Hypertrophic Cardiomyopathy in Northern Sweden
With special emphasis on molecular genetics

Stellan Mörner

Umeå 2004
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# ABBREVIATIONS

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<td>ACTC</td>
<td>cardiac α-actin gene</td>
</tr>
<tr>
<td>ANS</td>
<td>autonomic nervous system</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
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<tr>
<td>DHPLC</td>
<td>denaturing high performance liquid chromatography</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DTI</td>
<td>Doppler tissue imaging</td>
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<tr>
<td>ECG</td>
<td>electrocardiogram</td>
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<tr>
<td>EF</td>
<td>ejection fraction</td>
</tr>
<tr>
<td>ET</td>
<td>ejection time</td>
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<tr>
<td>FAP</td>
<td>familial amyloid polyneuropathy</td>
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<td>HCM</td>
<td>hypertrophic cardiomyopathy</td>
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<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>HRV</td>
<td>heart rate variability</td>
</tr>
<tr>
<td>ICT</td>
<td>isovolumic contraction time</td>
</tr>
<tr>
<td>IRT</td>
<td>isovolumic relaxation time</td>
</tr>
<tr>
<td>ICD</td>
<td>implantable cardioverter defibrillator</td>
</tr>
<tr>
<td>IVSD</td>
<td>interventricular septum dimension in end diastole</td>
</tr>
<tr>
<td>LA</td>
<td>left atrium</td>
</tr>
<tr>
<td>LV</td>
<td>left ventricular</td>
</tr>
<tr>
<td>LVEDD</td>
<td>left ventricular end diastolic diameter</td>
</tr>
<tr>
<td>LVESD</td>
<td>left ventricular end systolic diameter</td>
</tr>
<tr>
<td>LVIH</td>
<td>left ventricular hypertrophy</td>
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<tr>
<td>LVOT</td>
<td>left ventricular outflow tract</td>
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<tr>
<td>LVPWD</td>
<td>left ventricular posterior wall dimension in end diastole</td>
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<tr>
<td>MYBPC3</td>
<td>cardiac myosin binding protein C gene</td>
</tr>
<tr>
<td>MYH7</td>
<td>cardiac β-myosin heavy chain gene</td>
</tr>
<tr>
<td>MYL2</td>
<td>cardiac regulatory myosin light chain gene</td>
</tr>
<tr>
<td>MYL3</td>
<td>cardiac essential myosin light chain gene</td>
</tr>
<tr>
<td>NSVT</td>
<td>non-sustained ventricular tachycardia</td>
</tr>
<tr>
<td>PCG</td>
<td>phonocardiogram</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PRKAG2</td>
<td>protein kinase A (γ2 subunit) gene</td>
</tr>
<tr>
<td>RV</td>
<td>right ventricular</td>
</tr>
<tr>
<td>RVWT</td>
<td>right ventricular wall thickness</td>
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<tr>
<td>SAM</td>
<td>systolic anterior motion</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
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<tr>
<td>SCD</td>
<td>sudden cardiac death</td>
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<tr>
<td>SSCP</td>
<td>single-stranded conformation polymorphism</td>
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<tr>
<td>TAPSE</td>
<td>tricuspid annular plane systolic excursion</td>
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<tr>
<td>TNNC1</td>
<td>cardiac troponin C gene</td>
</tr>
<tr>
<td>TNNI3</td>
<td>cardiac troponin I gene</td>
</tr>
<tr>
<td>TNNT2</td>
<td>cardiac troponin T gene</td>
</tr>
<tr>
<td>TPM1</td>
<td>α-tropomyosin gene</td>
</tr>
<tr>
<td>TTN</td>
<td>titin gene</td>
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<tr>
<td>TTR</td>
<td>transthyretin gene</td>
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ABSTRACT

Hypertrophic cardiomyopathy (HCM) is a heterogeneous, often familial disease, characterized by cardiac hypertrophy, predominantly affecting the interventricular septum. To date, no study has systematically analysed the genetic and phenotypic aspects of the disease in a Swedish population. The aim of this thesis was to identify the genotypes causing HCM in northern Sweden, to characterize the disease phenotypes and correlate these findings.

Forty-six patients were recruited for the genetic studies (21 women), 11 familial and 35 sporadic cases. Eight sarcomeric protein genes were screened for mutations. A total of 11 different disease causing mutations were found in four genes. Six of the mutations were previously not described. A novel mutation (a 33 base pair deletion) in the troponin I gene was found in one HCM family. Despite the severe genetic defect, the associated phenotype displayed only mild cardiac hypertrophy and few symptoms. Most mutations (64%) were identified in the myosin binding protein C gene, a gene considered to have a low penetrance. Mutations were identified in 10 of 11 familial HCM cases, but only in three of the 35 sporadic cases.

It was found that cardiac amyloidosis can sometimes present itself as HCM. Three HCM patients (7%) carried the ATTR Val30Met mutation, also found in Swedish patients with familial amyloid polyneuropathy (FAP). The patients had no symptoms of polyneuropathy, but cardiac amyloidosis as the cause of hypertrophy was verified by myocardial biopsy in an index case. Amyloid heart disease should therefore be considered as a differential diagnosis in patients with HCM.

By studying heart rate variability (HRV), it was found that young patients with HCM had signs of autonomic dysfunction, expressed as a reduced HRV. Treatment with beta-blockade attenuated these effects. Abnormal autonomic function might be a substrate for lethal arrhythmias, most often encountered in younger patients with HCM. The results suggest a possible protective effect of beta-blockade, remaining to be studied further.

Ventricular function is frequently abnormal in HCM. In particular, diastolic dysfunction has been demonstrated. The recently described myocardial performance index allows the assessment of cardiac function by combining systolic and diastolic performance. We found that patients with hypertrophic cardiomyopathy had evidence of global and regional right ventricular dysfunction, besides left ventricular dysfunction. Hypertrophic
cardiomyopathy is traditionally considered to be a disease of the left ventricle. The results show that hypertrophic cardiomyopathy should more be regarded as a biventricular disease.

In conclusion, the myosin binding protein C gene is the most common gene causing familial HCM in northern Sweden. This disease gene is considered to be associated with a mild, late-onset disease with \( \approx 50\% \) penetrance at 30 years of age. The low disease penetrance emphasizes the importance of adequate family screening when evaluating patients with HCM, since the familial nature of the disease might easily be overlooked. These particular disease features in northern Sweden contrast to most previous reports, which indicate another disease gene as the most frequent in HCM, associated with a much higher penetrance. Amyloid heart disease, requiring different treatment than HCM, should be kept in mind as a differential diagnosis in the management of patients with HCM.
SAMMANFATTNING PÅ SVENSKA

Hypertrofisk kardiomyopati är en hjärtsjukdom som leder till förtjockning av muskelväggarna i hjärtat. Framförallt brukar mellanskiljeväggen mellan vänster och höger kammare förtjockas. I ca 55% av fallen är sjukdomen ärflig, resten av fallen anses vara sporadiska. Hypertrofisk kardiomyopati har tidigare betraktats som mycket ovanlig, men senare undersökningar har visat att den kan förekomma i ett fall per 500 till 1000 invånare. Sjukdomsförföppet är mycket varierande, från plötslig död i unga år, till en normal livstid utan några som helst symtom. Ibland upptäcks sjukdomen av en slump när hjärtat undersöks av andra skäl, t.ex. hos idrottss gymnast.

Hypertrofisk kardiomyopati kan ge symptom i form av andfåddhet, kärlkramp, hjärtklappning, simningar och i värsta fall, plötslig död. Våra kunskaper om sjukdomen är begränsade och någon systematisk undersökning av de bakomliggande genetiska orsakerna har tidigare inte gjorts i Sverige.

I detta arbete har vi systematiskt studerat patienter i den norra sjukvårdsregionen i Sverige. Förutom läkarundersökning, ultraljudsundersökning av hjärtan samt olika EKG-undersökningar, har även genetiska analyser utförts för att bestämma vilka gener som orsakar sjukdomen. Dessa resultat har sedan sammanställts i fem olika delarbete.

I delarbete I studerades en familj med ärflig hypertrofisk kardiomyopati orsakad av en ovanlig mutation (genetisk avvikelse). Mutationen medför ett borttagande av 33 st DNA-baser i genen för troponin I, en av de kända sjukdomsgenerna. Trots den allvarliga gendefekten, visade sig sjukdomen vara tämligen mild, vilket tyder på att effekten på proteinets totala funktion var begränsad.

I delarbete II redovisas de olika sjukdomsgenerna i norra Sverige. Majoriteten av mutationerna (64%) hittas i en enda gen, myosinbindande protein C (MYBPC3), som ger ett senare insjuknande och en mindre allvarlig sjukdomsbild jämfört med vissa andra sjukdomsgener. Av detta kan man förvänta sig att en stor del av patienterna kommer att diagnostiseras först i medelåldern. Den sena sjukdomsdebuten kan också göra att ärfligheten lätt förbises. I familjer där gendefekten är känd, har genetisk vägledning nu blivit möjlig.

I delarbete III studerades hjärtfrekvensvariabilitet (variationer i frekvensen med vilket hjärtat slår). Denna styrs bl.a. från det autonoma (icke viljestyrda) nervsystemet. Sänkt hjärtfrekvensvariabilitet kan medföra risk för hjärtrytmrubningar, ibland med dödlig
utgång. Patienter med hypertrofisk kardiomyopati har en sänkt hjärtfrekvensvariabilitet, medan patienter som behandlades med en viss typ av hjärtmedicin, s.k. betablockerare hade mindre uttalade rubbningar, vilket antyder att dessa kan ha en skyddande effekt.


