Clinical and Genetic Aspects of

Hypertrophic Cardiomyopathy

Hannah Gillian van Velzen

Clinical and genetic aspects of hypertrophic cardiomyopathy

Academic thesis, Erasmus University Rotterdam

Printing of this thesis has been financially supported by:

Erasmus MC Thoraxcenter

Cardialysis B.V.

Cover design Merel de Boer *merel@studiowys.nl*

Printing Optima Grafische Communicatie *www.ogc.nl*

ISBN 978-94-6361-207-4

Clinical and Genetic Aspects of Hypertrophic Cardiomyopathy

Klinische en genetische aspecten van hypertrofische cardiomyopathie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. R.C.M.E. Engels

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

Woensdag 16 januari om 13.30 uur

Hannah Gillian van Velzen
geboren te Spijkenisse

Erasmus University Rotterdam
Make it happen.

PROMOTIECOMMISSIE

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Copromotoren:

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Dr. A.F.L. Schinkel

Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged

Voor mijn ouders

CONTENTS

Part III Clinical aspects of hypertrophic cardiomyopathy

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common inherited myocardial disease with an estimated prevalence of 1:500 to 1:200.(1, 2) It is defined by an increased left ventricular wall thickness that is not solely explained by abnormal loading conditions(3-5) (Figure 1). HCM is frequently caused by mutations in genes that encode proteins of the cardiac sarcomere; the smallest contractile unit of the cardiac muscle.(6) Classic microscopic features of HCM are muscle fiber disarray, microvascular remodeling, and interstitial fibrosis.(7, 8) The distribution of the hypertrophy is typically asymmetrical with a predilection for the septum and the anterior wall, but concentric hypertrophy is also observed and the hypertrophy can be located in other parts of the left or right ventricle including the papillary muscles.(7, 9, 10) Other abnormalities include systolic anterior motion (SAM) of the mitral valve, mitral regurgitation, outflow obstruction, impaired diastolic relaxation, and autonomic dysregulation.(4)

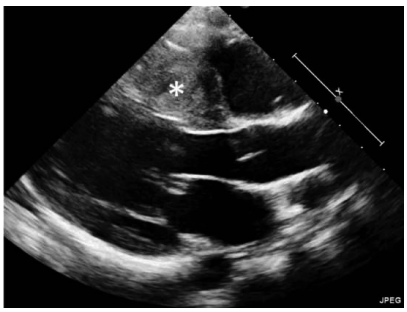

Figure 1. Parasternal long axis two-dimensional echocardiogram illustrating asymmetrical septal left ventricular hypertrophy (*) in a patient with hypertrophic cardiomyopathy.

HCM can present from infancy to the very elderly.(11) Symptoms associated with HCM include chest pain, exertional dyspnea, palpitations and syncope which have a range of causes such as microvascular ischemia, outflow obstruction, heart failure or arrhythmias.(3) The clinical course ranges from normal life expectancy to sudden cardiac death (SCD) at a young age, progressive heart failure, and atrial fibrillation with an increased risk of thromboembolism.(11, 12). Although overall the life expectancy of patients with HCM is good with many achieving advanced longevity, a proportion of the patients follow a distinctive pathway in which there is worsening systolic and diastolic function, increasing fibrosis, and elevated risk of ventricular and atrial arrhythmias.(7, 13) (Figure 2)

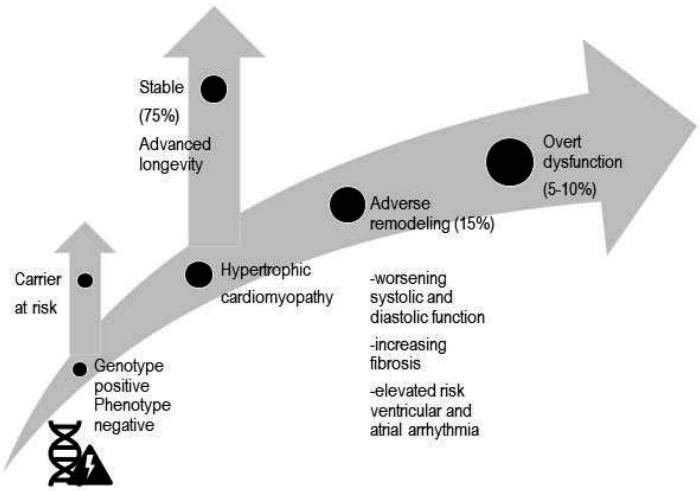

Figure 2. Stages of hypertrophic cardiomyopathy. Percentages in brackets represent prevalence of each stage. The prevalence of genotype-positive, phenotype-negative individuals is unknown. Figure adapted from figure 1. in reference 13.

A brief historical overview of the diagnosis and management

In the 1950s the first modern descriptions of HCM were published by Donald Teare, a forensic pathologist, describing the association of HCM with SCD in young people, the asymmetric distribution of the hypertrophy, and the muscle fiber disarray.(8) Russell Brock, Andrew Morrow and Eugene Braunwald recognized the presence of functional left ventricular outflow tract obstruction.(14, 15) In 1961, Morrow introduced the myotomy-myectomy procedure, in which excessive tissue in the outflow tract is excised during open heart surgery.(16) In 1995 a trans catheter approach to relieve outflow obstruction was introduced, the alcohol septal ablation.(17) Today, both procedures are offered to patients with drug-refractory symptoms and have similar effects on functional status and similar procedural mortality.(3) Overall, the procedure of choice depends largely on the mechanism of the outflow obstruction, the severity of the hypertrophy, the coronary artery anatomy, patient preference, and the experience of the referral center.(18)

The most feared complication of HCM is SCD, although it is relatively infrequent among patients with HCM (annual risk 0.5-1%).(12) It occurs most commonly in young asymptomatic patients < 35 years(4). In fact, a number of studies from the Unites States have reported that HCM is the most common cause of SCD in young athletes.(4, 19) Although rare, exercise can induce ventricular arrhythmias leading to SCD.(20) And so, current guidelines for patients with HCM advise against participation in competitive sports and intense physical activity.(3, 4) Furthermore, in certain countries

and major sporting federations it is the rationale for pre-participation cardiovascular screening of competitive athletes.(21) The impact, cost-effectiveness and preferred strategies are however debatable.(21)

During the past 50 years extensive research has been performed to identify clinical SCD risk factors. SCD risk stratification became especially relevant when in 1980 the implantable cardiac defibrillator (ICD) was introduced, a device that can effectively terminate malignant ventricular arrhythmias and thus save lives.(22, 23) Currently, there is an ongoing debate between Europe and the United States regarding the most appropriate SCD risk stratification and the indication for ICD implantation.(24-28)

Genetics and family screening

In the 1940s Evans had noticed the familial nature of HCM.(29) Family studies subsequently elucidated the autosomal dominant mode of inheritance.(30, 31) Pedigree analysis is an important aspect of the clinical management of patients with HCM (Figure 3). Since the year 2003 guidelines have encouraged family screening by electrocardiography and echocardiography.(3-5) Current guidelines recommend cardiac evaluation from age 10-12 years until 18-21 years of age, and every 2-5 years thereafter until advanced age.(3, 4, 32) Younger children can be screened in case of a severe family history, competitive sports participation, or when cardiac symptoms are present.(3)

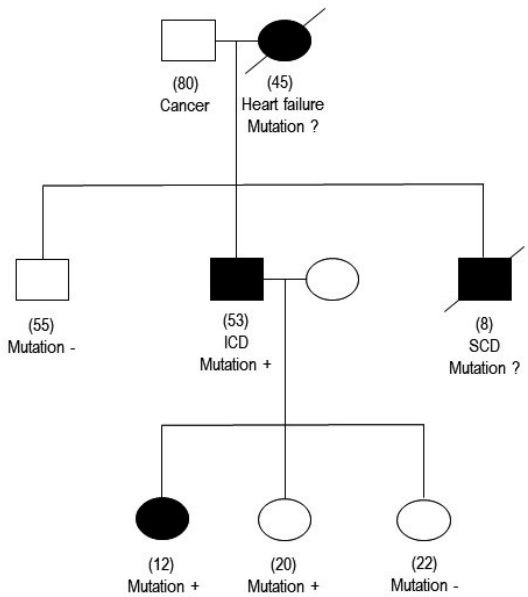

Figure 3. Example of pedigree analysis by using a family tree. Black filling of the squares (males) and circles (females) indicate a diagnosis of HCM. ICD, implantable cardiac defibrillator; SCD, sudden cardiac death.

In 1989 the genetic substrate of HCM was demonstrated by Seidman et al. and located on chromosome 14q1(6). Since then, more than 1500 mutations in at least 11 genes have been identified to cause HCM.(12) Most of these genes encode proteins of the myofilaments or Z-disc of the sarcomeres.(33) Mutations in the myosin-binding protein C (*MYBPC3*) gene and the ß-myosin heavy chain (*MYH7*) gene represent >70% of the mutations.(33) In the Netherlands, *MYBPC3* mutations are exceptionally frequent, due to the presence of three Dutch *MYBPC3* founder mutations. Roughly 35% of HCM is caused by one of these three Dutch *MYBPC3* founder mutations.(34, 35) These mutations cause C-terminally truncated protein leading to haplo-insufficiency.(36) Pathophysiologic studies have demonstrated that these mutations are associated with a reduced force generating capacity of cardiomyocytes, cardiomyocyte hypertrophy and reduced myofibril density.(37, 38)

The extreme genetic and clinical heterogeneity of HCM makes it challenging to assess genotype-phenotype associations.(39) Currently, asides from its use in the differentiation between HCM and phenocopies, genetic testing is mainly used for the evaluation of family members.(3, 32, 40) A pathogenic mutation is identified in 50-60% of patients with HCM.(41) Determining the pathogenicity of DNA variants involves several steps including the assessment of public databases, published data, co-segregation in families, and predicted effects on slicing and the protein.(42) Variants are then classified into 5 categories: (I) benign; (II) likely benign; (III) uncertain significance; (IV) likely pathogenic; and (V) pathogenic (Figure 4).(42) During the past decade, advances in DNA sequencing methodology has enabled us to offer multigene testing to all patients with HCM, but it also generates many variants with uncertain significance, which complicate the interpretation of the results.(33) Therefore, a multidisciplinary team composed of cardiologists, molecular biologists, bio-informaticians, clinical geneticists and genetic counsellors is crucial.(33, 43) Counselling is essential before and after genetic testing, due to the potential psychosocial, emotional and financial consequences of genetic testing.(32, 44)

Class I Benign	Class II Likely Benign	Class III VUS	Class IV Likely Pathogenic	Class V Pathogenic
Recognized neutral in the literature, mutation databases Previously reported	Sequence variation probably not causative Previously unreported	Sequence variation may or may not be causative Previously unreported Family studies? Structural effect on protein?	Sequence variation expected to be causative Previously unreported	Recognized causative in the literature, mutation databases

Figure 4. Classification of variants according to the American College of Medical Genetics and Genomics recommendations(42). VUS, variant with uncertain significance.

In case a pathogenic mutation is identified, relatives can undergo pre symptomatic genetic testing.(32) Relatives who have not inherited the mutation can be reassured and discharged from follow-up and there is no increased risk of transmitting the disease to offspring.(32) Relatives who have inherited the mutation but have no clinical expression of HCM are advised to undergo repeated clinical evaluation, because HCM can develop later in life i.e. there is age-related penetrance.(32) HCM mutation carriers without clinical expression of HCM are of tremendous interest to researchers, because it helps us understand the pathophysiological processes which occur before the expression of disease.(45) Subsequently, gene-therapy and other novel therapeutics might in the future be able to prevent the development of HCM instead of treating the symptoms after HCM has developed as is the case presently.(40, 46)

Outline of the thesis

An overview of the chapters and its content is presented in table 1. During the past 60 years the diagnosis and clinical management of HCM has undergone significant changes.(2, 33, 47-49) Septal reduction therapies, ICD implantations, heart transplantation, and catheter-based procedures have significantly improved the clinical outcome of patients with HCM to the point where it is now a treatable disease with many patients reaching extended longevity.(12) However, the impact of adverse outcomes associated with HCM (SCD, progressive heart failure, stroke) is huge for individual cases and families. Also, due to advances in diagnostic imaging, family screening, and an unexpected high prevalence of pathogenic sarcomere mutations in the general population, HCM is more prevalent than previously estimated.(2) With the discovery of the genetic substrate of HCM 20 years ago, we have entered a new era in which a more preventive approach is aspired.(40) Although we know that genotype influences the phenotype and the prognosis in HCM, its prognostic value is currently limited due to extreme clinical and genetic heterogeneity.(39) Since roughly 50-60% of the patients with HCM have a positive genotype, investigating the impact of a genotype-positive status might gain insight into the prognostic value of genetic test results. Therefore, in **chapter 1** we compare HCM patients with and without sarcomere mutations and investigate the association between a genotype-positive status and long-term clinical outcome. We continue to investigate genotype-phenotype associations in **chapter 2**, where we analyze the clinical characteristics and long-term outcome of HCM caused by Dutch *MYBPC3* founder mutations. In **chapter 3**, we investigate the results of HCM family screening including genetic testing. With the introduction of pre symptomatic genetic testing in relatives a new subgroup has emerged: HCM mutation carriers without clinical expression of HCM. These individuals are at risk of developing HCM. In the next two chapters we seek to determine preclinical markers of HCM by comparing these HCM mutation carriers with healthy controls and performing longitudinal follow-up of the mutation carriers. In **chapter 4**, we assess the prognostic significance of anterior mitral valve leaflet length for the development of HCM, and in **chapter 5**, we study the prognostic significance of global longitudinal strain using speckle tracking

echocardiography. In the HCM field, three-dimensional (3D) echocardiography is currently still primarily a research tool.(47) Studies have indeed demonstrated superior performance to two-dimensional echocardiography for the evaluation of myocardial hypertrophy, LV volumes, LV ejection fraction, and LV mass. In **chapter 6** we study the utility of 3D echocardiography for the assessment of LV hypertrophy and papillary muscle morphology. Atrial fibrillation (AF) is the most common arrhythmia in the HCM population and an important risk factor for heart failure and stroke. Its identification has direct implications for the management of HCM. Therefore, in **chapter 7** we assess the incidence and impact of device-detected AF in patients with HCM and a cardiac implantable electronic device. Currently, insufficient data is available regarding the impact of gender on the long-term outcomes of patients with HCM. In **chapter 8** we compare the clinical presentation, phenotype, genotype, and outcome between male and female patients with HCM. Finally, in **chapter 9** we go from bedside-to-bench by analyzing whether sex differences in the diastolic function of patients with HCM can be explained at a cellular level.

Table 1. Overview of the chapters (continues on the next page)

Chapter	Study population	Test	Outcome
1	Patients with HCM	Genetic testing	Mortality Interventions
2	*MYBPC3* founder mutation carriers	Clinical evaluation 2D echocardiography	Mortality Interventions
3	Relatives of patients with HCM	Clinical evaluation Genetic testing	Development of HCM Mortality Interventions
4	HCM mutation carriers and healthy controls	Clinical evaluation Electrocardiography 2D echocardiography	Development of HCM
5	HCM mutation carriers and healthy controls	Clinical evaluation Electrocardiography Speckle tracking echocardiography	Development of HCM Mortality
6	Patients with HCM	3D echocardiography	Left ventricular wall thickness Papillary muscle abnormalities

HCM, hypertrophic cardiomyopathy; *MYBPC3*, myosin-binding protein C; 2D, two-dimensional; 3D, three-dimensional

Table 1. Overview of the chapters (continued)

Chapter	Study population	Test	Outcome
7	Patients with HCM	CIED interrogation	Mortality
			Interventions
			Thromboembolism
8	Patients with HCM	Gender	Mortality
		Genetic testing	Interventions
			Nonfatal clinical events
9	Patients with HCM	2D echocardiography	Diastolic function
		Force measurements Protein	Passive tension
		analysis Histomorphometrical	Titin function
		analysis	Fibrosis

CIED, cardiac implantable electronic device; HCM, hypertrophic cardiomyopathy; 2D, two-dimensional

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PART I

Genetic testing and family screening in hypertrophic cardiomyopathy

CHAPTER 1

Value of genetic testing for the prediction of long-term outcome in hypertrophic cardiomyopathy

Hannah G. van Velzen
Pieter A. Vriesendorp
Rogier A. Oldenburg
Marjon A. van Slegtenhorst
Jolanda van der Velden
Arend F.L. Schinkel
Michelle Michels

Am J Cardiol. 2016 Sep 15;118(6):881-7

ABSTRACT

Pathogenic gene mutations are found in about 50 % of hypertrophic cardiomyopathy (HC) patients. Previous studies have shown an association between sarcomere mutations and medium-term outcome. The association with long-term outcome has not been described. The aim of this cohort study was to assess the long-term outcomes of genotype positive (G+) and genotype negative (G-) HC patients. The study population consisted of 626 HC patients (512 probands, and 114 relatives) who underwent phenotyping and genetic testing between 1985 and 2014. End points were: all-cause mortality, cardiovascular (CV) mortality, heart failure (HF) related mortality and sudden cardiac death/aborted sudden cardiac death (SCD/aborted SCD). Kaplan Meier and multivariate cox regression analyses were performed. A pathogenic mutation was detected in 327 (52%) patients. G+ probands were younger than G- probands (46±15 vs 55±15 years, $p<0.001$), had more non sustained ventricular tachycardia (34% vs 13%; $p<0.001$), more often a history of syncope (14% vs 7%; $p=0.016$), and more extreme hypertrophy (maximal wall thickness ≥ 30 mm 7% vs 1%; $p<0.001$). G- probands were more symptomatic (NYHA ≥ II 73% vs 53%, $p<0.001$) and had higher left ventricular outflow tract gradients (42±39 vs 29±33 mmHg, $p=0.001$). During 12±9 years follow-up, G+ status was an independent risk factor for all-cause mortality (HR 1.90; 95% CI 1.14 –3.15; $p=0.014$), CV mortality (HR 2.82; 95% CI 1.49–5.36; $p=0.002$), HF related mortality (HR 6.33; 95% CI 1.79–22.41; $p=0.004$), and SCD/aborted SCD (HR 2.88; 95% CI 1.23–6.71; $p=0.015$). In conclusion, during long-term follow-up, G+ HC patients are at increased risk of all-cause death, CV death, HF related death, and SCD/aborted SCD.

INTRODUCTION

Hypertrophic cardiomyopathy (HC) is the most common inherited myocardial disease, with an estimated prevalence of 1 in 500.(1) Although the majority of patients with HC have a good prognosis, a small minority may experience life-threatening complications, such as heart failure (HF), sudden cardiac death (SCD) and atrial fibrillation (AF) leading to stroke.(2) The difficulty in determining the prognosis of HC patients lies in the genetic and clinical heterogeneity. More than 1500 pathogenic mutations in at least 11 genes encoding thick and thin myofilament protein components of the sarcomere have been identified.(1) A pathogenic mutation is found in about 50% of HC patients.(3) Current guidelines advise to genotype HC patients in order to facilitate family screening.(4) The prognostic significance of genetic test results in patients with HC is still under debate. Previous studies have shown an association between sarcomere mutations and clinical outcome.(5-8) The follow-up duration in these studies varied from 1(5) to 6.6(7) years. Information on the value of genetic testing for the prediction of the long-term outcome in patients with HC is currently not available. Therefore, the aim of this study was to investigate the association between G+ status and long-term clinical outcome.

METHODS

This prospective cohort study included 626 HC patients (probands: n=512, 82%; relatives: n=114, 18%), who attended the cardio-genetic outpatient clinic between May 1985 and August 2014. Probands were defined as patients with HC who presented with signs or symptoms of HC. Relatives were defined as patients with HC who were identified via family screening. Each patient had an established diagnosis of HC based on maximal wall thickness (MWT) ≥ 15 mm unexplained by loading conditions, or ≥ 13 mm for relatives of HC patients. Patients with HC linked to other causes were excluded. The study conforms to the principles of the Declaration of Helsinki. All patients gave informed consent, and review board approval was obtained.

All patients underwent genetic counselling. Before the year 2012, DNA analysis consisted of direct sequencing of all coding intro-exon boundaries of the following genes: myosin binding protein C (*MYBPC3*), myosin heavy chain 7 (*MYH7*), regulatory myosin light chain 2 (*MYL2*), regulatory myosin light chain 3 (*MYL3*), troponin T (*TNNT2*), troponin I (*TNNI3*), cysteine and glycine-rich protein 3 (*CSRP3*), titin-cap/telethonin (*TCAP*), α-tropomyosin 1 (*TPM1*), cardiac muscle alpha actin (*ACTC1*), cardiac troponin C (*TNNC1*), and teneurin C-terminal associated peptides (*TCAP*). From 2012, next-generation-sequencing was used, covering the following genes: *ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CAV3, CRYAB, CSRP3, CTNNA3, DES, DSC2, DSG2, DSP, EMD, FHL1, GLA, JPH2, JUP, LAMA4, LAMP2, LDB3, LMNA, MIB1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, MYPN, NEXN, PKP2, PLN, PRDM16, PRKAG2, RBM20, SCN5A, TAZ, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR* and *VCL*. Variants were classified into classes: (I) benign;

(II) likely benign; (III) variant of unknown clinical significance; (IV) likely pathogenic; or (V) pathogenic, adapted from the classification proposed by Plon et al.(9) Patients were considered G+ when the mutation was classified as class IV or V.

Follow-up data were obtained in November 2014, and was complete for 99 % of patients. Mortality was retrieved from the civil register. An electrophysiologist evaluated ICD interventions. The study end points were: all-cause mortality, CV mortality, HF related mortality, and SCD/aborted SCD. Cardiac transplantation was considered HF related mortality. CV mortality consisted of HF related death, SCD/aborted SCD, postoperative death after a cardiac intervention and stroke related death. SCD/aborted SCD was defined as: (1) instantaneous and unexpected death in patients who were previously in a stable clinical condition, or nocturnal death with no history of worsening symptoms; (2) resuscitation after cardiac arrest; or (3) ICD intervention for ventricular fibrillation or for fast ventricular tachycardia (>200 beats/min). Syncope was defined according to the guidelines(4). Statistical analyses were performed using SPSS 21 (IBM, Armonk, New York) and Microsoft Access 2010 (version 14.0.7143.5000). Unpaired t-test or the chi-square test were used to compare variables. P values < 0.05 were considered significant. Multivariate analysis was performed with a model in which each variable with $p < 0.05$ (based on univariate analysis) was entered, with a maximum of 1 variable per 10 events. Survival curves were constructed according to the Kaplan Meier method, and compared using the log rank test. Due to a high prevalence of three *MYBPC3* founder mutations (c.2373dupG, c.2827C>T and c.2864_2865delCT)(10), we adjusted for the founder effect by including only the first enrolled proband with a founder mutation. Founder mutations were defined according to Alders et al.(11) All reported annual mortality rates are in 50-year survivors.

RESULTS

The baseline characteristics are presented in table 1. A pathogenic mutation was detected in 234 (46%) probands, and in 93 (82%) relatives. G+ probands were younger than G- probands (46±15 vs 55±15 years, p<0.001), had more AF (26% vs 15%; p<0.001), and a higher MWT (20±5 mm vs 18±4 mm; p<0.001). The following risk factors for SCD were more common in G+ probands: family history of SCD, non-sustained ventricular tachycardia, syncope, and MWT ≥ 30 mm. G- probands were more symptomatic (NYHA ≥ II 73% vs 53%, p=<0.001) and had higher LVOT gradients (42±39 vs 29±33 mmHg, p=0.001). Relatives presented to clinic primarily through familial evaluation (n=66, 58%) and through positive genetic screening (n=48, 42%). Compared to probands, relatives were younger (46±15 vs 51±15 y, p=0.003), had fewer AF (11% vs 20%, p=0.034), were less symptomatic (NYHA ≥ II 18% vs 64%, p<0.001), had a lower MWT (17±4 vs 19±5 mm, p<0.001), smaller left atria (41±7 vs 45±8, p<0.001), and had lower LVOT peak gradients (11±15 vs 36±37, p<0.001). Relatives more often had a family history of SCD (28% vs 12%, p<0.001). There were no significant differences between G+ and G- relatives (table 1).

Table 1. Baseline characteristics of probands and relatives with hypertrophic cardiomyopathy.

Variable	Entire cohort (n=626)	Probands (n=512)			Relatives (n=114)		
		Genotype + (n=234)	Genotype - (n=278)	p-value	Genotype + (n=93)	Genotype - (n=21)	p-value
Male	404 (65%)	159 (68%)	171 (62%)	0.130	61 (66%)	13 (62%)	0.749
Age (years)	51±15	46±15	55±15	<0.001	45±15	51±13	0.092
AF (by history)	115 (18%)	61 (26%)	41 (15%)	0.001	1 (12%)	2 (10%)	0.764
NYHA II or higher	216 (55%)	81 (53%)	121 (73%)	<0.001	11 (16%)	3 (25%)	0.473
Maximal wall thickness	18±5	20±5	18±4	<0.001	17±4	17±4	0.806
Left atrial size	44±8	45±8	45±7	0.996	43±8	41±7	0.340
LV end diastolic diameter	46±6	45±6	46±7	0.438	46±5	47±7	0.541
Apical morphology	31 (5%)	4 (2%)	22 (8%)	0.001	3 (3%)	2 (10%)	0.203
LVOT peak gradient	32±16	29±33	42±39	0.001	10±14	16±20	0.325
LVOT PG > 30 mmHg	178 (28%)	67 (29%)	106 (38%)	0.024	3 (3%)	2 (10%)	0.203
LV systolic dysfunction	70 (12%)	31 (15%)	31 (12%)	0.430	7 (8%)	1 (5%)	0.632
Family history of SCD	61 (12%)	46 (20%)	15 (6%)	<0.001	26 (30%)	5 (25%)	0.706
nsVT on Holter monitoring	111 (22%)	67 (34%)	26 (13%)	<0.001	14 (18%)	4 (22%)	0.675
Abnormal exercise BPR	79 (16%)	28 (14%)	41 (20%)	0.141	8 (10%)	2 (12%)	0.790
Syncope	52 (10%)	32 (14%)	20 (7%)	0.016	4 (4%)	1 (5%)	0.926
MWT ≥ 30 mm	18 (4%)	16 (7%)	2 (1%)	<0.001	0	0	-

All values are mean ± SD or number (%) AF = atrial fibrillation, BPR = blood pressure response, LV = left ventricle, LVOT = left ventricular outflow tract, MWT = maximal wall thickness, NYHA = New York Heart Association functional class, nsVT = non sustained ventricular tachycardia, PG = peak gradient, SCD = sudden cardiac death

The distribution of the affected genes are presented in figure 1. Next-generation sequencing was performed in 161 (26%) patients. Most patients had *MYBPC3* mutations (n=240; 73%), followed by *MYH7* mutations (n=47; 14%) and thin filament mutations (n=19; 6%). Figure 2 demonstrates the distribution of the *MYBPC3* founder mutations. *MYBPC3* founder mutations were present in 101 (47%) G+ probands and 53 (57%) G+ relatives. A detailed overview of the individual pathogenic mutations is presented in supplementary table 1 (online only). Three patients (1%) had multiple mutations: one compound heterozygous *MYBPC3* mutation in trans and two double heterozygous (*MYBPC3*/*MYL2* and *MYH7*/*MIB1*) mutations. Most mutations were truncating mutations (n=184; 56%) followed by missense (n=101; 31%) and splice site mutations (n=34; 10%). Supplementary table 2 (online only) illustrates the varying types of mutations in the patients who died from HF and

SCD/aborted SCD. The gene most commonly affected in SCD/aborted SCD was *MYBPC3* (founder: n=10, non-founder: n=6), followed by *MYH7* (n=2), and a double mutation carrier. SCD/aborted SCD did not occur among *TNNT2* mutation carriers (n=10; mean age 61±9).

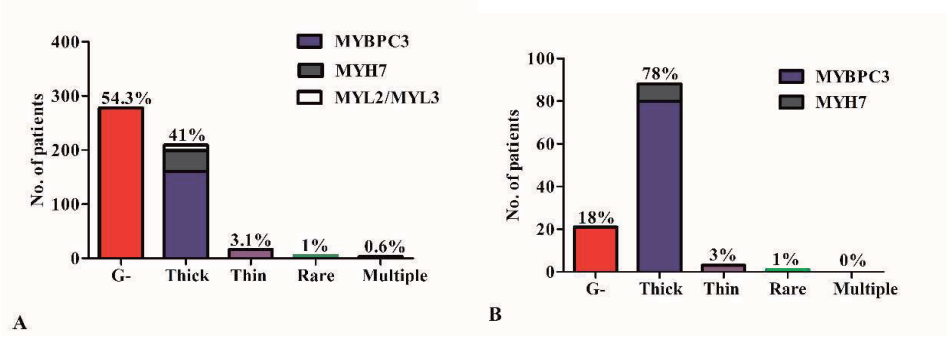

Figure 1. The distribution of pathogenic gene mutations in 512 probands (A) and 114 relatives (B). G- = genotype-negative HC patients. Thick = patients with thick filament associated gene mutations: myosin binding protein C (MYBPC3), myosin heavy chain (MYH7), regulatory myosin light chain 2 (MYL2) and regulatory myosin light chain 3 (MYL3). Thin = patients with thin filament associated gene mutations: troponin I, troponin T and α-tropomyosin 1. Rare = patients with rare mutations: calreticulin 3, cysteine and glycine-rich protein 3, and myopalladin. Multiple = patients with multiple mutations.

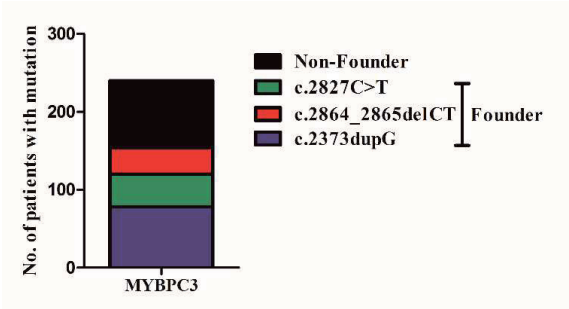

Figure 2. The distribution of founder and non-founder mutations in the myosin binding protein C (*MYBPC3*) gene. *MYBPC3* founder mutations include: c.2373dupG (purple); n=78 (33%), c.2827C>T (green); n=42 (18%) and c.2864_2865delCT (red); n=33 (14%). Non-founder *MYBPC3* mutations (black): n=86 (36%).

Mortality and interventions during follow-up are presented in table 2. During the mean follow-up period of 12±9 years, G+ probands had a greater probability of all end points: all-cause mortality, HF related mortality, CV mortality, and SCD/aborted SCD (figures 3 and 4). Annual rates for G+ vs G- patients were as follows: (1) all-cause mortality: 2.4% vs 1.0%, log rank p<0.001; (2) HF related

mortality: 0.9% vs 0.2%, log rank p<0.001; (3) CV mortality: 1.8% vs 0.4%, log rank p<0.001; and (4) SCD/aborted SCD: 1.1% vs 0.15%, log rank p=0.002. After adjustment for the founder effect, all of these differences remained significant. ICDs for primary prevention were implanted more often in G+ probands (16% vs 9%; p=0.019). There was no significant difference in the number of septal reduction therapies (both ASA and surgical myectomy) between G+ and G- probands (31% vs 33%; p=0.710). All-cause mortality for relatives was comparable to probands (10% vs 14%, p=0.247), with an annual all-cause mortality rate of 1.3%. Compared to probands, cardiovascular death trended lower in relatives (4% vs 9%, p=0.084). There were no significant differences between G+ and G- relatives. Multivariate cox regression analyses of G+ status in probands for the end points are presented in Table 3. G+ status was an independent predictor of all-cause mortality (HR 1.90, p=0.014), CV mortality (HR 2.82, p=0.002) and HF related mortality (HR 6.33, p=0.004). G+ status was also a predictor of SCD/aborted SCD, after adjusting for established risk factors for SCD as described in the guidelines from 2003(12) and 2011(13).

Table 2. Mortality and interventions during follow-up of probands and relatives

Variable	Entire cohort (n=626)	Probands (n=512)			Relatives (n=114)		
		Genotype + (n=234)	Genotype – (n=278)	p-value	Genotype + (n=93)	Genotype – (n=21)	p-value
All-cause mortality	81 (13%)	40 (17%)	30 (11%)	0.039	10 (11%)	1 (5%)	0.401
Age at death, y	62±14	62±16	64±11	0.488	58±14	49	0.550
Cardiovascular mortality	53 (9%)	32 (14%)	16 (6%)	0.002	4 (4%)	1 (5%)	0.926
Heart failure related mortality	20 (3%)	15 (6%)	3 (1%)	0.001	2 (2%)	0	0.498
Cardiac transplantation	7 (1%)	4 (2%)	2 (1%)	0.300	1 (1%)	0	0.633
SCD/aborted SCD	29 (5%)	17 (7%)	9 (3%)	0.039	2 (2%)	1 (5%)	0.500
True SCD	9 (1%)	7 (3%)	2 (1%)	0.051	0	0	
Aborted SCD	20 (3%)	10 (4%)	7 (3%)	0.269	2 (2%)	1(5%)	0.500
Stroke related death	2 (0.3%)	0	2 (0.7)	0.194	0	0	
Post procedural cardiac death	2 (0.3%)	0	2 (0.7)	0.194	0	0	
Septal reduction therapy	171 (27%)	73 (31%)	91 (33%)	0.710	7 (8%)	0	0.194
Alcohol septal ablation	53 (9%)	21 (9%)	32 (12%)	0.348	0	0	
Surgical myectomy	126 (20%)	53 (23%)	66 (24%)	0.771	7 (8%)	0	0.194
ICD	98 (16%)	49 (21%)	35 (13%)	0.011	10 (11%)	4 (19%)	0.296
For primary prevention	76 (12%)	38 (16%)	26 (9%)	0.019	8 (9%)	4 (19%)	0.159
For secondary prevention	22 (4%)	11 (5%)	9 (3%)	0.395	2 (2%)	0	0.498

All values are mean ± SD, median [Q1 – Q3] or number (%). ICD = implantable cardioverter defibrillator, SCD=sudden cardiac death

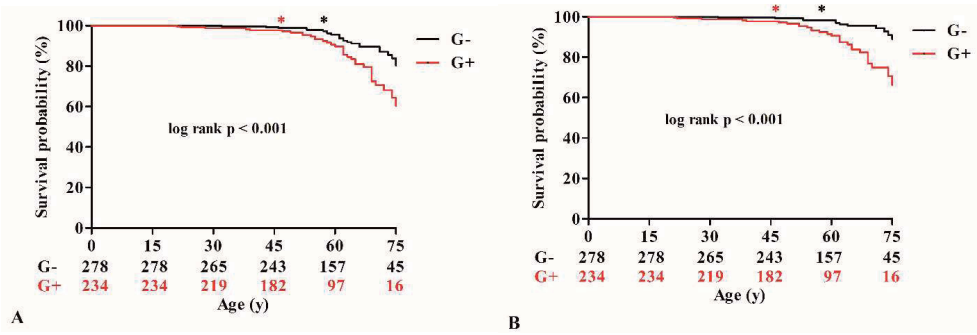

Figure 3. Kaplan-Meier analysis comparing (A) all-cause mortality in G+ probands and G- probands and (B) cardiovascular mortality in G+ probands and G- probands. * = age at presentation (red for G+ and black for G-). G+ = genotype-positive. G- = genotype-negative. Cardiovascular mortality is defined as death related to heart failure or stroke, sudden cardiac death or postoperative death after a cardiac intervention.

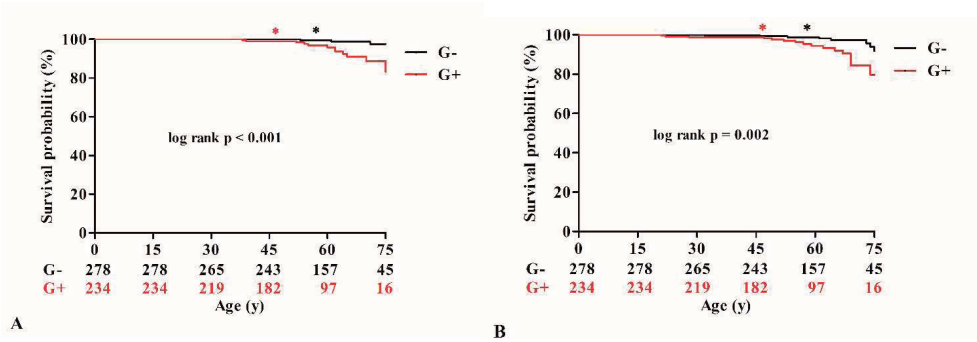

Figure 4. Kaplan-Meier analysis comparing (A) heart failure related mortality in G+ probands and G- probands and (B) sudden cardiac death/aborted sudden cardiac death in G+ probands and G- probands. * = age at presentation (red for G+ and black for G-). G+ = genotype-positive. G- = genotype-negative.

Table 3. Cox regression analysis of genotype-positive status for the clinical endpoints of 512 probands

End point	Predictor	HR (95% CI)	P-value
All-cause mortality	Genotype-positive status	1.90 (1.14-3.15)	0.014
	Atrial fibrillation	2.15 (1.30-3.56)	0.003
	Systolic left ventricular dysfunction	1.92 (1.07-3.47)	0.030
	Extreme hypertrophy (MWT ≥ 30 mm)	6.22 (2.33-16.60)	<0.001
Cardiovascular mortality	Genotype-positive status	2.82 (1.49-5.36)	0.002
	Atrial fibrillation	3.31 (1.81-6.06)	<0.001
	Systolic left ventricular dysfunction	2.33 (1.18-4.60)	0.015
	Extreme hypertrophy (MWT ≥ 30 mm)	10.23 (3.64-28.73)	<0.001

Table 3. Cox regression analysis of genotype-positive status for the clinical endpoints of 512 probands (continued)

End point	Predictor	HR (95% CI)	P-value
Heart failure related mortality	Genotype-positive status	6.33 (1.79-22.41)	0.004
	Atrial fibrillation	12.66 (3.63-44.20)	<0.001
SCD/aborted SCD *analysis 1*	Genotype-positive status	2.88 (1.23-6.71)	0.015
	≥ 2 established risk factors (2003 guidelines)	2.44 (0.99-6.01)	0.052
SCD/aborted SCD *analysis 2*	Genotype-positive status	2.88 (1.24-6.67)	0.014
	≥1 established risk factors (2011 guidelines)	2.32 (1.04-5.16)	0.039

Multivariate Cox proportional-hazards analysis was used. Established risk factors for sudden cardiac death according to the 2003 guidelines included: extreme hypertrophy (maximal wall thickness ≥ 30 mm), unexplained syncope, abnormal exercise blood pressure, non-sustained ventricular tachycardia, family history of sudden cardiac death. Established risk factors for sudden cardiac death according to the 2011 guidelines included: extreme hypertrophy (maximal wall thickness ≥ 30 mm), unexplained syncope and a family history of sudden cardiac death. MWT = maximal wall thickness. SCD = sudden cardiac death.

Kaplan Meier curves for HF related mortality in carriers of different types of mutations are presented in figure 5. Thin filament mutation carriers had a greater probability of HF related death than thick filament mutation carriers (16% vs 5%, log rank p=0.06), and missense mutation carriers had a greater probability of HF related death than truncating mutation carriers (7% vs 4%, log rank p=0.03).

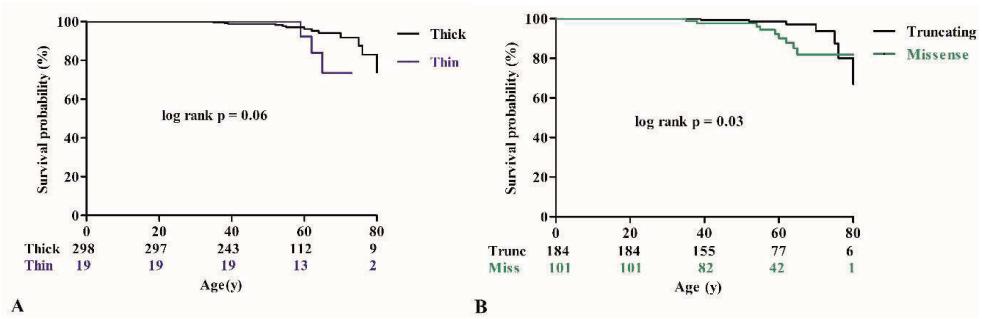

Figure 5. Kaplan-Meier analysis comparing heart failure related death in (A; top) HC patients with thick filament associated gene mutations and HC patients with thin filament associated gene mutations, and (B; bottom) HC patients with truncating gene mutations and HC patients with missense gene mutations.

DISCUSSION

This study compared the clinical outcome of G+ and G- patients with HC. During 12±9 years follow-up, multivariate analysis demonstrated that G+ status was an independent risk factor for all-cause mortality, CV mortality, HF related mortality, and SCD/aborted SCD.

Several previous studies have evaluated the impact of sarcomere mutations on clinical outcome. Olivotto et al (8) studied 203 patients (G+: 62%), and found a greater probability of severe left ventricular systolic and diastolic dysfunction (HR 2.1; 95% CI 1.1-4.0;p=0.02), during a median follow up of 4.5 years. Li. et al (7) studied 558 patients (G+: 35%), and demonstrated that G+ status was an independent predictor of HF events (HR 4.5; 95% CI 2.1-9.3; p<0.001), during a mean follow up of 6.6±6.3 years. Fujita et al.(5) studied 193 patients (G+: 47%), and reported more CV events in G+ HC, during 1 year follow up. Lopes et al.(6) studied 874 patients (G+: 44%), and reported a higher proportion of CV deaths and SCD events in G+ patients, during a mean follow up of 4.8±3.5 years. The mean follow-up period in these previous studies varied from 1 to 6.6 years. The present long-term follow-up study confirms and extends the findings from these previous studies.

G+ status in HC patients was an independent predictor of HF related mortality. The precise pathways through which sarcomere mutations lead to HF are unclear. In this study, 47% of G+ HC was caused by *MYBPC3* founder mutations. These mutations are responsible for ~35% of HC cases in the Netherlands.(10) The pathophysiological consequences of *MYBPC3* founder mutations have been investigated by van Dijk et al.(14) They reported a reduction of 33% in full-length cardiac MyBP-C protein, suggesting haploinsufficiency is part of the pathophysiology. In addition, the force generating capacity of cardiomyocytes was lower than myocardium from donor samples(14). This ''hypocontractile sarcomere phenotype'' seemed to be a common feature of HC patients, suggesting it is rather part of the remodeling process.(14, 15) This was investigated by correcting for a decrease in myofibril density.(16) After correction, values returned to normal for *MYBPC3* mutations, but not for *MYH7* mutations(16). And so, *MYH7* mutations seem to cause hypocontractile sarcomeres directly. Other pathophysiological mechanisms may be a reduced phosphorylation of sarcomeric proteins, and enhanced Ca^{2+}-sensitivity of the sarcomeres. Possibly, these early pathways involved in disease progression can be targets for future therapies.(3, 17)

This study demonstrates a significant relationship between G+ status and SCD/aborted SCD. The risk of SCD/aborted SCD was low in G- probands and relatives. Lopes et al.(6) similarly reported an increased incidence of SCD/aborted SCD in G+ HC. However, other studies(7, 8) did not show a relationship between G+ status and SCD, probably related to the low number of events, or relatively short follow-up duration. Ho et al.(18) demonstrated that myocardial collagen synthesis was increased in G+ individuals compared to control subjects. This suggests that sarcomere mutations lead to myocardial fibrosis, which is a substrate for SCD. Since myocardial fibrosis is believed to be visualized by cardiovascular magnetic resonance (CMR) with late gadolinium enhancement (LGE), it

was shown that the extent of LGE on CMR was associated with an increased risk of SCD events.(19, 20) Furthermore, an independent association between LGE and HF was reported(21).

In this cohort, G- probands were older, more symptomatic, and had higher LVOT gradients. During follow-up, 33% of G- probands underwent septal reduction therapy. Previous data have shown excellent long-term outcomes after septal reduction therapy in symptomatic patients with HC and severe LVOT obstruction.(22) The survival disadvantage associated with LVOT obstruction can be substantially decreased by appropriate invasive therapy.(22) The G+ probands in this study had a more advanced cardiomyopathy, which is indicated by a higher MWT, higher incidence of AF, higher incidence of non-sustained ventricular tachycardia, and more often a family history of SCD (table 1). Therefore, the G+ probands were at an increased risk of SCD and HF related death. Part of G- HC patients may have undiscovered pathogenic mutations. However, the additive genetic yield of next generation sequencing in HC seems limited.(23, 24) Possibly, whole-exome and whole-genome sequencing will add more value to the discovery of new mutations(3). However, such massive sequencing also generates many variants of unknown significance(3, 23). Determining of the clinical significance of these variants is a major challenge.(23)

In this study, relatives with HC were younger and had a more benign phenotype than probands. This can be explained by the way of presentation. It seems that family screening leads to the detection of disease in an earlier phase.(25) Although this was not reflected in a significantly better clinical outcome, a trend was found for fewer cardiovascular deaths among relatives. The lack of difference between G+ and G- relatives can be explained by the small number of G- relatives.

