Original Article

Genetic Testing in the Management of Relatives of Patients with Hypertrophic Cardiomyopathy

(echocardiography / DNA sequence analysis / cardiomyopathies)

P. TOMAŠOV[1], M. MINÁRIK[2], D. ZEMÁNEK[1], P. ČADOVÁ[1], S. HOMOLOVÁ[1], K. ČURILA[3], M. PĚNIČKA[3], L. BENEŠOVÁ[2], B. BELŠÁNOVÁ[2], P. GREGOR[3], J. VESELKA[1]

[1]Department of Cardiology, Second Faculty of Medicine, Charles University in Prague and University Hospital Motol, Prague, Czech Republic
[2]Laboratory for Molecular Genetics and Oncology, Genomac Research Institute, Prague, Czech Republic
[3]Third Department of Internal Medicine – Department of Cardiology, University Hospital Královské Vinohrady, Prague, Czech Republic

Abstract. Hypertrophic cardiomyopathy is the most common genetic cardiac disease with vast genetic heterogeneity. First-degree relatives of patients with HCM are at 50% risk of inheriting the disease-causing mutation. Genetic testing is helpful in identifying the relatives harbouring the mutations. When genetic testing is not available, relatives need to be examined regularly. We tested a cohort of 99 unrelated patients with HCM for mutations in *MYH7*, *MYBPC3*, *TNNI3* and *TNNT2* genes. In families with identified pathogenic mutation, we performed genetic and clinical examination in relatives to study the influence of genetic testing on the management of the relatives and to study the usefulness of echocardiographic criteria for distinguishing relatives with positive and negative genotype. We identified 38 genetic variants in 47 patients (47 %). Fifteen of these variants in 21 patients (21 %) were pathogenic mutations. We performed genetic testing in 52 relatives (18 of them (35 %) yielding positive results). Genetic testing of one HCM patient allowed us to omit 2.45–5.15 future cardiologic examinations of the relatives. None of the studied echocardiographic criteria were significantly different between the relatives with positive and negative genotypes, with the exception of a combined echocardiographic score (genotype positive vs. genotype negative, 3.316 vs. -0.489, P = 0.01). As a conclusion, our study of HCM patients and their relatives confirmed the role of genetic testing in the management of the relatives and found only limited benefit of the proposed echocardiographic parameters in identifying disease-causing mutation carriers.

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease with an estimated prevalence in general population of 1 : 500 (Niimura et al., 1998; Watkins et al., 1992). It is the leading cause of sudden cardiac death in young athletes and is associated with heart failure morbidity (Maron and Maron, 2013). HCM is inherited as an autosomal dominant trait with incomplete or age-related penetrance (Marian and Roberts, 1994). More than 1,400 mutations causing HCM have been identified in at least 12 genes for mainly sarcomeric proteins, many of them being private mutations identified only in a single family (Maron and Maron, 2013).

First-degree relatives of the patients are at 50% risk of carrying an HCM-causing mutation (Marian and Roberts, 1994). The clinical management of the relatives therefore consists of regular follow-up with electrocardiogram (ECG) and echocardiographic examination. Because of the age-related penetrance, cardiologic examination should be performed until the age of 50–60

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Corresponding author: Pavol Tomašov, Department of Cardiology, Second Faculty of Medicine, Charles University in Prague and University Hospital Motol, V Úvalu 84, 150 06 Prague 5, Czech Republic. Phone: (+420) 224 434 901; Fax: (+420) 224 434 920; e-mail: pavol.tomasov@fnmotol.cz

Abbreviations: ACCF/AHA – American College of Cardiology Foundation/American Heart Association, ASE – American Society of Echocardiography ECG – electrocardiogram, ESC – European Society of Cardiology, HCM – hypertrophic cardiomyopathy, LVH – left ventricular hypertrophy.

years (Charron et al., 2010). During this time, the relatives without phenotypic features of HCM cannot be given the certainty of not harbouring a disease-causing mutation.

The hallmark of the echocardiographic examination which distinguishes the affected and unaffected relatives is the presence of left ventricular hypertrophy (LVH) in the absence of other systemic or cardiovascular disease capable of causing such extent of hypertrophy (Charron et al., 1997). This feature, however, is unreliable as a detector of disease-causing mutation carrier. Several features of ECG and echocardiographic examination have been proposed to be able to identify mutation carriers before the development of LVH (Ho et al., 2002; Michels et al., 2009; Gandjbakhch et al., 2010). Furthermore, in some cases of HCM, especially with mutations in troponin T genes, LVH may not develop at all (Watkins et al., 1995; Varnava et al., 2001).