Sammanfattningsvis: Ärfiltig hypertrofisk kardiomyopati i norra Sverige orsakas till största delen av mutationer i en gen, myosinbindande protein C, som ger ett senare insjuknande (≈50% penetrans vid 30 års ålder) och en mindre allvarlig sjukdomsbild än vad som tidigare ansetts typiskt för denna sjukdom. Sjukdomens ärfiltighet kan lätt förbisas och man bör därför även undersöka patientens närmaste släktingar. Hjärtamyloidos kan ibland ge en klinisk bild liknande hypertrofisk kardiomyopati, vilket man bör ha i åtanke vid utredningen av dessa patienter.
Original papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


INTRODUCTION

The first case description of hypertrophic cardiomyopathy appeared already in the middle of the 19th century at the Hôpital de la Salpêtrière in Paris. In 1868, Alfred Vulpian described a case with "rétrécissement de l'orifice ventriculo-aortique" or "sub-aortic stricture" found at post mortem examination (1). There are a few additional early observations (2-4), but it was not until 1958 that hypertrophic cardiomyopathy was established as a diagnostic entity, following the reports of Brock, who described a "functional obstruction of the left ventricle" (5) and Teare, who described "asymmetrical hypertrophy of the heart in young adults" (6). The diagnosis was based on the presence of a primary, unexplained left ventricular hypertrophy.

Our changing conceptions of the disease have been strongly influenced by the diagnostic tools available at the time. The early descriptions of the disease were anatomic and consisted of the unanticipated finding of unexplained cardiac hypertrophy at autopsy, in young individuals who had died suddenly. In the early 1960s, invasive hemodynamic investigations focused attention on the unique pathophysiology of dynamic left ventricular (LV) outflow tract obstruction, in contrast to the more fixed obstruction to blood flow encountered in patients with valvular aortic stenosis (7). These studies also gave popularity to the acronym idiopathic hypertrophic subaortic stenosis (IHSS), one of the no fewer than 58 names under which this condition has been known (8).

In the early 1970s, studies with M-mode echocardiography refocused attention on the morphologic aspects of the disease, identifying asymmetric septal hypertrophy (ASH) as the pathognomonic anatomic abnormality (9), reconfirming the early autopsy findings. With the advent of two-dimensional echocardiography, it became apparent that many patients had myocardial hypertrophy confined to segments of the LV not detectable by M-mode echocardiography, giving clinical recognition to the morphologic diversity of the disease (10, 11).

The term hypertrophic cardiomyopathy (HCM) now becomes the preferred name for the disease, since it describes the overall disease spectrum without introducing misleading inferences that LV outflow tract obstruction is an invariable disease feature. Screening of larger populations, including relatives of index patients, showed that HCM was familial in approximately 55% of the cases, with predominantly autosomal dominant mode of inheritance (12). It was also shown that the LV outflow tract obstruction, the hallmark of
the disease in the invasive studies, was only present in a minority of patients, when large populations were investigated with two-dimensional echocardiography (12, 13).

A new era was entered 1989, when advances in molecular genetics allowed the use of linkage analysis to establish linkage of the disease to a locus on chromosome 14q1 in a large French-Canadian family (14). The results were confirmed in several families from North America (15) and eventually the cardiac β-myosin heavy chain gene (MYH7) was identified as the disease causing gene (16). Having reached this point, the observations first made by Vulpian in 1868, could now finally be explained at the molecular genetics level. In the following sections, a more comprehensive background will be given and some of the current understanding of HCM will be reviewed.
BACKGROUND AND CURRENT KNOWLEDGE

Nomenclature
In a report of the World Health Organization/International Society and Federation of Cardiology Task Force from 1980, cardiomyopathies were defined as "heart muscle diseases of unknown origin" and were differentiated from specific heart muscle disease of known cause (17). With increasing understanding of etiology and pathogenesis, the difference between cardiomyopathy and specific heart muscle disease has become indistinct. Cardiomyopathies are now defined as diseases of the myocardium associated with cardiac dysfunction and classified by the dominant pathophysiology or, if possible, by etiological/pathogenetic factors (18). They are classified as dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy.

Diagnosis
Hypertrophic cardiomyopathy is diagnosed by the presence of left ventricular hypertrophy (LVH), usually demonstrated by echocardiography, in the absence of other cardiac or systemic disease, such as hypertension or valvular heart disease, capable of producing the magnitude of hypertrophy evident (18). There is often predominant involvement of the interventricular septum, although any pattern of diffuse or segmental wall thickening may be present, including hypertrophy of the right ventricle. The LV cavity is non-dilated with normal or hyperdynamic global systolic function.

The usual clinical diagnostic criterion for HCM is a maximal LV wall thickness of ≥15 mm, but genotype-phenotype correlations have shown that any wall thickness, including those within normal range (≤12 mm), are compatible with the presence of a mutant gene. Therefore, modified major and minor diagnostic criteria have been proposed in familial HCM, by McKenna et al and are summarized in Table 1 (19). They are intended to be applied only to relatives of patients with known familial HCM, where the relative has a 50% likelihood of carrying the disease causing mutation. Based on these criteria, the diagnosis of hypertrophic cardiomyopathy in a first degree adult relative would be fulfilled by the presence of; a) One major criterion, or b) Two minor echocardiographic criteria, or c) One minor echocardiographic plus two minor electrocardiographic criteria.
Table 1. Diagnostic criteria in familial HCM

<table>
<thead>
<tr>
<th>Major criteria</th>
<th>Minor criteria</th>
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<tr>
<td><strong>Echocardiography</strong></td>
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<tr>
<td>Left ventricular wall thickness ≥13 mm in the anterior septum or posterior wall or ≥15 mm in the posterior septum or free wall</td>
<td>Left ventricular wall thickness 12 mm in the anterior septum or posterior wall or 14 mm in the posterior septum or free wall</td>
</tr>
<tr>
<td>Severe SAM (septal-leaflet contact)</td>
<td>Moderate SAM (no septal-leaflet contact)</td>
</tr>
<tr>
<td>Redundant MV leaflets</td>
<td></td>
</tr>
<tr>
<td><strong>Electrocardiography</strong></td>
<td></td>
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<tr>
<td>LVH + repolarisation changes (Romhilt-Estes) (20)</td>
<td>Complete BBB or (minor) interventricular conduction defect (in LV leads)</td>
</tr>
<tr>
<td>T wave inversions in leads I and aVL (≥3mm) (with QRS-T axis difference ≥30°), V3-V6 (≥3mm) or II, III and aVF (≥5mm)</td>
<td>Minor repolarisation changes in LV leads</td>
</tr>
<tr>
<td>Abnormal Q (&gt;40ms or &gt;25% of R wave in at least 2 leads from II, III, aVF (in absence of left anterior hemiblock), V1-V4; or I, aVL, V5-V6</td>
<td>Deep S in V2 (&gt;25 mm)</td>
</tr>
<tr>
<td>Unexplained chest pain, dyspnoea or syncope</td>
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</table>

SAM – systolic anterior motion (of the mitral valve); MV – mitral valve; BBB – bundle branch block; LV – left ventricular; LVH – Left ventricular hypertrophy. From McKenna et al, Heart 1997;77(2):130-2, reprinted with permission from the BMJ Publishing Group.

Thus, in the context of familial disease, a left ventricular wall thickness of ≥13 mm or major ECG abnormalities are considered sufficient for diagnosis. One must keep in mind that the first index case in a family (and all sporadic cases) must meet the traditional criterion of ≥15 mm wall thickness.

**Prevalence**

Hypertrophic cardiomyopathy was previously considered to be uncommon, although the frequency in the population was largely unknown. Most studies that have attempted to define the epidemiologic features of HCM, have been based on data from selected population subgroups, such as patients referred to tertiary medical institutions (21), cases identified at autopsy (21, 22) or identification through voluntary participation in industrial health screening program (23). In a study in western Denmark, the crude annual incidence of HCM was estimated by autopsy at 0.4/100 000 (21). In Iceland, the estimated
prevalence by autopsy was 33/100 000. In this study the authors assumed an annual case fatality for HCM of 3.5% (22). In a Japanese study, 12 841 adult workers were surveyed with ECG and subsequently with echocardiography in a subset of 12%, with a suggested HCM prevalence of 0.17% (22/12 841). Nine of the 22 affected subjects in this study also had arterial hypertension and were counted as a subset of HCM with hypertension. In a population-based study in Olmsted County, Minnesota, the incidence and prevalence of HCM in the entire population was estimated to be 2.5/100 000 person-years and 19.7/100 000, respectively (24). The figures were obtained by reviewing the complete medical records of all patients diagnosed in a 10-year period, which was possible since diagnosis codes from virtually every health care provider in the community was entered into one central data bank at the Mayo Clinic.

Only one study has estimated the prevalence of HCM defined by echocardiography in a large cohort of apparently healthy young adults. A total of 4111 individuals (25 to 35 years old) were selected from a community-based general population in four urban areas in the United States (25). The prevalence was found to be 0.17%, seven of the study subjects were found to have a ventricular septal thickness between 15 and 21 mm. Six of the cases had not been suspected of having any cardiovascular disease and were free of important symptoms. This study indicates that recognition of HCM by morphological criteria in the general population yields a prevalence up to 10 times greater than prior estimates based on symptomatic presentation, as in the Olmsted study.
Pathophysiology

Macroscopic anatomy
Structural heterogeneity in HCM is considerable, with no single typical pattern of LV hypertrophy. At least two-thirds of the patients are described to have an asymmetric distribution of myocardial hypertrophy that affects the interventricular septum more than the free walls of the left ventricle. In the remainder of the patients, the hypertrophy is concentric or affects only a small segment in the left ventricle. Right ventricular (RV) involvement is present in at least 30% of the cases. Apical hypertrophy is uncommon, except in Japanese and Chinese patients (26, 27).
In an extensive echocardiographic study of 600 HCM patients (7 to 79 years old), it was shown that the anterior part of the septum was the most frequently hypertrophied single segment of the left ventricle (96% of the cases) and was also the segment with most pronounced hypertrophy in 83% of the cases (28). Abnormalities of the mitral valve itself are also frequently found, with elongation of one or both leaflets or anomalous insertion of the papillary muscles into the main body of the leaflet (29, 30).