The current findings demonstrate that G+ HC patients are at increased risk of progression towards HF and SCD/aborted SCD. Previous studies have demonstrated that genetic test results are predictive of medium-term outcome, and the current study demonstrates that this also holds for the long-term outcome of patients with HC. Due to the heterogeneous nature of HC, the therapeutic implications of a G+ status are currently limited. Phenotypic characterization is currently still the most important factor for determining prognosis in HC patients. The clinical challenge is to incorporate genetic test results in contemporary risk prediction models. Fundamental research on the pathophysiological consequences of sarcomere mutations is crucial to develop genotype-specific risk-assessment and targeted therapies.

This study has several limitations. Patients who died and never presented to the clinic were missed in the analysis. Due to significant advances in DNA-sequencing methodology during the past decade, there was no homogenous genotyping over the whole period. The rate of complex genotype (1%) could be an underestimation of the real rate of complex genotype. Previous literature reported a rate of 5-7%(3).

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Supplementary table 1. Gene mutations associated with Hypertrophic Cardiomyopathy in 327 genotype-positive patients with hypertrophic cardiomyopathy

Nucleotide change	Protein change	Mutation type	No. of patients with mutation	Nucleotide change	Protein change	Mutation type	No. of patients with mutation
	MYBPC3 Gene (n=240)				***MYH7 Gene (n=47)***		
c.2373dupG	p.Trp792fs	frameshift	78	c.4130C>T	p.Thr1377Met	missense	4
c.2827C>T	p.Arg943*	nonsense	42	c.1816G>A	p.Val606Met	missense	4
c.2864_2865delCT	p.Pro955fs	frameshift	34	c.1207C>T	p.Arg403Trp	missense	3
c.1458-1G>C	p.?	splicesite	8	c.976G>C	p.Ala326Pro	missense	3
c.3776delA	p.Gln1259Argfs	frameshift	8	c.2156A>G	p.Arg719Gln	missense	3
c.481C>T	p.Pro161Ser	missense	8	c.1727A>G	p.His576Arg	missense	2
c.1624+1G>A	p.?	splicesite	5	c.1063G>A	p.Ala355Thr	missense	2
c.2149-2delA	p.?	splicesite	5	c.1987C>T	p.Arg663Cys	missense	2
c.927-2A>G	p.?	splicesite	5	c.2080C>T	p.Arg694Cys	missense	2
c.654+1G>A	p.?	splicesite	3	c.2783A>T	p.Asp928Val	missense	2
c.1831G>A	p.Glu611Lys	missense	4	c.2081G>A	p.Arg694His	missense	1
c.2308G>A	p.Asp770Asn	missense	2	c.1988G>A	p.Arg663His	missense	1
c.2391C>A	p.Tyr797*	nonsense	2	c.2104A>G	p.Arg719Gln	missense	1
c.688delC	p.Gln230fs	frameshift	2	c.2167C>T	p.Arg723Cys	missense	1
c.1484G>A	p.Arg495Gln	missense	2	c.2945T>C	p.Met982Thr	missense	1
c.772G>A	p.Glu258Lys	missense	3	c.2221G>C	p.Gly741Arg	missense	1
c.1696T>C	p.Cys566Arg	missense	2	c.3133C>T	p.Arg1045Cys	missense	1
c.897delG	p.Lys301fs	frameshift	2	c.3100-2A>C	p.?	splicesite	1
c.913_914delTT	p.Phe305fs	frameshift	1	c.3169G>A	p.Gly1057Ser	missense	1
c.1766G>A	p.Arg589His	missense	1	c.1357C>T	p.Arg453Cys	missense	1
c.1548-1G>A	p.?	splicesite	1	c.727C>T	p.Arg243Cys	missense	1
c.2543_2544dupCG	p.Val849fs	frameshift	1	c.728G>A	p.Arg243His	missense	1
c.2432A>G	p.Lys811Arg	missense	1	c.1532T>C	p.Ile511Thr	missense	1
c.3029delA	p.Glu1010fs	frameshift	1	c.5135G>A	p.Arg1712Gln	missense	3
c.2893C>T	p.Gln965*	nonsense	1	c.2146G>A	p.Gly716Arg	missense	1
c.3181C>T	p.Gln1061*	nonsense	1	c.5786C>T	p.Thr1929Met	missense	1
c.3640T>C	p.Trp1214Arg	missense	1	c.2306T>C	p.Leu769Pro	missense	1
				c.2788G>A	p.Glu930Lys	missense	1
c.3332_3335dup	p.Trp1112*	nonsense	1				

CSRP3 = cysteine and glycine-rich protein 3, *CALR3* = calreticulin 3, *MYBPC3* = myosin binding protein C, *MYH7* = myosin heavy chain 7, *MYL2* = regulatory myosin light chain 2, *MYL3* = regulatory myosin light chain 3, *MYPN* = myopalladin, *TNNT2* = troponin T, *TNNI3* = troponin I, *TPM1* = α-tropomyosin 1

Supplementary table 1. Gene mutations associated with Hypertrophic Cardiomyopathy in 327 genotype-positive patients with hypertrophic cardiomyopathy (continued)

Nucleotide change	Protein change	Mutation type	No. of patients with mutation	Nucleotide change	Protein change	Mutation type	No. of patients with mutation
MYBPC3 Gene (n=240)				***MYL2 gene (n=8)***			
c.3331-2A>G	p.?	splicesite	1	c.64G>A	p.Glu22Lys	missense	6
c.3392T>C	p.Ile1131Thr	missense	1	c.403-1G>C	p.?	splicesite	1
c.3814+1G>A	p.?	splicesite	1	c.286G>A	p.Glu96Lys	missense	1
c.442G>A	p.Gly148Arg	missense	1	***MYL3 gene (n=3)***			
c.1800delA	p.Lys600Asnfs	frameshift	1	c.452C>T	p.Ala151Val	missense	3
c.1404delG	p.Gln469fs	frameshift	1	***MYPN gene (n=1)***			
c.701ins26	unknown	frameshift	1	c.59A>G	p.Tyr20Cys	missense	1
c.208delG	p.Glu70fs	frameshift	1	***TNNI3 gene (n=7)***			
c.1053_1054delGCinsTT	p.Arg351_Leu352delinsSerPhe	complex	1	c.433C>T	p.Arg145Trp	missense	4
c.7191-1G>A	p.?	splicesite	1	c.497C>T	p.Ser166Phe	missense	1
c.821+1G>A	p.?	splicesite	1	c.114dupA	p.Ser39fs	frameshift	1
c.932C>A	p.Ser311*	nonsense	1	c.470C>T	p.Ala157Val	missense	1
del exon 23-26	p.?	splicesite	1	***TNNT2 gene (n=10)***			
c.1000G>T	p.Glu334*	nonsense	1	c.832C>T	p.Arg278Cys	missense	3
c.3490+1G>T	p.?	splicesite	1	c.856C>T	p.Arg286Cys	missense	3
				c.274C>T	p.Arg92Trp	missense	1
	Double (n=3)			c.421delC	p.Arg141fs	frameshift	1
c.1000G>T (*MYBPC3)* & c.64G>A (*MYBPC3)*	p.Glu334* & p.Glu22Lys		1	c.874C>T	p.Arg292Trp	missense	1
c.913_914delT (*MYBPC3)* & c.1468G>A (*MYL2)*	p.Phe305fs & p.Gly490Arg		1	c.853C>T	p.Arg285Cys	missense	1
c.5135G>A (*MYH7)* & c.2530_2532delTCTinsC (*MIB1)*	p.Arg1712Gln & p.Ser844fs		1	**TPM1 (n=2)**			
				c.184G>C	p.Glu62Gln	missense	2
	CSRP3 gene (n=2)			***CALR3 (n=4)***			
c.131T>C	p.Leu44Pro	missense	2	c.564delT	p.Gly189fs	frameshift	4

CSRP3 = cysteine and glycine-rich protein 3, *CALR3* = calreticulin 3, *MYBPC3* = myosin binding protein C, *MYH7* = myosin heavy chain 7, *MYL2* = regulatory myosin light chain 2, *MYL3* = regulatory myosin light chain 3, *MYPN* = myopalladin, *TNNT2* = troponin T, *TNNI3* = troponin I, *TPM1* = α-tropomyosin 1

Supplementary table 2. Patients with hypertrophic cardiomyopathy that died from heart failure or sudden cardiac death, presented per gene affected and type of mutation

Gene	Mutation type	No. of patients with mutation	Heart failure related death	Sudden cardiac death
Total		327	17 (5%)	19 (6%)
MYBPC3	Truncating	179	7 (4%)	12 (7%)
	Missense	27	2 (7%)	2 (7%)
	Splicesite	33	1 (3%)	2 (6%)
	Complex	1	0	0
MYH7	Missense	46	3 (7%)	2 (4%)
	Splicesite	1	0	0
MYL2	missense	7	1 (14%)	0
	splicesite	1	0	0
MYL3	missense	3	0	0
MYPN	missense	1	0	0
TNNI3	truncating	1	0	0
	missense	6	0	0
TNNT2	truncating	1		
	missense	9	1 (11%)	0
TPM1	missense	2	2 (100%)	0
CALR3	truncating	4	0	0
CSRP3	missense	2	0	0
Complex genotype		3	0	1 (33%)

All values are in number (%). *CSRP3* = cysteine and glycine-rich protein 3, *CALR3* = calreticulin 3, *MYBPC3* = myosin binding protein C, *MYH7* = myosin heavy chain 7, *MYL2* = regulatory myosin light chain 2, *MYL3* = regulatory myosin light chain 3, *MYPN* = myopalladin, *TNNT2* = troponin T, *TNNI3* = troponin I, *TPM1* = α-tropomyosin 1

CHAPTER 2

Clinical characteristics and long-term outcome of hypertrophic cardiomyopathy in individuals with a MYBPC3 founder mutation

Hannah G. van Velzen
Arend F.L. Schinkel
Rogier A. Oldenburg
Marjon A. van Slegtenhorst
Ingrid M.E. Frohn – Mulder
Jolanda van der Velden
Michelle Michels

Circ Cardiovasc Genet. 2017 Aug;10(4).

ABSTRACT

Background

Myosin-binding protein C (*MYBPC3*) founder mutations account for 35% of hypertrophic cardiomyopathy (HCM) cases in the Netherlands. We compared clinical characteristics and outcome of *MYBPC3* founder mutation (FG+) HCM with non-founder genotype positive (G+) and genotype negative (G-) HCM.

Methods and results

The study included 680 subjects: 271 FG+ carriers, 132 G+ probands with HCM and 277 G- probands with HCM. FG+ carriers included 134 FG+ probands with HCM, 54 FG+ relatives diagnosed with HCM after family screening, 74 FG+/phenotype-negative relatives, and 9 with non-compaction or dilated cardiomyopathy. The clinical phenotype of FG+ and G+ probands with HCM was similar. FG+ and G+ probands were younger with less LVOT obstruction than G- probands, however had more hypertrophy and non-sustained ventricular tachycardia. FG+ relatives with HCM had less hypertrophy, smaller left atria, and less systolic and diastolic dysfunction than FG+ probands with HCM. After 8±6 years, cardiovascular mortality in FG+ probands with HCM was similar to G+ HCM (22 vs 14%, log rank $p=0.14$), but higher than G- HCM (22 vs 6%, log rank $p<0.001$) and FG+ relatives with HCM (22 vs 4%, $p=0.009$). Cardiac events were absent in FG+/phenotype-negative relatives; subtle HCM developed in 11% during 6 years follow-up.

Conclusions

Clinical phenotype and outcome of FG+ HCM was similar to G+ HCM, but worse than G- HCM and FG+ HCM diagnosed in the context of family screening. These findings indicate the need for more intensive follow-up of FG+ and G+ HCM versus G- HCM and FG+ HCM in relatives.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease with an estimated prevalence of 1:500 to 1:200.(1) More than 1500 mutations in at least 11 genes have been described in association with HCM.(2) Myosin-binding protein C (*MYBPC3*) is the most frequently mutated gene, representing 30-40% of all HCM mutations.(2) In the Netherlands, 35% of HCM cases are caused by 3 *MYBPC3* founder mutations: c.2373dupG (p.Trp792Valfs*41), c.2827C>T (p.Arg943*), and c.2864_2865delCT (p.Pro955fs*95).(3-5) These mutations cause C-terminally truncated protein, leading to haploinsufficiency.(6-8) Pathophysiologic studies have demonstrated that these mutations are associated with a reduced force generating capacity of cardiomyocytes, cardiomyocyte hypertrophy and reduced myofibril density.(8, 9) The clinical phenotype of *MYBPC3* founder mutation (FG+) carriers varies.(10) Information on the clinical characteristics and long-term outcome of FG+ carriers is lacking. The aim of this study was to compare clinical characteristics and outcome of FG+ HCM with non-founder mutation genotype-positive (G+) HCM and genotype-negative (G-) HCM.

METHODS

Study design and population

This retrospective cohort study included a total of 680 subjects: 271 FG+ carriers (141 FG+ probands; 130 FG+ relatives) from 127 families, 132 G+ probands with HCM and 277 G- probands with HCM, who underwent clinical evaluation between 1985 and 2015. Probands were defined as patients with HCM, dilated cardiomyopathy (DCM), or non-compaction cardiomyopathy (NCCM), presenting with signs or symptoms. Relatives were defined as subjects who were evaluated in the context of family screening. The study conforms to the principles of the Declaration of Helsinki. All patients gave informed consent for inclusion in the registry and local institutional review board approval was obtained.

Genetic analysis

All subjects underwent genetic counselling. Before 2012, Sanger sequencing of all coding intron-exon boundaries of the following genes was available: *MYBPC3*, β-myosin heavy chain (*MYH7*), cardiac troponin C (*TNNC1*), cardiac troponin T (*TNNT2*), cardiac troponin I (*TNNI3*), cardiac-regulatory myosin light chain (*MYL2*), cardiac-essential myosin light chain (*MYL3*), cardiac α-actin (*ACTC1*), α-tropomyosin (*TPM1*), cysteine and glycine-rich protein 3 (*CSRP3*), and titin-cap/telethonin (*TCAP*). After the identification of a pathogenic mutation, genotyping was extended at the discretion of the treating physician. Since 2012, next-generation-sequencing covering 48 genes is used. Due to this change in DNA-analysis over time, subjects underwent either extensive genotyping (≥ 6 sarcomeric genes) or limited genotyping (*MYBPC3* only). Extensive genotyping was performed in 77 (57%) FG+ probands with HCM, 57 (42%) G+ probands with HCM and 244 (88%) G- probands with HCM.

Next-generation sequencing was performed in 15 (11%) FG+ probands with HCM, 30 (22%) G+ probands with HCM, and 116 (60%) G- probands with HCM. Seven out of 9 (78%) individuals with non-HCM phenotypes underwent extensive genotyping. Relatives in FG+ families were tested for the familial mutation and referred for cardiac screening if the mutation was present.(11) Genetic testing before adulthood was offered in families with severe HCM in childhood, when cardiac symptoms were present, or severe anxiety among parents existed.

Cardiac evaluation

All individuals underwent periodic cardiac evaluation including history, clinical examination, electrocardiography (ECG) and echocardiography. Echocardiographic studies were analyzed according to the American Society of Echocardiography guidelines.(12) In probands, HCM was diagnosed when maximal wall thickness (MWT) ≥ 15 mm; a cutoff value of 13 mm was used in relatives.(13) DCM was diagnosed based on left ventricular (LV) dilatation and LV systolic dysfunction.(14) NCCM diagnosis was based on the Jenni criteria.(15) MWT, LV end-diastolic diameter (LVEDD), left atrial (LA) size and LV outflow tract (LVOT) pressure velocity at rest and during provocation were measured. LVOT gradient was calculated with the Bernoulli equation. Systolic LV function (sLVF) was assessed using the wall-motion score index, and described as normal, mildly reduced, moderately reduced, or poor, which corresponded with left ventricular ejection fractions of ≥55%, 45-54%, 30-44%, and <30% respectively, according to echocardiography guidelines.(16) Systolic dysfunction was defined as mildly reduced, moderately reduced or poor systolic function. Diastolic LVF was described as normal, abnormal relaxation (stage I), pseudonormal (stage II) and restrictive filling (stage III).(17) Clinical disease stages were defined according to Olivotto et al: (1) non-hypertrophic (MWT < 13 mm); (2) classic phenotype (MWT ≥ 13 mm and normal sLVF); (3) adverse remodeling (mildly reduced sLVF); and (4) overt cardiac dysfunction (moderately reduced or poor sLVF).(18) After cardiomyopathy diagnosis cardiac evaluation was extended with exercise testing and 24 hour ambulatory ECG monitoring. Non sustained ventricular tachycardia was defined as ≥ 3 consecutive beats at ≥ 120/min lasting <30 seconds.(13)

Patient follow-up

Follow-up data were obtained in January 2016. Follow-up was complete for 99% of patients. Patients who were lost to follow-up were censored at time of last follow-up. Mortality was retrieved from the civil service register and cause of death from the medical chart or the general practitioner. Septal reduction therapy (SRT) and implantable cardioverter defibrillator (ICD) were registered. ICDs were implanted according to the guidelines.(13, 19, 20) Cardiovascular mortality was defined as the combined end point of sudden cardiac death (SCD)/aborted SCD, heart failure (HF) related death (including heart transplantation), stroke related death, coronary artery disease related death, and procedure-related cardiac death. SCD/aborted SCD was defined as (1) instantaneous and unexpected

death in patients who were previously in a stable condition, or nocturnal death with no antecedent history of worsening symptoms; (2) resuscitation after cardiac arrest; or (3) appropriate ICD intervention for ventricular fibrillation or ventricular tachycardia > 200 beats/min.

Statistical analysis

Calculations were performed using SPSS 21 (IBM, Armonk, New York) and R Statistical Software version 3.2.4.-using packages 'survival' and 'lme4'. Normally distributed continuous data are expressed as mean ± standard deviation (SD) and non-normally distributed data as median, followed by the interquartile range [IQ1-IQ3]. For comparing categorical variables Pearson's chi-square test was used. For comparing continuous variables, student t-test was used and Mann-Whitney test in the case of non-normally distributed data. In order to make comparisons between FG+ probands and FG+ relatives, generalized linear mixed models were used, with random intercepts for family to account for family relatedness. Survival curves were constructed according to the Kaplan Meier method. Comparisons of survival and other clinical outcomes between FG+ probands with HCM and FG+ relatives with HCM were performed by using Cox models. To estimate the standard errors of the hazard ratios while taking into account family relatedness, the grouped jackknife method was used. Fisher's exact test was used in case of a zero cell count in either of the groups. Log rank test was used for comparison of survival between FG+ patients and G+ or G- patients. For comparison of consecutive echocardiographic data, the paired t-test and in case of non-normally distributed data the Wilcoxon signed rank test were used. Statistical significance was defined by $p < 0.05$.

RESULTS

Study population

In 141 FG+ probands (age 45±14 y, 66% male), HCM was diagnosed in 134 (95%), NCCM in 4 (3%), and DCM in 3 (2%), In 130 FG+ relatives (age 42±15 y, 37% male), HCM was diagnosed in 54 (42%), NCCM in 1 (1%), and DCM in 1 (1%). The remaining 74 (57%) were FG+/phenotype-negative (FG+/Ph-). Baseline characteristics of the study population are presented in table 1 and 4.

Genetic test results

In FG+ carriers, the c.2373dupG mutation was most frequent (46%), followed by c.2827C>T (32%) and c.2864_2865delCT (22%). A complex genotype was present in 4 (1%); 3 probands and 1 relative. Complex genotypes were: compound heterozygosity for the c.2373dupG and c.2827C>T mutations; homozygosity for the c.2827C>T mutation; compound heterozygosity for the c.2373dupG and c.442G>A (G148R) mutations in the *MYBPC3* gene, and a digenic mutation (c.2827C>T in the *MYBPC3* gene and c.222dupA; p.Leu75fs in the Ankyrin Repeat Domain 1 [*ANKRD1*] gene).

In G+ probands, pathogenic mutations were present in the *MYBPC3* gene (45%), *MYH7* (29%), *TNNT2* (7%), *MYL2* (6%), *TNNI3* (5%), *CALR3* (2%), *MYL3* (2%), *CSRP3* (2%), and complex genotypes were present in 3 (2%). Complex genotypes were: (1) compound heterozygosity for the c.913_914delTT and c.1468G>A mutations in the *MYBPC3* gene, (2) digenic mutations c.5135G>A in the *MYH7* gene and c.2530_2532delTCTinsC in the *MIB1* gene, and (3) digenic mutations c.1000G>T in the *MYBPC3* gene and c.64G>A in the *MYL2* gene.

Table 1. Baseline characteristics of the study population.

Variable	FG+ probands with HCM (n=134)	FG+ relatives with HCM (n=54)	G+ HCM (n=132)	G- HCM (n=277)
Age, years (range)	44±14	47±16	47±15	55±15*†‡
<18 y	4 (3)	3 (6)	4 (4)	5 (2)
18-35 y	33 (25)	11 (20)	28 (21)	28 (10)*†‡
36-50 y	51 (38)	17 (32)	45 (35)	58 (21)*†
>50 y	46 (34)	23 (43)	55 (41)	187 (67)*†‡
Men, n (%)	91 (67)	31 (57)	89 (66)	171 (62)
Reasons for evaluation				
Symptoms, n (%)	75 (56)	0	70 (56)	181 (65)
Abnormal ECG, n (%)	23 (17)	0	27 (22)	36 (13)
Systolic murmur, n (%)	32 (24)	0	26 (21)	50 (18)
Sudden death, n (%)	3 (2)‖	0	1 (1)	1 (0.4)
Incidental finding, n (%)	1 (1)	0	1 (1)	3 (1)
Familial evaluation, n (%)	0	54 (100)	0	0
NYHA class ≥ II, n (%)	48 (48)	3 (8)*	71 (56)	164 (61)*‡
History of AF, n (%)	28 (21)	4 (7)*	36 (27)	41 (15)†
History of stroke, n (%)	12 (9)	4 (8)	11 (8)	22 (8)
MWT, mm	20±5	16±4*	20±5	17±4*†‡
LA size, mm	45±8	40±7*	45±7	45±7‡
Systolic dysfunction	23 (17)	0*§	22 (18)	30 (12)‡
Diastolic dysfunction	50 (56)	19 (38)*	53 (71)	159 (80)*‡
MWT ≥ 30 mm, n (%)	5 (4)	0§	11 (9)	2 (1)*†
LVOTO ≥ 30 mmHg	31 (28)	2 (4)*	36 (32)	101 (45)*†‡
LVOTO ≥ 50 mmHg	18 (16)	2 (4)	24 (21)	85 (38)*†‡
Non-sustained VT, n (%)	44 (42)	6 (16)*	35 (32)	26 (13)*†

Data are expressed as mean ± SD or as absolute and %. AF = atrial fibrillation, FG+ = Dutch *MYBPC3* founder mutation, LVOTO = left ventricular outflow tract obstruction at rest, MWT = maximal wall thickness, NYHA = New York Heart Association functional class, VT = ventricular tachycardia. * = $p<0.05$ versus FG+ probands with HCM. †= $p<0.05$ versus G+ HCM. ‡ $p<0.05$ vs FG+ relatives with HCM. § = Fisher's exact test was used because of zero cell count. ‖ = one was not successfully resuscitated.

Baseline clinical and echocardiographic characteristics

Main reasons for evaluation in probands were cardiac symptoms (61%), systolic murmur (20%), and abnormal ECG (16%). Five (1%) probands presented with cardiac arrest. All FG+ relatives were evaluated in the context of family screening. Men predominated in all groups.

The clinical phenotype of FG+ probands with HCM was similar to G+ probands with HCM. Both FG+ and G+ probands were younger than G- probands with HCM and had less LVOT obstruction, however they had more hypertrophy and non-sustained ventricular tachycardia. FG+ relatives with HCM had less hypertrophy, smaller left atria, and less systolic and diastolic dysfunction in comparison to FG+ probands with HCM. Among FG+ relatives with HCM, MWT was not different between males and females after indexing for body surface area (6.6±2.1 vs 6.4±2.6 mm/m^2, p=0.776).

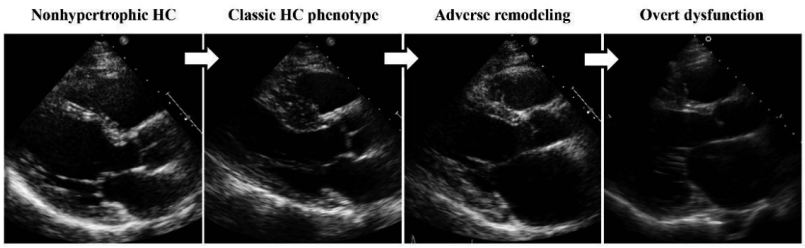
Echocardiographic findings during follow-up of FG+ carriers

Echocardiographic findings during 10±6 years follow-up of FG+ probands and FG+ relatives with HCM are presented in table 2. Figure 1 demonstrates the clinical HCM disease stages(18) at baseline and during follow-up of FG+ probands and FG+ relatives. A significant proportion of FG+ probands (29%) progressed to stage III (adverse remodeling), and 18% to stage IV (overt dysfunction); in FG+ relatives only 5% progressed to stage III and none to stage IV. Systolic dysfunction during follow-up was frequently present in G+ and FG+ HCM (46% vs 46%, log rank p=0.23), and less frequent in G- HCM than in FG+ HCM (31% vs 46%, log rank p<0.001). Moderate to severe systolic dysfunction was also frequently present in G+ and FG+ HCM (15% vs 25%, log rank p=0.54), and less frequent in in G- HCM than in FG+ HCM (7% vs 25%, log rank p<0.001).

Table 2. Echocardiographic findings during follow-up of FG+ probands and FG+ relatives with hypertrophic cardiomyopathy

Variable	FG+ probands with HCM			FG+ relatives with HCM		
	Baseline	Follow-up	P-value	Baseline	Follow-up	P-value
Maximal wall thickness, mm	20±5	17±4	<0.001	16±4	15±4	0.053
Left atrial size, mm	45±8	49±9	<0.001	40±7	41±6	0.044
LVEDD, mm	46±7	48±7	<0.001	47±5	45±6	0.107
LVOTO ≥ 30 mmHg, n (%)	32 (28)	6 (6)	<0.001	2 (4)	2 (4)	1.000
LVOTO ≥ 50 mmHg, n (%)	18 (16)	4 (4)	0.005	2 (4)	2 (4)	1.000
Systolic dysfunction, n (%)	23 (17)	47 (40)	<0.001	0	4 (8)	0.046
Diastolic dysfunction, n (%)	50 (56)	75 (84)	<0.001	18 (37)	26 (59)	0.011

Data are expressed as mean ± SD or as absolute and %. LVOTO = left ventricular outflow tract obstruction at rest; LVEDD = left ventricular end-diastolic diameter.



FG+ Probands	Baseline	0	83% (111/134)	10% (14/134)	7% (9/134)
	Follow-up	0	53% (51/96)	29% (28/96)	18% (17/96)
FG+ Relatives	Baseline	58% (74/128)	42% (54/128)	0	0
	Follow-up	44% (38/86)	51% (44/86)	5% (4/86)	0

Figure 1. Clinical characteristics of individuals with a Dutch *MYBPC3* founder mutation. Stage I: Non-hypertrophic stage in a c.2864_2865delCT mutation carrier. Stage II: Classic HCM phenotype in a c.2373dupG mutation carrier. Stage III: Adverse remodeling in a c.2373dupG mutation carrier. Stage IV: Overt dysfunction (hypokinetic dilated form) in a c.2373dupG mutation carrier.

Mortality and interventions during follow-up

Mortality and interventions are presented in table 3. Cardiovascular mortality was 21% in FG+ probands with HCM and 14% in G+ probands (p=0.14), and cardiovascular mortality was significantly lower in FG+ relatives with HCM (4%) and G- probands (7%) (figure 2). During 8±6 years follow-up, annual cardiovascular mortality was 2.1%, 1.6%, 1.0%, and 0.5% for FG+ probands with HCM, G+ probands, G- probands, and FG+ relatives with HCM respectively. By multivariate Cox analysis taking into account age, sex and family relatedness, FG+ relatives with HCM exhibited a lower risk of cardiovascular death than FG+ probands with HCM (hazard ratio 0.15 95% confidence interval 0.04; 0.64, p=0.01). Both HF related death and SCD/aborted SCDs were more frequent in FG+ HCM than in G- HCM (8% vs 1%, log rank p<0.001 and 14% vs 4%, log rank p<0.001 respectively). In FG+ HCM, SCD/aborted SCD occurred at ages ranging from 11 to 77 years, and HF-related death generally occurred after the age of 50 years. SRT was performed more often in G- HCM than in FG+ HCM (33% vs 23%, p=0.004). ICDs for the primary and secondary prevention of SCD were implanted with similar proportions in all groups.

Table 3. Long-term outcome of the study population

Variable	FG+ probands with HCM (n=134)	FG+ relatives with HCM (n=54)	G+ HCM (n=132)	G- HCM (n=277)
All-cause mortality, n (%)	39 (29)	3 (6)*	20 (15)*	38 (14)*†
CV mortality, n (%)	29 (22)	2 (4)*	19 (14)	20 (7)*
HF related deaths, n (%)	10 (8)	0‡	10 (8)	4 (1)*†
Cardiac transplants, n (%)	1 (0.7)	0‡	3 (2)	2 (1)
SCD/Aborted SCD, n (%)	18 (14)	2 (4)*	9 (7)	12 (4)*†
True SCD, n (%)	11 (8)	1 (2)	4 (3)	4 (1)*†
Stroke related deaths, n (%)	0	0	0	2 (1)
Procedure-related deaths, n (%)	0	0	0	2 (1)
CAD related deaths, n (%)	0	0	0	0
Non-cardiac deaths, n (%)	8 (6)	1 (2)	1 (1)*	15 (5)
SRT, n (%)	31 (23)	2 (4)*	39 (30)	91 (33)*
ICD 1st prevention, n (%)	26 (19)	5 (10)	16 (12)	26 (9)
ICD 2nd prevention, n (%)	5 (4)	2 (4)	6 (5)	7 (3)

Data are expressed as mean ± SD or as absolute and %. CAD = coronary artery disease, FG+ = Dutch *MYBPC3* founder mutation, HCM = hypertrophic cardiomyopathy, ICD = internal cardioverter defibrillator, SCD = sudden cardiac death, SRT = septal reduction therapy (alcohol septal ablation or surgical myectomy). * = p<0.05 vs FG+ probands with HCM †=log rank p<0.05 vs G+ HCM. ‡Cox model with grouped jackknife method did not converge because of insufficient number of events.

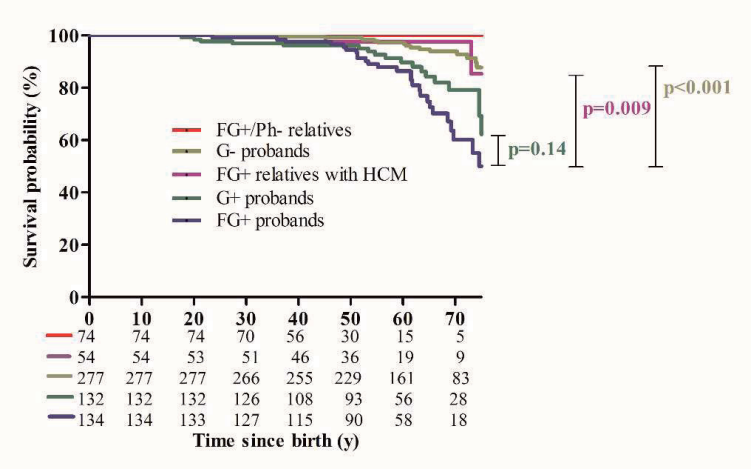


Figure 2. Kaplan-Meier survival analysis for cardiovascular mortality. P = p-value calculated with log rank test in FG+ versus G+ probands, and in FG+ versus G- probands. In FG+ probands versus FG+ relatives with HCM, the p-value was calculated with a cox model with grouped jackknife method. In FG+/phenotype negative relatives the Cox model with grouped jackknife method did not converge because of insufficient number of events.

FG+/phenotype-negative relatives

Characteristics and outcomes of FG+/Ph- relatives versus FG+ HCM are presented in table 4. FG+/Ph- relatives were predominantly female (63% vs 35%; p<0.001); there was no significant age difference. During 6±4 years follow-up, there were no cardiovascular deaths among FG+/Ph- relatives. Echocardiographic follow-up was available in 44 (59%) FG+/Ph- relatives (table 5). After 6±3 years, 5 (11%) FG+/Ph- subjects developed HCM. These 5 subjects were asymptomatic and without LVOT obstruction; conversion occurred at a median age of 37 (range 25-71) years. Baseline ECG abnormalities were present in 3 (2 pathological Q's inferior/lateral and 1 non-pathological Q inferior), and anterior mitral valve leaflet elongation ≥ 30 mm in 1 (20%). Systolic anterior motion and diastolic dysfunction were absent in all 5. Hypertrophy developed at a pace of a median 0.5 [0.2-0.8] mm per year during a median 6 [3.5-9] year follow-up, in which MWT increased from a median 11 [9.5-11.5] to 13 [13-13.5] mm.

Table 4. Characteristics of FG+/phenotype negative relatives versus FG+ HCM (probands and relatives combined)

Variable	FG+ HCM (n=188)	FG+/Ph- relatives (n=74)	P-value
Baseline			
Age, years (range)	45±15 (9-80)	42±15 (4-83)	0.077
<18 y	7 (4)	3 (4)	0.205
18-35 y	44 (23)	22 (30)	0.249
36-50 y	71 (38)	35 (47)	0.163
>50 y	66 (35)	14 (19)	0.013
Women, n (%)	66 (35)	47 (64)	< 0.001
NYHA ≥ II, n (%)	51 (37)	2 (3)	<0.001
History of stroke, n (%)	16 (9)	0	0.008*
History of AF, n (%)	32 (17)	1 (1)	0.008
Follow-up			
All-cause mortality, n (%)	43 (23)	1 (1)	0.036
Cardiovascular mortality, n (%)	31 (16)	0	†

Data are expressed as mean ± SD, or as absolute and %. AF = atrial fibrillation, FG+ = Dutch *MYBPC3* founder mutation, NYHA = New York Heart Association functional class. * = Fisher's exact test was used because of zero cell count. †Cox model with grouped jackknife method did not converge because of insufficient number of events.

Non-compaction and dilated cardiomyopathy in FG+ carriers

NCCM was diagnosed in 5 (3%) FG+ carriers; 4 probands and 1 relative. A complex genotype was present in 3 (60%): 2 FG+ probands who suffered HF related death within two months after birth and one 18-year-old asymptomatic FG+ relative. One NCCM patient suffered HF related death at 50 years of age. DCM was diagnosed in 4 (2%) FG+ carriers; 3 probands and 1 relative. A complex genotype was present in 1 (25%), leading to cardiac transplantation at the age of 8 years. Another FG+ proband with DCM died of HF at one year of age. Figure 3 presents cardiac magnetic resonance and echocardiographic imaging, demonstrating the overlap of HCM and NCCM within one family and the presence of NCCM in a FG+ carrier.

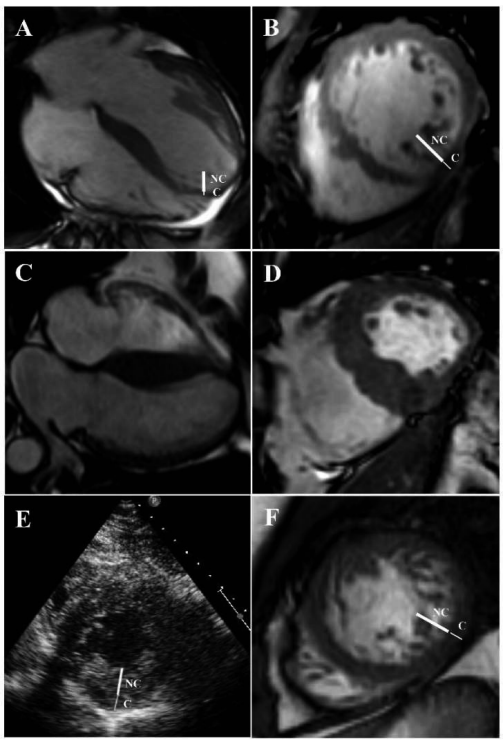


Figure 3. Cardiac magnetic resonance images of non-compaction cardiomyopathy and hypertrophic cardiomyopathy, measured in apical 3 chamber view and short-axis view at apical level during end-diastole. Non-compaction cardiomyopathy was diagnosed in an 18-year old FG+ relative (image A and B) after family screening; asides from the familial mutation c.2827C>T, next-generation sequencing revealed a second mutation c.222dupA in the Ankyrin Repeat Domain 1 gene. Mild hypertrophy (13 mm) of the left ventricular posterior wall was also present. His father (image C and D) had hypertrophic cardiomyopathy based on the c.2827C>T mutation. Another FG+ individual presented with non-compaction cardiomyopathy on echocardiography (image E) and cardiac magnetic resonance imaging (image F); this individual carried the c.2827C>T mutation. Left ventricular systolic function was poor.

Table 5. Echocardiographic follow-up of FG+/phenotype negative relatives

Variable	Baseline	Follow-up	P-value
MWT, mm	9.9±1.6	10.2±2.0	0.188
LA size, mm	37±4	36±6	0.715
LVEDD, mm	48±4	46±4	0.021
Maximal LVOT gradient, mmHg	5 [4-8]	6 [6-7]	0.072
Systolic dysfunction, n (%)	0	1 (2)	1.000
Diastolic dysfunction, n (%)	4 (10)	6 (15)	0.625

Data are expressed as mean ± SD, median [interquartile range], or as absolute and %. LA = left atrial; LVOT = left ventricular outflow tract, LVEDD = left ventricular end-diastolic diameter; MWT = maximal wall thickness.

DISCUSSION

Key findings from the current long-term follow-up study are: (1) clinical phenotype and outcome of FG+ HCM was similar to G+ HCM, but worse than G- HCM and FG+ HCM diagnosed in the context of family screening, and (2) cardiac events were absent in FG+/Ph- relatives; 11% of FG+/Ph- relatives developed HCM during 6±3 years follow-up.

FG+ HCM

This study demonstrates that the prognosis of FG+ carriers is primarily defined by the presenting phenotype and the reason for evaluation. Cardiovascular mortality was significantly higher in FG+ HCM than in FG+/Ph- relatives and significantly lower in FG+ relatives diagnosed with HCM in the context of family screening. Adverse remodeling and progression to end-stage HCM was highly prevalent among FG+ probands with HCM, resulting in significantly more HF related deaths in comparison to FG+ relatives with HCM. This finding is in line with the findings of Kubo et al.(21) We also observed a higher cardiovascular mortality rate in FG+ HCM in comparison to G- HCM. Several studies have previously demonstrated an increased risk of cardiac death in G+ versus G- HCM(22-24), including studies of *MYBPC3* founder mutations.(25, 26) G- probands in this study were older and more symptomatic most likely related to LVOT obstruction and diastolic dysfunction. Possibly, G- HCM represents a separate disease with a different pathophysiology. Unlike previous observations(27), we did not observe a lower complication rate in FG+ versus G+ patients.

Initial cardiac screening revealed HCM in 42% of FG+ relatives, which is comparable to previous studies (24% to 62%).(26, 28-31) Extreme hypertrophy was absent in FG+ relatives and there was less adverse remodeling. Identification of HCM leads to lifestyle modifications, periodic SCD risk stratification and close clinical follow-up, with the opportunity to implant an ICD for primary

prevention and timely referral for SRT. In the future, early disease identification might lead to novel therapies to prevent hypertrophy(32) or delay progression to advanced disease stages.(33)

In this study the clinical phenotypes of FG+ carriers showed substantial variation. The clinical heterogeneity in subjects carrying the same pathogenic mutation is intriguing. Basic studies have shown a decrease in the force generating capacity of cardiomyocytes in G+ HCM patients.(8, 9) For *MYBPC3* mutations, the force generating capacity normalized after correction for myofibril density.(9) The drop in force was associated with cardiomyocyte hypertrophy and reduced myofibril density, suggesting sarcomere dysfunction is secondary to cardiomyocyte remodeling.(9) Other triggers e.g. altered Ca^{2+} handling and disturbances in myocardial energetics(34) are additionally being investigated. The exact pathways from mutation to disease remain largely unknown. The relatively large population of FG+ carriers is useful for translational research in which clinical data are combined with data from basic research to further unravel the pathomechanism and identify secondary disease-modifiers such as additional (epi)genetic variations and environmental disease triggers.

FG+/Ph- individuals

Because of the known age-related penetrance in HCM(29), long-term follow-up of FG+/Ph- subjects is recommended.(13, 20, 35) The interval at which clinical evaluation should be repeated is subject to debate. The American guideline recommends a 1-2 year interval for family members aged 10-20 years and 2-5 year interval for those > 20 years, whereas the European guideline does not advise a specific interval.(13, 20) In this study, 11% of FG+/Ph- relatives developed a subtle form of HCM after 6 years follow-up. Additionally, comparable to previous studies(31, 36, 37), the prognosis of FG+/Ph- relatives was good. These findings support cardiac follow-up of adult FG+/Ph- relatives with a low frequency, as advised by the American guideline.(20) The number of family members aged < 18 years in our cohort was too small to propose screening intervals for this group. However, Jensen et al reported a similarly low manifestation rate (6%) in 36 at-risk relatives <18 years of age during 12 years follow-up.(36) The main advantage of genetic testing in relatives is reassurance in case the mutation is absent. However, the identification of FG+/Ph- subjects currently has limited therapeutic and prognostic consequences, because at present no therapy is available to retard or prevent the development of HCM(32, 33), and clinical manifestation cannot be predicted.(35) Moreover, a FG+/Ph- status may have psychological and socio-economic implications.(38) Clearly, cardio-genetic counseling of relatives should include a balanced discussion of the advantages and potential disadvantages of genetic testing.

NCCM and DCM in FG+ carriers

MYBPC3 mutations are associated with various forms of cardiomyopathies, such as DCM and NCCM.(5) In this study, a significantly poor outcome was observed in a minority of young NCCM

and DCM patients, partly explained by homozygous and compound heterozygous mutations.(39) Since *MYBPC3* founder mutations are truncating mutations leading to haploinsufficiency(8), compound heterozygous or homozygous mutations would theoretically result in human *MYBPC3* knockouts (no functional *MYBPC3* protein), leading to severe HF at a young age.(39) The likelihood of compound heterozygotes or homozygotes in countries with founder mutations is increased.(5) The finding of NCCM and DCM in the FG+ population and within one family, supports previous suggestions that the various cardiomyopathies are part of a cardiomyopathy spectrum with similar pathogenesis. Lorca et al. similarly described the overlapping of HCM and NCCM phenotypes within one family.(40)

Male predominance in HCM

The male predominance in HCM patients was previously partly explained by a referral bias.(41) In this study, there was also a male predominance in FG+ relatives with HCM eliminating referral bias. Other theories explaining gender differences in HCM include differential gene regulation and the protective effect of estrogens.(41) Another explanation might be the use of the 13 mm cutoff value to diagnose HCM in relatives.(13) In this study, the difference in MWT between male and female relatives disappeared after indexing for body surface area, suggesting that women are clinically underdiagnosed or men over diagnosed. Recommendations for cardiac chamber quantification report different normal ranges for septal and posterior wall thickness in males (6-10 mm) and females (6-9 mm).(42) Therefore, either indexing to body surface area or creating gender specific cutoff values for family members might lead to a higher diagnostic accuracy.

Limitations

This FG+ population is specific for the Netherlands, and therefore it might be difficult to extrapolate these findings to other countries. Follow-up echocardiography was not available in all subjects. Due to developments in genetic testing methodology over time, extended genotyping was not performed in all subjects. Because autopsy was not routinely performed in SCD cases, comorbidities such as coronary artery disease cannot be fully excluded.

CONCLUSION

Clinical phenotype and outcome of FG+ HCM was similar to G+ HCM, but worse than G- HCM and FG+ HCM diagnosed in the context of family screening. These findings indicate the need for more intensive follow-up of FG+ and G+ HCM versus G- HCM and FG+ HCM in relatives.

FUNDING

We acknowledge the support from the Netherlands Cardiovascular Research Initiative CVON2014-40 DOSIS, an initiative with support of the Dutch Heart Foundation.

Conflict of interest: none declared.

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CLINICAL PERSPECTIVE

In hypertrophic cardiomyopathy (HCM), extreme genetic and clinical heterogeneity challenge the use of genotype as a prognostic factor. The gene most frequently affected in HCM is myosin-binding protein C (*MYBPC3*). In the Netherlands, 3 *MYBPC3* founder mutations represent 35% of HCM cases. To investigate the impact of genotype on the clinical course of HCM, we compared clinical phenotype and outcome of *MYBPC3* founder mutation (FG+) HCM with non-founder mutation genotype-positive (G+) HCM and genotype-negative (G-) HCM. Also, a distinction was made between FG+ HCM in probands who presented with signs or symptoms of disease, and in relatives who were diagnosed with HCM in the context of family screening. We observed a more severe phenotype at a younger age in FG+ and G+ HCM than in G- HCM and FG+ HCM in relatives, including more hypertrophy and non-sustained ventricular tachycardia, and more adverse remodeling during follow-up. Cardiovascular mortality was more frequent in FG+ and G+ HCM than in G- HCM and FG+ HCM in relatives. FG+ relatives without a phenotype suffered no cardiac events; although 11% developed subtle HCM during 6 years follow-up. The findings of the current study are important for the practicing clinician because it contradicts previous reports that observed a more benign clinical course in FG+ HCM. The results indicate the need for more intensive follow-up of FG+ and G+ HCM versus G- HCM and FG+ HCM in relatives. Moreover, adult FG+ carriers without a phenotype can be screened at low frequency.

