The Clinical Characteristics of Families with Hypertrophic Cardiomyopathy Associated with Mutations of Cardiac Myosin Binding Protein C

Thesis for the degree of MD(Res)
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I, Stephen Page, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

This thesis is dedicated to the memory of my father, Dr Richard L Page DM FRCP
in whose footsteps I follow.

Abstract

Introduction: Mutations in cardiac myosin binding protein-C (MYBPC3), the most common genetic cause of hypertrophic cardiomyopathy (HCM), have been reported to cause a comparatively benign and late-onset form of the disease with incomplete penetrance. Based upon selected families with small numbers of mutations, these early reports may be misleading however. This study aimed to redefine the clinical characteristics of HCM related to MYBPC3 by evaluating a large cohort of unselected patients and their families, in whom an MYBPC3 mutation had been identified.

Methods: Index cases and their families underwent history, physical examination, electrocardiogram (ECG), transthoracic echocardiography, ambulatory ECG monitoring, metabolic exercise testing and mutation analysis. Long-term follow up data was collected where available.

Results: 44 MYBPC3 mutations were identified in 59 index cases. 26 of 59 (44%) were missense with 11 (19%) insertions/deletions, 11 (19%) intronic, and 5 (8%) nonsense mutations. A further 6 (10%) had complex genetic status with two different sequence variations identified. Nine families shared the R502W missense mutation and haplotype analysis confirmed a common founder, the first to be described in a UK cohort. A further 111 mutation carriers were identified, of which 39 were clinically affected - disease penetrance was therefore incomplete (58%) and related to age and gender but not mutation type. Mean age at diagnosis was 40.1 +/- 15.9 years with a wide range (5-76); 91.8% of affected mutation carriers were diagnosed over the age of 20 years. Most had asymmetric septal hypertrophy (86.4%) and mean maximal wall thickness was 20 +/-

5.8mm. Families sharing identical mutations showed significant variability in disease penetrance, age at diagnosis and risk of sudden death, suggesting that modifying factors play a significant role in disease development. No clinically useful markers of early disease expression were apparent from tissue Doppler studies in unaffected genotyped relatives. During long term follow up (mean 7.9 +/- 4.5 years) 1 individual developed hypertrophy as an adult, 5 individuals died (3 suddenly) and overall survival was 94%.

Discussion: The broad spectrum of mutations, disease severity and natural history of disease suggests that earlier reports of late-onset, benign disease related to MYBPC3 mutations were premature. In this study disease expression is broadly similar to non-genotyped HCM cohorts with disease severity ranging from mild to severe, risk of sudden death ranging from low to high and clinical disease being diagnosed in all decades of life. Such variance is not adequately explained by the sarcomeric protein gene or specific mutation per se and other genetic and environmental factors influence disease penetrance, severity and prognosis. The next generation of genotype-phenotype studies require a shift in focus from single gene analysis to include other genetic and environmental variables and an international collaborative database is recommended.

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ACKNOWLEDGEMENTS

This thesis, describing the relationship between genotype and phenotype in families with hypertrophic cardiomyopathy related to mutations in the MYBPC3 gene, represents the candidate's own original work. A cohort of patients with HCM and a sequence variation in the MYBPC3 gene had previously been identified as part of a British Heart Foundation programme grant and formed the starting point for the current work. The candidate's specific roles in the study were as follows:

- Contacting index cases for inclusion in the study
- Counselling and arranging clinical and genetic screening for family members at risk of inheriting a disease causing mutation
- Establishing the likely pathogenicity of sequence variations
- Obtaining informed written consent
- Performing clinical evaluation including full history and physical examination for index cases and their relatives
- Obtaining venous blood samples from relatives for DNA analysis
- Arranging appropriate investigations including ECG, Echo studies, Holter monitoring, exercise testing and CMR assessment
- Interpretation of investigations including ECG analysis, and off-line Echo analysis
- Providing routine clinical care to index cases and their families, including discussing test results and determining management strategies

- Data collection and statistical analysis

This study would not have been possible however without the help and support of many individuals who are gratefully acknowledged below.

I am extremely grateful to the British Heart Foundation for funding a Junior Research Fellowship and providing additional funding for consumables. Without this support, this work would not have been completed

Professor William McKenna and Dr Perry Elliott were an inspiration in providing insight into the fascinating world of heart muscle disease and were a source of constant support and guidance during the preparation of the manuscript.

Dr Michael Christiansen, Paal Skytt Andersen, Rune Frank Hansen (Statens Serum Institue, Copenhagen) and Petros Syrris (University College London) conducted DNA and RNA genetic analysis, identified mutations and provided technical and advisory support for all genetic aspects of the project.

Stavros Kounas and Camelia Demetrescu performed Echo studies, James Moon provided cardiac MR assessment, and Brian Mist conducted cardiopulmonary exercise testing. The Heart Hospital cardiac physiologists performed Holter monitoring analysis.

Finally, I would like to thank my wife Emma who has provided such encouragement, support and understanding over the last 4 years.

INTRODUCTION

Pre-amble

Since the initial description of ‘asymmetric hypertrophy of the heart in young adults’ by Donald Teare in 1958 [Teare 1958], the condition now known as Hypertrophic Cardiomyopathy (HCM) has captured the imagination of clinicians, pathologists, geneticists and patients alike. The first descriptions linked the finding of severe cardiac hypertrophy with sudden cardiac death, and demonstrated the familial nature of this condition [Goodwin 1960][Pare 1961][Braunwald 1964]. Our understanding of HCM however has developed at great pace with the advent of molecular genetics and advanced cardiac imaging, but we are beginning to understand that the complexities of this condition are great and numerous. Over the last 15 years or so, genetic mutation analysis has been particularly helpful giving us insight into the molecular pathogenesis of HCM – ‘a disease of the sarcomere’ and providing an opportunity for pre-clinical diagnosis in some families. The relationship between genotype and phenotype is not straightforward however and has received considerable attention by investigators striving to explain the clinical heterogeneity of this condition. The focus of this thesis is to provide further insights into the relationship between genotype and phenotype in patients with mutations in the gene encoding cardiac myosin binding protein-C (MYBPC3).

Overview of Hypertrophic Cardiomyopathy

Definition and Diagnosis

Hypertrophic cardiomyopathy (HCM) is defined clinically by ventricular hypertrophy in the absence of an identifiable cause [Maron 2003a][Maron 2006]. Most investigators would consider this to be a maximal wall thickness ≥13 mm, or more than 2 standard deviations from the mean corrected for age, size and gender [Maron 2003a] [Elliott 2004]. This definition, while useful clinically, lacks precision and sensitivity as certain forms of HCM are associated with minimal hypertrophy [Thierfelder 1994], and it excludes family members who have inherited the family mutation with signs of early disease expression, although diagnostic criteria for family members have been proposed [McKenna 1997]. The diagnosis is traditionally best made with 2-dimensional transthoracic echocardiography (TTE) but recommendations for including electrocardiographic (ECG) abnormalities in the diagnostic criteria exist [McKenna 1997].

Epidemiology

The population prevalence of HCM in adults is estimated to be approximately 1 in 500 (0.2%) [Maron 1995] based on echocardiographic screening in a young adult population, but this may be an underestimate if a broader definition of HCM is used.

Pathology

HCM is characterized macroscopically by left ventricular hypertrophy. The pattern and degree of ventricular hypertrophy is highly variable. It is usually predominant in the left ventricle (but can be biventricular) and usually affects the interventricular septum (the 'classical' asymmetric septal hypertrophy). It may also be concentric however, or eccentric involving any other ventricular wall including the apex [Maron 1983]. Hypertrophy may be associated with anterior displacement of the papillary muscles and mitral valve abnormalities leading to systolic anterior motion of the leaflets. If this anterior motion is complete and prolonged, obstruction to systolic ejection may occur in the left ventricular outflow tract with a resulting pressure gradient between the left ventricular cavity and the aorta. This dynamic process called left ventricular outflow tract obstruction (LVOTO) is present in perhaps 25% of individuals at rest and up to 70% of individuals on exertion or altered loading conditions, and is a major cause of morbidity in the HCM population [Maron 2006]. Other important abnormalities include diastolic dysfunction, left atrial enlargement and mitral regurgitation.

On a histological level the classical pathological features of HCM are those of interstitial fibrosis with myocyte hypertrophy and disarray [Ferrans 1972][Factor 1991][Shirani 2000][Varnava 2001]. Disarray is patchy and consists of obliquely aligned and irregular hypertrophied myocytes. There is nuclear enlargement, polychromasia and pleomorphism [Hughes 2004] and an increase in interstitial fibrosis and with altered collagen fibre morphology [Shirani 2000]. Intramural arteriolar hypertrophy and dysplasia is seen and may account for reduced coronary flow and small vessel ischaemia [Maron 1986a] [Varnava 2000].

Aetiology and Pathogenesis

Genetic linkage studies and candidate-gene screening techniques have identified that mutations in sarcomeric protein genes account for approximately 60% of cases of HCM [see Table 1 for references]. Other molecular abnormalities include metabolic disorders such as Anderson-Fabry disease, AMP-kinase disease and glycogen storage disorders.

Table 1 Spectrum of pathogenic genes identified in HCM

	Abbreviation	Reference
Sarcomeric Protein disease		
Cardiac myosin binding protein C	MYBPC3	Bonne 1995 Watkins 1995a
Beta-myosin heavy chain	MYH7	Geisterfer-Lowrance 1990
Cardiac troponin T	TNNT2	Thierfelder 1994
Cardiac troponin I	TNNI3	Watkins 1993 Kimura 1997
Alpha-tropomyosin	TPM1	Watkins 1995b
Essential myosin light chain	MYL3	Poetter 1996
Regulatory myosin light chain	MYL2	Poetter 1996
Alpha cardiac actin	ACTC	Mogensen 1999
Alpha-myosin heavy chain	MYH6	Carniel 2005 Niimura 2002
Titin	TTN	Satoh 1999
Cardiac troponin C	TNNC1	Hoffman 2001
Sarcomere Associated Protein Genes		
Muscle LIM protein	CSRP3	Geier 2003
Myosin light chain kinase 2	MYLK2	Davis 2001
LIM binding domain 3	LDB3	Theis 2006
Telethonin	TCAP	Hayashi 2004
Vinculin/metavinculin	VCL	Vasile 2006a Vasile 2006b
α-Actinin 2	ACTN2	Theis 2006
Phospholamban	PLN	Haghighi 2006
Myozenin 2	MYOZ2	Osio 2007
Junctophillin 2	JPH2	Lansdtrom 2007
Metabolic disorders		
AMP-activated protein kinase	PRKAG2	AMP Kinase disease Blair 2001
α-Galactosidase A	GLA	Anderson Fabry disease Sachdev 2002
Acid α-1,4-glucosidase	GAA	Pompe disease Amato 2000
Amylo-1, 6-glucosidase	AGL	Forbe's disease Amato 2000
Lysosome-associated membrane protein 2	LAMP2	Danon disease Amato 2000
Mitochondrial disorders	Various genes	MELAS, LOHN, MERRF DiMauro 2003
Syndromic disorders		
Tyrosine phosphatase SHP-2	PTPN11	LEOPARD syndrome, Noonan syndrome Tartaglia 2002 Sarkozy 2003
Frataxin	FRDA	Friedrich's ataxia Palau 2001

The link between genetic mutation and the clinical phenotype of HCM is poorly understood. A number of different mechanisms may play a part including ‘poison polypeptide’ or ‘dominant negative’ effect where the mutated allele affects function of the wild-type allele, or haploinsufficiency where the wild-type allele is unable to produce sufficient normal functional protein. This may be an important mechanism in some families with MYBPC3 related disease where the post-translational protein product is so small that effective incorporation into the sarcomere would be impossible [Andersen 2004].

The mechanism by which such molecular abnormalities cause the clinical features of HCM is varied and poorly understood. It is thought that the hallmark finding of hypertrophy is a secondary phenomenon to underlying abnormalities in myocyte function [Redwood 1999] and abnormal myocardial bioenergentics may play a pivotal role [Watkins 2003].

Clinical Disease Expression

The earliest descriptions of the disease necessarily focused on cases with severe hypertrophy and an obvious clinical phenotype [Teare 1958][Goodwin 1960][Pare 1961]. Sudden cardiac death was prominent leading health professionals and the lay public alike to fear the disease [Braunwald 1964]. However these early descriptions exaggerated the severity of disease and gloomy prognosis, as severe clinically obvious cases were overrepresented in these series. In fact the majority of patients in the community with HCM remain undiagnosed and are without symptoms with near normal life-expectancies.

There exists therefore a remarkably heterogeneous spectrum of disease severity. Presentations vary from neonatal heart failure [Lekanne Deprez 2006], sudden cardiac death in young adults, symptomatic left ventricular outflow tract obstruction, an incidental ECG abnormality, and asymptomatic elderly relatives diagnosed during family screening [Maron 2002].

While many patients are asymptomatic, the spectrum of clinical symptoms when present includes exertional chest pain, breathlessness, palpitations, syncope and pre-syncope. There are a number of different pathophysiological mechanisms responsible for causing symptoms which may co-exist in the same patient. Hypertrophy (the hallmark of clinical diagnosis) results in abnormal diastolic ventricular function and a consequent increase in left ventricular end-diastolic pressure. The resultant increase in left atrial pressure contributes to left atrial dilatation and predisposes the individual to atrial tachyarrhythmias. Arteriolar smooth muscle hyperplasia may induce ischaemia with scarring and fibrosis predisposing to ventricular arrhythmias [Varnava 2000].

Left ventricular outflow tract obstruction (LVOTO) occurs in 25% of individuals at rest (and up to 70% on exercise) [Maron 2006] and is an important cause of both symptoms and mortality [Elliott 2006a]. LVOTO occurs in the presence of systolic anterior motion of the mitral valve and is highly labile. The pressure gradient in the LVOT is susceptible to changes in pre-load and after-load and may be inducible with manoeuvres such as Valsalva or exercise. Modifying LVOTO has been a focus of therapeutic strategies for many years.

Early reports described a high incidence of sudden death in selected cohorts of patients with HCM [Braunwald 1964]. Sudden death remains an important, but relatively uncommon complication of this disease with an annual mortality rate of approximately 1% in unselected cohorts [Maron 2000a]. HCM however remains the leading cause of sudden cardiac death in athletes [Maron 1996]. It is likely that a combination of a predisposing arrhythmic substrate (such as severe hypertrophy, fibrosis and disarray) and subsequent triggers (such as exercise or ischaemia) are responsible for inducing ventricular arrhythmias [Frenneaux 2000] which is frequently the terminal event in individuals dying suddenly with HCM [Maron 2000b].

Accurately identifying individuals 'at risk' has received considerable interest from investigators and algorithms exist to risk stratify patients and guide prophylactic therapies [Elliott 2000][Maron 2002a][Frenneaux 2004]. Such algorithms are imperfect however and such decisions should be individualized as much as possible. Current consensus guidelines [Maron 2003a] base risk stratification for sudden cardiac death risk upon 5 established risk factors: a family history of sudden premature cardiac death, maximal wall thickness $\geq$ 30mm, an abnormal blood pressure response on exercise, unexplained syncope and non-sustained VT on Holter monitoring [Elliott 2000]. The relative risk of sudden death is related to the number of risk factors present in an individual. It should be noted that the significance of a risk factor is age dependent - the presence of non-sustained VT in a child is significantly more predictive than in a 60 year old for example. Risk factors for sudden cardiac death and stroke are listed in Tables 2 and 3.

Table 2 Predictors and risk factors for Sudden Cardiac Death in HCM

Risk Factor	Definitions
Major risk factors*	
Previous cardiac arrest	Previous documented cardiac arrest with successful resuscitation
Family history of sudden premature cardiac death	
Severe hypertrophy	Maximal wall thickness ≥ 30mm
Abnormal blood pressure response in patients < 40 years of age	Failure to rise > 25mmHg from baseline to end of exercise OR a fall > 15mmHg from peak exercise to end exercise
Unexplained syncope	Syncope in absence of identifiable cause (e.g. LVOT obstruction)
Non-sustained VT	3 or more beats of VT (rate > 120 bpm)
Other proposed risk factors	
Left ventricular outflow tract obstruction	
Myocardial ischaemia	
Late gadolinium enhancement on magnetic resonance imaging	
Mutation specific risk	

* *Elliott 2000*

Stroke is also an important complication in HCM with an annual incidence of approximately 0.8% [Maron 2002a] and is a leading cause of death (with sudden death and heart failure) in the HCM population. Risk factors for stroke include female gender, increasing age, left atrial enlargement and congestive heart failure but the major risk factor is atrial fibrillation with 88% of all strokes occurring in individuals with a history of atrial fibrillation [Maron 2002a].

Table 3 Risk factors for Stroke in HCM*

Atrial fibrillation
Left atrial enlargement
Congestive symptoms
Increasing age

* Maron 2002a

Table 4 Clinical symptoms, signs and complications in HCM

Symptoms	
	Chest pain
	Breathlessness
	Syncope / Pre-syncope
	Fatigue
	Palpitations
	Fluid retention
Clinical Signs	
	Sustained apical impulse
	Rapid upstroke pulse
	Ejection systolic murmur related to outflow tract obstruction
	Pansystolic murmur related to mitral regurgitation
Complications	
	Atrial arrhythmias
	Ventricular arrhythmias and sudden death
	Systemic thromboembolism including stroke
	Infective endocarditis
	Diastolic dysfunction and heart failure
	Left ventricular systolic dysfunction and heart failure

Management

The majority of individuals with HCM are asymptomatic. In these individuals management is limited to lifestyle advice, education, genetic counseling and often reassurance. In the remainder a number of strategies exist to modify symptoms, however few randomized trial data exist to guide management decisions.

A major cause of symptoms in HCM is LVOTO and gradient reduction therapy has received considerable interest as a therapeutic measure in symptomatic patients. The mainstay of therapy are agents aimed at reducing inotropic work: beta-blockers, calcium channel blockers such as verapamil, and class 1 agents such as disopyramide [Sherrid 2005] have been used effectively in reducing LVOTO [Maron 2003a]. While such drugs may be useful for symptomatic relief it must be borne in mind that no data exist to support their use in asymptomatic patients, despite the theoretical potential benefits in reducing LVOT gradient. In patients in whom medical therapy has been unsuccessful a

number of percutaneous and surgical strategies exist aimed at reducing LVOT gradient. Alcohol septal ablation, in which a portion of the interventricular septum is 'ablated' with intracoronary absolute alcohol has been performed for over 13 years and has become a well established method of gradient reduction therapy [Sigwart 1995] [Knight 1997][Faber 2000]. In some centres surgical myomectomy has been performed safely and effectively although the requirement for cardiopulmonary bypass carries limitations and associated risk [Koch 1980]. Programmed ventricular pacing has also received attention as a therapeutic option especially in those unsuitable for more aggressive forms of gradient reduction therapy, or in whom a device was indicated for another reason [Gadler 1999]. While some patients benefit symptomatically, randomized trials have been contradictory and the use of pacing is not widespread [Kappenberger 1997][Nishimura 1997][Kappenberger 1999].

In individuals without LVOTO therapeutic strategies may be aimed at reducing arrhythmic burden with beta-blockers or amiodarone and alleviating heart failure symptoms. It is yet unknown whether the natural history of disease can be modified in asymptomatic individuals and randomized trials are urgently required in this group of patients.

Prognosis

Although the common perception amongst lay people and health professionals is that HCM is a feared diagnosis, the overall prognosis in unselected cohorts is relatively good. In a large cohort of unselected patients followed up for a mean 8.0 +/- 7 years HCM-related death during follow up occurred in 12% with three modes of death accounting for the majority [Maron 2000a] - sudden death, heart failure death and fatal stroke. Mode of

death appears to be age related with sudden death being more prominent in the young and heart failure and stroke deaths being more prevalent later in life. Previous studies have suggested that an increased risk of sudden death is associated with specific genotypes (such as TNNT2 mutations [Watkins 1995b][Moolman 1997]) while others may carry a more benign prognosis (such as MYBPC3 mutations [Charron 1998a][Niimura 1998]) but whether these initial observations extend to large numbers of unselected individuals remains to be seen.

In published series to date, the overall annual incidence of major complications is as follows:

- sudden cardiac death - less than 1% [Maron 2000a][Maron 2002b] (although some individuals are at a much higher individual risk).
- stroke – approximately 0.8% [Maron 2002a].
- infective endocarditis - approximately 0.1% [Spirito 1999].
- end-stage disease (related to left ventricular systolic dysfunction) - 0.87% [Thaman 2005].

The incidence of sudden death may be modified however with implantable cardioverter-defibrillator implantation [Maron 2008] which can successfully cardiovert ventricular tachycardia or fibrillation and abort sudden death [Maron 2007].

Genotype-Phenotype Correlations

Hypertrophic cardiomyopathy is a diverse heart muscle disease:

- Mutations in one of many different genes encoding both sarcomeric and non-sarcomeric proteins cause the disease

- The spectrum of disease is broad – it can present at any stage in life with a wide range of symptoms and complications

Such clinical diversity makes managing patients and families with the condition challenging as the clinician has to educate, inform and predict outcome for his patients based on knowledge about the disease which often appear conflicting.

In order to examine determinants of the clinical diversity, early studies focused on whether the clinical variation between individuals could be explained by differences in the molecular genetic abnormality. A few early studies have led to accepted views on differences in patterns of disease expression that are gene-specific. These are summarized in Table 5.

Table 5 Summary of Published Genotype-Phenotype Correlation Studies in HCM

Gene	Observed phenotype	References
MYBPC3	Late-onset, low penetrance, benign clinical course	Niimura 1998 Charron 1998a
MYH7	Severe hypertrophy with high risk of sudden death	Anan 1994
TNNT2	Mild hypertrophy, high risk of sudden cardiac death	Moolman 1997 Watkins 1995b
TNNI3	Variable ? restrictive cardiomyopathy	Mogensen 2004 Doolan 2005 Kubo 2007
TPM1	Variable, sudden death reported	Watkins 1995b
MYL3	Variable, skeletal myopathy	Poetter 1996
MYL2	Variable, skeletal myopathy	Poetter 1996
ACTC	Rare, apical hypertrophy	Mogensen 1999
MYH6	Rare, ? late onset	Carniel 2005 Niimura 2002
TTN	Rare, typical HCM	Satoh 1999
TNNC1	Rare, Typical	Hoffman 2001
Others	Rare	See references in Table 1.

While these early observations were welcome – purporting to explain to some extent the clinical diversity seen in HCM – there are a number of important limitations when considering these genotype-phenotype studies:

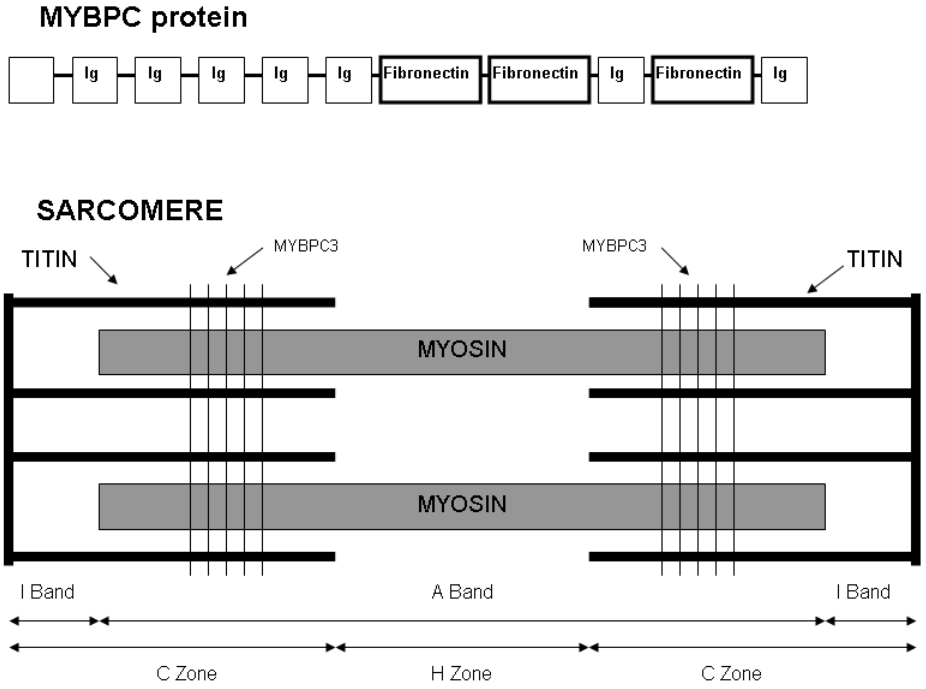
- Reports were based upon small numbers of mutations for each gene which may not be representative of the overall population

- In some cases the majority of data was obtained from small numbers of families which introduces selection bias
- Most studies are conducted at large tertiary referral centres which include an element of referral bias.
- Although the statistical mean for a given parameter may differ between different genes the variation in data may be similar and the differences in the calculated mean may not be clinically relevant.

Cardiac Myosin Binding Protein C

In most cross-sectional series of individuals with HCM, MYBPC3 mutations are the most commonly identified (Van Driest 2005). The mechanisms underlying the pathogenic effects of MYBPC3 mutations are complex however and poorly understood. Cardiac myosin binding protein C (MyBP-C) is a polypeptide with a molecular weight of approximately 135 kDa [Winegard 1999]. It is a thick-filament associated protein which is localized to the C zone of the A band of the sarcomere [Robbins 2002] (Figure 1). It has 10 globular domains of which 7 are immunoglobulin I-like and 3 are fibronectin-3 like. MyBP-C binds to myosin via the terminal C10 domain and titin via C9 or C10 domains.

Figure 1 Structure of the Cardiac Sarcomere and MYBPC Protein



Its role in sarcomeric contraction is poorly defined but is thought to include the assembly, maintenance of structural integrity and regulation of contractile activity of the sarcomere [Flashman 2004][Oakley 2004]. Interestingly knock-out mice (with no functional MyBP-C) are viable and assembly of the sarcomere is possible, albeit disordered, suggesting that MyBP-C is not absolutely essential for sarcomere formation [Harris 2002]. Cardiac hypertrophy and contractile dysfunction still occurred however.

The gene encoding cardiac myosin binding protein C (MYBPC3) is located on the short arm of chromosome 11 (11p11.2) and comprises 35 exons of which two are unusually small (3 base pairs each) [Carrier 1997]. Pathogenic mutations have been reported throughout the entire length of the protein (for an on-line list of reported mutations see http://genetics.med.harvard.edu/~seidman/cg3/muts/MYBPC3_mutations_TOC.html).

In contrast to other sarcomeric protein genes, mutations in MYBPC3 commonly result in a truncated protein product suggesting that haploinsufficiency may play an important role in disease pathogenesis in some families. Moolman et al. examined protein expression in a large family sharing a single base insertion in exon 25 resulting in premature truncation and loss of the terminal myosin and titin binding sites [Moolman 2000]. Abnormal mutated protein was not demonstrated in human myocardial tissue obtained from surgical myectomy suggesting that the mechanism leading to disease development in this instance was haploinsufficiency (i.e. absence of normal protein) rather than the 'dominant-negative' effect proposed for missense mutations, observed commonly in other sarcomeric protein genes. Haploinsufficiency has been proposed by others as an important mechanism of disease development, at least in some families, with HCM related to MYBPC3 mutations [Rottbauer 1997][Flashman 2004] [Andersen 2004].

The Spectrum of Clinical Disease in Patients with MYBPC3 Mutations

A Historical Perspective

In 1990 affected family members of a large French-Canadian family (originally described by Pare et al. in 1961 [Pare 1961]) were found to be heterozygous for the R403Q mutation in the MYH7 sarcomeric protein gene [Geisterfer-Lowrance 1990]. Subsequent studies identified mutations in other sarcomeric protein genes - TNNI3 [Watkins 1993], TNNT2 [Thierfelder 1994] and TPM1 [Watkins 1995b].