Microscopic anatomy
At the histological level, HCM is characterized by myocyte disarray (31, 32), interstitial fibrosis (33) and abnormalities of the intramyocardial small vessels (34, 35).

Disarray
Myocyte disarray comprises; hypertrophy of myocytes and increased variations in their size and shape, abnormally arranged myocytes, often at oblique angles, with multiple intercellular junctions, causing a disruption of the LV myocardial architecture (Figure 1). Myocyte disarray is not a unique finding in HCM, it can also be present in other cardiac conditions, such as coronary or valvular heart disease and arterial hypertension, although to a lesser extent (usually <5% of the myocardium). It is probable that disarray can serve as a substrate for arrhythmias.
In HCM, disarray may be widely distributed, often in a patchy manner, such that severely disarrayed myocytes can lie adjacent to normally sized and normally aligned myocytes. In average, disarray is present in 28-35% of the LV wall (32, 36). It is interesting to note that
disarray is present in all parts of the left ventricle and also in the right ventricle (36), suggesting that HCM is a biventricular disease and not confined to (a limited part of) the left ventricle. It has been shown that the degree of disarray has clinical impact, since it is more extensive in young patients with fatal outcome of the disease (32, 37). This is particularly true for patients with mutations in the troponin T gene, who are at high risk of premature death and where severe myocyte disarray is present, in spite of the relatively mild hypertrophy associated with this particular disease gene (38).

Small vessel disease
Patients with HCM may exhibit symptoms consistent with ischemia in the absence of coronary atherosclerosis (39, 40). Functional studies on myocardial perfusion using positron-emission tomography have demonstrated reduced coronary vasodilator reserve, a feature that recently was found to be associated with poor outcome (41). Abnormal intramural coronary arteries may partly be responsible for this impairment of coronary reserve (40, 42, 43).

These arteries are characterized by thickening of the vessel wall and a decrease in luminal size, due to proliferation of medial and/or intimal components, particularly smooth muscle cells and collagen. This can be regarded as a form of small vessel disease (34, 35) and has been identified very early in the disease process, often seen already in children and infants affected with HCM (34, 36). There is not only a reduction in the lumen of small vessels, arteriolar density is also reduced (43) as well as capillary density (44) in the myocardium of patients with HCM. It has previously been reported that small vessel abnormalities are particularly prominent in areas of marked fibrosis (34) and suggested that these small arteries, by compromising blood flow, trigger ischemia leading to cell death and replacement by fibrosis, causing scarring of the myocardium. In contrast, more recent studies have failed to demonstrate the relation between fibrosis and small vessel disease (36, 37). The extent of fibrosis has been correlated to the occurrence of non-sustained ventricular tachycardia (37). Thus, it seems probable that ischemia, myocardial disarray and fibrosis serve as substrates for life-threatening arrhythmias.
Left ventricular systolic function
In a large proportion of patients with HCM, the left ventricle appears to be hypercontractile, as indicated by an increased ejection fraction (45, 46). However, the increase in ventricular wall thickness results in a decrease in wall stress during systole, compatible with a normal or even decreased contractility (dP/dt). It has been proposed that a decrease in afterload explains the high ejection fraction in the presence of a normal or decreased contractility and the left ventricle is thus better described as hyperdynamic than truly hypercontractile (46, 47).

Regional systolic function may still be abnormal, particularly in the septum, which often displays reduced circumferential and longitudinal shortening (48). Assessment of systolic function by measuring the atrioventricular plane displacement will lead to underestimation of ejection fraction (EF) in patients with cardiac hypertrophy (48). Late in the disease process, a small subset of patients (~5%) develop a progressive dilatation with systolic dysfunction and wall thinning, the so-called “end-stage” or “dilated” phase of HCM. Drug treatment in this phase includes ACE inhibitors, beta-blockers, diuretics, digoxin and spironolactone, much like the treatment of systolic heart failure of other causes. These patients may eventually become candidates for heart transplantation.

Left ventricular diastolic function
Hypertrophic cardiomyopathy is sometimes considered as a “model disease” for diastolic dysfunction. Exertional dyspnoea often occurs in the presence of preserved systolic LV function and is usually attributed to mainly a diastolic dysfunction (49-51). Diastolic LV filling is impaired due to abnormal dilatation and reduced compliance (increased chamber stiffness), leading to increased atrial and LV end-diastolic pressures, pulmonary congestion and impaired exercise capacity with reduced oxygen consumption at peak exercise (52-55).

Abnormal LV relaxation is caused by myocyte disarray, myocardial ischemia, abnormal intracellular calcium flux and nonuniformity in the regional ventricular function. Passive diastolic properties (compliance) are altered as a consequence of hypertrophy and increased interstitial fibrosis. Invasive studies have shown an increase in LV end-diastolic pressures and impaired filling (56, 57). Noninvasive assessment by echocardiography, radionuclide angiography and magnetic resonance imaging have shown a wide variety of
diastolic abnormalities, including prolonged isovolumic relaxation time (IRT) and time to peak early filling. Reduced early filling and increased atrial contribution to the transmitral flow has also been described, although not by all authors (51, 58-61). No single pattern of diastolic filling is typical in HCM. The transmitral filling velocities have often been used to assess LV diastolic function, but overall, there has been a failure to show significant correlation between the flow profiles and important clinical parameters like LVH, symptoms, exercise capacity or mean left atrial pressure in patients with HCM (50, 59, 62-64).

In patients with HCM, conventional Doppler estimates are unreliable, probably because of their dependence on loading conditions. The clinical usefulness is therefore limited and attempts have been made to find other markers of diastolic function, less dependent on the loading conditions in the heart.

**Left ventricular outflow obstruction**

It is essential to distinguish between the obstructive and nonobstructive forms of HCM, since important clinical decisions depend on the presence or absence of outflow obstruction. Approximately 25% of the patients have an LV outflow tract gradient during resting conditions (12, 13), which is nearly always associated with systolic anterior motion (SAM) (Figure 2). A harsh systolic ejection murmur is often heard at the lower left sternal border and apex. Obstruction may be located in the LV outflow tract or, less commonly, in the midventricular region when excessive midventricular hypertrophy causes muscular apposition in systole.

The clinical significance of LV outflow obstruction has been a subject of controversy for many years. The hypothesis that the pressure gradient reflects a true mechanical impedance to outflow has been advocated by some authors (7, 49), questioned by others (65-67) and its prognostic importance has been uncertain. Those who object to equating the subaortic pressure gradient with a “true” obstruction argue that the gradient is an effect of a rapid and complete emptying (cavity obliteration) of the hyperdynamic ventricle, with elevated intraventricular pressures being created by continued isometric contraction of the virtually empty ventricle (65). The same authors point out that this mechanism is not comparable with the high pressures that can be seen when the catheter
is embedded in the myocardium, creating false, artefactual pressure waves ("catheter entrapment").

By most authors, it is now believed that the outflow pressure gradient does represent true obstruction to LV ejection (7, 13, 49, 68), based on the following observations: a) The subaortic gradient develops simultaneously with SAM (septal contact). b) The severity of the gradient correlates with the onset and duration of SAM. c) Aortic blood flow shows a rapid midsystolic deceleration at the time of SAM (septal contact). d) LV ejection continues after the development of the pressure gradient, with a significant amount of blood being ejected in the presence of subaortic obstruction.

The dynamic nature of the LV obstruction is characteristic for HCM, in some cases highly labile, with no gradient at rest, which may develop rapidly with provocation in the laboratory setting. The gradient has been found to vary considerably from day to day when measured repeatedly in stable patients and may also respond to physiological alterations such as food intake and ingestion of small amounts of alcohol (69, 70).

**Right ventricular function**

Much less is known about the RV systolic and diastolic function, compared to the extensive knowledge of the left ventricle. Systolic function has previously been shown to be normal. Two studies have assessed the tricuspid annular plane systolic excursion (TAPSE), a noninvasive index of systolic RV function, and found normal values in HCM (71, 72). One invasive study has shown preserved RV systolic function with increased systolic pressure, contractility (dP/dt) and ejection fraction in patients with HCM. Relaxation, however, was clearly impaired with prolonged time to peak filling rate and prolonged time constant of isovolumic pressure decay. Right ventricular end diastolic pressures were significantly elevated and pressure-volume relations were shifted upwards, compared to controls, implying decreased diastolic compliance (73). Using conventional Doppler, other authors have also demonstrated diastolic abnormalities, including a reduced transtricuspid E/A-ratio and deceleration time, as well as prolonged IRT (72, 74).

**Cardiac autonomic nervous system**

Parasympathetic supply to the heart runs in the vagus nerves, providing rich innervation to the sino-atrial node, atrioventricular conducting pathways and atrial myocardium. The
question as to whether there is a substantial vagal efferent supply to the mammalian ventricular muscle remains controversial (75). Activity in the efferent vagal nerves slows the heart rate, which is achieved by hyperpolarising the pacemaker cells and slowing their rate of spontaneous depolarisation.

Sympathetic innervation supplies all regions of the heart; pacemaker and conducting tissue, as well as atrial and ventricular myocardium. Increased sympathetic activity increases heart rate by increasing the rate of depolarisation of pacemaker cells. The cardiac chambers are richly innervated with afferent fibres from both parasympathetic and sympathetic divisions. The heart is thus able to sense both the extent of its filling and the pressure that it generates and can initiate appropriate regulatory responses. The afferent innervation of the ventricles is almost entirely by non-myelinated nerves and most of these supply the left ventricle (76).

Mechanosensitive ventricular receptors may be excited by increases in ventricular pressure (particularly end diastolic pressure) or mechanical stimulation, resulting in reflex depressor responses (77). Discharges from ventricular afferents are also be influenced by the cardiac inotropic state (78) (79).

There is less knowledge about the effects of sympathetic afferents from the heart. They are believed to mediate cardiac pain and the reflex responses probably include increases in heart rate and blood pressure (77).