CHAPTER 3

Outcomes of contemporary family screening in hypertrophic cardiomyopathy

Hannah G. van Velzen
Arend F.L. Schinkel
Sara J. Baart
Rogier A. Oldenburg
Ingrid M.E. Frohn – Mulder
Marjon A. van Slegtenhorst
Michelle Michels

Circ Genom Precis Med. 2018 Apr;11(4)

ABSTRACT

Background

Contemporary hypertrophic cardiomyopathy (HCM) family screening includes clinical evaluation and genetic testing (GT). This screening strategy requires the identification of a pathogenic mutation in the proband. Our aim was to examine the results of this HCM screening strategy.

Methods and Results

Between 1985 and 2016, 777 relatives of 209 probands were assessed in the context of HCM screening. A pathogenic mutation was identified in 72% of probands. After counseling, GT was performed in 620 (80%) relatives: 264 (43%) were genotype-positive (G+), and 356 (57%) were genotype-negative (G-). G+ relatives (n=264, age 41±18 y) and relatives without GT (n=157, age 30±17 y) underwent clinical screening. In G- relatives (age 48±17 y) mortality was assessed. At first screening, HCM was diagnosed in 98 (37%) G+ relatives and 28 (17%) relatives without GT ($p<0.001$). During 9 years follow-up of relatives diagnosed with HCM, 8 (6%) underwent septal reduction therapy, 16 (16%) received primary prevention ICDs, and cardiac mortality was 0.3%/year. During 7 years follow-up of relatives without HCM, 29 (16%) developed HCM. Survival at 5/10 years was 99%/95% in G+ relatives, 97%/94% in G- relatives ($p=0.8$), and 100%/100% in relatives without GT.

Conclusions

HCM was identified in 30% of relatives at first screening, and 16% developed HCM during 7 years of repeated evaluation. GT led to a discharge from clinical follow-up in 46% of the study population. Survival in the relatives was good.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disease with a prevalence of 1:500 to 1:200.(1) It is mainly transmitted in an autosomal dominant pattern, and the primary presentation may be sudden cardiac death (SCD).(2) Guidelines have encouraged family screening by electrocardiography (ECG) and echocardiography since 2003.(2-4) The latest European guidelines recommend to include genetic testing (GT) in the screening strategy.(2) However, in several countries, the routine use of GT is hampered by insufficient health care insurance coverage. In the Netherlands, GT in HCM is covered by the national basic health-care plan, and therefore can be routinely used for family screening. This strategy requires the identification of a pathogenic mutation in the proband, which is fulfilled in 40-60% of cases.(2) Genotype-positive (G+) relatives and relatives without GT are advised to undergo repeated cardiac evaluation. Genotype-negative (G-) relatives can be discharged.(2) Data regarding results of HCM screening including GT are scarce.(5, 6) The aim of this study was to examine the results of contemporary family screening in HCM, using GT and repeated cardiac evaluation.

METHODS

Study design and population

This retrospective cohort study included 777 relatives from 209 unrelated probands with HCM who visited the cardio-genetic outpatient clinic of the Erasmus Medical Center for screening purposes between 1985 and 2016. A median of 1 (interquartile range, 1-2) relative per proband was screened with a range of 1 to 17 relatives per proband. Relatives who presented symptomatically were excluded from the study. Relatives who were screened in another center and subsequently referred to our center were included. Families with HCM caused by Anderson-Fabry disease, Danon disease, Noonan syndrome or amyloidosis were excluded. The study conforms to the principles of the Declaration of Helsinki. Subjects gave informed consent for inclusion in the Erasmus MC Inherited Cardiomyopathy registry and local institutional review board approval was obtained. The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. Requests for collaboration will be handled on an individual basis.

Genetic analysis and family screening

Genetic counseling and testing was offered to all probands with HCM. Before the year 2012, genetic analysis consisted of direct sequencing of all coding intro-exon boundaries of the following genes: myosin binding protein C (*MYBPC3*), ß-myosin heavy chain (*MYH7*), cardiac-regulatory myosin light chain (*MYL2*), cardiac-essential myosin light chain (*MYL3*), cardiac troponin T (*TNNT2*), cardiac troponin I (*TNNI3*), cysteine and glycine-rich protein 3 (*CSRP3*), titin-cap/telethonin (*TCAP*), α-tropomyosin (*TPM1*), cardiac muscle α-actin (*ACTC1*), and cardiac troponin C (*TNNC1*). From the year 2012, next-generation-sequencing covering 48 cardiomyopathy-associated genes was used.

Classification of variants was done at time of initial testing. Variants were interpreted using a protocol adapted from the American College of Medical Genetics and Genomics recommendations(7), and classified into 5 categories: (I) benign; (II) likely benign; (III) uncertain significance; (IV) likely pathogenic; and (V) pathogenic. The potential pathogenicity of variants was assessed using Alamut Visual software (Interactive Biosoftware, Rouen, France), that integrates data from several large-scale population studies, evolutionary conservation of nucleotides and amino acids, in silico missense predictions (Align GVGD, SIFT, MutationTaster and PolyPhen-2) and splicing prediction modules (SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder). The criteria for classification of variants included the allele frequency in the dbSNP/ESP/ExAC/GoNL (cutoff minor allele frequency 1% in at least 300 ethnically matched control alleles equals benign), predicted effects on splicing, the in silico prediction of effect on the protein, and previously described links to disease. Furthermore, segregation analysis in families with more affected individuals and information considering presence in Human Gene Mutation Database (HGMD®) Professional 2017.3 (Qiagen) is taken into account. Variant reclassifications during follow-up were registered, and variant classification as assessed at the end of follow-up was used for the analyses. Patients with a reclassified variant were informed about the reclassification and if applicable about the indication for renewed evaluation. Patients were considered G+ when the mutation was classified as likely pathogenic or pathogenic (class IV and V). First-degree relatives were informed by means of a family letter provided by the proband about the presence of HCM in the family including the indication for cardiac evaluation, and if applicable about the possibility of GT. GT was offered to relatives of probands with likely pathogenic or pathogenic variants, and to relatives of probands without pathogenic mutations only in case HCM was revealed in the relative during cardiac screening. Based on this family letter relatives with interest for screening were referred to the clinical geneticist by their general practitioner for counseling. G+ relatives and relatives who refused GT were referred for cardiac evaluation. G- relatives did not routinely undergo cardiac evaluation. Cardiac evaluation < 10 years of age or GT before adulthood was offered in families with severe HCM in childhood, when cardiac symptoms were present, or severe anxiety among parents existed. Cardiac evaluation was repeated every 2-4 years in phenotype-negative children and every 3-5 years in phenotype-negative adults. Genetic and cardiac screening was extended after identification of a G+ relative and/or HCM diagnosis in a relative (cascade screening).

Cardiac evaluation

Cardiac evaluation included medical history, clinical examination, ECG and echocardiography. Cardiac symptoms were defined as ≥ 1 of the following: (1) Typical or atypical angina (with disregard of non-anginal chest pain); (2) New York Heart Association functional class ≥ 2; or (3) a history of cardiac syncope. The diagnosis of HCM in relatives was based on a maximal wall thickness ≥ 13 mm or z-score > 2, unexplained by loading conditions.(2) Maximal wall thickness, left atrial size, left

ventricular (LV) end-diastolic diameter, and LV outflow tract (LVOT) gradient at rest and during provocation were measured, according to the guidelines.(8) LVOT gradient was calculated with the Bernoulli equation, and LVOT obstruction defined as a gradient ≥ 30 mmHg at rest or during provocation. Systolic dysfunction was visually assessed and defined as mildly reduced, moderately reduced, or poor function. Diastolic dysfunction was defined as abnormal relaxation, pseudonormal filling, or restrictive filling(9), based on the Doppler mitral inflow pattern parameters including early (E) and late (A) LV filling velocities, E/A ratio, and tissue Doppler imaging-derived septal early diastolic velocities (e').

Follow-up and outcome measures

Follow-up data were obtained in July 2017, and was available in 99% of cases. Mortality was retrieved from the civil service register and cause of death from the medical chart or the general practitioner. Survival of the Dutch general population was obtained from the Central Agency for Statistics.(10) Cardiac mortality was defined as the combined end point of SCD, appropriate implantable cardioverter defibrillator (ICD) shock, and heart failure related death (including cardiac transplantation). SCD was defined as instantaneous death in individuals who were previously in a stable condition or successful resuscitation after cardiac arrest. Appropriate ICD shock was defined as shock or antitachycardia pacing for ventricular fibrillation or ventricular tachycardia >200/min. Septal reduction therapy and ICD implantation for primary or secondary prevention of SCD were registered.

Statistical analysis

Calculations were performed using SPSS 21 (IBM, Armonk, New York) and R Statistical Software version 3.4.1 using packages nlme, lme4 and survival. Normally distributed continuous data are expressed as mean ± standard deviation and non-normally distributed data as median followed by interquartile range (IQR). Relationship to the patient was categorized into sibling, child, parent and other, and the overall p-value of this categorical variable was examined. To compare baseline characteristics between G+ relatives and relatives without GT, and between subjects with and without HCM at initial evaluation, generalized linear mixed models were used with random intercepts for family to account for family relatedness. For covariates with suspicion of collinearity, we screened for multicollinearity and ran the multivariate model twice. Fisher's exact test was used in case of a zero cell count in either of the groups. For comparison of consecutive echocardiographic data the paired t-test was used and the Wilcoxon signed rank test in case of non-normally distributed data. Survival analyses were performed using the jackknife version of the Cox proportional hazards model to account for correlations caused by family relatedness. Results are presented with hazard ratio (HR) and 95% confidence interval (CI). All analyses were two-sided; P-values < 0.05 were considered significant.

RESULTS

Genetic testing

Figure 1 presents a flowchart of the results of GT in probands and relatives. GT was performed in 196 (94%) probands: 149 (76%) were G+, 33 (17%) had a variant with uncertain significance, and 14 (7%) were G-. GT was not performed in 13 (6%) probands, due to premature death in 7 and refusal in 6. Figure 2A presents the affected genes in G+ probands. A complex genotype was present in 8 (5%), including 4 homozygous, 2 digenic, and 2 compound heterozygous mutations. During follow-up, the following 3 (2%) pathogenic mutations were reclassified into variants with uncertain significance after data sharing between different centers in the Netherlands: (1) *CALR3* gene mutation c.564delT, (2) *ABCC9* gene mutation c.4537G>A, and (3) *MYPN* gene mutation c.59A>G. An overview of pathogenic mutations and complex genotypes is provided in supplementary tables 1 and 2 respectively. In the 47 (24%) probands without an identified pathogenic mutation ≥ 8 sarcomeric genes were tested, and next-generation-sequencing was performed in 34 (72%). In 24 (12%) probands without an identified pathogenic mutation there was no family history of HCM. GT was performed in 620 (80%) relatives: 264 (43%) were G+, and 356 (57%) were G-. Figure 2B presents the affected genes in G+ relatives. A complex genotype was found in 6 (2%); 3 digenic and 3 homozygous mutations. GT was not performed in 157 relatives due to the following reasons: (1) G- proband (n=78, 50%), (2) refusal of GT despite the presence of a pathogenic mutation in the proband (n=35, 22%), (3) GT not offered due to age < 18 years (n=25, 16%), and (4) no GT performed in the proband (n=19, 12%). Relatives without GT included 39 (25%) relatives who related to probands without an identified pathogenic mutation and no family history of HCM. Relatives who refused GT had a mean age of 29±11 years at time of evaluation.

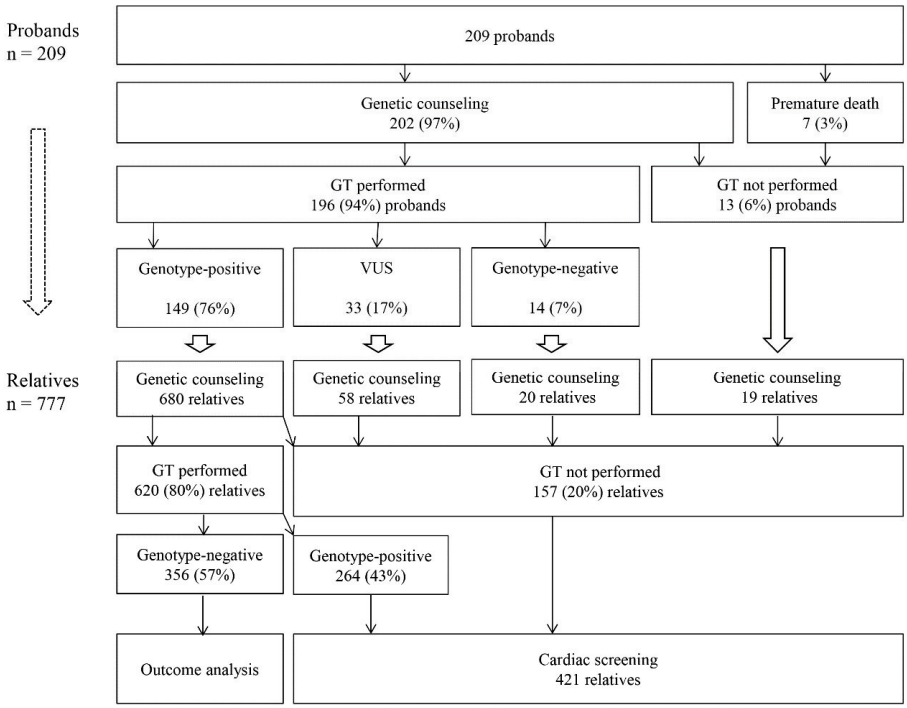

Figure 1. Flowchart of the study population. GT = genetic testing, VUS = variant with uncertain significance

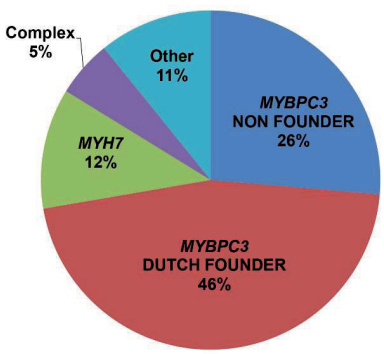

Figure 2a. Distribution of affected genes in 149 genotype-positive probands. *MYBPC3* = myosin-binding protein C; *MYH7* = β-myosin heavy chain.

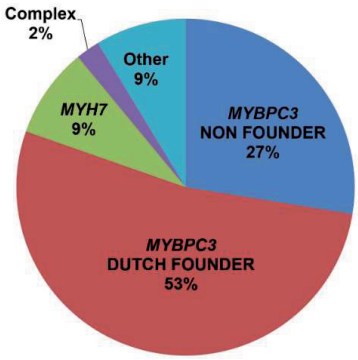

Figure 2b. Distribution of affected genes in 264 genotype-positive relatives. *MYBPC3* = myosin-binding protein C; *MYH7* = β-myosin heavy chain

First cardiac screening

Cardiac screening was performed in 421 relatives: 264 G+ relatives (age 41±18 y, 46% male) and 157 relatives without GT (age 30±17 y, 48% male) (table 1). A small proportion of male and female relatives had cardiac symptoms (6% vs 11%, p=0.09). At first evaluation, HCM was diagnosed in 126 (30%) relatives: 98 (37%) G+ and 28 (17%) relatives without GT (p<0.001). Mean age at HCM diagnosis was 44±16 (range 1-75) years and 57% was male. MWT was ≥ 20 mm in 11 (9%) and ≥ 30 mm in none. Four (6%) had LVOT obstruction, and 41 (40%) had diastolic dysfunction. One (0.2%) relative with a non-founder *MYBPC3* mutation had end-stage HCM, and another (with a digenic mutation) had non-compaction cardiomyopathy.

Table 1. Baseline characteristics of the study population

Variable	Whole cohort (n=421)	G+ relatives (n=264)	Relatives without GT (n=157)	P-value
Age (y)	37±18	41±18	30±17	<0.001
- Age < 12 years (n, %)	41 (10)	20 (8)	21 (13)	
- Age 12 - 18 years (n, %)	42 (10)	13 (5)	29 (19)	
- Age 19 - 35 years (n, %)	113 (27)	67 (25)	46 (29)	
- Age 36 - 50 years (n, %)	127 (30)	90 (34)	37 (24)	
- Age > 50 years (n, %)	98 (23)	74 (28)	24 (15)	
Men (n, %)	198 (47)	122 (46)	76 (48)	0.66
Proband age at diagnosis (y)	40±18	39±18	41±17	0.29
Cardiac symptoms	31 (9)	21 (9)	10 (8)	0.56
- Angina	12 (3)	9 (4)	3 (2)	0.38
- NYHA ≥ 2	15 (4)	11 (5)	4 (3)	0.40
- Cardiac syncope	8 (2)	4 (2)	4 (3)	0.47
Relationship to proband				0.002
- Sibling (n, %)	121 (29)	82 (31)	39 (25)	
- Child (n, %)	169 (40)	86 (33)	83 (53)	
- Parent (n, %)	44 (10)	32 (12)	12 (8)	
- Other (n, %)	87 (21)	64 (24)	23 (15)	

Data are expressed as mean ± standard deviation, or as absolute n (%). G+ = genotype-positive, GT = genetic testing, NYHA = New York Heart Association functional class.

Among G+ relatives, the HCM prevalence was higher in non-founder *MYBPC3* mutation carriers than in Dutch founder *MYBPC3* mutation carriers (49% vs 35%, p=0.04), despite similar age (43±17 vs 41±18 y, p=0.56). In 16 (57%) relatives with HCM, extensive GT in the proband failed to identify a pathogenic mutation. As presented in figure 3, the HCM prevalence increased with advancing age. In

41 children who were screened < 12 years, 6 (15%) had HCM. All 6 children were asymptomatic; 5 had a cardiac murmur, and 3 had a malignant family history of HCM.

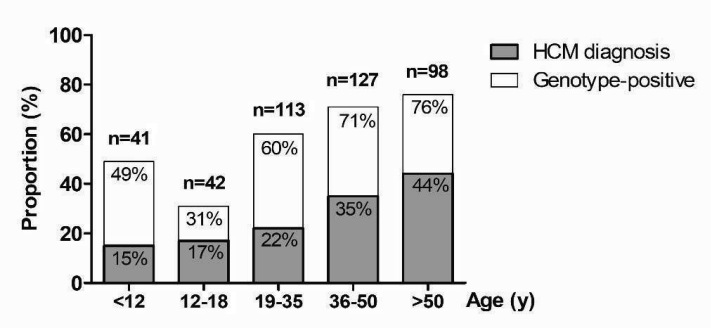

Figure 3. The proportion of genotype-positive relatives and the proportion of relatives diagnosed with hypertrophic cardiomyopathy at first screening are shown in different age groups. HCM = hypertrophic cardiomyopathy.

Predictors of HCM during first screening

Characteristics of relatives diagnosed with HCM versus those without HCM, are summarized in table 2. Relatives diagnosed with HCM were more likely G+, were older at time of screening (44±16 vs 34±18 years, p<0.001), more likely male, more frequently symptomatic, related to probands with a younger age at diagnosis (37±17 vs 41±18 years, p=0.045), and were differently related to the proband (e.g. sibling, child, parent, other). Multivariate analysis demonstrates that G+ status (adjusted odds ratio 4.23; 95% CI 2.88-5.58; p<0.001), male sex (adjusted odds ratio 2.13; 95% CI 1.51-2.76; p=0.02), proband age at diagnosis (adjusted odds ratio 0.98; 95% CI 0.96-0.99, p=0.02) and age at time of screening (adjusted odds ratio 1.04; 95% CI 1.02-1.06; p<0.001) were independent predictors of HCM diagnosis. Symptomatic status lost significance in the multivariate analysis. The results of the multivariable model did not differ with inclusion of relation to the proband and age had a more significant effect in the models than relation to the proband.

Table 2. Characteristics of subjects with and without hypertrophic cardiomyopathy at initial evaluation

Variable	HCM at initial evaluation?		P-value
	YES (n=126)	NO (n=295)	
Age, y (range)	44 ± 16 (1-75)	34 ± 18 (1-83)	<0.001
Men, n (%)	72 (57)	126 (43)	0.01
Proband age at diagnosis (y)	37±17	41±18	0.045
Cardiac symptoms	13 (14)	18 (7)	0.04
Relationship to proband			<0.001
- Sibling, n (%)	51 (40)	70 (24)	
- Child, n (%)	29 (23)	140 (47)	
- Parent, n (%)	21 (17)	23 (8)	
- Other, n (%)	25 (20)	62 (21)	
Genotype-positive status, n (%)	98 (78)	166 (56)	<0.001
Maximal wall thickness, mm (range)	16±3 (10-25)	9±2 (5-12)	<0.001
LVEDD, mm (range)	47±5 (33-60)	47±5 (32-61)	0.96
LA size, mm (range)	41±6 (27-60)	35±5 (21-53)	<0.001
LVOT obstruction ≥ 30 mmHg	4 (4)	0 (0)	0.008*
Diastolic dysfunction, n (%)	41 (41)	33 (12)	<0.001
Systolic dysfunction, n (%)	1 (1)	2 (1)	0.81

Data are expressed as mean ± standard deviation, or as absolute n (%). HCM = hypertrophic cardiomyopathy; LA = left atrial; LVEDD = left ventricular end-diastolic diameter; LVOT = left ventricular outflow tract. * Fisher's exact test was used because of zero cell count.

Clinical outcome of relatives with HCM

Mortality and interventions are presented in table 3. Relatives with HCM at baseline were followed for a median 9 (IQR 6, 12) years. Annual all-cause and cardiac mortality was 0.7% and 0.3% respectively. Cardiac mortality occurred in 7 (7%) relatives with HCM (all were G+), and included 3 cardiac transplants, 3 SCDs, and 1 appropriate ICD shock. There was no difference in either all-cause or cardiac mortality between Dutch founder *MYBPC3* and non-founder *MYBPC3* mutation carriers (p=0.7 and p=0.6 respectively). Septal reduction therapy was performed in 8 (6%) relatives with HCM. ICDs for the primary prevention of SCD were implanted in 12 (12%) G+/HCM+ and 4 (14%) HCM+ relatives without GT (p=0.9). There was one appropriate ICD shock in a G+/HCM+ relative 3 years after implantation.

Echocardiographic follow-up with a median of 6.6 (IQR 3.2, 10.8) years was available in 103 (82%) relatives with HCM. LV end-diastolic diameter decreased (47±5 to 44±4 mm; p=0.006), presence of diastolic dysfunction (40% to 68%; p=0.003) and systolic dysfunction (1% to 17%; p=0.001) increased. Seven (6%) patients developed end-stage HCM. Maximal wall thickness was overall stable (16±3 to 16±3 mm; p=0.54).

Table 3. Medium-term clinical outcome of relatives with and without hypertrophic cardiomyopathy

Variable	HCM diagnosis		No HCM diagnosis	
	G+	Without GT	G+	Without GT
	(n=98)	(n=28)	(n=165)	(n=129)
All-cause mortality (n, %)	13 (13)	0	4 (2)	0
Cardiac mortality (n, %)	7 (7)	0	1 (0.6)	0
SCD (n, %)	3 (3)*	0	1 (0.6)	0
Appropriate ICD shock (n, %)	1 (1)	0	0	0
Cardiac transplant (n, %)	3 (2)	0	0	0
Septal reduction therapy (n, %)	6 (6)	2 (7)	0	0
ICD (n, %)	14 (14)	4 (14)	5 (3)†	0
- primary prevention (n, %)	12 (12)	4 (14)	5 (3)	0
- secondary prevention (n, %)	2 (2)	0	0	0

Data are expressed as absolute n (%).* two were successfully resuscitated. † 3 (60%) received an ICD after HCM had developed during follow-up. G+ = genotype-positive, GT = genetic testing. HCM = hypertrophic cardiomyopathy; ICD = implantable cardioverter defibrillator; SCD = sudden cardiac death. P-values are not presented due to low number of events.

Clinical outcome of relatives without HCM

Relatives without HCM at baseline were followed for a median 8 (IQR 3-11) years. Annual all-cause mortality was 0.1%. Annual cardiac mortality in G+/HCM- subjects was 0.06% (1 SCD event) and 0% in HCM- relatives without GT (table 3). SCD occurred in a 21-year old non-founder (c.1624+1G>A, p.?) *MYBPC3* mutation carrier. Autopsy confirmed the absence of HCM. Post mortem analysis revealed a pathogenic mutation (c.1708G>T, p.Ala570Ser) in the *KCNH2* (Potassium Voltage-Gated Channel Subfamily H member 2) gene associated with long-QT syndrome. The ECG demonstrated no evidence of long-QT and there was no family history of long-QT syndrome. Two (1%) G+/HCM- subjects received an ICD for primary prevention; 1 had symptomatic non-sustained ventricular tachycardia, the other a troponin T mutation and family history of SCD. During a median 3 (IQR 2, 11) year follow-up, there were no appropriate ICD shocks and 1 inappropriate ICD shock due to lead failure.

Echocardiographic follow-up was available in 178 relatives without HCM (age 32±19 y, 46% male, 65% G+). During a median 7 (IQR 4, 10) years follow-up, HCM developed in 29 (16%) relatives at the mean age of 40±20 (range 9-77) years. The majority, 24 (83%) were G+ (20 *MYBPC3* carriers, 2 *MYH7* carriers, 1 *MYL2* carrier and 1 digenic *MYBPC3/MYL2* carrier). G+ relatives were more likely to develop HCM than relatives without GT (HR 2.44, 95% CI 1.02-5.85, p=0.04) (figure 4a); however, not after adjusting for age (HR 1.20, 95% CI 0.46-3.14, p=0.71) (figure 4b).

Adults (n=126, age 41±14 y, 44% male, 77% G+) were followed for 6±4 years and 19 (15%) developed HCM at mean age 50±16 (range 21-77) years. The majority, 15 (79%) were G+ (14 *MYBPC3* carriers; 1 digenic *MYBPC3/MYL2* carrier) and 68% was male. G+ adults were not more likely to develop HCM than adults without GT (HR 1.44, 95% CI 0.53-3.92, p=0.48). Maximal wall thickness increased from a median of 12 (IQR 10, 12) mm to a median of 14 (IQR 13, 15) mm at an average rate of 0.55 (0.2, 1.0) mm/year. Baseline median maximal wall thickness was higher in adults who developed HCM than in those who did not (12 (IQR 10, 12) vs 10 (IQR 8, 11) mm, p=0.004), and diastolic dysfunction was equally present (19% vs 13%, p=0.49).

Children (n=52, age 10±5 y, 49% male, 36% G+) were followed for 14±8 years and 10 (19%) developed HCM at mean age 20 ± 8 (range 9-31) years. Conversion mostly occurred between 19 and 31 years of age (n=7; 70%), and did not occur between 12 and 18 years of age. The majority, 9 (90%) were G+ (6 *MYBPC3* carriers, 2 *MYH7* carriers and 1 *MYL2* carrier), and 70% was male. G+ children were not more likely to develop HCM than children without GT (HR 3.26, 95% CI 0.59-17.97, p=0.17).

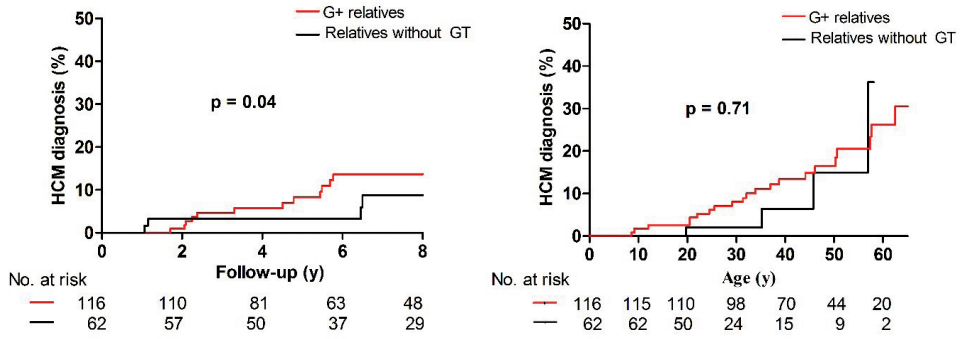

Figure 4. (A) Kaplan Meier curve demonstrating the cumulative hypertrophic cardiomyopathy incidence during follow-up of relatives without hypertrophic cardiomyopathy at baseline, stratified by genotype. (B) Kaplan Meier curve demonstrating the cumulative hypertrophic cardiomyopathy incidence during follow-up of relatives without hypertrophic cardiomyopathy at baseline, stratified by genotype and age. P-values are calculated using the jackknife version of the Cox model to account for family relatedness. G+ = genotype-positive; GT = genetic testing; HCM = hypertrophic cardiomyopathy.

Survival of G- relatives

All-cause mortality in G- relatives (age 48±17 y, 45% male) was assessed 7.5±3.6 years after GT. Eighteen (5%) G- relatives died at the mean age of 69±15 [range 49-88] years. As seen in figure 5,

survival from all-cause mortality in G- relatives (97% at 5 years, 94% at 10 years), G+ relatives (99% at 5 years, 95% at 10 years), and relatives without GT (100% at 5 and 10 years) was similar. In multivariate cox regression analysis adjusting for age, gender, and family relatedness, survival from all-cause mortality in G- relatives was not significantly different from that in G+ relatives (adjusted HR 0.92, 95% CI 0.43-1.94, p=0.8). There was an insufficient number of events in the relatives without GT (n=0) to compare survival between these relatives and G+ and G- relatives.

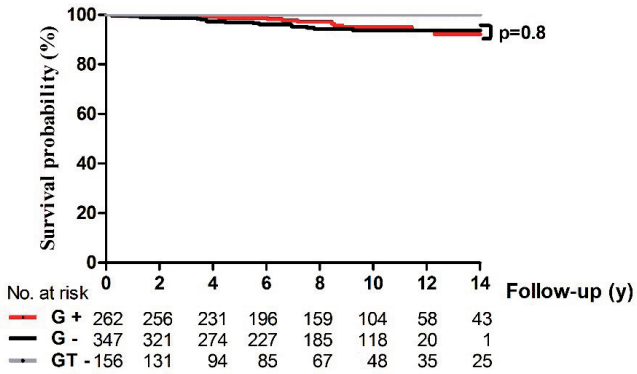

Figure 5. Survival of G+ relatives, G- relatives, and relatives without genetic testing.. P-value is calculated using the jackknife version of the multivariable Cox model to account for family relatedness, age, and gender. There was an insufficient number of events (n=0) in the relatives without GT to compare survival with G+ and G- relatives. G+ = genotype-positive; G- = genotype-negative, GT - = without genetic testing

DISCUSSION

The key findings of this study on HCM family screening are: (1) at first cardiac screening, HCM was diagnosed in 37% of G+ relatives and 17% of the relatives without GT (p<0.001), (2) G+ status, male sex, age at time of screening, and proband age at diagnosis were independent predictors of HCM diagnosis, (3) 16% of relatives without abnormalities at baseline developed HCM during 7 years of follow-up, and (4) survival in G- relatives, G+ relatives, and relatives without GT was good.

GT facilitates HCM screening

In the current study, 93% of probands and 80% of relatives underwent GT, which led to the reassurance of 356 G- relatives (46% of the total study population), and significantly reduced the number of clinical screening visits to the outpatient clinic. This supports previous studies that demonstrated the cost-effectiveness of family screening including GT.(1, 2) As expected, the HCM prevalence was higher in G+ relatives due to the exclusion of G- relatives from cardiac evaluation.

The genetic yield in the probands was high (76%) in comparison to previous studies.(3, 4) This could partly be due to the large Dutch *MYBPC3* founder population,(5) and partly due to a referral bias, because relatives from G+ families are informed about the confirmed heritability of HCM, and the HCM burden in G+ families is likely to be higher.(4) The rate of complex genotypes was relatively high (5%) in comparison with recent studies which used contemporary variant classification methods.(6, 7) This might be related to the high prevalence of Dutch *MYBPC3* founder mutations, the tertiary nature of our center, and the inclusion of children with more severe phenotypes.

HCM was found to be familial in families without an identified pathogenic mutation despite extensive genotyping. This finding suggests that genetic causes are still unrecognized. This information can be useful for counselling purposes. Unfortunately, expanded gene panels using next generation sequencing have offered limited additional pathogenic variants,(8) and have increased the prevalence of variants with uncertain significance.(9) Potentially, whole exome sequencing will have added value in the identification of novel HCM-causing genes, although this approach also has major challenges.(9) In 12% of the families, HCM was not familial and no pathogenic mutation was identified. Relatives in these families may not have a mendelian risk of disease, and so the utility of clinical screening may be impacted.(10) The inclusion of GT in family screening strategies allows the identification of this nonfamilial HCM subtype, which was reported to have later disease-onset and better clinical outcome.(10, 11)

It is important to be aware of the potential for variant reclassification. In this study, variants were classified according the most recent knowledge. Reclassification occurred during follow-up after data sharing between different centers in the Netherlands. All G- relatives who were initially discharged from clinical evaluation, should be informed about a reclassification and the indication for a renewed evaluation.

GT was refused by 6% of the relatives aged 29±11 years, likely due to socio-economic consequences. The Dutch Medical Examination Act protects unaffected HCM mutation carriers for life insurance below 268.000 Euro(12); above this amount carriers will have to disclose their HCM risk status, potentially resulting in an increased life insurance premium.(13, 14) Since health care insurance is obligatory in the Netherlands and covers the costs of GT, a lack of insurance coverage is not an issue. Other reasons for GT refusal include limited therapeutic implications. In the future, novel therapies(15) might increase the implications of GT.

Predictors of HCM during screening

More male than female relatives were diagnosed with HCM, a finding that is in line with the described male predominance in HCM cohorts.(3, 16) Previous studies suggest that the underrepresentation of women may reflect reduced patient awareness regarding cardiovascular risk, referral bias, or clinician

bias.(16) Referral bias in the current study is probably limited, because all relatives presented in the context of HCM screening. It was left to the decision of the relative whether to accept the invitation for screening, which is influenced by many factors including family and personal characteristics (symptomatic status, gender, race, education).(17) Other possible explanations for male predominance in HCM may be related to genetic and endocrine factors. Studies in mice models of HCM-associated mutations have shown that male sex predisposes to an earlier onset of disease, however the underlying pathophysiological mechanisms are not fully understood.(18, 19) HCM diagnosis was associated with advancing age, a feature that is in line with the described age-related penetrance of HCM.(20, 21) A younger age at diagnosis in the proband was associated with HCM in the relative. This illustrates the use of obtaining a family history and indicates a higher yield of screening in those families with more severe disease expression.

Clinical outcome of relatives with HCM and G+/HCM- relatives

In relatives diagnosed with HCM during screening, cardiac mortality was low (0.3%/y) in comparison to the reported cardiac mortality in probands with HCM (1-2%/y).(3) Since relatives are referred by familial evaluation instead of signs or symptoms, HCM in relatives is probably diagnosed in an earlier disease stage(22). Lifestyle modifications, periodic SCD risk evaluation and close clinical follow-up, resulting in ICD implantations, and timely referral for septal reduction therapies might have led to a better clinical outcome.(23) Given the low event rate in HCM larger numbers and longer follow-up are needed to really show an impact on clinical outcome. One G+/HCM- relative died suddenly and a long-QT mutation was identified post-mortem.

Family screening strategies evaluated

Current guidelines(3, 24, 25) recommend cardiac evaluation from age 10-12 years and repeat evaluation every 1-2 years until 18-21 years of age, and every 2-5 years thereafter. Younger children can be screened in case of a severe family history, competitive sports participation, or when cardiac symptoms are present.(3) In this cohort, 6 out of 41 (15%) children < 12 years of age were diagnosed with HCM during clinical screening, which is more than expected and may be related to the tertiary nature of our center. Three of these cases did not have a severe family history, cardiac symptoms, or competed in sports, and so these cases would have been missed if current guidelines were followed. Simple cardiac auscultation would have raised suspicion because a cardiac murmur was present in almost all cases. The prevalence and prognosis of sarcomeric childhood HCM is currently unknown and requires investigation.(3) A multigenerational pedigree study demonstrated an increased mortality risk for untreated relatives aged 10-19 years with a 0.5 probability of truncating *MYBPC3* carriership.(26) This supports clinical evaluations from 10 years of age. Interestingly, our data demonstrates no conversion during adolescence but rather < 12 and > 18 years of age. This questions the need for more frequent evaluations during adolescence(24), rather illustrates the need for earlier

screening i.e. at the age of 8 years, and continued screening into adulthood, similar to the conclusions of Jensen et al.(20) (3) Whether or not to perform GT in children is disputable. Although we demonstrated in this study that GT has an impact on clinical screening strategies, the impact on management and lifestyle is limited due to the lack of prognostic value of genotype for disease-onset and risk. Moreover, due to the potential social, emotional, psychosocial, educational and financial consequences of GT and the advantage of integrating patients into medical decision making(27) we prefer to postpone GT until the legal age of 18 years. However, there are some situations in which the psychological or social benefits outweigh the risks of GT. For example, parents who cannot deal with the anxiety of 'not knowing' might have a more negative impact on the child than would complying with the request for GT. In addition, early knowledge of carrier status might increase coping with the information.(27) Our HCM program makes decisions on a case-by-case basis after extensive counseling of the family and the child, including psychological support and taking all of the above considerations into account. We feel that screening frequencies should not be different in G+ children or children without GT, because untested children may have a 50% probability of carriership.(26) In adults, we support intervals with a low frequency e.g. 5-yearly, as is advised by the 2011 American guidelines(24), because the HCM incidence was low, and the development of hypertrophy slow.

Limitations

The efficiency of clinical screening is dependent on the uptake of GT in probands and relatives. The uptake of GT in the current study was high, because in the Netherlands GT is covered by the national basic health care insurance, and because all probands were seen at our cardio-genetic outpatient clinic where cardiac evaluation and genetic counseling and testing is offered simultaneously. This limits generalizability of results to other countries with different financial and organizational approaches regarding GT in the HCM population. The high proportion of Dutch *MYBPC3* founder mutation carriers limits representation of broader populations with HCM. The number of screened children was relatively small. Due to significant advances in DNA-sequencing methodology during the past decade, there was no homogenous genotyping over the whole period. Since 12% of the study population was referred after HCM was diagnosed in another center, this has created a selection bias. Clinical phenotyping was not performed in G- relatives, limiting the ability to identify non-penetrance and/or second variants, which may be an important caveat in some families.

CONCLUSION

HCM was identified in 30% of relatives at first screening, and 16% developed HCM during 7 years of repeated cardiac evaluation. GT led to a discharge from clinical follow-up in 46% of the study population. Survival in the relatives was good.

Acknowledgements

Thank you to Isabella Kardys, MD PhD, for her help with the statistical analysis.

Conflict of interest: none declared.

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CLINICAL PERSPECTIVE

In hypertrophic cardiomyopathy (HCM) family screening, guidelines recommend repeated clinical evaluations from age 10-12 years until advanced age. The latest European guidelines recommend to include genetic testing (GT) in the screening strategy. GT allows the identification of genotype-positive relatives without HCM and the reassurance and discharge of genotype-negative relatives. We retrospectively analyzed the results of this contemporary family screening strategy in 777 relatives of 209 probands who were evaluated at our cardio-genetic outpatient clinic where cardiac evaluation and genetic counseling and testing is offered simultaneously. After performing GT in 94% of the probands and 80% of the relatives, we were able to reassure 356 (46%) genotype-negative relatives, thereby significantly reducing the number of clinical screening visits. First cardiac evaluation in genotype-positive relatives (n=264) and relatives without GT (n=157) revealed HCM in 37% and 17% respectively. During follow-up, cardiac mortality among relatives with HCM was low (0.3%/year) reflecting early disease stages. One genotype-positive relative without HCM died suddenly and a long-QT mutation was identified post-mortem. During 7 years follow-up 16% of the relatives without HCM at first evaluation developed subtle HCM. The findings of the current study are important for the practicing clinician, because it demonstrates the impact of GT on the HCM clinical screening process, and it shows current challenges associated with GT in families with HCM. Moreover, evaluating the HCM prevalence at first evaluation and after repeated evaluations in adults and children and reporting the prognosis of relatives with and without HCM helps to determine the preferred clinical screening strategy.

PART II

Imaging in hypertrophic cardiomyopathy

CHAPTER 4

Prognostic significance of anterior mitral valve leaflet length in individuals with a hypertrophic cardiomyopathy gene mutation without hypertrophic changes

Hannah G. van Velzen
Arend F.L. Schinkel
Myrthe E. Menting
Annemien E. van den Bosch
Michelle Michels

J Ultrasound. 2018 Jun 6.

ABSTRACT

Purpose

Previous studies suggest that anterior mitral valve leaflet (AMVL) elongation is a primary phenotypic feature in hypertrophic cardiomyopathy (HCM). Our aim was to assess AMVL length in individuals with HCM gene mutations and in healthy controls and to identify predictors of the development of HCM during follow-up.

Methods

A total of 133 HCM mutation carriers and 135 controls underwent cardiac examination including electro- and echocardiography. AMVL length was measured in the parasternal long axis and apical 3 chamber view during diastole. Univariate and multivariable cox proportional hazard regression analyses were performed to identify predictors of HCM.

Results

There were no significant differences between HCM mutation carriers and controls regarding age and sex. In the parasternal long axis view, AMVL length was similar in mutation carriers and controls (24±4 vs 24±4 mm, p=0.8). In the apical 3 chamber view, AMVL were shorter in mutation carriers (29±4 vs 30±4 mm, p=0.02). When averaged for both views, AMVL length was similar in mutation carriers and controls (27±3 vs 27±3 mm, p=0.2). During 5.8±3.0 years follow-up, 13 (14%) HCM mutation carriers developed HCM. Pathological Q wave (hazard ratio 9.89, p=0.004), E/e' ratio (hazard ratio 2.52, p=0.001), and maximal wall thickness (hazard ratio 2.15, p=0.001) were independent predictors of HCM. AMVL length was not predictive of the development of HCM.

Conclusions

AMVL length is similar in HCM mutation carriers and controls. AMVL length is not predictive of the development of HCM, in contrast to pathological Q wave, E/e' ratio, and maximal wall thickness.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a genetic cardiac disease with an estimated prevalence of 1:500 to 1:200.(1-3) The diagnosis is based on the presence of a maximal wall thickness ≥ 15 mm in index patients and ≥ 13 mm in relatives, that is not solely explained by abnormal loading conditions.(2) A pathogenic HCM mutation is identified in 40-60% of patients with HCM.(2, 4) Presymptomatic genetic testing of relatives has led to the identification of HCM gene mutation carriers who do not fulfill the echocardiographic criterion of HCM.(5) HCM mutation carriers are at risk of developing HCM.(5) Conflicting data exists on whether the anterior mitral valve leaflets (AMVL) are elongated in mutation carriers and whether AMVL elongation is a predictor of the development of HCM during follow-up.(6-11) The aim of this study was to assess AMVL length in HCM mutation carriers and healthy controls and to determine the prognostic significance of AMVL length in HCM mutation carriers for the development of HCM during follow-up.

METHODS

Study design and population

This single-center retrospective case-control and cohort study included 133 HCM mutation carriers without clinical expression of HCM who were clinically evaluated at our cardio-genetic outpatient clinic between the years 2004-2017. Genetic assessment and the family screening strategy at our center have been described previously.(12, 13) For comparison, 135 healthy controls underwent cardiac evaluation.(14) Controls were recruited via an advertisement. Inclusion criteria were normal physical examination, normal electrocardiography (ECG), and left ventricular (LV) ejection fraction > 51%; exclusion criteria were prior cardiovascular disease or risk factors consisting of hypertension, diabetes mellitus, and hypercholesterolemia, systemic disease, medication known to influence cardiac function including thyroid medication (with the exception of asthma inhalers), professional athletes, body mass index > 40 and women with breast implants.(14) The study conforms to the principles of the Declaration of Helsinki. All patients gave informed consent for inclusion in the registry and local institutional review board approval was obtained.

Clinical evaluation

Clinical evaluation included medical history, physical examination, ECG, and transthoracic echocardiography. Standard 12-lead ECG was performed in the supine position during quiet respiration. LV hypertrophy was evaluated with the Romhilt-Estes criteria. Pathological Q waves were defined as duration > 40 ms or depth > 30% R wave in ≥ 2 leads. T-wave inversion was defined as ≥ 3 mm in ≥ 2 leads. Echocardiographic studies were analyzed according to the guidelines.(15, 16) Maximal wall thickness, left atrial size, leaflet and chordal systolic anterior motion of the mitral valve, and resting LV outflow tract peak velocity were assessed. LV outflow tract gradient was calculated

with the Bernoulli equation. LV systolic function was categorized as: good (LV ejection fraction > 51%), mildly reduced (LV ejection fraction 41% to 51%), moderately reduced (LV ejection fraction 30% to 40%), and poor (LV ejection fraction < 30%).(16) LV diastolic function was defined as normal, abnormal relaxation, pseudonormal or restrictive filling, based on Doppler mitral inflow pattern parameters including early (E) and late (A) LV filling velocities, E/A ratio, and tissue Doppler imaging-derived septal early diastolic velocities (e').(17) HCM during follow-up was defined as a maximal wall thickness ≥ 13 mm according to the guidelines.(2)

AMVL measurements

AMVL length was measured in the parasternal long axis (PLAX) view and in the apical 3 chamber (A3C) view, during diastole and with the leaflet maximally extended. In the PLAX view, leaflet length was defined as the distance from the tip of the leaflet to the junction between the anterior leaflet and the posterior aortic wall (hinge point), according to Klues et al.(18) In the A3C view, leaflet length was defined as the distance from the tip of the leaflet to the insertion of the noncoronary aortic leaflet, according to Alhaj et al.(19) Examples of AMVL measurements in both views are shown in figure 1. All AMVL measurements were performed by one reader. For intraobserver variability, one reader independently measured 40 AMVLs in the PLAX view and 40 AMVLs in the A3C view in an identical fashion on 2 occasions. For interobserver variability, 2 readers independently measured 20 AMVLs in the PLAX view and 20 AMVLs in the A3C view.