In 1993, Carrier et al. had mapped a different familial HCM locus to chromosome 11 [Carrier 1993]. Using a large multi-generational family with multiple affected family members they were able to map the probable locus using micro satellite markers and linkage techniques. Although few clinical data were reported, the authors felt that this particular family was not noticeably different to other families with MYH7 mutations they had seen, leading them to conclude that mutations at this locus caused 'the same phenotype as that described previously'. It is important to understand however, that in order to perform linkage studies large families with multiple affected family members are required – not necessarily typical of the broader HCM population. Two years later, the same group were able to identify a splice acceptor site mutation in the MYBPC3 gene at this locus on chromosome 11 in the same family [Bonne 1995]. Interestingly the mutation was also present in several clinically unaffected family members raising the possibility of incomplete penetrance – a challenging concept for the clinician and geneticist. Previously mutations in the MYH7 gene were thought to be highly penetrant [Anan 1994] although reports of incomplete penetrance had been made [Fannapazir 1994]. Simultaneously, and reported in the same edition of Nature Genetics, Watkins et al. identified a splice donor

mutation and duplication mutation in MYBPC3 in two large multigenerational families [Watkins 1995a]. These families also both showed high but incomplete disease penetrance.

In 1997, Carrier et al. characterized the entire sequence for the MYBPC3 gene and identified a further 6 mutations from 7 families with familial HCM. Of 43 mutation carriers, only 29 were clinically affected confirming previous reports of incomplete disease penetrance (in this case 67%).

As further sarcomeric genes were identified as disease causing in HCM, and further individual mutations were identified, the focus of research switched from gene identification towards genotype-phenotype correlation studies in an attempt to explain the marked diversity in clinical disease expression - a discussion of some of the important themes relating to MYBPC3 mutations follows.

Prevalence of MYBPC3 Mutations in Patients with HCM

A number of studies have systematically screened patients with HCM for mutations in genes encoding sarcomeric proteins to estimate the relative frequency of mutations for each gene. This is of importance to the clinician or geneticist, especially if phenotypic differences exist between each particular genotype. Geographical or ethnic variations may exist and it is important that a wide selection of cohorts in different patient populations is examined to highlight any important differences.

Erdmann et al. screened 110 unrelated patients from Germany and Turkey with HCM (defined as maximal wall thickness > 15mm) for mutations in MYBPC3 [Erdman 2001].

13 mutations in 15 families were found accounting for approximately 15% of the overall HCM population. No other sarcomeric protein genes were screened however.
Niimura et al. examined the spectrum of mutations in a defined group of elderly patients with late-onset HCM (diagnosed over the age of 40) [Niimura 2002]. 31 individuals (18 female, 13 male) were screened. Mutations were identified in MYBPC3 (5 mutations), TNNI3 (2 mutations) and MYH6 (1 mutation). MYBPC3 mutations accounted for only 16% of the overall population, but 62.5% of those in whom a mutation was identified.

Alders et al. reported a founder mutation 2373insG in MYBPC3 which was identified in 23% of patients with HCM in the Netherlands [Alders 2003]. Initially they screened 22 patients for sarcomeric protein gene mutations and found an unexpectedly high proportion of unrelated individuals having this mutation. They then examined the DNA of a further 237 unrelated Dutch patients with HCM and found a further 50 carriers of the 2373insG mutation giving an overall prevalence of 23% throughout the whole Dutch HCM population. Haplotype analysis confirmed the presence of a founder effect over 25 generations previously. No clinical data was reported in this study.

Morner et al. examined the spectrum of sarcomeric protein gene mutations in a Swedish population of 46 patients with familial or sporadic HCM [Morner 2003]. 7 MYBPC3 mutations were identified in 10 families with double heterozygosity occurring in one family with a MYH7 mutation. 5 missense, 1 nonsense and 1 frameshift mutation were identified. The overall prevalence of MYBPC3 in this cohort was 21.7%. This study

demonstrated the geographical and phylogenetic variation in different patient populations as MYBPC3 was the most common disease gene in this Swedish population.

Richard et al. screened 197 unrelated index cases in a French population for sarcomeric protein gene mutations. Disease causing mutations were identified in 124 index cases (64%) [Richard 1999]. MYBPC3 and MYH7 mutations accounted for 42% and 40% respectively of those in whom a mutation was identified but MYBPC3 mutations accounted for 26.4% of the overall population. Of the 39 MYBPC3 mutations, 26 (66.7%) were frameshift or nonsense mutations including splice site mutations. The remainder (33.3%) were missense mutations. An important additional finding in this study was a high prevalence of families with complex genetic status (either compound or double heterozygotes or homozygotes) which occurred in 6% of cases.

Erdmann et al. examined the spectrum of sarcomeric protein gene mutations in a German population [Erdmann 2003]. 108 families were screened for sarcomeric protein gene mutations. 18 MYBPC3 mutations were identified in 20 families (18.5%). Little clinical data was reported, but this study demonstrated that MYBPC3 mutations are the most common genetic cause of HCM in this German population.

Andersen et al. examined the spectrum of MYBPC3 mutations in a Danish population of 81 consecutive patients referred to a tertiary centre in Copenhagen [Andersen 2004]. They also screened 7 other sarcomeric protein genes. 9 mutations were detected in 10 families with one mutation present in two families (12.3% of the overall population).

Three families were found to be double heterozygotes with mutations occurring in both MYBPC3 and MYH7 – a high proportion (30%). Ectopic messenger RNA (mRNA) expression was studied using peripheral blood leukocytes. At least 3 of the 9 mutations were felt likely to cause haploinsufficiency - as the product of translation would be so short as to prevent effective incorporation into the sarcomere.

Jaaskelainen et al. screened 37 Finnish patients with HCM for MYBPC3 mutations and identified 4 disease causing mutations, one of which was a founder mutation (Gln1061X) [Jaaskelainen 2002]. Penetrance was variable within the 6 families with the founder mutation and clinical disease severity varied with most individuals having mild symptoms but one patient requiring cardiac transplantation. Although the data was unpublished they demonstrated that MYBPC3 mutations are the most prevalent cause of HCM in Finland accounting for 38% of cases of familial HCM.

Van Driest et al. screened 389 HCM patients at the Mayo Clinic for mutations in the sarcomeric protein genes MYBPC3, MYH7, MYL2, MYL3, TNNT2, TNNI3, TPM1 and ACTC [Van Driest 2004]. 46 MYBPC3 mutations were identified in 71 individuals. The overall prevalence of MYBPC3 mutations in this population was 18.3% with MYH7 mutations accounting for 13.8%. Of the MYBPC3 mutations, 46% were missense, 33% frameshift, 13% nonsense, 7% splice site and 2% in-frame deletions. It should be noted however that the patient population at the Mayo Clinic is subject to referral bias as it is a tertiary referral surgical centre with a reputation for surgical myomectomy. Their HCM population is not necessarily representative of the broader population of HCM patients with and without LVOT obstruction therefore.

Song et al. screened for mutations in MYBPC3, MYH7 and TNNT2 in a Chinese HCM population [Song 2005]. Of 100 patients with HCM, 25 mutations were found in 34 families, including 9 MYBPC3 mutations (9%).

Ingles et al examined the spectrum of sarcomeric mutations in an Australian HCM cohort [Ingles 2005]. Of 80 unrelated probands, 10 MYBPC3 mutations were identified in 11 families (13.8%). 5% of the overall cohort had complex genetic status and were either double (MYH7 + MYBPC3) or compound (MYBPC3 + MYBPC3) heterozygotes.
In summary, the prevalence of MYBPC3 mutations in unrelated, unselected patients with both familial and sporadic HCM ranges between 15 and 26.4%. MYBPC3 is the most commonly mutated gene in most series, and accounts for up to 50% of all identified mutations. In series of familial HCM this proportion is even higher. The clinical importance of MYBPC3 mutations therefore is their reported frequency in the overall HCM population, in that approximately 1 in 5 of the HCM population harbor a mutation in this gene.

Penetrance of Disease

In the first large scale study of its kind, Niimura et al. described the penetrance of disease and basic clinical characteristics of 228 gene positive individuals from 16 families with familial HCM in whom 12 different mutations in the MYBPC3 gene had been identified [Niimura 1998]. HCM was defined as a left ventricular wall thickness > 13mm in the absence of an identifiable cause. Mutations were considered pathogenic if absent in more than 200 chromosomes from control subjects, and were predicted to cause a biological effect. Of 212 gene carrying family members, 121 (57.1%) had evidence of clinical

disease expression. Symptom severity, and the pattern and extent of hypertrophy were similar to those observed in patients with other HCM causing mutations (data not published). Ninety one individuals did not fulfill clinical diagnostic criteria for HCM, of which 53 were adults. The main findings of this study were therefore:

1) Disease penetrance in adult gene carriers was incomplete in the 16 families studied (70.9% overall and 57.1% in relatives assessed at family screening)

2) Disease penetrance appeared to increase with age

3) Prognosis appeared better than for families with MYH7 and TNNT2 mutations (data not published)

The main limitation of this study is that the data is from a small number of families with familial HCM and over 50% of the data is derived from 3 large families sharing the same mutation. The possibility of referral bias also exists as such large families with multiple affected family members (while useful for linkage studies) are not necessarily representative of the broad population of HCM. The conclusions drawn suggesting that MYBPC3 disease is late-onset and benign are strongly worded and supported only by indirect or unpublished data.

The impact of this paper however has been significant and long-lasting - current consensus guidelines recommend periodic screening throughout adulthood to detect patients with late-onset disease with major implications on resource allocation as well as causing potential anxiety for families attending for serial clinical examination [Maron 2003a]. In addition, this study for the first time suggested that at least part of the phenotypic diversity seen by the clinician could be explained by genetic heterogeneity

and that mutation specific advice could potentially play a role in managing patients and families with HCM.

In smaller studies involving a limited number of families disease penetrance has varied. Moolman et al. reported disease penetrance of only 35% in a large South African family [Moolman 2000] while estimates of 90-100% were reported by other groups [Rottbauer 1997][Moolman-Smook 1998][Doi 1999]. Disease penetrance was calculated at 71% in a large French cohort [Charron 1998a] and 86% in a large German/Turkish population [Erdmann 2001]. Two further studies estimated penetrance at 57% in a Danish cohort [Andersen 2004] and 76.9% in a Japanese cohort [Kubo 2005].

While an average disease penetrance statistic is useful it doesn't reflect the variation between families – i.e. some families are fully penetrant [Anan 2001] whilst others are incompletely penetrant [Moolman 2000]. The factors which affect disease penetrance however are poorly understood. Niimura et al. suggested that disease penetrance was age-related – i.e. penetrance increases with increasing age [Niimura 1998]. By calculating the proportion of individuals who have clinical disease from the total number of mutation carriers in a particular age group, it was shown that this proportion increased with increasing age, implying a relationship between disease penetrance and age. These data were derived from cross-sectional data and not from longitudinal follow-up however and provide only indirect evidence that disease may develop at any stage throughout life, and is not limited to adolescence and young adulthood as previously thought [Maron 1986b]. Anecdotal evidence of disease developing during adulthood has occasionally been demonstrated however [Maron 2001]. Maron et al. described the development of echocardiographic left ventricular hypertrophy in genotyped adults [Maron 2001]. Sixty

one individuals from 7 families were found to be mutation carriers, of which 12 were clinically unaffected. 5 of these individuals were followed up prospectively, 3 (2 females, 1 male) of which developed echocardiographic evidence of LVH during follow up at ages 33, 35 and 42. One individual had an abnormal ECG on initial evaluation but the other two had normal ECGs at both the initial and follow-up evaluation. While this study did not determine the frequency of late-onset disease expression it did suggest that it can occur and they concluded that adult relatives without hypertrophy can no longer be reassured and require further follow up. These data support the theory that disease penetrance is age-related and that disease may develop in adults.

Age at Diagnosis in Patients with MYBPC3 Mutations

From the early studies examining the clinical spectrum of disease the general belief is that HCM develops in adolescence or early adulthood [Maron 1986b]. Following reports that MYBPC3 mutations may cause late-onset disease however [Niimura 1998] the relationship between age and disease onset has received considerable interest. Difficulty arises however because the age at clinical evaluation or first presentation does not necessarily correspond to the age at which the disease develops. Most studies which use a cross-sectional design are therefore unable to reliably identify at what age disease developed and unfortunately longitudinal studies are lacking.

Assuming these limitations are constant however it is possible to compare the age at diagnosis or age at symptom onset for each sarcomeric gene.

Estimates of mean age at diagnosis range from 33.0 years in a German population [Erdmann 2001] to 48.4 years in a Chinese population [Song 2005]. Wide confidence

intervals of the mean exist however. In a study which pooled published data [Van Driest 2005] from the United States, France, Germany, Sweden, Finland and Spain the overall age at diagnosis in patients with a MYBPC3 mutation was 37.9 which was statistically no different from any of the other major sarcomeric protein genes evaluated (MYH7 = 38.4, TNNT2 = 35.5, TNNI3 = 46.7, MYL2 = 35.1). The only longitudinal study to date which has reported disease development in adults demonstrated the development of hypertrophy *de novo* at the ages of 33, 35 and 42 [Maron 2001]. Disease development was defined in that study as the development of hypertrophy on transthoracic Echo which raises more complex questions regarding the definition of disease and whether ECG abnormalities constitute disease development or not.

Clinical Characteristics of Disease in Patients with MYBPC3 Mutations

Although much interest has been focused towards genotype-phenotype correlations, clinical data from patients with MYBPC3 mutations in the published literature is limited. A broad spectrum of HCM phenotypes have been reported in association with MYBPC3 mutations including neonatal heart failure [Lekanne-Deprez 2006], premature sudden cardiac death [Elliott 2000], severe outflow tract obstruction requiring surgical correction [Elliott 2006a], heart failure [Thaman 2005], thromboembolic stroke [Maron 2002] and mild disease with little restriction on daily activities [Niimura 1998].

Although the first reports of MYBPC3 mutations were found in families with both sudden cardiac death and severe hypertrophy [Watkins 1995a], the concept that MYBPC3 mutations caused a benign and mild phenotype arose from the first large published genotype-phenotype correlation study [Niimura 1998]. Although the ‘clinical

expression' of patients in this study was 'similar to other genetic causes of hypertrophic cardiomyopathy' the calculated Kaplan-Meier survival curves suggested improved survival compared to TNNT2 or 'malignant' MYH7 mutations (raw data unpublished). Once again it should be pointed out that over 50% of the data in this study was derived from 3 families with the same single base pair insertion mutation. No other clinical data was reported in this study.

Charron et al described the phenotype of 33 individuals carrying a splice acceptor mutation in intron 20 of the MYBPC3 gene from 2 unrelated families [Charron 1998b]. Their clinical data were compared with data from 3 families with mutations in the MYH7 gene. Maximal wall thickness was 17.6 +/- 6.3mm. No clinically important differences were apparent between the MYBPC3 individuals and the MYH7 individuals. Long term survival in patients with the MYBPC3 mutation was significantly reduced however, (survival to ages 20, 50 and 60 was 100%, 90% and 44% respectively) but better in comparison to a MYH7 mutation. It was not clear whether the prognosis was similar in both the families with the same MYBPC3 mutation or whether the premature, disease-related deaths occurred in one of the two families.

This same data was included in a larger study of MYBPC3 mutations by the same group later that year [Charron 1998a]. This study aimed to compare disease expression both between MYBPC3 and other mutations and also within the same gene (MYBPC3). Almost 50% of the data in this study however was that published in the earlier paper by the same group. In a comparison between pooled data from 69 patients with MYBPC3

mutations and pooled data from 45 individuals with MYH7 mutations the important findings were:

1) age of onset of symptoms was higher in patients with MYBPC3 mutation (40.9 +/- 19 years vs 34.6 +/- 17, $p<0.02$).

2) mean age of death was higher in patients with MYBPC3 mutations (59.6 +/- 10 years vs 38.5 +/- 16, $p<0.002$)

3) Kaplan-Meier product-limit curves showed better prognosis for patients with MYBPC3 mutations ($p<0.0001$).

These data, accepting the limitations noted above, were used to support the hypothesis that MYBPC3 mutations were associated with a relatively mild form of the disease.

Erdmann et al. screened 110 patients with HCM for mutations in MYBPC3 [Erdman 2001]. 13 mutations in 15 families were found and 14 other family members were found to be gene carriers (4 of which were unaffected). Two interesting observations were made. Firstly there was a trend towards protein truncation mutations causing earlier more severe disease than missense mutations, although this didn't reach statistical significance. Secondly, comparing the age of onset, severity of hypertrophy, and need for ICD implantation or myectomy with a group of 11 families with MYH7 mutations (unpublished data) there was no significant difference.

Richard et al screened 197 index cases for sarcomeric protein gene mutations [Richard 2003]. Disease causing mutations were identified in 124 index cases (64%). MYBPC3 and MYH7 mutations accounted for 42% and 40% respectively. Of the 39 MYBPC3

mutations, 26 were insertions/deletions, nonsense mutations or splice site mutations. The remainder were missense mutations. Index cases were classified into benign, intermediate or malignant groups according a history of adverse cardiac events within each family. MYBPC3 mutations were less prevalent in families with a malignant prognosis compared to MYH7 mutations, although MYBPC3 mutations were more common in patients with an intermediate prognosis than MYH7 mutations. MYBPC3 and MYH7 mutations were equally prevalent in families with a benign prognosis. Families with complex mutations (either compound or double heterozygotes or homozygotes) occurred in 6% of cases).

In a large study of 389 HCM patients at the Mayo Clinic [Van Driest 2004], there were no apparent clinical differences between the different mutation types. Comparing MYBPC3 mutations to patients harboring MYH7, MYL2 or MYL3 mutations (thick filament) or ACTC, TNNT2, TNNI3 or TPM1 (thin filament) mutations there were no differences in terms in of age on onset, severity of hypertrophy, need for myectomy or ICD implantation. The mean age at diagnosis for patients with an MYBPC3 mutation was 37.6 +/- 15 years. Interestingly 7% of the genotypes cohort (2.9% of the overall HCM cohort) had multiple mutations either as compound or double heterozygotes. These individuals were younger, more hypertrophied and required more interventions (myectomy or ICD) than any other subgroup. The HCM population at the Mayo Clinic however is subject to tertiary referral bias as it is specialist surgical centre offering surgical myectomy. Over 35% of patients had already undergone surgical myectomy prior to enrollment and 29% had an ICD. While screening this population has clear

benefit in itself, the results cannot be widely extrapolated to the HCM population in general.

Lekanne Deprez et al [Lekanne Deprez 2006] described two cases of fatal neonatal HCM, both of whom were compound heterozygotes for MYBPC3 mutations. The first child carried the paternally inherited splice site mutation c.1624+1G>A and the maternally inherited c.2373_2374insG mutation. The second child carried the paternally inherited nonsense mutation Arg943X and the maternally inherited Glu1096fsX92. No cardiac tissue was available for proteomic analysis but the authors felt that given the existing knowledge of the functional significance of at least one of the mutations and speculation as to the significance of the others it was likely that both patients could be considered as true MYBPC3 knockouts – i.e. no functional MYBPC3 protein expressed in the cardiac tissue - hence the severe phenotype.

A number of smaller studies have reported clinical data on individuals or small families with MYBPC3 mutations with clinical data not dissimilar to that seen in the larger studies [Rottbauer 1997][Moolman-Smook 1998][Richard 1998][Doi 1999][Waldmuller 2003] [Morner 2003][Konno 2003a][Song 2005].

Summary:

Over the last 10 years we have learned a great deal about the relationship between genotype and phenotype and the existing literature can be summarized as follows:

- The prevalence of MYBPC3 mutations in genotyped HCM cohorts is approximately 15-25%
- Disease penetrance is incomplete and ranges between 60-80% in most large series and may be age-related
- Mean age at diagnosis from pooled data is approximately 37.9 years [Van Driest 2005]
- The pattern and degree of hypertrophy is highly variable
- Prognosis is variable with both normal life expectancy and sudden premature death reported in different cohorts but few longitudinal data exist. While early studies suggested that MYBPC3 disease was associated with a more favorable prognosis, subsequent studies have observed a more varied clinical course.

Despite the considerable interest in MYBPC3 related disease important questions therefore remain unanswered. This thesis attempts to add to the existing knowledge base by combining genetic and clinical evaluation of a large cohort of unselected individuals with MYBPC3 mutations; the specific aims and objectives of which are listed in the next section.

METHODS AND SUBJECTS

Aims and Objectives

The overall objective of this study was to provide new insights into the relationship between genotype and phenotype in patients with HCM and a mutation in the MYBPC3 gene.

More specifically this study aimed to:

- Determine the penetrance of disease expression associated with MYBPC3 mutations in a large unselected population
- Describe the spectrum of disease and clinical disease severity in affected mutation carriers
- Evaluate markers of early disease expression in a genotyped population
- Determine the long-term effects of MYBPC3 mutations by serial clinical evaluation
- Determine the spectrum of disease expression in families sharing an identical mutation

Methodology

Study Design

A cross-sectional design was used to determine the penetrance of disease and clinical disease severity. A longitudinal cohort design was used to determine the long-term effects of MYBPC3 mutations in individuals with HCM.

A number of individuals were involved in data collection:

- Steve Page (SP)
- Stavros Kounas (SK)

- Brian Mist (BM)
- Michael Christiansen (MC)
- Paal Skytt Andersen (PSA)

Case Selection

A previous research programme at our unit had identified a cohort of 59 patients with HCM and a mutation in the MYBPC3 gene (BHF Programme Grant RG/2000009). These individuals had been identified from a larger cohort of 585 consecutive patients with HCM referred to our unit for evaluation who were systematically screened for genetic mutations in sarcomeric protein genes. All individuals fulfilled diagnostic criteria for HCM and the main reasons for referral included diagnostic clarification, clinical management, risk stratification and to facilitate family screening.

Of these 585 individuals a total of 44 likely disease causing sequence variations were identified in 59 individuals. These individuals were either under active follow-up in our unit (n= 31) or had been seen previously and were now under local follow-up (n=28). Index cases were contacted (SP) to discuss whether they would be interested in participating in the study. They were provided with verbal and written information regarding the study (SP) and given time to consider whether they wished to take part. Participants were invited to attend the Inherited Cardiovascular Diseases Unit, The Heart Hospital, London for clinical evaluation. Participants were also asked to invite 1st degree relatives to attend for clinical and genetic evaluation. If relatives were willing to participate they were provided with verbal and written information and written informed consent was obtained (SP - information sheet and consent form are found in the

Appendix). If clinical evidence of was demonstrated in a 1st degree relative, or if that individuals was found to be a mutation carrier, cascade screening was then offered in turn to their 1st degree relatives and so on.

Clinical Evaluation

All individuals (index cases and relatives) were invited to attend the Inherited Cardiovascular Diseases Unit, The Heart Hospital, London for clinical evaluation. Demographic details and full clinical history were obtained and combined with a physical examination (SP).

Blood pressure measurements were obtained using a mannual sphygmomanometer. Systolic pressure was defined as the pressure at which the first Korotkoff sound first appeared and the fifth Korotkov sound was used to define diastolic pressure.

A 12-lead electrocardiogram recording (ECG) by either SP or a cardiac physiologist. ECG recordings were made in the supine position at rest. Standard bipolar limb lead electrodes were connected to the right arm, left arm and left leg with a neutral electrode attached to the right leg. Augmented limb lead electrodes were recorded using Wilson's central terminal. Chest leads were positioned in the following sequence.

- V1 4th intercostals space just right of the sternum
- V2 4th intercostals space just left of the sternum
- V3 midway between V2 and V4
- V4 apex
- V5 5th intercostals space anterior axillary line

- V6 5th intercostals space mid axillary line

A paper spead of 25mm/s and calibration of 10mm/mV was used.

ECGs were analysed by SP for the following:

- Rhythm
- Atrial morphology - biphasic P wave in V1 with terminal deflection > 1mm and >0.04s duration
- PR interval
- Presence or absence of Q waves (a pathological Q wave was defined as a negative deflection with a duration >40ms and greater than 1/3 ensuing R wave height, and a non-pathological defined as a negative deflection with a duration < 40ms and less than 1/3 ensuing R wave height).
- QRS duration
- Presence of left ventricular hypertrophy using Romhilt Estes score, Sokolow Lyon criteria and Cornell criteria (Table 6 for definitions).
- T wave morphology – either flat or inverted below the isoelectric line

Table 6 Definitions used for ECG LVH Measurements

Parameter	Definition		Score
Romhilt Estes Score	Voltage Criteria (any of):		Scored out of 14. Scores > 4 considered to represent LVH
	R or S in limb lead > 20mm S in V1 or V2 > 30mm R in V5 or V6 > 30mm	3 points	
	ST-T Abnormalities		
	With Digoxin	1 point	
	Without Digoxin	3 points	
	Left Atrial Enlargement in V1	3 points	
	Left axis deviation	2 points	
	QRS duration > 90ms	1 point	
	Intrinsicoid deflection in V5 or V6 > 50ms	1 point	
Sokolow Lyon Criteria	S in V1 + R in V5 or V6 $\geq$ 35 mm		Yes or No
Cornell Criteria	S in V3 + R in aVL > 24 mm (men) S in V3 + R in aVL > 20 mm (women)		Yes or No

Individuals also had a transthoracic echocardiogram using a dedicated departmental protocol. Studies were performed on Vivid 7 machines (GE Healthcare, Little Chalfont, UK) using a 3.5Hz probe. In addition to standard views and measurements [Gardin 2002], the following measurements were made: maximal left ventricular wall thickness measurements at 12, 3, 6 and 9-o'clock positions at mitral valve, papillary muscle and apical levels (12- and 6 o'clock only) using leading-edge to leading-edge technique. Right ventricular wall thickness (also using leading-edge to leading-edge technique) was assessed in the parasternal long axis view, the parasternal short axis view at the level of the RVOT, the right ventricular free wall in the apical 4-chamber view and the free wall in the sub-costal view [Foale 1986][McKenna 1988]. Pulsed tissue Doppler imaging was used to record the systolic, early and late diastolic velocities at mitral valve annulus at the septum and lateral wall (in the apical 4-chamber view) and anterior and inferior walls (in the apical 2-chamber view) when possible. Colour flow mapping was used to detect flow acceleration in the LV cavity and pulsed wave and continuous wave Doppler was used to estimate peak velocity in the LV outflow tract and mid-cavity. If no outflow tract obstruction was noted at rest the measurements would be repeated following a Valsalva manoeuvre. Left atrial diameter was recorded in the parasternal long-axis view and apical 4-chamber view. The right ventricle was carefully assessed for evidence of hypertrophy and pulmonary artery systolic pressures were calculated. Transthoracic echo studies were performed by highly trained echocardiographers with a specialist interest in imaging patients with cardiomyopathy. Studies were reviewed off-line by SP and SK.

When possible individuals also underwent metabolic exercise testing using a bicycle ramp protocol. Tests were symptom limited and supervised by a trained exercise physiologist (BM). Gas exchange data, symptoms, arrhythmias and blood pressure response were recorded at baseline and throughout the exercise protocol into the recovery phase. A failure of the systolic blood pressure to rise at least 25mmHg above baseline was considered an abnormal blood pressure response (ABPR) [Elliott 2000]. Individuals underwent 24 hour ambulatory Holter monitoring if symptoms of palpitations or syncope were reported, or routinely in individuals with HCM as part of risk stratification for sudden cardiac death. Data was analysed manually by a cardiac physiologist and atrial and ventricular premature complexes, supraventricular and ventricular arrhythmias, bradycardia, mean, minimum and maximum heart rate were recorded. Patients were supplied with a symptom diary card to allow correlation of symptoms to arrhythmias. Non-sustained ventricular tachycardia (NSVT) was defined as three or more consecutive beats with a ventricular origin at a rate greater or equal to 120 beats per minute.

Other cardiac investigations such as coronary angiography, cardiac magnetic resonance imaging and myocardial perfusion imaging were performed on the basis of clinical need. If individuals were unable to travel to The Heart Hospital local arrangements for clinical screening were made with investigations (ECG and Echo) forwarded for analysis (SP).

Clinical Management

All individuals fulfilling diagnostic criteria for HCM were managed according to Clinical Expert Consensus Documents [Maron 2003a] and local clinical expertise. The main

principles of management were to reduce symptoms and to prevent sudden complications such as sudden cardiac death and stroke. Gradient reduction therapies included beta-blockers, calcium channel blockers, disopyramide, alcohol septal ablation, surgical left ventricular myomectomy and dual chamber pacemaker implantation. Risk factors for sudden cardiac death were determined systematically in all individuals with HCM. Major risk factors were: previous cardiac arrest, unexplained syncope, family history of sudden cardiac death, left ventricular wall thickness greater than 30mm, non-sustained VT on Holter monitoring and an abnormal blood pressure response on bicycle exercise testing. In patients with 2 or more risk factors, consideration for ICD implantation was made. In those with only 1 risk factor, decisions were made on an individual basis. Patients felt to be at increased risk of thromboembolic stroke were considered for anticoagulation with Warfarin. Risk factors included atrial fibrillation, left atrial diameter greater than 50mm in the parasternal long axis view, heart failure and increasing age.

