). As a quality control, several of the sequence variants detected by SSCP were also analysed by DHPLC. All the abnormal profiles seen at SSCP were readily detected by DHPLC.

2.3. Statistical analysis

Values are given as mean ± S.D. Differences between two groups were compared using the Student's *t*-test.

3. Results

DNA from the 46 individuals diagnosed with HCM was analysed for the presence of sequence variations in eight sarcomeric protein genes. In 13 individuals (referred to by roman numerals in Table 1), nine men and four women, mutations were found in four different genes (Table 1). In these genes, a total of 11 different mutations were detected, seven in the *MYBPC3* gene, two in the *MYH7* gene and one in the *MYL2* and *TNNI3* genes, respectively (Table 2). Six of them are not previously described. Of the detected mutations, eight were missense mutations, two represent frameshift mutations (*MYBPC3* and *TNNI3*) and one was a nonsense mutation (*MYBPC3*). No mutation was found in the *TNNT2*, *TPM1*, *MYL3* or *ACTC* genes. Furthermore, three common polymorphisms resulting in amino acid substitution were detected, which definitely did not associate with HCM. The Val158Met and Ser236Gly polymorphisms in *MYBPC3* were found in eight and 16 of the 46 individuals, whereas the Lys253Arg variant in *TNNT2* was found in 15 individuals.

The associated phenotypes were those of classical HCM, with asymmetric septal or concentric hypertrophy. Only one sporadic case had prominent apical hypertrophy. The sporadic cases had more dyspnoea than the familial cases (74% vs. 36%, $P = 0.025$), but there were no other significant differences in clinical or echocardiographic parameters between these groups. Mean septal thickness in the 46 patients was 18.6 ± 3.8 mm, 46% had signs of left ventricular hypertrophy in the ECG (defined as a Romhilt-Estes score ≥4) and 39% had left ventricular outflow obstruction. In this small sample, there were no significant differences in phenotypic expression between the different mutated genes.

3.1. Cardiac myosin-binding protein C (MYBPC3) mutations

Seven mutations were found in the *MYBPC3* gene, of which five were missense mutations, leading to the substitution of a single amino acid residue. An A→C nucleotide transversion in exon 6 replaces a highly conserved tyrosine with serine at amino acid residue 237 (Tyr237Ser). The

Table 1
Clinical characteristics of probands with familial HCM or positive genotype

Individual number	Gender	Age (years)	Age at diagnosis (years)	Gene	Mutation	Familial/ sporadic case	Symtoms	LA (mm)	IVSD (mm)	LVPWD (mm)	IVSD/ LVPWD	LVEDD (mm)	LVESD (mm)	FS (%)	LVOT-obstruction *	Myectomy	Sust VT or ICD
I	Male	53	37	MYBPC3	Tyr 237 Ser	Familial	None	36	17	14	1.3	46	31	32	Yes	Yes	No
II	Male	68	67	MYBPC3	Arg 326 Gln	Familial	D, P	45	17	16	1.1	45	20	55	No	No	No
III	Male	45	30	MYBPC3	Arg 668 His	Sporadic	D, P	53	22	18	1.2	52	24	53	Yes	No	No
IV	Female	80	72	MYBPC3	Arg 668 His	Familial	None	42	19	11	1.8	46	27	41	Yes	No	No
V	Male	44	42	MYBPC3	Ala 833 Thr	Familial	D, P	37	17	15	1.1	44	23	48	Yes	No	No
VI	Female	57	47	MYBPC3	Lys 565 Stop	Familial	P, S	39	16	8	2.0	48	29	39	Yes	Yes	No
VII	Male	26	25	MYBPC3	(del CG, ins TCT 852)	Sporadic	P, S	39	28	12	2.4	36	18	52	No	No	No
VIII	Male	62	43	MYBPC3	(del CG, ins TCT 852)	Familial	P	39	19	17	1.1	33	13	62	No	No	No
IX	Male	44	43	MYBPC3	(del CG, ins TCT 852)	Familial	AP, P	34	23	10	2.2	46	31	33	No	No	No
X	Male	73	55	MYH7	Ala 430 Glu	Sporadic	P	64	18	14	1.3	50	28	45	No	No	No
XI	Female	67	58	MYH7 + MYBPC3	Glu 924 Lys + Val 896 Met	Familial	D, P	38	14 #	9	1.5	41	26	37	Yes	No	No
XII	Female	67	31	MYL2	Arg 58 Gln	Familial	D, AP, P, S	51	15	13	1.2	47	38	19	No	No	No
XIII	Male	64	44	TNNI3	del 33 nt, 202	Familial	None	49	16	14	1.1	48	25	48	No	No	No
XIV	Male	47	30	?	Not found	Familial	P	47	16	14	1.1	39	21	46	No	No	No