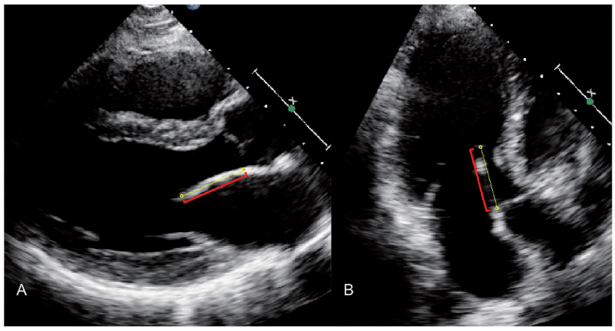

Fig. 1 Example of anterior mitral valve leaflet length (AMVL) measurements in a hypertrophic cardiomyopathy gene mutation carrier without hypertrophic changes. In the parasternal long-axis view (**a**), the AMVL measured 26 mm, and in the apical 3 chamber view (**b**), the AMVL measured 26 mm

Statistical methods

The statistical analysis was performed using SPSS 21 (IBM, Armonk, New York). Normally distributed continuous data are expressed as mean ± standard deviation and non-normally distributed

data as median followed by interquartile range. For comparing categorical variables Pearson's chi-square test was used. For comparing continuous variables t-test was used, and Mann-Whitney U in case of non-normally distributed data. All analyses were two-sided; P-values < 0.05 were considered significant. Inter-observer and intra-observer agreement was defined as the mean of the difference between two measurements ± standard deviation. Univariate and multivariable cox proportional hazard regression was performed to determine hazard ratios (HR) and 95% confidence intervals (CI). After screening for multicollinearity, the univariate significant variables with the highest HR were entered into the multivariable regression model. To calculate the allowed number of variables for inclusion in the multivariable analysis, the square root of the number of events was used. This is an alternative method to determine the number of variables allowed for inclusion in the multivariable analysis.(20)

RESULTS

Clinical evaluation

HCM gene mutation carriers represented mutations in 10 different genes. The *MYBPC3* gene was most frequently affected (77%), followed by the *MYH7* gene (11%). Other genes affected were *TNNT2* (3%), *MYL2* (2%), *FHL1* (2%), *ALPK3* (2%) *MIB1* (0.75%), *TNNI3* (0.75%), *TPM1* (0.75%), and *MYL3* (0.75%). Clinical and echocardiographic characteristics in mutation carriers and controls are presented in table 1. Mutation carriers and controls had similar age, gender, and body surface area. Compared to controls, more mutation carriers had pathological Q waves (4% vs 0%, p=0.02), and mutation carriers had a higher E/e' ratio (8.2±1.9 vs 7.7±1.9, p=0.03), and a higher maximal wall thickness (8.9±1.9 vs 8.0±1.8 mm, p=0.001).

Table 1. Clinical and echocardiographic characteristics of the study population (continues on the next page)

Variable	Mutation carrier (n=133)	Control (n=135)	P-value
Age, (y)	41±14	44±14	0.11
Female gender, n (%)	85 (64)	73 (54)	0.18
Body surface area (m^2)	1.9±0.2	1.9±0.2	0.86
Electrocardiography			
Romhilt-Estes ≥ 4, n (%)	10 (8)	4 (3)	0.09
T wave inversion, n (%)	1 (1)	0 (0)	0.31
Pathological Q wave, n (%)	5 (4)	0 (0)	0.02
Echocardiography			
Maximal wall thickness (mm)	8.9±2.0	8.0±1.8	<0.001
Left atrial size (mm)	34±5	34±4	0.24
LVOT gradient ≥ 30 mmHg*, n (%)	0 (0)	0 (0)	0.50
AMVL, PLAX (mm)	24±4	24±4	0.85
AMVL, A3C (mm)	29±4	30±4	0.02
AMVL, averaged (mm)	27±3	27±3	0.17
Chordal systolic anterior motion, n (%)	5 (4)	1 (1)	0.09
Leaflet systolic anterior motion, n (%)	0 (0)	0 (0)	0.50

Table 1. Clinical and echocardiographic characteristics of the study population (continued).

Variable	Mutation carrier (n=133)	Control (n=135)	P-value
E/A ratio	1.4±0.5	1.6±0.7	0.03
E/e' ratio	8.2±1.9	7.7±1.9	0.03
Septal e' (cm/s)	9.5±2.5	9.6±2.6	0.61
Diastolic function			
Normal, n (%)	105 (83)	111 (85)	0.66
Abnormal relaxation, n (%)	10 (8)	7 (5)	0.39
Pseudonormal filling, n (%)	11 (9)	13 (10)	0.76
Restrictive filling, n (%)	0 (0)	0 (0)	0.50
Systolic function			
Good, n (%)	132 (99)	135 (100)	0.31
Mildly reduced, n (%)	1 (1)	0 (0)	0.31
Moderately reduced, n (%)	0 (0)	0 (0)	0.50
Poor, n (%)	0 (0)	0 (0)	0.50

Data are expressed as mean ± standard deviation or as absolute and %. AMVL = anterior mitral valve leaflet; A3C = apical 3 chamber view; LVOT = left ventricular outflow tract; PLAX = parasternal long-axis view; *at rest

AMVL measurements

Beeswarm plots of AMVL measurements in the PLAX and the A3C view are presented in figure 2. In the PLAX view, AMVL length did not differ between mutation carriers and controls (24±4 vs 24±4 mm, p=0.8). In the A3C view, AMVL were shorter in the mutation carriers (29±4 vs 30±4 mm, p=0.02). When averaged for both views, AMVL length was similar in mutation carriers and controls (27±3 vs 27±3 mm, p=0.2). Overall, AMVL were significantly longer in the A3C view than in the PLAX view (30±4 vs 24±4 mm, p<0.001).

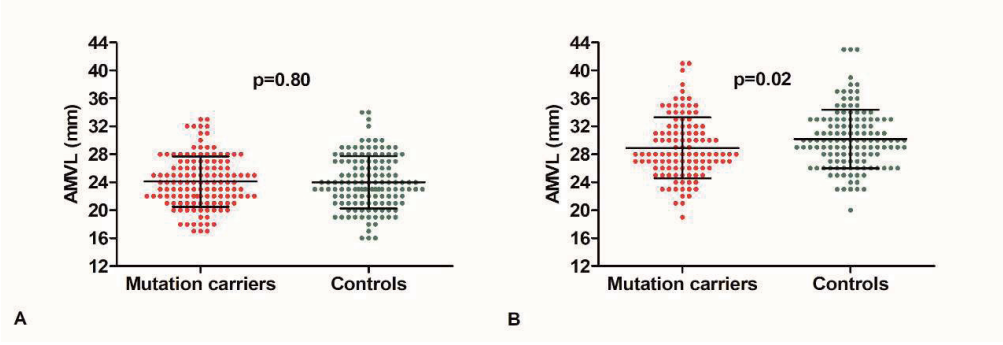

Fig. 2 Beeswarm plot of anterior mitral valve leaflet (AMVL) length measurements in hypertrophic cardiomyopathy gene mutation carriers without hypertrophic changes versus healthy controls, assessed with transthoracic echocardiography in the **(a)** parasternal long-axis view and **(b)** apical 3 chamber view

Intra-observer and inter-observer agreement

In the PLAX view, the inter-observer agreement was -2.7±2.6 mm and the intra-observer agreement was -1.0±3.5 mm. In the A3C view, the inter-observer agreement was 2.0±2.5 mm and the intra-observer agreement was 0.5±2.6 mm.

Follow-up

During 5.8±3.0 years follow-up, 13 (14%) mutation carriers developed HCM. Mean age at HCM diagnosis was 52±17 years. In these 13 mutation carriers, maximal wall thickness increased from a median 10 (interquartile range 8, 11) mm to 13 (interquartile range 13, 14) mm, with a mean rate of 0.7±0.3 mm/year. Table 2 presents the baseline characteristics in those who developed HCM during follow-up and those who did not. Univariate significant predictors of the development of HCM were pathological Q wave (HR 9.74, 95% CI 2.53-37.46, p=0.001), maximal wall thickness (HR 1.64, 95% CI 1.12-2.39, p=0.01), E/e' ratio (HR 1.63, 95% CI 1.20-2.23, p=0.002), left atrial size (HR 1.16, 95% CI 1.03-1.31, p=0.01), and age (HR 1.06, 95% CI 1.02-1.11, p=0.01). AMVL length was not predictive of the development of HCM in the PLAX view (HR 1.03, 95% CI 0.87-1.22, p=0.72) or in the A3C view (HR 0.99, 95% CI 0.86-1.15, p=0.92). Multivariable cox regression analysis which included 3 variables demonstrates that pathological Q wave (adjusted HR 9.89, 95% CI 2.09-46.95, p=0.004), E/e' ratio (adjusted HR 2.52, 95% CI 1.48-4.29, p=0.001), and maximal wall thickness (adjusted HR 2.15, 95% CI 1.36-3.42, p=0.001) all were independent predictors of HCM during follow-up.

Table 2. Baseline characteristics of HCM gene mutation carriers who did and did not develop hypertrophic cardiomyopathy during follow-up.

Variable	Developed HCM		
	YES (n=13)	NO (n=77)	P-value
Age (y)	47±19	39±13	0.05
Male gender, n (%)	8 (62)	25 (33)	0.04
Romhilt-Estes ≥ 4, n (%)	1 (8)	5 (7)	0.87
T wave inversion, n (%)	1 (8)	0 (0)	0.01
Pathological Q, n (%)	3 (23)	2 (3)	0.003
Left atrial size (mm)	39±5	33±5	<0.001
Maximal wall thickness (mm)	10±2	9±2	0.02
AMVL, PLAX (mm)	25±5	24±3	0.47
AMVL, A3C (mm)	29±5	29±4	0.90
Chordal systolic anterior motion, n (%)	1 (8)	3 (4)	0.54
E/e' ratio	9.3±1.8	8.1±1.7	0.03
Septal e' (cm/s)	8.5±2.7	9.5±2.6	0.20
Abnormal diastolic function, n (%)	4 (31)	11 (16)	0.21

Data are expressed as mean ± standard deviation or as absolute and %. AMVL = anterior mitral valve leaflet; A3C = apical 3 chamber view; HCM = hypertrophic cardiomyopathy; PLAX = parasternal long axis view.

DISCUSSION

During HCM family screening, individuals who carry a HCM gene mutation may not fulfil the echocardiographic diagnostic criterion of HCM.(5) Because of the age-related penetrance of HCM, long-term clinical follow-up including ECG and echocardiography is recommended.(2, 3) Currently, it is unclear which HCM mutation carriers will develop HCM.(2, 11) We aimed to assess AMVL length in mutation carriers and controls, and determine the prognostic value of AMVL length for the development of HCM during follow-up. Our main findings are: (1) AMVL length is similar in mutation carriers and controls, (2) AMVL length is not predictive of the development of HCM, and (3) pathological Q wave, E/e' ratio, and maximal wall thickness are independent predictors of the development of HCM.

AMVL elongation is not a primary phenotypic feature of HCM

In patients with HCM, AMVL elongation has been demonstrated pathologically, on echocardiography, and on cardiovascular magnetic resonance imaging.(10, 18, 21-23) Among other factors, AMVL elongation contributes to systolic anterior motion of the mitral valve and LV outflow tract obstruction.(23-25) The etiology of AMVL elongation in patients with HCM is unclear. Both the pathological study of Klues et al. and the in vivo study of Kim et al. found that AMVL elongation is not secondary to LV outflow tract obstruction or systolic anterior motion of the mitral valve, since it also occurs in patients without LV outflow tract obstruction or systolic anterior motion.(19, 21, 22) Therefore, it was suggested that AMVL elongation is a primary phenotypic expression of HCM. Several studies have indeed reported AMVL elongation in mutation carriers as measured by magnetic resonance imaging(6, 7, 10), and echocardiography.(9) The current study contradicts these findings. In line with our findings, a recent magnetic resonance imaging study similarly reported no difference in AMVL length between mutation carriers and controls.(8) The discrepancy between the studies may be related to the small number of participants, different imaging modalities used, different distribution of genetic mutations, or different methodologies used for AMVL measurements.

For several reasons, we believe it is unlikely that HCM gene mutations cause AMVL elongation. First, there are no sarcomeric proteins in the mitral valve leaflet.(25) Second, a HCM animal model including heterozygous cardiac myosin-binding protein C targeted knock-out mice embryos did not show mitral leaflet elongation.(26) Third, Captur et al. observed AMVL elongation in genotype-negative patients with HCM.(10) And finally, most morphological studies demonstrate that mitral leaflets are intrinsically normal.(21, 27) Other potential etiologies of AMVL elongation are being investigated, such as the paracrine effects from the abnormal LV wall which influences valvulogenesis, or the abnormal differentiation of pluripotent epicardial-derived cells into fibroblast-cells with increased synthesis of periostin which might drive leaflet elongation.(25)

Predicting the development of HCM

The current study demonstrates that AMVL length had no predictive value for the development of HCM. Hence, AMVL length cannot be used as a preclinical marker of the development of HCM. Similar observations were made in a prior smaller study by Ho et al.(28) Pathological Q wave had a high predictive value for the development of HCM. Indeed, prior investigation of ECGs in genotyped HCM populations demonstrated that Q waves and repolarization abnormalities are the most distinguishing ECG manifestations of sarcomere mutations.(29) However, the clinical utility of Q waves is probably limited because of the low negative predictive value; 10 out of 13 mutation who developed HCM did not have pathological Q waves at baseline. Our study did not demonstrate a prognostic value of septal e', in contrast to Ho et al.(28) The age difference between the studies (16 vs 41 y) and differences in genetic mutations might explain this discrepancy. However, we did observe a predictive value of E/e' ratio, which supports the suggestion that diastolic dysfunction is a primary phenotypic feature of HCM.(28, 30)

Technical challenges associated with AMVL measurement by echocardiography

Previous studies most commonly used magnetic resonance imaging to measure AMVL length.(6-8, 10) Since transthoracic echocardiography is the advised imaging modality in HCM clinical screening strategies and has a higher spatial and temporal resolution than cardiac magnetic resonance imaging(31, 32), we used echocardiography to determine AMVL length. Overall, inter-observer variability in both views was 2 to 3 mm, similar to previous studies.(6, 8, 18) The difference between observers may be explained by the technical difficulty of distinguishing the mitral leaflet from the chordae tendineae, and by the frame-to-frame variability in AMVL length caused by AMVL movement during diastole and respiration. Intra-observer agreement was best for the A3C view, which was unexpected because in the PLAX view the distance to the transducer is shorter. It may be explained by the landmarks that were used; in the A3C view the insertion of the noncoronary aortic leaflet is more easily identifiable in comparison to the hinge point in the PLAX view. Finally, AMVL were significantly longer in the A3C view than in the PLAX view; the measurement in the A3C view included the intervalvular fibrosa.

Limitations

This study has several limitations. First, although the study population is large compared to previous studies, a higher sample size would reduce the risk of sampling error. Second, the proportion of HCM gene mutation carriers that developed HCM during follow-up was limited.

CONCLUSIONS

AMVL length is similar in HCM mutation carriers and healthy controls. AMVL length is not a predictor of the development of HCM during follow-up, in contrast to pathological Q wave, E/e' ratio, and maximal wall thickness.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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CHAPTER 5

Five-year prognostic significance of global longitudinal strain in individuals with a hypertrophic cardiomyopathy gene mutation without hypertrophic changes

Hannah G. van Velzen
Arend F.L. Schinkel
Roderick W. J. van Grootel
Marjon A. van Slegtenhorst
Jolanda van der Velden
Mihai Strachinaru
Michelle Michels

Accepted in Netherlands Heart Journal 2018

ABSTRACT

Previous studies report that global longitudinal strain (GLS) is reduced in patients with hypertrophic cardiomyopathy (HCM) while left ventricular (LV) ejection fraction is normal. Our aim was to assess GLS in individuals with HCM gene mutations without hypertrophic changes and determine its prognostic value for the development of HCM. This retrospective case-control and cohort study included 120 HCM mutation carriers and 110 healthy controls. GLS and LV ejection fraction were assessed with Tomtec Imaging software. Mutation carriers and controls had similar age, gender and body surface area. Compared to controls, mutation carriers had a higher maximal wall thickness (9±2 vs 8±2 mm, p<0.001) and LV ejection fraction (60±5 vs 58±4%, p<0.001). GLS was higher in mutation carriers than in controls (-21.4±2.3% vs -20.3±2.2%, p<0.001). Regionally, this difference was observed in the mid-LV (-21.5±2.5% vs -19.9±2.5%, p<0.001) and the apex (-24.1±3.5% vs -22.1±3.4%, p<0.001), but not in the base of the LV (-20.0±3.3% vs -20.0±2.6%, p=0.9). Echocardiographic follow-up was performed in 80 mutation carriers. During 5.6±2.9 years follow-up, 13 (16%) mutation carriers developed HCM. In cox regression analysis, age (HR 1.08, p=0.01), pathological Q wave (HR 8.56; p=0.01), and maximal wall thickness (HR 1.94; p=0.01) were independent predictors of the development of HCM. GLS was not predictive of the development of HCM (HR 0.78, p=0.07). In conclusion, GLS is increased in HCM mutation carriers without hypertrophic changes. GLS provided no clear prognostic value for the development of HCM during follow-up, in contrast to age, pathological Q waves and maximal wall thickness.

BACKGROUND

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease caused by mutations in genes that encode proteins of the cardiac sarcomere.(1, 2) Genetic testing allows the identification of individuals carrying HCM mutations who do not fulfill the echocardiographic criterion of HCM.(1, 2) Due to age-related penetrance, these mutation carriers are advised to undergo periodic follow-up including electrocardiography (ECG) and echocardiography.(1, 2) Currently, we are unable to predict the development of HCM.(3) In patients with overt HCM, studies have revealed a reduced global longitudinal strain (GLS) assessed with speckle-tracking echocardiography (STE), despite a normal left ventricular ejection fraction (LVEF).(4) Several HCM cohort studies have shown that GLS is predictive of outcome.(5-7) Data regarding GLS in HCM gene mutation carriers without hypertrophic changes is limited.(8-11) Our aim was to assess GLS in mutation carriers and healthy controls, and to determine its predictive value for the development of HCM during long-term follow-up.

METHODS

This single-center retrospective case-control and cohort study included 120 HCM mutation carriers without hypertrophic changes and 110 healthy controls, who were clinically evaluated at our cardio-genetic outpatient clinic between the years 2004-2017. For the cases, inclusion criteria were individuals with a pathogenic HCM gene mutation not fulfilling the echocardiographic criterion of HCM and with a LVEF ≥ 50% according to the guidelines(1, 2, 12) Exclusion criteria were a tracking feasibility score < 2 in ≥ 2 apical views and prior cardiovascular surgery. In total, 138 HCM mutation carriers without HCM were identified. Sixteen (12%) were excluded due to insufficient tracking feasibility and 2 (1%) because of prior cardiovascular surgery. Variants classified as class IV or V were considered pathogenic.(13) Controls were recruited via an advertisement.(14) For the controls, inclusion criteria were normal physical examination, normal ECG, and a LVEF ≥50%. Exclusion criteria were prior cardiovascular disease or risk factors, systemic disease, medication known to influence cardiac function, sports participants exercising for 6 hours or more per week on a regular basis and aiming to improve themselves, body mass index > 40 and women with breast implants.(14) The study conforms to the principles of the Declaration of Helsinki. Of the 138 healthy controls who were clinically evaluated, 110 (80%) were included. Ten (7%) were excluded due to insufficient tracking feasibility and 18 (13%) were randomly excluded for age- and sex-matching. All patients and controls gave informed consent for inclusion in the registry and local institutional review board approval was obtained.

Clinical assessment included medical history, physical examination, ECG, and transthoracic echocardiography. Studies were performed using commercially available echocardiography systems (Philips). LV hypertrophy was evaluated with the Romhilt-Estes criteria. Pathological Q waves were

defined as duration > 40 ms or depth > 30% R wave in ≥ 2 leads. Maximal wall thickness, left atrial dimension, and LV end-diastolic dimension were measured according to the guidelines.(2, 12)

Standard 4-chamber, 2-chamber, and 3-chamber views were obtained for STE analysis at frame rates of ≥ 50 frames/s. All STE measurements were performed by a single observer using Tomtec Imaging Systems, 2D-CPA, Build No. 1.3.0.91, UnterSchleissheim, Germany. First, the cardiac cycle with the best image quality was selected. Cardiac cycles were defined by the positioning of R-waves. End-systole and end-diastole were defined by the frame with the smallest respectively largest LV diameter, and by determining the aortic valve closure. After manual tracing of 3 by the software-designated points in the LV on an end-systolic frame, the software automatically traced the endocardial border. Tracking feasibility in each apical view was rated with a tracking feasibility score of 3 when, on visual inspection, tracking of all myocardial segments appeared correct; 2 if it failed in one segment; and 1 if tracking was insufficient in ≥ 2 segments.(15) In views with foreshortening the tracking was deemed insufficient for that view. The software automatically divided the LV wall into 18 segments (6 basal, 6 mid-LV, and 6 apical) and calculated the longitudinal strain in all segments individually, after which the appropriate segments were averaged according to the defined region (base, mid-LV, apex) (figure 1). Peak systolic longitudinal strain for each individual segment was defined as the peak value on the curve during the ejection phase of one cardiac cycle. The software calculated the GLS automatically. For intra-observer variability, one reader independently performed STE analysis on 20 cases in an identical fashion on 2 occasions with a two-month period in between the measurements. For inter-observer variability, 2 readers independently performed STE analysis on 20 cases. LV end-diastolic and end-systolic volumes, and LVEF were assessed with the biplane method of the disks using the Tomtec software.

Mortality data were obtained from the civil service register in May 2017, and was complete in 99% of the cases. Cause of death was obtained from the medical chart or the general practitioner. Echocardiographic follow-up was available in 80 mutation carriers. HCM during follow-up was defined as a maximal wall thickness ≥ 13 mm according to the guidelines.(2)

Calculations were performed using SPSS 24 (IBM, Armonk, New York). Normally distributed continuous data are expressed as mean ± standard deviation and non-normally distributed data as median followed by interquartile range. For comparing categorical variables Pearson's chi-square test was used. For comparing continuous variables t-test was used, and Mann-Whitney U in case of non-normally distributed data. For comparison of consecutive echocardiographic data, the paired t-test and in case of non-normally distributed data the Wilcoxon signed rank test were used. All analyses were two-sided; P-values < 0.05 were considered significant. Univariate and multivariate cox regression analyses were performed and expressed as hazard ratio (HR) and 95% confidence interval (CI). Univariate significant variables were entered into the multivariable regression model.

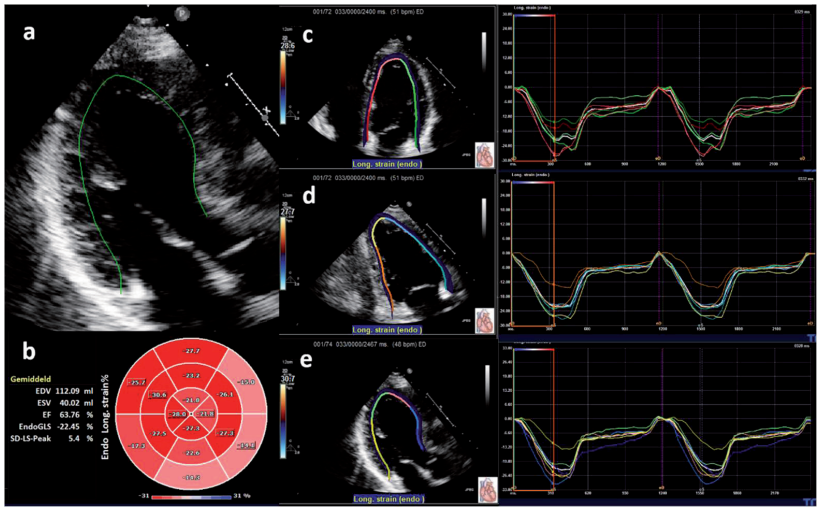

Figure 1. Example of left ventricular longitudinal strain measurements in a mutation carrier. The software automatically divides the left ventricular wall into 18 segments. **(A)** demonstrates the tracing in the 3-chamber view, **(B)** demonstrates the bull's eye in which the segmental values are plotted. On the left of the bull's eye, the end-diastolic volume (EDV), end-systolic volume (ESV), left ventricular ejection fraction (EF), and global longitudinal strain (EndoGLS) values are presented, **(C)** demonstrates the segmental strain curves in the 4-chamber view, **(D)** demonstrates the segmental strain curves in the 2-chamber view, and **(E)** demonstrates the segmental strain curves in the 3-chamber view

RESULTS

Clinical characteristics of the study population are presented in table 1. HCM gene mutation carriers represented 40 different mutations in 11 genes. The *MYBPC3* gene was most frequently affected (77%), followed by the *MYH7* gene (10%). Other genes affected were *FHL1* (3%), *TNNT2* (3%), *MYL2* (1.5%), *ALPK3* (1.5%) *MIB1* (0.8%), *MYH6* (0.8%), *TNNI3* (0.8%), *TPM1* (0.8%), and *MYL3* (0.8%).

The mean tracking feasibility score was highest in the 4-chamber view (2.69±1.21), followed by the 3-chamber view (2.66±0.58), and the 2-chamber view (2.17±0.74). GLS was based on measurements from 3 apical views in 79 (64%) mutation carriers and 86 (78%) controls (p=0.02); the remaining were based on 2 apical views. 2-chamber views were most often excluded (21%) in comparison to 3-chamber (6%) and 4-chamber views (2%). The intra-observer agreement was 0.3±1.2% and the inter-observer agreement was -0.4±1.6%.

Conventional echocardiographic and STE measurements are presented in table 2. GLS was significantly higher in mutation carriers than in controls. When only individuals were assessed in whom all 3 apical views were analyzable, GLS was also higher in the mutation carriers (-21.1±2.3% vs -20.4±2.3%, p=0.04). There were significantly more mutation carriers with a GLS ≥ 24.7% (mean control + 2*standard deviation) than controls (8% vs 2%, p=0.04). There was considerable overlap

between the individual GLS measurements (figure 2). In both mutation carriers and controls, longitudinal strain significantly increased from the base to the apex. When compared to controls, the longitudinal strain was higher in the mid-LV and apex but similar to controls in the base of the LV.

Table 1. Clinical characteristics of the study population

	Mutation carriers (n=120)	Controls (n=110)	P-value
Age (y)	41±13	44±13	0.15
Female gender, n (%)	83 (69)	66 (60)	0.15
Body surface area (m²)	1.9±0.2	1.9±0.2	0.89
Systolic BP (mmHg)	124±18	125±13	0.43
Diastolic BP (mmHg)	76±9	79±8	0.02
Mean arterial pressure (mmHg)	92±11	94±9	0.07
Medical history			
Arterial hypertension, n (%)	9 (8)	0 (0)	0.003
Atrial fibrillation, n (%)	0 (0)	0 (0)	0.50
Diabetes Mellitus, n (%)	2 (2)	0 (0)	0.17
Hypercholesterolemia, n (%)	1 (1)	0 (0)	0.34
Medication			
Antihypertensive, n (%)*	8 (7)	0 (0)	0.01
Statin, n (%)	4 (3)	0 (0)	0.05
Antidiabetic, n (%)	2 (2)	0 (0)	0.17
Antiplatelet, n (%)	2 (2)	0 (0)	0.17
Oral anticoagulation, n (%)	1 (1)	0 (0)	0.34
Electrocardiography			
Sinus rhythm, n (%)	120 (100)	110 (100)	0.50
Heart rate (b/m)	67±13	59±9	<0.001
Romhilt Estes ≥ 4, n (%)	11 (9)	2 (2)	0.02
Pathological Q wave, n (%)	3 (3)	0 (0)	0.10
T wave inversion, n (%)	1 (0.8)	0 (0)	0.34

Data are expressed as mean ± standard deviation or as absolute and %. * includes diuretic (n=3), beta-blocker (n=3), ace-inhibitor (n=2), angiotensin-2-antagonist (n=2), calcium antagonist (n=2)

Table 2. Findings during conventional echocardiography and speckle tracking echocardiography

	Mutation carriers (n=120)	Controls (n=110)	P-value
Conventional echocardiography			
Maximal wall thickness (mm)	9.4±1.7	7.9±1.7	<0.001
Left atrial dimension (mm)	36±4	34±4	0.001
LV end-diastolic dimension (mm)	46±5	46±4	0.39
E-wave (m/s)	0.77±0.17	0.71±0.16	0.01
A-wave (m/s)	0.57±0.17	0.49±0.15	<0.001
E/A ratio	1.49±0.57	1.61±0.67	0.16
Deceleration time (ms)	187±47	189±41	0.73

Table 2. Findings during conventional echocardiography and speckle tracking echocardiography (continued)

	Mutation carriers (n=120)	Controls (n=110)	P-value
Conventional echocardiography			
E' (cm/s)	9.4±2.4	9.6±2.6	0.66
E/E' ratio	8.5±2.1	7.7±2.0	0.004
Diastolic function, n (%)			
Normal, n (%)	97 (89)	89 (86)	0.57
Impaired relaxation, n (%)	8 (7)	5 (5)	0.45
Pseudo normal filling, n (%)	4 (4)	9 (9)	0.12
Restrictive filling, n (%)	0 (0)	0 (0)	0.50
Speckle tracking echocardiography			
Global longitudinal strain (%)	-21.4±2.3	-20.3±2.2	<0.001
Basal anteroseptal strain (%)	-20.1±4.9	-20.3±4.2	0.68
Base longitudinal strain (%)	-20.0±3.3	-20.0±2.6	0.87
Mid-LV longitudinal strain (%)	-21.5±2.5*	-19.9±2.5	<0.001
Apex longitudinal strain (%)	-24.1±3.5*†	-22.1±3.4*†	<0.001
LV ejection fraction (%)	60±5	58±4	<0.001
End-diastolic volume (ml)	107±30	116±26	0.02
End-systolic volume (ml)	42±14	49±13	<0.001

Data are expressed as mean ± standard deviation or absolute and %. LV = left ventricular *=p<0.05 vs base longitudinal strain. †=p<0.05 vs mid-left ventricular longitudinal strain. ‡=P<0.05 vs controls.

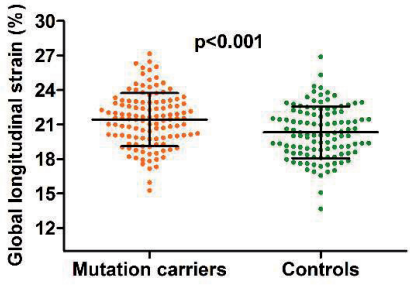

Figure 2. Bee swarm plot of the individual global longitudinal strain measurements in the hypertrophic cardiomyopathy mutation carriers without hypertrophic changes and in the healthy controls

During 6.8±3.1 (range 0.6-12.1) years follow-up, one (0.8%) mutation carrier died of a non-cardiac cause. Echocardiographic follow-up was performed in 80 mutation carriers (supplementary table). During 5.6±2.9 (range 1.3-11.5) years follow-up, 13 (16%) mutation carriers developed HCM. Those who developed HCM had a higher baseline maximal wall thickness and more ECG

abnormalities (table 3). GLS did not differ between the groups. In univariate cox regression analysis, GLS was not a predictor of the development of HCM (HR 0.78; 95% CI 0.60-1.02; p=0.07); neither was LVEF (HR 1.00; 95% CI 0.89-1.12; p=0.95). In multivariable cox regression analysis, age (adjusted HR 1.08, 95% CI 1.02-1.13; p=0.01), pathological Q wave (adjusted HR 8.56; 95% CI 1.63-44.92; p=0.01), and maximal wall thickness (HR 1.94; 95% CI 1.16-3.27; p=0.01) were independent predictors of the development of HCM.

Table 3. Characteristics in hypertrophic cardiomyopathy mutation carriers and the occurrence of hypertrophic cardiomyopathy during follow-up

	HCM DURING FOLLOW-UP (5.6±2.9 years)		
Variable	**YES (n=13)**	**NO (n=67)**	**P-value**
Age (y)	45±18	39±13	0.15
Female sex, n (%)	6 (46)	48 (72)	0.07
Romhilt-Estes ≥ 4, n (%)	2 (15)	5 (8)	0.36
Pathological Q wave, n (%)	2 (15)	1 (2)	0.02
T wave inversion, n (%)	1 (8)	0 (0)	0.02
Maximal wall thickness (mm)	10.6±1.4	9.3±1.8	0.01
Left atrial dimension (mm)	37±5	36±4	0.26
LV end-diastolic diameter (mm)	46±5	46±5	0.89
E wave (m/s)	0.77±0.16	0.77±0.18	0.94
A wave (m/s)	0.57±0.17	0.57±0.17	0.99
E/A ratio	1.49±0.52	1.48±0.58	0.97
Deceleration time (ms)	176±31	180±46	0.81
e' (cm/s)	9.0±2.7	9.4±2.5	0.62
E/e' ratio	8.8±1.7	8.5±2.1	0.58
Abnormal diastolic function, n (%)	3 (23)	7 (11)	0.25
Global longitudinal strain (%)	-21.4±2.5	-21.5±2.3	0.81
Basal longitudinal strain (%)	-20.4±3.0	-20.3±3.5	0.93
Mid-LV longitudinal strain (%)	-21.2±2.9	-21.7±2.5	0.53
Apex longitudinal strain (%)	-25.4±3.1	-24.0±3.6	0.21
LV ejection fraction (%)	63±5	60±5	0.08

Data are expressed as mean ± standard deviation or absolute and %. ECG = electrocardiography; LV = left ventricular; HCM = hypertrophic cardiomyopathy.

DISCUSSION

The main findings of the study are: (1) GLS is increased in mutation carriers, and (2) GLS is not predictive of the development of HCM during a 5-year follow-up, in contrast to age, pathological Q wave, and maximal wall thickness.

In patients with HCM, multiple strain imaging studies have demonstrated an impaired longitudinal systolic function while LVEF is normal.(4, 9, 11, 16, 17) In mutation carriers without

hypertrophy, results are contradictory. Some tissue Doppler and strain imaging studies reported lower myocardial longitudinal velocities and deformation in mutation carriers(8, 18, 19) while other studies observed no difference.(9-11, 20, 21) In the past assessment of GLS was hampered by a lack of imaging standard. In this study, the Tomtec Imaging Systems 2D-CPA Build No. 1.3.0.91, was used which was developed after publication of the consensus document of the EACVI/ASE/Industry Task force.(22) This allows the results to be reproducible by others and directly comparable as numbers. The current study demonstrates that GLS is increased in mutation carriers. Previously, Ho et al. similarly observed a higher GLS in *MYH7* mutation carriers(11), and De et al. reported higher tissue doppler derived systolic velocities implying supranormal myocardial contractility.(10) In the current study, the GLS difference between mutation carriers and controls was statistically significant. However, the clinical relevance of this difference is not sufficient in order to use GLS as discriminating parameter, because the difference was small (~1%) and there was a large overlap of the measurements. Similar to the conclusion of Yiu et al(9), this suggests that the assessment of GLS is not helpful for the identification of mutation carriers when genetic testing is not available.

There are multiple factors which may cause an increased GLS in HCM gene mutation carriers without hypertrophy. In line with previous studies(14, 23), we observed an increasing longitudinal strain from the base of the LV towards the apex. In comparison with controls, strain was increased in the mid-LV and the apex but not in the base of the LV. This indicates a regional variation in the LV contraction pattern. GLS may be increased as a compensatory mechanism due to subclinical dysfunction in the base of the LV. Previous studies have reported a reduced septal strain in mutation carriers.(9, 10) We analyzed the basal anteroseptal wall separately but found no difference between mutation carriers and controls in this region. A reduced systolic function in mutation carriers would suggest that the myocardium is diseased (i.e. coronary arteriole remodeling and muscle fiber disarray). Currently, there is no data regarding the histopathology of the myocardium in mutation carriers. However, in vivo mouse models and in vivo human studies have demonstrated a disturbance in the myocardial energy efficiency in mutation carriers without hypertrophic changes.(24, 25) Changes in myocardial efficiency may represent a primary trigger for the development of the HCM phenotype. In the future, gene-specific metabolic treatment may improve myocardial energetics and slow the progression to heart failure.(26)

Another factor that might explain the increased GLS is mutation-induced cardiomyocyte hypercontractility leading to enhanced systolic function. Biophysical studies on isolated sarcomeric protein and myofilaments have demonstrated that HCM mutations increase contractility, evident from a higher actin sliding velocity, higher actomyosin ATPase activity, and increased myofilament Ca^{2+}-sensitivity resulting in a higher cardiomyocyte force at physiologic [Ca^{2+}].(27, 28) A study that used myectomy samples from HCM patients harboring sarcomere mutations demonstrated the opposite, namely a reduced force.(29) Due to the presence of cellular remodeling in tissues obtained during myectomy, it is difficult to interpret the primary consequences of the mutation. In patients with *MYH7*

mutations the force generation was reduced irrespective of cellular remodeling, suggesting these mutations directly cause hypocontractility.(29) Whether HCM gene mutations cause hyper- or hypocontractility of the cardiomyocyte is subject to ongoing investigations.(28) Hypothetically, HCM mutations may initially cause hypercontractility which then could lead to exhaustion of the cardiomyocyte in a later disease stage. Future studies exploring the temporal relation of GLS at baseline and at follow-up in mutation carriers might shed more light on this issue.

The current study is the first to evaluate the predictive value of GLS for the development of HCM, and report no clear prognostic value during a 5-year follow-up. Nevertheless, GLS trended to be lower in subjects who developed HCM during follow-up. Future studies, preferably prospective multicenter studies with larger patient numbers are necessary to evaluate the precise role of GLS in this specific patient cohort. A multivariate analysis demonstrated that age, pathological Q waves and maximal wall thickness were independent predictors of HCM during follow-up. The finding that advancing age is predictive supports current recommendations to perform cautionary long-term evaluation of mutation carriers without HCM.(2) The clinical utility of pathological Q wave is probably limited, because 11 out of 13 subjects who developed HCM had no pathological Q waves at initial evaluation. The association between maximal wall thickness and the development of HCM suggests extra attention for mild abnormalities indicative of HCM would be beneficial in cases with borderline wall thickness. However, the clinical significance of these mild abnormalities is probably limited.(2) This is also demonstrated by the excellent prognosis in this cohort (no cardiac deaths in 120 mutation carriers during 6.8±3.1 years follow-up). Numerous studies have reported impaired diastolic indices in mutation carriers, suggesting diastolic dysfunction is an early phenotypic marker of HCM.(9, 10, 18-21) Ho et al. reported lower baseline E' velocities in subjects who developed HCM during follow-up in comparison to subjects who did not develop HCM.(30) According to our data, diastolic indices including E' had no predictive value for the development of HCM. This may be due to the fact that the mean E' was relatively low in the whole group of mutation carriers, which is probably related to aging. In this study, 9 HCM mutation carriers had a history of arterial hypertension. Since the blood pressure was sufficiently controlled using antihypertensive medication, it is unlikely that the LV wall thickening and diastolic impairment in the HCM mutation carriers were induced by arterial hypertension.

This study has several limitations. Firstly, the study population was relatively small, and echocardiographic follow-up was not performed in controls and a proportion (33%) of the mutation carriers. Secondly, GLS was based on measurements from only 2 apical views in a significant proportion of the subjects (36% of mutation carriers; 22% of controls), which was caused by insufficient image quality. Third, previous studies mostly used GE software which provides mid-myocardial GLS values. The software we used only provides endocardial GLS values limiting comparability between the studies. And finally, the majority of individuals had a mutation in the

myosin-binding protein C gene (77%) and therefore results may not be applicable to carriers of other mutations.

CONCLUSION

GLS is increased in HCM mutation carriers without hypertrophic changes as compared to normal individuals. GLS provided no clear prognostic value for the development of HCM during follow-up, in contrast to age, pathological Q waves and maximal wall thickness.

FUNDING

This work was supported by the Netherlands Cardiovascular Research Initiative CVON2014-40 DOSIS, an initiative with support from the Dutch Heart Foundation.

Conflict of Interest: none declared.

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CHAPTER 6

Three-dimensional echocardiography for the assessment of left ventricular geometry and papillary muscle morphology in hypertrophic cardiomyopathy

Mustafa Erden
Hannah G. van Velzen
Myrthe E. Menting
Annemien E. van den Bosch
Claire Ben Ren
Michelle Michels
Wim B. Vletter
Ron T. Van Domburg
Arend F.L. Schinkel

J Ultrasound. 2018 Jan 6.

ABSTRACT

Background

Hypertrophic cardiomyopathy (HC) is characterized by left ventricular (LV) hypertrophy and associated with papillary muscle (PM) abnormalities. The aim of this study was to evaluate the utility of three-dimensional echocardiography (3DE) for the geometric assessment of LV hypertrophy and PM morphology.

Hypothesis

3DE allows assessment of PM morphology and LV hypertrophy in HC

Methods

The study included 24 patients with an established diagnosis of HC and 31 healthy controls. 3DE was performed using an iE33 or EPIQ 7C ultrasound system with an X5-1 transducer. QLAB software was used for the 3D analysis of LV wall thickness (LVWT) and PM morphology and hypertrophy; the number and cross-sectional area (CSA) of anterolateral and posteromedial PMs; and the presence of bifid or accessory PMs.

Results

Patients with HC had a larger LVWT compared to controls in all segments ($P<0.001$), and LVWT was largest in the mid-ventricular septal segment (2.12±0.68 cm). The maximum LVWT followed a spiral pattern from the LV base to the apex. The CSA of both anterolateral and posteromedial PMs was larger in patients with HC than in controls (1.92 vs. 1.15 cm^2; $P=0.001$ and 1.46 vs. 1.08 cm^2; $P=0.033$ respectively). The CSA of the posteromedial PM was larger in patients with LVOT obstruction than in those without (2.64 vs 1.16 cm^2, $p=0.021$).

Conclusions

3DE allows the assessment of LV geometry and PM abnormalities in patients with HC. 3DE demonstrated that the maximum hypertrophy was variable and generally located in a spiral from the LV base to the apex.

INTRODUCTION

Hypertrophic cardiomyopathy (HC) is the most common inherited cardiac disease, with an estimated prevalence of 1 in 500(1). HC is characterized by a broad clinical and morphological spectrum including left ventricular (LV) hypertrophy and abnormal LV papillary muscle (PM) morphology and thickness(2-7). Currently, two-dimensional echocardiography (2DE) is the most frequently used imaging modality for the diagnosis and follow-up of patients with HC.(8, 9) Clearly, 2DE has inherent limitations that may be overcome by three-dimensional echocardiography (3DE). There are indications that 3DE allows a better geometric assessment of PM morphology and LV hypertrophy, and may provide information that alters clinical decision making.(9, 10) The aim of this study was to assess the utility of 3DE for the assessment of LV geometry and PM abnormalities in patients with HC as compared to healthy controls. Additionally the relation between PM abnormalities and left ventricular outflow tract (LVOT) obstruction was studied.

METHODS

Patient population and study protocol

This study included 24 consecutive patients with an established diagnosis of HC and 31 age-matched healthy controls. All patients underwent standard 2DE in conjunction with 3DE. Only patients with sufficient image quality were included in this study. The diagnosis of HC was based on a LV wall thickness (LVWT) ≥15 mm, that was not explained by loading conditions. LVOT obstruction was defined as a resting or provocable gradient ≥30 mmHg assessed by Doppler echocardiography. Patients with HC linked to Noonan's syndrome, Fabry's disease, or congenital heart defects were excluded. Approval from the local ethics committee was obtained. The study was conducted according to the Declaration of Helsinki. All subjects consented participation in this study.

Echocardiography

3DE was performed using an iE33 or EPIQ 7C ultrasound system (Philips, Best, The Netherlands) with a X5-1t matrix-array transducer. Electrocardiographically gated full volume datasets of the LV (built from 4 subvolumes) were acquired in the parasternal long-axis and apical views during breath-hold. Care was taken to include the complete LV including the PM, and septum within the imaging volume throughout the acquisition by adjusting the lateral and elevation widths of the acquisition sector. Each full-volume dataset was digitally stored and exported to QLAB 3DQA software (Philips, Best, The Netherlands) for offline analysis.