Genetic Evaluation

The cohort of index cases had been previously identified from a larger cohort of 585 individuals fulfilling diagnostic criteria for HCM referred to our unit for evaluation. Each individual had provided a blood sample for mutation analysis. Each sample was screened for mutations in the following genes:

- MYBPC3
- MYH7
- TNNT2
- TNNI3
- MYL2

- MYL3
- TPM1
- PRAKG2 (if clinically indicated – premature conduction tissue disease or pseudo pre-excitation pattern on ECG)
- GLA (if clinically indicated – concentric hypertrophy, X-linked inheritance, associated non-cardiac symptoms such as angiokeratoderma and anhydrosis)

All coding regions were screened systematically for sequence variations by direct sequencing and fluorescent (F)-SSCP analysis by MC, PSA and RFH according to standard techniques. All genes were screened even once a sequence variation had been identified. Sequence variations were considered to be pathogenic mutations on the basis of the following criteria with reference to published criteria [Cotton 1998].

- The sequence variation cosegregated with clinical disease within the family (determined from clinical evaluation performed by SP)
- The sequence variation occurred within a region of conserved DNA across different species (for missense mutations)
- The sequence variation was absent in commercially available control alleles
- The sequence variation was predicted to cause a mutated protein with biological effect either confirmed by RNA functional studies or by inference in the case of non-sense or frameshift mutations

Relatives were invited to provide a venous blood sample for mutation analysis for the family mutation. No other sarcomeric protein genes were screened in relatives. Relatives were counselled by either a clinical genetics counsellor or by SP. Counselling was performed face to face when possible or over the telephone if the patient was unable to

travel to The Heart Hospital. Relatives were provided with both verbal and written information (see Appendix) and informed written consent was obtained.

Venous blood samples from relatives were collected (SP) into 2x4.5ml EDTA tubes which were immediately refrigerated and subsequently stored at -80 °C for subsequent batched DNA analysis. Samples were analysed for the known family mutation only and an individual could therefore either be wild type or a mutation carrier.

If relatives were unable to attend The Heart Hospital individuals were offered genetic screening remotely with verbal and written information provided and genetic counselling performed by telephone. Venous blood samples were collected locally, and posted to The Heart Hospital for further processing.

Definitions

A number of terms are used frequently in this thesis and are defined below.

A *diagnosis of HCM* was defined by ventricular hypertrophy greater or equal to 13mm in the absence of a haemodynamic cause [McKenna 1997]

An *index case* was defined as an individual fulfilling diagnostic criteria for HCM, from the original cohort in whom a MYBPC3 mutation had been identified.

An *affected mutation carrier* was an individual who carried the family mutation and had echocardiographic hypertrophy with a maximal wall thickness of at least 13mm.

An *unaffected mutation carrier* was an individual who carried the family mutation but had a maximal wall thickness of less than 13mm.

Sudden cardiac death was defined as death occurring within 1 hour of the onset of new symptoms, or nocturnal death without an antecedent history of worsening symptoms.

A *family history of sudden cardiac death* was defined as sudden cardiac death in two or more first degree relatives less than 40 years old.

Disease penetrance was defined as the proportion of mutation carriers who demonstrated evidence of clinical disease expression sufficient to fulfil diagnostic criteria for HCM

Statistics

Data was recorded using Microsoft Excel spreadsheets and statistical analysis was performed using SPSS software. Mean values with standard deviations were calculated for continuous variables with ranges given where appropriate. Comparison of means between groups was made using Student's t-test and ANOVA when more than two groups were being compared, assuming the data was normally distributed. For categorical variables the Chi-square test was used. A p-value of < 0.05 was considered statistically significant.

Ethical Approval and Funding

This clinical study was an observational, cross-sectional study in which the selection of patients and clinical management decisions were based upon clinical need rather than specifically for research. Local Ethical Committee approval was obtained as part of an ongoing larger British Heart Foundation Programme Grant (BHF Programme Grant RG/2000009) into genotype-phenotype correlations in HCM.

Ethical approval was obtained for the larger study 'Hypertrophic cardiomyopathy (HCM): Clinical and Genetic Investigation of a Hereditary Heart Disease'. This ethical approval was obtained in 2003 by Professor WJ McKenna – approved by the Wandsworth Local Research Ethics Committee, South London REC, St George's Hospital, London (Ref 01.78.10). Following the departmental move to the Heart Hospital, UCLH, London, ethical approval was also sought from the UCLH REC (Ref 03/0196).

Participation in the study was voluntary and individuals could withdraw from the study at any stage. All individuals providing blood for DNA analysis provided written informed consent. Funding for this study was provided by the British Heart Foundation who awarded a Junior Research Fellowship to SP.

THE SPECTRUM OF MYBPC3 MUTATIONS IN FAMILIES WITH HCM

Introduction

HCM is characterised by an autosomal dominant pattern of inheritance with a mutation in one of the sarcomeric protein genes identified in up to 60% of individuals [Richard 2003]. For a complete list of genes associated with HCM see Table 1. The most common sarcomeric protein gene in which mutations are found is MYBPC3, accounting for approximately 20% of HCM cohorts [Van Driest 2005]. Most mutations are novel and 'private' although founder mutations have been identified in some populations [Moolman-Smook 1999][Waldmuller 2003][Richard 2003][Alders 2003][Konno 2003][Jaaskelainen 2004][Kubo 2005].

In most large series missense and insertions/deletions account for the majority of MYBPC3 mutations, with fewer nonsense and splice site mutations [Richard 2003][Erdman 2003][Van Driest 2004].

In this section the spectrum of mutation identified in this study are described.

Methods

A previous study, supported by the British Heart Foundation (BHF Programme Grant RG/2000009) systematically screened a cohort of 585 consecutive individuals fulfilling diagnostic criteria for HCM for mutations in sarcomeric protein genes. DNA analysis was performed in Copenhagen, at the Statens Serum Institute by PSA and MC.
DNA sequencing techniques and screening methods are described in the Methods Section.

Mutations located in the flanking introns of the MYBPC3 micro-exons were examined using in silico methods. Ectopic expression of mRNA in blood leukocytes in the respective patients was examined using reverse transcription-PCR.

Results

Index cases

A total of 44 MYBPC3 mutations were identified in 59 of 585 index cases (10.1%) which were believed to be disease causing. These mutations are described in Table 7. Of the 44 mutations identified 28 were novel (63.6%).

Table 7 Disease Causing MYBPC3 Mutations in 59 Index Cases with HCM

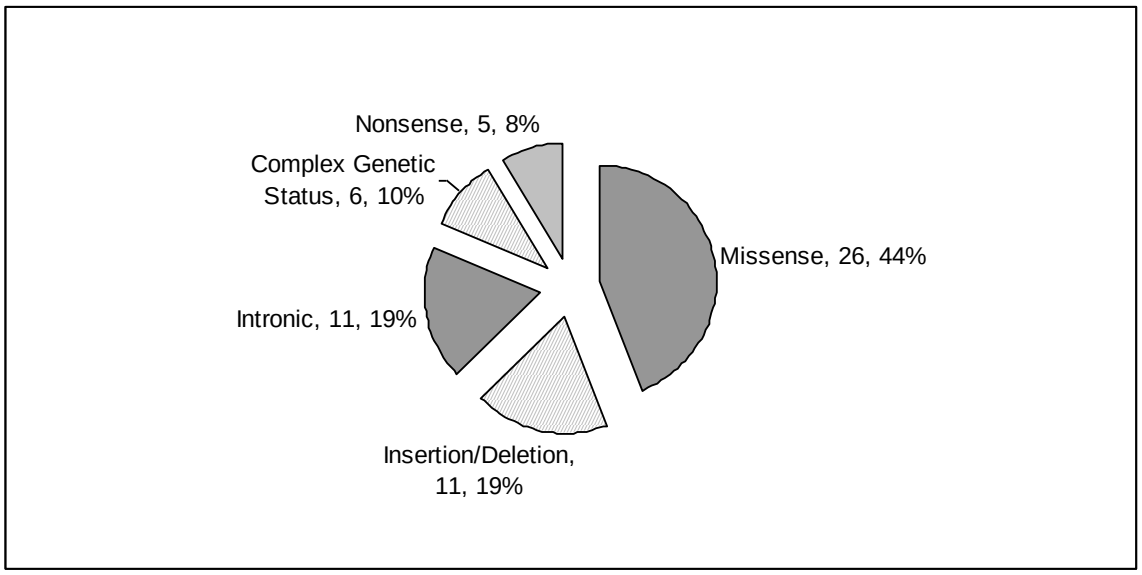
Mutation Type	Mutation Description
Nonsense (4 mutations in 5 families)	Q425X
	Q969X *
	K1055X
	R943X
Deletions/Insertions (10 in 11 families)	g7040_7041delTT
	g14271delC
	g14274delC
	g15919insG
	g16225delG
	g16190_16196delGCGTCTA
	g20350insT *
	g14291insA
	698delC
	g18567delCT
Missense (15 in 26 families)	R502W *
	R502Q *
	E258K *
	G341R
	R495G *
	P873Q
	E542Q
	D605N
	G490R
	R820Q
	T750M
	G148R
	F1177L
	C1266Y
	T957S
Intronic (9 in 11 families)	IVS20-2A>G
	IVS7+1G>A
	IVS13-2A>G
	IVS14-13G>A
	IVS1-2A>G
	IVS27+1G>A
	IVS13-19G>A *
	IVS17+4A>T
	IVS9-36G>A
Complex Genetic Status (6 in 6 families)	MYBPC3 G148R AND GLA N215S
	MYBPC3 IVS14-13G>A* AND MYH7 T1854M
	MYBPC3 R502W * AND MYH7 N602S
	MYBPC3 G490R AND MYBPC3 Q642X
	MYBPC3 V1125M AND MYBPC3 IVS9-1G>C
	MYBPC3 IVS18+7G>A AND MYBPC3 D880D
Sequence Variations of Uncertain Significance (9 in 9 families)	IVS26-52G>A
	IVS1-2A>G
	IVS20-23A>G
	IVS16-26C>G
	IVS8-37C>T
	IVS25-10C>T
	IVS2-51 T>C
	g15096C>T, G758G
	g21033C>T, I1193I

* - Mutation found in more than 1 family

A further 9 sequence variations were identified in which the pathogenicity was uncertain or unlikely. These mutations either did not co-segregate with clinical disease or were of uncertain functional significance. Data from these families was excluded from analysis.

Mutations were grouped by type (Figure 2). The most common type were missense mutations (26, 44%). Insertions or deletions accounted for 11 (19%). Five (8%) were nonsense mutations producing a premature stop codon. Eleven (19%) were intronic mutations. Interestingly 10% of the genotyped cohort had complex genetic status. In one index case, mutations were identified in MYBPC3 and GLA. This individual had clinical features consistent with Anderson Fabry disease with other similarly affected individuals on the maternal side of the family. The MYBPC3 mutation had been inherited from the paternal side of the family. In 2 families, mutations were identified in MYBPC3 and MYH7 (double heterozygotes) and in 3 families two mutations were identified in MYBPC3 (compound heterozygotes). One of these families had both an IVS18+7G>A mutation and a single base substitution (g17675 C>T) resulting in D880D. The significance of this second sequence variation is uncertain and it was not possible to study the functional consequences of this mutation as no relatives were found to be carriers of this mutation alone.

Figure 2 Spectrum of mutations in 59 index cases



A total of seven mutations were discovered in the introns flanking the two micro-exons 10 and 14, but none were found in introns flanking exon 11.

Familial Mutation Analysis

In addition to the 59 index cases, familial mutation analysis identified a further 111 mutation carriers and 118 relatives who were wild type for the family mutation. The total number of mutation carriers (index cases and relatives) was 170.

Discussion

The main findings can be summarised as follows:

- The prevalence of MYBPC3 mutations in our HCM cohort was 10.1%
- More than 60% were novel mutations
- Missense mutations were the most frequent accounting for 44% of all mutations identified
- The previously described R502W mutation was found in 9 families with evidence of a founder effect following haplotype analysis
- Intronic mutations were common and accounted for 18.6% of mutations identified
- Complex genetic status was common and occurred in 10.2% of index cases with an MYBPC3 mutation

The spectrum of mutation type seen in this study is broadly similar to that seen in other cohorts of MYBPC3 mutations (Figure 3) [Richard 2003][Erdman 2003][Van Driest 2004].

A pathogenic mutation in MYBPC3 was identified in 10.1% of the overall HCM cohort which is slightly lower than previously reported [Van Driest 2005]. This may represent differences in mutation detection methods, although in this study all exons (including 3 micro-exons) and intronic sequences were analysed. It may be that the strict criteria used in this study to define a disease causing mutation led to a lower yield than in previously published data.

Similar to previous series, most identified mutations were novel. The phenomenon of so-called 'private mutations' lends added complexity to the clinical genetics of HCM, as mutation specific data are usually lacking. Consequently 'proof of pathogenicity' becomes more challenging and providing mutation specific advice when counseling families becomes impossible. This phenomenon is not specific to MYBPC3 mutations however.

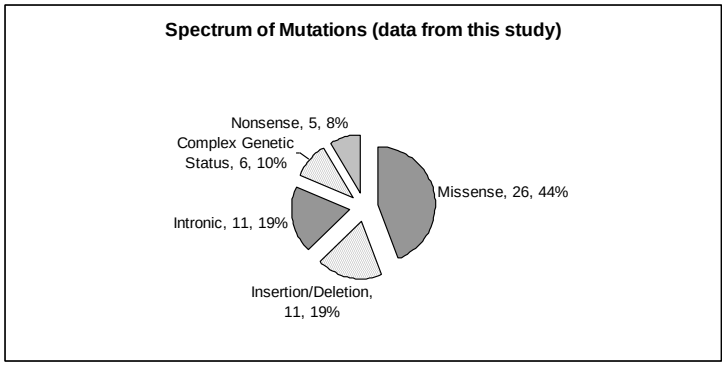
The finding of a founder effect in 9 families with the R502W mutation is interesting as this is the first founder mutation to be described in an UK population. Founder MYBPC3 mutations have been described previously in HCM cohorts, including a highly prevalent 2373insG mutation in the Dutch population, found in 23.2% of all HCM index cases [Alders 2003). Identifying several families with an identical mutation provides a unique opportunity to examine phenotypic heterogeneity having controlled for a specific mutation. This data is discussed later in Chapter: Disease Expression in Families Sharing Identical Mutations.

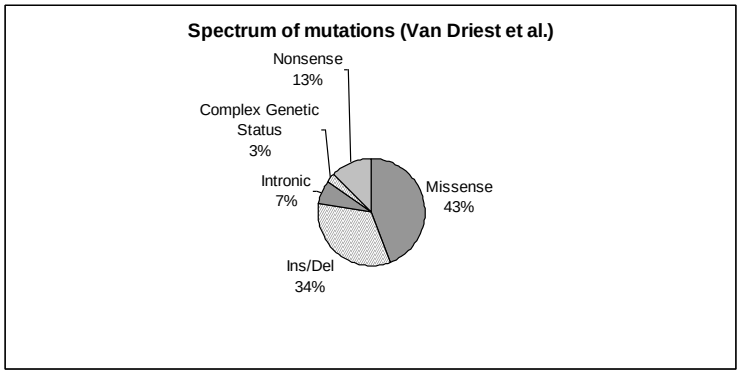
Of note was a high proportion of individuals with complex genetic status with both double heterozygotes and compound heterozygotes seen. This has major implications for DNA screening strategies - a high incidence of complex genetic status affects the accuracy and reliability of genetic predictive testing and has implications for calculating the risk of inheriting a mutation for children in families with two pathogenic mutations. Whether the mutations are compound or double and whether the mutations occur on the same allele (for compound mutations) can greatly affect the risk that should be discussed with the parents when family planning. Furthermore, a more severe phenotype has been

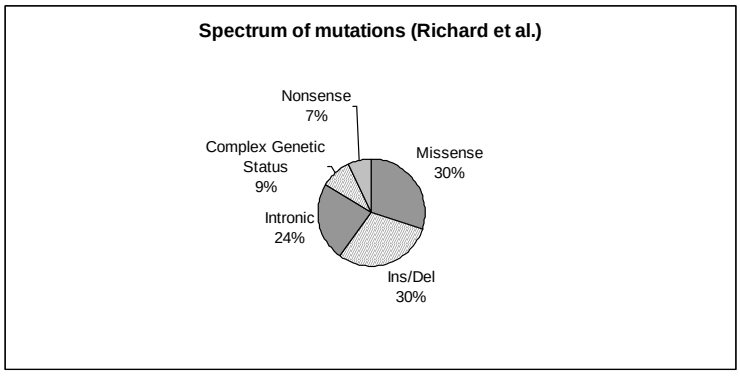
described in certain individuals with complex genetic status [Van Driest 2004][Richard 2003] including neonatal hypertrophy [Lekanne Deprez 2006] and severe hypertrophy in a female homozygous for a A627V missense mutation [Garcia-Castro 2005]. The high incidence of complex genetic status in this study supports a protocol of full sequencing of all sarcomeric protein genes [Ingles 2005], rather than targeted selective DNA screening as advocated by some [Girolami 2006]. This concept is further supported by the novel identification of pathogenic mutations in the intronic flanking regions of the two micro-exons 10 and 14 [Frank-Hansen 2008].

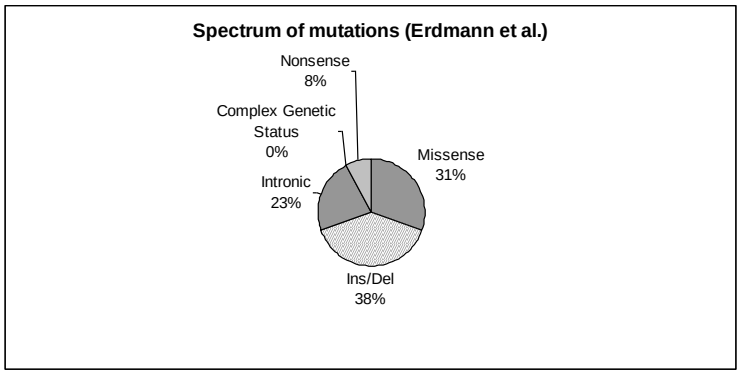
In summary the spectrum of mutations in this cohort is broadly similar to previous studies but highlights the wide genetic heterogeneity typically seen in HCM. The remainder of this thesis describes in detail the clinical characterisation of index cases and their families with the MYBPC3 mutations identified during this study.

Figure 3 Spectrum of MYBPC3 mutations in different cohorts









DISEASE PENETRANCE, AGE AND GENDER IN FAMILIES WITH MYBPC3 MUTATIONS

Introduction

In common with many autosomal dominant conditions, disease expression in HCM is highly variable, and for most reported mutations disease penetrance is incomplete (i.e. not all mutation carriers manifest clinical disease) [Niimura 1998][Charron 1998a]. As described in the Introduction, estimates of disease penetrance in families with MYBPC3 mutations vary but in most studies of reasonable size, range between 57% [Andersen 2004] and 86% [Erdmann 2001].

The factors that influence disease penetrance are poorly understood. Niimura et al. suggested that low penetrance was characteristic of MYBPC3 mutations per se, and may be age-dependent [Niimura 1998]. Historically HCM has been considered to be a disease that develops in adolescence and early adulthood [Maron 1986b], but in a cross-sectional study of families with MYBPC3 mutations, Niimura et al. observed a linear relationship between increasing age and increasing disease penetrance with almost complete penetrance by the 7th decade [Niimura 1998]. This was in contrast to families with mutations in MYH7 and TNNT2 who appeared to have almost complete penetrance by the 4th decade. This observation provided indirect evidence that disease can develop in adulthood, and so called ‘late-onset’ disease was felt to be a phenomenon associated only with MYBPC3 mutations. Cross-sectional studies however are inherently limited in their ability to identify ‘late-onset’ disease rather than ‘late-diagnosis’ – a distinction with important implications for how clinicians screen HCM families . Another study suggested that MYBPC3 mutations are predominant in a group of patients with disease diagnosed > 40 years, although the proportion was not dissimilar to the overall genotyped HCM population (16%) [Niimura 2002].

The relationship between penetrance and gender is unknown. Despite an autosomal dominant pattern of inheritance, most HCM cohorts demonstrate a slight male predominance of approximately 3:2, consistent amongst cohorts from different genetic and racial backgrounds [Maron 2003][Olivotto 2005]. Although data on gender differences in HCM are limited, a number of observations have been reported including: 1) an inverse relationship between age and maximal wall thickness has been reported in females [Maron 2003a], 2) females are older at first diagnosis [Maron 2003b], 3) females are more symptomatic and have a higher prevalence of left ventricular outflow tract obstruction [Olivotto 2005], 4) overall mortality and risk of sudden cardiac death appear similar between males and females however [Elliott 2000]. If gender differences in clinical disease expression do exist it is possible that gender plays a role in determining disease penetrance.

This section examines disease penetrance with particular reference to age and gender.

Methods

59 index cases found to have mutations in MYBPC3 were evaluated. Relatives were invited for clinical and genetic evaluation as described in Chapter 2. HCM was defined in relatives as unexplained LVH (maximal wall thickness ≥13mm) [McKenna 1997]. Disease penetrance was calculated as the proportion of all mutation carriers who fulfilled diagnostic criteria for HCM (index cases and relatives).

Clinical markers of disease expression (including prevalence of symptoms and echocardiographic parameters) were compared between males and females. The

relationship between disease penetrance and age was also examined. Age at diagnosis was defined as the age (to the nearest year) at which a diagnosis of HCM was first made, rather than age at initial evaluation at our institution.

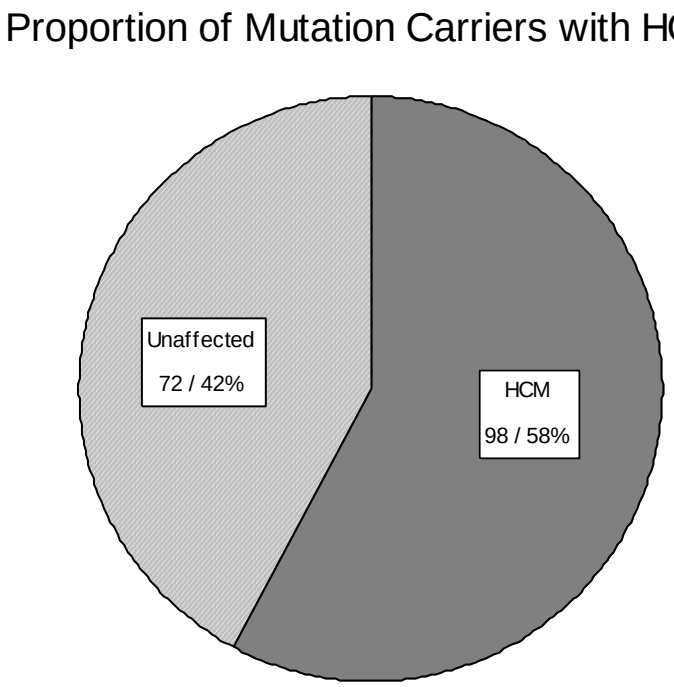
Comparisons between groups were made using ANOVA or independent Students t-test for continuous variables and Chi-square test for categorical data. A p-value of <0.05 was considered statistically significant.

Results

Disease Penetrance

In addition to the 59 index cases, family screening identified a further 111 mutation carriers and 119 relatives who were negative for the family mutation. The total number of mutation carriers (index cases and relatives) was 170. At the last clinical evaluation, 98 fulfilled diagnostic criteria for HCM and 72 were unaffected mutation carriers. Disease penetrance for the overall cohort was therefore 58% (Figure 4).

Figure 4 Proportion of individuals fulfilling diagnostic criteria in 170 mutation carriers

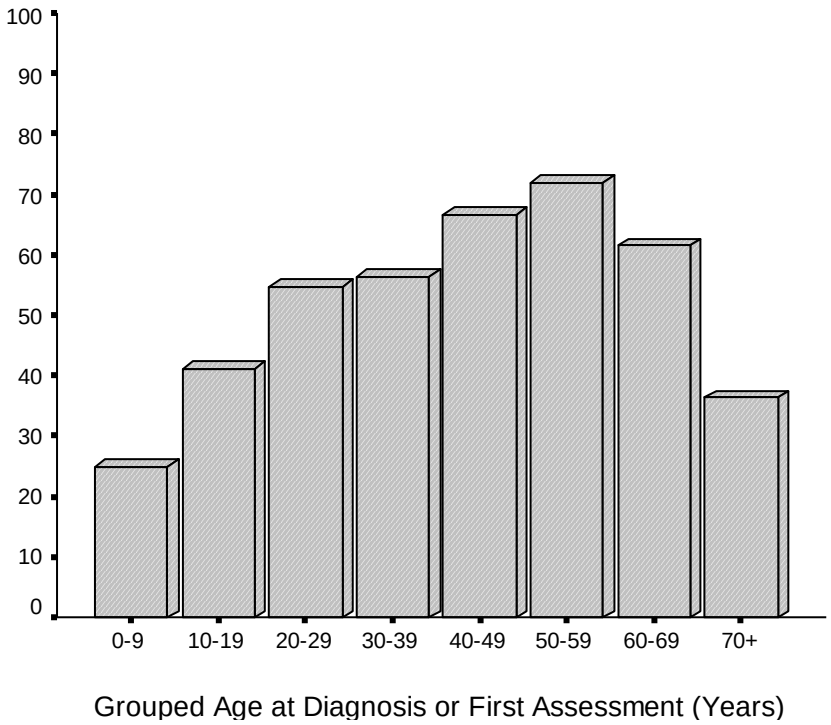


Family screening identified an additional 39 affected mutation carriers from 234 family members screened (a yield of 16.7%).

Disease Penetrance and Age

The relationship between disease penetrance and age at initial evaluation is shown in Figure 5.

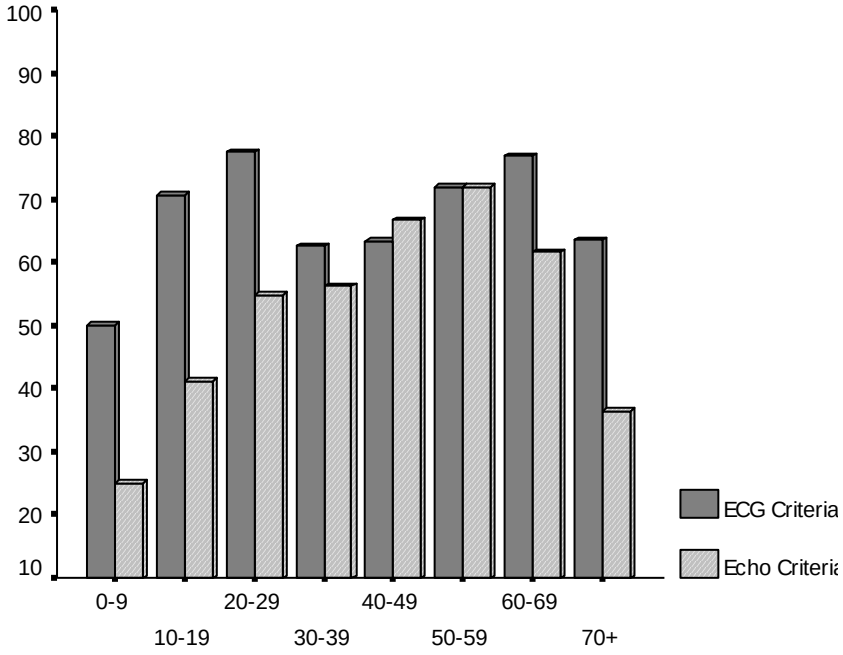
Figure 5 Disease Penetrance According to Age in 170 Mutation Carriers



Disease penetrance appeared to increase with age until the 6th decade providing indirect but supportive evidence that the diagnostic hallmark of HCM (echocardiographic evidence of ventricular hypertrophy) may develop in adulthood. Interestingly, disease penetrance appears to plateau (or even fall in the 7th and 8th decades – perhaps due to reduced survival in affected individuals), demonstrating that a proportion of mutation carriers will never develop clinical evidence of disease. Of the 7 unaffected mutation carriers aged ≥70 years the spectrum of mutations was as follows: 1 nonsense, 1 deletion,

3 missense, 1 intronic and 1 compound heterozygote – i.e. a broad spectrum of mutation types is demonstrated in this sub-group of individuals. When disease penetrance is defined in terms of an abnormal ECG (defined as either RE score >4, abnormal T wave inversion or abnormal Q waves), rather than echocardiographic LVH, it is apparent that ECG abnormalities pre-date the development of LVH on echocardiography and using ECG criteria for evidence of clinical disease expression is more sensitive than criteria based upon echocardiography alone (Figure 6). It can be seen that disease penetrance, as defined by an abnormal ECG, is also incomplete - 71.2% overall – i.e. a proportion of mutation carriers (approximately 30%) have a normal ECG even at advanced age.