P: palpitations, S: syncope, D: dysponea, AP: angina pectoris, LA: left atrium, IVSD; interventricular septum diameter in end-diastole, LVPWD: left ventricular posterior wall diameter in end-diastole, LVEDD; left ventricular end-diastolic diameter, LVESD: left ventricular end-systolic diameter, FS; fractional shortening, Sust VT: ventricular tachycardia >30 s, ICD: implantable cardioverter-defibrillator, LVOT: left ventricular outflow tract, *: >30 mmHg at rest or >50 mmHg under stress; #: maximal wall thickness 20 mm, in lateral free wall.

Table 2
Mutations in probands with HCM

Gene	Mutation/type	Exon	Nucleic acid change	Novel/known	Disease association	Number of probands	Family history
Missense							
MYBPC3	Tyr 237 Ser	6	TAC → TCC	Novel	Strong	1	Familial
MYBPC3	Arg 326 Gln	12	CGG → CAG	known	Possible	1	Familial
MYBPC3	Arg 668 His	21	CGT → CAT	Novel	Strong	2	1 Fam. 1 Spor.
MYBPC3	Ala 833 Thr	25	GCG → ACG	Novel	Possible	1	Familial
MYBPC3	Val 896 Met	26	GTG → ATG	Known	Weak	1	Familial *
MYH7	Ala 430 Glu	14	GCA → GAA	Novel	Strong	1	Sporadic
MYH7	Glu 924 Lys	23	GAG → AAG	Known	Strong	1	Familial
MYL2	Arg 58 Gln	4	CGA → CAA	Known	Strong	1	Familial
Nonsense							
MYBPC3	Lys 565 Stop	18	AAA → TAA	Novel	Strong	1	Familial
Frameshift mutations							
MYBPC3	(del CG, ins TCT 852)	25	CG → TCT	Novel	Strong	3	2 Fam. 1 Spor.
TNNI3	del 33 nt, 202	8	del 33 nt	known (29)	Strong	1	Familial

* The proband also carries a mutation in *MYH7*

proband carrying this mutation, a 53-year-old man (Individual No. I in Table 1), had 11 first-degree relatives, seven were studied, of which one (a 49-year-old brother), was shown to be affected. The mutation was not found in the controls and is considered to have a strong disease association. A G→A transition in exon 12, found in a 68-year-old man (Individual No. II), replaces a conserved arginine with glutamine at residue 326 (Arg326Gln). Five of seven close relatives were studied and one affected brother was found. In exon 21, a G→A transition alters a highly conserved arginine residue, which is replaced by histidine (Arg668His). This mutation was found in two unrelated individuals, No. III and IV. Individual No. III was a 45-year-old man without known family history of HCM. All five close family members were studied, a 50-year-old sister with arterial hypertension had mild hypertrophy (septum 14 mm) and was shown to carry the mutation, as well as a 49-year-old brother with mild systolic anterior motion (SAM) of the mitral valve as only positive finding. Individual No. IV was an 80-year old asymptomatic woman, diagnosed with HCM 8 years previously because of a systolic murmur. The condition was judged to be familial due to the occurrence of exercise-related sudden death in a 16-year-old son. All living first-degree relatives, three children 54, 40 and 34 years old, were examined. The 54-year-old son (without children) with a completely normal ECG and echocardiography examination carried the mutation, whereas two unaffected daughters were genotype negative. Another G→A transition at residue 833 in exon 25, found in a 44-year-old man (Individual No. V), replaces a conserved alanine with threonine (Ala833Thr). All six family members were examined. The mutation was present in a 42-year-old brother with mild hypertrophy of the basal septum (15 mm) and SAM of the mitral valve, as well as the 69-year-old father with mild septal hypertrophy (14 mm), who also had atrial fibrillation, arterial hypertension and diabetes mellitus. In exon 26, a G→A transition in a less conserved residue alters valine to methionine (Val896Met), found in a 67-year-old woman (Individual No. XI), who was also found to carry a mutation in *MYH7* (Glu924Lys). The Ala833Thr variant was also found in one healthy control subject, and the Val896Met was identified in three of the controls. The other three missense mutations, Tyr237Ser, Arg326Gln and Arg668His, were not found in the control samples.