The full volume LV 3DE dataset was displayed as 3 orthogonal multiplanar reconstruction views. For the analysis of end-diastolic LVWT, a 16-segment model was used, according to EAE/ASE recommendations.(11) The short-axis end-diastolic frames that provided the best visualization of the endocardial and epicardial borders were selected. The LVWT was assessed at the center of each

myocardial segment from the leading endocardial edge to the leading epicardial edge. The geometric pattern of LV hypertrophy was determined by the location and extent of hypertrophy at basal, midventricular and apical level. For the PM analysis, the anterolateral PM (ALPM) and posteromedial PM (PPM) were identified. Subsequently, morphology of the PMs was assessed: individual PMs were assessed using long and short axis images on different levels to identify bifid, double bifid and accessory PMs. The cross-sectional area (CSA) of each PM was measured in the short-axis view at midventricular level, and total CSA was calculated for the ALPM and PPM.

Statistical analyses

Statistical analyses were performed using SPSS 21 (IBM, Armonk, New York). Continuous variables are reported as mean ± standard deviation. Categorical variables are expressed as number (%). Mann-Whitney *U* test was used to compare the continuous variables and the Chi-square test was used to compare categorical variables. A p value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

The clinical characteristics of patients with HC and controls are summarized in table 1. The age of the patients with HC and controls was comparable (35±14 vs 33±7 y, p=0.173), and the patient group included significantly more men (88% vs 42%, p<0.05).

Table 1: Characteristics of patients with hypertrophic cardiomyopathy (HC) and healthy controls

Variable	Patients with HC (n=24)	Controls (n=31)	P-value
Age (years)	35 ± 14	33 ± 7	0.173
Men	21 (88%)	13 (42%)	<0.05
BMI (kg/m^2)	23 ± 5	23 ± 2	
BSA (m^2)	1.83 ± 0.32	1.85 ± 0.18	
Angina	3 (13%)	0	
Dyspnea	6 (25%)	0	
NYHA class ≥ II	6 (25%)	0	
Palpitations	6 (25%)	0	
LVOT obstruction (≥30 mmHg)	6 (25%)	0	
Pathogenic mutations			
Myosin-binding protein C	8 (33%)	-	
Myosin heavy chain	2 (8%)	-	
Troponin I	1 (4%)	-	
Mitochondrial DNA	1 (4%)	-	

Data are presented as mean ± standard deviation or number (%). BSA = body surface area, LVOT = left ventricular outflow tract, NYHA = New York Heart Association functional class.

Echocardiographic findings

Measurements of LVWT obtained from 3DE are presented in table 2. Overall, LVWT in all 16 segments was larger in patients with HC than in controls ($p<0.001$). The maximal LVWT in patients with HC at basal LV level was observed in the inferoseptal segments (1.87 ± 0.62 cm) and anteroseptal segments (1.87 ± 0.38 cm); at midventricular level in the anteroseptal segments (2.18 ± 0.67 cm); and at apical level in the lateral segment (1.76 ± 0.55 cm). The segments with maximal hypertrophy formed a spiral pattern from the base to the apex of the LV.

Table 2: Left ventricular wall thickness (LVWT) assessed by three-dimensional echocardiography (3DE)

Variable		Patients with HC (n = 24)	Controls (n = 31)	P-value
Basal level	Anterior	1.43 ± 0.37	0.84 ± 0.12	< 0.001
	Anteroseptal	1.87 ± 0.38	0.86 ± 0.21	< 0.001
	Inferoseptal	1.87 ± 0.62	0.85 ± 0.16	< 0.001
	Inferior	1.41 ± 0.49	0.80 ± 0.15	< 0.001
	Inferolateral	1.25 ± 0.26	0.76 ± 0.15	< 0.001
	Anterolateral	1.25 ± 0.31	0.73 ± 0.14	< 0.001
Midventricular level	Anterior	1.54 ± 0.36	0.86 ± 0.17	< 0.001
	Anteroseptal	2.18 ± 0.67	0.87 ± 0.20	< 0.001
	Inferoseptal	2.17 ± 0.73	0.89 ± 0.14	< 0.001
	Inferior	1.47 ± 0.42	0.85 ± 0.16	< 0.001
	Inferolateral	1.53 ± 0.51	0.75 ± 0.14	< 0.001
	Anterolateral	1.46 ± 0.46	0.73 ± 0.15	< 0.001
Apical level	Anterior	1.52 ± 0.28	1.03 ± 0.43	< 0.001
	Septal	1.69 ± 0.71	1.01 ± 0.23	< 0.001
	Inferior	1.49 ± 0.45	0.85 ± 0.21	< 0.001
	Lateral	1.76 ± 0.55	0.94 ± 0.35	< 0.001

Data are presented as mean ± standard deviation in centimeter. HC = hypertrophic cardiomyopathy

Measurements of PMs obtained from 3DE are presented in table 3. The total ALPM and total PPM CSA were significantly larger in patients with HC than in controls (1.92 ± 0.81 vs. 1.15 ± 0.47 cm^2; P = 0.001 and 1.46 ± 0.62 vs. 1.08 ± 0.37 cm^2; P = 0.031 respectively). Figure 1 demonstrates an example of the 3DE analysis of the PM area. There was no significant difference in the number of ALPMs and PPMs between patients with HC and controls. Moreover, bifid and accessory PMs were not observed more frequently in patients with HC than in controls. Figure 2 shows an example of a patient with HC and a bifid PM and figure 3 demonstrates an example of an accessory PM visualized by 3DE.

Table 3. Papillary muscle (PM) evaluated by three-dimensional echocardiography (3DE)

Variable		Patients with HC (n = 24)	Controls (n = 31)	P-value
ALPM	Number	1.7 ± 0.5	1.5 ± 0.6	0.172
	CSA (cm^2)	1.92 ± 0.81	1.15 ± 0.47	0.001
	Bifid appearance	3 (13%)	2 (6%)	0.352
PPM	Number	1.9 ± 0.4	2.1 ± 0.4	0.288
	CSA (cm^2)	1.46 ± 0.62	1.08 ± 0.37	0.031
	Bifid appearance	3 (13%)	1 (3%)	0.144
Accessory PM		5 (21%)	3 (10%)	0.276

Data are presented as mean ± standard deviation or number (%). ALPM = anterolateral papillary muscle, CSA = cross-sectional area, HC = hypertrophic cardiomyopathy, PM = papillary muscle, PPM = posteromedial papillary muscle

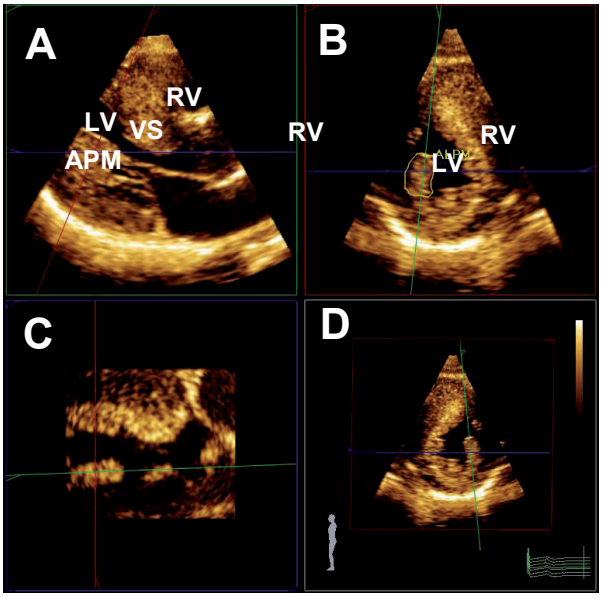

Figure 1. 3DE assessment of anterolateral papillary muscle (ALPM) cross-sectional area in a patient with HC. **a** Long-axis plane; **b** Short-axis plane; **c** Coronal plane; **d** Alternative real time 3D. *ALPM* anterolateral papillary muscle, *LV* left ventricle, *RV* right ventricle, *VS* ventricular septum

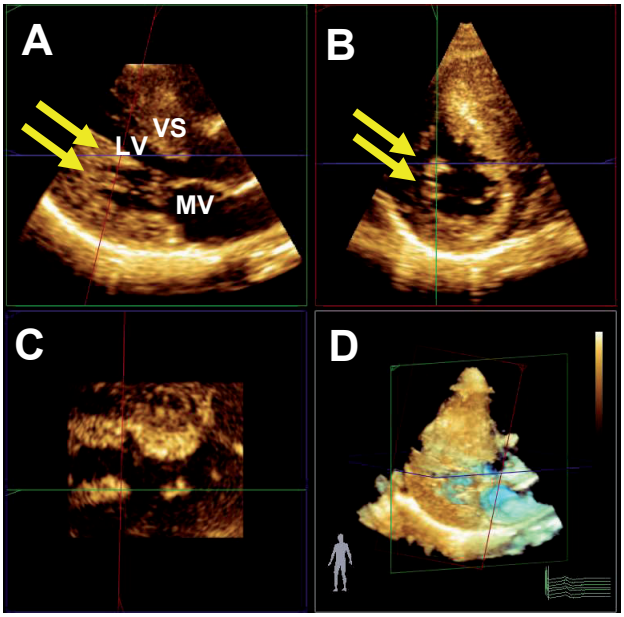

Figure 2. 3DE assessment of a bifid PM in a patient with HC (yellow arrows). **a** Long-axis plane, **b** Short-axis plane; **c** Coronal plane; **d** Real time 3D. *LV* left ventricle, *MV* mitral valve, *VS* ventricular septum

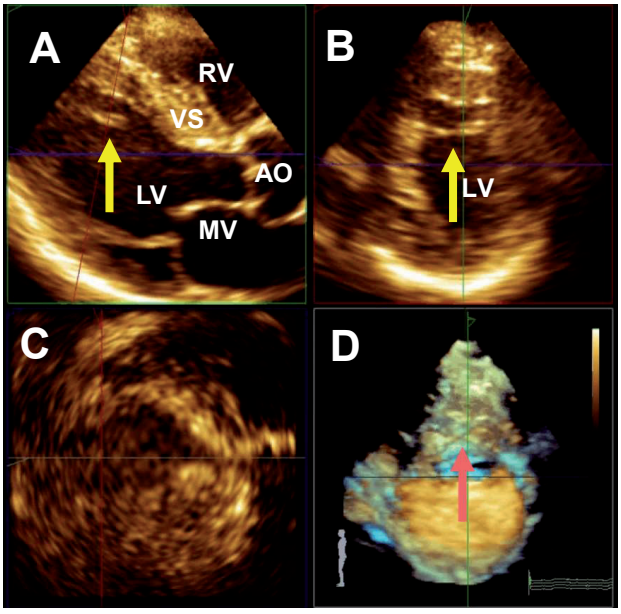

Figure 3. 3DE assessment of an accessory papillary muscle in a patient with HC (yellow and red arrows). **a** Long-axis plane; **b** Short-axis plane; **c** Coronal Plane; **d** Real time 3D. *Ao*ascending aorta, *LV* left ventricle, *MV* mitral valve, *RV* right ventricle, *VS* ventricular septum

In the group of 24 patients with HC, the median maximal LVOT gradient was 7 [IQR 4-30] mmHg. Six (25%) patients with HC demonstrated significant LVOT obstruction (≥30 mmHg). The PPM CSA was larger in patients with LVOT obstruction than in patients without (median 2.64 vs 1.16 cm^2; p=0.021). The ALPM CSA was not different between patients with and without LVOT obstruction (2.00 cm^2 vs 1.92 cm^2, p=0.88).

DISCUSSION

This study demonstrates the utility of 3DE for the assessment of the geometric pattern of LV hypertrophy and PM morphology in patients with HC. Maximal LV hypertrophy was present at basal level in the infero- and anteroseptal segments, at midventricular level in the anteroseptal segments, and at apical level in the lateral segments. The hypertrophy in patients with HC followed a spiral pattern from the LV base to the apex. 3DE allowed evaluation of the PM morphology and hypertrophy, and showed that patients with HC had significantly hypertrophied PMs as compared to healthy controls. Moreover, the amount of hypertrophy of the PPM was related to significant LVOT obstruction.

Previous studies have demonstrated that 3DE may be superior to 2DE, and has a comparable accuracy to that of CMR for determining LV volumes, mass, and ejection fraction in various patient groups (10, 12-14). Shimada and Shiota(15) performed a meta-analysis of the accuracy of 3DE for the measurement of LV mass, including 18 articles with 25 studies. The meta-analysis showed that a significant improvement of the accuracy of the 3DE technique has been achieved over time. The improved accuracy in most recent studies on 3DE is probably related to an improvement in the temporal and spatial resolution of the probe, and a more developed data analysis method using updated software. The meta-analysis included 2 studies on the use of 3DE for the assessment of LV mass in patients with HC(10, 16). Oe et al.(16) studied the accuracy of 3DE in 21 patients with LV hypertrophy (17 with HC, and 4 patients with hypertensive heart disease) using CMR as a reference technique. LV mass was estimated accurately and easily by 3DE, whereas LV mass assessed by 2DE correlated less well with CMR. Bicudo et al.(10) studied 20 patients with HC who underwent 2DE, 3DE and CMR. In that study, 3DE had a better performance than 2DE for the evaluation of LV hypertrophy, volumes, ejection fraction, and mass when compared to CMR. The current study extends the findings from these previous studies and demonstrates the utility of 3DE in patients with HC for the assessment of the geometric pattern of LV hypertrophy and PM morphology and hypertrophy. Hence, 3DE allows both the assessment of global LV hypertrophy (as measured by LV mass) as well as local hypertrophy (as measured by LV segmental wall thickness and PM CSA).

In this study, the amount and location of LV hypertrophy varied among patients with HC. Generally, a longitudinal spiral trajectory of the hypertrophy was observed. This is in line with a recent study by Florian et al.(17), who studied the geometry of hypertrophy on CMR in 132 patients with HC. Using 3D analysis, the majority of patients exhibited a spiral pattern of hypertrophy. As in the present

study, the magnitude of hypertrophy and rotation was variable. This spiral distribution of the hypertrophy may be caused by predominant involvement of the subendocardial layers of the myocardium. These findings are in agreement with necropsy studies in patients who died from HC, showing that the greatest cellular hypertrophy was in the layers closest to the cavity.(18) Likely, the distribution of hypertrophy is related to the stress distribution in the LV endocardium and sub endocardium.

Approximately 30% to 60% of the patients with HC has a resting or provable LVOT obstruction, which may cause symptoms(19). LVOT obstruction in patients with HC may be caused by several factors. Clearly, basal septal hypertrophy and systolic anterior movement of the mitral valve leaflet may cause LVOT obstruction. But also anatomical variations and hypertrophy of the PM may contribute.(20) Anatomical variations such as anterior displacement of the PM, the presence of a double bifid PM, anomalous insertion of the PM onto the anterior mitral valve leaflet, or fusion of the PM and the septum or LV free wall, may have hemodynamic consequences.

Traditionally, 2DE has been used to assess the factors causing LVOT obstruction, but additional information may be obtained by 3DE and CMR. Our study demonstrates that the PPM CSA was larger in HC patients with LVOT obstruction than in those without. Previous studies have reported similar findings. Harrigan et al.(21) obtained CMR images in 201 patients with HC and 43 controls, in order to characterize PM morphology. PM mass index was significantly increased in patients with HC compared with controls, and PM hypertrophy was most severe in patients with LVOT obstruction. Furthermore, Kwon et al.(22) studied 56 patients with HC and 30 controls using CMR. The presence of PM abnormalities on CMR was correlated with resting LVOT gradients obtained by Doppler echocardiography. Patients with HC and abnormal PMs had significantly higher resting LVOT gradients, independent of septal LVWT. The identification of PM abnormalities may be relevant to understand the pathophysiology of LVOT obstruction and could have therapeutic consequences in patients with HC and significant LVOT obstruction who are considered for septal reduction surgery. Kwon et al(23) have suggested that symptomatic patients with HC and significant LVOT obstruction with abnormal PM morphology may need surgical PM reorientation instead of or combined with standard surgical procedures. According to a large surgical series reported by Minakata et al(6), patients with HC associated with anomalous PMs or chordae, including accessory PMs, can be successfully treated by surgical relief of morphological anomalies and an extended septal myectomy without mitral valve replacement. Finally, the utility of 3DE was also shown for the measurement of mitral leaflet surface area and subvalvular geometry in patients with HC. (24) Kim et al. studied 47 patients with HC and 32 controls using 3DE, and demonstrated that abnormal PM-mitral valve geometry assessed by 3DE may provide reasonable new targets for individualized surgical intervention.

This study has several limitations. First, the study population was relatively small, which may have affected the results. Second, only patients with sufficient image quality were included in the

study. Third, the study was conducted in a referral center for patients with HC, and may have therefore caused a bias. Fourth, the patient group included significantly more men than the control group. Finally, research is needed to integrate the findings from 3DE imaging in clinical decision making in order to improve outcome of patients with HC.

In conclusion, 3DE allows the assessment of LV geometry and PM abnormalities in patients with HC. 3DE demonstrated that the maximum hypertrophy was variable and generally located in a spiral from the LV base to the apex. A better visualization of these structures may improve the understanding of the pathophysiology and may influence the surgical treatment of left ventricular outflow tract obstruction in these patients.

Conflict of interest: none declared.

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PART III

Clinical aspects of hypertrophic cardiomyopathy

CHAPTER 7

Device-detected atrial fibrillation and long-term outcomes in patients with hypertrophic cardiomyopathy

Hannah G. van Velzen
Dominic A.M.J. Theuns
Sing-Chien Yap
Michelle Michels
Arend F.L. Schinkel

Am J Cardiol. 2017 Jan 1;119(1):100-105.

ABSTRACT

Atrial fibrillation (AF) is a common complication of hypertrophic cardiomyopathy (HC), and associated with adverse clinical outcomes, such as thromboembolisms. Cardiac implantable electronic devices (CIED) enable early detection of AF. The aim of this study was to assess the incidence of device-detected AF and the impact on long-term outcomes in patients with HC. The cohort consisted of 132 patients (63% male, mean age 52±16 years) with a diagnosis of HC and a CIED. Follow-up started at the date of CIED implantation to assess the incidence of device-detected AF. Patients with persistent AF at the time of implantation were excluded from the analysis of the incidence of AF. End points were all-cause and cardiac mortality, device-detected AF, and thromboembolism (stroke, transient ischemic attack or peripheral arterial embolism). In total, 114 patients were in sinus rhythm at time of CIED implantation. During the median 2.8 [1.2-5.4] year follow-up, device-detected AF occurred in 29 (25%) patients, resulting in an annual incidence of 7.0%/year. Device-detected AF led to a change in the clinical management in 22 (76%) patients. Anticoagulation therapy was started in 13 (45%), anti-arrhythmic medication in 9 (31%), and 8 (28%) patients underwent electrical cardioversion. Six (5%) patients suffered a thromboembolic complication. All-cause mortality was 27 (20%), and cardiac mortality was 21 (16%). A history of AF at time of implantation was an independent predictor of cardiac death (HR 4.7, p=0.003). In conclusion, the incidence of device-detected AF in patients with HC was 7.0%/year, leading to a change in clinical management in the majority (76%) of cases in order to reduce the risk of thromboembolic complications. These findings stress the importance of AF detection in HC and advocate vigilant interrogation of the device.

INTRODUCTION

Hypertrophic cardiomyopathy (HC) is the most prevalent genetic cardiac disease.(1) Atrial fibrillation (AF) is a common complication of HC, and associated with adverse clinical outcomes, such as stroke and heart failure.(2, 3) Not just symptomatic, but also subclinical AF is associated with a substantially increased risk of stroke.(4) Since the incidence of stroke is high in patients with HC and AF, current guidelines recommend lifelong treatment with oral anticoagulants (OAC), even when sinus rhythm is restored.(5) The detection of AF may be challenging, because AF is often asymptomatic, and the diagnosis may be missed using traditional intermittent monitoring strategies.(6) A subset of patients with HC has a cardiac implantable electronic device (CIED), allowing the detection of AF. This patient group frequently has advanced disease and is at increased risk of developing AF.(2, 7, 8) The aim of this study was to assess the incidence of device-detected AF and the impact on long-term outcomes in patients with HC.

METHODS

The study population consisted of 132 consecutive patients with HC who received a CIED (implantable cardioverter defibrillator [ICD] in 116 and a pacemaker [PM] in 16 patients) between 1988 and 2015. The study protocol conforms to the Declaration of Helsinki.(9) The study was approved by the institutional review board and all patients gave informed consent. Each patient had an established diagnosis of HC, based on unexplained left ventricular (LV) hypertrophy of ≥ 15 mm. Patients with HC linked to Noonan's syndrome, Fabry's disease, mitochondrial disease, or congenital heart defects were excluded.

The indication for CIED implantation was made by a team consisting of ≥ 1 electrophysiologist and ≥ 1 cardiologist dedicated to the care of patients with HC. ICDs were implanted for primary prevention of sudden cardiac death (SCD) based on the presence of established major risk factors for SCD, or for secondary prevention of SCD in patients with a history of ventricular fibrillation or sustained VT.(5, 10) PMs were implanted in patients with a third or second degree type 2 atrioventricular block and in patients with symptomatic sinus node disease.(11) The selection of the CIED was made according to the practice guidelines.(5, 10, 11) Patients received single or dual-chamber ICDs or PMs, or biventricular ICDs if cardiac resynchronization therapy (CRT) was indicated, with transvenous lead systems. The rate cutoff for detection of ventricular fibrillation or ventricular tachycardia and activation of antitachycardia pacing was set at the discretion of the treating electrophysiologist. A select group of patients received a subcutaneous ICD system in case no bradycardia or tachycardia pacing, or CRT was indicated.

Device-detected AF was defined as AF (paroxysmal or persistent), which was detected by interrogation of the device or using the home-monitoring function of the device during follow-up. Patients with persistent AF at the time of CIED implantation were excluded from the analysis of the

incidence of AF. An electrophysiologist evaluated all high-rate episodes (>180 beats per minute) lasting more than 30 seconds(12, 13) documented by intracardiac electrograms. AF detected only by device diagnostics (without electrograms) was not considered for the present study. Inappropriate ICD intervention triggered by AF was also considered device-detected AF. Atrial high-rate episodes were classified as AF, atrial flutter, atrial tachycardia, and sinus tachycardia. AF was assumed to occur if the atrial electrogram showed a changing morphology. The diagnosis of atrial flutter was based on regular AA intervals and no changes in morphology of the atrial electrogram. The prerequisite of sinus and atrial tachycardia was an atrial electrogram preceding the ventricular electrogram. Sinus tachycardia was diagnosed if the ventricular rhythm showed a gradual increase in heart rate with unchanged morphology of the atrial and ventricular electrogram. The diagnosis of atrial tachycardia was based on a sudden increase of the ventricular rate and a change in morpholology of the atrial electrogram. All electrocardiograms (ECG), ambulatory ECG monitoring results, telemetry reports and hospital patient records were investigated for AF in all patients with single-chamber ICDs and PMs, and subcutaneous ICDs. The decision to start OAC was made at the discretion of the treating physician.

Follow-up started at the time of CIED implantation. End points were all-cause and cardiac mortality, device-detected AF, thromboembolism (defined as stroke, transient ischemic attack or peripheral arterial embolism), and appropriate and inappropriate ICD intervention. End points were retrieved from hospital patient records, from civil service population registers, and from information provided by general practitioners. Cardiac mortality was defined as SCD, death from stroke, death from end-stage heart failure and heart transplantation.

SPSS version 21 (IBM, Armonk, NY) was used for statistical analyses. Normality of the distribution was assessed using the Shapiro-Wilks test. Normally distributed continuous data are expressed as mean ± standard deviation and non-normally distributed data as median [interquartile range]. For comparing variables either χ^2-test or unpaired t-test were used, for categorical and continuous data respectively. The Mann-Whitney U test was used in case the data was non-normally distributed. Estimated survival and actuarial event-free rates from device-detected AF were calculated according to the Kaplan-Meier method. Patients who underwent heart transplantation were censored on the day of transplantation. Cox regression analysis was used to determine predictors of outcome. Variables were selected for multivariable analysis if univariate P value was <0.10. Variables were expressed as hazard ratio (HR) with 95% confidence interval. $P < .05$ was considered statistically significant.

RESULTS

A flowchart of the study population is presented in figure 1. Overall, 132 patients (63% male) were included in the study. The majority (116, 87%) had an ICD and the remaining 16 (13%) patients had a PM. The indication for ICD therapy was primary prevention in 99 (85%) patients and secondary

prevention in 17 (15%). The implanted ICD systems were: DDD (45; 39%), VVI (39; 34%), VDD (16; 14%), subcutaneous ICD (12; 10%), and CRT (4; 3%). The implanted PM systems were: DDD (10, 65%) and VVI (6, 35%). The baseline characteristics of the study population are presented in table 1. The mean age at CIED implantation was 52±16 (range 8-85) years, and 21 (16%) patients were in NYHA class ≥ III. Twenty-four (18%) patients had a history of surgical septal myectomy, and 23 (17%) alcohol septal ablation. A history of stroke was noted in 10 (8%) patients and transient ischemic attack in 4 (3%) patients.

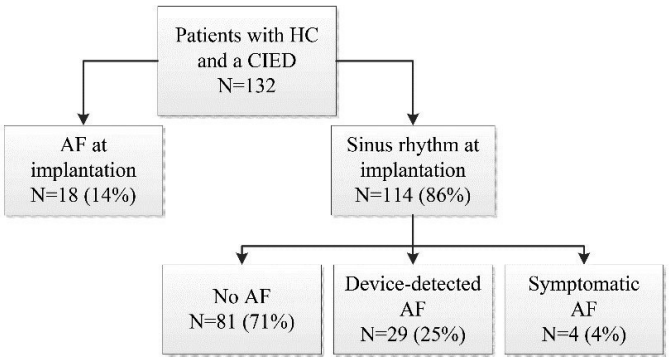

Figure 1. Flowchart of the study population.

In total, 114 (86%) patients were in sinus rhythm at time of implantation. Twenty-two (17%) of these patients had a history of paroxysmal AF, 18 of whom (78%) were using OAC at the time of implantation. During the median follow-up period of 2.8 [1.2-5.4] years, 33 (29%) patients had AF. Twenty-nine (88%) of cases were device-detected. The systems that detected AF were the DDD-ICD (n=17), the DDD-PM (n=5), the VDD-ICD (n=2), and the CRT (n=2), and 3 cases of AF were detected by inappropriate ICD intervention because of AF (2 had a VVI-ICD and 1 had a subcutaneous ICD). Four (12%) cases of AF were not device-detected (all had VVI systems), but were detected through evaluation of symptoms with ECG. In the 29 patients with device-detected AF, 10 (34%) developed permanent AF, and 19 (66%) had a median of 3 [interquartile range 1-5.5] paroxysms of AF during follow-up, with a median duration of 188 [interquartile range 3.5-930] minutes. From the 18 patients with AF at implant, 15 (78%) had permanent AF. The other 3 (22%) converted to sinus rhythm during follow-up (1 after pulmonary venous isolation, 1 after defibrillator threshold testing, 1 spontaneously).

Table 1. Baseline characteristics of the study population

Variable	Total (n=132)	AF During Follow-up YES	NO	P-value
Age (years)	52±16	58±15	48±15	<0.001
Men	83 (63%)	17 (52%)	57 (70%)	0.056
NYHA class ≥ II	59 (45%)	19 (58%)	26 (32%)	0.012
NYHA class ≥ III	21 (16%)	3 (9%)	12 (15%)	0.412
LV ejection fraction	48%±12%	41%±12%	51%±11%	0.008
Left atrial size, mm	48±10	49±7	44±7	0.001
Hypertension	38 (29%)	10 (30%)	20 (25%)	0.537
Stroke	10 (8%)	2 (6%)	4 (5%)	0.808
Transient ischemic attack	4 (3%)	1 (3%)	1 (1%)	0.508
Coronary artery disease	10 (8%)	2 (6%)	7 (9%)	0.643
Diabetes Mellitus	9 (7%)	4 (12%)	4 (5%)	0.173
Surgical septal myectomy	24 (18%)	10 (30%)	9 (11%)	0.013
Alcohol septal ablation	23 (17%)	7 (21%)	15 (19%)	0.741
Vitamin K antagonist	43 (33%)	16 (49%)	9 (11%)	<0.001
Aspirin	27 (21%)	5 (15%)	21 (26%)	0.214
Beta blocker	72 (55%)	18 (55%)	45 (56%)	0.922
Calcium antagonist	23 (17%)	4 (12%)	16 (21%)	0.281
Digoxin	5 (4%)	1 (3%)	0	0.116
Sotalol	11 (8%)	6 (18%)	3 (4%)	0.009
Amiodarone	16 (12%)	6 (18%)	7 (9%)	0.146
Diuretic	34 (29%)	19 (58%)	10 (12%)	<0.001
Ace inhibitor	40 (30%)	14 (42%)	17 (21%)	0.020
Statin	21 (18%)	5 (19%)	14 (18%)	0.969

All values are mean ± SD or number (%).

LV = left ventricular, NYHA = New York Heart Association functional class

Figure 2 demonstrates the event-free survival from device-detected atrial fibrillation during follow-up. The annualized incidence of device-detected AF was 7.0%/year, and of symptomatic AF 0.7%/year. The annualized incidence of de novo AF in 92 patients without either persistent or paroxysmal AF at baseline was 4.4%/year. Characteristics of patients with AF and patients without AF are presented in table 1. Patients with AF were older (58±15 vs 48±15 y, p<0.001), had more symptoms of heart failure (NYHA class ≥ II 58% vs 32%, p=0.012), lower LV ejection fraction (41%±12% vs 51%±11%, p=0.008), larger left atria (49±7 vs 44±7 mm, p=0.001) and more often had a history of surgical myectomy (30% vs 11%, p=0.013).

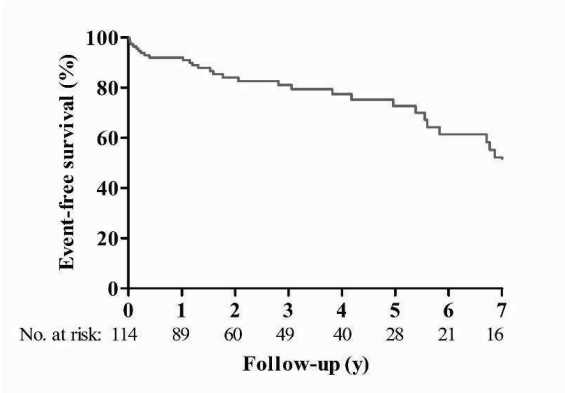

Figure 2. Kaplan Meier analysis demonstrating event-free survival from device-detected atrial fibrillation during follow-up, among patients with hypertrophic cardiomyopathy who were in sinus rhythm at the time of implantation of the device.

The occurrence of device-detected AF led to a change in the clinical management in 22 (76%) patients. OAC was initiated in 13 (45%) patients, anti-arrhythmic therapy was initiated or adjusted in 9 (31%) patients, and 8 (28%) underwent electrical cardioversion. One (3%) patient was prescribed low molecular weight heparin instead of OAC because of pregnancy, and 15 (52%) were already using OAC. Figure 3 presents the intracardiac electrogram of a 56 year old male with a VDD-ICD system, in whom paroxysmal AF was detected using the home-monitoring function of the device. New OAC (dabigatran) was started.

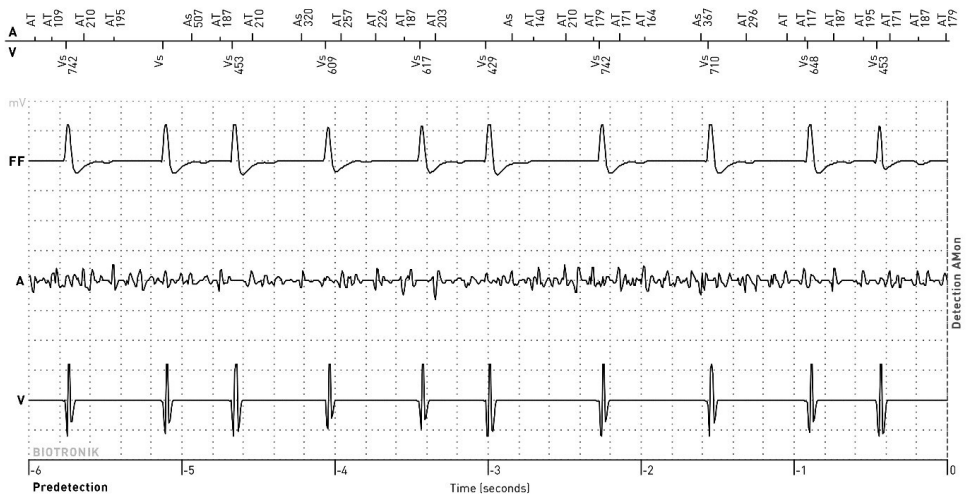

Figure 3. An intracardiac electrogram of a 56 year old male with a VDD-ICD system, in whom paroxysmal atrial fibrillation was detected using the home-monitoring function of the device. New oral anticoagulation (dabigatran) was started.

Long term outcomes of the patients are summarized in table 2. During 2.8 [1.2-5.4] years follow-up, the all-cause mortality was 27 (20%), and cardiac mortality was 21 (16%). The dominant cause of death was heart failure, which was the cause of death in 17 (63%) patients. Eight (6%) patients received a heart transplant, two of whom died due to transplant-related complications (rejection and infection).

Table 2. Long-term outcomes during follow-up of the study population (n=132)

	Crude incidence	**Annualized incidence**
All-cause mortality	27 (20%)	5.1%
Cardiac mortality	21 (16%)	4.1%
Thromboembolism	6 (5%)	1.3%
Stroke	3 (2%)	0.7%
Transient ischemic attack	2 (2%)	0.5%
Peripheral arterial embolism	1 (1%)	0.2%
Heart transplant	8 (6%)	1.5%
Appropriate ICD intervention	26 (22%)	5.6%
Inappropriate ICD intervention	18 (16%)	4.1%
Electrical cardioversion	12 (10%)	2.6%
Pulmonary venous isolation	1 (1%)	0.3%

All values are number (%). ICD = implantable cardioverter defibrillator

A thromboembolic event occurred in 6 (5%) patients. Characteristics of these patients at the time of the event are presented in table 3. Two patients had no atrial high rate episodes at the time of the thromboembolic event or during follow-up. The etiology of the embolic event in these two patients was unknown. The annual incidence of thromboembolic events was 1.3%/year (0.65% for patients with AF and 0.65% for patients without AF).

Table 3. Characteristics of the 6 patients with a thromboembolic event

Patient	**Gender, age**	**AF at implant**	**AF during follow-up**	**Oral anticoagulation**	**Antiarrhythmic therapy**	**Event**
1	Female, 54	-	+*	+	+	Ischemic stroke
2	Female, 43	+†	+	+	+	Ischemic stroke
3	Female, 43	-	+‡	-	-	Peripheral arterial embolism
4	Male, 52	-	+‡	-	+	Transient ischemic attack
5	Female, 48	-	-	-	-	Ischemic stroke
6	Male, 73	-	-	-	+	Transient ischemic attack

+ = present, - = absent, * = symptomatic paroxysmal AF, † = permanent AF, ‡ = device-detected paroxysmal AF after the event

Appropriate ICD intervention occurred in 26 (22%) patients (shock in 9 patients; antitachycardia pacing in 16 patients; both in 1 patient). Appropriate ICD intervention did not occur more often in patients with AF (7 out of 29 [24%] vs 15 out of 85 [18%], p=0.444), or in patients using sotalol (3 out of 11 [27%] vs 23 out of 121 [19%], p=0.509). Inappropriate ICD intervention occurred in 18 (16%) patients. Besides AF in 3 (17%) patients, reasons for inappropriate ICD intervention were atrial tachycardia in 6 (33%), sinus tachycardia in 4 (22%), noise in 3 (17%), and T-top oversensing in 2 (11%) patients.

Cardiac death occurred more often in patients with a history of paroxysmal or persistent AF at the time of implantation (40% vs 7%, p<0.001). A history of paroxysmal or persistent AF at the time of implantation was a predictor of cardiac death during follow-up (HR 4.7, 95% CI 1.7-12.8, p=0.003), independent of age and gender.

DISCUSSION

This study analyzed the incidence of device-detected AF and its impact on long-term outcomes among patients with HC and a CIED. The main findings of this study are that the incidence of device-detected AF was 7.0%/year, and the incidence of symptomatic AF was 0.7%/year. Hence, most cases (88%) of AF were subclinical and device-detected. The occurrence of device-detected AF led to a change in the clinical management in 76% of the patients. A history of paroxysmal or persistent AF at the time of implantation was an independent predictor of cardiac death during 2.8 [1.2-5.4] years follow-up. The annualized rate of thromboembolic complications was relatively low (1.3%), possibly because the majority of patients with device-detected AF were treated with OAC and due to the young age of the study population.

Previous studies have addressed the incidence of AF and thromboembolic complications in patients with HC.(12, 14, 15) The annualized incidence of AF in this study was higher than the pooled annualized incidence of 3.1% that was reported in a meta-analysis by Guttman et al.(15) Recently, Wilke et al. reported an annualized incidence of de novo AF of 33% in 30 patients with HC and a CIED.(12) The high incidence of AF in this study and the study of Wilke et al. can be explained by several factors. First, the type of population that we studied consisted of patients with HC and a CIED. Patients with HC and a CIED generally have an advanced form of cardiomyopathy and are at a substantially increased risk of developing AF.(2, 7, 8) Second, the majority of the cases (88%) were device-detected. These cases probably would have been missed if surveillance was performed by routine ECG or traditional intermittent monitoring strategies.(6) Subclinical AF was previously shown to be associated with an increased risk of ischemic stroke or systemic embolism (HR 2.49, p=0.007).(4) Therefore, the detection of subclinical AF appears to be of equal relevance as the detection of symptomatic AF. However, subclinical AF is not always detected before the thromboembolic event. In this study, in 2 out of 6 cases with a thromboembolic event, AF was

detected after the event. This is in line with the findings of the ASSERT trial(16) which studied the temporal relationship of subclinical AF and embolic events in 51 patients with a CIED. In that study subclinical AF was associated with an increased risk of ischemic stroke and systemic embolism, although very few patients had subclinical AF in the month before their stroke. This suggests that AF is a risk marker, but not always a direct cause of stroke and embolism.

Patients with HC are at increased risk of developing AF because of several factors, including diastolic dysfunction with increased left atrial pressure and size, LV outflow tract obstruction and mitral regurgitation.(5) Olivotto et al. analyzed the prognostic implications of AF in 480 patients with HC.(3) During 9.1±6.4 years follow-up, patients with AF had increased risk for HC-related death (3%/year vs 1%/year in controls) because of excess heart failure-related mortality. AF patients were also at increased risk of stroke (21% vs 2.6%) and functional deterioration to NYHA class III and IV.(3). Stroke is associated with significant mortality and morbidity.(3, 17) Maron et al. reported that 10 out of 44 patients (23%) with HC and a stroke died within 4 months, and that among the 34 survivors, 11 (32%) had permanent neurologic impairment such as aphasia or hemiparesis.(17) Moreover, stroke has a major burden on health care systems and society.(18) Detecting AF with a device may therefore be important in the clinical management of patients with HC in order to initiate OAC and possibly antiarrhythmic therapy. An early detection of AF by a CIED may reduce the incidence of thromboembolic complications including stroke.

Overall, the incidence of thromboembolism in this cohort was relatively low (1.3%/year) compared to previous studies (3.8%/year).(15) Possibly, the detection of AF with a device reduced the incidence of thromboembolisms, since therapy with OAC was initiated in the majority of cases. Current guidelines recommend lifelong therapy with OAC in all patients with HC and AF (regardless of the CHADSVASC score), even when sinus rhythm is restored.(5) This stresses the need for attention for device-detected AF in patients with HC and a CIED. Clearly, a good collaboration between the device-specialist or electrophysiologist and the treating physician is needed to further improve the clinical management of patients with device-detected AF.

ICD implantation is recommended for secondary prophylaxis of SCD in patients with a history of cardiac arrest or sustained ventricular tachycardia, and for primary prophylaxis in patients who are at high risk for SCD.(5, 10) The risk for SCD can be estimated through evaluation of the known risk factors(10) or using the recently introduced HCM Risk-SCD calculator.(5) Current guidelines recommend a single ventricular lead system, unless there is an indication for dual-chamber pacing or CRT in case of reduced LV function.(5, 10) However, a device that is capable of atrial sensing may be considered, since atrial electrograms facilitate the detection of AF. Disadvantages of systems with atrial leads are longer procedure times and higher complication risk.(19-24) Therefore, a novel single-chamber system was developed containing a floating bipole in the atrium allowing atrial sensing without the need for an atrial lead.(25) Sticherling et al. reported that this device was non-inferior to a dual-chamber device with regard to the detection and therapy of ventricular tachycardia and

supraventricular tachycardia.(25) Further research is needed to assess the value of this device in detection of AF in patients with HC.

The study has some limitations. First, the study population is relatively small. Second, patients with single-chamber devices and subcutaneous ICDs were included in the study. Since these devices do not have atrial sensing capabilities, an underestimation of the incidence of AF might have occurred. Third, this study was performed in a referral center for patients with HC, and most of these patients had an advanced cardiomyopathy. Therefore the cohort was at an increased risk of developing AF.

Conflicts of interest: none.

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CHAPTER 8

Effect of gender and genetic mutations on outcomes in patients with hypertrophic cardiomyopathy

Hannah G. van Velzen
Arend F.L. Schinkel
Sara J. Baart
Roy Huurman
Isabella Kardys
Michelle Michels

Am J Cardiol. 2018 Sep 8.

ABSTRACT

Gender has been proposed to impact the phenotype and prognosis of hypertrophic cardiomyopathy (HC). Our aims were to study gender differences in the clinical presentation, phenotype, genotype, and outcome of HC. This retrospective single-center cohort study included 1007 patients with HC (62% male, 80% genotyped) evaluated between 1977 and 2017. Hazard ratios (HR) were calculated using multivariable Cox proportional hazard regression models. At first evaluation, female patients presented more often with symptoms (43% vs 35%, p=0.01), were older than male patients (56±16 vs 49±15 y, p<0.001), and more frequently had hypertension (38% vs 27%, p<0.001), left ventricular outflow tract obstruction (37% vs 27%, p<0.001) and impaired left ventricular systolic (17% vs 11%, p=0.01) and diastolic (77% vs 62%, p<0.001) function. Overall, the genetic yield was similar between genders (54% vs 51%, p=0.4), however in patients ≥ 70 years the genetic yield was less in females (15% vs 36%, p=0.03). During 6.8 (interquartile range, 3.2-10.9) years follow-up, female gender was not independently associated with all-cause mortality (HR 1.25 [0.91 - 1.73]), cardiovascular mortality (HR 1.22 [0.83 - 1.79]), heart failure related mortality (HR 1.77 [0.95 - 3.27]), or sudden cardiac death/aborted sudden cardiac death (HR 0.75 [0.44 - 1.30]). Interventions and nonfatal clinical events did not differ between the genders. In conclusion, female patients with HC present at a more advanced age with a different clinical, phenotypic, and genetic status. There is no independent association between female gender and all-cause, cardiovascular, heart failure related, or sudden cardiac death.

INTRODUCTION

Hypertrophic cardiomyopathy (HC) is a heterogeneous monogenic cardiac disease known to lead to sudden cardiac death (SCD), heart failure (HF), and atrial fibrillation with the increased risk of stroke.(1, 2) Gender has been proposed to impact the age of onset and the phenotype of HC.(3-14) Studies which assessed gender and clinical outcome of HC report conflicting results.(5, 15-17) Some studies report an independent association between female sex and all-cause mortality(16, 17) or heart failure related events.(15, 16, 18, 19) Genotype has been shown to impact the phenotypic expression and clinical outcome of HC.(7, 20-22) In the Netherlands, genetic counselling and testing is offered to all patients with HC, because it is covered by the national basic health-care program. The aim of this study was to assess gender-related differences in the genetic test results, clinical presentation, phenotype, and outcome of HC.

METHODS

This single-center retrospective cohort study included 1007 patients with HC who were evaluated at the Erasmus Medical Center in Rotterdam, the Netherlands, between the years 1977 and 2017. The diagnosis of HC was based on a maximal wall thickness (MWT) ≥ 15 mm in probands, ≥ 13 mm in relatives, and a z-score > 2 in children, not solely explained by loading conditions. Patients with HC caused by Anderson-Fabry disease, Danon disease, Noonan syndrome, amyloidosis, or other confirmed metabolic or mitochondrial disorders or malformation syndromes were excluded. The study conforms to the principles of the Declaration of Helsinki. All patients gave informed consent for inclusion in the registry and local institutional review board approval was obtained.