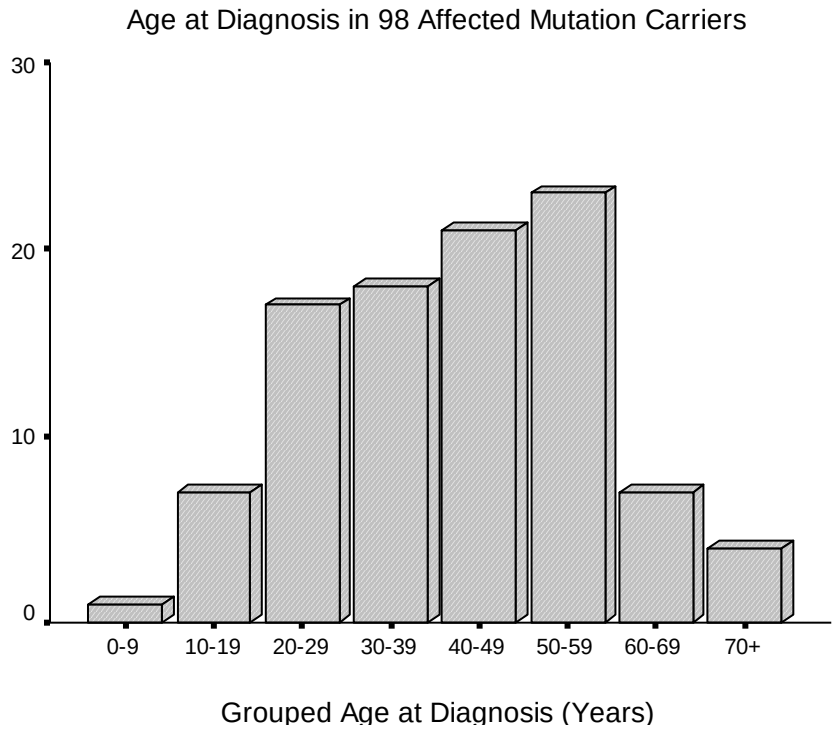
Figure 6 **Disease Penetrance According to ECG or Echo Criteria**



Of 98 affected mutation carriers only 8 were diagnosed before the age of 20 years (8.2%) (Figure 7) – i.e. over 90% of individuals were diagnosed after the age of 20 years. Indeed

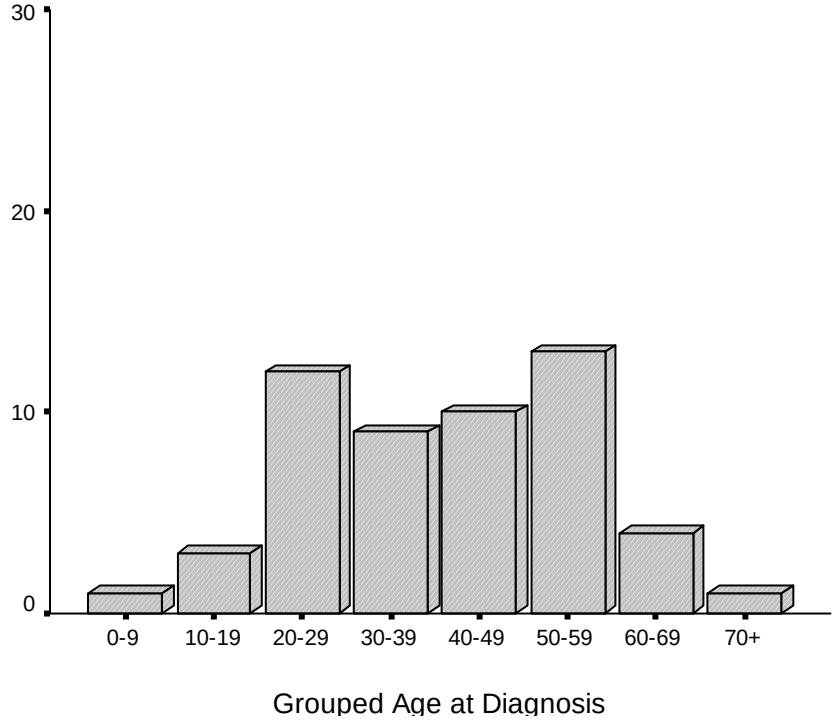
Figure 7 demonstrates that HCM related to MYBPC3 mutations can be diagnosed in any decade of life. Of the 4 individuals diagnosed age 70+, 3 were diagnosed in their 70s and 1 in his 80s.

Figure 7 Age at Diagnosis in 98 Affected Mutation Carriers



If all those diagnosed at family screening (in this group, age is dependent on the strategy of the screening hospital) were excluded, 4 of 49 (8.2%) were diagnosed before age 20 years and 91.8% of index cases were diagnosed after the age of 20 years (Figure 8).

Figure 8 Age at Diagnosis in 49 Affected Mutation Carriers Excluding those Diagnosed at Family Screening



When affected individuals were grouped into early-diagnosis (< 40 years) and late-diagnosis (≥ 40 years) there were no apparent important clinical differences in terms of septal thickness, maximal wall thickness, LV cavity size, or % symptomatic (Table 8). The age of 40 years was chosen as it has been used previously in studies to define early-onset and late-onset disease [Niimura 2002]. The data was also analysed defining late-onset disease as > 30 years, > 50 years, > 60 years and > 70 years. No significant differences were observed however in any of the variables shown in Table 8, and it was not possible to identify a clinically useful definition of late-onset disease.

The prevalence of confirmed familial disease (defined as at least 1 other affected relative) was no more common in those with early-diagnosis (76.7% v 69.1, p=0.4).

Table 8 Comparison between individuals with early and late diagnosis

Parameter	Early Diagnosis (< 40 Years)	Late Diagnosis (≥40 Years)	P – value
Number of Individuals	43	55	-
Mean Age at Diagnosis (years)	23.0 +/- 7.8	53.2 +/- 10.1	0.000
Male (%)	65.1	54.5	$\chi 2 = 1.1$, 1df, p=0.291
Symptomatic (%)	60.0	59.2	$\chi 2 = 0.006$, 1 df, p=0.938
Echo parameters			
LA diameter (mm)	40.3 +/- 8.4	42.2 +/- 6.2	0.220
LVEDD (mm)	42.6 +/- 7.3	44.7 +/- 6.0	0.136
LVESD (mm)	24.5 +/- 6.3	27.2 +/- 6.4	0.052
FS (%)	43.9 +/- 9.5	41.6 +/- 8.6	0.384
IVSd (mm)	17.7 +/- 6.0	18.0 +/- 4.8	0.782
PWd (mm)	9.8 +/- 2.4	10.8 +/- 2.5	0.059
MWT (mm)	18.6 +/- 5.8	18.7 +/- 5.2	0.922
Mean number of Risk factors	0.9 +/- 0.8	0.8 +/- 0.8	0.490
Confirmed familial disease (%)	76.7	69.1	$\chi 2 = 0.7$, 1 df, p=0.4

In three individuals disease developed during prospective follow up. H610.1 (mutation Cys698del) and H62.2 (mutation Arg502Gln) were diagnosed shortly after initial assessment at ages 13 and 14 respectively. Only H610.8 (mutation 698delC) clearly developed HCM as an adult which was diagnosed age 53. He was first assessed in 1993, age 40, as part of family screening and had a normal ECG and Echo at that stage. In 2006, age 53, he underwent a routine medical. ECG showed LVH with widespread repolarisation abnormalities and a Romhilt Estes score of 7. Transthoracic Echo showed asymmetrical septal hypertrophy with a maximal wall thickness of 15mm. He had had no interim assessment in between so the age at which LVH developed is not precisely known, but occurred between the ages 40 and 53. He was not known to be hypertensive and although fit and active, had never undergone sustained athletic training.

Disease Penetrance and Gender

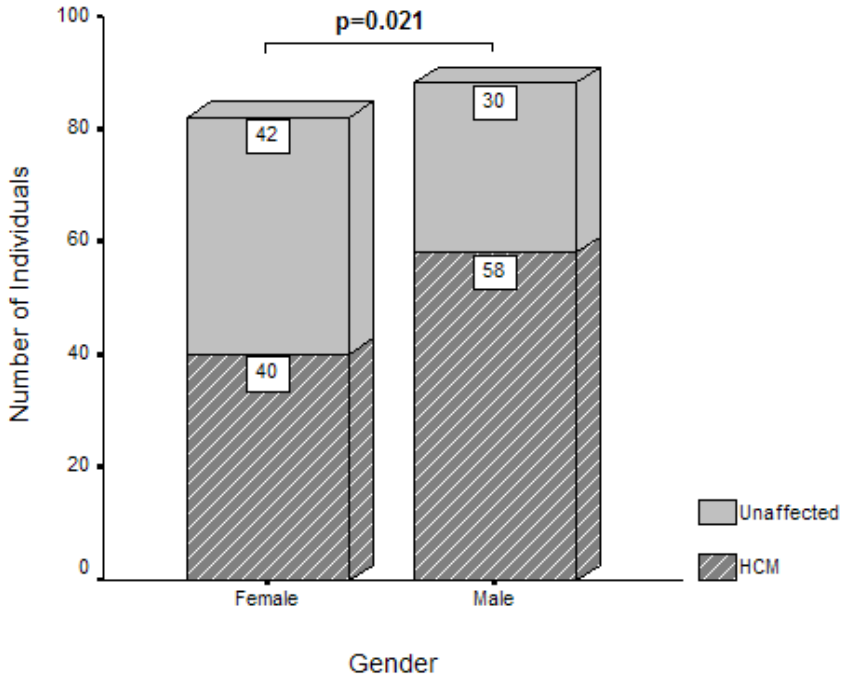
Of 170 mutation carriers, 88 (51.8%) were male and 82 (48.2%) were female. Disease penetrance was higher in males than in females (65.9% v 48.8%, χ^2=5.35, 1df, p= 0.021) (Figure 9). Mean age at diagnosis in affected individuals was no different between males

and females (40.6 +/- 15.1 years v 43.3 +/- 17.2, p=0.411). When all 170 mutation carriers are considered, males have greater LA diameter (40.7 v 36.5, p=0.001), greater LV cavity dimensions (LVEDD 46.4 v 43.1, p=0.001, LVESD 28.9 v 26.1, p=0.006) and greater degrees of hypertrophy (septal thickness 15.5 v 12.7, p=0.004, maximal wall thickness 16.1 v 13.6, p=0.012) (Table 9). When only affected individuals are considered however these gender differences no longer exist and are therefore explained by the higher disease penetrance in males.

Table 9 Gender Differences in 170 Mutation Carriers

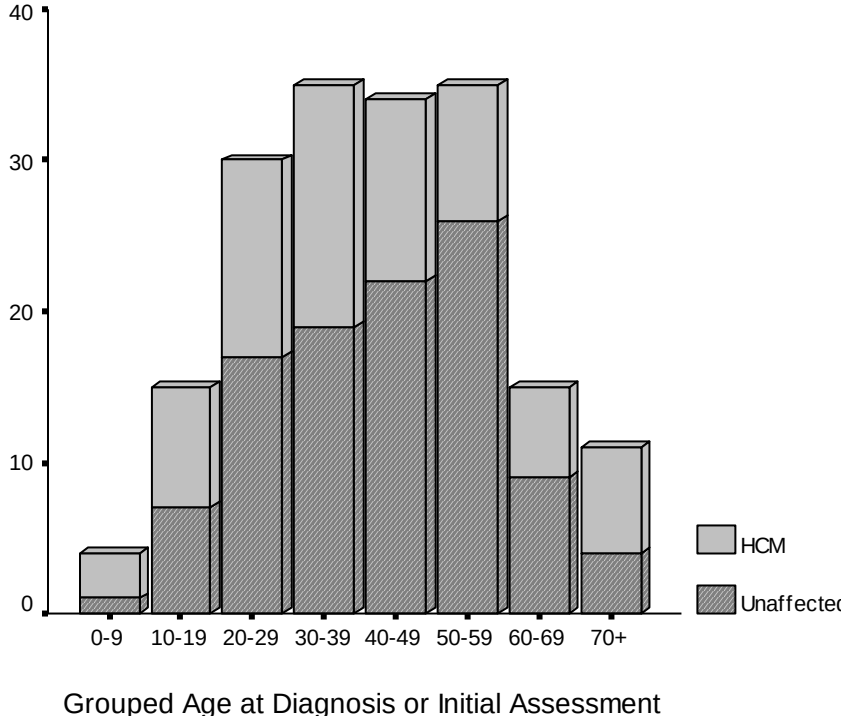
Parameter	Male	Female	p Value
Number of Patients	88	82	-
Age at diagnosis (affected only, years)	40.6 +/- 15.1	43.3 +/- 17.2	0.411
% Affected	65.9	48.8	0.021
% Symptomatic	38.6	36.8	$\chi 2 = 0.049$, 1 df, p=0.824
% Predicted VO2	78.9 +/- 23.0	75.4 +/- 3.2	0.484
Echocardiographic parameters			
LA dimension (mm)	40.7 +/- 7.2	36.5 +/- 7.4	0.001
LVEDD (mm)	46.4 +/- 6.5	43.1 +/- 6.1	0.001
LVESD (mm)	28.9 +/- 6.1	26.1 +/- 5.7	0.006
FS (%)	39.2 +/- 7.8	40.3 +/- 9.5	0.517
Septal thickness (mm)	15.5 +/- 6.0	12.7 +/- 5.9	0.004
Posterior wall thickness (mm)	10.0 +/- 2.0	9.1 +/- 2.8	0.024
Maximal wall thickness (mm)	16.1 +/- 6.2	13.6 +/- 6.2	0.012

Figure 9 **Disease Penetrance in Males and Females**



Another way of looking at the data is calculating the proportion of individuals fulfilling diagnostic criteria for HCM (maximal wall thickness ≥13mm) for grouped age at diagnosis or initial assessment (Figure 10). It can be seen that in all age groups disease penetrance is incomplete.

Figure 10 Number of individuals in each age group showing proportion fulfilling diagnostic criteria for HCM

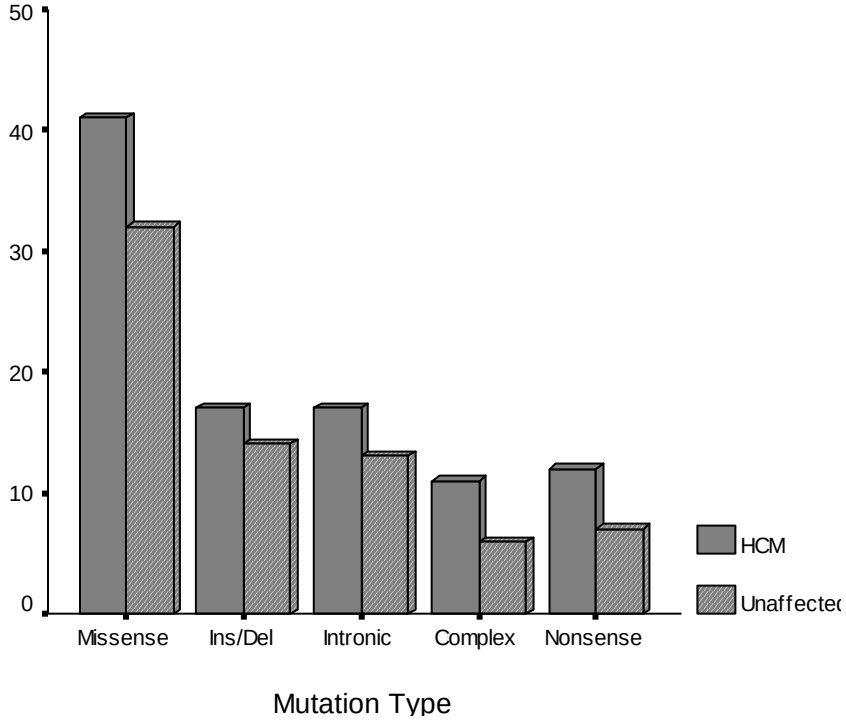


Disease Penetrance and Mutation Type

Disease penetrance was incomplete for all mutation types (Figure 11). Of 38 families with at least 2 mutation carriers in adults aged 18 years or more, 33 showed incomplete penetrance and 5 showed fully penetrant disease (2 missense, 1 intronic, 2 complex genetic status). Disease penetrance was also incomplete in families with complex genetic status - 13 individuals inherited two possible disease causing mutations, of which 11 fulfilled diagnostic criteria for HCM (84.6%). Two individuals were unaffected – one was a 17 year old female with a 2 base-pair deletion and cryptic splice site mutation in

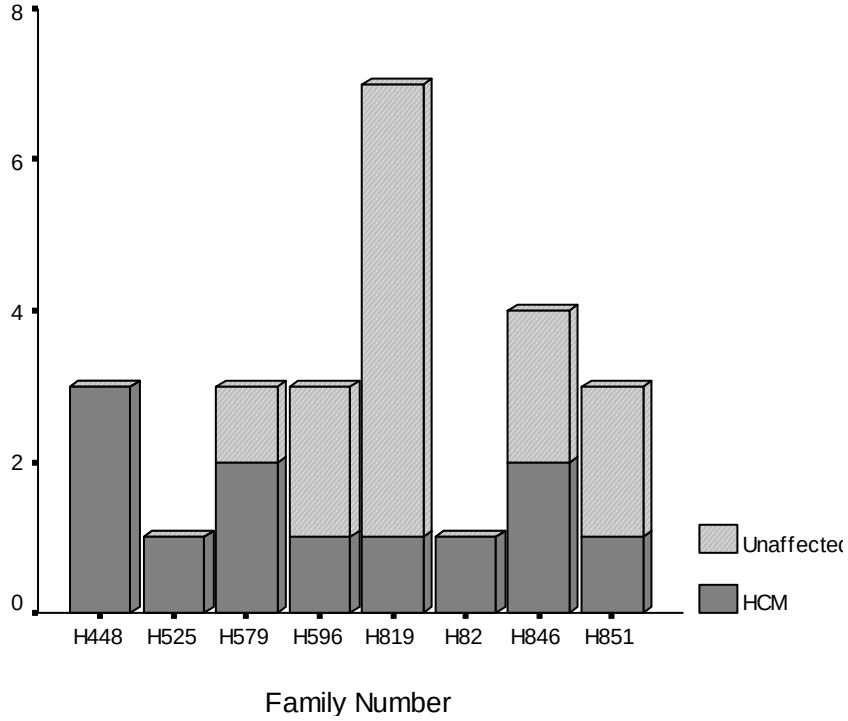
MYBPC3. The other was a 51 year old female with missense mutations in MYBPC3 and MYH7.

Figure 11 **Disease penetrance according to mutation type**



Penetrance appeared to be independent of the specific mutation. A total of 8 families shared the same R502W mutation: of the 6 families with at least 2 mutation carriers, 4 families had incomplete penetrance while in 2 families all mutation carriers examined fulfilled diagnostic criteria for HCM (Figure 12).

Figure 12 **Disease penetrance in 25 R502W mutation carriers**



Discussion

The main findings relating to disease penetrance can be summarized as follows:

1. Disease penetrance is incomplete (57.6%) in this cohort of 170 MYBPC3 mutations carriers
2. Disease penetrance is related to age, however…
3. Incomplete penetrance is seen in all groups even at advanced age
4. Disease penetrance is independent of mutation type
5. Disease penetrance is independent of specific mutation
6. Disease penetrance is higher in males than in females

Several important conclusions can be drawn from these data.

Previous data have suggested that disease penetrance increases with age, and is complete by the later decades, with no plateau effect, implying that clinical evidence of disease will develop at some stage during an individual's lifetime [Niimura 1998]. While a relationship between increasing age and increased disease penetrance was also seen in this study, in contrast to previous data, a plateau effect (around the 5^{th} or 6^{th} decade) was observed suggesting that up to 25-30% of individuals may never develop clinical evidence of disease even at advanced age. This has important implications for counseling unaffected mutation carriers. Increased penetrance with increasing age indirectly supports the hypothesis of 'late-onset' disease. Indirect evidence from cross-sectional data, does not prove this theory however and longitudinal cohort studies are required to accurately define the risk of developing disease in an individual. Our data suggests that in practice

this is an uncommon occurrence – 'late-onset' disease was only demonstrated in 1 adult despite moderately rigorous serial evaluations (see also Results: Longitudinal Follow-Up).

Disease penetrance appears to be unrelated to mutation type. This is perhaps surprising as different mutation types might be expected to confer different mechanisms disease expression. It suggests a common final pathway leading to the development of hypertrophy independent of mutation type. It is particularly interesting that penetrance is incomplete even in individuals with complex genetic status and two disease causing mutations. Reports of severe neonatal HCM have been seen in families with complex genetic status with two mutations (insertion + splice site mutation in one neonate and deletion + nonsense mutation in another), and severe hypertrophy has been reported in a 47 year old male homozygous for a MYBPC3 missense mutation [Garcia-Castro 2005]. In contrast we demonstrated two individuals without clinical evidence of disease despite carrying two disease causing mutations, supporting the role of powerful disease modifying factors.

These modifying factors (genetic or environmental) apparently influence disease development independent of specific mutation, mutation type or age. This study is the first to report a gender difference in disease penetrance with a higher proportion of male mutation carriers affected than female. This observation is consistent with males being overrepresented in HCM cohorts [Maron 2003b][Olivotto 2005]. There are several possible explanations for this observation, including sex-chromosome factors or sex-hormone differences which affect disease expression, environmental triggers or modifiers

such as athletic training may be more common in males than females. Gender specific differences in hypertrophy development have been observed in animal models [Witt 2008] and gender differences have been reported in ECG derived hypertrophy regression in response to antihypertensive therapy [Okin 2008]. Identifying gender specific differences in HCM is important for at least two reasons: firstly it may guide investigators in a direction for further study which may improve our understanding of how, when and why disease develops and possible determinants of disease expression in this disease, and secondly it may lead to more directed and individual counselling for unaffected relatives in terms of the likelihood of developing disease in the future. Recent interest is focusing on single nucleotide polymorphisms (SNPs) and the role they might play in modifying disease penetrance and disease expression [Marian 2002]. Angiotensin converting enzyme (ACE) polymorphisms have been demonstrated to affect magnitude of LVH in individuals with HCM related to MYBPC3 mutations [Perkins 2005], but the effect on disease penetrance has not been studied. A similar study in 26 individuals from a single family with an insertion mutation in MYBPC3 suggested that the development of LVH occurred in those family members with 'pro-LVH' genotypes (in ACE, angiotensinogen, angiotensin II receptor type 1, chymase A and aldosterone synthase genes) and not in those without [Ortlepp 2002]. This was true for LV mass, septal thickness and the prevalence of an abnormal ECG. While providing useful insight into the potential role of modifier mutations, by controlling for the main disease causing mutation, the clinical significance of identifying such modifiers is uncertain. The age at diagnosis in this cohort of affected individuals with MYBPC3 mutations is very wide and normally distributed. From these data there are no apparent clinically important

differences between individuals diagnosed early or late – they appear to have a similar pattern and magnitude of hypertrophy, and the types of mutation identified appeared to be similar. As age at diagnosis is normally distributed, the concept of late-onset disease is difficult to define and in this cohort has little clinical relevance.

It must be remembered that age at diagnosis is not equivalent to the age at which hypertrophy develops which requires serial evaluation. Without control groups using individuals with other sarcomeric protein gene mutations, this study is unable to directly compare how these data are applicable to the overall HCM population.

CLINICAL DISEASE EXPRESSION IN AFFECTED INDIVIDUALS WITH MYBPC3 MUTATIONS

Introduction

Detailed clinical data is lacking in cohorts of families with MYBPC3 mutations. Charron et al. found no significant differences in symptoms, ECG abnormalities, or pattern or degree of hypertrophy between two families (33 mutation carriers) sharing an identical splice acceptor site mutation (SAS Int20) and families with MYH7 mutations [Charron 1998b]. A larger study (76 MYBPC3 mutation carriers, including the 33 SAS Int20 carriers) by the same group compared symptoms, ECG and Echo parameters with families with MYH7 mutations. MYBPC3 mutation carriers were slightly older (41 v 35, p=0.02) and there were fewer deaths reported in this group (10 v 18, p=0.004). Mean septal thickness was 15 +/- 4mm, 30% had SAM and LVOT obstruction greater than 30mmHg was present in 14% [Charron 1998a]. Erdmann et al reported a variable clinical course in 25 MYBPC3 mutation carriers but suggested that disease onset was earlier and invasive procedures (including ICD and septal ablation) were more common in families with protein truncations compared to those with missense or inframe deletions/insertions [Erdmann 2001]. In a large US study from the Mayo Clinic, index cases (i.e. no family screening was performed) with MYBPC3 mutations did not differ from patients with thick filament-HCM, thin filament-HCM, or mutation negative HCM with respect to age at diagnosis, severity of hypertrophy or incidence of major complications [Van Driest JACC 2004]. They did find more severe disease in patients with complex genetic status however. As discussed previously, data from the Mayo clinic is skewed towards individuals with obstructive HCM as it is a centre of expertise in surgical myomectomy, and such patients are over-represented in their cohort.

Data is somewhat conflicting therefore and requires clarification. In this section detailed clinical assessment of 98 affected MYBPC3 mutation carriers is reported.

Methods

Index cases and affected relatives were evaluated as described in the Methods section. Demographic data, symptomatology, ECG, Echo, Holter monitoring and exercise data were recorded and analysed for 98 affected mutation carriers. Data is presented as number of individuals, with percentages in parentheses. Mean +/- SD is reported where relevant. In group comparisons, categorical data are compared using Chi-square test and continuous variables compared using independent sample Student's t-test. A p-value of <0.05 was considered statistically significant.