Two mutations are predicted to result in a truncated myosin-binding protein C, both are considered to have a strong disease association. An A→T transversion in exon 18 replaces a lysine residue with a termination signal (Lys565Stop), which was found in a 57-year-old woman (Individual No. VI). The mutation was also present in her two affected daughters, but not in any of her six unaffected siblings. A frameshift mutation in exon 25, found in three unrelated individuals (No. VII, VIII and IX), is predicted to alter the reading frame and create a premature stop codon in exon 26 (delCG/insTCT852). Individual No. VII was a 26-year-old man, without apparent family history of HCM. Two brothers and the father had normal phenotypes, although one brother (27 years old) was a mutation carrier. Since the father was not carrying the mutation, it should have been inherited from the mother. She did not have any known cardiac condition and died of a severe infectious disease. Individual No. VIII was a 62-year-old man, the proband in a family with nine affected individuals (Fig. 1). He had an affected son and an unaffected daughter. His affected father died (63 years old) suddenly and had been symptomatic for many years. One affected brother and two affected sisters died suddenly at young age. Two sons (27 and 29 years old) of another affected brother had mild hypertrophy of the septum (13 mm) and were both shown to carry the mutation, as well as their 19-year-old sister who had no sign of cardiac hypertrophy. Individual No. IX was a 44-year-old man, proband in a small family, where the asymptomatic mother (63 years) was shown to be affected. The Arg326Gln and Val896Met mutations are previously described, whereas the other five mutations are novel [32,33].

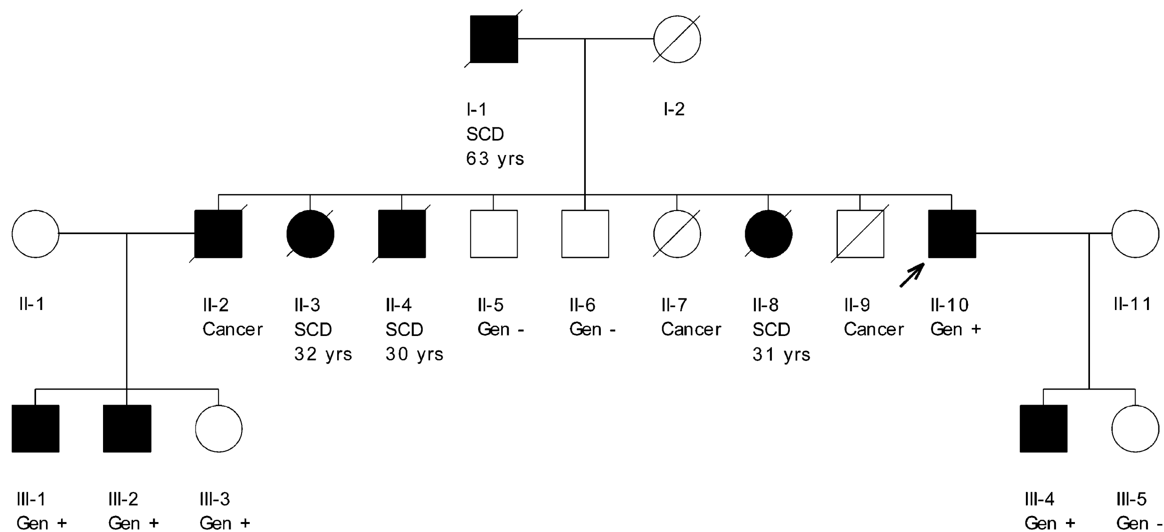

Fig. 1. A family with a frameshift mutation in exon 25 of *MYBPC3* (del CG, ins TCT 852). Squares, males; circles, females; filled symbols, clinically affected subjects; SCD, sudden cardiac death; Gen+, genotype positive; Gen–, genotsype negative. Proband indicated by arrow.