Overall, the most sensitive and specific examination for detecting mutation-carrying relatives who are at risk of developing full phenotype of HCM is genetic testing. Genetic testing in relatives is feasible in case that a disease-causing mutation has been identified in the family's index patient. Indication for genetic testing in HCM patients is controversial and the topic is discussed in a recent European Society of Cardiology (ESC) position statement and it is a class IIa indication in the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) Guidelines for the diagnosis and treatment of HCM when used in the index patient of a family to facilitate the identification of relatives at risk of developing HCM (Charron et al., 2010; Gersh et al., 2011). The result of genetic testing in relatives has a major impact on their clinical management, with no further follow-up needed when the results are negative and a full work-up when the results are positive (Charron et al., 2010; Gersh et al., 2011).

We tried to identify the influence of genetic testing on the routine clinical management of HCM patients' relatives in a setting of two tertiary cardiology centres. We also studied the proposed echocardiographic criteria for distinguishing disease-causing mutation carriers in our population (Ho et al., 2002; Michels et al., 2009; Gandjbakhch et al., 2010).

Material and Methods

Study population

We performed genetic screening of 99 consecutive unrelated HCM patients of Caucasian origin examined at our institutions between the dates September 2006 – December 2007. The diagnosis of HCM was based on the presence of LVH (assessed by echocardiography) in the absence of other systemic or cardiovascular disease capable of causing such hypertrophy. The demographic, clinical and echocardiographic data of the patients are summarized in Table 1. In families with positive results of genetic testing, we suggested all the first-degree relatives to undergo genetic testing (apart from the usual clinical examination) as well. All procedures and examinations performed met the ethical requirements of the respective Institutional Review Boards.

Genetic analysis

After signing informed consent with genetic testing, the patients' peripheral blood was drawn and the genomic DNA was isolated using standard spin-column method with QIAQuick DNA mini kits (Qiagen GmbH, Hilden, Germany) or JetQuick DNA Isolation kits (GENOMED GmbH, Loehne, Germany). Primer pairs were designed to amplify the coding exons and flanking intronic sequences of the genes *MYH7* (β-myosin heavy chain), *MYBPC3* (myosin-binding protein C), *TNNI3*

Table 1. Demographic, clinical and echocardiographic parameters of the studied HCM patient group

Variable	HCM patients (N = 99) Median [interquartile range] or %	Variable	HCM patients (N = 99) Median [interquartile range] or %
Age (years)	55 [49-62.75]	IVS (mm)	17 [14.75-21]
Gender (male)	58 %	PWT (mm)	13 [12-14.5]
BSA (m^2)	1.91 [1.745-2.185]	LVEDD (mm)	44 [40-47]
BP systolic (mm Hg)	130 [110-140]	LVESD (mm)	26 [23-29]
BP diastolic (mm Hg)	80 [70-90]	LVEF (%)	75 [73-80]
NYHA class	2 [1-2.375]	LA diameter (mm)	43 [39-48]
Beta blockers (%)	77 %	Peak E velocity (m/s)	0.675 [0.5875-0.91]
ACE inhibitors (%)	26 %	Peak A velocity (m/s)	0.78 [0.69-0.91]
AT receptor antagonists (%)	6 %	LVOT rest (mm Hg)	13 [7.25-34.75]
Calcium channel antagonists (%)	23 %	LVOT provocation (mm Hg)	29 [11.75-83.25]
Diuretics (%)	26 %		

ACE – angiotensin converting enzyme, AT – angiotensin, BP – blood pressure, BSA – body surface area, IVS – interventricular septum, LA – left atrium, LVEDD – left ventricular end-diastolic diameter, LVEF – left ventricular ejection fraction, LVESD – left ventricular end-systolic diameter, LVOT – left ventricular outflow tract, NYHA – New York Heart Association, PWT – posterior wall thickness

(cardiac troponin I) and *TNNT2* (cardiac troponin T) according to CardioGenomics – Sarcomere Protein Gene Mutation Database (http://genepath.med.harvard.edu/~seidman/cg3/). PCR amplifications were performed according to standard protocols with QiaAmp kits (Qiagen GmbH) and Taq-Purple PCR Master Mix kits (Top-bio, Prague, Czech Republic). The amplicons were analysed by direct sequencing using DYEnamic terminator chemistry and run in a MegaBACE 1000 device (GE Biosciences, Piscataway, NJ) as per manufacturer's instructions. Detected variants in a sample were confirmed at least in two independent PCR and sequencing runs. Sequencher software (Gene Codes, Ann Arbor, MI) was used to facilitate data analysis and mutation identification followed by visual inspection of individual sequencing traces.