Results

Index Cases

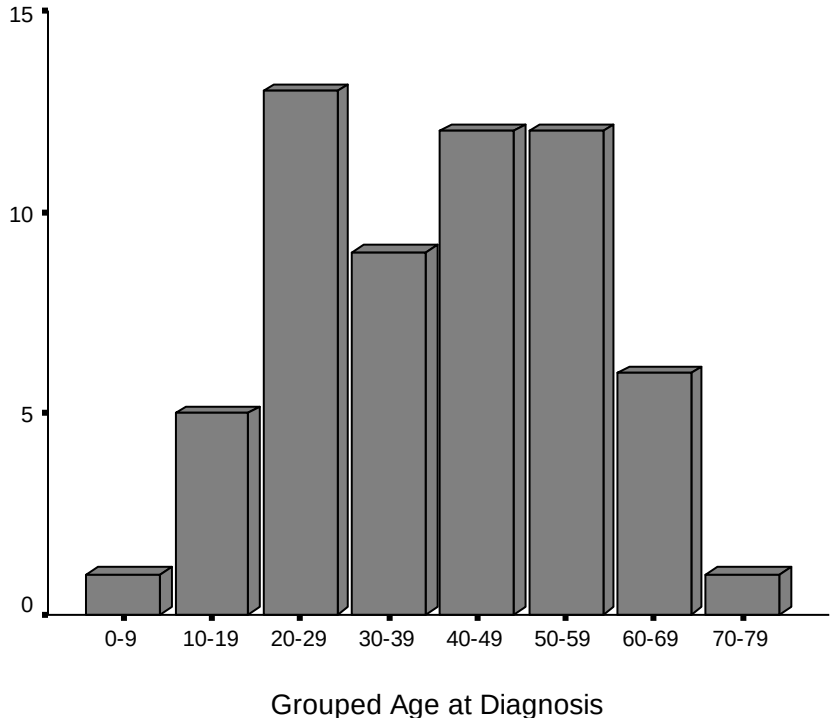
34 of 59 individuals were male (57.6%) – Table 10. Mean age at diagnosis was 40.1 +/- 15.9 years, range 5-76 (Figure 13). Reason for diagnosis were as follows: Symptoms 32 patients (54.2%), incidental finding 16 (27.1%), family screening 10 (16.9%) and in 1 individual (1.7%) the diagnosis was made at autopsy following sudden cardiac death. 54 of 59 index cases (91.5%) were of White European ethnic origin, 2 (3.4%) were Black and 3 (5.1%) were Asian. Ethnic mix in the cohort was broadly similar to our overall HCM cohort (including other sarcomeric protein mutations and non-genotyped

individuals) – 91% White European, 2% Black and 6% Asian. Ethnic mix was also broadly similar to the ethnic composition of the general population derived from the last national census in 2001 – 92.1% White European, 2% Black, 4.0% Asian – [Census 2001]

Table 10 Clinical data for 59 index cases with mutations in MYBPC3

	Total	SD	Range
Demographics			
Total individuals (n (%))	59(100)		
Male (n (%))	35(59.3)		
Age at diagnosis (years)	40.0	15.9	5-76
White Ethnicity (n (%))	54(91.5)		
Symptomatic (n (%))	42(71.2)		
Initial ECG			
Sinus Rhythm (n (%))	55(93.2)		
LA enlargement (n (%))	36(61)		
PR interval (ms)	167	26	108-224
QRSd (ms)	97	17	80-160
Mean RE score	6.2	3.4	0-12
First Echo			
LA dimension (mm)	42	7.2	24-60
LVEDD (mm)	43	6.7	25-60
LVESD (mm)	25	6.2	15-48
FS (%)	44	8.5	20-63
IVSd (mm)	19	5.6	8-37
PWd (mm)	10	2.4	6-18
MWT (mm)	20	5.8	9-37
ASH (n (%))	51(86.4)		
Severe LVH ≥ 30mm (n (%))	2(3.4)		
Mid-systolic obliteration (n (%))	2(3.4)		
RVH (n (%))	3(5.1)		
LVOTG > 30mmHg (n (%))	18(30.5)		
Metabolic exercise test			
Peak VO2 ml/Kg/min	26.2	11.2	9.5-57.9
% Predicted VO2 (%)	75	22	37-128
Holter			
NSVT (n (%))	13(22)		

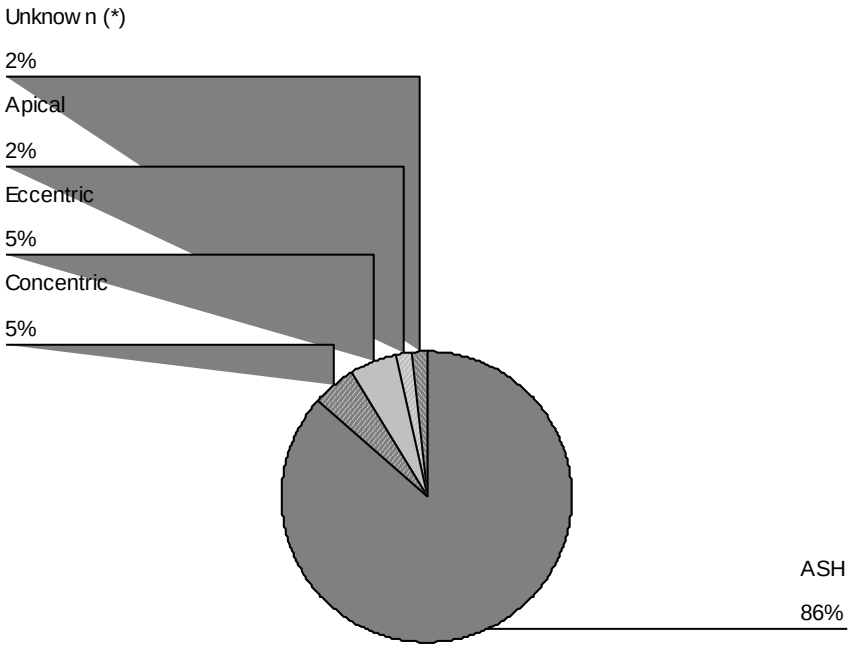
Figure 13 **Age at Diagnosis in 59 Index Cases**



55 of 59 index cases were in sinus rhythm at diagnosis (93.2%) with 2 (3.4%) in atrial fibrillation, 1 in an AV synchronous paced rhythm, and 1 unknown (presented with sudden cardiac death). Non-sustained ventricular tachycardia (NSVT – defined as 3 or more ventricular premature complexes at a rate > 120 bpm) on 24 hour Holter monitoring was present in 13 (22.0%) individuals. 36 (61.0%) had left atrial enlargement on ECG, 29 (49.2%) fulfilled Sokolow Lyon criteria for LVH, 2 (3.4%) had bundle branch block and 41 (62.5%) had a Romhilt Estes score ≥4.

Most individuals had asymmetrical septal hypertrophy (86.4%) but other patterns of hypertrophy were observed including concentric (5.1%), eccentric (5.1%), apical (1.7%) and 1 (1.7%) undetermined (presented with sudden cardiac death). Mean septal thickness was 19 +/- 5.6mm, posterior wall 10 +/- 2.4mm and maximal wall thickness 20 +/- 5.8mm (Figure 14).

Figure 14 **Pattern of Hypertrophy in 59 Index Cases**



* Patient presented with sudden death, so pattern of hypertrophy not identified ante-mortem

One female (28 years old at diagnosis) had progressive wall thinning and predominant restrictive physiology requiring cardiac transplantation age 43. Left ventricular outflow tract obstruction (peak gradient ≥ 30mmHg) was seen in 18 (30.5%) individuals, with mid-cavity obstruction in 2 individuals (3.4%) and biventricular ventricular hypertrophy in 3 (5.1%). There were no differences in age at diagnosis or maximal wall thickness between different mutation types (Figures 15&16 and Table 11).

Figure 15 **Age at Diagnosis in 59 Index Cases According to Mutation Type**

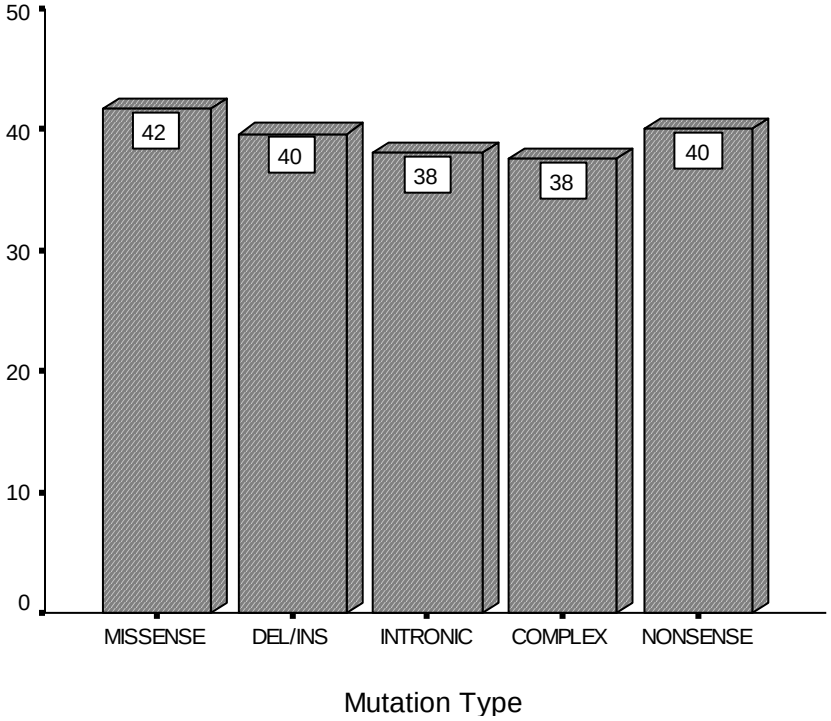
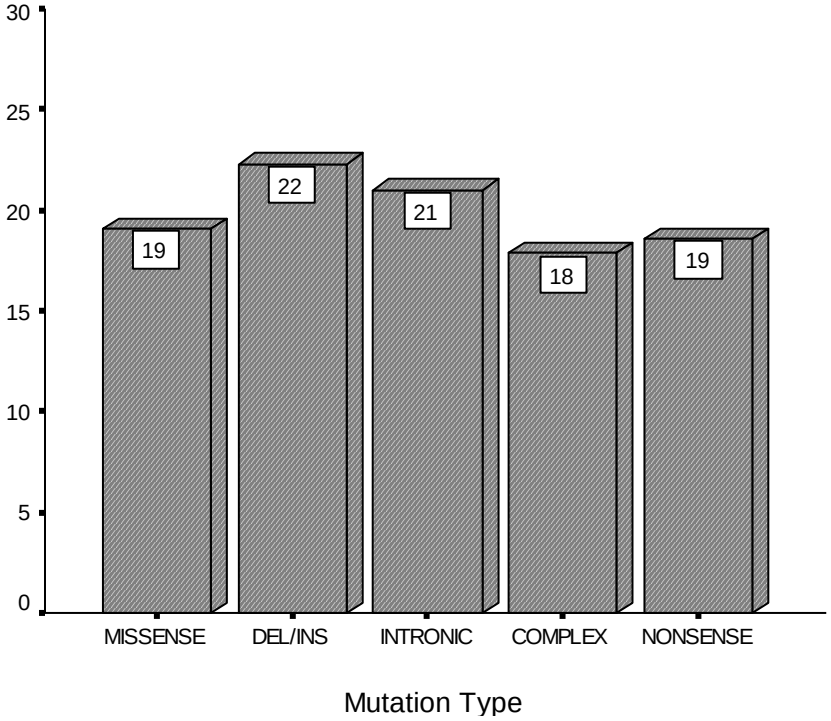


Figure 16 **Maximal Wall Thickness in 59 Index Cases According to Mutation Type**



There were no statistically significant differences between different types of mutation in terms of age at diagnosis (p=0.970), % in sinus rhythm (p=0.521), LV cavity size (p=0.387) or maximal wall thickness (p=0.781).

Table 11 Clinical and Demographic Data for 59 Index Cases with HCM According to Mutation Type

	Nonsense	Ins/Del	Missense	Intronic	Complex Status	Total	P – value
Number of Individuals	5	10	26	10	8	61	-
Male (n(%))	4 (80)	8 (80)	16 (61.5)	6 (60)	2 (25)	36 (59.0)	0.097
Age at diagnosis (yrs)	40.0	38.5	41.4	37.1	40.4	40.0	0.970
White Ethnicity (n(%))	4 (80)	9 (90)	25 (96.2)	9 (90)	6 (75)	55 (90.2)	0.431
Symptomatic (n(%))	4 (80)	8 (80)	13 (50)	9 (90)	7 (87.5)	42 (70)	0.064
Initial ECG							
Sinus Rhythm (n(%))	4 (80)	10 (100)	25 (96.2)	9 (90)	8 (100)	56 (91.8)	0.521
First Echo							
LA dimension (mm)	43.8	45.5	41.1	42.1	41.0	42.1	0.703
LVEDD (mm)	42.4	42.3	45.2	40.6	41.7	43.3	0.387
LVESD (mm)	24.5	23.2	26.3	23.3	25.6	25.0	0.749
FS (%)	45.3	46.0	43.8	44.5	38.3	43.5	0.615
IVSd (mm)	18.0	22.4	17.5	20.1	18.1	18.8	0.294
PWd (mm)	10.9	11.1	10.2	10.6	10.1	10.5	0.804
MWT (mm)	18.6	22.0	19.2	21.1	19.0	20.0	0.781

All Affected Mutation Carriers

In addition to the 59 index cases, family screening identified a further 111 mutation carriers of whom a further 39 fulfilled diagnostic criteria for HCM. Therefore, of 170 mutation carriers (index cases and relatives) a total of 98 individuals fulfilled diagnostic criteria for HCM. Clinical data is shown in Table 12.

Table 12 Clinical data for 98 affected mutation carriers

	Total	SD	Range
Demographics			
Total individuals (n(%))	98(100)		
Male (n(%))	58(59.2)		
Age at diagnosis (years)	42.1	16.1	5-81
White Ethnicity (n(%))	92(93.9)		
Symptomatic (n(%))	53(54.1)		
Initial ECG			
Sinus Rhythm (n(%))	94(95.3)		
PR interval (ms)	169	32	108-333
QRSd (ms)	96	17	72-160
Mean RE score	5.4	3.6	0-12
First Echo			
LA dimension (mm)	41.4	7.3	22-60
LVEDD (mm)	43.7	6.7	25-60
LVESD (mm)	26.0	6.5	10-48
FS (%)	42.5	9.0	20-67
IVSd (mm)	17.9	5.3	6-37
PWd (mm)	10.3	2.5	5-18
MWT (mm)	18.7	5.4	6-37

Mean age at diagnosis was 42.1 +/- 16.1 years (range 5-81) (Figure 17). Mean septal thickness was 17.9 +/- 5.3mm, posterior wall thickness 10.3 +/- 2.5mm and maximal wall thickness was 18.7 +/- 5.4mm. 84 (85.7%) had ASH with 7(7.1%) concentric, 3(3.1%) eccentric and 3(3.1%) apical patterns of hypertrophy. In one individual the pattern of hypertrophy was unknown as he had presented with sudden cardiac death and the diagnosis of HCM was made at autopsy.

Maximal wall thickness did not appear to be related to age (Figure 18). Note that there was only one individual diagnosed between the ages 0-9 who had a maximal wall thickness of 24mm.

Figure 17 **Age at Diagnosis in 98 Affected Mutation Carriers**

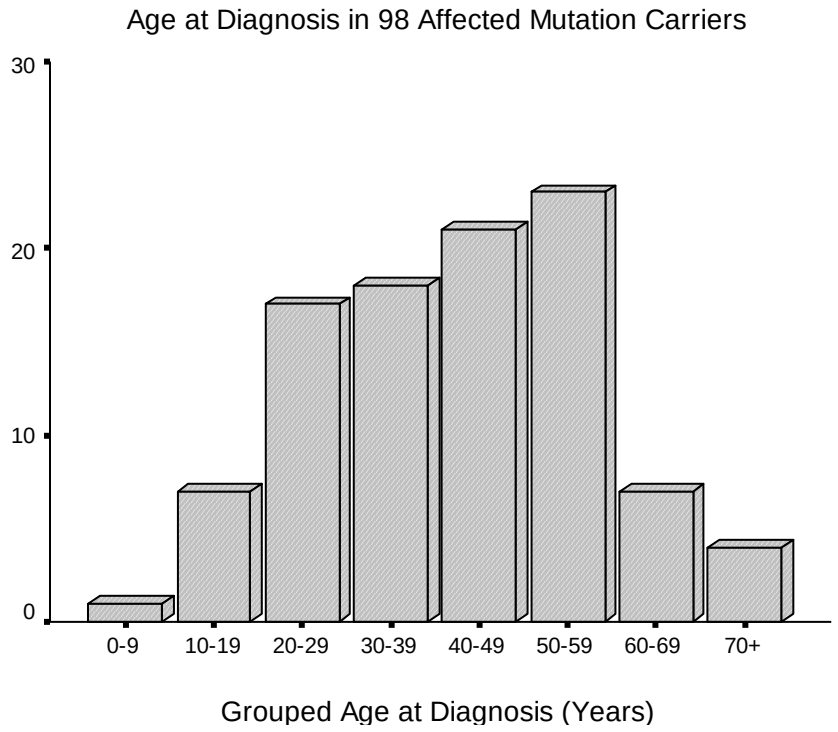
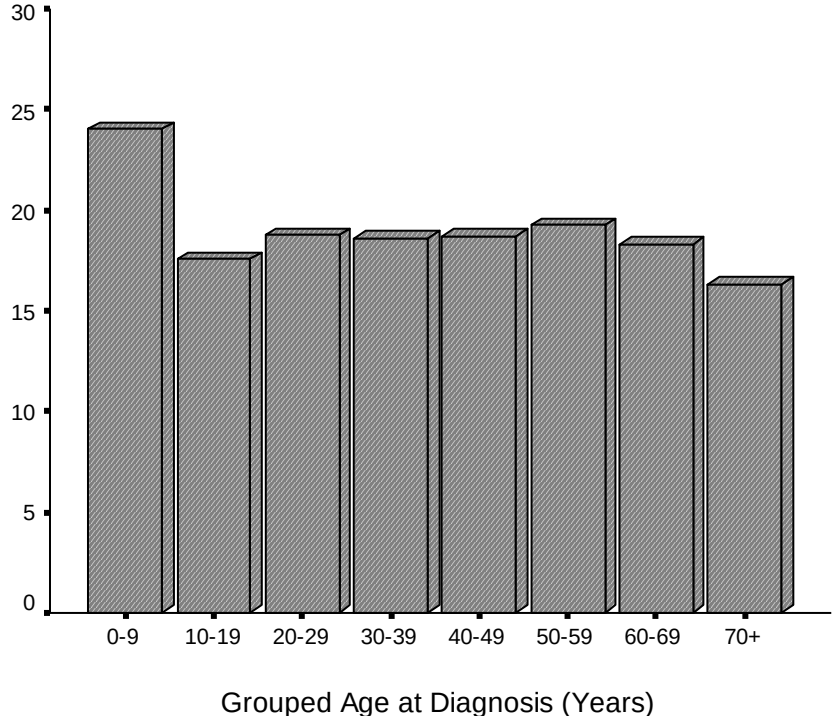
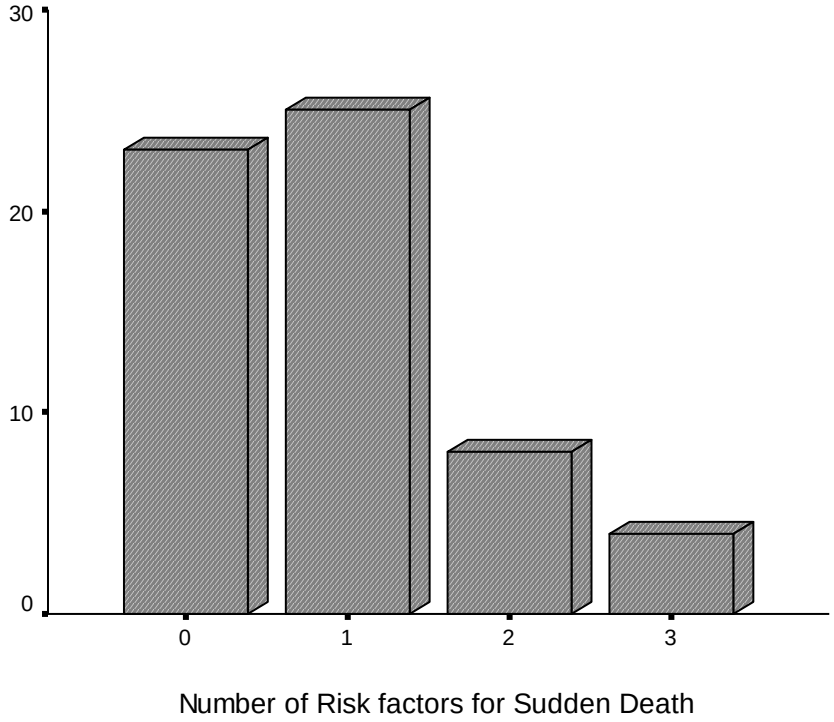


Figure 18 **Mean Maximal Wall Thickness in 98 Affected Mutation Carriers According to Age**



In terms of risk factors for sudden cardiac death: 10 (10.2%) had previous history of unexplained syncope, 1 (1%) had maximal wall thickness > 30mm, 29 (29.6%) had a family history of sudden premature death in a relative < 40 years, 22 (22.4%) had NSVT on Holter monitoring and 12 (12.2%) had an abnormal blood pressure response on exercise. Sixty of 98 affected mutation carriers had a complete data set for risk stratification for sudden cardiac death (symptom and family history, Echo, 48 hour Holter and metabolic exercise test). The majority of these 60 individuals were index cases. The remaining 38 were largely affected relatives diagnosed during family screening who were due to be followed up at their local cardiac centre rather than at the Heart Hospital. Of the 60 with complete data, 12 (20%) had 2 or more risk factors for sudden death, with the remaining 48 (80%) being considered at low or intermediate risk (≤1 risk factor) (Figure 19). This is similar to the overall HCM cohort at the Heart Hospital with 83% being at low or intermediate risk (1 risk factor) and 17% having 2 or more risk factors (data derived from HCM database representing an unselected HCM cohort).

Figure 19 Risk factor profile at first assessment in 60 affected mutation carriers with complete data



Index Cases Compared to Relatives

Compared to index cases, relatives diagnosed via family screening had less severe septal hypertrophy (16 v 19mm, p=0.002), less severe maximal wall thickness (17 v 21mm, p<0.001), greater LV cavity size (LVEDD 46 v 42mm, p=0.012, LVESD 28 v 24, p=0.006) and mean Romhilt Estes score was less (4.4 v 6.3, p=0.018) (Table 13).

Table 13 Comparison between index cases and relatives diagnosed during family screening

	Index cases	Relatives	P-value
Demographics			
Total individuals (n)	59	39	-
Male (n(%))	35(59.3)	23(59.0)	0.973
Age at diagnosis (years)	39.8 +/- 15.7	44.6 +/- 16.3	0.141
White Ethnicity (n(%))	54(91.5)	1(2.6)	0.412
Symptomatic (n(%))	40(67.8)	11(28.3)	0.002
Initial ECG			
PR interval (ms)	170 +/- 26	168 +/- 38	0.746
QRSd (ms)	98 +/- 18	93 +/- 15	0.151
Mean RE score	6.3 +/- 3.3	4.4 +/- 3.8	0.018
First Echo			
LA dimension (mm)	42.3 +/- 7.8	40.2 +/- 6.5	0.167
LVEDD (mm)	42.2 +/- 6.8	45.7 +/- 6.0	0.012
LVESD (mm)	24.3 +/- 6.5	28.1 +/- 5.8	0.006
FS (%)	44.0 +/- 9.1	40.2 +/- 8.6	0.105
IVSd (mm)	19.5 +/- 5.5	16.0 +/- 4.5	0.002
PWd (mm)	10.5 +/- 2.4	10.1 +/- 2.6	0.490
MWT (mm)	20.5 +/- 5.6	16.6 +/- 4.5	0.000

Discussion

In this section a number of clinical data on 59 index cases and 39 affected relatives have been presented. The main findings relating to MYBPC3 mutations in this cohort can be summarized as follows:

1. Disease may be diagnosed at any stage in life (5-81 years) and affected individuals are more commonly male (57.6%)
2. There is a wide spectrum of disease in terms of pattern and magnitude of hypertrophy and symptomatic status
3. Affected relatives were less symptomatic with milder hypertrophy than index cases
4. Mutation type was not related to age at diagnosis or magnitude of hypertrophy

Previous studies have attempted to describe sarcomeric protein gene mutations in terms of particular phenotype, by comparing summary statistics between different cohorts [Charron 1998b]. While this may highlight statistically significant differences in particular clinical parameters, it does not necessarily reflect the spectrum and variation of disease that may be encountered for any given genotype.

The summary data from this study are broadly similar to those data published previously [Moolman 2000][Richard 2003][Van Driest 2004][Song 2005] however detailed clinical data in affected individuals with MYBPC3 mutations are lacking, and are obscured by the inclusion of data from unaffected mutation carriers [Charron 1998a][Charron 1998b], or such data are lacking [Niimura 1998][Doi 1999][Erdmann 2001][Andersen 2004]. Study

cohorts were similar in terms of age at diagnosis and maximal wall thickness (Table 14) suggesting that the individuals in this cohort are similar to the broad population of families with MYBPC3 mutations.

Table 14 Summary data for affected MYBPC3 mutation carriers in this and previously published studies

	This study	Van Driest	Richard	Song	Moolman
Number of Individuals	98	63	48	16	10
% Male	58	65	48	-	40
Mean age at diagnosis (years)	42	38	40	48	-
Mean maximal wall thickness (mm)	19	23	-	20	21

Data from this study demonstrate a broad spectrum of disease. MYBPC3 mutation may cause severe hypertrophy at age 5 (H851) or sub-clinical disease diagnosed at family screening age 81 (H151). Some families have multiple cases of sudden cardiac death (H936) whereas other families appear to have a more benign clinical course (H544). Even within families sharing the same mutation, the spectrum of disease appears wide (see Disease Expression in Families Sharing Identical Mutations). For many of these important parameters, ranges are broad and standard deviations are wide.

This study lacks a directly comparable control group, although it is possible to compare to the overall HCM population at The Heart Hospital, and previously published data would suggest that MYBPC3 related disease is not dissimilar to disease associated with other sarcomeric protein gene mutations. Van Driest et al compared clinical data from 63 index cases with data from cohorts of thick filament (MYH7, MYL2 and MYL3) and thin filament (TNNT2, TNNI3, TPM1 and ACTC) mutations [Van Driest 2004]. No differences in age at diagnosis, severity of hypertrophy, incidence of myectomy or family history of HCM or sudden death were apparent between the groups.

Whether the broad spectrum of disease seen in our cohort is applicable to the wider HCM population is unproven.

DISEASE EXPRESSION IN FAMILIES SHARING IDENTICAL MUTATIONS

Introduction

Following the recognition that mutations in sarcomeric protein genes are found in approximately 50% of cases of HCM [Richard 2003], interest has focussed on whether gene-specific, or mutation-specific phenotypes exist that may guide family counselling, clinical management and further our understanding of the pathogenesis of HCM.

Early reports, necessarily based upon highly selected families suitable for linkage analysis, suggested that a specific phenotype could be attributed to a specific sarcomeric protein gene [Anan 1994][Watkins 1995b][Poetter 1996][Moolman 1997][Niimura 1998][Charron 1998a][Mogensen 1999][Satoh 1999 [Hoffman 2001][Niimura 2002][Mogensen 2004][Carniel 2005][Kubo 2007].
Moreover, early studies suggested that mutation specific differences exist, and reports of "malignant" and "benign" mutations in MYH7 and also in TNNT2 suggested a role for genetic analysis in risk stratification [Watkins 1992][Anan 1994][Consevage 1994][Marian 1995a][Coviello 1997][Moolman 1997][Hwang 1998][Marian 1998][Ho 2000][Roberts 2001][Van Driest 2002][Ackerman 2002].

While this is an attractive concept, potentially allowing the clinician or geneticist to provide a family with mutation specific advice, most mutations are private and novel and large numbers of individuals from separate families are required to confirm or refute the original claims regarding disease expression for that specific mutation. Van Driest et al. performed a meta-analysis examining gene specific differences pooling data from several large studies published between 1998 and 2004 and found no statistical differences in

terms of age at diagnosis, or severity of hypertrophy between populations with different sarcomeric protein gene mutations [Van Driest 2005]. While certain specific MYH7 mutations are felt to have an adverse prognosis [Marian 1995a] it is less clear whether risk in MYBPC3 families is mutation specific.

Systematic genetic screening programmes in unselected HCM cohorts allows comparison of families sharing the same mutation. While this study is limited to MYBPC3 mutations and therefore precludes gene specific comparisons, it does allow mutation specific comparisons to be made between families sharing the same MYBPC3 mutation.

Methods

Six mutations were identified in at least 2 unrelated families (Table 15.). Clinical data was compared between families sharing each identical mutation and is reported below.

Table 15 Spectrum of mutations shared by more than one family

Mutation	Families	Total Number of Gene Carriers	Previously Reported Mutation?
Arg495Gly	H481, H600	3	No
Arg502Gln	H62, H151	15	Yes
Arg502Trp	H82, H448, H525, H579, H596, H819, H846, H851, H908	25	Yes
Gln969X	H652, H936	9	Yes
Glu258Lys	H432, H621, H919	8	Yes
IVS13-23G>A	H692, H896, H920	6	No

Results

Arg495Gly Mutation

Two families shared the novel Arg495Gly mutation. In one family (H481) the proband presented age 67 with dilated phase HCM and incessant atrial arrhythmias. He died age

82 secondary to heart failure. No other family members were known to be affected, and his two children refused clinical and genetic screening. In another family (H600) with the same Arg495Gly mutation, the proband was diagnosed incidentally age 17 and found to have asymmetric septal hypertrophy (MWT 22mm) and paroxysmal AF treated with amiodarone. An ICD was implanted as primary prophylaxis for unexplained syncope and an abnormal blood pressure response to upright exercise. Her mother was diagnosed during family screening age 37 and found to have asymmetric septal hypertrophy (MWT 16mm). No other family members were affected and four relatives were negative for the family mutation.