3.2. Cardiac β-myosin heavy chain (MYH7) mutations

Two missense mutations were found. A 67-year-old woman (Individual No. XI) with familial HCM was found to carry a known mutation in exon 23 of *MYH7*, where glutamic acid is replaced by lysine in a highly conserved residue (Glu924Lys) [34]. This individual was found to be double heterozygous, with a known missense mutation in *MYBPC3* (Val896Met) as well. Three first-degree relatives, a brother and two children, were clinically affected but not available for genotyping. A novel mutation was found in exon 14 in a sporadic case, a 73-year-old man (Individual No. X), where a C→A transversion replaces a highly conserved alanine with glutamate at residue 430 (Ala430Glu). This residue was found to be conserved in nine different species and isoforms (data not shown). First-degree relatives were two older brothers and a sister, they had all reached 75 years of age or more without being diagnosed with HCM, although only one unaffected brother (83 years old) agreed to participate in the study. None of the β-myosin heavy chain mutations were found in normal controls.

3.3. Cardiac regulatory myosin light chain (MYL2) mutation

A G→A transition in exon 4 was found in the proband of a family of three generations, a 67-year-old woman (Individual No. XII). It is a known missense mutation (Arg58Gln) of the cardiac regulatory myosin light chain [35]. Interestingly, her 44-year-old daughter carried the mutation, without any clinical sign of HCM, while the 18-year-old granddaughter was genotype positive and had developed obstructive HCM, requiring pacemaker treatment.

3.4. Cardiac troponin I (TNNI3) mutation

A frameshift mutation was found in the 64-year-old male proband (Individual No. XIII) in a family, where nine members in three generations were studied, published earlier by our group [29]. Three subjects were affected, two brothers (61 and 64 years old) and their mother. A 27-year-old daughter was a healthy carrier of the mutation. It is a deletion of 33 nucleotides including the stop codon in exon 8 of the cardiac troponin I, predicted to delete the last eight amino acids in the C-terminal part of the cardiac troponin I. This deletion was not present in the normal controls.

4. Discussion

4.1. Familial cases

Almost all familial HCM in northern Sweden is caused by four of the known HCM genes, the *MYBPC3*, *MYH7*, *MYL2* and *TNNI3* genes. A mutation was identified in 10 of 11 cases that had a definite family history of HCM. The *MYBPC3* is the major gene for familial HCM in northern Sweden, accounting for 64% of the different mutations found in four of the eight HCM genes that were studied. Two of the *MYBPC3* mutations detected in the familial cases were also found in individuals with sporadic HCM. The population in northern Sweden is genetically homogenous, and the high frequency of mutations in *MYBPC3* might partly be explained by founder effects. One mutation, the delCG/insTCT 852, was found in three unrelated individuals (No. VII, VIII and IX) and a subsequent haplotype analysis with five multi-allelic microsatellite markers showed that they shared the same haplotype, suggesting a common founder. Furthermore, one missense *MYBPC3* mutation (Arg668His) was found in two unrelated individuals (No. III and IV) from different geographic regions.

These results challenge the present view that the *MYH7* gene is the most common cause of familial HCM, while in this study it only represented 9% of the mutations found in familial cases. This is in concordance with a recent study

from Finland, which also showed that the *MYH7* gene was not the predominant gene, but instead the *MYBPC3* gene was more common in the population in eastern Finland [24]. The results in this study are based on observations in 46 individuals. Respecting the exclusion criteria of the study, another 14 cases could have been eligible, but were not included mainly because some patients hesitated when they lived far away from our centre. Thus, the majority of correctly diagnosed HCM patients seen by cardiologists in northern Sweden participated in the study. Patients only seen by non-cardiologists would not be identified by the search system used and is a limitation of the study.