**Possible role of autonomic nervous system in pathogenesis**

It has been hypothesized that the autonomic nervous system (ANS) may play a role in the development of HCM, with increased sympathetic activity in the heart. A downregulation of myocardial beta adrenoreceptors has previously been observed in these patients (80), probably due to locally increased levels of noradrenaline (81), and was shown to be associated with a reduced catecholamine reuptake by myocardial sympathetic nerve terminals (82). Unlike patients with congestive heart failure, no significant increase in circulating catecholamines has been found in patients with HCM (80, 82), which further supports the idea that locally increased neurotransmitter concentrations are important in the downregulation of myocardial beta adrenoreceptors. These pathophysiological findings suggest the presence of an increased sympathetic drive in the myocardium of patients with HCM. Whether this is a primary or secondary
phenomenon is not fully understood. Schwartz et al. found that increased afferent sympathetic firing in anaesthetized cats was associated with reduced efferent vagal-cardiac firing (83), indicating that locally released catecholamines in HCM patients also could result in reduced efferent vagal-cardiac firing. There are also a number of other factors that may influence the autonomic balance, such as LVH, myocardial ischemia and the activity of mechanosensitive ventricular receptors (77-79, 84, 85).
Clinical considerations

Clinical presentation
Hypertrophic cardiomyopathy may clinically present itself during any phase of life, from infancy to old age (>90 years) (68). This marked heterogeneity, both in genotype and in phenotype, is characteristic for the disease. It is considered to be hereditary in more than half of the cases and genetically affects men and women equally, although men are overrepresented among patients with clinically developed HCM. Historically, much of the knowledge about HCM has come from large referral centers, with an overrepresentation of severely symptomatic or high-risk patients (86, 87). Annual case fatality rates of 3% to 6% were reported (88, 89). Clinically stable, asymptomatic and elderly patients were underrepresented. A more balanced perspective regarding prognosis has evolved recently. It is now recognized that many patients are asymptomatic and the annual case fatality in an unselected patient population is ≤1% (86, 90, 91).

The clinical course in patients with HCM can follow different pathways. Many patients remain stable over long periods of time, without signs of clinical deterioration. Adverse events that may appear are typically those of sudden death, embolic stroke and development of heart failure (92, 93). Assessment of the risk of sudden death is one of the cornerstones in the clinical management. Embolic stroke is usually related to the presence of atrial fibrillation, not uncommon in patients with HCM. Symptoms of heart failure may develop in the presence of a preserved systolic LV function (attributed to mainly diastolic dysfunction) or as an end-stage phenomenon with progressive LV dilatation and systolic heart failure. About 25% of the patients have LV outflow obstruction and a minority of these will eventually require specific therapy directed at alleviating the obstruction.

Clinical investigation
Patient history should be taken with emphasis on the occurrence of HCM in the family and presence of HCM related sudden death in relatives. Episodes of syncope and paroxysmal arrhythmia should be inquired for, as well as symptoms of heart failure and chest pain. The clinical investigation includes:

a) Physical examination; Often completely normal. A harsh systolic ejection murmur is sometimes heard at the lower left sternal border and apex, which
may indicate LV outflow obstruction and sometimes mitral regurgitation. A fourth heart sound may be heard.

b) Standard 12-lead ECG, which is usually abnormal with a variety of changes from T-wave abnormalities to signs of LVH.

c) Echocardiography (two-dimensional, M-mode and Doppler), to assess site and extent of cardiac hypertrophy, ventricular function, SAM and LV obstruction.

d) Exercise test, mainly to assess blood pressure response and exercise tolerance.

e) Holter ECG (24 or 48 hours), mainly to detect ventricular arrhythmias.

Symptoms
In the early reports of selected patient populations, most patients were found to be symptomatic (94-96). More recent studies of relatively unselected patient populations, not followed in tertiary referral centers, have shown that most of the patients (~90%) have only mild or no symptoms of heart failure (NYHA functional class I or II) at the time of diagnosis (90, 91, 97, 98).

In symptomatic patients, dyspnoea is the most common symptom, reported in ~40% of all patients but angina pectoris (~25%), syncope (~15%) and palpitations also constitute typical symptoms in HCM. Exertional dyspnoea often occurs in the presence of preserved systolic LV function and seems to be caused by mainly a diastolic dysfunction (49-51, 92, 99).

Chest pain is often present in the absence of significant atherosclerotic lesions in the major coronary arteries and may be typical of angina pectoris or atypical in character. There is substantial evidence for the occurrence of myocardial ischemia in HCM, demonstrated by the finding of reversible thallium-scintigraphic perfusion defects, reduced myocardial lactate consumption during atrial pacing and impaired coronary vasodilator capacity (42, 100, 101). Myocardial ischemia is usually attributed to the presence of “small vessel disease” or an imbalance between oxygen supply and demand due to increased cardiac muscle mass (34, 35, 43). Elevated diastolic filling pressures may also contribute to (subendocardial) ischemia.

Syncope and presyncope can be caused by paroxysmal arrhythmia, conduction disturbances, exercise induced development of substantial LV outflow gradient or
abnormal vascular response during exercise (102). In many cases, no underlying cause can be identified.

**Electrocardiography**

The ECG is usually abnormal in patients with HCM, but there are no patterns that are specific to the disease. ST-depression and negative T-waves are most common, followed by evidence of LVH (defined by the point-score system by Romhilt and Estes (20), typically with increased QRS-voltage and repolarisation abnormalities in LV leads). Pathological Q waves, often in inferior and lateral leads, and left atrial enlargement are also common findings (103). The ECG abnormalities may show progressive signs of hypertrophy over time. A normal ECG is found in 15% of the patients, usually when only a localized left ventricular hypertrophy is present.

Sometimes the ECG changes precede the echocardiographic findings, which has been possible to study in families where a disease causing mutation is known. In one study, seven subjects carrying a pathogenic mutation in the cardiac ß-myosin heavy chain gene were found to have various ECG abnormalities, but still had normal echocardiograms (104), emphasizing the importance of the ECG. In a larger study with 155 adults, of which 77 were affected with a mutation in the cardiac ß-myosin heavy chain or cardiac myosin binding protein C gene, the diagnostic value of major ECG criteria (LVH, abnormal Q waves or marked T wave inversions) versus major echocardiographic criterion (LV wall thickness >13 mm) were evaluated (105). A positive genotype was the diagnostic reference criterion for HCM. Sensitivity and specificity was quite similar for both methods (ECG; sens. 61%, spec. 97%. Echocardiography; sens. 62%, spec. 100%). Sensitivity was increased by combining ECG and echocardiography (sens. 70%, spec. 97%), or by taking minor criteria into account, at the price of lower specificity. In that study, three patients had major ECG abnormalities, but normal echocardiograms and two patients had normal ECG, but abnormal echocardiograms.

If only ECG or echocardiography is used as a diagnostic tool, the correct diagnosis will be missed in a minority of the cases. Therefore, it is preferred to use both methods if possible when screening for HCM in clinical practice.
Echocardiography
Echocardiography (two-dimensional and M-mode) remains the key investigation for the diagnosis of HCM, demonstrating LVH, typically with asymmetric hypertrophy of the interventricular septum (Figure 3). The anterior part of the septum is involved in 96% of the cases. However, it is now recognized that virtually any pattern of hypertrophy is consistent with the diagnosis of HCM, including concentric hypertrophy (Figure 4) and hypertrophy confined to a single segment of the left ventricle. Left ventricular cavity dimension is often small (or normal) with normal or hyperdynamic global systolic function. However, regional systolic function may be abnormal, particularly in the septum, which often displays reduced circumferential and longitudinal shortening (48).

Different scoring systems have been proposed to classify the severity of hypertrophy. Spirito and Maron calculated an index by dividing the left ventricle into four segments in the parasternal short axis view (anterior, posterior, lateral and medial) and adding the greatest wall thickness measurement obtained in each segment (106). The calculated score is a quantitative expression of the overall magnitude of LV hypertrophy and has mainly been used within the context of research.

The different patterns of hypertrophy of the LV seen on echocardiography have also been classified by Maron et al into four types (Figure 5) (11). As shown, type 1 hypertrophy is confined to the anterior part of the septum. Type 2 hypertrophy involves both the anterior and posterior part of the septum. Type 3 hypertrophy involves a substantial part of the septum (most often the entire septum) as well as the lateral free wall. Type 3 hypertrophy may also involve the entire LV circumference. Type 4 hypertrophy is most uncommon and involves LV regions other than the anterior septum. The distribution of different patterns of LVH in patients with HCM has been assessed in two major studies (11, 28). In the more recent and larger study with 600 patients (28), it was found that 25% of the patients had type 1 hypertrophy, 30% had type 2 hypertrophy, 41% had type 3 hypertrophy and 4% had type 4 hypertrophy.

Systolic anterior motion (Figure 2) of the mitral valve leaflets typically occurs in patients with LV obstruction (obstructive HCM). SAM is caused by the action of LV flow on the protruding mitral valve leaflet, probably by a drag effect, or possibly a suction (Venturi) phenomenon and is the cause of subaortic obstruction, sometimes with premature closure of the aortic valve and (often mild) mitral regurgitation due to incomplete apposition of
Figure 1. Myocyte disarray

Myocardial section (hematoxylin and eosin stain) demonstrating myocyte disarray. Individual myocytes vary in length and diameter and contain abnormal nuclei. There are abnormal intercellular connections, and cells form circles around areas of increased connective tissue. Reprinted from Crawford and DiMarco: Cardiology, 2001, with permission from Elsevier.

Figure 2. Systolic anterior motion (SAM) of the mitral valve

Echocardiographic M-mode image of mitral valve in parasternal long axis view. In systole, the mitral valve leaflets move in anterior direction (upwards) towards the septum, thereby causing obstruction to LV outflow.
Figure 3. Asymmetric septal hypertrophy

Echocardiographic two-dimensional image, parasternal long axis view. The septum (IVS) is thicker than the posterior wall (PW). Left atrium (LA) is enlarged.