The identified variants were divided into categories as described in the ACCF/AHA guidelines: pathogenic mutation (as listed in CardioGenomics – Sarcomere Protein Gene Mutation Database), probably pathogenic mutation, variants of uncertain significance and intronic variants (Gersh et al., 2011).

Relatives who signed informed consent with genetic testing were examined after obtaining their DNA from the bucal mucosa. The DNA was then tested for the presence of the variant found in the index patient of the respective family.

Echocardiographic studies

Echocardiographic studies were performed with a Philips Sonos 5500 ultrasound system (Philips Healthcare, Best, Netherlands) in standard two-dimensional (2D) mode, M-Mode, pulsed Doppler and Tissue Doppler Imaging (TDI), according to the American Society of Echocardiography (ASE) guidelines (Lang et al., 2005). The following data were collected in respective modes and views: M-mode in long-axis parasternal view: end-diastolic LV IVS and LPW thickness, LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), end-diastolic left atrial (LA) diameter and aortic root (AO) diameter; pulsed Doppler in apical four chamber view: early (E) and late (A) transmitral peak velocities, pulsed TDI in apical four chamber view: systolic (Sa), early diastolic (Ea) and late diastolic (Aa) myocardial velocities at septal margin of mitral annulus. Left ventricular ejection fraction was calculated according to the Teichholz formula. The E/A ratio, E/septal Ea ratio, IVS/PWT ratio and the relative wall thickness (RWT), defined as (IVS + PWT)/LVEDD, were calculated from the obtained values. The left ventricular outflow tract obstruction in HCM patients was assessed as described previously (Dimitrow et al., 2009; Zemanek et al., 2011).

Statistical analysis

When comparing the variables between the genetically affected and unaffected relatives we used a non parametric Mann-Whitney test for continuous variables, as it was impossible to test normality of distribution due to the low number of subjects, and Fisher's exact test for categorical variables. Two-sided P value of < 0.05 was considered statistically significant. The statistical software GraphPad PRISM version 5.01 (GraphPad Software Inc., CA) was used for all analyses.

Results

We identified 38 genetic variants inside the coding exons and flanking intronic regions of the *MYH7, MYBPC3, TNNI3* and *TNNT2* genes in 47 of 99 HCM patients (47 %). Fifteen of these variants found in 21 patients (21 %, one of them being a compound heterozygote) were pathogenic mutations. Others were probably pathogenic mutations, variants of uncertain significance, or variants in flanking intronic sequence (Figs. 1, 2). In the 21 families with known pathogenic mutations, we managed to perform genetic testing in 52 relatives (18 of them (35 %) yielding positive results) and clinical examination including ECG and echocardiography in 20 relatives (50 % of these examined relatives harboured a disease-causing mutation and 50 % did not). We then analysed the number of future cardiologic examinations

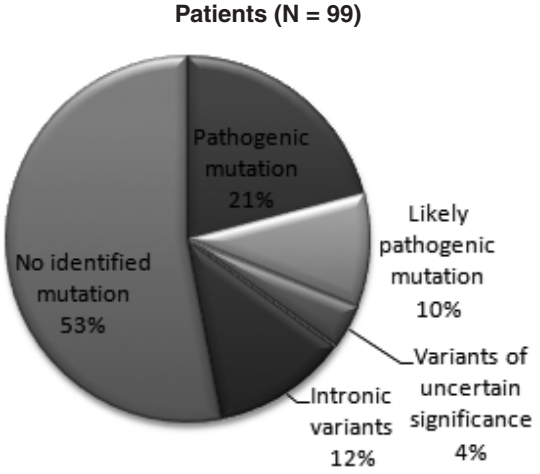

Fig. 1. Types of genetic variants identified in HCM patients' cohort

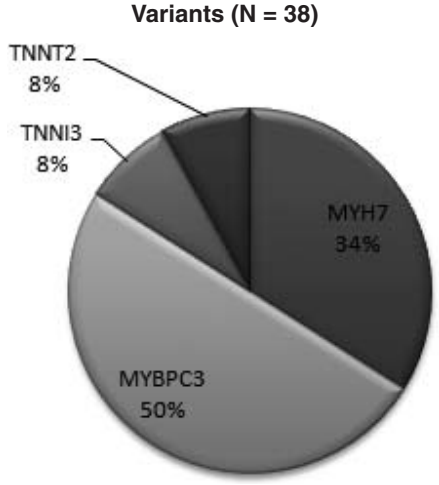

Fig. 2. Distribution of identified variants in the studied genes

Table 2. Influence of genetic testing of HCM patients on the management of relatives from families with identified pathogenic mutation