4.2. Sporadic cases

Only three of the 35 sporadic cases (Individuals No. III, VII and X) were found to have a mutation in one of the HCM genes (Table 1). Two individuals carried a mutation in *MYBPC3* (Arg668His and delCG/insTCT852), and one had a mutation in the *MYH7* (Ala430Glu) gene. The delCG/insTCT852 found in Individual No. VII was also found in an asymptomatic brother with a normal phenotype. This mutation was also present in two of the familial cases (No. VIII and IX) but not in the healthy controls and is considered to have a strong disease association. The Arg668His found in Individual No. III was also found in a hypertensive sister with mild cardiac hypertrophy and a brother with only one minor criterion (mild SAM of the mitral valve) for HCM. The initial designation as a sporadic case was not changed, since the siblings did not fulfil the strict criteria for HCM, according to McKenna et al. [26]. This mutation was also detected in a familial case (Individual No. IV), where a healthy carrier of the mutation was also found in the family. The mutation affects a highly conserved amino acid residue, it was found in two unrelated probands in the study, but not in the healthy controls and, therefore, is considered to have a strong disease association. The novel *MYH7* mutation (Ala430Glu) found in Individual No. X is also considered to have a strong disease association, since it affects a highly conserved amino acid residue and was not found in the healthy controls.

In the majority of the cases, 17 women and 16 men, no mutation was identified. However, the *TTN*, *TNNC1* and *PRKAG2* genes were not investigated and the relevance of these specific genes in northern Sweden remains unknown. They are regarded as uncommon causes of HCM, the *TTN* is a very large gene to study and *PRKAG2* has only been found in combination with electrophysiologic abnormalities. Furthermore, there is also a limitation in the techniques used for mutation detection, SSCP has a sensitivity of about 80% and DHPLC approximately 90%. In a recent study of 31 sporadic, elderly-onset HCM cases, six sarcomeric protein genes were screened for mutations. In four cases, *MYBPC3* mutations were found, but in the majority of the cases, as in the present study, no mutation was found [32]. The proportion of sporadic cases in this study was larger than expected and the reason for this is not clear. When including patients in the study, an effort was made to include all available cases, both sporadic and familial. It is possible that some of the patients in this study represent the subset of elderly-onset HCM, 11 patients were >65 years old at the time of diagnosis. One must also bear in mind that the families were not completely studied in every case, which is a limitation of the study.

The phenotypic expression was quite similar in sporadic and familial cases. Except for dyspnoea, which was more common in sporadic cases, there were no other clinical or echocardiographic parameters that distinguished individuals with sporadic or familial disease.

4.3. Mutations

The clinical significance of a missense mutation should be interpreted with some caution, especially in the *MYBPC3* gene, since the distinction between a rare polymorphism and disease-causing mutation is not always clear. The information about each mutation and its possible disease association is summarised in Table 2, taking into account:

1. if the mutation co-segregates with the disease, keeping in mind that some families are small and mutations can also be found in seemingly sporadic cases;
2. if the mutation has been found in the healthy controls in this or other published studies;
3. the degree of conservation in the amino acid sequence.

Three of the *MYBPC3* mutations found, Arg326Gln, Ala833Thr and Val896Met, deserve further comment (see below).

The Arg326Gln mutation was found in two affected brothers with familial HCM (family of Individual No. II). It was recently described in a case with elderly-onset, sporadic HCM, as well as one healthy individual [32]. In another study, it was possibly not associated with HCM, since it was found in healthy controls as well as affected subjects [24]. In the present study, this variant was not found in the normal control subjects. The amino acid sequences from 10 mammalian, avian and amphibian species flanking the residues, where the missense mutations were found in *MYBPC3* are shown in Table 3. The arginine at residue 326 is conserved in three species and the mutation is possibly disease associated in this family.

The novel Ala833Thr mutation was found in a 44-year-old male proband in a family with HCM (Individual No. V). A brother with mild hypertrophy and systolic anterior motion of the mitral valve also carried the mutation, which was shown to come from the father who had mild cardiac hypertrophy. Both parents had arterial hypertension, obscuring the phenotype assessment. One of the healthy controls, a 19-year-old man, also had this variant. The mutated residue is conserved in three species, supporting evidence of it being possibly disease associated, although the possibility of a rare polymorphism cannot be ruled out.