Figure 4. Symmetric (concentric) left ventricular hypertrophy

Echocardiographic two-dimensional image, parasternal short axis view at the mid-ventricular level. Hypertrophy is similar in the septum (IVS) and the posterior wall (PW).
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the mitral valve leaflets. The underlying mechanism causing SAM remains a subject of unresolved debate. Previously, it was hypothesized that SAM is caused by a Venturi effect, whereby high velocity flow in the outflow tract lifts the mitral valve towards the septum (49, 107). Recently it has been shown that blood flow velocities in the outflow tract are normal at the time of SAM onset. In two-thirds of the patients, SAM began even before the onset of Doppler LV ejection, supporting the theory that blood flow at relatively low speed pushes (drags) the protruding mitral valve leaflet into the outflow tract (108).

**Doppler tissue imaging (DTI)**

Doppler tissue imaging is a recent echocardiographic development, allowing the assessment of myocardial tissue velocities, which are substantially lower than the blood flow velocities recorded by conventional pulsed wave (PW) and continuous wave (CW) Doppler (109, 110). This technique has enabled the characterization of regional myocardial systolic and diastolic function, both by recording myocardial velocities and different time indices in the cardiac cycle. Regional myocardial function, assessed by Doppler tissue imaging, is abnormal in patients with HCM. Both systolic and diastolic functional parameters are altered in the LV (111, 112) and in the right ventricle, evidence of diastolic abnormalities have been found (72).

One of the applications has been to identify indices of diastolic function that are less dependent on the loading conditions, compared to the conventional Doppler indices used. Indeed, it has been found that early diastolic LV lateral annular velocity (E_m) can be used as a preload independent index of LV filling pressure in patients with HCM. Conventional Doppler transmitral E velocity/E_m was shown to correlate well with pre-atrial LV pressure (62). Important clinical parameters in HCM, like NYHA functional class and exercise capacity have also been found to correlate to mitral E/E_m.

Another application of the technique has been to identify markers that are present early in the disease, even preceding the development of cardiac hypertrophy. Again, the E_m (at the lateral, septal, anterior and inferior LV base) was shown to be a sensitive index, showing reduced velocities in patients carrying a mutation for HCM, before any sign of hypertrophy or abnormality in any of the conventional Doppler measurements was evident (113). In another study, systolic LV lateral annular velocities (S_m) were also
Thus, DTI seems to be more sensitive than conventional echocardiographic techniques and can provide a novel means for early diagnosis in HCM.

**Clinical importance of outflow obstruction**

The clinical importance of obstruction has now been recognized in two recent large studies (with 225 and 1101 HCM patients), where obstruction to LV outflow was an independent predictor of death and progressive heart failure (90, 98). There was no further elevation in risk for patients with a gradient increased above the threshold of 30 mmHg during resting conditions (98). It is therefore useful to divide HCM patients into subgroups, based on the LV outflow gradient assessed with continuous wave (CW) Doppler. Different gradient cut-offs have been used to determine the presence of obstruction, usually 30 or 50 mmHg at rest, with the higher value required for surgical intervention to be proposed (99). Based on recent studies, it has now been suggested that HCM patients be divided into three hemodynamic subgroups (68): a) Obstructive, with a resting gradient ≥ 30 mmHg. b) Latent (dynamic) obstructive, with a resting gradient ≤ 30 mmHg, but a gradient ≥ 30 mmHg with provocation. c) Nonobstructive, with a gradient ≤ 30 mmHg at rest and provocation.

Although the presence of LV obstruction has now been identified as an independent risk factor for adverse events, the data is still insufficient to abruptly change the management of patients with obstructive HCM. Septal myectomy and alcohol septal ablation are today the procedures that relieve the outflow gradient most reliably, but both are associated with some risk of complications and death (93). Therefore, such interventions should not be performed in asymptomatic or mildly symptomatic patients (NYHA functional class I or II). However, since patients with outflow obstruction have been shown to be at significantly increased risk of progressive heart failure and death (98), they should be continuously monitored for the development of aggravating symptoms. Thus, the presence of LV obstruction justifies intervention to reduce or abolish subaortic gradients in symptomatic patients who do not respond to maximum pharmacological therapy.

**Differentiation from hypertensive heart disease**

The strict definition of HCM does not allow the diagnosis to be made in individuals with other potential causes of cardiac hypertrophy, such as hypertension. However, it is
obvious that a condition as common as hypertension, especially in the elderly, must sometimes be found in patients with “true” HCM. In a patient with cardiac hypertrophy of ≥15mm and concomitant hypertension, the difficulty lies in the differentiation between HCM and hypertension with secondary cardiac hypertrophy. There are no absolute criteria to govern this differentiation, but some guidelines may be given.

HCM is more likely in a patient with pronounced hypertrophy (>20 mm), isolated distal hypertrophy and if asymmetric septal hypertrophy is present. Concentric hypertrophy is more common in hypertensive patients. SAM occurs in both conditions, but complete SAM (septal contact in systole) with outflow obstruction is more typical for HCM. In the literature, authors have dealt with this problem in different ways, some have excluded all patients with hypertension and some have regarded hypertensive individuals as a subset of HCM, if the hypertrophy has been judged to be to severe to be explained by the elevation in blood pressure.

In a study by Karam et al, the impact of hypertension on the degree of cardiac hypertrophy in HCM was assessed in two patient groups (115). The patients were initially identified by the echocardiographic diagnosis of HCM. They were then divided into two groups, HCM with and without hypertension. The echocardiographic findings were almost identical in the hypertensive and nonhypertensive groups, with similar incidence of SAM, septal thickness >20 mm and outflow tract gradient. However, increased posterior wall thickness was more common in the hypertensive group. The authors concluded that hypertension can slightly increase the hypertrophy already present in HCM, but is not the primary cause of the cardiomyopathy and that HCM with hypertension appears to be a part of the diverse spectrum of idiopathic HCM. One might object to the fact that patients in this study initially were given a diagnosis of HCM by echocardiography, despite the presence of hypertension. The hypertensive group in that study might represent an extreme of the phenotypic spectrum that might be seen in hypertension, with severe cardiac hypertrophy caused by hypertension. Normally, only a minority of patients with mild to moderate hypertension will develop clinically detectable LVH (116), but it has been suggested that some patients may have a predisposed myocardial sensitivity to the development of hypertrophy in the setting of hypertension (117). The issue of differential diagnosis is not yet settled. In clinical practice, there is a category of patients, mostly
elderly, where the differentiation of HCM from hypertensive heart disease with pronounced hypertrophy remains uncertain.

This discussion should not be confused with hypertensive cardiomyopathy, an entity of its own, which often presents with LVH in association with features of dilated or restrictive cardiomyopathy with cardiac failure (18).

**Hypertrophic cardiomyopathy in the elderly**

Early descriptions of HCM indicated that it is a disease of the young (6, 7). However, it has been recognized that HCM may also be present in the elderly, and patients aged 75 years or older may constitute as much as 25% of a cohort of HCM patients (86). Elderly patients have a more favourable long-term prognosis than the young (118, 119). Left ventricular outflow obstruction is frequently found, but only a minority of the patients have severe heart failure, suggesting that subaortic obstruction may be present for long periods, without clinical deterioration.

Age related differences in cardiac morphology have also been seen (120, 121). The left ventricle in young patients is often crescent shaped with reversed septal curvature, whereas the elderly patients more often show an ellipsoid LV cavity, often with milder hypertrophy. The LV outflow tract is often more narrow in the elderly, associated with anterior displacement of the whole mitral valve apparatus and mitral annulus calcification.

HCM in the elderly is most often regarded a sporadic form of the disease, in contrast to younger patients, where the familial form is more common. Interestingly, it has recently been shown that approximately 20% of elderly cases, without known family history of HCM, were caused by mutations in sarcomeric proteins, resembling the situation in younger cases. The frequencies of different disease causing genes were different compared to the young, most frequently with mutations in the cardiac myosin binding protein C gene (MYBPC3) and cardiac troponin I gene (TNNI3) (122).

**Differentiation from cardiac amyloidosis**

Amyloidosis is caused by deposits of misfolded proteins derived from different plasma proteins. Cardiac amyloidosis is an infiltrative, restrictive cardiomyopathy, characterized by left and right ventricular wall thickening, due to extracellular deposition of amyloid. Typically the distortion and replacement of myocytes results in a decrease in the voltage
of the ECG. Cardiac amyloidosis may appear late in life as senile cardiac amyloidosis, where the amyloid deposits are derived from normal transthyretin (123). The heart may also be affected in systemic primary (AL) or a secondary (AA) amyloidosis (124). Cardiac amyloidosis may also be a feature of hereditary transthyretin (TTR) amyloidosis, caused by mutations in the TTR gene. Several TTR mutations with predominant heart involvement are known (125). However, cardiac amyloid deposits may also be noted in TTR mutations in which neuropathy is the predominant symptom, such as familial amyloidotic polyneuropathy (FAP), Portuguese type (ATTR Val 30 Met). The typical phenotype of FAP is characterized by a progressive somatic and autonomic polyneuropathy, with complications from several other organ systems such as the gastrointestinal tract, kidneys, eyes and heart (126). Typical cardiac symptoms in FAP are related to conduction defects (127), but some patients develop isolated progressive cardiac hypertrophy (128-130). Thus, the cardiac manifestations of cardiac amyloidosis may closely resemble those of HCM.

Low QRS-voltage in the ECG, the presence of pericardial effusion, granular sparkling myocardial echoes and thickened heart valves on echocardiographic examination suggest the presence of cardiac amyloidosis (131). However, cardiac amyloidosis may be confused with hypertrophic cardiomyopathy, especially if other symptoms related to the amyloidosis are insignificant (132).

**Differentiation from athlete’s heart**

Long-term athletic training leads to an increase in LV mass, due to increase in the cavity dimension, wall thickness, or both. In the vast majority of highly trained athletes, left ventricular wall thickness is normal or only slightly increased. In a large study, only 2.2% of male elite athletes had a wall thickness ≥13 mm (133), all of them having increased LV end diastolic cavity dimensions as well, 55-63 mm. The hypertrophic response is most pronounced in endurance sports like rowing, canoeing and cycling. Cross-country skiing or running does not provoke the same response, nor does isometric activities (like weightlifting).