	Tested relatives (N = 52)
Genotype + (%)	35 %
Genotype - (%)	65 %
Total number of examinations omitted due to negative genetic analysis (2-year model)	510
Total number of examinations omitted due to negative genetic analysis (5-year model)	243
Number of examinations omitted thanks to genetic testing of one HCM patient (2-year model)	5.15
Number of examinations omitted thanks to genetic testing of one HCM patient (5-year model)	2.45

omitted in the relatives with negative results of genetic testing using two extremes of ESC recommendation for serial follow-up (i.e. every 2 years and every 5 years between the age of 20–60). Thus, genetic testing of one HCM patient in this setting allowed us to omit 2.45–5.15 future cardiologic examinations including ECG and echocardiographic study of the relatives as summarized in Table 2.

After excluding relatives with phenotypic features of HCM on their echocardiographic examination (LVH ≥ 12 mm), we compared the echocardiographic parameters in seven relatives with disease-causing mutation and 10 relatives without disease-causing mutation. Due to the small size of our cohorts, none of the studied echocardiographic parameters showed a significant difference between the groups when using a non-parametric test, with the exception of the combined echocardiographic score proposed by Gandjbakhch et al. (2010, genotype positive vs. genotype negative 3.316 vs. -0.489, P = 0.01, Table 3). Variables with significant difference in other studies are shown in box-whisker graphs in Figs. 3–6.

Discussion

Genetic testing is an important achievement and a valuable tool in managing patients with hereditary diseases. In the setting of HCM with vast genetic heterogeneity, the feasibility and usefulness of routine genetic testing is impaired by the large number of genes to be studied, resulting in a time-consuming and costly process (Charron et al., 2010). Nevertheless, genetic testing of HCM patients is becoming part of the clinical practice and it provides information which mainly influences the management of relatives (Charron et al., 2010; Gersh et al., 2011), providing an example of translation of originally basic research into clinical practice. Several recommendations regarding the use of genetic testing of HCM patients have been published, including an ESC position statement and ACCF/AHA guidelines (Charron et al., 2010; Gersh et al., 2011). The indications for genetic testing of the patients are limited and most of the obtained data are from research projects. Once the genetic testing yields a positive result, however, there are simple rules for performing genetic testing in relatives and modifying their management according to the results (Charron et al., 2010; Gersh et al., 2011).

Novel techniques in molecular diagnostics derived from the whole-genome micro-array studies have the potential of significant time reduction in testing for genetically heterogeneous diseases such as HCM and may increase the number of tested patients (Faita et al., 2012).

When results of genetic testing are not available or are inconclusive, the relatives of HCM patients need to undergo serial clinical follow-up due to the incomplete,

Table 3. Comparison of demographic and echocardiographic parameters of genotype positive and genotype negative relatives from families with identified pathogenic mutation

Variable	**Genotype + (N = 7) Median [interquartile range] or %**	**Genotype - (N = 10) Median [interquartile range] or %**	**P value**
Age (years)	31 [24.5-40.5]	25.5 [15.25-56]	0.74
Gender (male)	29%	70%	0.15
IVS (mm)	9 [8-11]	8.5 [7.75-9.25]	0.52
PWT (mm)	9 [8-10]	8 [7-8.25]	0.20
LVEDD (mm)	43 [40-47]	45 [40.5-47.5]	0.81
IVS/PWT ratio	1 [1-1.1]	1.065 [1-1.133]	0.72
RWT	0.447 [0.333-0.476]	0.369 [0.346-0.422]	0.47
LVEF (%)	65 [64-68.75]	64 [62-66]	0.59
LA diameter (mm)	30 [29-33]	34.5 [27.75-40.25]	0.35
Peak E velocity (m/s)	0.80 [0.73-0.88]	0.745 [0.53-1.01]	0.73
Peak A velocity (m/s)	0.46 [0.45-0.60]	0.57 [0.495-0.795]	0.40
Septal Ea velocity (cm/s)	11.4 [8.38-11.70]	12.05 [6.525-15]	0.81
Septal Aa velocity (cm/s)	9 [7.3-10.1]	8.145 [6.515-12.65]	0.94
Septal Sa velocity (cm/s)	7.8 [7.1-9.1]	8.9 [8.04-9.7]	0.37
E/Ea ratio	8 [6.5-9.5]	7 [6 -9]	0.61
ECHO index by Gandjbakhch et al. (2010)	3.316 [2.950-3.531]	-0.489 [-1.679-1.827]	**0.01**