Genetic counseling and testing was offered to all patients. Before the year 2012, genetic analysis consisted of direct sequencing of all coding exons and intron-exon boundaries of the following eight genes: myosin binding protein C (*MYBPC3*), ß-myosin heavy chain (*MYH7*), cardiac-regulatory myosin light chain (*MYL2*), cardiac troponin T (*TNNT2*), cardiac troponin I (*TNNI3*), cysteine and glycine-rich protein 3 (*CSRP3*), titin-cap/telethonin (*TCAP*), and α-tropomyosin (*TPM1*). From the year 2012, a next-generation-sequencing targeted approach including 48-52 cardiomyopathy-associated genes was used. Classification of variants was done at time of initial testing. Variants were interpreted using a protocol adapted from the American College of Medical Genetics and Genomics recommendations(23), and classified into 5 categories: (I) benign; (II) likely benign; (III) uncertain significance; (IV) likely pathogenic; and (V) pathogenic. The potential pathogenicity of variants was assessed using Alamut Visual software (Interactive Biosoftware, Rouen, France), that integrates data from several large-scale population studies, evolutionary conservation of nucleotides and amino acids, in silico missense predictions (Align GVGD, SIFT, MutationTaster and PolyPhen-2) and splicing prediction modules (SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder). The criteria for classification of variants included the allele frequency in the

dbSNP/ESP/ExAC/GoNL (cutoff minor allele frequency 1% in at least 300 ethnically matched control alleles equals benign), predicted effects on splicing, the in silico prediction of effect on the protein, and previously described links to disease. Furthermore, segregation analysis in families with more affected individuals and information considering presence in Human Gene Mutation Database (HGMD®) Professional 2017.3 (Qiagen) is taken into account. Variant reclassifications during follow-up were registered, and variant classification as assessed at the end of follow-up was used for the analyses. Patients with a reclassified variant were informed about the reclassification and if applicable about the indication for renewed evaluation. Patients were considered genotype-positive when the mutation was classified as likely pathogenic or pathogenic (class IV and V).

Clinical assessment included medical history, physical examination, electrocardiography and transthoracic echocardiography. Echocardiographic studies were analyzed according to the guidelines.(1, 24, 25) MWT, left atrial dimension, left ventricular (LV) end-diastolic diameter, and resting LV outflow tract velocity were assessed.(1, 24) LV outflow tract gradient was calculated with the Bernoulli equation. LV systolic function was categorized as: good (LV ejection fraction > 51%), mildly reduced (LV ejection fraction 41% to 51%), moderately reduced (LV ejection fraction 30% to 40%), and poor (LV ejection fraction < 30%).(25) LV diastolic function was defined as normal, abnormal relaxation, pseudonormal or restrictive filling, based on Doppler mitral inflow pattern parameters including early (E) and late (A) LV filling velocities, E/A ratio, and tissue Doppler imaging-derived septal early diastolic velocities (e').(26) Body surface area was calculated with the Du Bois & Du Bois formula.

Mortality data was retrieved from the civil service register in August 2017. Patients were followed for a median 6.8 (interquartile range 3.2-10.9) years (7 363 total personyears; 0.01% missing due to loss of follow-up). Patients who were lost to follow-up were censored at time of last follow-up. The cause of death was retrieved from the medical chart or the general practitioner and was obtained in 171 (87%) of mortality cases. Those with unknown causes of death were classified as all-cause mortality. Cardiovascular mortality included SCD/aborted SCD, HF related death, postoperative death after a cardiac intervention and stroke related death. SCD/aborted SCD was defined as: (1) instantaneous and unexpected death in patients who were previously in a stable clinical condition, or nocturnal death with no antecedent history of worsening symptoms; (2) resuscitation after cardiac arrest; or (3) appropriate implantable cardioverter defibrillator (ICD) intervention. Appropriate ICD intervention was defined as shock or antitachycardia pacing for ventricular fibrillation or ventricular tachycardia >200/min. Cardiac transplantation was considered HF related mortality and patients were censored at the time of transplantation. The following nonfatal clinical events and interventions were registered: atrial fibrillation (paroxysmal, persistent or permanent), stroke, transient ischemic attack, hospital admission for HF, septal reduction therapy (surgical myectomy and alcohol septal ablation), and ICD and pacemaker implantations. ICDs and pacemakers were implanted according to the guidelines.(1, 24).

Calculations were performed using SPSS 21 (IBM, Armonk, New York) and R statistical Software version 3.4.2 using packages nlme, lme4, survival, and smcfcs. Normally distributed continuous data are expressed as mean ± standard deviation and non-normally distributed data as median followed by interquartile range. In order to make comparisons between male and female patients, generalized linear mixed models were used, with random intercepts for family to account for family relatedness. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using univariable and multivariate Cox proportional hazard regression models with adjustment for family relatedness. For this purpose the grouped jackknife method was used. Missing values of variables included in the multivariable analyses were imputed using 10 imputed datasets. All analyses were two-tailed; P-values < 0.05 were considered significant.

RESULTS

Baseline characteristics are presented in table 1. Overall, there was a male predominance of 62%. The male predominance was present in all age groups, except in patients ≥ 70 years where females predominated (figure 1). Male patients presented more often via routine medical examinations, and female patients presented more often with symptoms (table 2). Female patients were significantly older than male patients both at time of diagnosis and at first evaluation (table 1), also after excluding patients who presented via routine medical examinations (51±18 vs 46±17 y, p<0.001 and 55±17 vs 49±16 y, p<0.001 respectively). Female patients more frequently had a history of hypertension and stroke/transient ischemic attack.

Table 1. Baseline characteristics according to gender (continues on the next page)

Variable	Overall (n=1007)	Male (n=620)	Female (n=387)	P-value
Age at evaluation (years)	52±16	49±15	56±16	<0.001
< 30	102 (10%)	68 (11%)	34 (9%)	0.26
30 - 50	338 (34%)	241 (39%)	97 (25%)	<0.001
> 50	567 (56%)	311 (50%)	256 (66%)	<0.001
Age at diagnosis (years)	46±17	44±16	50±19	<0.001
BSA (mm/m2)	1.94±0.23	2.05±0.17	1.80±0.17	<0.001
Arterial hypertension	310 (31%)	164 (27%)	146 (38%)	<0.001
Coronary artery disease	62 (6%)	43 (7%)	19 (5%)	0.21
Atrial fibrillation	213 (21%)	123 (20%)	90 (23%)	0.41
Septal reduction therapy	51 (5%)	30 (5%)	21 (5%)	0.67
ICD/PM implantation	47 (5%)	24 (4%)	23 (6%)	0.14
Stroke/TIA	61 (6%)	25 (4%)	36 (9%)	0.02
HF admission	24 (4%)	11 (3%)	13 (5%)	0.17
SCD/aborted SCD	16 (2%)	11 (2%)	5 (1%)	0.33
Medication				
Betablockers	497 (49%)	292 (47%)	205 (53%)	0.07

Table 1. Baseline characteristics according to gender (continued)

Variable	Overall (n=1007)	Male (n=620)	Female (n=387)	P-value
Other anti-arrhythmic*	58 (6%)	31 (5%)	27 (7%)	0.19
Calcium antagonists	298 (30%)	183 (30%)	115 (30%)	0.95
Statins	196 (20%)	110 (18%)	86 (22%)	0.08
Diuretics	188 (19%)	84 (14%)	104 (27%)	<0.001
Aspirin	159 (16%)	80 (13%)	79 (20%)	0.001
Oral anticoagulants†	123 (12%)	64 (10%)	59 (15%)	0.02
ACE-i	124 (12%)	72 (12%)	52 (13%)	0.39
ATIIA	102 (10%)	54 (9%)	48 (12%)	0.06
ACE-i / ATIIA	222 (22%)	123 (20%)	99 (26%)	0.03
Genetic testing performed	810 (80%)	511 (82%)	299 (77%)	0.05
Pathogenic mutation	430 (53%)	277 (54%)	153 (51%)	0.39
Echocardiography				
MWT (mm)	19±4	19±4	18±4	0.03
< 13**	8 (1%)	5 (1%)	3 (1%)	0.96
13 - 15	208 (21%)	108 (18%)	100 (26%)	0.001
16 - 19	428 (43%)	271 (45%)	157 (41%)	0.33
20 - 24	253 (26%)	167 (27%)	86 (23%)	0.09
25 - 29	68 (7%)	41 (7%)	27 (7%)	0.82
≥ 30	24 (2%)	17 (3%)	7 (2%)	0.35
MWT/BSA (mm/m2)	9.6±2.3	9.2±2.0	10.3±2.6	<0.001
LA (mm)	45±8	45±8	44±8	0.001
LA/BSA (mm/m2)	23.2±4.1	22.5±3.9	24.5±4.1	<0.001
LVEDD (mm)	46±6	47±6	44±6	<0.001
LVEDD/BSA (mm/m2)	23.3±3.4	22.7±3.2	24.3±3.5	<0.001
LVOT ≥ 30 mmHg‡	300 (31%)	160 (27%)	140 (37%)	<0.001
Diastolic function				
Normal	285 (32%)	206 (38%)	79 (23%)	<0.001
Impaired relaxation	276 (31%)	147 (27%)	129 (38%)	<0.001
Pseudonormal filling	269 (30%)	169 (31%)	100 (30%)	0.60
Restrictive filling	55 (6%)	25 (5%)	30 (9%)	0.01
Systolic function				
Good	857 (87%)	543 (89%)	314 (83%)	0.01
Mildly reduced	95 (10%)	51 (8%)	44 (12%)	0.10
Moderately reduced	24 (2%)	8 (1%)	16 (4%)	0.01
Severely reduced	11 (1%)	7 (1%)	4 (1%)	0.87

Data are expressed as mean ± standard deviation or as absolute n (%). Generalized linear mixed models were used, with random intercepts for family to account for family relatedness. * = includes flecainide, amiodarone, disopyramide, and ritmoforin. † = includes 1 new oral anticoagulant. ** = end stage hypertrophic cardiomyopathy or post septal reduction therapy. ‡ = at rest. ATIIA, angiotensin II antagonist; ACE-i, ACE inhibitor; BSA, body surface area; HF, heart failure; LA, left atrial size; LVEDD, left ventricular end diastolic diameter; LVOT, left ventricular outflow tract gradient; MWT, maximal wall thickness; ICD, implantable cardioverter defibrillator; PM, pacemaker; SCD, sudden cardiac death; TIA, transient ischemic attack.

Table 2. Triggers for diagnosis in male and female patients with hypertrophic cardiomyopathy

Variable	Overall (n=1007)	Male (n=620)	Female (n=387)	P-value
Precordial murmur	149 (18%)	106 (20%)	43 (14%)	0.03
Abnormal ECG	111 (13%)	76 (15%)	35 (11%)	0.20
Other*	33 (4%)	20 (4%)	13 (4%)	0.75
Chest pain	145 (18%)	88 (17%)	57 (19%)	0.52
Dyspnea	112 (14%)	61 (12%)	51 (16%)	0.04
Palpitations	65 (8%)	30 (6%)	35 (12%)	0.004
Dizziness	37 (5%)	21 (4%)	16 (5%)	0.45
Syncope	39 (5%)	29 (6%)	10 (3%)	0.14
Fatigue	65 (8%)	27 (5%)	38 (13%)	<0.001
Sudden cardiac death†	11 (1%)	9 (2%)	2 (1%)	0.19
Atrial fibrillation	21 (3%)	11 (2%)	10 (3%)	0.33
Heart failure	5 (0.6%)	3 (0.6%)	2 (0.7%)	0.95
Acute myocardial infarction	11 (1%)	7 (1%)	4 (1%)	0.99
Stroke/TIA/embolism	5 (0.6%)	4 (0.8%)	1 (0.3%)	0.45
HC family screening	165 (20%)	103 (20%)	62 (20%)	0.81

Data are expressed as absolute n (%). Generalized linear mixed models were used, with random intercepts for family to account for family relatedness. * = during preoperative screening, prescan, cardiac echo for other cardiac diseases. † = two sudden cardiac deaths were not successfully resuscitated. ECG, electrocardiography; HC, hypertrophic cardiomyopathy; TIA, transient ischemic attack.

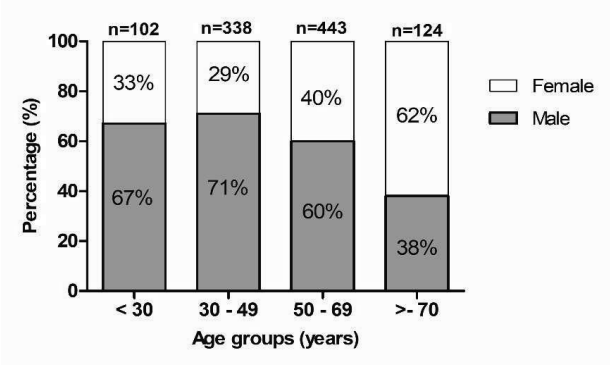

Figure 1. Male/female distribution among several age groups.

In patients ≥ 70 years old, the genetic yield was significantly less in female patients than in male patients (15% vs 36%, p=0.03) (figure 2). Genes most frequently affected were *MYBPC3* (74%) and *MYH7* (14%) (figure 3). Other genes affected were *TNNI3* (3%), *TNNT2* (3%), *MYL2* (2%), *ALPK3* (1%), *TPM1* (0.7%), *MYL3* (0.7%), *CSRP3* (0.7%), *FHL1* (0.5%), *MIB1* (0.2%), and *TNNC1* (0.2%). There was no significant difference regarding the proportion of *MYBPC3* mutations (77% vs 69%, p=0.08) or *MYH7* mutations (12% vs 18%, p=0.09) in male or female patients respectively.

A complex genotype was present in 8 (3%) female patients and 8 (2%) male patients (p=0.3), and included 8 homozygous mutations, 4 digenic, and 4 compound heterozygous mutations (supplementary table 1).

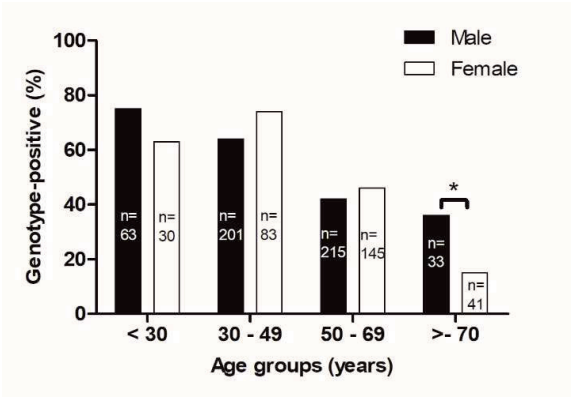

Figure 2. Genetic yield in male and female patients among several age groups. * indicates statistical significance with a p<0.05.

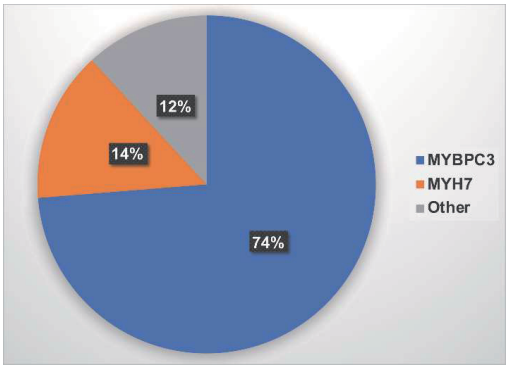

Figure 3. Distribution of pathogenic mutations in genotype-positive patients. *MYBPC3*, myosin binding protein C; *MYH7*, ß-myosin heavy chain. *Other* includes mutations in cardiac troponin T (3%), cardiac troponin I (3%), cardiac-regulatory myosin light chain (2%), alpha-protein kinase 3 (1%), cysteine and glycine-rich protein 3 (0.7%), cardiac-essential myosin light chain (0.7%), α-tropomyosin (0.7%), four and a half LIM domains protein 1 (0.5%), mindbomb E3 ubiquitin protein ligase 1 (0.2%), and cardiac troponin C (0.2%).

The MWT was higher in male patients, but the MWT corrected for body surface area was higher in female patients. Similar observations were made for left atrial dimension and LV end-diastolic

diameter. A greater proportion of female patients had LV outflow tract obstruction, and systolic and diastolic function was more often impaired in female patients.

Mortality during a median 6.8 (interquartile range, 3.2-10.9) year follow-up is presented in table 3. In multivariable analysis (tables 4 and 5), there was no independent association between gender and all-cause mortality (HR 1.25, p=0.16), cardiovascular mortality (HR 1.22, p=0.31), HF related death (HR 1.77, p=0.08) or SCD/aborted SCD (HR 0.75, p=0.31). Missing values for the following variables were imputed: pathogenic mutation (20%), diagnosis by routine examination (18%), and body surface area (19%). Clinical follow-up was performed in 691 (69%) patients; the remaining 316 (31%) patients were followed up in other hospitals. Interventions and nonfatal clinical events did not differ significantly between male and female patients (table 6).

Table 3. Outcome differences in males and females with hypertrophic cardiomyopathy

Variable	Overall (n=1005)	Male (n=618)	Female (n=387)	HR (95% CI)	P-value
Follow-up	6.8 [3.2-10.9]	7.7 [3.5-11.1]	5.8 [2.3-10.1]	-	0.003
All-cause mortality	183 (19%)	91 (15%)	92 (24%)	1.85 (1.40-2.44)	<0.001
Cardiovascular mortality	110 (11%)	56 (9%)	54 (15%)	1.76 (1.22-2.54)	0.002
SCD/Aborted SCD	57 (6%)	37 (6%)	20 (5%)	0.99 (0.57-1.71)	0.97
Appropriate ICD shock	20 (2%)	15 (3%)	5 (1%)	0.63 (0.23-1.71)	0.36
Cardiac arrest	37 (4%)	22 (4%)	15 (4%)	1.23 (0.64-2.39)	0.53
HF related mortality	46 (5%)	19 (3%)	27 (7%)	2.50 (1.42-4.39)	0.001
Cardiac transplantation	16 (2%)	5 (1%)	11 (3%)	3.65 (1.22-10.9)	0.02
Stroke related death	4 (0.4%)	1 (0.2%)	3 (0.8%)	5.57 (0.55-56.8)	0.15
CIRD	6 (0.6%)	0 (0%)	6 (2%)	*	
Non-cardiac mortality	47 (5%)	22 (4%)	25 (7%)	2.11 (1.21-3.69)	0.009

Data are expressed as absolute n (%). Hazard ratios (HR) were calculated using univariable Cox proportional hazard regression models with adjustment for family relatedness. *Hazard ratio is not presented due to low number of events. CIRD, cardiac intervention related death; HF, heart failure; ICD, implantable cardioverter defibrillator; SCD, sudden cardiac death; For all-cause mortality, cardiovascular mortality, SCD/Aborted SCD, and intervention-related death survival analyses the patients with a history of SCD/Aborted SCD were excluded.

Table 4. Multivariate cox proportional hazard regression analyses for all-cause and cardiovascular mortality (continues on the next page)

Variable	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Female gender	1.21 (0.88 - 1.66)	0.23	1.21 (0.82 - 1.78)	0.33
Age at evaluation	1.03 (1.01 - 1.04)	<0.001	0.99 (0.98 - 1.02)	0.98
Diagnosis by routine examination	0.83 (0.58 - 1.19)	0.31	0.73 (0.46 - 1.15)	0.17
Arterial hypertension	0.88 (0.63 - 1.23)	0.47	0.79 (0.51 - 1.24)	0.31

CI, confidence interval; HR, hazard ratio

Table 4. Multivariate cox proportional hazard regression analyses for all-cause and cardiovascular mortality (continued)

Variable	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Atrial fibrillation	1.31 (0.96 - 1.80)	0.09	1.99 (1.35 - 2.94)	<0.001
Abnormal systolic function	2.83 (1.97 - 4.08)	<0.001	3.19 (2.04 - 4.98)	<0.001
MWT/BSA (mm/m2)	1.03 (0.95 - 1.10)	0.49	1.02 (0.94 - 1.102)	0.67
Left atrial size/BSA (mm/m2)	1.05 (1.01 - 1.09)	0.01	1.07 (1.02 - 1.11)	0.003
Pathogenic mutation	1.01 (0.72 - 1.43)	0.94	1.07 (0.68 - 1.69)	0.75

BSA, body surface area; CI, confidence interval; HR, hazard ratio; MWT, maximal wall thickness

Table 5. Multivariate cox proportional hazard regression analyses for SCD/Aborted SCD and HF related mortality.

Variable	SCD/Aborted SCD		HF related mortality	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Female gender	0.75 (0.44 - 1.28)	0.29	1.73 (0.92 - 3.24)	0.09
Age at evaluation	0.99 (0.97 - 1.01)	0.27	1.00 (0.97 - 1.03)	0.99
Atrial fibrillation	1.39 (0.78 - 2.48)	0.27	3.65 (1.80 - 7.41)	<0.001
Abnormal systolic function	2.60 (1.36 - 4.95)	0.004	6.56 (3.34 - 12.85)	<0.001
MWT/BSA (mm/m2)	1.06 (0.95 - 1.17)	0.29	-	
LA size/BSA (mm/m2)	1.05 (0.99 - 1.11)	0.08	1.09 (1.02 - 1.17)	0.009
Pathogenic mutation	1.21 (0.66 - 2.22)	0.54	1.32 (0.67 - 2.60)	0.43

BSA, body surface area; CI, confidence interval; HF, heart failure; HR, hazard ratio; MWT, maximal wall thickness; SCD, sudden cardiac death

Table 6. Differences in interventions and nonfatal clinical events in male and female patients with hypertrophic cardiomyopathy during follow-up

Variables	Overall (n=691)	Male (n=431)	Female (n=260)	HR (95% CI)	P-value
Septal reduction therapy	223 (32%)	131 (30%)	92 (36%)	1.29 (0.98-1.69)	0.07
Surgical myectomy	173 (25%)	101 (23%)	72 (28%)	1.32 (0.97-1.79)	0.08
Alcohol septal ablation	63 (9%)	38 (9%)	25 (10%)	1.13 (0.68-1.87)	0.64
ICD implantation	155 (23%)	102 (24%)	53 (21%)	0.89 (0.64-1.24)	0.49
PM implantation	29 (4%)	14 (3%)	15 (6%)	1.96 (0.95-4.01)	0.07
AF de novo	49 (9%)	36 (11%)	13 (7%)	0.66 (0.36-1.23)	0.19
Stroke	22 (3%)	13 (3%)	9 (4%)	1.22 (0.52-2.85)	0.65
TIA	23 (3%)	17 (4%)	6 (2%)	0.57 (0.23-1.42)	0.23
HF admission	44 (6%)	22 (5%)	22 (9%)	1.71 (0.95-3.07)	0.07

Data are expressed as absolute n (%). Hazard ratios (HR) were calculated using univariable Cox proportional hazard regression models with adjustment for family relatedness. AF, atrial fibrillation; HF, heart failure; ICD, implantable cardioverter defibrillator; PM, pacemaker; TIA, transient ischemic attack.

DISCUSSION

In this study we report the following gender differences in patients with HC: (1) at presentation female patients were older, more frequently had a history of hypertension, and presented more frequently with symptoms; (2) female patients more frequently had an impaired systolic and diastolic function and more frequently exhibited LV outflow tract obstruction; (3) in the whole cohort there was a male predominance, however among patients ≥ 70 years old females predominated in whom the genetic yield was significantly lower than in male patients, and (4) during 6.8 years follow-up, there was no independent association between gender and all-cause mortality, cardiovascular mortality, HF related mortality, or SCD/aborted SCD.

In this study, female patients had a delayed clinical presentation in comparison to male patients, also after excluding those who presented via routine medical examinations. Several previous studies have similarly reported a delayed clinical presentation in female patients with HC.(3, 7, 12, 15-17, 19) Olivotto et al. studied gender differences among 969 patients with HC and reported that female patients were 9 years older at time of initial evaluation(15), similar to Bos et al. who reported a 9 year delay in female patients among 382 patients with HC.(7) Wang et al. reported a 3 year delay in female patients among 621 patients with HC(16) and recently Geske et al. reported a 7 year delay in female patients in a large cohort of 3673 HC patients.(17) Sociocultural processes (i.e. lack of attention to early clinical signs in women or diagnostic bias) may account for the delay. However, Dimitrow et al. reported that not just diagnosis but also the onset of symptoms was delayed in females with HC.(3) Therefore, differences in sexual hormones and gene expression may play a role.(27)

Female patients were older and had different clinical and phenotypic features including more hypertension, more LV outflow obstruction, and a higher indexed MWT. Indeed, hypertensive HC is known to occur predominantly in the elderly, particularly female.(28) Krumholz et al. reported that women adapt differently to hypertension than men, namely women develop concentric hypertrophy with normal or reduced LV size and men develop LV dilatation without increased LV wall thickness.(29) It may be due to these differences that LV outflow obstruction was more common in female patients. In addition, the underlying HC mutation most likely has an important impact on the phenotypic expression of HC. Bos et al. demonstrated that patients with sigmoidal HC were generally older women with hypertension and LV outflow obstruction.(7) The majority of these patients were mutation-negative, in contrast to patients with reverse curve HC where 80% was mutation-positive.(30) This study extends these findings by showing that women ≥ 70 years old had a significantly lower genetic yield in comparison to men. Mutation-negative HC might culminate from a multifactorial process involving undefined genetic and environmental factors.(20)

At baseline, female patients showed more signs of adverse remodeling than male patients (systolic and diastolic impairment, larger indexed left atria and larger LV). Whether female patients with HC are indeed at a higher risk of HF is currently unknown. Unlike previous studies(15, 16, 18,

19), we did not observe an increased risk of HF related mortality or hospital admission for HF in female patients during follow-up. The discrepancy with previous studies may be caused by the use of different end points. Studies which assessed LV contractility in similarly aged male and female patients with HC also reported conflicting results. Dimitrow et al. measured fractional shortening in 77 males and 52 females with HC, and found no gender difference (45% vs 44%, p>0.05).(6) Kubo et al. described a higher fractional shortening in 88 female patients versus 173 male patients (43% vs 40%, p=0.01).(11)

In the current study, there was no independent association between gender and all-cause mortality, cardiovascular mortality, HF related death, or SCD/aborted SCD. Important predictors of outcome were age at evaluation, abnormal systolic function, left atrial size adjusted for body surface area, and atrial fibrillation. Previous studies have also demonstrated a prognostic value for these variables in patients with HC.(31-33) The variables combined represent part of a distinct disease pathway termed ‘’stage III, adverse remodeling’’.(34) About 15-20% of the patients with HC follow this pathway and are at increased risk of death.(34) Previous studies that assessed gender and mortality in HC have reported conflicting results.(5, 15-17) Similar to our results, Olivotto et al. found no association between gender and all-cause mortality, HC-related death or SCD among 969 patients with HC after 6.2 years follow-up.(15) However, they found an association between female gender and the combined end point of progression to NYHA classes III or IV or death from HF or stroke.(15) Terauchi et al. studied gender differences among 50 patients with HC caused by *MYBPC3* mutations and reported more HF events in female patients, however no gender difference regarding survival.(19) Dimitrow et al. reported no survival difference between 111 male and 70 female patients with HC during 7 years follow-up.(5) In contrast to these studies, Wang et al. found female gender to be independently associated with all-cause mortality (HR 2.19, p=0.01), cardiovascular death (HR 2.19, p=0.01), and progression to HF (HR 1.73, p=0.01) during 4 years follow-up of 621 patients with HC.(16) Of note, in that study patients with HF at baseline were excluded. Geske et al. demonstrated that female sex was an independent predictor of all-cause mortality (HR 1.13, p=0.01) during 11 years follow-up of 3673 patients with HC.(17) The discrepancy with previous studies was suggested to be caused by their larger, sicker cohort.(17)

Overall, the findings in the current study illustrate that there is a significant delay in the clinical presentation of female patients with HC and that female patients present with more advanced disease than male patients. Similar observations were made for patients with coronary artery disease, which is partly attributable to sex-specific differences in the sensitivity of diagnostic procedures.(27) In the current study, adjusting echocardiographic parameters to body surface area revealed a worse phenotype than we suspected based on unadjusted parameters, suggesting a diagnostic bias. By applying gender- or body surface area-adjusted parameters, we may be able to recognize disease progression earlier, resulting in more intense follow-up and management and potentially a better outcome. Future studies are needed to investigate this further.(9)

This study has several limitations. First, this is a retrospective study which has inherent limitations. Second, the patients were referred to a tertiary center for cardiomyopathy, which may have caused a selection bias. Third, the prevalence of Dutch *MYBPC3* founder mutations in the Netherlands is relatively high(35) which may affect extrapolation of the findings to other countries. And fourth, follow-up for the occurrence of nonfatal clinical events was available in only 69%, due to follow-up in other hospitals.

In conclusion, female patients with HC present at a more advanced age with a different clinical, phenotypic, and genetic status. There is no independent association between female gender and all-cause, cardiovascular, heart failure related, or sudden cardiac death.

Disclosures: None.

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cases in the Netherlands. Eur Heart J. 2003;24(20):1848-53.

Supplementary table 1. Complex genotypes in patients with hypertrophic cardiomyopathy

Case	Complex type	Gender	Gene (s)	Nucleotide change
1	Homozygous	female	*MYBPC3*	c.1765C>G
2	Homozygous	female	*MYBPC3*	c.1765C>G
3	Homozygous	female	*MYBPC3*	c.1765C>G
4	Homozygous	female	*MYBPC3*	c.1765C>G
5	Homozygous	female	*MYBPC3*	c.1765C>G
6	Homozygous	male	*MYBPC3*	c.1831G>A
7	Homozygous	male	*ALPK3*	c.5294G>A
8	Homozygous	female	*ALPK3*	c.3781C>T
9	Digenic	male	*MYBPC3* and *MYL2*	c.3065G>C and c.52T>C
10	Digenic	male	*MYBPC3* and *MYL2*	c.1000G>T and c.64G>A
11	Digenic	male	*MYH7* and *MIB1*	c.5135G>A and c.2530_2532delTCTinsC
12	Digenic	female	*MYL3* and *TNNT2*	c.452C>T and c.832C>T
13	Compound heterozygous	male	*MYBPC3*	c.913_914delTT and c.1468G>A
14	Compound heterozygous	female	*MYBPC3*	c.932C>A and c.442G>A
15	Compound heterozygous	male	*MYBPC3*	c.2373insG and c.2827C>T
16	Compound heterozygous	male	*MYH7*	c.3100-2A>C and c.5135G>A

ALPK3, alpha kinase 3; *MIB1*, Mindbomb E3 ubiquitin protein ligase; *MYBPC3*, myosin binding protein C; *MYL2*, cardiac-regulatory myosin light chain; *MYH7*, ß-myosin heavy chain; *MYL3*, cardiac-essential myosin light chain.

CHAPTER 9

Sex-differences at the time of myectomy in hypertrophic cardiomyopathy

Louise L.A.M. Nijenkamp
Ilse A.E. Bollen
Hannah G. van Velzen
Jessica A. Regan
Marjon van Slegtenhorst
Hans W.M. Niessen
Arend F.L. Schinkel
Martina Krüger
Corrado Poggesi
Carolyn Y. Ho
Diederik W.D. Kuster
Michelle Michels
Jolanda van der Velden

Circ Heart Fail. 2018 Jun;11(6)

ABSTRACT

Background

One of the first clinically detectable alterations in heart function in hypertrophic cardiomyopathy (HCM) is a decline in diastolic function. Diastolic dysfunction is caused by changes in intrinsic properties of cardiomyocytes and/or an increase in fibrosis. We investigated if clinical and cellular parameters of diastolic function are different between male and female HCM patients at the time of myectomy.

Methods and Results

Cardiac tissue from the interventricular septum of HCM patients (27 female; 44 male) was obtained during myectomy preceded by echocardiography. At myectomy, female patients were 7 years older than male patients, and showed more advanced diastolic dysfunction than men evident from significantly higher values for E/e' ratio, LV filling pattern, TR velocity and left atrial diameter indexed for body surface. While most male patients (56%) showed mild (grade I) diastolic dysfunction, 50% of female patients showed grade III diastolic dysfunction. Passive tension in HCM cardiomyocytes was comparable to controls, and myofilament calcium-sensitivity was higher in HCM compared to controls, but no sex-differences were observed in myofilament function. In female HCM titin was more compliant and more fibrosis was present compared to males. Differences between female and male HCM patients remained significant after correction for age.

Conclusion

Female HCM patients are older at time of myectomy and show greater impairment of diastolic function. Furthermore, LV and LA remodeling is increased in women when corrected for body surface area. At cellular level HCM women showed increased compliant titin and a larger degree of interstitial fibrosis.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular (LV) hypertrophy.[1] It is the most prevalent monogenetic inherited cardiac disease, with a prevalence of 200 up to 500 per 100,000 individuals.[2] In approximately 65% of patients with HCM, a causative mutation is identified which is inherited in an autosomal dominant fashion.[3] Approximately 80% of identified mutations are located in two genes that encode the thick filament proteins, cardiac myosin binding protein C (cMyBP-C; gene *MYBPC3*) and β-myosin heavy chain (β-MyHC; gene *MYH7*).[4] Despite increased knowledge of the HCM-causing mutations, the exact path from genetic defect to cardiomyopathy is still largely unknown.

LV hypertrophy in HCM patients is often asymmetric and mainly affects the interventricular septum (IVS), which causes LV outflow tract obstruction (LVOTO). HCM is defined by a wall thickness ≥ 15 mm, although a cut-off value of 13 mm applies to first-degree relatives.[1] The hypertrophied myocardium is characterized by cardiomyocyte and myofilament disarray, reduced myofibrillar density, increased interstitial fibrosis and vascular abnormalities.[5,6] While these structural changes are part of the more advanced (hypertrophied) stage of the disease, impaired relaxation is present at an early disease stage in mutation carriers without evident cardiac hypertrophy.[7–9] Interestingly, female HCM patients have been described to show less ventricular remodeling compared to male patients,[10,11] while several studies reported more severe diastolic dysfunction in women than in men with HCM.[12,13] Within the latest cohort studies, women represent a minority of HCM patients, with percentages ranging from 26% to 45%, suggesting lower disease penetrance in women.[14,15] Moreover, women are on average 9 years older than men at the time of HCM diagnosis.[14,16] To better understand sex-differences in HCM pathology, we studied tissue properties of cardiac samples obtained during surgical myectomy from a clinically well-characterized group of female and male HCM patients carrying a thick filament (*MYH7* or *MYBPC3*) mutation. A comparison was made between female and male HCM patients at the time of cardiac surgery.

Our study shows that women are on average 7 years older than men at the time of surgery. Greater diastolic dysfunction was present in female compared to male HCM patients evident by diastolic grading. The sex-difference in diastolic function was not explained by intrinsic properties of the sarcomeres as similar increases in myofilament Ca^{2+}-sensitivity and no changes in passive stiffness were observed in single cardiomyocytes isolated from female and male HCM hearts compared to non-failing controls. A higher level of fibrosis in women compared to men may underlie the more severe impaired diastolic function in females, which was associated with an apparent compensatory shift towards more compliant titin isoform expression in female HCM hearts. Overall we see more severe changes in diastolic properties of the heart in female HCM patients compared to male HCM patients at the time of myectomy. Because the diagnostic criteria of LV wall thickness ≥15 mm does not take into

account body surface area, disease severity might be underestimated in female HCM patients and warrants future research.

MATERIALS AND METHODS

Myocardial Samples

Our study included patients carrying mutations in *MYH7* (n=17) and *MYBPC3* (n=54), of which 38% were female (mean age 50±13 ranging from 6 to 72). Cardiac tissue from the IVS was obtained during myectomy surgery to relieve LVOTO. LV tissue from 37 non-failing donors without a history of cardiac abnormalities was used as control (51% female, mean age of 41±14 ranging from 14 to 65). All samples were immediately frozen and stored in liquid nitrogen. Not all samples were sufficiently large to perform all analysis. Clinical cut-off values are indicated by dotted lines in Figure 1. The average values of control cardiac samples are indicated by the dotted lines in Figures 2 to 5; data for male and female control samples are shown in Supplemental Figure 1. The study protocol was approved by the local Ethics Committees, and written consent was obtained.

Echocardiographic measurements

Echocardiographic studies were done with commercially available systems and analyzed according to the American Society of Echocardiography guidelines.[17] Maximal wall thickness, left atrial diameter (LAD), LV end-diastolic diameter (LVEDD), and LVOTO gradient were measured. LVOTO was defined as a gradient ≥ 30 mmHg at rest or during provocation. Mitral valve inflow was recorded using pulsed wave Doppler from the apical four chamber view. Mitral E and A velocity (cm/s) and deceleration time (ms) were measured. Pulsed wave tissue Doppler imaging was used to measure septal e' velocity (cm/s). Continuous wave Doppler in the parasternal and apical four chamber was used to measure tricuspid regurgitation (TR) velocity (m/s).

Diastolic dysfunction was graded as follows: grade I when E/A ratio ≤ 0.8 and E peak velocity ≤ 50 cm/s; grade III when E/A ratio ≥ 2. In patients with E/A ratio ≤ 0.8 and E peak velocity > 50 cm/s or E/A ratio > 0.8 but < 2, the E/e' ratio (>14), LADi (>24) and TR velocity (> 2.8 m/s) were used to further differentiate diastolic function. When ≥ 2 out of 3 variables were abnormal, LA pressure was elevated and grade II diastolic dysfunction was present. When 1 out of 3 variables was abnormal, grade I diastolic dysfunction was present.[18]

Isometric Force Measurements

Force measurements were performed in mechanically isolated single membrane-permeabilized cardiomyocytes as described previously.[19] In short, we measured passive tension at four sarcomere lengths (SL) ranging from 1.8 to 2.4 μm. All passive forces were normalized to cross-sectional area (CSA) (i.e. CSA = width x depth x π/4). To determine myofilament calcium-sensitivity (pCa_{50}), active tension was measured at SL 2.2 μm, with different calcium concentrations.

Protein analyses

Titin isoform gel electrophoresis was performed on patient samples as previously described.[20] Samples were measured in triplicate, of which the mean was used. Phosphorylation of titin was assessed as previously described.[21] Proteins were transferred to PVDF membrane (Roth) and these were blocked with 3% bovine serum albumin (BSA, Sigma). For assessment of titin phosphorylation, site-specific antibodies directed to Serine 4010 (N2Bunique sequence (N2Bus) domain; PKA and extracellular signal-regulated kinase 2 (ERK2) target), and Serine 11878 (PEVK domain; protein kinase C (PKC) and Ca^{2+}/calmodulin dependent protein kinase II (CaMKIId) target) were used (Eurogentec Belgium), visualized with enhanced chemiluminescence detection kit (Amersham) and scanned with Amersham Imager 600. After stripping (RestoreTM Western Blot Stripping Buffer, Thermo Scientific), and blocking in 3% BSA membranes were incubated with an antibody directed to total titin (Eurogentech, Belgium), visualized with enhanced chemiluminescence detection kit (Amersham) and scanned with Amersham Imager 600.

To determine cMyBP-C protein level, proteins were separated on 4-15% pre-cast Tris-HCl gels (BioRad) and stained with SYPRO Ruby. The level of cMyBP-C was expressed relative to α-actinin. The same gels were stained with ProQ Diamond to determine phosphorylation of cMyBP-C.[22] The phosphorylation level of cMyBP-C was normalized to cMyBP-C expression and values given as a fraction of controls, which were set to 1. Phosphorylation of PKA sites in cardiac troponin I (cTnI) was analyzed using a specific antibody directed against serine 23 and 24 (Cell Signaling). The distribution of phosphorylated forms of cTnI was analyzed using Phos-tag acrylamide gels.[23]

Expression levels Ca^{2+}-handling proteins phospholamban (PLN, Abcam) and sarcoplasmic reticulum Ca^{2+}-ATPase 2 (SERCA2) were determined by Western blot analysis using specific antibodies and normalized to actin.

Histomorphometrical analysis

Histomorphological analysis was performed on 5 μm cryosections. Tissue was stained using Picro-Sirius Red staining to determine the extent of interstitial and replacement fibrosis, expressed as collagen volume fraction (CVF %). Cardiomyocyte myofibril density (MFD) was determined in a subset of patients of all groups using Electron microscopy (EM) as described previously.[24] The sum of myofibril area relative to cardiomyocyte area was expressed as a percentage.[24,25]

Data Analysis

Data in figures are presented as mean±standard error of the mean (SEM) per group. Data in tables is presented as mean±standard deviation (SD) per group. If data was normally distributed means were compared with a student's T-test, a Mann-Whitney test was used when data was not normally distributed. To test sex-differences in abnormality of clinical data a Chi-square test was performed. Correlations were tested by means of linear regression. $P<0.05$ was considered significant, sex-

differences are indicated by a hashtag (#) and differences in comparison to controls are indicated by an asterisk (*). As female HCM patients are on average older than male HCM patients additional analyses were performed in which we corrected for age at myectomy. For this we used a linear regression model with sex and age at myectomy as independent variables. Normality of residuals was checked. In case, residuals were not normally distributed a log-transformation was used for the dependent variable. The data and analytic methods are available to other researchers upon request for purposes of reproducing the results or replicating the procedure.

RESULTS

Female HCM patients are on average 7 years older at the time of myectomy

Echocardiographic analysis was performed to study sex-differences in cardiac diastolic parameters (Table 1). Detailed characteristics and parameters per patient are provided in supplemental Table 1. Female HCM patients were on average 7 years older than their male counterparts at time of surgery (50±13 vs 43±13 years; $p<0.05$). IVS thickness was increased in all patients, meeting the wall thickness criteria of ≥ 15 mm, with a mean of 23±7 mm (Figure 1A). No difference was seen in absolute IVS thickness between women and men. LVEDD in our patient population was within normal range[26] and showed no sex-difference (Table 1). LAD was increased compared to control values,[26] but did not show a sex-difference.

Greater impairment of in vivo diastolic function in female HCM patients at time of myectomy

As diastolic impairment is one of the hallmarks of HCM, we looked for sex-differences in *in vivo* diastolic function assessed by echocardiography at the time of myectomy. Echocardiographic measurements of diastolic function included E/e' ratio, E/A ratio and TR velocity (Table 1, Figure 1).[18,27]

E/e' ratio is the ratio between early peak diastolic mitral valve flow (E in cm/s) and the movement of the mitral valve annulus during early diastole (e' in cm/s). This ratio is a good indicator of LV filling pressure.[28] An E/e' ratio <8 implies normal filling pressure, while an E/e' ratio >15 indicates increased filling pressure.[29] In 42 HCM patients E/e' ratio was determined, of which 26 showed an increased E/e' ratio (92% of women and 48% of men; $p<0.01$). The average E/e' ratio was also significantly higher in the female HCM group compared to males (Figure 1B; 23.8±7.8 vs 14.8±3.4 respectively; $p<0.0001$). The normal range of LV filling pattern (E/A ratio) is between 0.8 and 2, and only 33% of our female patients fit within this range compared to 67% of our male patients (Figure 1C; $p=0.10$). TR velocity showed higher values in female compared to male HCM patients (Figure 1D; 2.61±0.54 vs 2.15±0.54 respectively; $p<0.05$). Overall, LV filling pressure, LV filling pattern and TR velocity were more abnormal in HCM women than men (Figure 1). The differences in diastolic cardiac properties between female and male HCM patients remained even after correction for age (Table 1, P values corrected for age).

Table 1. Patient characteristics and echo parameters of diastolic function

Sex	Female (27)	Male (44)	N	P value	Distribution	P value corrected for age
Age at myectomy, years	50±13 (27)	43±13 (44)	71	<0.05	Normal	
IVS, mm	23±9 (26)	22±6 (40)	66	0.65	Not normal	0.33[1]
LVOTO, mmHg	66±31 (23)	58±32 (39)	62	0.34	Normal	0.39
LVEDD, mm	43±5 (22)	43±6 (37)	59	0.81	Normal	0.77
LAD, mm	48±7 (20)	45±7 (33)	53	0.26	Normal	0.10
E, cm/s	95±35 (18)	74±21 (38)	56	<0.01	Normal	0.071
E/A ratio	1.8±1.0 (15)	1.3±0.5 (33)	48	0.10	Normal	<0.05
E/e' ratio	23.8±7.8 (13)	14.8±3.4 (29)	42	<0.0001	Normal	<0.0001
TR velocity, cm/s	2.61±0.54 (11)	2.15±0.54 (18)	29	<0.05	Normal	<0.05

Table 1 shows the patient characteristics and echocardiographic parameters reflecting diastolic function grouped by sex as mean±SD. [1]Linear regression on log(IVS). Residuals were normally distributed. Abbreviations: N (number of patients), IVS (inter ventricular septum); LVOTO (left ventricular outflow tract obstruction); LVEDD (left ventricular end-diastolic diameter); LAD (left atrial diameter); E (mitral E velocity); TR (tricuspid regurgitation) velocity. Detailed characteristics and parameters per patient are shown in supplemental Table 1, 2 and 3

Sex-differences in cardiac remodeling may be obscured by body size. IVS, LVEDD and LAD were therefore corrected for body surface area (BSA). After correction for BSA, indexed IVS thickness (IVSi) was significantly greater in HCM women compared to men with a difference of 2 mm/m^2 (Table 2). This might imply a more severe phenotype in our female HCM group. Correction of LVEDD for BSA did not reveal a sex-difference, while LA remodeling was affected more in female patients than in male patients evident from a significantly higher indexed LAD (LADi)(Table 2). Longstanding diastolic dysfunction leads to enlargement of the LA, with an LADi ≥ 24 considered to be abnormal.[26] An LADi above this cut-off value was seen in 44% of our HCM patient cohort who were predominantly female (65%; p<0.01). The average LADi of male patients (22±3 mm/m^2) falls within the normal range (15 to 24 mm/m^2), while we observed a significantly larger average LADi in females (Figure 1E; 26±3 mm/m^2; p<0.001). After correction for age, IVSi and LADi values were still significantly higher in female compared to male HCM patients (Table 2, P values corrected for age).