The upper normal limit of LV hypertrophy attributable to physical training in men seems to be 16 mm (133), sometimes creating a diagnostic dilemma between physiological hypertrophy and HCM with mild hypertrophy. Female elite athletes do not show the same
hypertrophic response to intensive training. In a study of 600 elite female athletes, LV wall thickness was within normal values, ranging from 6-12 mm, but LV end diastolic cavity dimension was increased in 8% of the subjects (134).

In the differential diagnosis between athlete’s heart and HCM in the “grey zone” of 13-16 mm LV wall thickness, some clinical and echocardiographic features may be used (135, 136);

a) In athletes, the hypertrophy of the LV is typically uniform, engaging the entire ventricle. In HCM, the hypertrophy is often asymmetric, with dominance of the septum or sometimes other segments in the LV. Furthermore, in HCM, contiguous portions of the LV often show striking differences in wall thickness, with abrupt transition between such areas.

b) Systolic anterior motion of the mitral valve with outflow obstruction is not seen in athletes.

c) LV end diastolic cavity dimension of < 45 mm favours the diagnosis of HCM, whereas a dimension of > 55 mm is often seen in athletes.

d) In females, even mild hypertrophy is not seen in athletes, and most likely represents HCM.

e) Regression of LV hypertrophy with deconditioning. In athletes with LVH, a regression in wall thickness (2 to 5 mm) may be seen within 3 months after training has ceased and is inconsistent with the presence of HCM.

f) Family history. If HCM has been diagnosed in other family members, it is highly likely that the hypertrophy seen in the athlete represents a case of HCM. Absence of HCM in the family, however, does not exclude HCM in the athlete, since the disease can be sporadic.

g) ECG. Wide variations of ECG abnormalities are seen in both athletes and patients with HCM, rendering the ECG less useful for the purpose of differential diagnosis. Athletes most often have increased voltage in precordial leads and mild T-wave inversions. Such ECG changes are also seen in patients with HCM, but the presence of pathological Q-waves, ST segment depression and deep, negative T waves are rare in athletes and more suggestive of HCM.

h) Doppler tissue imaging. Systolic and early diastolic LV lateral annular velocities are reduced in patients with HCM compared to athletes (137).
i) Type of sport training. It is mainly some endurance sports that have been associated with cardiac hypertrophy.

**Recommendations for participation in sports**

It is generally accepted that strenuous physical activity is associated with an increased risk of sudden death in patients with HCM (138). This includes endurance sports, sports with burst exercise (e.g., sprinting) and heavy isometric exercise (e.g., weight lifting). The magnitude of the increase in risk associated with exercise is difficult to quantify. Not all athletes with HCM will die suddenly during competition and overall, only some of the sudden deaths in HCM occur during exercise. Nevertheless, patients with HCM are recommended to discontinue competitive athletic activity to reduce the risk of sudden death and this modification of lifestyle can be regarded as a treatment per se (139).

**Heart rate variability**

During the last decades, analysis of HRV has become an accepted method for assessing autonomic modulation of the heart rate. Analysis of HRV is either based on ambulatory long-term recordings (up to 24 hours) or short-term recordings (30-60 minutes) performed in laboratories and then often combined with physiological or pharmacological tests (140). Both methods have empirically been shown to give an estimate of the overall function of the cardiac autonomic nervous system (ANS) and also information on the function of the sympathetic and parasympathetic components, respectively. The relationship between the ANS and cardiovascular mortality has been extensively studied (141, 142). There is experimental evidence for an association between a propensity for lethal arrhythmias and signs of changes in sympathetic and/or parasympathetic activity, which has encouraged the development of quantitative markers of autonomic activity (140).

**Evidence of autonomic dysfunction in hypertrophic cardiomyopathy**

A number of studies have suggested that autonomic function is abnormal in patients with HCM, although they have shown wide discrepancies and even contradictory results. Apparent problems in comparisons between HRV studies are the lack of standardisation of the methods (140), the varying severity of the disease in the patients from different
studies and the possible residual effect of cardioactive drugs such as beta-blockade. Several authors have reported a reduction in parasympathetic activity in patients with HCM (143-147), whereas others have demonstrated a decreased sympathetic tone (148, 149) (Table 2). It is, however, with these methods, not possible to locate the anatomical level of the dysfunction, which might be within the brain, in peripheral ganglia or nerves or in the target organ receptors. The mechanisms by which HRV is altered in HCM have not been defined but are likely to involve derangements in the neural activity of cardiac origin.

Autonomic control mechanisms are characterized by adequate variability of heart rate under physiological conditions. Distortion of these mechanisms, as evidenced by reduced HRV, has been shown to be a risk factor for adverse events in heart failure and after myocardial infarction (150, 151) but is so far not a proven risk indicator in HCM. Therefore, the role of HRV assessment in the management of HCM patients is at present unclear.

**Assessment of risk for sudden death**

Sudden death may be the initial presentation of the disease, most often in young people. Most patients with HCM will not be at increased risk for sudden death. One of the clinical challenges lies in the identification of the small group of patients with a high risk for adverse events.

Patients who have experienced a prior cardiac arrest or have spontaneous sustained ventricular tachycardia are considered to be at high risk of sudden death and should be treated with an implantable cardioverter defibrillator (ICD).

For patients without such dramatic events, a number of risk markers for sudden death have been identified:

- A family history of HCM-related sudden death, in one or more close relatives.
- Unexplained syncope, particularly if recurrent or in young people.
- Nonsustained ventricular tachycardia (NSVT) on ambulatory ECG monitoring.
- Abnormal blood pressure response during exercise test (≤25 mmHg increase in systolic blood pressure).
- Identification of a high-risk mutation.
- Severe left ventricular hypertrophy, >30mm.
Table 2. Previous HRV-studies in hypertrophic cardiomyopathy

<table>
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<tr>
<th>Study (Author, year)</th>
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<th>Controls (n)</th>
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<td>LF ↓ (ms), HF ↓ (ms), SDNN ↓, SDANN ↓, RMSSD ↓, pNN50 ↓</td>
<td>Comparison of symptomatic vs asymptomatic patients. Freq. domain in ms.</td>
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<td>31 31</td>
<td>24-Hour ECG</td>
<td>LF ↓ (n.u.), HF ↑ (n.u.)</td>
<td>Values after normalisation (n.u.) or CCV-correction presented.</td>
<td></td>
</tr>
<tr>
<td>Tanabe et al, 1995 (144)</td>
<td>35 early 21 advanced</td>
<td>24-Hour ECG</td>
<td>Early HCM; No differences. Advanced HCM; HF ↓ (CCV), HF ↓ (n.u.), LF ↑ (n.u.).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bonaduce et al, 1997 (146)</td>
<td>33 33</td>
<td>24-Hour ECG</td>
<td>HF ↓, LF ↓, LF/HF ↑</td>
<td>HF component = 0.15-0.34 Hz</td>
<td></td>
</tr>
<tr>
<td>Limbruno et al, 1998 (149)</td>
<td>22 HNCM</td>
<td>28 Short-time ECG</td>
<td>No significant changes, rest/tilt</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28 HOCM</td>
<td>28 Short-time ECG</td>
<td>LF ↓ (n.u.), at rest. LF ↓, LF/HF ↓, during tilt.</td>
<td>No difference in LF at rest without normalisation.</td>
<td></td>
</tr>
<tr>
<td>Döven et al, 2001 (147)</td>
<td>23 18</td>
<td>24-Hour ECG</td>
<td>SDNN ↓, RMSSD ↓, pNN50 ↓</td>
<td>No spectral analysis performed.</td>
<td></td>
</tr>
</tbody>
</table>

HNCM – Hypertrophic non obstructive cardiomyopathy, HOCM – Hypertrophic obstructive cardiomyopathy, VLF - Very low-frequency power spectrum (0.003-0.04 Hz), LF - Low-frequency power spectrum (0.04-0.15 Hz), HF - High-frequency power spectrum (0.15-0.45 Hz), SDNN - Standard deviation of all the NN (normal-to-normal) R-R intervals, TINN - Baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of NN intervals, SDANN - Standard deviation of all 5-minute NN interval means, RMSSD - Square root of the mean squared differences of successive NN intervals, pNN50 - the ratio of NN interval differences of successive NN intervals greater than 50 ms to the total number of NN intervals, n.u. – Normalised units, CCV - Coefficient of component variance.

Treatment with ICD or antiarrhythmic therapy (amiodarone) are the available options. The ICD is a more reliable treatment option and should be considered in the presence of multiple risk factors. One problem is that these risk markers are limited by their low positive predictive value (=15-30%) (102). Nonsustained ventricular tachycardia is seen
in ≈25% of patients with HCM. On the other hand, their negative predictive value is high (=90%) and their absence can be used to identify patients at low risk. Patients without risk factors constitute an important part of the overall HCM population and do not require medical treatment, except for symptomatic relief. Patients with one risk factor must be assessed individually, but are usually not candidates for aggressive medical treatment, like amiodarone or ICD.

Recent attention has been focused on the magnitude of hypertrophy as a risk factor for sudden death. A correlation between the extent of hypertrophy and risk for sudden death has been shown in one study, with an annual risk of 2% for patients with a wall thickness >30mm (152). In another study, the magnitude of hypertrophy had predictive power only when combined with other risk factors (153). Severe LVH is less frequent in older patients, suggesting that these patients are at risk for sudden death at young age, or the presence of LV remodelling with age. Severe LVH (>30 mm wall thickness) as a single risk factor is in general not sufficient to justify treatment with ICD, but may be considered in young patients.
Molecular genetics

Hypertrophic cardiomyopathy occurs in a sporadic form and in a familial form, inherited in an autosomal dominant fashion. The characteristics of this type of inheritance is shown in Figure 6. Physical examination, ECG, or echocardiography does not distinguish between the sporadic and familial forms. Approximately 55% of the cases have been suggested to be familial (12). This conception, however, derives from a time when molecular genetics was not yet introduced and the actual percentage of familial cases might be higher.