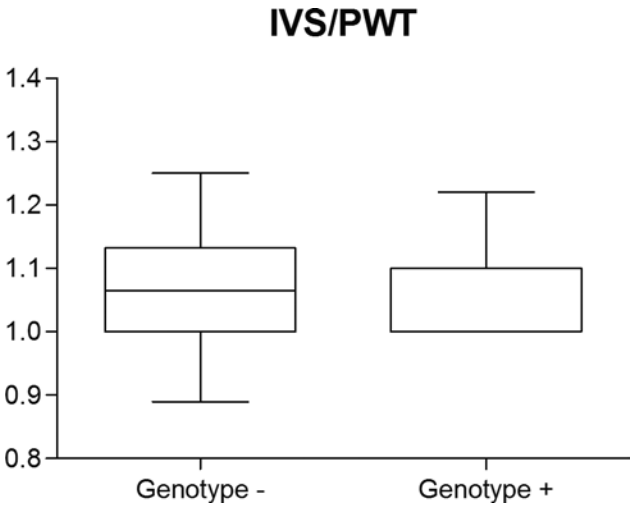

Fig. 3. Comparison of the IVS/PWT ratio in genotype negative and genotype positive relatives

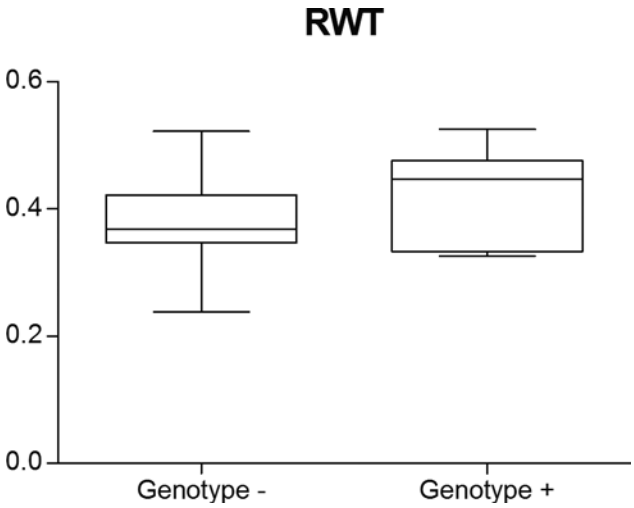

Fig. 4. Comparison of the RWT in genotype negative and genotype positive relatives

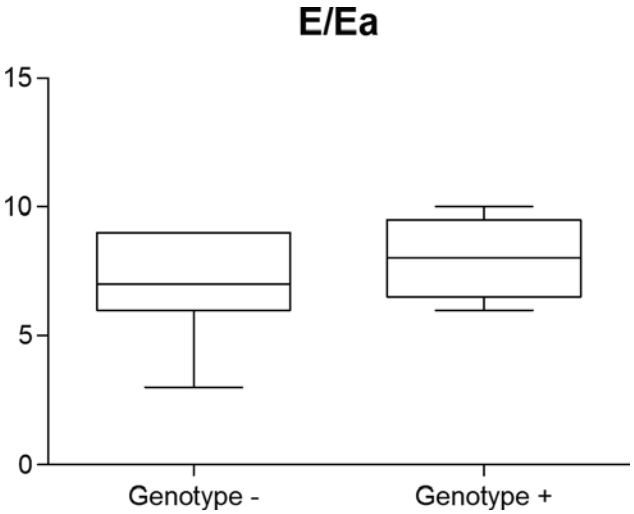

Fig. 5. Comparison of the E/Ea ratio in genotype negative and genotype positive relatives