Diastolic dysfunction can be graded into three groups: Abnormal (grade I), pseudo normal (grade II) and restrictive relaxation (grade III). We graded our patients and found a significant difference in distribution between female (n=15) and male (n=30) HCM patients (Figure 1F; p<0.0001). 50% of our female patients showed grade III diastolic dysfunction, while 56% of our male patients showed grade I diastolic dysfunction. Taken together, the echocardiographic data indicate a higher degree of diastolic dysfunction in HCM women at the time of myectomy.

Table 2. LV and LA dimensions indexed for body surface area

Sex	Female	Male	N	P value	Distribution	P value corrected for Age
BSA, m^2	1.8±0.3 (19)	2.0±0.1 (34)	53	<0.001	Normal	
IVSi, mm/m^2	13.3±5.3 (19)	10.5±1.8 (32)	51	<0.05	Not normal	<0.01[1]
LVEDDi, mm/m^2	23±3 (16)	21±3 (32)	48	0.12	Normal	0.10
LADi, mm/m^2	26±3 (17)	22±3 (28)	45	<0.001	Normal	<0.01

Indexed LV and LA dimension as mean±SD. [1]Linear regression on IVSi. Residuals were normally distributed. Abbreviations: BSA, body surface area, IVSi (indexed interventricular septum thickness), LVEDDi (indexed left ventricular end-diastolic diameter), LADi (indexed left atrial diameter). Detailed characteristics and parameters per patient are shown in supplemental Table 1, 2 and 3.

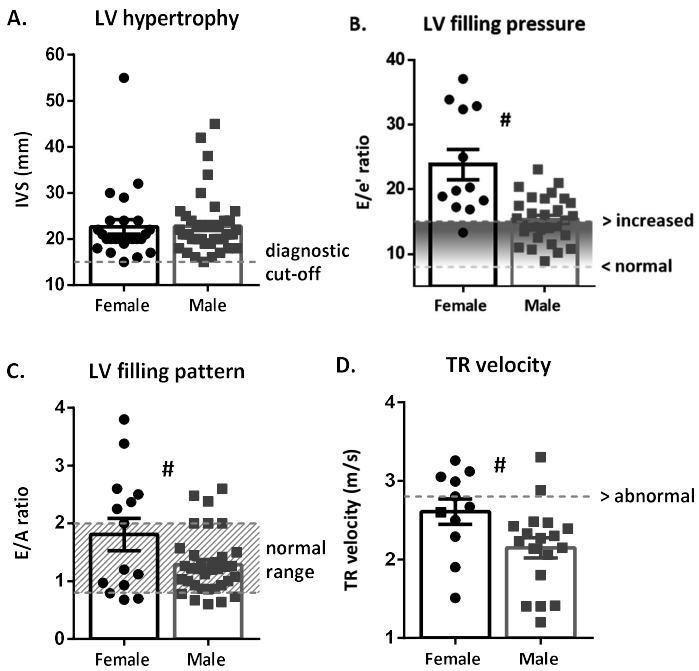


Figure 1. Influence of sex on echocardiographic parameters. A. Wall thickness of the interventricular septum was independent of sex (n=25(F)/44(M)). **B.** Female patients showed a higher E/e' ratio compared to men (p<0.0001)(n=13(F)/29(M)). **C.** LV filling pattern determined by the E/A ratio was more abnormal in female HCM patients (p<0.05)(n=15(F)/33(M)). **D.** Tricuspid regurgitation (TR) velocity was higher in female than in male HCM patients (p<0.05)(n=11(F)/18(M)).

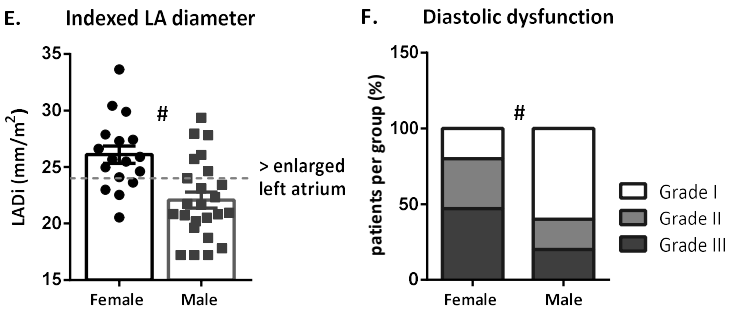


Figure 1 (continued). Influence of sex on echocardiographic parameters. E. Indexed left atrial diameter was higher in female compared to male HCM patients ($p<0.001$)(n=17(F)/28(M)). **F.** Diastolic dysfunction graded per patient: Female patients show greater impairment compared to men ($p<0.0001$)(n=15(F)/30(M)). Significant sex-differences are depicted by #. HCM values are illustrated relative to published values in the general population (i.e. normal values, indicated by dotted lines and marked areas).[1,18,29]

Cardiomyocyte stiffness is independent of sex

Impaired relaxation may be caused by mutation-mediated changes in the intrinsic properties of cardiomyocytes, hypertrophy and/or an increase in fibrosis.[8,30] At the cellular level, diastolic function can be influenced by passive tension of sarcomeres, which was assessed by single cardiomyocyte measurements. Data was compared to non-failing donor tissue to determine if the observed parameters deviate from control levels. Figure 2A demonstrates similar length-dependency of passive tension in female and male samples. Passive tension was not different between women (1.95±0.16 kN/m^2) and men (1.91±0.10 kN/m^2)(Figure 2B). Moreover, the HCM group did not significantly differ from control values (2.15±0.32 kN/m^2).

The number of myofibrils determine cellular passive tension and may be decreased as a result of hypertrophy.[31] Therefore, passive tension was corrected for myofibril density (MFD). Figure 2C and D shows representative EM images of HCM tissue used to determine MFD. In line with our previous study,[31] we found a significant decrease in MFD in HCM patients compared to controls (Figure 2E; 49±1% vs 65±2%; $p<0.0001$). No sex-difference in MFD was found in the HCM patient group. To test the hypothesis that the normal passive tension observed in HCM patients is caused by fewer but stiffer myofibrils, passive tension was corrected for MFD in a subset of patients (Figure 2F). Corrected passive tension was higher in HCM patients compared to controls, although not significantly (3.73±0.23 vs 2.88±0.42 respectively). Hence, passive tension of cardiomyocytes cannot explain the sex-difference in the degree of diastolic dysfunction.

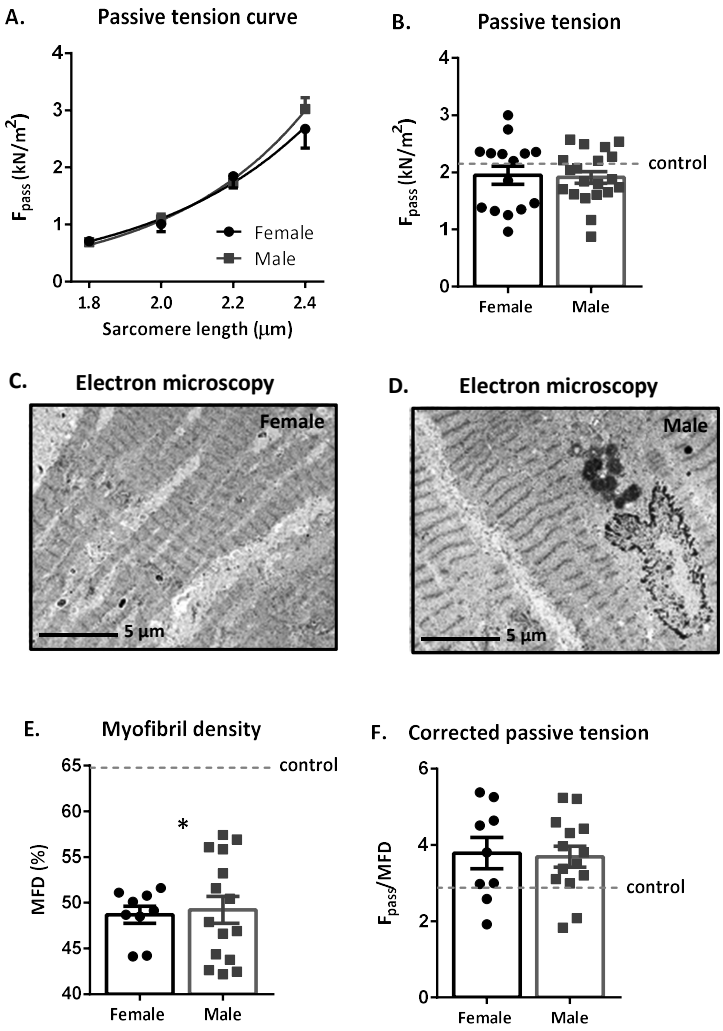


Figure 2. Myofilament passive tension. A. Passive tension (F_{pass}) measured at different sarcomere lengths in female (n=9) and male (n=10) HCM samples. B. Passive tension was measured at a sarcomere length of 2.2 µm in a larger group of samples (15 female and 20 male HCM samples; 15 controls). No sex-differences were observed in F_{pass}. **C and D.** Representative electron microscopy picture of a female and male HCM samples. **E.** Myofibril density was decreased in HCM patients (n=9(F)/15(M) in comparison to controls (n=9)($p<0.0001$). **F.** Passive tension at a sarcomere length of 2.2 µm corrected for myofibril density showed a trend towards an increase in HCM patients in comparison to controls ($p=0.0623$), but no sex-difference. Each data point reflects a mean of cardiomyocytes/EM pictures measured per patient. Significant differences between HCM and controls are depicted by *.

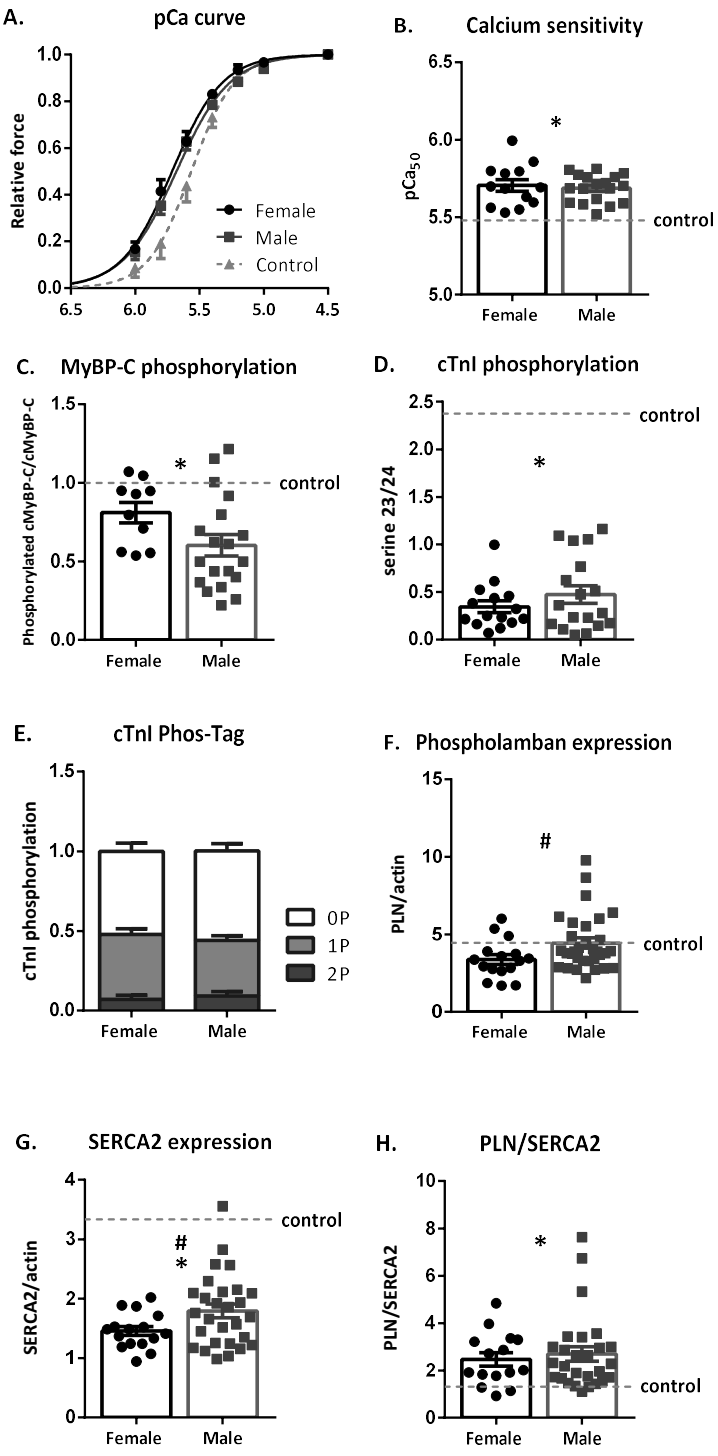
Absence sex-difference in myofilament calcium-sensitivity

Apart from high passive tension, high calcium-sensitivity may underlie impaired cellular relaxation.[32] From previous studies, it is known that calcium-sensitivity is increased in cardiomyocytes from HCM patients.[33] Consistent with previous studies, an increase in myofilament calcium-sensitivity was observed in the HCM patient group compared to controls (Figure 3A and B; $p<0.0001$), but no influence of sex on calcium-sensitivity was found.

Disease-related changes in myofilament protein composition and phosphorylation may underlie altered myofilament function. Mutations in *MYBPC3* were associated with reduced cMyBP-C expression (i.e. haploinsufficiency).[34,35] Accordingly, in the present study we observed cMyBP-C haploinsufficiency in HCM patients carrying *MYBPC3* mutations, which was independent of sex (females: 0.55±0.02 and males 0.55± 0.03 vs controls 0.81± 0.03; $p<0.0001$). Protein kinase A (PKA)-mediated phosphorylation, via activation of the ß-adrenergic receptor pathway, is down-regulated in HCM.[36] cMyBP-C and cTnI are sarcomeric proteins that are phosphorylated by PKA and thereby may influence myofilament function (i.e. reduction in myofilament calcium-sensitivity). In line with reduced PKA-mediated phosphorylation, phosphorylation levels of cMyBP-C were decreased in HCM patients (female 0.81± 0.07 and male 0.60± 0.07) compared to controls (1.00± 0.04; $p<0.001$). The sex-difference in overall cMyBP-C phosphorylation may be explained by site-specific phosphorylation differences between female and male as cMyBP-C is phosphorylated at multiple sites which are target for different kinases.[37] The decrease was largest in male patients (Figure 3D). A decrease was also found in cTnI phosphorylation in HCM patients compared to controls (Figure 3D; F 0.35± 0.06 and M 0.47± 0.09 vs controls 2.38± 0.49; $p<0.0001$). No sex-difference was found on cTnI phosphorylation. Accordingly, Phos-tag analyses of the different forms of cTnI phosphorylation (un-, mono-, and bis-phosphorylated) showed a similar pattern in women and men (Figure 3F).

Lower expression of Ca^{2+}-handling proteins in myectomy samples from female compared to male HCM patients

PLN and SERCA2 are key regulators of myocardial relaxation during diastole. PLN expression levels did not differ between HCM patients and controls (4.45±0.66). There was however a lower expression of PLN in female compared to male HCM patients (Figure 3F; 3.38±0.31 vs 4.44±0.33; $p<0.05$). SERCA2 expression was considerably lower in HCM patients (female 1.46±0.08 and male 1.79±0.12) compared to controls (Figure 3G; 3.34±0.24; $p<0.0001$). SERCA2 also showed a sex-difference with lower expression levels in female compared to male HCM patients ($p<0.05$). PLN/SERCA2 ratio was higher in HCM patients compared to controls (Figure 3G: female: 2.47±0.29 and male: 2.70±0.31 versus controls: 1.32±0.18) but did not show a sex-difference.


A.
pCa curve
Relative force
1.0
0.8
0.6
0.4
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6.5
6.0
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4.5
Female
Male
Control
B.
Calcium sensitivity
pCa50
6.5
6.0
5.5
5.0
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control
Female
Male
C.
MyBP-C phosphorylation
Phosphorylated cMyBP-C/cMyBP-C
1.5
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Female
Male
D.
cTnI phosphorylation
serine 23/24
2.5
2.0
1.5
1.0
0.5
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control
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Female
Male
E.
cTnI Phos-Tag
cTnI phosphorylation
1.5
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0.5
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0P
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2P
Female
Male
F.
Phospholamban expression
PLN/actin
15
10
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control
Female
Male
G.
SERCA2 expression
SERCA2/actin
4
3
2
1
0
control
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Female
Male
H.
PLN/SERCA2
PLN/SERCA2
10
8
6
4
2
0
*
control
Female
Male

Figure 3. Myofilament calcium-sensitivity. A. Calcium-force relationships at a sarcomere length of 2.2 μm of female (n=11) and male (n=15) HCM samples are shifted to the left in comparison to controls (n=7). **B.** Myofilament calcium-sensitivity (pCa_{50}) was significantly higher in HCM patients ($p<0.0001$; n=13(F)/18(M)) compared to controls (n=13), while no sex-difference was observed. Each data point reflects a mean of cardiomyocytes measured per patient. **C.** MyBP-C phosphorylation ($p<0.001$) was decreased in HCM patients (10 females/19 males) compared to controls (12). D **and E.** In comparison to controls (n=17), phosphorylation of cTnI was decreased in HCM patients (15 females/18 males), with an even distribution in (un)phosphorylated cTnI forms between women and men. F**.** Phospholamban (PLN) expression was lower in female (n=16) than in male (n=30) HCM patients. **G.** SERCA2 expression was lower in HCM patients compared to controls ($p<0.0001$; n=11) with an even larger decrease in female than in male patients ($p<0.05$; 16 female /28 male). **H.** The PLN/SERCA2 ratio was higher in HCM patients compared to controls ($p<0.05$). Significant sex-differences are depicted by #; Significant differences between HCM and controls are depicted by *.

More compliant titin in female HCM patients

Another important contributor to diastolic function is titin.[38,39] Titin functions as a molecular spring thereby modulating passive stiffness of cardiomyocytes, and has been shown to regulate passive stiffness in an isoform-dependent manner.[39] In cardiac muscle two major isoforms are identified: a short, more stiff isoform (N2B) and a long, more compliant isoform (N2BA). Both isoforms are co-expressed within the human heart and can be displayed in a N2BA/N2B ratio to describe titin-based passive stiffness.

A representative titin gel is depicted in Figure 4A. Sex had a profound effect on titin isoform composition in HCM patients, as women showed more compliant titin compared to men (Figure 4B; $p<0.05$). This sex-effect on titin composition was not seen in controls (0.58±0.06 (F) versus 0.70±0.09 (M); mean value controls 0.63±0.20). The male HCM group (0.74±0.04) showed no difference compared to controls, but female HCM patients had more compliant titin isoform (1.00±0.10) compared to controls ($p<0.01$). The titin N2BA/N2B ratio significantly correlates with diastolic dysfunction (Figure 4C; $p<0.01$; R^2 0.29).

Phosphorylation of titin also influences passive stiffness of the cardiomyocytes. PKA-mediated phosphorylation at serine 4010 decreases passive tension while PKC-mediated phosphorylation at serine 11878 increases passive tension.[40,41] Representative blots of both phosphorylation sites are shown in Figure 4D. PKA phosphorylation did not show sex-differences (Figure 4E; female (n=10) 0.95±0.05 versus male (n=13) 0.96±0.07). In addition, no difference was observed in PKC phosphorylation of titin (Figure 4F; female (n=5) 1.07±0.19 versus male (n=6) 1.21±0.31).

Our data show that titin isoform changed in a sex-dependent manner with a shift towards a more compliant isoform in women, while no sex-differences in phosphorylation are present. The increase in compliant titin isoform appears to be related with the degree of diastolic dysfunction.

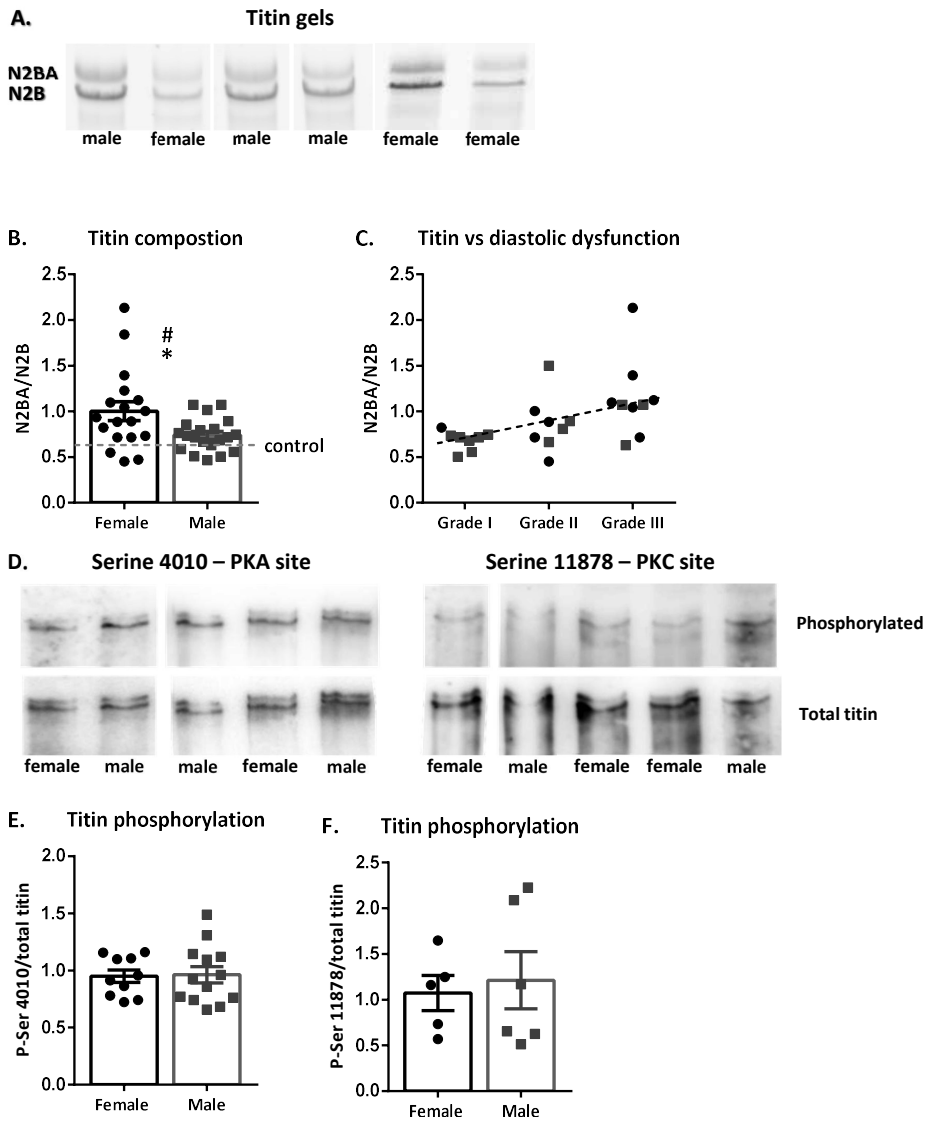

Figure 4. Increased compliant titin isoform in female HCM patients. A. Representative titin gel showing N2BA and N2B titin isoforms of HCM patients. **B.** N2BA/N2B ratio was increased in female (n=18) HCM patients in comparison to controls ($p<0.01$; n=15) and significantly higher than in male (n=20) HCM patients ($p<0.05$). **C.** Scatterplot showing a significant correlation (linear regression) between N2BA/N2B ratio and the grade of diastolic dysfunction ($p<0.01$; R^2: 0.29) **D.** Representative titin phosphorylation blots of PKA and PKC sites on titin. **E and F.** Phosphorylation of both the PKA (serine 4010) and PKC (serine 11878) site did not show sex-differences. Each data point reflects a mean titin ratio measured per HCM patient and control. Significant sex-differences are depicted by #; Significant differences between HCM and controls are depicted by *.

More fibrosis in female HCM patients

Stiffness of the cardiomyocyte is dependent on the cellular components mentioned above. However, changes in the extracellular matrix can influence cardiac stiffness as well. Both replacement-, and interstitial fibrosis are known to increase in HCM patients during remodeling, and negatively influence cardiac compliance.[42,43] Figure 5A shows representative images of Picro-Sirius red stainings, from which CVF was determined. As expected our controls showed little fibrosis (1.21±0.23%), and no differences were found between women and men. CVF in HCM patients was significantly increased compared to control values ($p<0.0001$), with a significantly larger increase in female HCM patients compared to males (Figure 5B; 6.42±0.95 versus 4.18±0.66%; $p<0.05$).

Sex-differences in titin isoform composition and fibrosis in the HCM patient group remained after correction for age, while no significant sex-difference was present for the Ca^{2+}-handling proteins SERCA2 and PLN after age correction (Table 3).

Table 3: Protein analyses: differences between males and females before and after correction for age.

Protein	N (female/male)	P value	Distribution	P value corrected for age
PLN/actin	16/30	<0.05	Not normal	0.090
SERCA2/actin	16/28	<0.05	Normal	0.054
Titin N2BA/N2B	18/20	<0.05	Normal	<0.05
Fibrosis	11/13	<0.05	Not normal	<0.05

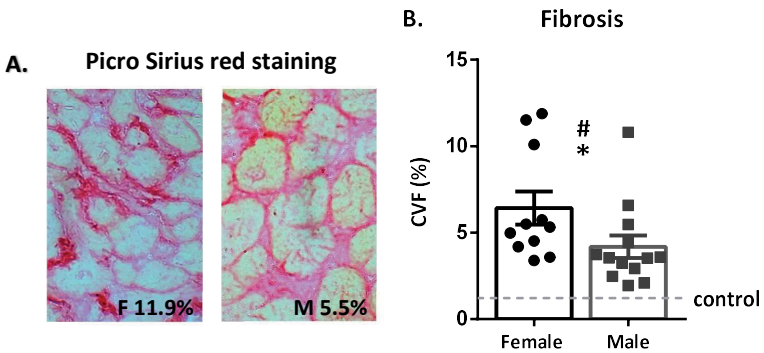

Figure 5. Higher amounts of fibrosis in HCM women. A. Representative Picro-Sirius red staining on a female (11.9%) and male (4.4%) HCM sample. The deep red staining depicts fibrosis. **B.** Fibrosis was higher in female compared to male HCM patients ($p<0.05$). Furthermore, the HCM group in total showed more fibrosis compared to controls ($p<0.0001$). Each data point reflects a mean collagen volume fraction measured per HCM patient. Significant sex-differences are depicted by #; Significant differences between HCM and controls are depicted by *.

DISCUSSION

Here, we investigated if changes in diastolic characteristics of the heart in HCM patients at the time of cardiac surgery are sex-dependent. Main findings of the study are that women with HCM have greater diastolic dysfunction than men at the time of surgery, apparent from the increased LV filling pressures, altered filling pattern, greater TR velocities and more pronounced LA remodeling. Factors contributing to impaired relaxation in HCM are increased calcium-sensitivity and fibrosis, with fibrosis being higher in women. Furthermore, the compliant titin isoform is increased in female HCM patients, suggesting a sex-difference in the compensatory response to the progression of diastolic dysfunction.

In this study, female HCM patients that underwent myectomy were on average 7 years older at time of surgery than men. *Olivotto et al.* found an age difference of 9 years at time of diagnosis and initial evaluation, with more advanced symptoms in female HCM patients.[14] A number of other studies have confirmed these findings.[15,44,45] Women are underrepresented in the patient population in the present (38%) and multiple previous HCM studies (~40%).[10,14–16,43–48] We found greater diastolic dysfunction in our female HCM patients, which is in line with a previous study.[12] The preload-independent measure for diastolic function (E/e' ratio) was in the pathological range in 92% of our female HCM patient group compared to 48% of the male group. Increases in LAD reflect the chronic burden of increased diastolic pressures on the LA.[46] In most of our HCM population LAD was increased, confirming previous studies.[49,50] We did not find a sex-difference in the absolute values of LAD, but after indexing LAD for BSA, LADi was significantly higher in women than in men. LADi values in our female HCM group range from moderately to severely abnormal, while in our male group values range from normal to mildly abnormal.[26] LV wall thickness of ≥15 mm is currently the diagnostic criteria for HCM, without adjustment for BSA or sex.[1] In line with earlier published data, absolute IVS thickness is independent of sex.[47,48] However, when we indexed IVS thickness for BSA, female patients show a greater IVSi compared to male patients. The increases of LADi and IVSi in our female patients could simply be due to the more advanced age. However, there was no correlation between age and IVSi and only a weak positive correlation between age and LADi ($p<0.01$;$R^2=0.20$). There were only two parameters that significantly correlated with age: LV filling pressure (E/e') increases with age, while phospholamban expression decreases with age in both males and females (Supplemental Figure 2). In addition, after correction for age *in vivo* echocardiographic parameters of diastolic dysfunction and cardiac remodeling (IVS, and LADi) remained significantly different between female and male HCM patients (Tables 1 and 2). Overall, our data show a more severe stage of cardiac dysfunction and remodeling in females than in males at the time of myectomy.

On a cellular level, titin is an important contributor to cardiomyocyte stiffness and thereby diastolic function. Specifically the ratio between the stiffer N2B isoform and the more compliant N2BA isoform.[51,52] Our group of non-failing control samples had a titin isoform ratio of 0.63±0.20 which is comparable to previously published data.[53–55] We showed for the first time a higher

N2BA/N2B ratio in female compared to male HCM patients. However, titin phosphorylation by PKA and PKC did not show sex-differences. As no sex-difference in passive tension was observed, other post-translational modifications of titin (e.g. induced by oxidative stress)[56] may counterbalance the higher level of compliant titin isoform in women. A correlation, independent of sex, was found between diastolic dysfunction and titin composition, which implies that the switch to longer titin isoforms is an attempt to compensate for the impaired relaxation. Despite the titin isoform change, women still show more severe diastolic dysfunction. A rat study showed that under metabolic stress female rats show an increase of the compliant titin isoform.[38] Furthermore, ovariectomized rats show an even greater increase, suggesting an influence of female hormones on titin isoform switch during stress. Estrogen does not seem to have a direct effect on titin isoform expression but could, during stress, act as a modulator enabling isoform switch.[38] The increase in titin compliance could also be a reaction to the increase in interstitial fibrosis seen in HCM patients, a correlation, however, was not found. Previous clinical studies in HCM patients showed more fibrosis in men,[10,57] except for *Chen et al.*[12] who showed no sex-difference. These studies have measured fibrosis through late gadolinium enhancement. Measuring interstitial fibrosis with the latter technology is not possible and might explain the contrast to our results.

Although more compliant titin isoform was observed in female compared to male HCM, we did not observe a sex-difference in cardiomyocyte passive tension. Moreover, HCM values did not differ from control values. In line with our results, *Hoskins et al.* did not find a difference in passive tension between HCM cardiomyocytes and controls.[58] Correction for MFD also did not reveal a significant sex-difference in passive tension. The increase in myofilament calcium-sensitivity in HCM cardiomyocytes could partly explain the diastolic dysfunction, although our data suggests that sex is not an influencing factor regarding calcium-sensitivity. The lower expression of PLN and SERCA2 in female compared to male HCM patients may underlie the sex-difference in diastolic dysfunction, although the PLN/SERCA2 ratio did not differ between female and male patients.

Limitations and clinical implications

Our study focused on patients who underwent myectomy, while many HCM patients receive alcohol septal ablation (ASA) to relieve LVOTO. As age and disease severity may differ between ASA and myectomy patients, the gender difference observed in the present study may not apply to the ASA group. Most HCM patient samples included in our study came from the Erasmus Medical Center. In a 20-year period (1996-2016), 23% of patients received ASA at the Erasmus Medical Center. There was no age difference between the ASA and myectomy group (52±16 vs 52±15 years), and in both groups 60% was male. Previous single and multicenter studies which compared outcome in ASA and myectomy patients showed that the age at the time of intervention was slightly higher in the ASA than in the myectomy group, but no differences were present in baseline functional/anatomical characteristics of the heart (similar maximal wall thickness, LVOT gradient, systolic and diastolic

dysfunction).[59–61] Thus, currently there are no indications that disease severity is different between ASA and myectomy patients.

The number of *MYBPC3* mutations in our study is high compared to the previously reported average *MYBPC3* mutation frequency (~20%).[62] In the Netherlands, HCM is dominated by 3 *MYBPC3* founder mutations,[63] which is why the percentage of *MYBPC3* compared to *MYH7* is relatively high. This may limit translation of our observations to the general HCM population, because of differences in disease onset and progression between mutation groups. However, no difference was found in disease penetrance between *MYBPC3* founder mutations and other *MYBPC3* mutations.[63] A recent meta-analysis by Sedaghat-Hamedani and colleagues reported an average mutation frequency of 20% in *MYBPC3* and 14% in *MYH7*.[62] The meta-analysis showed a lower mean age of onset in *MYH7* (35 years) compared to *MYBPC3* (39 years), and a higher risk of ventricular tachycardia in *MYH7* compared to *MYBPC3*, while no differences were observed in mean IVS thickness and LVOTO. The age of onset showed large heterogeneity in all mutation groups which is characteristic for HCM. Nannenberg and colleagues showed increased mortality risk in specific age categories (ranging from 10-19 years to 50-59 years) in HCM families with *MYBPC3* mutations.[64] These studies show that disease onset and progression are highly variable in all mutation groups. In our patient group, no difference was observed in age of onset, LVOTO and IVS thickness between *MYH7* and *MYBPC3* mutation groups, indicating that the clinical indications for myectomy were similar in both groups. Moreover, both mutation groups had a similar percentage of women, respectively 41% in the *MYH7* mutation group (7 out of 17) and 37% in the *MYBPC3* mutation group (20 out of 54). The average age distribution was also comparable in both mutation groups (mean age: *MYH7* group: 49 years in females, 44 years in males; *MYBPC3* group: 48 years in females, 44 years in males).

To conclude, our clinical data shows that women are older at time of operation and have more advanced diastolic dysfunction, even upon correction for age. We found increased titin compliance, lower PLN and SERCA2 expression and more interstitial fibrosis in female compared to male HCM patients. Data from our HCM patient group suggests that disease severity may be underestimated in women with a similar IVS, but higher IVSi compared to men.

Acknowledgments

We would like to give special thanks to Dirk de Jong who has been a great help by digitalizing all of our EM pictures. We thank Peter van de Ven for statistical analyses. We acknowledge the support from the Netherlands Cardiovascular Research Initiative, an initiative with support of the Dutch Heart Foundation, CVON2011-11 ARENA.

Disclosures: none.

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CLINICAL PERSPECTIVE

What is new? This study shows sex-differences in HCM patients at the time of myectomy with respect to diastolic (dys)function and parameters influencing passive stiffness on a cellular level. Female HCM patients showed a higher degree of diastolic dysfunction (measured by echocardiography) at the time of myectomy. At a cellular level female HCM patients showed a higher amount of fibrosis and more compliant titin compared to male HCM patients. One of the diagnostic criteria for HCM is a septal thickness of >15 mm, which was independent of sex. However, when corrected for body mass index, we found a higher indexed IVS thickness in female compared to male HCM patients.

What are the clinical implications? The higher indexed IVS thickness in women implies a more progressed state of HCM in women than in men at time of surgery. The degree of diastolic dysfunction and sex-related cellular differences may be explained by the progression of the disease. Future research is needed to investigate if sex-specific diagnostic protocols (indexed for body mass) are warranted.

EPILOGUE

Summary and future perspectives

Nederlandse samenvatting

List of publications

PhD portfolio

About the author

Dankwoord

SUMMARY AND FUTURE PERSPECTIVES

SUMMARY

Genetic testing and family screening

A pathogenic mutation is identified in 50-60% of patients with HCM.(1) Information on the prognostic value of genotype is currently limited.(2) Therefore, in **chapter 1** we studied the clinical phenotype and outcome of 234 genotype-positive (G+) and 278 genotype-negative (G-) patients with HCM. At first evaluation, G+ probands were younger than G- probands, had more non-sustained ventricular tachycardia, more often a history of syncope and more extreme hypertrophy. In contrast, G- probands were more symptomatic and had higher left ventricular outflow tract gradients. Most likely, other genetic variants and lifestyle factors (hypertension) are involved in the pathophysiological process of G- HCM.(3-5) In comparison to probands, relatives with HCM who were identified via family screening were younger and had a more benign phenotype. It seems that family screening leads to the detection of disease in an earlier stage.(6) During 12±9 years follow-up of HCM probands, G+ status was an independent risk factor of all-cause mortality, cardiovascular mortality, heart failure related mortality, and sudden cardiac death (SCD). These findings are consistent with prior studies that demonstrate a predictive value of G+ status for adverse outcome.(7-10) In order to develop genotype-specific risk-assessment and targeted therapies, fundamental research on the pathophysiological consequences of sarcomere mutations is crucial.(11, 12)

Myosin-binding protein C (*MYBPC3)* founder mutations have been identified in populations in Iceland, Italy, Finland, Japan, France, and the Netherlands(13) and have arisen from common ancestors many generations ago.(14, 15). In the Netherlands, 35% of HCM cases are caused by 3 *MYBPC3* founder mutations: c.2373dupG (p.Trp792Valfs*41), c.2827C>T (p.Arg943*), and c.2864_2865delCT (p.Pro955fs*95).(13, 14, 16) They cause C-terminally truncated protein leading to haploinsufficiency(17-19), and are associated with a reduced force generating capacity of cardiomyocytes, cardiomyocyte hypertrophy and reduced myofibril density.(17, 20) In **chapter 2**, we analyzed 271 individuals with Dutch *MYBPC3* founder mutations (134 probands and 128 relatives), and compared the clinical findings and outcome with that of 132 nonfounder G+ probands with HCM and 277 G- probands with HCM. There was no difference between *MYBPC3* founder mutation HCM and nonfounder mutation G+ HCM, contradicting the existing notion that *MYBPC3* founder HCM is more benign than HCM caused by other mutations.(15, 16, 21, 22) In this study we also compared patients with HCM who presented with signs or symptoms of disease and relatives who presented in the context of family screening. Relatives with HCM had significantly better clinical outcome, most likely reflecting earlier disease stages. This illustrates that the way of presentation has important prognostic value. Again, our study demonstrated the extreme phenotypic variability in individuals with the same mutation. In fact, a small proportion of the individuals with *MYBPC3* founder mutations had non-compaction or dilated cardiomyopathy instead of HCM. This was partly explained by multiple sarcomere mutations. Indeed, the phenotypic variability which we observe in genetically homogeneous

groups is most likely explained by additional sarcomere or nonsarcomere mutations, and otherwise by nongenetic factors.(23) Further collaborative bench-to-bedside investigation is needed to unravel this genotype-phenotype ‘’black box’ (figure 1).

Figure 1. The genotype-phenotype ‘’black box’’.

When HCM is diagnosed in a patient, cardiologists need to inform the patient about the genetic aspect of the disease and organize appropriate cardiac evaluation of their relatives.(24) Guidelines have encouraged family screening by electrocardiography and echocardiography since 2003, with evaluations starting at the age of 10 years and repeating evaluations until advanced age.(25-28) Recent European guidelines recommend to include genetic testing (GT) in the screening strategy(25); a cost-effective approach (29, 30) which allows the reassurance and discharge of genotype-negative relatives and the identification of G+ relatives at risk for the development of HCM.(24) In **chapter 3**, we evaluated the results of this contemporary screening strategy by analyzing GT results and clinical findings in 777 relatives descending from 209 patients with HCM. GT was performed in the majority of patients (93%) and relatives (80%) leading to the reassurance and discharge of 356 (46%) genotype-negative relatives. First cardiac evaluation in 264 G+ relatives and 157 relatives without GT revealed HCM in 37% and 17% respectively. During follow-up, cardiac mortality among relatives with HCM was lower than is generally observed in patients with HCM (0.3%/y vs 1-2%/y) reflecting early disease stages and possibly, the effect of lifestyle modifications, periodic SCD risk evaluation and close clinical follow-up. In the 165 G+ relatives without HCM only one SCD occurred which was attributable to a long-QT mutation, illustrating a benign prognosis. During 7 years of echocardiographic follow-up in 178 relatives without HCM (65% G+), 29 (16%) developed subtle HCM (24 G+). Interestingly, the development of HCM was not observed during adolescence, which is thought to be a notorious phase for the development of HCM due to hormonal changes.(31) Conversion was rather observed before the age of 12 years and between the ages of 20 and 30 years. Therefore, we propose cardiac evaluations to be initiated earlier i.e. at the age of 8 years, repeating them every 2-4 years in children and once every 5 years in adults, which may be continued until advanced age. From the results in this study we conclude that GT facilitates HCM family screening by

reducing the number of clinical screening visits to the outpatient clinic. However, the feasibility of this screening strategy depends on on the genetic yield in the probands and on the uptake of GT in the families, both of which were relatively high in this study. The high genetic yield may partly be explained by the high prevalence of *MYBPC3* founder mutations in the Netherlands(14, 32), and partly by a referral bias since relatives from G+ families are informed about the confirmed heritability of HCM and the HCM burden in G+ families is likely to be higher.(33) The high uptake of GT can be explained by the fact that GT is covered by our national basic health care insurance and because cardiac evaluation and genetic counselling and testing is offered simultaneously at our cardio-genetic outpatient clinic. Other countries have different financial and organizational approaches regarding GT in the HCM population. We must be aware of the potential psychosocial, emotional, and financial consequences of GT, especially since the prognostic value of genotype for disease-onset and risk is currently still limited.(2) In children, we generally do not perform GT before the age of 18 years.

Imaging in hypertrophic cardiomyopathy

Due to the age-related penetrance of sarcomere mutations, lifelong follow-up is advised in G+ relatives who do not express the clinical HCM phenotype (‘’HCM mutation carriers’’).(25, 26) Currently, we are unable to predict the development of HCM in HCM mutation carriers. Prior cross-sectional investigations have identified pre-phenotypic markers, such as diastolic dysfunction(34-36), altered myocardial energetics(37), electrocardiography abnormalities(38), myocardial crypts(39-41), and mitral leaflet elongation(39, 42-44). However, longitudinal follow-up studies of HCM mutation carriers are scarce.(45) Since the development of HCM in HCM mutation carriers is generally very slow and occurs infrequently, it takes a considerable amount of time before enough data is collected. In the following two chapters we studied several functional and morphological features in HCM mutation carriers and performed longitudinal follow-up in order to assess its predictive value for the development of HCM. In **chapter 4**, we used two-dimensional echocardiography to measure anterior mitral valve leaflet (AMVL) length in 133 HCM mutation carriers and 135 healthy controls. AMVL length did not differ between the groups. In the 13/80 mutation carriers who developed HCM, AMVL length had no predictive value in contrast to pathological Q waves, E/e’ ratio and maximal wall thickness. These findings contradict the concept that AMVL elongation is a primary phenotypic feature of HCM.(39, 42-44) The etiology of AMVL elongation in patients with HCM is still unclear.(46) In **chapter 5**, we used speckle tracking echocardiography to assess the global longitudinal strain (GLS) in 120 HCM mutation carriers and 110 healthy controls. In patients with HCM, previous studies consistently demonstrate a reduced GLS indicating subclinical systolic dysfunction.(47-51) GLS was statistically higher in HCM mutation carriers than in controls, however many of the measurements overlap. In the 13/80 mutation carriers who developed HCM during follow-up, GLS had no predictive value. And so, GLS is not a useful parameter to discriminate HCM mutation carriers from normal individuals nor a useful predictor for the development of HCM. Why GLS is increased in

HCM mutation carriers is currently unclear. It might be a result of mutation-induced cardiomyocyte hypercontractility or a compensatory mechanism for regional hypocontractility.(52, 53) Indeed, whether sarcomere mutations cause hyper- or hypocontractility of the cardiomyocyte is subject to ongoing investigations.(54) Fundamental research has an important role in identifying primary triggers for the development of HCM.

The usefulness of three-dimensional echocardiography (3DE) has been demonstrated in the evaluation of LV volumes and mass and visualization of heart valves.(55-58) In **chapter 6**, we investigated the usefulness of 3DE for the assessment of LV hypertrophy and papillary muscle (PM) morphology. We obtained 3DE images in 24 patients with HCM and 31 healthy controls, and identified a spiral pattern of LV hypertrophy in the patients with HCM. Also, we found that both PMs were significantly larger in patients with HCM than in controls, and enlargement of the posteromedial PM was associated with LV outflow obstruction. Since the location and morphology of the PM is important for determining the mechanism causing LV outflow obstruction and thus for determining the appropriate technique to relieve outflow obstruction(59), 3DE might in the future be of clinical value. Additional research is however needed to study the feasibility and reproducibility of 3DE imaging in the evaluation of PM morphology.