Arg502Gln Mutation

Two families shared the Arg502Gln mutation. The proband of family H151 was diagnosed incidentally age 14. Investigations showed severe septal hypertrophy (MWT 37mm) but the patient remains asymptomatic during follow up. His sister is a gene carrier with an abnormal ECG but has shown no evidence of hypertrophy on Echo during 17 years of follow up. Their father was diagnosed with HCM during family screening (asymmetric septal hypertrophy MWT 24mm) and is asymptomatic. Four other family members were found to be gene carriers. Two of these fulfilled diagnostic criteria for HCM (45 year old man with mild ASH and an 81 year old man with mild concentric LVH) and two did not fulfill diagnostic criteria (53 year old woman with abnormal ECG and normal Echo and 23 year old woman with normal ECG and Echo). There were no sudden deaths or strokes in this family. In contrast in family H62, the proband was diagnosed with HCM at post mortem having died suddenly age 16. Seven other adult family members were gene carriers. Of these 3 were clinically affected – 51 year old man

with end-stage HCM and heart failure, 14 year old asymptomatic girl with apical hypertrophy and 54 year old man with hypertension and mild ASH which regressed during follow up. He died of a haemorrhagic stroke age 64. The other 4 had no evidence of disease expression during 18 years of follow up (now aged 57, 39, 37 and 34).

R502W Mutation

This mutation was identified in 9 apparently unrelated families (8 as the sole mutation and 1 in whom the MYH7 H602S mutation was also identified). The data described below is only for those individuals with a single R502W mutation.

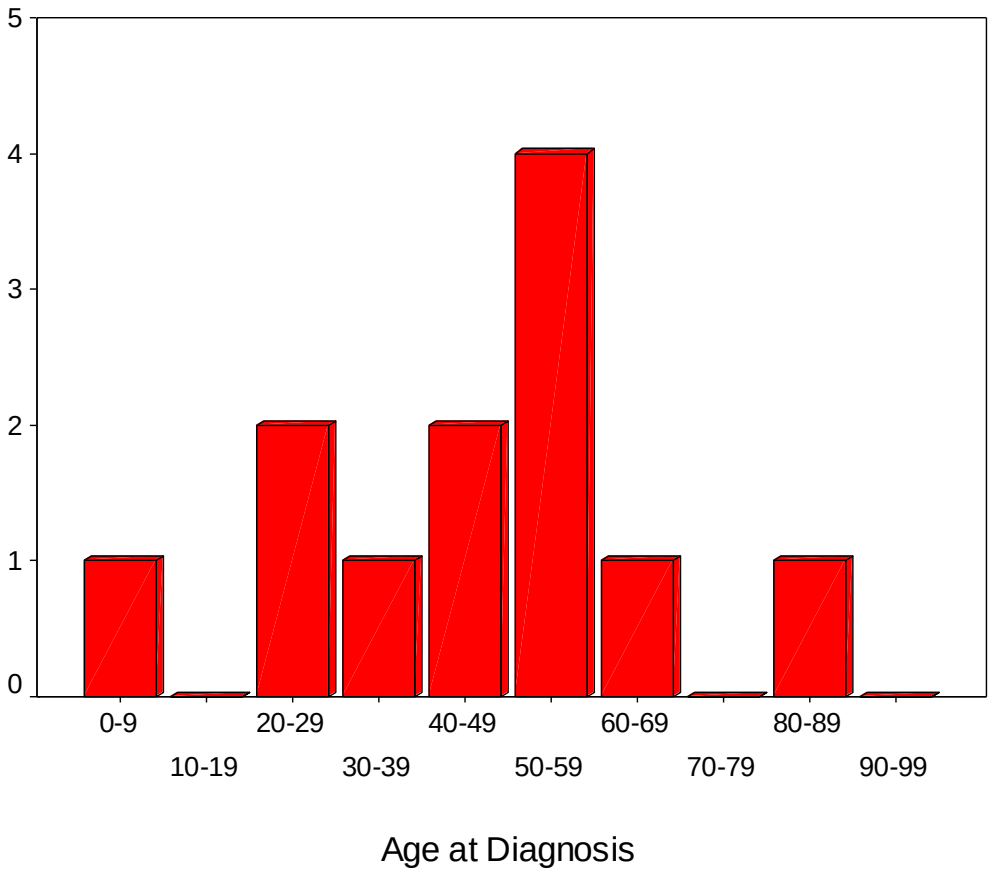
All 9 families were Caucasian and haplotype analysis identified a probable founder effect, the first to described in a British HCM population.

Of 25 mutation carriers, 12 fulfilled diagnostic criteria for HCM and 13 were unaffected. The overall penetrance was 48%. In one family (H448) all three mutation carriers were clinically affected (100% penetrant), whereas in 3 other families with at least 2 adult mutation carriers the penetrance was incomplete (H851, H819 and H846). Of the 12 affected individuals, 3 were diagnosed incidentally, 4 presented with symptoms and 5 were diagnosed at family screening. Age at diagnosis was very different between the families with one male (H851.1) presenting age 5 with breathlessness, and one female (H579.6) presenting age 80 with an abnormal ECG. Age at diagnosis is shown in Figure 20. Summary data for these families is shown in Table 16.

Table 16 Summary data for affected patients with R502W mutation

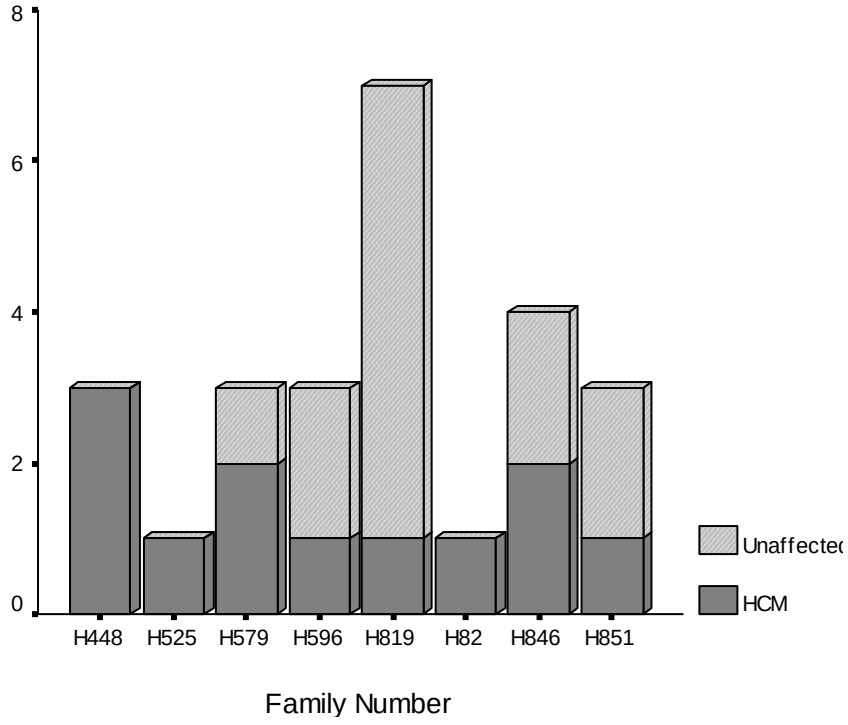
	Mean	Range	Standard deviation
Age at diagnosis (years)	41.9	5-80	18.8
% Male (%)	50		
Echo parameters			
LA diameter (mm)	37.3	24-45	1.8
IVSd (mm)	15.1	10-24	1.1
PWd (mm)	9.8	6-16	0.8
MWT (mm)	16.9	13-24	1.1
LVEDD (mm)	45.5	25-55	2.4
LVESD (mm)	27.5	15-35	2.0
FS (%)	39.6	26-63	3.1

Figure 20 Age at diagnosis for individuals with the R502W mutation



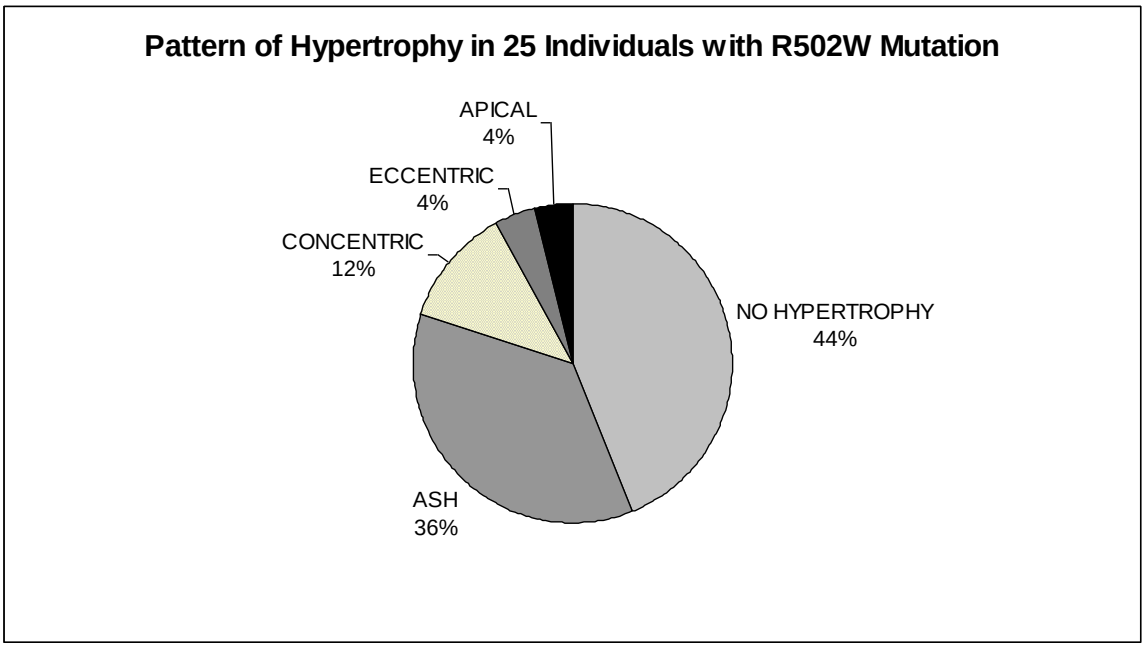
Disease penetrance varied between families sharing the R502W mutation (Figure 21). In H448 for example all 3 mutation carriers were affected whereas in H819 only 1 of 7 mutation carriers was affected.

Figure 21 Disease Penetrance in Families Sharing the R502W Mutation



Pattern and extent of hypertrophy varied significantly: One individual had severe asymmetric septal hypertrophy with a maximal wall thickness of 38mm, while other patients had either mild ASH or other patterns of hypertrophy (see Figure 22.)

Figure 22 Pattern of Hypertrophy in 25 mutation carriers with the R502W mutation.



Prognosis appeared to vary between families with multiple premature sudden cardiac deaths occurring in two families (H82 and H846), and a more benign prognosis in other families. In H82, H82.3 died suddenly age 36 in 1992. The post mortem noted pulmonary oedema, marked left ventricular hypertrophy with a heart weight of 400g and evidence of viral myocarditis on initial histology. Review of his histology 14 years later during this study however revealed widespread interstitial fibrosis and marked myocyte disarray. DNA was extracted from tissue blocks preserved at the time of death and attempts at DNA analysis would suggest that he was not a carrier for the R502W mutation. Due to the lack of a whole heart specimen and poor DNA quality the diagnosis and mutation status in this individual is not clear and limits the conclusions that can be drawn. His sister also died suddenly at the age of 12 in 1965. No post mortem was performed. The surviving sibling H82.2 is overweight with hypertension and was clinically unaffected. He was also wild type for the R502W mutation. This raises the possibility that another mutation was present in this family that may have contributed to the instances of sudden

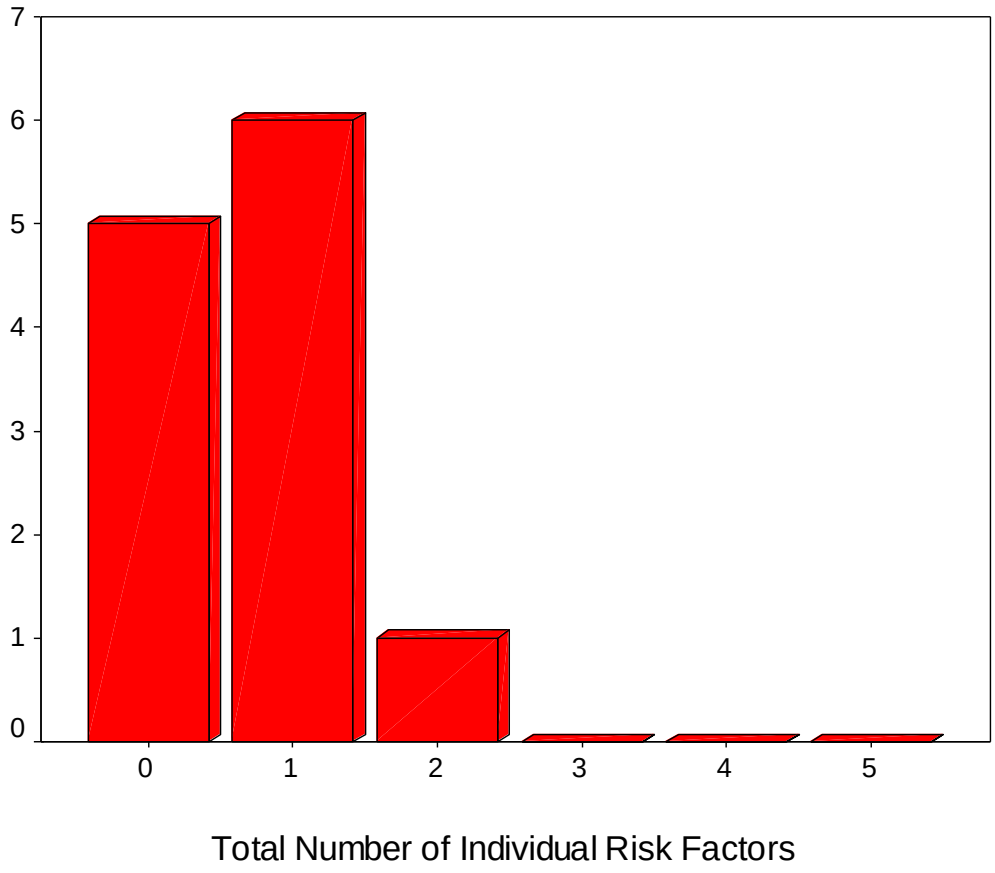
cardiac death, possibly paternally inherited. Interestingly the father of these three children was clinically unaffected.

Another family (H846) also had 3 instances of premature death although details were lacking. One male child died suddenly age 4 and was reported to have Tetralogy of Fallot (unconfirmed). Another female died suddenly age 15 at a bus stop and was reported to have 'aortic coarctation and myocardial degeneration' – also unconfirmed. One other female died less than 12 months old of unknown causes. Interestingly, 2 second degree relatives in this family are clinically affected but do not carry the R502W mutation. In the case of H846.3 all other sarcomeric protein genes have been screened and no other mutations identified. This also raises the possibility that another (unidentified) mutation is present in this family and may be relevant to the reported cases of premature sudden cardiac death (albeit not confirmed to be related to HCM).

The risk factors for sudden cardiac death in those affected individuals were assessed. Complete risk stratification was complete in only 8 out of 12 individuals with no metabolic exercise test data available for 4 individuals, and no Holter data available for 2 individuals.

Risk factor profiles are shown in Figure 23.

Figure 23 Spectrum of Risk Factors in Individuals with the R502W Mutation



During long term follow up of affected mutation carriers (mean 6.6 years +/- 1.3), 1 woman developed heart failure, there were no sudden cardiac deaths or cardiac arrests, no individuals underwent LV myomectomy or alcohol septal ablation and 2 prophylactic ICDs were implanted. No appropriate ICD therapies were recorded during follow-up.

The R502W mutation has been reported previously in several studies [Richard 2003][Van Driest 2004][Ingles 2005] but unfortunately no mutation specific data has been reported. It is therefore not possible to compare the observed phenotype in this study to the published literature.

Gln969X Mutation

Two Caucasian families shared this nonsense mutation causing a premature stop codon. Once again the two families appear to have a very different clinical course. H652 is a large family, the index case of which (H652.1) presented in 1985 with effort intolerance and breathlessness. He was subsequently found to have ASH with provokable mid-cavity obstruction but no LVOTO, and severe diastolic dysfunction with restrictive filling pattern using transmitral pulsed wave Doppler. He remains limited by exertional symptoms despite medical therapy. Attempts at cardiac resynchronization therapy were unsuccessful due to phrenic nerve stimulation. Three siblings and his mother were found to be wild type for the Gln969X mutation. There are no other family members know to be affected, and no family history of sudden cardiac death. It is possible that the mutation has arisen *de novo* in the index case.

In contrast, H936 manifests a particularly severe phenotype with multiple sudden cardiac deaths. The index case in the family (H936.1) presented age 29 with exertional breathlessness and was found to have ASH without outflow tract obstruction. His mother is affected with a mild phenotype but two of her siblings died suddenly at a young age and a further sibling survived a cardiac arrest and underwent ICD implantation. Three other siblings are clinically affected with a variable phenotype (H939.9 – asymptomatic with non-obstructive ASH and H936.7 – very limited with left ventricular outflow tract obstruction, heart failure, previous stroke and atrial fibrillation). Two other relatives are unaffected mutation carriers. A 3 year old girl is asymptomatic with a normal ECG and

Echo for her age and a 43 year old woman is also asymptomatic with a normal ECG and Echo. The overall penetrance of disease in adults in this family is 86%.

Glu258Lys Mutation

Three Caucasian families shared this missense mutation.

In H621 the index case presented age 26 in 1991 with a murmur found at a routine medical examination. He was found to have ASH without LVOTO and is in permanent atrial fibrillation. His mother was an obligate carrier for the mutation (as both her children are carriers, and her husband is wild type for the family mutation) and died suddenly on the waiting list for a heart-lung transplant. She had ASH with restrictive physiology and developed severe pulmonary hypertension and right sided heart failure.

H919 is a family with fully penetrant disease expression in all four mutation carriers. The index case (H919.1) presented age 21 with chest pain. She was found to have ASH without obstruction and has no risk factors for sudden cardiac death. Her brother and mother are both gene carriers with mild disease expression but are both asymptomatic. H919.6 died age 79 with post-mortem showing evidence of left ventricular hypertrophy and DNA analysis showing her to be a mutation carrier. She was asymptomatic prior to death and not known to have HCM ante-mortem.

In H432 the index case (H432.1) presented for family screening following the sudden cardiac death of her son age 19. He didn't have a post-mortem examination but had been diagnosed with HCM 7 weeks prior to his death in 1996. At family screening the only other mutation carrier identified was his mother (H432.1) who was asymptomatic but had mild ASH on transthoracic echo. She remains asymptomatic 10 years later.

IVS13-23G>A Mutation

Three families shared this intronic mutation which creates a de novo acceptor splice site, extending the transcript by 17 nucleotides, creating a frameshift and ultimately a premature stop codon in exon 15 [Frank-Hansen 2008]. They were of different ethnic origins (1 White British family, 1 Greek Cypriot family and 1 Asian family).

H920.1 was a highly trained athlete who presented with syncope age 15. He was found to have ASH with a maximal wall thickness 17mm and no outflow tract obstruction. Other family members declined screening.

H692.1 presented age 43 with chest pain and was found to have an eccentric pattern of hypertrophy involving the lateral wall and apex predominantly. No other mutation carriers have been identified in the family.

H896.1 presented in 2001 with longstanding history of breathlessness and fatigue. He was found to have ASH, with a maximal wall thickness of 18mm and severe outflow tract obstruction with a peak resting gradient of 140mmHg. He subsequently underwent surgical myomectomy in 2001 and remains asymptomatic during follow up. Three other mutation carriers were identified in this family. His brother (age 25) and father (age 60) are both asymptomatic and unaffected. His paternal uncle however is also a mutation carrier and is clinically affected with ASH but is asymptomatic.

Discussion

Although the number of individuals sharing a particular mutation in this study is small, it is evident that disease expression can be very different between individuals with an identical mutation. This is true for age at diagnosis (compare family H851 vs H579), family history of sudden cardiac death (H82 and H846 vs H448 and H579), and pattern and extent of hypertrophy (H851 vs H579). It is of particular interest that the pattern of hypertrophy can be so variable between individuals with the same (R502W) mutation with all types of ventricular hypertrophy seen in this sub-cohort. This suggests that factors independent of the mutation specific molecular abnormality in these families influence how, when and where hypertrophy develops.

A major limitation of studying disease expression in families is the cross sectional design – HCM is a chronic disease and therefore longitudinal cohort studies are required to identify age-dependent trends. Longitudinal follow up of these individuals is beyond the scope of this thesis but may provide important insights into disease expression in the future.

The major clinical implication of these data is that counseling relatives in families in which the mutation is known and has been previously reported in the literature should remain a general discussion of the principles of HCM inheritance and not try to include mutation specific information based upon limited published data.

As individual institutions will always be limited by the infrequency of a particular mutation in their own cohort it is likely that international databases are required to report clinical data for a given mutation, increasing the statistical power, to allow important

trends (if they exist) to emerge. Even if such mutation specific trends do exist, it is evident from this study that at least some of the variance in disease expression is related to factors independent of the family mutation. It is likely that a combination of genetic factors (complex genetic status, disease modifier genes, polymorphisms and possibly gender (see Disease Penetrance, Age and Gender in Families with MYBPC3 Mutations) and environmental factors such as physical training and hypertension may play a role in determining the eventual phenotype in any individual.

EARLY DIAGNOSTIC MARKERS OF DISEASE EXPRESSION

Introduction

In a disease state with age-related and incomplete penetrance it is theoretically desirable to be able to predict which individuals are likely to develop disease in the future as this may allow early intervention to reduce the risk of adverse events.

Data from a transgenic rabbit model of HCM has suggested that reduced myocardial Doppler velocities may identify mutation carriers irrespective of ventricular hypertrophy [Nagueh 2000]. Further studies in humans have supported the role of tissue Doppler imaging in identifying unaffected mutation carriers [Nagueh 2001] and even predicting which individuals go on to develop HCM during longitudinal follow up [Nagueh 2003]. Data from other investigators have been inconsistent however [Ho 2002][Cardim 2002]. In order to study this issue further, a number of clinically derived parameters were assessed in a large genotyped population in order to assess whether it is possible to accurately identify unaffected mutation carriers from control subjects from clinical data alone.

Methods

As part of an ongoing study we have identified a cohort of 59 index cases fulfilling diagnostic criteria for HCM and a disease causing mutation in the MYBPC3 gene. First and second degree relatives were invited to participate in this study and undergo clinical and genetic evaluation. Evaluation included history, clinical examination, 12-lead electrocardiogram (ECG) and transthoracic echocardiography (Echo). All at risk relatives were considered eligible and were only excluded if their Echo data was of insufficient quality to allow detailed analysis as described below.

ECGs were analysed (blinded to genotype) for the presence of pathological and non-pathological Q waves, Romhilt Estes score, Sokolow Lyon Criteria, T wave inversion (> 1mm in two consecutive leads), bundle branch block (QRS duration > 0.12s), axis deviation and atrial enlargement. Definitions used in ECG analysis are shown in Table 17.

Table 17 Definitions used in ECG analysis

Parameter	Definition		Score
Romhilt Estes Score	Voltage Criteria (any of):		Scored out of 14. Scores > 4 considered to represent LVH
	R or S in limb lead > 20mm		
	S in V1 or V2 > 30mm	3 points	
	R in V5 or V6 > 30mm		
	ST-T Abnormalities		
	With Digoxin	1 point	
	Without Digoxin	3 points	
	Left Atrial Enlargement in V1	3 points	
	Left axis deviation	2 points	
	QRS duration > 90ms	1 point	
	Intrinsicoid deflection in V5 or V6 > 50ms	1 point	
Sokolow Lyon Criteria	S in V1 + R in V5 or V6 $\geq$ 35 mm		Yes or No
Cornell Criteria	S in V3 + R in aVL > 24 mm (men) S in V3 + R in aVL > 20 mm (women)		Yes or No
Abnormal Q Wave	Duration > 40ms and greater than 1/3 ensuing R wave height		Yes or No
Left atrial enlargement	biphasic P wave in V1 with terminal deflection > 1mm and >0.04s duration		Yes or No
T wave abnormality	Flat or inverted T wave		Yes or No

Konno 2005b

Transthoracic echo data was analysed off-line (EchoPAC, GE Healthcare, Little Chalfont, UK) according to published criteria [Gardin 2002]. Septal and posterior wall thicknesses were measured at end-diastole by M-mode in the parasternal long-axis view as well as LV end-diastolic and end-systolic dimensions. LA diameter was measured in the parasternal long and short axis, and apical views. Mitral valve inflow pulsed wave Doppler was recorded to measure E and A wave velocity, E/A ratio, E wave deceleration, A wave duration, time velocity integral and isovolumic relaxation time. Pulmonary vein Doppler was recorded to measure S, D and A wave velocities, D wave deceleration time and time velocity integrals. Pulsed tissue Doppler recordings were made at the mitral annulus at the septum and lateral wall, and anterior and inferior walls when possible. All

measurements were made by one observer (SK) who was blinded to the genetic status of each individual.

Longitudinal strain and strain rate were calculated from colour Doppler myocardial imaging data of the apical 4chamber view. Due to Doppler angle dependence, analysis was performed only at the basal and mid segments of the septal and lateral wall using high frame rate acquisitions (>140fps). Sample volume size was 10x2mm with continuous wall tracking and maximum measurements were averaged over 3 consecutive cardiac cycles. Technically adequate recordings obtained from 10 unaffected mutation carriers were matched for age and gender with 10 normal controls.

Statistics

Relatives were categorised into 3 groups on the basis of their genetic and clinical status: Mutation negative control subjects (Group 1), unaffected mutation carriers who did not fulfil diagnostic criteria for HCM (maximal LV wall thickness <13mm) (Group 2) and mutation carriers fulfilling diagnostic criteria for HCM (LV wall thickness >13mm) (Group 3). Means and standard deviations were calculated for continuous variables and Student's t-test (comparing groups 1 and 2) and ANOVA (comparing all three groups) were used to compare differences between groups. For categorical variables the Chi-square test was used. A p-value < 0.05 was considered statistically significant.

Results

A total of 96 individuals were included in the study including 56 mutation carriers with 24 mutations, from 27 families. In two families more than one disease causing mutation was identified (complex genetic status). The spectrum of mutations is shown in Table 18.

Table 18 Spectrum of MYBPC3 Mutations in Early Diagnosis Study

Mutation	Mutation Type	Number of Mutation Carriers
g_18567delCT	Insertion/deletion	1
R502Q	Missense	7
R502W	Missense	12
698delC	Insertion/deletion	2
g14271delC	Insertion/deletion	5
g16190_16196delGCGTCTA	Insertion/deletion	1
Q969X	Nonsense	2
E258K	Missense	2
G341R	Missense	1
IVS1-2A>G	Intronic	2
IVS13-2A>G	Intronic	1
IVS14-13G>A	Intronic	1
IVS18+7G>A + D880D	Complex	1
IVS20-2A>G	Intronic	3
K1055X	Nonsense	3
IVS9-36G>A	Intronic	1
V1125M + IVS9-1G>C	Complex	1
P873Q	Missense	2
Q425X	Nonsense	1
R943X	Nonsense	2
T750M	Missense	3
T957S	Missense	2

There were 40 mutation negative control subjects (Group 1), 39 unaffected mutation carriers (Group 2) and 17 mutation carriers fulfilling diagnostic criteria for HCM (Group 3). The yield in terms of identifying individuals who fulfil diagnostic criteria for HCM from the screening programme was 17.7%. Baseline clinical and echo data is shown in Table 18. Although there were slightly more females than males in Group 2 this was not statistically significant (χ^2 =1.0, 1 df, p=0.311). Affected individuals were older and more symptomatic. Mutation types were similar between the three groups (data not shown).

There were no significant differences in terms of LV cavity dimensions (LVEDD 48.5 v 47.7, p=NS), septal thickness (8.5 v 9.1, p=NS) or maximal wall thickness (8.8 v 9.5, p=NS) between mutation negative individuals and unaffected mutation carriers.