Familial disease is more common in the young, compared to elderly cases. More than 150 different mutations in ten sarcomeric protein genes have been identified in families with HCM. These sarcomeric protein genes are the cardiac β-myosin heavy chain (MYH7) (16), the cardiac myosin binding protein C (MYBPC3) (154-156), the cardiac troponin T (TNNT2) (157), the α-tropomyosin (TPM1) (157), the cardiac regulatory and essential myosin light chains (MYL2 and MYL3) (158), the cardiac α-actin (ACTC) (159), the cardiac troponin I (TNNT1) (160), the cardiac troponin C (TNNC1) (161) and the titin (TTN) (162). Mutations in a non-sarcomeric protein gene; the γ2 subunit of the protein kinase A (PRKAG2) have been described in HCM associated with electrophysiological abnormalities, particularly the Wolf-Parkinson-White syndrome (163, 164). The role of this gene as a cause of HCM is not yet clear, it has been suggested that mutations in PRKAG2 cause glycogen storage disease in the heart, mimicking HCM (165), or that an inefficient use of energy, caused by mutations in this gene, plays a central role in the pathogenesis of HCM in these cases (164). It may therefore be prudent to distinguish the disease caused by PRKAG2 mutations from HCM caused by the sarcomeric protein mutations.

There are other disease states in which LVH resembling HCM can occur in children and adults, e.g., Noonan’s syndrome (166), mitochondrial myopathies, Friedrich’s ataxia (167), Anderson-Fabry disease (168) and cardiac amyloidosis (169).

Despite the genetic heterogeneity, HCM can be regarded as a single disease entity and a primary disorder of the sarcomere. Three of the disease causing genes have been found to predominate in frequency; the MYH7, MYBPC3 and TNNT2. Most mutations found are missense mutations, leading to the substitution of a single amino acid in the sarcomeric protein. The exception from this rule is the MYBPC3 gene, in which frameshift mutations
are much more common, leading to a truncated protein product. Frameshift mutations are caused by insertions or deletions of DNA-nucleotides, disrupting the translational reading frame. The numerous mutations identified to date mean that each affected family often have their own “private” mutation, but there are also “hot spots” for mutations in several genes, where a number of unrelated families may share a common mutation. Sometimes, founder effects can be important, leading to an overrepresentation of a certain mutation in the population, even in a whole country (170). The mechanisms by which disease causing mutations cause LV hypertrophy are largely unknown, but several hypotheses have been suggested.

Figure 6.

Example pedigree with autosomal dominant inheritance. Typical features of this mode of inheritance are: The disease is present in every generation. Equal numbers of males and females are affected. The risk of inheriting the mutated gene from an affected parent is 50% for every child. On average, half of the children in each generation will then be genetically affected.

Current hypotheses

The identification of missense mutations in HCM have led to the ”Poison polypeptide” hypothesis, which proposes that mutant sarcomeric proteins incorporate into the myofibrils and act as dominant negative proteins (16). Another theory is the ”haplo-insufficiency” hypothesis, which suggests that HCM results from an insufficient amount of the sarcomeric protein. The identification of frameshift mutations leading to truncated proteins supports this hypothesis, since such proteins are not expected to be expressed or incorporated into the myofibrils (156, 171). A fundamental question is how a missense or frameshift mutation leads to cardiac hypertrophy, myocyte disarray and interstitial
fibrosis. It has been proposed that the sarcomeric protein gene mutation leads to impaired cardiac myocyte contractile performance, inducing stress-responsive growth factors which stimulate myocyte hypertrophy and fibroblast proliferation. The hypertrophy would then be a compensatory response. Experimental data from isolated muscle fibres, expressing the mutant protein have shown diverse results, both impaired and increased contractility has been shown (172).

Calcium sensitivity has also been shown to be abnormal in HCM. Binding of Ca\(^{2+}\) to cardiac troponin C initiates the acto-myosin interaction, leading to muscle contraction. Several reports have shown increased calcium sensitivity (92, 173, 174), but the opposite has also been found (175).

A more recent theory suggests that myocardial energy depletion is the key underlying factor in the development of HCM (176). According to this theory, mutations in \textit{MYH7} would lead to increased energy demand by the mutated globular head of the myosin heavy chain, not contributing to the power stroke, but still consuming ATP. Mutations in \textit{TNNT2} are predicted to accelerate the acto-myosin detachment, decreasing the power output for each ATP hydrolyzed. The unifying dysfunction in HCM would then be increased energy demand owing to inefficient sarcomeric energy utilization. The fact that mutations in \textit{PRKAG2} and mitochondrial disorders both can result in severe cardiac hypertrophy supports the theory.

Despite an abundance of data, the exact mechanisms at the molecular level, leading to the HCM phenotype are yet to be determined.

**Variable penetrance and expression**

Not all individuals with a mutation in one of the HCM genes will express the clinical features of HCM during their lifetime. The penetrance is highly variable and seems to depend on which gene that is affected (177). Mutations in the \textit{MYH7} gene seem to be most penetrant, reaching more than 90% penetrance at 20 years of age. \textit{TNNT2} conveys an intermediate penetrance, approximately \(\approx 50\%\) at 20 years of age and \textit{MYBPC3} has been associated with the lowest penetrance, approximately \(\approx 50\%\) at 30 years of age. There is, however, considerable variation in penetrance between different mutations within the same gene and even between close relatives in the same family, all carrying the same specific mutation. With increasing knowledge in molecular genetics and increasing
numbers of reported families with different genetic defects, the estimated figures of penetrance might very well change in the future. For the majority of disease causing HCM genes, the reported number of patients are too low to be able to give accurate estimates of penetrance.

Clinical importance of variable penetrance
The question of penetrance has considerable clinical impact. Traditionally, it has been assumed that HCM develops during adolescence, to reach full morphologic disease expression at the time of physical maturity (=18 years). Studies in molecular genetics have certainly proved that the disease is much more complex. There is, in fact, no maximum age, when it is completely safe to say that the risk of developing HCM has been passed. This is important when counselling asymptomatic individuals, in a family with HCM, about their risk of developing the disease and also the risk of transmitting the disease to their children. Identifying a disease causing mutation by DNA-analysis (genotyping) is the most definite way to assess these risks in asymptomatic family members, it is also the most definite way to establish the diagnosis of HCM in a patient with LVH. Due to the methodological difficulties associated with identifying a single disease causing mutation among 10 different genes, genotyping is not yet available in the routine care of HCM patients, it is mainly confined to a few research laboratories.

Not only the penetrance, but also the clinical expression shows great variations, which can partly be attributed to the genetic heterogeneity. Patients with \textit{TNNT2} mutations appear to have relatively mild hypertrophy, but a poor prognosis. On the other hand, mutations in the \textit{MYBPC3} gene are generally associated with a favourable prognosis and late onset disease. Mutations in the \textit{MYH7} gene have been associated with variable clinical outcomes, both poor and favourable. These conclusions are based on observations of large patient groups and are difficult to apply at the individual patient level, due to the heterogeneity of the disease.

Family screening
Once the diagnosis has been established in a patient, a family history should be obtained. Due to the variable penetrance and often small family sizes, the familial nature of the disease might easily be overlooked. The patient should be informed about the (often)
familial nature and screening of first-degree relatives and sometimes other family members should be encouraged. Such screening should include patient history, physical examination, ECG and echocardiography. Asymptomatic family members younger than 12 years are not screened on a routine basis, since disease manifestations are rarely seen before that age. Between 12 and 18 years of age, screening should be offered at 1 or 2-year intervals, and thereafter, asymptomatic adults at risk of having inherited a mutated gene, should be offered continued screening every 5 years.

**Genetic counselling**
Except in a very few cases, the genetic information has no direct prognostic value for the individual HCM patient. Genetic analysis is not performed in the routine management of patients with HCM. Such analyses are usually done within the context of research. However, once the disease causing mutation has been identified in a family, direct mutation analysis and genetic counselling about the future risk of developing HCM and risk of transmission to the children could be possible for the family members. In families where the disease causing mutation is not known, it is still possible to inform about the genetic aspects of the disease in general terms.
Treatment

Treatment of HCM basically follows two principles; drug treatment for nonobstructive patients (initially also for obstructive patients) and specific treatment for outflow obstruction. Treatment strategies are outlined in Figure 7. In general, treatment is more successful in patients with symptoms caused by LV outflow obstruction. Apart from these principles of treatment, each patient diagnosed with HCM should also be assessed for the risk of sudden death.

Pharmacological treatment

Pharmacological treatment is aimed at relieving symptoms of heart failure, exertional dyspnoea, with or without associated chest pain. Treatment is to a large extent empirical since there are very few randomised trials comparing the different drugs in HCM. For the majority of patients with nonobstructive HCM, treatment with drugs is the only option to gain symptom relief.

Beta-blockers

The first drug to be used in HCM, in the 1960s, was propranolol. Since then, nadolol, atenolol and metoprolol have also been used (99, 178, 179). Beta-blockers are still usually the drug of choice, when starting pharmacological therapy in HCM. They can be used in obstructive and nonobstructive patients, in standard doses. Treatment with beta-blockers has been shown to reduce provocable outflow gradients, present during exercise when sympathetic tone is high. The beneficial effects on heart failure symptoms and exercise intolerance seem to be associated with a decrease in heart rate and consequent improvement in passive ventricular filling. A reduced myocardial oxygen demand might also be important.