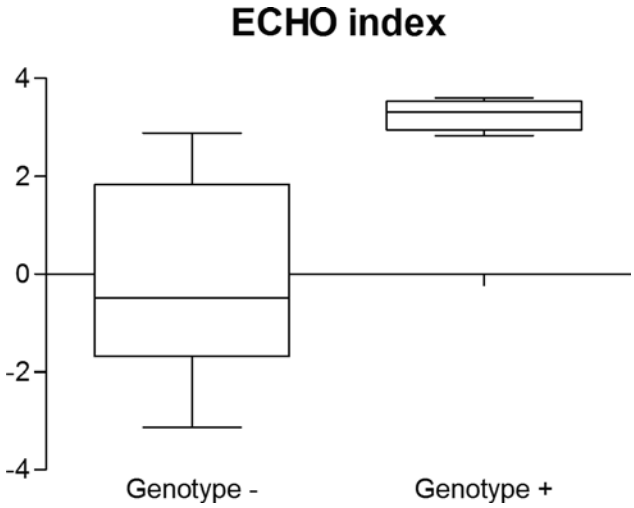

Fig. 6. Comparison of the echocardiographic index by Gandjbakhch et al. (2010) in genotype negative and genotype positive relatives

or rather age-related penetrance of HCM (Charron et al., 2010; Gersh et al., 2011). The inability to state the risk of developing HCM carries a certain psychological burden as well (Burkett and Hershberger, 2005). Several parameters predicting the risk of developing full phenotype of HCM in relatives without LVH have been identified. First in animal models and later in humans, mitral annulus velocities measured by TDI have been shown to identify the disease-causing mutation carriers with high sensitivity and specificity (Marian et al., 1997; Charron et al., 1998; Kim et al., 1999; Marian, 2000; Maron et al., 2001). Later studies, however, did not prove the high value of mitral annulus velocities, and this topic remains controversial (Ho et al., 2002; Michels et al., 2009). One of the confounding factors for TDI parameters could be the age of the relatives, which was similar or higher in our study when compared with other reports; however, comparison of several studies shows significant variations in mitral annulus velocities irrespective of the age of the subjects (Ho et al., 2002; Michels et al., 2009; Gandjbakhch et al., 2010).

Other studies combined morphologic criteria (IVS/PWT ratio, RWT) and TDI measurements of mitral annulus velocities (E/Ea ratio) to identify disease-causing mutation carriers (Gandjbakhch et al., 2010). Our study implies a confirmation of the usefulness of the combined echocardiographic score by Gandjbakhch et al. (2010); however, the proposed cut-off value of 0.45 with excellent specificity in the original study showed a moderate specificity (60 %) and high sensitivity (100 %) in our work, showing different distribution of the studied variables in distinct populations (Gandjbakhch et al., 2010). Such a score has the advantage of containing information about both left ventricular morphology (as a prequel to overt hypertrophy) and diastolic dysfunction. By combining more variables in one score there is also greater chance to prove significant differences between populations, as shown in our study.

Naturally, neither of the studied parameters reaches the accuracy of genetic testing in identifying the disease-causing mutation carriers. On the other hand, even when testing for all the known mutations, about 40 % of HCM patients yield negative results (presumably due to unknown mutations in sarcomeric and non-sarcomeric genes or even mitochondrial DNA, Charron et al., 2010; Gersh et al., 2011; Palecek et al., 2012). Therefore, in a considerable part of the families, clinicians need to rely on using mainly echocardiographic and ECG parameters to be able to identify the relatives at risk of developing the HCM phenotype and influence their clinical management. None of these parameters is perfect, as shown by the controversy of the previous reports as well as the results of our cohort of relatives, implying perhaps a need for stratification scores combining more variables.

Limitations

Our study was not sufficiently powered to prove a small statistical difference in the parameters distinguishing the affected and unaffected relatives. We rather observed the distribution of previously proposed variables in our cohort of genotyped relatives to test their usefulness for routine clinical management.

The positive results of genetic testing were obtained in fewer patients of our cohort than could be expected according to previously published studies. This could be caused by several factors. We only tested our cohort in four genes, which, however, constitute the majority of identified mutations, and the expected loss of positive results due to not examining other genes should not exceed 10 %. The Czech population may well carry different mutations than populations previously studied and many of the newly identified variants in our study may yet prove to be true disease-causing mutations. Finally, our HCM patients' cohort may be biased due to attracting patients suitable for alcohol septal ablation to our institutions. These patients, although meeting the ESC criteria for HCM, may in some cases not suffer from a true allelic disorder with disease-causing mutation in sarcomeric genes.

Conclusion

Our study of a well-characterized group of HCM patients and their relatives confirms the role of genetic testing in the management of relatives and the benefit provided to both carriers and non-carriers of a disease--causing mutation. In the scenario of families with negative results of genetic testing, our study found only limited benefit of the proposed echocardiographic parameters in identifying disease-causing mutation carriers.

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