Table 19 Clinical and demographic data of 96 individuals screened for HCM

	Group 1 Mutation Negative	Group 2 Unaffected Mutation Carriers	Group 3 HCM	p-value Group1 vs Group2
Number of Individuals (n)	40	39	17	-
% Male	55.0	43.6	58.8	NS
Mean Age (Years)	38.5 +/- 16.7	37.4 +/- 16.8	47.3 +/- 15.3	NS
Transthoracic Echo Measurements				
LA size (mm)	34.3 +/- 4.3	35.0 +/- 4.8	37.7 +/- 7.0	NS
LVEDD (mm)	48.5 +/- 5.0	47.7 +/- 5.2	44.0 +/- 4.5	NS
LVESD (mm)	31.4 +/- 4.4	30.9 +/- 4.0	29.0 +/- 5.6	NS
IVSd (mm)	8.5 +/- 1.4	9.1 +/- 1.6	13.9 +/- 4.6	NS
PWd (mm)	8.1 +/- 1.3	8.6 +/- 1.5	8.7 +/- 1.4	NS
Maximal wall thickness (mm)	8.8 +/- 1.5	9.5 +/- 1.6	15.3 +/- 4.3	NS
FS (%)	48.1 +/- 4.5	47.4 +/- 4.8	43.4 +/- 4.5	NS

* Students t-test comparing Groups 1 and 2 only.

ECG Measurements

Categorical and continuous ECG variables were compared between mutation negative (Group 1) and unaffected mutation carriers (Group 2) (Tables 20&21). Six mutation negative individuals had an abnormal ECG (5 were hypertensive and 1 had a history of previous anterior myocardial infarction). The prevalence of LA enlargement (20.5% v 2.5%, p=0.012) and non-pathological Q waves (64.1% v 27.5, p=0.001) were more common in Group 2 (non-pathological Q wave: defined as a negative deflection with a duration < 40ms and an amplitude < 1/3 the height of the ensuing R wave). Unaffected mutation carriers had higher mean Romhilt Estes scores (1.4 vs 0.8, p=0.042), but a RE score > 4 was no more common in Group 2 (7.8% v 5.0, p=0.623). Mean S wave amplitudes were greater in Group 2 for leads V2 (16.6 v 12.4mV, p=0.023) and V3 (12.7mm v 9.3, p=0.023). The proportion fulfilling Cornell criteria for LVH were more

common in Group 2 but this did not reach statistical significance (17.9% v 5.0, p=0.070). Eight (21%) unaffected mutation carriers had T wave inversion on the ECG (7 lateral or high lateral leads and 1 anteriorly) compared to 3 (8%) mutation negative controls (p=0.095).

Overall, 15 of 39 (38.5%) unaffected mutation carriers had an abnormal ECG (defined as fulfilling either Cornell criteria, Sokolow Lyon criteria or a Romhilt Estes score ≥4). In comparison 6 of 40 (15%) mutation negative controls had an abnormal ECG (Chi square 5.57, 1 df, p=0.18)

Table 20 ECG measurements

	Group 1 Mutation Negative	Group 2 Unaffected Mutation Carriers	Group 3 HCM	p-value Group 1 v Group 2
Heart rate (bpm)	69 +/- 14	69 +/- 12	66.5 +/- 14.2	0.907
PR Interval (ms)	157 +/- 19	155 +/- 21	166 +/- 49	0.570
QRS duration (ms)	86 +/- 10	86 +/- 10	89 +/- 12	0.926
Mean Romhilt Estes Score	0.8 +/- 1.2	1.4 +/- 1.6	3.1 +/- 3.9	0.042
Total QRS Voltage (mV)	136 +/- 45	149 +/- 48	146 +/- 48	0.208
S wave V1	9.6 +/- 5.2	10.9 +/- 4.7	10.5 +/- 4.7	0.229
S wave V2	12.4 +/- 8.2	16.6 +/- 7.9	12.7 +/- 6.6	0.023
S wave V3	9.3 +/- 6.1	12.7 +/- 6.9	13.1 +/- 6.1	0.023
R Wave V5	12.0 +/- 5.2	11.8 +/- 4.6	9.8 +/- 4.2	0.835
R Wave V6	9.9 +/- 3.8	9.8 +/- 3.9	8.9 +/- 4.2	0.950
R Wave aVL	3.6 +/- 3.2	3.9 +/- 2.7	6.1 +/- 5.8	0.606

Data presented as mean +/- SD

Table 21 Sensitivity and Specificity for Categorical ECG Parameters

	Group 1 Mutation Negative	Group 2 Unaffected Mutation Carriers	Sensitivity (%)	Specificity (%)	P-value Chi-square
LA Enlargement (n(%))	1(3)	8(21)	21	98	0.012
Pathological Q Waves (n(%))	1(3)	0(0)	0	98	0.320
Non-pathological Q Waves (n(%))	11(28)	25(64)	64	73	0.001

Sokolow Lyon Criteria (n(%))	2(5)	1(3)	3	95	0.571
Cornell Criteria (n(%))	2(5)	7(18)	18	95	0.070
Romhilt Estes Score > 4 (n(%))	2(5)	3(8)	8	95	0.623
Abnormal T Wave Inversion (n(%))	3(8)	8(21)	21	93	0.095

Echo Measurements

There were no differences in standard measurements of cavity size and wall thickness between groups 1 and 2 (Table 22). LA width measured in the apical 4 chamber view was greater in Group 2 however (37.2mm v 34.3, p=0.017) although other measurements of LA dimensions were no different. Indices of mitral valve pulsed wave Doppler were no different between the two groups. Pulmonary vein Doppler indices were also evaluated. AR wave duration was greater in Group 2 (115ms v 105, p=0.047) and D wave deceleration time was shorter (203ms v 232, p=0.038). All other indices were similar between the two groups.

Table 22 2D and Doppler Echo Measurements

	Group 1 Mutation Negative	Group 2 Unaffected Mutation Carriers	Group 3 HCM	p-value Group 1 v Group 2 **(t-test)**
LA dimension PLAX (mm)	34.3 +/- 4.3	35.7 +/- 4.9	38.4 +/- 6.0	0.184
LA dimension SAX (mm)	34.5 +/- 5.0	35.3 +/- 5.2	39.7 +/- 7.8	0.474
LA depth A4C (mm)	45.7 +/- 5.8	47.2 +/- 5.6	50.2 +/- 5.9	0.254
LA width A4C (mm)	34.3 +/- 4.8	37.2 +/- 5.7	36.8 +/- 6.3	0.017
E Velocity (cm/s)	0.8 +/- 0.2	0.8 +/- 0.1	0.8 +/- 0.2	0.524
A Velocity (cm/s)	0.6 +/- 0.2	0.6 +/- 0.2	0.7 +/- 0.3	0.866
EA ratio	1.5 +/- 0.7	1.5 +/- 0.5	1.2 +/- 0.5	0.824
E Deceleration time (ms)	195 +/- 35	190 +/- 36	203 +/- 54	0.591
A Duration (ms)	124 +/- 16	123 +/- 19	117 +/- 22	0.680
Isovolumic relaxation time (ms)	94 +/- 19	90 +/- 17	105 +/- 27	0.264
E Wave VTI (cm/s)	12.0 +/- 2.8	12.8 +/- 2.5	13.7 +/- 4.4	0.166
A Wave VTI (cm/s)	5.4 +/- 2.0	5.1 +/- 2.0	6.3 +/- 2.8	0.510

S wave velocity (cm/s)	59.0 +/- 9.3	55.1 +/- 7.7	54.8 +/- 14.6	0.057
D wave velocity (cm/s)	53.0 +/- 17.0	53.4 +/- 12.3	44.5 +/- 10.0	0.869
AR velocity (cm/s)	24.9 +/- 5.6	26.1 +/- 7.1	31.0 +/- 9.3	0.421
AR duration (ms)	105 +/- 21	115 +/- 22	114 +/- 21	0.047
D Wave deceleration time (ms)	232 +/- 58	203 +/- 56	239 +/- 71	0.038
S wave VTI (cm/s)	16.5 +/- 3.1	16.1 +/- 4.0	15.4 +/- 2.6	0.642
D wave VTI (cm/s)	12.4 +/- 3.7	12.1 +/- 3.5	11.2 +/- 3.2	0.764
AR wave VTI (cm/s)	2.4 +/- 0.7	2.7 +/- 0.8	3.1 +/- 1.0	0.167

Tissue Doppler velocities at the mitral valve annulus were also compared. Late-diastolic velocities at the lateral annulus were greater in Group 2 (Aa 8.9 cm/s v 7.5, p=0.005) but all other indices were similar between the two groups (Table 23).

Table 23 Tissue Doppler Measurements

	Group 1 Mutation Negative	Group 2 Unaffected Mutation Carriers	Group 3 HCM	P-value Group 1 v Group 2
Sa Lateral Annulus (cm/s)	9.8 +/- 2.6	10.1 +/- 2.4	9.4 +/- 2.5	0.598
Ea Lateral Annulus (cm/s)	13.4 +/- 4.9	13.3 +/- 3.9	9.4 +/- 3.7	0.972
Aa Lateral Annulus (cm/s)	7.5 +/- 1.9	8.9 +/- 2.3	9.1 +/- 3.5	**0.005**
E/Ea Ratio Lateral	6.0 +/- 1.8	5.8 +/- 1.2	9.6 +/- 8.6	0.615
IVRT Lateral Annulus (ms)	75.8 +/- 17.5	81.3 +/- 16.3	92.5 +/- 19.2	0.173
Sa Septum (cm/s)*	8.1 +/- 1.4	7.9 +/- 1.6	7.0 +/- 1.9	0.716
Ea Septum (cm/s)*	10.1 +/- 2.7	9.0 +/- 2.8	7.9 +/- 4.3	0.328
Aa Septum (cm/s)*	8.1 +/- 21.1	7.3 +/- 1.3	7.9 +/- 1.1	0.251
IVRT septum (ms)*	92.0 +/- 21.1	92.5 +/- 13.8	106 +/- 39.8	0.952

* - Due to suboptimal image quality TDI data at the septum was measured in 18 individuals in Group 1, 9 Group 2 and 7 Group 3

Having excluded those with an abnormal ECG (6 from Group 1 and 9 from Group 2, in order to assess the role of Echo in the context of a normal ECG) the observed differences persisted between the two groups in terms of 2D, Doppler and tissue Doppler indices: LA

4-chamber width (p=0.018), D deceleration time (p=0.041), Lateral Aa velocity (p=0.021), AR duration was no longer different between the two groups however (p=0.257).

Strain and Strain-rate Imaging

A sub-group of individuals with adequate image quality were selected for strain and strain-rate imaging analysis off-line. Data from 10 unaffected mutation carriers and 10 age and sex matched mutation negative control subjects were evaluated (Table 24). No significant differences were observed for any parameters between the two groups.

Table 24 Strain and Strain-rate Imaging in sub-groups of Groups 1 and 2

Parameter	Group 1 (n=10)	Group2 (n=10)	P – value
Mean age	37.4 +/- 13.9	36.0 +/- 16.3	0.837
% Male	50.0	50.0	-
Strain			
Sa septum (%)	5.5 +/- 0.8	5.7 +/- 0.9	0.616
Ea septum (%)	8.6 +/- 2.0	7.4 +/- 2.6	0.252
Aa septum (%)	6.0 +/- 1.3	6.2 +/- 1.9	0.864
Ea/Aa ratio septum (%)	1.6 +/- 0.7	1.4 +/- 0.9	0.603
Sa lateral (%)	6.3 +/- 1.9	7.1 +/- 2.0	0.339
Ea lateral (%)	10.5 +/- 4.2	9.8 +/- 3.3	0.722
Aa lateral (%)	4.7 +/- 1.1	6.6 +/- 2.2	**0.026**
Ea/Aa lateral (%)	2.4 +/- 1.1	1.8 +/- 1.2	0.281
Basal septum (%)	21.9 +/- 7.4	14.7 +/- 6.4	**0.042**
Mid septum (%)	24.5 +/- 8.5	21.0 +/- 4.6	0.277
Basal lateral (%)	20.6 +/- 7.1	20.6 +/- 7.7	0.992
Mid lateral (%)	14.9 +/- 5.1	16.2 +/- 6.0	0.639
Strain rate			
Basal septal (s^{-1})	1.4 +/- 0.9	1.1 +/- 0.5	0.372
Mid septal (s^{-1})	1.7 +/- 0.8	1.4 +/- 0.5	0.229
Basal lateral (s^{-1})	1.4 +/- 0.4	1.6 +/- 0.6	0.511
Mid lateral (s^{-1})	1.1 +/- 0.3	1.2 +/- 0.5	0.675

Lateral Aa velocity was greater in Group 2 (6.6 v 4.7, p=0.026) and basal septal strain was reduced (14.7 v 21.9, p=0.042).

Discussion

In this study the clinical value of a large number of ECG and Echo measurements in predicting genetic status in a large cohort of unaffected HCM family members has been assessed. The main findings were as follows:

- Several ECG abnormalities including LA enlargement and deep S waves in V1 and V2 were more common in unaffected mutation carriers compare to controls
- Tissue Doppler indices were clinically unhelpful in predicting genetic status and previous data suggesting useful role for reduced Ea and Sa velocities were not confirmed in this study

Previous studies have examined the ability to predict genotype from clinically derived parameters. Hagege et al. examined ECG criteria and 2D and M-mode Echo parameters in 100 genotyped individuals (20 unaffected mutation carriers) [Hagege 1998]. 50% had an abnormal ECG. When compared to gene negative individuals, unaffected mutation carriers had greater maximal wall thickness (9.7 vs 8.9mm), greater LV mass indexed for body surface area (107 vs 97g/m^2) and larger left atrial volume (27 vs 23 mm^3). They concluded that disease expression is a continuous spectrum from normal cardiac structure to typical ventricular hypertrophy.

Following early experimental data from transgenic rabbits, Nagueh et al examined the role of tissue Doppler imaging in 3 groups of highly selected, age and sex matched individuals: 1) control subjects 2) mutation carriers without LVH and 3) mutation carriers

fulfilling diagnostic criteria for HCM [Nagueh 2001]. There were 13 unaffected mutation carriers with mutations in MYH7, TNNT2 and MYBPC3 genes and had no evidence of LVH on ECG or Echo. Systolic (Sa) and early diastolic (Ea) velocities were reduced compared to normal controls at both the septum and lateral mitral annulus, but to a lesser extent than in patients with HCM. E/Ea ratio (a marker of LV filling pressure) at both the septum and lateral mitral annulus were increased. Late diastolic velocities (Aa) were similar in both unaffected mutation carriers and controls. Not only were the statistical means different between groups but lateral Sa velocity of <13cm/s had a sensitivity of 100% and specificity of 93% for differentiating between unaffected mutation carriers and controls. Similarly a lateral Ea velocity < 14 cm/s had a 100% sensitivity and 90% specificity. Septal Sa <12 cm/s and Ea < 13 cm/s both had 100% sensitivity and 90% specificity. The authors concluded that tissue Doppler derived data was able to reliably distinguish between unaffected mutation carriers and mutation negative controls and could thus prove useful in families with familial HCM where the mutation was unknown.

In a second study the same group published follow-up data from 12 of the 13 unaffected mutation carriers over a two year period (age 17-51) [Nagueh 2003]. 6 of these 12 individuals developed maximal LV wall thickness > 13mm, thus fulfilling diagnostic criteria for HCM. No significant changes between baseline and follow-up were noted in transmitral Doppler indices. Tissue Doppler parameters were also measured at baseline and at follow-up. Systolic (Sa) and early diastolic (Ea) velocities at both the septum and lateral mitral annulus were significantly reduced compared to controls at follow up. E/Ea ratio at the septum and lateral annulus remained higher at follow up compared to

controls. The authors concluded that these data confirmed the potential role of tissue Doppler imaging to identify unaffected mutation carriers who may subsequently develop left ventricular hypertrophy.

Ho et al. examined tissue Doppler parameters in 18 unaffected MYH7 mutation carriers from 5 families and controls [Ho 2002]. Most mutation carriers (Asp 906Gly) came from a single family however (13 of 18 individuals). Ages were similar and transmitral Doppler indices were no different between the two groups. There were no statistical differences in ECG parameters between the two groups. Unaffected mutation carriers had greater LV ejection fraction (71 vs 64%, $p<0.0001$). Early diastolic (Ea) velocities were reduced at the lateral, septal and inferior mitral annulus in unaffected mutation carriers, while systolic (Sa) and late diastolic (Aa) velocities were no different between the two groups. The authors remarked that there was considerable overlap of values between the two groups and cut-off values were neither sensitive nor specific for predicting genotype. Cardim et al. assessed tissue Doppler indices in 5 unaffected mutation carriers (Arg502Gln mutation in MYBPC3 gene) [Cardim 2002]. Compared with 10 normal controls subjects, mutation carriers had lower LV systolic velocities (Sa) and lower early diastolic (Ea) velocities, although there was no clinically useful cut-off value which accurately identified mutation carriers.

The results from our study are somewhat inconsistent with those published previously. We identified several ECG abnormalities which were were more prevalent in unaffected mutation carriers compared to mutation negative controls. LA enlargement is a common

abnormality in affected individuals and therefore LA enlargement on an ECG may represent evidence of early disease development. However while the specificity of LA enlargement on ECG was specific (98%) it was not sensitive in identifying unaffected mutation carriers. Unaffected mutation carriers also had higher mean Romhilt Estes scores (1.4 +/- 1.6 v 0.8 +/- 1.2), deeper S waves in V2 (16.6 +/- 7.9 v 12.4 +/-9.2mm) and deeper S waves in V3 (12.7 +/- 6.9 v 9.3 +/- 6.1mm) compared to mutation negative controls. Combined, these data suggest that ECG abnormalities occur early in disease development - and before hypertrophy develops. No ECG abnormality was sensitive however in identifying unaffected mutation carriers. Therefore while it is interesting to observe ECG abnormalities predating the development of hypertrophy (as detected by cardiac imaging), the ECG is unhelpful clinically in reliably predicting genotype, and does not allow the accurate identification of unaffected mutation carriers in this cohort. While Nagueh et al. [Nagueh2001] and Ho et al. [Ho 2002] reported reduced Sa and Ea velocities at the mitral annulus these results were not confirmed in this study. In our larger study, a cut-off of lateral Ea velocity < 14 had 40% sensitivity and 50% specificity in identifying mutation carriers. Lateral Sa velocity < 13 had sensitivity 50%, specificity 50%. A septal Sa < 12 was 100% but 0% specific and septal Ea <13 was 70% sensitive and 30% specific, although adequate TDI data from the septum was obtained in a small number of individuals. There was considerable overlap between the two groups however and no indices reliably or consistently identified mutation carriers. In our study, the Aa velocity at the lateral annulus was increased in unaffected mutation carriers compared to control subjects. This result was not observed in other published studies and contradicts

the expected finding of reduced Aa velocities in subjects with clinically evident hypertrophy. This may therefore represent a Type 2 statistical error.

There are a number of explanations for different findings between our study and other published data. Although systolic TDI velocities are independent of age, diastolic velocities (Ea and Aa) are highly age dependent [Henein 2002]. The mean ages in affected mutation carriers and controls in the study by Ho et al. were 24 and 26 years respectively (NS) and 35 and 36 years (NS) in the study by Nagueh. In our study the mean age of unaffected mutation carriers (Group 2) was 37 and 39 (NS) for mutation negative controls. It is possible, but unlikely therefore, that differences in age explain the different findings. In our study 44% of mutation carriers were male compared to 55% mutation negative controls, compared to 40% and 60% in the study by Ho et al. and 23% and 23% in the study by Nagueh et al. As the findings by Nagueh et al were largely in women it is possible therefore that gender specific differences in disease penetrance account for the lack of positive result in our study. The TDI velocities, especially at the lateral annulus were lower in our control group than one might expect [Henein 2002]. Within this group however were individuals with other pathologies such as hypertension and ischaemic heart disease. We specifically chose to assess the role of early diagnostic markers in a practical, clinical setting however and therefore did not exclude such individuals.

This study was limited to data from families with mutations in the MYBPC3 gene. This is the most common gene in which disease causing mutations occur [Van Driest 2004] and by limiting the study in this way gene specific differences should be avoided. Importantly

this study included a large range of mutations, representative of the overall cohort of MYBPC3 related disease. It is possible however that our findings are not necessarily applicable to the wider HCM population.

Not previously reported is the role of deformation imaging in predicting genotype in this context. We found no significant differences between unaffected mutation carriers and controls in this regard. Although there are technical and methodological difficulties associated with deformation imaging [Teske 2007], the overall quality of data was high in this study and the two groups were well matched for age and sex, the lack of a positive result is likely to be real.

In the absence of a known mutation in a family with HCM there are several theoretical advantages in identifying individuals before hypertrophy develops. Firstly in families in which the mutation is unknown, it could allow more focussed screening by selecting those individuals likely to, or at risk of, developing hypertrophy in the future. This has the potential advantage of avoiding unnecessary clinical visits by mutation negative individuals, which is beneficial both in terms of their own peace of mind and also of resource allocation. Secondly it could provide an opportunity to intervene at an early stage of the disease. Although to date there are no interventions that modify disease expression in humans, prophylactic ICD implantation in at risk mutation carriers is theoretically advantageous. Although sudden cardiac death in unaffected mutation carriers has been reported [McKenna 1990][Maron 1990], its incidence is poorly defined and how to best assess risk in unaffected mutation carriers is not known.

The strengths of this study lie in the large number of individuals examined (greater than the combined published data to date) and the blinded design. Subtle statistically significant differences were identified between unaffected mutation carriers and controls, but no clinically useful cut-off values were identified to allow accurate blinded identification of mutation carriers with no clinical evidence of disease expression. While careful, technical evaluation of tissue Doppler indices may elucidate subtle differences (as published by Nagueh et al. and Ho et al.) in mutation carriers this remains of academic interest at present. It is interesting for example to note that identifying abnormalities of contractile function before hypertrophy is evident, supports the hypothesis that ventricular hypertrophy in HCM is compensatory. At present however tissue Doppler derived data do not allow the accurate identification of unaffected mutation carriers and thus do not allow a more focussed family screening strategy

In summary, previously identified abnormalities of systolic and diastolic myocardial function in unaffected mutation carriers were not consistently confirmed in this study. While small studies in highly selected groups of individuals have suggested a role for tissue Doppler imaging in accurately identifying mutation carriers from controls, the application of such parameters does not appear to be relevant to clinical practice in this larger, less selected group of individuals. Family screening programmes ought to therefore continue to evaluate all relatives at risk of inheriting disease causing mutations for the time being.

LONGTERM FOLLOW-UP DATA

Introduction

There are relatively few data describing the natural history of HCM in relation to MYBPC3 mutations. Most series of genotyped patients have used a cross-sectional design and detailed longitudinal cohort studies are lacking. Such data are important when trying to manage patients and their families, and in trying to anticipate and prevent complications in the future. As has already been discussed, the issue of whether echocardiographically detectable hypertrophy develops commonly in adulthood is particularly important. Existing longitudinal data relating to HCM in individuals and families with MYBPC3 mutations are summarised below.

Maron et al described for the first time the development of ventricular hypertrophy in adults [Maron 2001]. Of 12 adult unaffected mutation carriers at initial evaluation, 5 were followed up prospectively, 3 (1 male) of whom developed echocardiographic ventricular hypertrophy at ages 33, 35 and 42. One individual had incomplete systolic anterior motion at initial evaluation age 29 with partial RBBB on ECG however, and another individual had an abnormal ECG with Q waves and T wave inversion at baseline age 27. In only one individual did hypertrophy develop with a normal ECG and Echo at baseline (age 38).

Nagueh et al described LVH developing in 6 of 12 adult mutation carriers unaffected at baseline assessment. 7 of these 12 shared the InsG791 mutation in MYBPC3 although it is not clear how many of these 7 developed HCM during 2 years of follow-up [Nagueh 2003].

Kubo et al described longitudinal follow-up in 15 families sharing an identical Japanese founder mutation (V592fs/8) in MYBPC3 [Kubo 2005]. 39 mutation carriers were identified of which 30 fulfilled diagnostic criteria at initial assessment. During a mean follow-up period of 9.2 +/- 5.5 years AF was detected in 33% (3.6%/year). LA size increased from 40 +/- 8.3 mm to 46 +/- 9.0mm (p-value not reported). At last follow-up 7 individuals had developed "end-stage" HCM defined as LV systolic dysfunction, cavity dilatation and irreversible heart failure. 6 of these 7 patients were 60 years or older. There were 2 stroke deaths, 1 heart failure death and 1 ICD discharge during follow-up (overall combined risk 1.4% per year).

This part of the study examines the natural history of clinical disease related to MYBPC3 mutations for both affected and unaffected mutation carriers.

Methods

Clinical data was compared at baseline and at last follow-up assessment for all mutation carriers with at least two clinical evaluations at least 12 months apart. Paired sample t-tests were used to compare means. Data are presented as mean +/- standard deviation, or as percentages. A p-value < 0.05 was considered statistically significant.

Results

All Mutation Carriers

Longitudinal follow up data was available for 84 mutation carriers. Of these, 67 were clinically affected at baseline. Mean age at start of follow up was 37.1 +/-16.4 years (range 5-76). Mean duration of follow-up was 7.9 +/- 4.5 years (range 1-19).

Overall survival was 94.0% - there were 5 deaths (3 sudden, 1 heart failure, 1 stroke). The 3 sudden deaths occurred in the following individuals:

- A 32 year old female (H588.2 with the IVS14-13G>A mutation) was diagnosed age 28 and followed up for 4 years. She had a maximal wall thickness of 22mm and had evidence of NSVT on Holter monitoring and an episode of syncope which occurred during physical exercise. Before prophylactic ICD implantation could be arranged she died suddenly while dancing.
- A 58 year old man (H697.1 with the K1055X mutation) was diagnosed with obstructive HCM age 51 following an incidental finding of a cardiac murmur. He was asymptomatic until he developed AF at which stage he developed heart failure. He was treated with amiodarone. He had a maximal wall thickness of 26mm, a positive family history of premature sudden cardiac death, and a borderline blood pressure response on exercise. He died suddenly age 58.
- A 31 year old man (H934.1 with the g14274delC mutation) presented age 30 with acute pulmonary oedema while swimming. Subsequent investigations demonstrated asymmetric septal hypertrophy (maximal wall thickness 24mm)

with incomplete SAM and NSVT on exercise. He died suddenly shortly afterwards.

One individual (H608.1 with the intronic splice site IVS1-2A>G mutation) had progressive wall thinning and predominantly restrictive physiology and underwent cardiac transplantation for intractable heart failure. One individual was lost to follow up. The overall annual mortality rate was therefore 0.75% and annual risk of sudden death 0.45%. The proportion of patients who were symptomatic was no different at baseline compared to follow-up (65.7% and 64.7%). 4 individuals underwent alcohol septal ablation and 2 underwent surgical myomectomy for symptomatic outflow tract obstruction. 13 individuals underwent ICD implantation (11 prophylactic, 2 following aborted sudden death). No appropriate ICD therapies (either anti-tachycardia pacing or DC shock therapy) were detected in 3.0 +/- 1.7 years (range 1-6). There were 5 strokes (1 fatal, annual risk of stroke 0.75%) Arrhythmias were common however with 28.8% of individuals having documented NSVT on Holter monitoring and 18.3% developing at least one episode of AF during follow-up.

Table 25 Event rates in 84 mutation carriers

	Number (%)
Symptom-free survival	35 (43.2)
Alive	79 (94.0)
Complications	
Cardiac transplantation	1 (1.2)
Heart Failure Death	1 (1.2)
Sudden death	3 (3.7)
Heart Failure	9 (11.0)
Atrial fibrillation	15 (18.3)
NSVT or VT	23 (28.8)
Survived Cardiac Arrest	1 (1.3)
Stroke	5 (6.1)
Infective Endocarditis	1 (1.2)
Interventions	
Pacemaker	7 (8.4)
Cardiac resynchronisation	1 (1.2)
ICD	13 (15.7)
Surgical myomectomy	2 (2.4)
Alcohol septal ablation	4 (4.8)

Affected Mutation Carriers

Echocardiographic data for affected individuals at baseline and at follow-up was compared (Table 26 and Figures 24-31). Longitudinal follow up was associated with a reduction in septal thickness (16.5 at baseline v 15.2mm, p=0.033), increased LV end-systolic diameter (25.5 v 28.6mm, p=0.000), reduced fractional shortening (41.6 v 37.0%, p=0.000) and increase in LA diameter (39.8 v 42.3 v mm, p=0.001). 13.4 % developed clinical symptoms of heart failure during follow up and dilated-phase HCM (defined as LVEDD > 55mm and FS < 25%) occurred in 2 individuals (3.0%).