The Val896Met mutation was found in a 67-year-old woman (Individual No. XI) with obstructive, familial HCM, who was found to be double heterozygous, with a missense mutation in *MYH7* as well. In this particular case, the Val896Met mutation in the *MYBPC3* gene was previously

Table 3
Comparison of amino acid sequences in cardiac and skeletal myosin-binding protein C of different species, flanking the residues where missense mutations have been found (extent of homology.shown in boxes)

Cardiac myosin binding protein C	Tyr 237 Ser	Arg 326 Gln	Arg 668 His	Ala 833 Thr	Val 896 Met
Human cardiac	AFTGS[Y]RCEVS	VWEIL[R]Q-APPS	AGNKL[R]LDVPI	ELSHE[A]RRMIE	RPPER[V]GAGGL
Mouse cardiac	TSAGG[Y]RCEVS	VWEIL[R]Q-APPS	TGNKL[R]LDVPI	ELSHE[A]RRMIE	RPPER[V]GAGGL
Chicken cardiac	TFAGG[Y]RCEVS	VWEIL[R]K-APPS	AGNKL[R]LDVPI	ELTYE[A]KRMIE	RPPERIGAGGL
Frog cardiac	TYAGG[Y]RCEVS	VWEILKK-APPS	AGNKL[R]LDVPI	ELTYESKRMIE	RPPERIGAGGL
Axolotl cardiac	TYAGG[Y]RCEAV	MSGKSEG-ASPS	AGNKL[R]LDVPI	-----------	-----------
Human slow skeletal	NFAGN[Y]RCEVT	VWELLKN-AKPS	AGNKL[R]LEIPI	ETTFEPKKMIE	RPPNRIGAGGI
Human fast skeletal	GDRGY[Y]RLEVK	IWELLKG-AKKS	AGNKL[R]LDVSI	ETTYESTKMIE	RPPDHIGAAGL
Chicken fast skeletal	GDRGD[Y]RCEVT	IWELLKGVTKKS	AGNKV[R]LDVPI	DTTYESTKMIE	RPPERIGAGGV
Mouse fast skeletal	GDRGD[Y]RLEIK	IWELLKG-AKKS	AGNKL[R]LDVAI	DTTYESTKMIE	RPPDRIGAGGI
Rat slow skeletal	-----------	------------	AGSKL[R]LEIPV	ETTFEPKKMIE	RPPDQIGAAGL

published as disease causing [33], but in another study, an unaffected individual was found to be homozygous for the mutation and five healthy controls were heterozygous [24]. In the present study, this mutation was found in three of the healthy controls, making probable that it represents a non-disease related polymorphism in the population of northern Sweden. This is also supported by the fact that the magnitude of hypertrophy in the proband was not exceptional, which has often been the case in other reports of families with double mutations [36]. Interestingly, this residue is less conserved (two species) than the other mutations found. The other mutation in this individual, a known Glu924Lys mutation in a highly conserved residue in the *MYH7* gene was not found in the healthy controls and is most likely disease associated in this case [34]. The other family members had moved out of our catchment area and were not available for genotyping, therefore the contribution to the phenotypes of each mutation could not be assessed further, although it was clearly a familial condition from the medical records, with two children and one brother affected.

The majority of *MYBPC3* mutations in this study were missense mutations (five of seven mutations), while in the literature, mutations resulting in a truncated protein are more often described in this gene. However, one of the missense mutations found in this study is a likely polymorphism unrelated to disease (Val896Met) and two mutations lack definite proof of being disease causing (Arg326Gln and Ala833Thr). If these three missense mutations of uncertain clinical importance are excluded, two of the remaining four mutations in the *MYBPC3* would be truncating. Mutations in *MYBPC3* are associated with a late-onset disease, which in combination with small family sizes can make it difficult to establish co-segregation between the mutation and the disease. This emphasises the need for adequate control studies before giving an *MYBPC3* missense mutation the status of a disease-causing mutation.