Clinical aspects of hypertrophic cardiomyopathy

Atrial fibrillation (AF) is the most common arrhythmia in patients with HCM and an independent predictor of morbidity and mortality, mainly heart failure or stroke-related.(60, 61) Due to the high incidence of stroke, lifelong therapy with oral anticoagulants is recommended in all patients with HCM and AF, even when sinus rhythm is restored and irrespective of the CHA2DS2-VASc score.(25, 26) AF can be difficult to detect, due to the often episodic and paroxysmal nature. Guidelines recommend 24-48 hour ambulatory electrocardiographic monitoring every 6-12 months, depending on the size of the left atrium.(25) Patients with a cardiac implantable electronic device (CIED) often have continuous monitoring of atrial activity, allowing for the detection of subclinical AF. In **chapter 7** we studied the incidence and impact of device-detected AF in 114 patients with HCM and a CIED. During 2.8 years follow-up, the annual incidence of device-detected AF was 7%, which is high in comparison to the previously reported incidence of AF in patients with HCM (2-3%).(62) This might be related to the fact that patients with a CIED have more advanced disease. Also, the device may have detected more AF episodes in comparison to traditional intermittent monitoring strategies. Since some of the patients included in the study had devices without atrial sensing capabilities, an underestimation of the incidence might have occurred. The incidence of thromboembolism was relatively low (1.3%), possibly because oral anticoagulants were initiated, and the majority of AF cases were consequently treated with antiarrhythmic medication or electrocardioversion. The findings in this study demonstrate the added value of CIEDs in the detection of AF and the implications for management. It might be a

stimulus for physicians to consider devices which allow atrial sensing, such as a single-chamber system with a floating bipole in the atrium.(63)

Gender differences have been observed in many cardiovascular diseases, such as ischemic heart disease, heart failure, hypertension, and aortic valve stenosis.(64) In HCM, gender has been proposed to impact the age of onset and the phenotype.(4, 65-75) In addition, some studies found an independent association between female sex and all-cause mortality(76, 77), while other studies did not.(67, 78, 79). In **chapter 8** we analyzed the clinical presentation, phenotype, genotype, and outcomes of 1007 patients (620 male, 387 female) who were evaluated between 1977 and 2017. We found that at presentation female patients were older, more frequently had a history of hypertension, and presented more frequently with symptoms. In addition, female patients more frequently had an impaired systolic and diastolic function and more frequently exhibited LVOT obstruction. During 6.8 years follow-up, female patients had increased all-cause mortality, cardiovascular mortality, and HF related mortality. Multivariable cox regression analysis demonstrated that the worse survival in women was attributable to the worse baseline status. We did not find an independent association between female sex and mortality. Why female patients present at a more advanced age in a worse condition is currently unclear. Sociocultural processes (lack of attention to early clinical signs in women or diagnostic bias) or differences in sexual hormones and gene expression possibly account for the delay.(64, 65, 78, 80, 81) Interestingly, we found that adjusting echocardiographic indices to body surface area (more specifically, maximal wall thickness, left atrial size, and left ventricular end-diastolic dimension) changed the clinical picture of females versus males dramatically, so that it illustrated a worse phenotype in females than we would suspect based on unadjusted indices. Currently, indices are not adjusted to body surface area which may have caused undertreatment of women by underestimation of disease severity. Additional gender studies are needed to elaborate on these findings. Future studies are needed to investigate further the cause of delayed diagnosis in women in order to try to advance diagnosis in women and thus improve outcome.

Finally, in **chapter 9** we go from bedside-to-bench by studying sex-differences in the echocardiographic (E/e' ratio, E/A ratio, tricuspid regurgitation, left atrial size) and cellular (passive tension, myofilament Ca^{2+} sensitivity, expression of Ca^{2+} handling proteins, titin isoform expression, interstitial fibrosis) parameters of diastolic function in patients with HCM. Cardiac tissue was obtained during surgical myectomy in 71 patients (38% female) preceded by echocardiography. Females were significantly older at the time of myectomy, and diastolic function was more severely affected than in males. The cellular analyses revealed more interstitial fibrosis in females, but no sex-difference regarding passive tension or myofilament Ca^{2+} sensitivity. Compliant titin isoform was increased in women, possibly reflecting a compensatory mechanism. Overall, the data showed a more severe stage of cardiac remodeling in females than in males at the time of myectomy. In addition, the current study

supports previous literature which states that women with HCM have a delayed clinical presentation.(65, 78) Factors causing this delay are unclear and warrant additional investigations. Again, since wall thickness was significantly higher in women than in men after correction for body surface area but appeared to be similar to men before correction to body surface area, we postulate that maybe disease severity is underestimated in women due to the fact that currently HCM diagnosis is based on absolute cutoff values of wall thickness without adjusting to gender or body surface area.

FUTURE PERSPECTIVES

Ultimate goals in the HCM scientific field are to prevent the development of HCM, improve quality of life and decrease symptoms associated with HCM, and to prevent life-threatening events (sudden cardiac death, stroke, heart failure). In order to realize this, we need to define all causes of HCM, unravel the pathophysiological pathways which lead from mutation to disease, and improve the risk stratification for sudden cardiac death and heart failure.

Advances in genetic testing

Currently, a pathogenic mutation is identified in about 50-60% of patients with HCM.(1) Whole-exome and whole-genome sequencing may uncover novel disease-causing genes. These new technologies demand major efforts regarding the handling of data and variant interpretation.(82) This includes the time-consuming manual analysis of large amounts of variants in order to select those most likely to have clinical relevance.(82) Subsequently, variants are classified into one of 5 classes (pathogenic, likely pathogenic, etcetera), a process which involves several steps including a literature review, co-segregation analysis, the use of predictive tools and in-vitro studies.(83) Genotype-negative HCM (in particular, the nonfamilial HCM subtype(84)) has a better prognosis and different clinical features (chapter 1). In order to fully understand the pathophysiology of genotype-negative HCM, future studies investigating disease modifiers such as (epi)genetic variations and environmental triggers are needed.(11) Also, the extreme phenotypic variability observed among individuals carrying the same pathogenic mutation (chapter 2) underlines the complexity of the HCM pathophysiology. Further collaborative bench-to-bedside investigation is needed to unravel this genotype-phenotype ‘’black box’. (11, 12, 85)

Improved phenotyping

Advanced imaging studies using speckle-tracking and three-dimensional echocardiography, cardiac magnetic resonance, and positron emission tomography(86), may reveal structural or functional disease manifestations of sarcomere mutations. Genotype-positive, phenotype-negative family members are ideal for gaining insight into the early manifestations of HCM (chapters 4 and 5). Future longitudinal follow-up studies are needed to further improve risk stratification. Since in HCM most follow-up studies are limited by little outcome-related events, large multicenter collaborations such as

the HCMR study(87) and the SHARE registry (https://theshareregistry.org/) are needed to identify novel risk markers, such as late gadolinium enhancement, T1 mapping (extracellular volume), genetics, and biomarkers (markers of fibrosis).

Gender differences

Women with HCM present later in life with more advanced disease in comparison to men. Adjusting echocardiographic parameters to body surface area revealed a worse phenotype in women than was suspected based on unadjusted parameters (chapters 8 and 9). Whether diagnostic bias or other sociocultural processes are accountable or if hormones and gene expression cause this delay is still unknown.(64) Therefore, future gender studies are needed to investigate further the cause of delayed diagnosis in women and assess whether using gender- or body surface area-adjusted parameters would improve the treatment and outcome of women with HCM.

Future therapeutic strategies

Unraveling the pathophysiology of HCM is necessary for the design of novel drugs which can prevent the onset and progression of HCM. For example, studies in human cardiac muscle revealed that the myocardial energetics were impaired, suggesting there is a therapeutic potential for myocardial energy modulators.(88) The ENERGY trial will investigate whether energy deficiency could be a therapeutic target to prevent the onset of HCM (http://www.amsterdamresearch.org/web/instituut-1/nieuws/tonenop/400k-grant-for-research-into-hypertrophic-cardiomyopathy.htm). Other examples of potential therapies include gene correction(89), myosin inhibitors(90), late sodium current inhibitors(91), and calcium and sodium channel blockers(92).(12) Large multicenter collaborations with long-term follow-up, such as the LIBERTY-HCM trial(93) and the EXPLORER-HCM trial (https://clinicaltrials.gov/ct2/show/NCT03470545) are needed for randomized controlled trials to have enough power to demonstrate alterations in disease progression or survival.

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NEDERLANDSE SAMENVATTING

Hypertrofische cardiomyopathie (HCM) is de meest voorkomende erfelijke hartziekte met een prevalentie van 1:500 tot 1:200.(1, 2) De definitie van HCM is een hartspierverdikking dat niet veroorzaakt wordt door hoge bloeddruk of aortaklepvernauwing.(3) HCM wordt veroorzaakt door mutaties in genen die coderen voor eiwitten met een functie in het sarcomeer; de kleinste zich herhalende structuur in spiervezels die zorgen voor het samentrekken van de spier.(3)

Genetisch onderzoek en familiescreening in hypertrofische cardiomyopathie

In ongeveer 50-60% van de patienten met HCM die genetisch onderzoek ondergaan wordt een pathogene mutatie ontdekt.(4) Een mutatie is pathogeen als zeker is dat het de ziekte veroorzaakt na raadpleging van de literatuur en databases.(5) De prognostische waarde van genotype (i.e. het wel of niet hebben van een pathogene mutatie) is momenteel beperkt.(6) In **hoofdstuk 1** analyseerden wij de klinische kenmerken en lange termijn uitkomsten van 234 genotype-positieve (G+) en 278 genotype-negatieve (G-) patienten met HCM. Ten tijde van de eerste cardiale evaluatie waren G+ patienten jonger dan G- patienten en vertoonden zij meer kenmerken die geassocieerd zijn met een verhoogd risico op plotse hartdood. Zo hadden zij meer non-sustained ventriculaire tachycardieen, waren zij vaker gecollabeerd in het verleden en waren er meer gevallen van extreme hypertrofie. G- patienten waren symptomatischer en hadden hogere drukgradienten over de uitstroombaan van het linker ventrikel. Waarschijnlijk spelen nog onontdekte genetische varianten en leefstijl factoren (hypertensie) een rol in de pathofysiologie van G- HCM.(7-9) Familieleden met HCM die zich presenteerden via familieonderzoek waren jonger en hadden een betere prognose dan patienten met HCM die zich presenteerden met signalen of symptomen van HCM. Waarschijnlijk is de HCM in de familieleden in een vroeger ziektestadium ontdekt.(10) Gedurende 12±9 jaar follow-up was G+ status een onafhankelijke risicofactor voor algehele mortaliteit, cardiovasculaire mortaliteit, hartfalen-gerelateerde mortaliteit en plotse hartdood. Deze bevindingen zijn overeenkomstig met voorgaande onderzoeken.(11-14) Fundamenteel onderzoek naar de pathofysiologische gevolgen van sarcomeer mutaties is cruciaal voor de ontwikkeling van genotype-specifieke risicostratificatie en gerichte therapieen.(15, 16)

Myosin-binding protein C (*MYBPC3*) founder mutaties zijn geidentificeerd in IJsland, Italie, Finland, Japan, Frankrijk en Nederland.(17) Het zijn mutaties die meerdere generaties oud zijn en een gezamenlijke voorouder hebben.(18, 19) In Nederland wordt maar liefst 35% van de HCM gevallen veroorzaakt door 1 van de 3 *MYBPC3* founder mutaties: c.2373dupG (p.Trp792Valfs*41), c.2827C>T (p.Arg943*), en c.2864_2865delCT (p.Pro955fs*95).(17, 18, 20) De mutaties leiden tot inkorting van de C-terminus van het *MYBPC3* eiwit en haploinsufficientie.(21-23) Hartspierweefselonderzoek van patienten met HCM laat een associatie zien tussen *MYBPC3* founder mutaties en een verminderde kracht genererende capaciteit van de cardiomyocyt, cardiomyocyt hypertrofie en een verlaagde myofibril dichtheid.(21, 24) In **hoofdstuk 2** vergeleken wij de klinische kenmerken en lange termijn

uitkomsten tussen 271 individuen met een *MYBPC3* founder mutatie (134 patienten, 128 familieleden), 132 nonfounder G+ patienten met HCM en 277 G- patienten met HCM. Het fenotype en de prognose van *MYBPC3* founder HCM was vergelijkbaar met dat van nonfounder G+ HCM; een bevinding die in strijd is met de heersende gedachte dat *MYBPC3* founder HCM een goedaardiger beloop kent.(19, 20, 25, 26) Familieleden die via familiescreening waren gediagnosticeerd met HCM hadden een betere prognose dan patienten die zich hadden gepresenteerd wegens signalen of symptomen van HCM, meest waarschijnlijk doordat familieleden zich in een vroeger ziektestadium bevinden. Tot slot laat deze *MYBPC3* founder mutatie populatie een extreme fenotypische heterogeniteit zien. Sommige individuen waren gediagnosticeerd met non-compactie of dilaterende cardiomyopathie. Additionele sarcomeermutaties of andere nongenetische factoren zullen een rol spelen.(27) Toekomstig translationeel onderzoek is nodig om deze genotype-fenotype ‘’black box’’ te ontrafelen (figuur 1).

Figuur 1. De genotype-fenotype ‘’black box’’.

Wanneer HCM wordt gediagnosticeerd bij een patient dient de behandelend cardioloog de patient te informeren over het genetische aspect van de ziekte en familieonderzoek aan te bieden aan zijn of haar familieleden.(28) Sinds het jaar 2003 adviseert de HCM richtlijn om familieonderzoek te organiseren door gebruik te maken van electro- en echocardiografie.(3, 29-31) Hierbij wordt aangeraden om de evaluaties aan te bieden vanaf de leeftijd van 10 jaar en deze te herhalen tot vergevorderde leeftijd. De recente Europese HCM richtlijn raadt aan om genetisch onderzoek (GO) toe te voegen aan de screening strategie(3); een kost-effectieve benadering.(32, 33) Deze strategie maakt het mogelijk om G- familieleden gerust te stellen en te ontslaan uit de screening. Daarnaast bewerktstelligt het de identificatie van G+ familieleden die een verhoogd risico lopen om HCM te ontwikkelen.(28) In **hoofdstuk 3** hebben wij deze hedendaagse screening strategie geevalueerd door de resultaten van het GO en de klinische bevindingen te analyseren in 777 familieleden die van 209 patienten met HCM afstammen. De meerderheid van de patienten (93%) en familieleden (80%) hebben GO ondergaan. Al met al heeft deze benadering geleid to de geruststelling van 356 (46%) familieleden. De cardiale evaluatie in 264 G+ familieleden en 157 familieleden zonder GO onthulde HCM in 37% en 17% respectievelijk. De cardiale mortaliteit tijdens follow-up van familieleden met HCM was lager dan de cardiale mortaliteit dat over het algemeen beschreven is bij patienten met HCM (0.3%/jaar t.o.v. 1-

2%/jaar). Dit reflecteert het vroegere ziektestadium waarin familieleden zich bevinden en mogelijk het effect van leefstijladviezen en intense follow-up met periodieke risicostratificatie voor plotse hart dood. De prognose van de 165 G+ familieleden zonder HCM was benigne. Één G+ familielid zonder HCM overleed plotseling waarbij GO een mutatie aantoonde dat geassocieerd is met het lang-QT syndroom. Gedurende 7 jaar echocardiografische follow-up van 178 familieleden zonder HCM (65% G+) ontwikkelden 29 (16%) een milde vorm van HCM (24 G+). Opmerkelijk genoeg ontwikkelde HCM zich niet tijdens de adolescentie, een periode dat berucht zou zijn voor de ontwikkeling van HCM door hormonale veranderingen.(34) HCM ontwikkelde zich met name voor de leeftijd van 12 jaar en tussen de leeftijd van 20 en 30 jaar. Daarom stellen wij voor om cardiale evaluaties vroeger te initiëren (vanaf 8 jaar) om ze vervolgens iedere 2-4 jaar te herhalen in kinderen en adolescenten en iedere 5 jaar in volwassenen en dit te continueren tot vergevorderde leeftijd. Gezien de resultaten van de huidige studie concluderen wij dat GO het HCM familieonderzoek faciliteert doordat het het aantal polikliniekbezoeken bedoeld voor cardiale screening aanzienlijk reduceert. De haalbaarheid van deze strategie hangt af van het aantal mensen dat GO wilt ondergaan en de opbrengst van het GO in de patienten. In onze patientenpopulatie was de genetische opbrengst relatief hoog, hetgeen verklaard kan worden door de hoge prevalentie van *MYBPC3* founder mutaties in Nederland.(18, 35) Tevens kan er een vertekening door verwijzing zijn opgetreden doordat familieleden van G+ families zijn geinformeerd over de erfelijkheid van HCM; de ziektelast in G+ families is waarschijnlijk ook hoger.(36) Daarnaast waren in onze patientenpopulatie relatief veel mensen bereid om GO te ondergaan, waarschijnlijk doordat in Nederland de kosten worden vergoed door de zorgverzekering en doordat genetische counselling, GO en cardiale evaluatie simultaan worden aangeboden. Andere landen hebben diverse financiele en organisatorische benaderingen omtrent GO in de HCM populatie. We moeten ons bewust zijn van de potentiële psychosociale, emotionele en financiele consequenties van GO, met name omdat de prognostische waarde van genotype beperkt is.(6) We bieden over het algemeen geen GO aan aan individuen jonger dan 18 jaar.

Beeldvorming van hypertrofische cardiomyopathie

Wegens leeftijdsgerelateerde penetrantie van sarcomeer mutaties wordt levenslange follow-up geadviseerd aan G+ familieleden zonder HCM (‘’mutatiedragers’’).(3, 30) Momenteel kunnen wij niet voorspellen welke mutatiedrager HCM gaat ontwikkelen. Eerdere studies hebben pre-fenotypische kenmerken geïdentificeerd zoals diastolische disfunctie(37-39), aantasting van de energiehuishouding van het myocard(40), electrocardiografische afwijkingen(41), myocard crypten(42-44) en mitralisklep verlenging(42, 45-47). Longitudinale follow-up studies van mutatiedragers zijn schaars.(48) Doordat HCM zich langzaam en infrequent ontwikkelt kan het lang duren voordat genoeg data is verzameld. In **hoofdstuk 4** hebben wij door middel van twee-dimensionale echocardiografie de lengte van het voorste mitralis klepblad (VMK) in 133 mutatiedragers en 135 gezonde controles gemeten; er was geen verschil. In de 13/80 mutatiedragers die HCM ontwikkelden tijdens follow-up had VMK lengte

geen voorspellende waarde, in tegenstelling tot pathologische Q golven, E/e' ratio en maximale wanddikte. Deze bevindingen zijn tegenstrijdig met voorgaande studies die rapporteren dat de VMK verlenging een primaire uiting is van HCM.(42, 45-47) De etiologie van VMK verlenging in patienten met HCM is nog onduidelijk.(49) In **hoofdstuk 5** gebruikten we speckle-tracking echocardiografie om de globale longitudinale strain (GLS) in 120 mutatiedragers en 110 gezonde controles te meten. Eerdere studies hebben consequent aangetoond dat de GLS verminderd is in patienten met HCM en een normale linker ventrikel ejectiefractie, hetgeen subklinische systolische disfunctie aanduidt.(50-54) In onze studiepopulatie was de GLS statistisch significant hoger in de mutatiedragers, echter de individuele metingen vertoonden veel overlap. GLS had geen voorspellende waarde voor de ontwikkeling van HCM in de 13/80 mutatiedragers die HCM hebben ontwikkeld tijdens follow-up. GLS is dus geen geschikte parameter om mutatiedragers te onderscheiden van gezonde mensen noch een bruikbare voorspeller voor de ontwikkeling van HCM. Waarom de GLS hoger is in mutatiedragers is onduidelijk. Hypercontractiliteit zou geinduceerd kunnen zijn door de mutatie.(55) Anderzijds zou het een compensatiemechanisme kunnen zijn voor regionale hypocontractiliteit.(56) Of sarcomeer mutaties hyper- of hypocontractiliteit veroorzaken wordt aanhoudend onderzocht.(57) Fundamenteel onderzoek heeft een belangrijke rol in de identificatie van primaire triggers voor de ontwikkeling van HCM.

De bruikbaarheid van drie-dimensionale echocardiografie (3DE) is aangetoond voor de evaluatie van linker ventrikel volume en massa en voor de visualisatie van hartkleppen.(58-61) In **hoofdstuk 6** onderzochten wij de bruikbaarheid van 3DE voor het beoordelen van linker ventrikel hypertrofie en papillairspier morfologie. Door middel van 3DE analyse in 24 patienten met HCM en 31 gezonde controles identificeerden wij een spiraalvormig patroon van de linker ventrikel hypertrofie in de patienten. Voorts waren de papillairspieren significant groter in de patienten dan in de controles. Vergroting van de posteromediale papillairspier was geassocieerd met linker ventrikel uitstroombaan obstructie. 3DE heeft potentieel klinische waarde in deze patientenpopulatie omdat het beoordelen van de locatie en morfologie van de papillairspier belangrijk is om het mechanisme van linker ventrikel uitstroombaan obstructie te beoordelen. Dit bepaalt namelijk onder andere welke techniek gewenst is om de obstructie te verhelpen.(62) Toekomstig onderzoek is verreist om de haalbaarheid en reproducibiliteit van 3DE bij patienten met HCM te evalueren.

Klinische aspecten van hypertrofische cardiomyopathie

Atriumfibrilleren (AF) is de meest voorkomende ritmestoornis onder patienten met HCM en een onafhankelijke risicofactor voor morbiditeit en mortaliteit, voornamelijk gerelateerd aan hartfalen en thrombo embolische complicaties.(63, 64) Vanwege de hoge incidentie van AF in de HCM populatie wordt levenslange therapie met orale antistolling geadviseerd aan alle patienten met HCM en AF, zelfs bij hersteld sinusritme en onafhankelijk van de CHA2DS2-VASc score.(3, 30) Het is vaak moeilijk om AF te detecteren vanwege het episodisch en paroxysmaal optreden. Richtlijnen adviseren 24-48 uur Holtermonitoring iedere 6-12 maanden, afhankelijk van de grootte van het linker atrium.(3) Patienten met een cardiaal inwendig elektronisch apparaat (CIEA) hebben vaak een continue bewaking van de atriale activiteit waardoor AF opgespoord kan worden. In **hoofdstuk 7** bestudeerden wij de incidentie en impact van CIEA-gedecteerde AF. Gedurende 2.8 jaar follow-up was de jaarlijkse incidentie van CIEA-gedecteerde AF 7%. Dit is hoog in vergelijking met eerder gerapporteerde incidenties van AF in patienten met HCM (2-3%).(65) Het zou gerelateerd kunnen zijn aan het feit dat HCM patienten met een CIEA zich vaak in een verder gevorderd ziektestadium bevinden. Daarnaast zal de CIEA meer AF episoden detecteren in vergelijking met traditionele intermitterende monitoring strategieen. Er heeft mogelijk een onderschatting van de echte incidentie plaats gevonden, omdat sommige apparaten niet het vermogen hadden om atriaal te sensen. De incidentie van thrombo embolische complicaties was relatief laag (1.3%), mogelijk ten gevolge van tijdige behandeling met orale anticoagulantia of doordat de meerderheid van de AF gevallen behandeld werden met anti-aritmische medicatie of electrocardioversie. De bevindingen in deze studie tonen de toegevoegde waarde van CIEAs aan voor de detectie van AF en de gevolgen dat het opsporen van AF heeft voor de behandeling. Het zou een stimulus kunnen zijn voor cardiologen om CIEDs met atriale sensing te overwegen indien er een indicatie is voor CIEA implantatie, zoals bijvoorbeeld een enkelkamer apparaat met een zwevende electrode in het atrium.(66)

Geslachtsverschillen zijn geobserveerd in meerdere cardiovasculaire aandoeningen zoals ischemisch hartlijden, hartfalen, hypertensie en aortaklepstenose.(67) In patienten met HCM is meermaals gerapporteerd dat geslacht de leeftijd van presentatie en het fenotype beinvloedt.(8, 68-78) Sommige studies beschrijven een onafhankelijke associatie tussen vrouwelijke geslacht en algehele mortaliteit(79, 80), in tegenstelling tot andere studies.(70, 81, 82) In **hoofdstuk 8** analyseerden wij de klinische presentatie, het fenotype, genotype en de uitkomsten van 1007 patienten met HCM (620 man, 387 vrouw) die klinisch beoordeeld zijn tussen 1977 en 2017. Ten tijde van de eerste evaluatie waren vrouwen ouder, symptomatischer en hadden zij vaker hypertensie. Ook hadden vrouwen vaker obstructie van de linker ventrikel uitstroombaan en was de systolische en diastolische linker ventrikel functie vaker aangetast. Multivariate cox regressie analyse gedurende 6.8 jaar follow-up liet geen onafhankelijke relatie zien tussen geslacht en algehele mortaliteit, cardiovasculaire mortaliteit, hartfalen-gerelateerde mortaliteit of plotse hartdood. Waarom vrouwen met HCM zich later

presenteren in een verdergevorderd ziektestadium is onduidelijk. Socioculturele processen (het negeren van vroege symptomen, diagnostische onnauwkeurigheid) of geslachtshormonen danwel verschillen in genexpressie zouden eraan ten grondslag kunnen liggen.(67, 68, 81, 83, 84) In de huidige studie observeerden we een verschuiving van het klinisch beeld als wij echocardiografische parameters corrigeren voor lichaamsoppervlakte. Waar de maximale wanddikte, linker atrium grootte en linker ventrikel eind-diastolische dimensie een relatief milder fenotype in de vrouwen ten opzichte van de mannen suggereerden bleek na correctie voor lichaamsoppervlakte het fenotype juist ernstiger. Het is dus mogelijk dat wij de ernst van het ziektebeeld in vrouwen onderschatten door ongecorrigeerde echocardiografische parameters te gebruiken. Toekomstige studies zullen moeten aantonen of corrigeren voor lichaamsoppervlakte danwel geslacht de diagnostiek en behandeling van HCM verbetert.

In **hoofdstuk 9** bestudeerden wij geslachtsverschillen in de echocardiografische (E/e' ratio, E/A ratio, tricuspidalisklepregurgitatie, linker atrium grootte) en cellulaire (passieve spanning, myofilament Ca^{2+} gevoeligheid, expressie van eiwitten die met Ca^{2+} werken, expressie van titine isoform, interstitiele fibrose) parameters van diastolische functie in patienten met HCM. Hartspierweefsel werd verkregen via chirurgische myectomie in 71 patienten met HCM (38% vrouw) voorafgegaan door echocardiografie. Vrouwen waren significant ouder ten tijde van de myectomie en de diastolische functie was vaker aangetast in de vrouwen. Voorts was er meer sprake van interstitiele fibrose in de vrouwen. Er waren geen verschillen aangaande passieve spanning of Ca^{2+} gevoeligheid. Buigzaam titine isoform was verhoogd in de vrouwen hetgeen mogelijk een compensatie mechanisme reflecteert. Over het algemeen toont de data ernstiger cardiale remodeling in vrouwen dan in mannen ten tijde van de myectomie. Daarnaast ondersteunt de data voorgaande studies die rapporteren dat vrouwen een verlate klinische presentatie hebben.(68, 81) De factoren die dit veroorzaken zijn vooralsnog onduidelijk en behoeven aanvullend onderzoek. Eveneens wordt in de vrouwen een relatief hogere maximale wanddikte gezien na correctie voor lichaamsoppervlakte. Dit suggereert wederom dat het ziektestadium van HCM mogelijk wordt onderschat in vrouwen indien ongecorrigeerde parameters worden gebruikt.

TOEKOMSTPERSPECTIEVEN

Het uiteindelijke doel van wetenschappelijk onderzoek op het gebied van HCM is om de klinische expressie van HCM te voorkomen, de kwaliteit van leven van patienten met HCM te verbeteren, symptomen te verminderen en om levensbedreigende complicaties van HCM te voorkomen (plotse hart dood, thrombo embolische complicaties, hartfalen). Om dit te realiseren willen we alle oorzaken van HCM achterhalen, de pathofysiologische processen ontrafelen en de risicostratificatie voor plotse hartdood en hartfalen verbeteren.

Vooruitgang in de genetische diagnostiek

Momenteel wordt in ongeveer 50-60% van de patienten met HCM die genetisch onderzoek ondergaan een pathogene mutatie ontdekt.(4) Met behulp van exoom- en genoomsequencing worden mogelijk nieuwe mutaties ontdekt die HCM veroorzaken. Deze nieuwe technologieen vereisen intensieve inspanningen om de data te analyseren en varianten te interpreteren.(85) Grote hoeveelheden varianten moeten handmatig geanalyseerd worden om degenen te selecteren die klinische relevantie hebben.(85) Vervolgens worden de varianten ingedeeld in 5 groepen (pathogeen, waarschijnlijk pathogeen, etcetera). Dit proces vereist het nakijken van de literatuur, co-segregatie onderzoek, predictie software en in-vitro studies.(5) Genotype-negatieve HCM (met name niet-familiaire HCM(86)) heeft een betere prognose en andere klinische kenmerken (hoofdstuk 1). Om de pathofysiologie van genotype-negatieve HCM te begrijpen zijn toekomstige studies nodig die modificerende factoren zoals (epi)genetische variaties en omgevingsfactoren onderzoeken.(15) De extreem fenotypische heterogeniteit in personen met dezelfde pathogene mutatie (hoofdstuk 2) benadrukt tevens de complexiteit van de HCM pathofysiologie. Translationeel onderzoek is cruciaal om de genotype-fenotype ‘’black box’’ verder te ontrafelen.(15, 16, 87)

Verbetering van de fenotypering

Geavanceerde beeldvorming zoals speckle-tracking en drie-dimensionale echocardiografie, magnetisch resonantie imaging en positron emissie tomografie(88) kan potentieel structurele en functionele manifestaties van sarcomeer mutaties aantonen. Om inzicht te krijgen in de vroege manifestaties van HCM vormen genotype-positief, fenotype-negatieve familieleden een ideale testpopulatie (hoofdstukken 4 en 5). Lange termijn follow-up studies zijn belangrijk om de risicostratificatie te verbeteren. In de HCM populatie worden follow-up studies vaak gelimiteerd door het beperkt aantal levensbedreigende complicaties. Daarom zijn grote internationale samenwerkingen zoals de HCMR studie(89) en de SHARE registratie (https://thesharereregistry.org/) essentieel voor de identificatie van nieuwe risicofactoren zoals late aankleuring met gadolinium, T1 mapping (extracellulair volume), genetica en biomarkers (markers van fibrose).

Geslachtsverschillen

In vergelijking met mannen presenteren vrouwen met HCM zich later in het leven in een verdergevorderd ziektestadium. Het corrigeren van echocardiografische parameters voor lichaamsoppervlakte liet een ernstiger fenotype zien in vrouwen dan beoordeeld werd op basis van ongecorrigeerde parameters (hoofdstukken 8 en 9). Het is onduidelijk of diagnostische onnauwkeurigheid, andere socioculturele processen, hormonen of genexpressie verantwoordelijk zijn voor de vertraagde klinische presentatie onder vrouwen.(67) Aanvullende studies zijn nodig om de oorzaak te achterhalen en om te analyseren of het corrigeren voor geslacht of lichaamsoppervlakte de diagnostiek en behandeling van vrouwen met HCM verbetert.

Toekomstige therapeutische strategieen

Om nieuwe medicijnen te creëren die de ontwikkeling of progressie van HCM kunnen voorkomen, is het noodzakelijk om de pathofysiologie van HCM te begrijpen. Hartspierweefselonderzoek liet bijvoorbeeld zien dat de energiehuishouding van het myocard is aangetast, hetgeen mogelijk een doelwit kan zijn om de ontwikkeling van HCM te voorkomen.(90) De ENERGY trial zal dit verder onderzoeken. (http://www.amsterdamresearch.org/web/instituut-1/nieuws/tonenop/400k-grant-for-research-into-hypertrophic-cardiomyopathy.htm). Andere voorbeelden van potentiële nieuwe therapieen zijn gencorrectie(91), myosineremmers(92), late natrium-instroom remmers(93), en calcium en natrium kanaal blokkers(94). Teneinde voldoende power te hebben om een verandering aan te tonen in de progressie of overleving van de ziekte zijn grote internationale samenwerkingen met lange termijn follow-up nodig. Voorbeelden hiervan zijn de LIBERTY-HCM trial(95) en de EXPLORER-HCM trial (https://clinicaltrials.gov/ct2/show/NCT03470545).

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LIST OF PUBLICATIONS

Liebregts M, Steggerda RC, Vriesendorp PA, **van Velzen H,** Schinkel AF, Willems R, van Cleemput J, van den Berg MP, Michels M, ten Berg JM. Long-Term Outcome of Alcohol Septal Ablation for Obstructive Hypertrophic Cardiomyopathy in the Young and the Elderly. JACC Cardiovasc Interv. 2016 Mar 14;9(5):463-9.

van Velzen HG, Vriesendorp PA, Oldenburg RA, van Slegtenhorst MA, van der Velden J, Schinkel AF, Michels M. Value of Genetic Testing for the Prediction of Long-Term Outcome in Patients With Hypertrophic Cardiomyopathy. Am J Cardiol. 2016 Sep 15;118(6):881-7.

van Velzen HG, Theuns DA, Yap SC, Michels M, Schinkel AF. Incidence of Device-Detected Atrial Fibrillation and Long-Term Outcomes in Patients With Hypertrophic Cardiomyopathy. Am J Cardiol. 2017 Jan 1;119(1):100-105.

van Velzen HG, Schinkel AF, Oldenburg RA, van Slegtenhorst MA, Frohn-Mulder IM, van der Velden J, Michels M. Clinical Characteristics and Long-Term Outcome of Hypertrophic Cardiomyopathy in Individuals with a MYBPC3 Founder Mutation. Circ Cardiovasc Genet. 2017 Aug;10(4).

Erden M, **van Velzen HG,** Menting M, van den Bosch AE, Ren B, Michels M, Vletter WB, van Domburg RT, Schinkel AF. Utility of three-dimensional echocardiography for the assessment of left ventricular geometry and papillary muscle morphology in hypertrophic cardiomyopathy. J Ultrasound. 2018 Jan 6.

van Velzen HG, Schinkel AF, Oldenburg RA, Frohn-Mulder IM, van Slegtenhorst MA, Michels M. Outcomes of contemporary family screening in hypertrophic cardiomyopathy. Circ Genom Precis Med. 2018 Apr;11(4)

van Velzen HG, Schinkel AFL, Menting ME, van den Bosch AE, Michels M. Prognostic significance of anterior mitral valve leaflet length in individuals with a hypertrophic cardiomyopathy gene mutation without hypertrophic changes. J Ultrasound. 2018 Jun 6.

Nijenkamp LLAM, Bollen IAE, **van Velzen HG**, Regan JA, van Slegtenhorst M, Niessen HWM, Schinkel AFL, Krüger M, Poggesi C, Ho CY, Kuster DWD, Michels M, van der Velden J. Sex Differences at the Time of Myectomy in Hypertrophic Cardiomyopathy. Circ Heart Fail. 2018 Jun;11(6)

van Velzen HG, Schinkel AFL, Kardys I, Baart S, Michels M. Effect of Gender and Genetic Mutations on Outcomes in Patients with Hypertrophic Cardiomyopathy. Am J Cardiol. 2018 Sep 8.

van Velzen HG, Schinkel AFL, van Grootel RWJ, van Slegtenhorst MA, van der Velden J, Strachinaru M, Michels M. Five-year prognostic significance of global longitudinal strain in individuals with a hypertrophic cardiomyopathy gene mutation without hypertrophic changes. Accepted in Netherlands Heart Journal 2018.

PHD PORTFOLIO

Name PhD student:	H.G. van Velzen
Erasmus MC department:	Cardiology
Research school:	COEUR
PhD period:	2014 – 2018
Promotor:	F. Zijlstra
Co-promotors:	M. Michels, A.F.L. Schinkel

Year	*Title/Name*	*ECTS*	*Location*
PhD Training		**33.3**	
In-depth courses			
General courses			
2014	Nihes Biostatistical Methods I: Basic Principles	5.7	Rotterdam, the Netherlands
2015	Molmed Photoshop	0.2	Rotterdam, the Netherlands
2015	BROK Regulations & Organization for Clinical Researchers	1.8	Rotterdam, the Netherlands
2015	Biomedical English Writing and Communication	3.0	Rotterdam, the Netherlands
2016	Research Integrity	0.6	Rotterdam, the Netherlands
Specific courses			
2015	COEUR Imaging and Diagnostics	1.5	Rotterdam, the Netherlands
2015	COEUR Congenital Heart Disease	1.5	Rotterdam, the Netherlands
2015	COEUR Cardiovascular Pharmacology	1.5	Rotterdam, the Netherlands
Teaching			
Lectures			
2014	Interns Educational Meeting ''The 2014 ESC hypertrophic cardiomyopathy guidelines''	0.6	Rotterdam, the Netherlands
2015	Cardiogenetics Meeting ''Clinical course of Dutch MYBPC3 founder mutation carriers''	0.6	Rotterdam, the Netherlands
2015	VITHAS Symposium ''Diagnosis and management of hypertrophic cardiomyopathy''	0.6	Leusden, the Netherlands
2015	Staff lunch ''Value of genetic testing in hypertrophic cardiomyopathy''	0.6	Rotterdam, the Netherlands
2015	CVON DOSIS Meeting ''Prognosis in Dutch MYBPC3 founder mutation carriers''	0.6	Utrecht, the Netherlands
2015	COEUR Seminar ''Prognostic significance of sarcomeric mutations''	0.6	Rotterdam, the Netherlands
2016	Cardiogenetics Meeting ''Yield and long-term outcome of family screening in hypertrophic cardiomyopathy''	0.6	Rotterdam, the Netherlands
2017	Cardiogenetics Meeting ''How can we predict the development of hypertrophic cardiomyopathy in mutation carriers without hypertrophic changes?''	0.6	Rotterdam, the Netherlands
2017	Staff Lunch ''Does global longitudinal strain predict the development of hypertrophic cardiomyopathy?	0.6	Rotterdam, the Netherlands
Supervision			
2014	Medical students ''What is the value of family screening in hypertrophic cardiomyopathy?''	0.6	Rotterdam, the Netherlands
2015	Medical students ''Risk factors for sudden cardiac death in hypertrophic cardiomyopathy''	0.6	Rotterdam, the Netherlands
2016	Medical students ''Doppler imaging for the identification of hypertrophic cardiomyopathy mutation carriers''	0.6	Rotterdam, the Netherlands
2016	Junior Med School students ''Hypertrophic cardiomyopathy: combatting gender-specific differences with a new relative diagnostic value''	0.6	Rotterdam, the Netherlands

Conferences and symposia

Oral Presentations

2015	American College of Cardiology Conference ''Patients with genotype-positive hypertrophic cardiomyopathy are at increased risk of heart failure related death''	2.1	San Diego, USA
2015	Dutch Society of Cardiology Congress ''Value of genetic testing in hypertrophic cardiomyopathy''	1.2	Papendal, the Netherlands
2017	EuroEcho-Imaging Cogress ''Global longitudinal strain in hypertrophic cardiomyopathy gene mutation carriers without hypertrophic changes	1.8	Lisbon, Portugal

Poster Presentations

2015	Dutch Society of Cardiology Spring Congress ''Prognosis in Dutch MYBPC3 founder mutation carriers is defined by phenotype''	0.6	Noordwijkerhout,the Netherlands
2015	European Society of Cardiology Congress ''Prognosis in Dutch MYBPC3 founder mutation carriers is defined by phenotype''	0.6	London, United Kingdom
2015	Florence International Symposium on Advances in Cardiomyopathies ''The yield of genetic and clinical screening of relatives for hypertrophic cardiomyopathy''	0.6	Florence, Italy
2017	American College of Cardiology Congress ''Outcomes of contemporary family screening in hypertrophic cardiomyopathy''	0.6	Washington D.C., USA
2017	European Society of Cardiology Congress ''Prognostic significance of anterior mitral valve leaflet length in sarcomere gene mutation carriers without hypertrophic cardiomyopathy''	0.6	Barcelona, Spain

Attended

2014	Juniorkamerdag ''Devices''	0.2	Gouda, the Netherlands
2014	Cardiology Club Rijnmond ''Tricuspid insufficiency''	0.2	Rotterdam, the Netherlands
2014	Symposium ''Research in hypertrophic cardiomyopathy; from bench to bedside''	0.2	Amsterdam, the Netherlands
2014	COEUR Symposium ''Imaging of cardiac arrhythmia's''	0.2	Rotterdam, the Netherlands
2015	Cardiology Club Rijnmond ''Pacing for dummies''	0.2	Rotterdam, the Netherlands
2015	Albert Schweitzer Hospital clinical teaching ''ultrasound in hypertrophic cardiomyopathy''	0.2	Dordrecht, the Netherlands
2015	Investigator Meeting Gilead Sciences ''Liberty hypertrophic cardiomyopathy randomized trial''	0.2	Milan, Italy
2015	Cardiology Club Rijnmond ''Anniversary symposium''	0.2	Rotterdam, the Netherlands

ABOUT THE AUTHOR

Hannah Gillian van Velzen was born on July 13th, 1986 in Spijkenisse, the Netherlands. After graduating high school (Penta College C.S.G. Angelus Merula, Spijkenisse), she started medical school at the Erasmus University Rotterdam. During her studies, she spent two internships abroad (Malawi, Africa; London, United Kingdom) and she backpacked through Australia and New Zealand. After obtaining her Medical Doctor's degree, she started working as a resident (Anios) at the department of Internal Medicine in the Amstelland Hospital Amstelveen and in geriatric medicine at Aafje healthcare facility Rotterdam. In 2014, she started the PhD project described in this thesis ''Clinical and genetic aspects of hypertrophic cardiomyopathy'' at the Erasmus Medical Center in Rotterdam, supervised by Prof. dr. F. Zijlstra, Dr. M. Michels, and Dr. A. Schinkel. During this PhD project, she had the opportunity to present her work on several national and international conferences and publish manuscripts in peer reviewed international journals.

DANKWOORD

Dit proefschrift was er niet geweest zonder de steun en hulp van velen. Graag wil ik een aantal personen in het bijzonder bedanken.

Allereerst mijn copromotoren Michelle en Arend. Zonder jullie sturing en input was het me niet gelukt om dit boek te schrijven. Jullie gaven mij de ruimte en vrijheid om zelf het meeste uit mijn promotie te halen. Dank voor de coaching en adviezen die jullie mij gegeven hebben, de praktische tips als ik vastliep en jullie vertrouwen in mij.

Mijn promotor, Prof. dr. Zijlstra, dank voor het bieden van de mogelijkheid om onderzoek te doen. Het heeft mij onder andere geleerd om kritisch te denken, een waardevolle eigenschap. Overige leden van de commissie; Prof. dr. van der Velden, Prof. dr. Asselbergs, Prof. dr. Deckers., hartelijk dank voor het feit dat u in de leescommissie hebt willen plaatsnemen. Dr. Galema, ik heb onze gesprekken zeer gewaardeerd.

Dr. van Domburg, Ron, bedankt dat ik altijd even binnen mocht lopen als ik vragen had. Isabella en Sara, bedankt voor jullie waardevolle hulp met de statistische analyses in R. Zonder jullie had ik de artikelen niet op tijd af kunnen krijgen. Maarten, Connie en Saskie, bedankt voor jullie hulp met Access. Marjon en Rogier, bedankt voor de fijne samenwerking.

Mijn kamergenoten op Ba-302, bedankt voor de fijne sfeer. René, ik heb echt veel met je gelachen, dank daarvoor en voor je hulp met Powerpoint en Photoshop. Jackie, bedankt voor de goede gesprekken en voor je hulp met het analyseren van echobeelden. Mihai, ik vond het erg leuk om met je van gedachten te wisselen en veel dank voor het meedenken wanneer ik vastliep met mijn onderzoek. Lennart, knap dat je in zo een korte tijd je promotie wist te voltooien en in opleiding wist te komen. Dank voor je gezelligheid. Claire, thanks for the many fun times we had.

Marja, bedankt dat je altijd benaderbaar was voor vragen over HCM. Ik vond het ook erg gezellig met je, vooral toen we op congres waren in Florence. Iedereen van de echoafdeling; Ellen, Linda, Debbie, Lourus, Saskia, Lisanne, Beata, Marcel, en Annemien, bedankt voor jullie toegankelijkheid bij vragen, de gezelligheid en alle lekkere taartjes. Jaap, ik was erg blij met jou als cardiomyopathie maatje. Veel succes in de kliniek. Raluca, I will never forget our awesome USA road trip.

Zonder de Ba-308 promovendi had ik een veel minder leuke promotietijd gehad. Lidia, Lucia, Carlijn, Allard, Vivan, Roderick en Iris, bedankt voor jullie gezelligheid in het EMC en op congressen. Roderick, bedankt voor je hulp met het speckle stuk.

Merel, dank voor al je inzet, het meedenken en je creativiteit. Het boek is super mooi geworden dankzij jou.

Degenen die mij van jongs af aan hebben gemotiveerd om het meeste uit mezelf te halen zijn mijn ouders. Dankjewel pap en mam voor al jullie steun en dat jullie er altijd voor me zijn. Joella, Michelle, Rolf en Mathijs, dankjewel voor al jullie goede adviezen en dat jullie zo lief voor me zijn. En de kleine Melle, Maas, Vic en Ben, jullie zijn sowieso top. Opa en Oma, Peter en Isabella, Hans, Carla, Djordie en Danique, bedankt voor jullie gezelligheid en alle steun. Niek, Trudy, Willem, Martijn en Mardi, bedankt dat ik me zo welkom voel in jullie warme nest. En dank voor al jullie support. Anika, dank voor onze fijne vriendschap! Mijn paranimfen, Mardi en Cheryl, dank voor jullie vriendschap en jullie onmisbare mentale ondersteuning in de maanden voorafgaand aan en op de dag van de verdediging.

Lieve Wouter, al die keren dat het niet vorderde of als ik tegenslagen had, dan wist jij me weer op te vrolijken. Sinds ik jou ken ben ik super gelukkig. Ik kijk erg uit naar onze toekomst samen.