Table 26 Echocardiographic parameters at baseline and at last follow up in 67 affected mutation carriers

	Baseline	Follow-up	P-value *
Age (years)	37.1 +/- 16.4	47.0 +/- 16.9	0.000
IVSd (mm)	16.5 +/- 6.6	15.2 +/- 6.4	0.033
PWd (mm)	9.8 +/- 2.7	9.2 +/- 2.6	0.122
MWT (mm)	17.6 +/- 6.7	16.8 +/- 6.2	0.166
LVEDD (mm)	43.5 +/- 7.2	44.7 +/- 6.9	0.086
LVESD (mm)	25.5 +/- 7.1	28.6 +/- 6.8	0.000
FS (%)	41.6 +/- 9.1	37.0 +/- 8.2	0.000
LA (mm)	39.8 +/- 8.2	42.3 +/- 8.6	0.001

*Using paired-samples T-test.

These data are shown graphically in Figures 24-31. Boxes represent the arithmetic mean with bars representing 95% confidence intervals.

Figure 24 Aortic Diameter at Baseline and Last Follow up

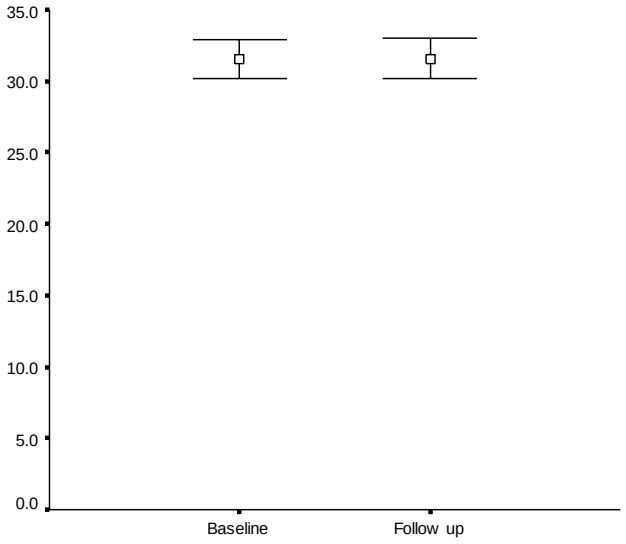


Figure 25 LA Diameter at Baseline and Last Follow up

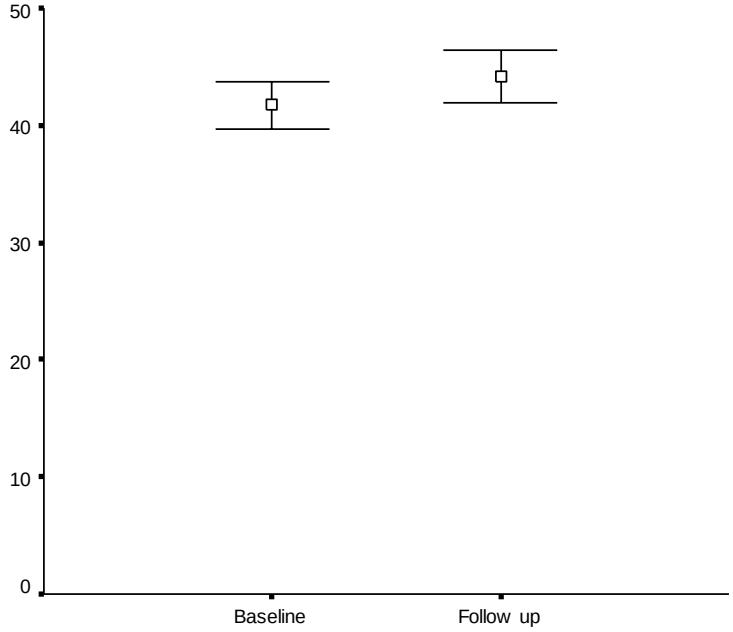


Figure 26 LVEDD at Baseline and Last Follow up

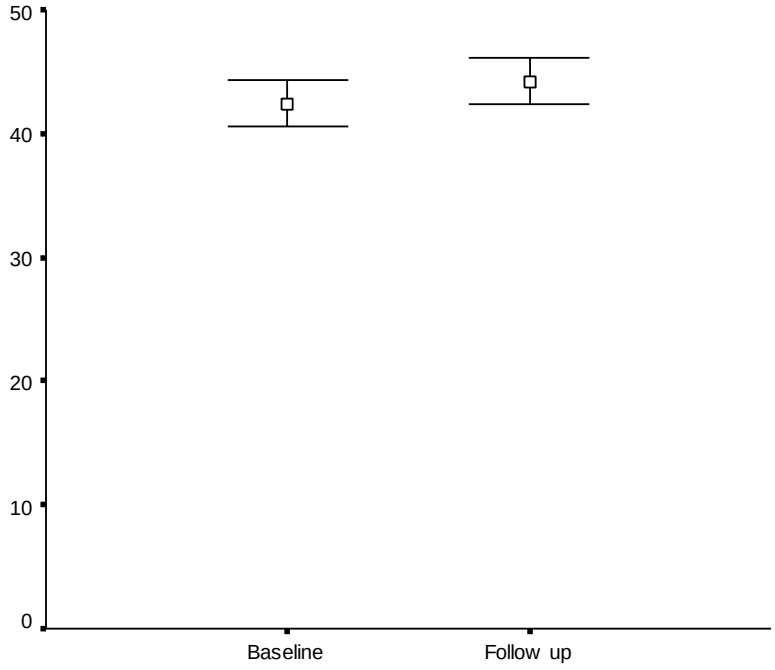


Figure 27 LVESD at Baseline and Last Follow up

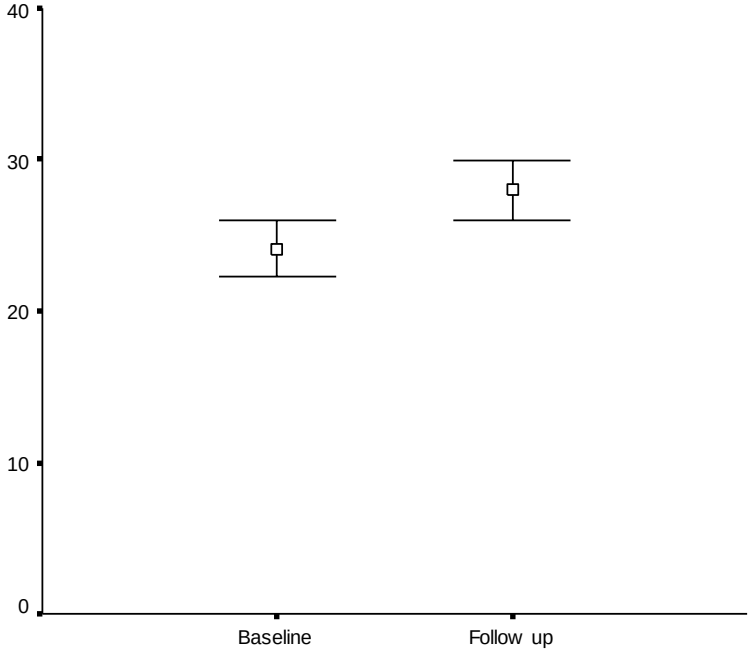


Figure 28 **Septal Thickness at Baseline and Last Follow up**

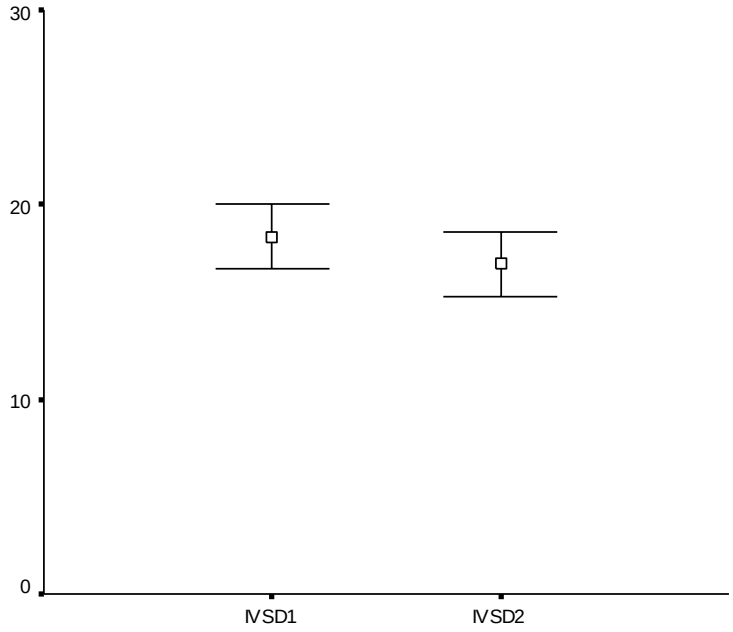


Figure 29 **Posterior Wall Thickness at Baseline and Last Follow up**

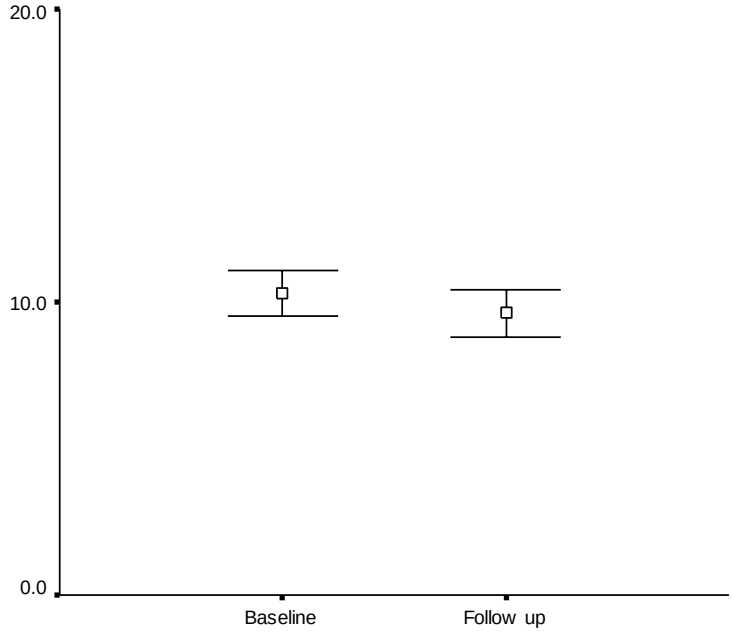


Figure 30 **Maximal Wall Thickness at Baseline and Last Follow up**

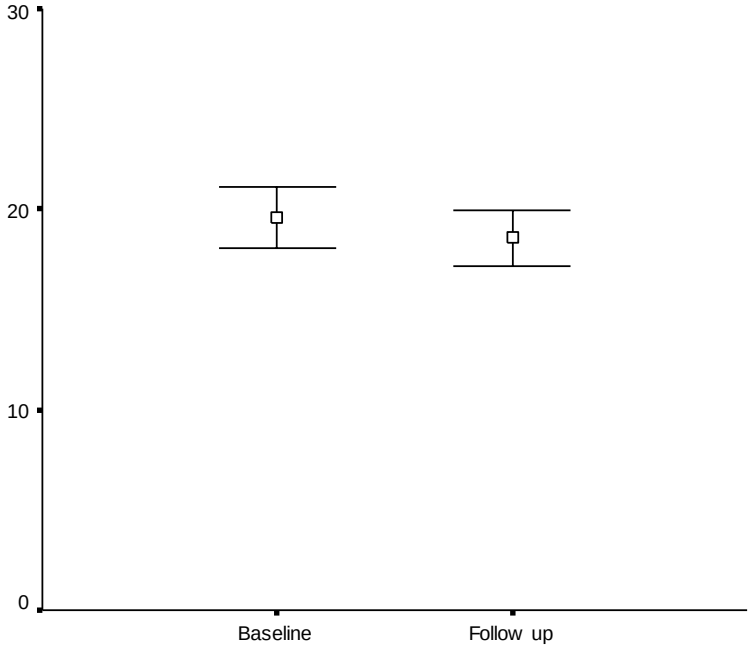
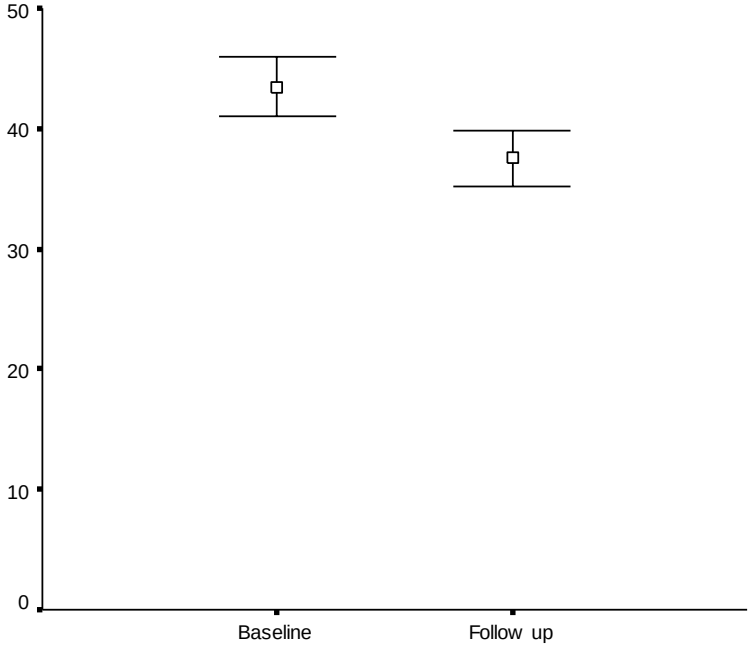


Figure 31 **Fractional Shortening at Baseline and Last Follow up**



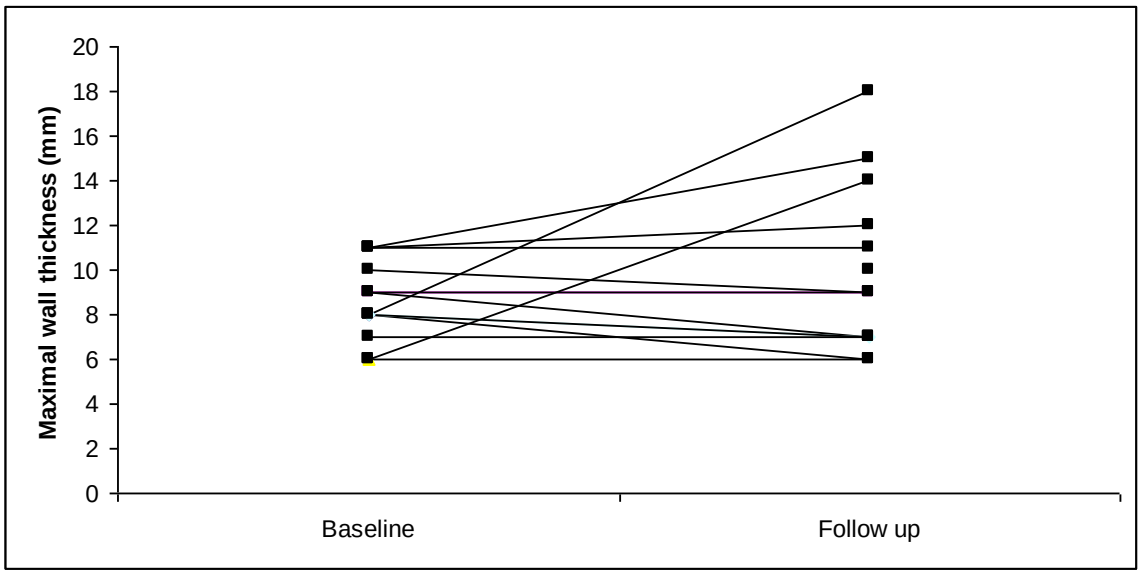
Unaffected Mutation Carriers

17 individuals who were clinically unaffected at baseline were followed up for a mean 9.4 +/- 7.1 years (range 1-18). Mean age at baseline was 25.0 +/- 14.3 years (range 10-53) and 33.1+/- 14.8 years (range 11-66) at second evaluation. Of these 17 individuals 3 (17.6%) developed clinical evidence of disease expression during follow up (Table 27). 2 of the 3 developed evidence of disease expression at ages 13 and 14. The third individual (H610.8) (5.9% of those followed up) was first evaluated at age 39 and found to have a normal ECG and Echo. He came from a large family with a deletion mutation (698delC) with multiple affected family members. He was subsequently diagnosed with HCM during a routine health check age 53 on the basis of T wave inversion in the anterior chest leads and asymmetric septal hypertrophy on transthoracic echocardiography (maximal wall thickness 15mm). He had not been evaluated in the interim period so it is not known at what age the hypertrophy developed. There was no history of hypertension or athletic training and he remains asymptomatic.

Table 27 Clinical Data for 3 Individuals developing LVH during the course of follow up.

Individual	Mutation	Gender	Age 1 (years)	Age 2 (years)	MWT 1 (mm)	MWT 2 (mm)	ECG 1	ECG 2
H610.13	Cys698del	F	13	21	6	14 (eccentric)	Nonpathological Inferolateral Q waves	Partial RBBB, pathological inferolateral Q waves
H62.2	Arg502Gln	F	14	27	8	18 (apical)	NA	TWI V1-V4
H610.8	Cys698del	M	39	53	11	15 (ASH)	NA	TWI V2-V6

Figure 32 **Maximal wall thickness in unaffected mutation carriers at initial evaluation**



Summary Echo data is shown in Table 28. There were no statistically significant differences in Echo derived parameters at baseline and at last follow-up assessment in 15 unaffected mutation carriers, despite three individuals developing hypertrophy during follow up. Changes in maximal wall thickness with time are shown in Figure 32.

Table 28 **Echo parameters in unaffected mutation carriers at initial evaluation**

	1st Assessment	Last Assessment	P value
Age (years)	25.5 +/- 15.2	33.5 +/- 15.5	0.001
LA size (mm)	31.8 +/- 5.1	34.0 +/- 5.0	0.174
LVEDD (mm)	49.5 +/- 5.8	47.3 +/- 5.9	0.272
LVESD (mm)	32.7 +/- 3.7	31.8 +/- 4.4	0.253
FS (%)	32.1 +/- 3.7	31.9 +/- 2.9	0.887
IVSd (mm)	7.8 +/- 1.3	7.3 +/- 1.6	0.214
PWd (mm)	7.7 +/- 1.6	7.5 +/- 1.6	0.723
MWT (mm)	8.6 +/- 1.6	9.7 +/- 3.6	0.262

Discussion

The main findings of this part of the study were as follows:

1. Annual mortality amongst mutation carriers was 0.75% per year
2. Annual sudden death rates were 0.45% per year
3. Left atrial diameter increases with age
4. LV end-systolic diameter increases and fractional shortening decreases with age
5. There is septal thinning but no decrease in maximal wall thickness with age
6. Hypertrophy can develop in adulthood but appears to be an uncommon event

Previous studies have calculated annual complication rates amongst non-genotyped cohorts of patients with HCM (i.e. affected patients only).

- The incidence of stroke is approximately 0.8% [Maron 2002a] – in our study the incidence of stroke amongst affected mutation carriers was 0.94%.
- The incidence of sudden death is approximately 1.02% [Elliott 2006b] – in our study the incidence of sudden death was 0.57%.
- The published incidence of endocarditis is approximately 0.38 [Spirito 1999] – in our study the incidence was 0.19

The observed complication rates in our cohort are therefore similar to published rates for non-genotyped cohorts therefore, which is contrary to previous suggestions that MYBPC3 mutations are associated with a relatively good prognosis [Niimura 1998][Charron 1998a][Charron 1998b][Richard 2003]. The event rates in this study are

low however and the actual complication rates may be significantly different if the numbers studied were greater.

Our study identified one patient who developed echocardiographic LVH during follow up. While this phenomenon has been described before [Maron 2001][Nagueh 2003], it appears to be an uncommon event and may not justify the current recommendations for periodic lifelong screening of unaffected adult relatives [Maron 2003a]. Large longitudinal follow-up studies are required in unaffected mutation carriers to better understand the frequency of hypertrophy development in adulthood. This study has now identified a cohort of unaffected mutation carriers who will continue to be followed up long-term and hopefully provide an insight into this important aspect of HCM epidemiology.

CONCLUSIONS

Conclusions

Over the last 50 years since Donald Teare's early description [Teare 1958] our understanding of hypertrophic cardiomyopathy has increased significantly. Modern techniques of DNA analysis and advanced cardiac imaging have enhanced our knowledge of this fascinating condition and the great complexities of this condition have become apparent. While elements of clinical management are fluid, with imaging and therapeutic techniques coming in and out of fashion, certain 'established facts' regarding HCM have been established. One such 'fact' is the role of sarcomeric proteins in disease pathogenesis, and mutations in the gene encoding cardiac myosin binding protein-C are of central importance. Studying genotyped cohorts therefore provides the opportunity to gain further insights into the condition.

In this thesis the largest cohort of HCM index cases with MYBPC3 mutations, and their families, have been described. Several important themes have emerged:

- Complex genetic status is common which has major implications for mutation detection strategies and counselling families.
- The spectrum of clinical disease is broad and even with individuals sharing a specific mutation, implying that disease modifying factors influence disease development and severity to a powerful degree.
- Disease penetrance is incomplete and may be related to gender suggesting an area for further study

- Clinical disease may present at any stage in life indicating that HCM should be part of the differential diagnosis in a patient presenting with cardiac symptoms at any age, and should be considered in anyone with affected family members.
- Although age-related penetrance is suggested by cross-sectional data, disease developing de novo is rare during adulthood. Do family screening strategies need to accommodate this apparently rare event? In asymptomatic individuals, serial clinical assessment may be associated with a low yield in detecting new cases of HCM
- Although the overall prognosis for this cohort is good, individual risk is highly variable and clinical management decisions need to reflect this
- Clinically useful markers of early disease expression and genotype prediction is not possible with currently available indices

This study has a number of strengths. This study describes the largest cohort of index cases to date with MYBPC3 mutations. The total number of mutation carriers (including family members) is the second largest to date with only one study [Niimura 1998] including more genotyped individuals. These data therefore add considerably to the body of knowledge available. Detailed clinical characterisation of such a cohort has never been previously performed and the longitudinal data presented are novel. Additionally, the evaluation of markers of early disease expression is in the largest genotyped cohort to date.

Establishing accurate genotype-phenotype correlations requires large numbers of patients, multiple different mutations, and need to be repeated in different patient

populations. The initial descriptions of MYBPC3 mutations, in highly selected families, may have over-emphasised the frequency and clinical relevance of 'late-onset' disease therefore, leading to premature assumptions of late-onset and benign disease. A major strength of this study therefore is that families were unselected and included a broad spectrum of mutations that is representative of MYBPC3 related disease as a whole.

There are a number of limitations to this study however.

Tertiary referral centres inevitably introduce an element of referral bias (such as the over-representation of obstructive HCM at the Mayo Clinic). While this is true, the reputation of the Inherited Cardiovascular Disease Unit at The Heart Hospital, London, and previously at St George's Hospital, London, is not based upon a particular clinical problem such as surgical myomectomy, and the reasons for referral are broad and include a wide range of patients. Indeed the international reputation of the Inherited Cardiovascular Disease Unit indicates that an inclusive demographic cohort is described. Therefore both patients with complex clinical problems, referred for expert opinion, and patients referred routinely for assessment from the local catchment area are represented.

This study lacks a comparative group, or a cohort of patients with mutations in another sarcomeric protein gene. It would clearly be advantageous to directly compare clinical indices between cohorts of patients with other sarcomeric protein mutations, but detailed clinical evaluation of such cohorts is beyond the scope of this study. This study is

therefore limited to comparisons with previously published data regarding MYBPC3 mutations and unselected cohorts of HCM index cases.

A further limitation was that due to limited clinical resources we were unable to fully clinically evaluate unaffected family members with ambulatory ECG monitoring and metabolic exercise testing. While there is not a strong clinical indiciation to evaluate unaffected family members in this way, having a cohort of unaffected mutation carriers provides a unique opportunity to fully investigate the phenotype of HCM. Unfortunately it was not possible to perform ambulatory ECG monitoring and metabolic exercise testing in all patients due to limited clinical availability.

What might the future hold?

The role of single gene genotype-phenotype studies is probably coming to an end [Ackermann 2005]. To data studies have been limited by small family size, a low frequency of each specific mutation and inability to assess and control other disease modifying factors [Marian 2001]. More sophisticated models of disease that include a monogenic mutation with other genetic and environmental variables are required. The Human Genome Project allows a new era of genetic evaluation to begin with single nucleotide polymorphisms (SNPs) and their effect on monogenic disorders to be investigated [Marian 2002]. Studying families sharing an identical mutation provides a good opportunity to study candidate variables [Marian 2002] and controlling mutation specific variation by studying founder mutations is an attractive direction for future studies. Large longitudinal cohort studies of genotyped families are required to determine

the frequency of adult-onset disease and gain insights into the natural history of hypertrophic cardiomyopathy. Given the limitations of individual centres, establishing international databases and a collaborative approach is desirable [Arbustini 2002].

Although classifying, and establishing diagnostic criteria for HCM has proved problematic [McKenna 1997][Richardson 1996][Maron 2003a][Maron 2006][, redefining HCM in the genetic era will be required to include mutation carriers not fulfilling diagnostic criteria – whether these individuals are at risk of sudden complication remains unclear, but even if this is the case this risk is probably not great.

The significant body of literature published to date has probably raised as many questions as it has answered, but with careful systematic clinical and genetic evaluation our knowledge can only be enhanced. It is hoped that the data presented in this thesis contributes, in a small part, to this body of knowledge.

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APPENDIX

The appendix contains the following:

1. Information sheet provided for relatives prior to obtaining informed consent.
2. Informed consent sheets used for aquiring blood samples for genetic analysis in relatives.

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Hypertrophic Cardiomyopathy -

An information sheet for relatives

What is Hypertrophic Cardiomyopathy?
People who are affected by hypertrophic cardiomyopathy (HCM) often have thickening of the heart muscle. Some do not have symptoms of disease while others may experience episodes of breathlessness, chest pain, and palpitations or have attacks of dizziness or fainting.

HCM is hereditary (inherited), and is therefore likely to run in families. In recent years changes in certain genes have been found to cause HCM. These genes make proteins in the sarcomere. Sarcomeres are fibres of the muscle that make the heart contract to pump blood around the body. Someone who carries a change in one of these genes has a 50% risk of passing the disease onto each of their children. Fortunately, a substantial number of the individuals carrying the gene change are healthy and have no major symptoms from the disease.

Why am I giving blood?
A relative of yours has HCM and has previously seen Professor McKenna in London. Genetic investigations showed a change in one of the genes causing HCM. Based on the nature of the specific change found we assume that this change is the disease causing agent within your family. There is a chance that you also have this change and by giving a blood sample we can test for this change. This will help us decide whether you have HCM or not.

What other tests do I need?
We would also like you to have a simple heart trace (ECG) and a heart scan (Echo). These tests are to look for evidence of heart muscle thickening that is part of the disease.

What happens next?
The results for the genetic test will take several months to arrive. When we have the results, we can compare your result with the result of the ECG and Echo scan. We will base any decision making upon your ECG and Echo scan, but will let you know your genetic result which may help us to understand the condition in your family better.

If you have any questions, please do not hesitate to get in touch.

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Consent form for Genetic Testing

Purpose(s) of test:

☐ To discover if the patient carries a gene alteration
☑ To enable relatives to benefit from genetic testing
☐ To clarify the implications of a previous test result
☐ To help make a diagnosis
☐ Other:_____

Blood sample to be analysed for: _____

Patient Statement: **(Please delete where appropriate)**

I agree/do not agree to the test on this form

I understand that

- This test is voluntary and I can withdraw from the testing process at any time.
- In the future my genetic test results may affect the ability of me or my family members to obtain some types of insurance.
- The test result will not be revealed or made available to any other person/organisation, except with my consent or when disclosure is required by law.
- The sample may be sent to another laboratory outside UCL/UCLH for testing. If testing is currently unavailable, the sample will be stored in UCL/UCLH for possible future use.
- The sample will be transported and stored in good faith but we cannot guarantee its suitability for testing.
- My sample will be disposed of in accordance with standard laboratory practices/regulatory requirements.

I give/do not give permission for my blood sample to be tested in the future if new genes are found to be associated with_____(Insert name of condition)

I give/do not give permission for my blood sample to be used in the future to benefit my family members.

Name of Patient	Date	Signature
Name of Doctor/Counsellor/Nurse	Date	Signature

Next of kin:

Name	Relationship	Telephone

1 copy for patient; 1 copy for hospital notes