5. Conclusion

Due to the heterogeneity of the disease, today one cannot use the genotype as a tool in the everyday management of patients with HCM. However, data concerning genotype-phenotype relationship is emerging, indicating that *MYBPC3* mutations are associated with a milder, late-onset disease compared with *MYH7* mutations. Adequate family screening is important when evaluating new cases with HCM, since the familial nature of the disease might easily be overlooked, especially in the presence of a gene with low penetrance. This is relevant for the situation in northern Sweden, where *MYBPC3* is the dominant gene. Information about the genotype in a family could be useful, especially when dealing with healthy family members, in doubt about their risk of developing the disease. The knowledge of the actual HCM genes present in a particular population can, therefore, be of interest. Indeed, the present study has prompted the initiation of a genetic counselling programme at our hospital, now available to HCM families, where the disease-causing mutation is known.

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References

[1] Towbin J, Roberts R. Cardiovascular diseases due to genetic abnormalities. 8th ed. In: Schlant RC, Alexander RW, editors. Hurst's the heart: arteries and veins. New York: McGraw-Hill Inc; 1994. p. 1725–59.

[2] Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA study. Coronary artery risk development in (young) adults [see comments]. Circulation 1995;92(4):785–9.

[3] Elliott PM, McKenna WJ. Hypertrophic cardiomyopathy. In: Crawford MH, DiMarco JP, editors. Cardiology. London: Mosby International limited; 2001. p. 12.1–12.12.

[4] Shah PM, Adelman AG, Wigle ED, Gobel FL, Burchell HB, Hardarson T, et al. The natural (and unnatural) history of hypertrophic obstructive cardiomyopathy. Circ Res 1974;35(2)(Suppl II):179–95.

[5] Hardarson T, De la Calzada CS, Curiel R, Goodwin JF. Prognosis and mortality of hypertrophic obstructive cardiomyopathy. Lancet 1973; 2(7844):1462–7.

[6] McKenna WJ. The natural history of hypertrophic cardiomyopathy. Cardiovasc Clin 1988;19(1):135–48.

[7] Cannan CR, Reeder GS, Bailey KR, Melton III LJ, Gersh BJ. Natural history of hypertrophic cardiomyopathy. A population-based study, 1976 through 1990. Circulation 1995;92(9):2488–95.

[8] Cecchi F, Olivotto I, Montereggi A, Santoro G, Dolara A, Maron BJ. Hypertrophic cardiomyopathy in Tuscany: clinical course and outcome in an unselected regional population. J Am Coll Cardiol 1995; 26(6):1529–36.

[9] Maron BJ, Nichols PFD, Pickle LW, Wesley YE, Mulvihill JJ. Patterns of inheritance in hypertrophic cardiomyopathy: assessment by M- mode and two-dimensional echocardiography. Am J Cardiol 1984; 53(8):1087–94.

[10] Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, et al. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell 1990;62(5):999–1006.

[11] Watkins H, Conner D, Thierfelder L, Jarcho JA, MacRae C, McKenna WJ, et al. Mutations in the cardiac myosin-binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. Nat Genet 1995;11(4):434–7.

[12] Bonne G, Carrier L, Bercovici J, Cruaud C, Richard P, Hainque B, et al. Cardiac myosin-binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. Nat Genet 1995;11(4):438–40.

[13] Carrier L, Bonne G, Bahrend E, Yu B, Richard P, Niel F, et al. Organization and sequence of human cardiac myosin-binding protein C gene (MYBPC3) and identification of mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy [see comments]. Circ Res 1997;80(3):427–34.

[14] Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP, et al. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. Cell 1994;77(5):701–12.

[15] Poetter K, Jiang H, Hassanzadeh S, Master SR, Chang A, Dalakas MC, et al. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. Nat Genet 1996;13(1):63–9.

[16] Mogensen J, Klausen IC, Pedersen AK, Egeblad H, Bross P, Kruse TA, et al. Alpha-cardiac actin is a novel disease gene in familial hypertrophic cardiomyopathy. J Clin Invest 1999;103(10):R39–43.

[17] Kimura A, Harada H, Park JE, Nishi H, Satoh M, Takahashi M, et al. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. Nat Genet 1997;16(4):379–82.

[18] Hoffmann B, Schmidt-Traub H, Perrot A, Osterziel KJ, Gessner R. First mutation in cardiac troponin C, L29Q, in a patient with hypertrophic cardiomyopathy. Hum Mutat 2001;17(6):524.

[19] Satoh M, Takahashi M, Sakamoto T, Hiroe M, Marumo F, Kimura A. Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene. Biochem Biophys Res Commun 1999;262(2):411–7.

[20] Gollob MH, Green MS, Tang AS, Gollob T, Karibe A, Ali Hassan AS, et al. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. N Engl J Med 2001;344(24): 1823–31.

[21] Blair E, Redwood C, Ashrafian H, Oliveira M, Broxholme J, Kerr B, et al. Mutations in the gamma(2) subunit of AMP-activated protein kinase cause familial hypertrophic cardiomyopathy: evidence for the central role of energy compromise in disease pathogenesis. Hum Mol Genet 2001;10(11):1215–20.

[22] Arad M, Benson DW, Perez-Atayde AR, McKenna WJ, Sparks EA, Kanter RJ, et al. Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy. J Clin Invest 2002;109(3):357–62.

[23] Marian AJ, Roberts R. The molecular genetic basis for hypertrophic cardiomyopathy. J Mol Cell Cardiol 2001;33(4):655–70.

[24] Jaaskelainen P, Kuusisto J, Miettinen R, Karkkainen P, Karkkainen S, Heikkinen S, et al. Mutations in the cardiac myosin-binding protein C gene are the predominant cause of familial hypertrophic cardiomyopathy in eastern Finland. J Mol Med 2002;80(7):412–22.

[25] Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task force on the definition and classification of cardiomyopathies. Circulation 1996;93(5):841–2.

[26] McKenna WJ, Spirito P, Desnos M, Dubourg O, Komajda M. Experience from clinical genetics in hypertrophic cardiomyopathy: proposal for new diagnostic criteria in adult members of affected families. Heart 1997;77(2):130–2.

[27] Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58(6):1072–83.

[28] Bonne G, Carrier L, Richard P, Hainque B, Schwartz K. Familial hypertrophic cardiomyopathy: from mutations to functional defects. Circ Res 1998;83(6):580–93.

[29] Morner S, Richard P, Kazzam E, Hainque B, Schwartz K, Waldenstrom A. Deletion in the cardiac troponin I gene in a family from northern Sweden with hypertrophic cardiomyopathy. J Mol Cell Cardiol 2000;32(3):521–5.

[30] Xiao W, Oefner PJ. Denaturing high-performance liquid chromatography: a review. Hum Mutat 2001;17(6):439–74.

[31] Tesson F, Dufour C, Moolman JC, Carrier L, al-Mahdawi S, Chojnowska L, et al. The influence of the angiotensin I converting enzyme genotype in familial hypertrophic cardiomyopathy varies with the disease gene mutation. J Mol Cell Cardiol 1997;29(2):831–8.

[32] Niimura H, Patton KK, McKenna WJ, Soults J, Maron BJ, Seidman JG, et al. Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. Circulation 2002;105(4):446–51.

[33] Moolman-Smook JC, De Lange WJ, Bruwer EC, Brink PA, Corfield VA. The origins of hypertrophic cardiomyopathy-causing mutations in two South African subpopulations: a unique profile of both independent and founder events. Am J Hum Genet 1999;65(5): 1308–20.

[34] Watkins H, Thierfelder L, Hwang DS, McKenna W, Seidman JG, Seidman CE. Sporadic hypertrophic cardiomyopathy due to de novo myosin mutations. J Clin Invest 1992;90(5):1666–71.

[35] Flavigny J, Richard P, Isnard R, Carrier L, Charron P, Bonne G, et al. Identification of two novel mutations in the ventricular regulatory myosin light chain gene (MYL2) associated with familial and classical forms of hypertrophic cardiomyopathy. J Mol Med 1998;76(3–4): 208–14.

[36] Richard P, Isnard R, Carrier L, Dubourg O, Donatien Y, Mathieu B, et al. Double heterozygosity for mutations in the beta-myosin heavy chain and in the cardiac myosin binding protein C genes in a family with hypertrophic cardiomyopathy. J Med Genet 1999;36(7):542–5.