There is a lack of standardization in therapy and patient responses are highly variable. If symptom relief is not achieved, beta-blockade may be substituted for verapamil, a combination of both drugs has not proven to be superior. Beta-blockade may be combined with disopyramide to relieve outflow obstruction. There is no evidence that beta-blockade reduces sudden death in adults with HCM.
**Verapamil**

Verapamil has been used empirically, like beta-blockade, in patients with and without LV obstruction, with a reported benefit for many patients, including those with a component of chest pain (180-182). Doses up to 480 mg/day have been used. The favourable effects seem related to improved ventricular relaxation and reduced myocardial ischemia. In a few severely symptomatic patients with outflow obstruction, verapamil has proven

(For picture see printed edition of this dissertation)
deleterious, probably because of predominating vasodilating effect, leading to increased outflow obstruction. Such patients should therefore be treated with caution. Verapamil is often used in patients with asthma and in those who do not benefit sufficiently from beta-blockade. There is no evidence that verapamil reduces sudden death.

**Disopyramide**

Like beta-blockers and verapamil, disopyramide is also a negative inotropic drug, used in HCM patients with resting LV obstruction in doses of 300-600 mg/day, with a dose-response effect (183-185). Symptomatic benefit is related to a decrease in SAM, outflow obstruction and mitral regurgitation. Anticholinergic side effects may be troublesome. A combination with beta-blockade is recommended, since disopyramide can facilitate atrioventricular conduction, with increased ventricular rate during atrial fibrillation. Disopyramide is not indicated in patients without outflow obstruction and is usually administered when beta-blockers and verapamil first have been tried.

**Other drugs**

Diuretics may be used when necessary, with caution in the presence of outflow obstruction. Many patients may require high filling pressures to achieve adequate ventricular filling. Digoxin and vasodilating substances, like nifedipine, nitroglycerine and ACE inhibitors should be avoided in the presence of outflow obstruction.

**Treatment of outflow obstruction**

A small subgroup of patients with outflow obstruction, only about 5% of the overall HCM population, are candidates for more definitive treatment. They are severely symptomatic, usually with dyspnoea and chest pain, despite optimal medical treatment. These patients usually have outflow gradients of 50 mmHg or more. Specific treatment of outflow obstruction involves surgery (septal myectomy), dual-chamber pacing or percutaneous alcohol septal ablation.

**Surgery**

Septal myectomy has been performed during the last 40 years and extensive experience from this procedure has been gained (at least 2000 cases). It is performed through an
aortotomy and involves resection of 5 to 10 g of muscle from the proximal septum. Mitral valve replacement or repair is only performed in the presence of intrinsic abnormalities of the mitral valve. In experienced hands, operative mortality is low (≤ 2%) and 70% of the patient experience symptomatic improvement for 5 years or longer. Complications such as complete heart block, requiring pacemaker or septal perforation occurs in 1-2% of the cases. The outflow gradient is abolished in >90% of the cases.

**Dual-chamber pacing**

Pacing from the right ventricular apical position, using a short AV delay (60-80 ms) to ensure full ventricular capture has been evaluated as an alternative treatment for outflow obstruction. The suggested mechanism of action is a pacing induced inversion of the septal activation sequence (186). Early studies without control groups, showed positive long-term results of DDD pacing in obstructive HCM, with substantial decrease in outflow gradient and symptom relief (187). Investigations with double-blind, crossover study design have shown more variable results. In one study there was no improvement in subjective or objective measures of symptoms or exercise capacity during pacing (188). A modest reduction in outflow gradient was achieved in most patients. During unblinded pacing, there was an improvement in functional class and quality of life, interpreted as mainly a placebo effect. Another study, on the other hand, showed significant reduction in outflow gradient, improvement in functional class, symptoms and exercise tolerance. The authors concluded that dual-chamber pacing is a treatment option in obstructive HCM (189).

Dual-chamber pacing can be regarded as an alternative particularly in patients where an alternative to surgery is desired (e.g. patients >65 years). Some patients may hesitate to accept surgery, but find pacemaker therapy more acceptable. The effect of pacing on the outflow tract gradient may be tested in the catheterization laboratory, prior to implantation. However, an immediate reduction in gradient does not guarantee a long-term efficacy of pacing.

**Alcohol septal ablation**

This treatment was first described in 1995 and has gained rapid popularity, it is estimated that ≈3000 cases have already been treated with this method. The treatment is performed
by catheter interventional technique and involves injection of a small amount of absolute alcohol into a septal perforator branch of the left anterior descending coronary artery. This produces a myocardial infarction in the proximal septum, leading to a reduction in septal thickness. Successful ablation may trigger a rapid decrease in outflow gradient, but often, a progressive decrease is seen during the following year. Overall, the results are very similar to those seen after surgery. There is a similar improvement in symptoms (190). Procedure-related mortality has been reported in 1% to 4%. Need for permanent pacemaker implantation has been reported in 5% to 30% (191-194), but the technique has been modified with use of less alcohol in recent studies, leading to less complications. One concern is the limited time of follow-up yet available for patients treated with this technique (≈5 years).
AIMS OF THE STUDIES

Hypertrophic cardiomyopathy is a very complex disease, characterized by a high degree of heterogeneity, both in its genotype and in its phenotypic expressions. In the background section an attempt has been made to review some of the current knowledge in hypertrophic cardiomyopathy. There are, however, still unresolved questions in many areas concerning the disease. To this date, no study has systematically analysed the genetic and phenotypic aspects of the disease in a Swedish population.

In view of this background, the present thesis aimed at gaining further knowledge about hypertrophic cardiomyopathy in northern Sweden.

The specific aims of the thesis were:

- To identify the genes causing hypertrophic cardiomyopathy in northern Sweden.
- To systematically characterize the phenotypic expressions of hypertrophic cardiomyopathy in northern Sweden.
- To correlate clinically important phenotypic findings to the underlying genotype.
- To evaluate arrhythmogenicity in hypertrophic cardiomyopathy.
- To study global and regional ventricular function in hypertrophic cardiomyopathy.
MATERIAL AND METHODS

Case identification (papers I, II, III, IV and V)
The region of northern Sweden has a population of 883,000 inhabitants. Specialized
cardiac care is provided by the Heart Center at Umeå University hospital. The Hospital
Discharge Register of the National Board of Health and Welfare in Stockholm was used
to identify patients that had been hospitalised with HCM and 50 possible cases were
identified. Moreover, the physicians in charge of cardiology at the 12 other hospitals in
the region were contacted to obtain information about known patients with HCM and
another 60 possible cases were found. Criterion for the diagnosis of HCM was left
ventricular hypertrophy demonstrated at echocardiography, with a wall thickness of $\geq 15$
mm (18). For the family members of an affected proband, the diagnostic criteria
suggested by McKenna et al. were used (19). Exclusion criteria were arterial
hypertension, defined as blood pressure $>160/90$ mmHg or ongoing antihypertensive
treatment, significant valvular disease or known systemic disease capable of producing
cardiac hypertrophy. All patients $>18$ years old were considered for the study. When
medical records were scrutinised, 64 cases were excluded; 25 hypertensive individuals
and 2 with aortic stenosis. In 8 cases with other conditions, the HCM diagnosis was
incorrect by documentation error in the medical records (wrong ICD10 diagnosis code
given) and 29 cases were not included for various reasons (not giving consent to the
study, age $<18$ years, non-Swedish ethnic background, previous cardiac transplant, other
illnesses, e.g., psychiatric disease). Thus, 46 patients were available for the studies in paper
I, II and IV (although only one patient and his family was used for the study in paper I).
For the study in paper III, the 46 patients mentioned were available, as well as two
patients of non-Swedish ethnic background and another five patients recruited after the
study in paper II. Ten patients were excluded because of arrhythmias. For the study in
paper V, the 46 patients were available, and by further investigation of the families, 20
more cases were found. A total of 16 were excluded because of arrhythmias or conduction
disturbances.

Definition of familial vs. sporadic HCM
Familial HCM is defined as the finding of at least two affected subjects in the same
family, including deceased individuals where a positive diagnosis can be established from the medical records. In sporadic cases, the other family members are not affected.

**Study population**

Paper I: Nine individuals in a family with familial HCM.

Paper II and IV: Forty-six unrelated individuals of Swedish ethnic background with familial or sporadic HCM.

Paper III: Forty-three individuals with familial or sporadic HCM.

Paper V: Fifty individuals with familial or sporadic HCM.

**Controls (papers III and V)**

The 121 control subjects (64 men and 57 women) in paper III were derived from a large local study focused on echocardiography in healthy individuals (ages 20 to 90 years). The subjects were randomly selected from the population register and examined with echocardiography, ECG, dynamic spirometry, clinical investigation, and were not taking any medication known to interfere with cardiac function. Exclusion criteria were: arterial hypertension (>160/90 mmHg), LVH (≥13 mm) on echocardiography, pathological spirometry or ECG (conduction defects or absence of sinus rhythm), pulmonary hypertension >35 mmHg and significant valvular heart disease. In paper V, 250 control subjects (127 men and 123 women) were used from the same study as in paper III.

**Echocardiography (papers I, II, III, IV and V)**

Two-dimensional, M-mode, and Doppler echocardiography was performed with an Acuson xp/10 or Acuson Sequoia ultrasound system (Acuson, Mountain View, CA, USA). Views of the heart were obtained from the parasternal, apical, and subcostal positions. M-Mode and Doppler tracings were all recorded at sweep speeds of 50 and 100 mm/sec. The examinations were recorded on a S-VHS video tape recorder and/or digitally stored on magneto optical discs. All measurements were done according to the standards of the American Society of Echocardiography (195). Doppler tissue imaging was performed from the apical four-chamber view, with the sample volume placed at the LV lateral and septal annulus and lateral tricuspid annulus. From the same position, systolic
atrioventricular plane displacement was measured at the LV lateral and septal annulus and lateral tricuspid annulus

**Genetic analysis (papers I, II and IV)**

A venous blood sample was obtained from each subject. DNA was extracted from peripheral blood leucocytes by standard protocol. The *MYH7, MYBPC3, MYL2* and the *TNNI3* genes were screened for mutations using single-stranded conformation polymorphism (SSCP) (196, 197). In principle, this method identifies mutations by abnormal migration properties of mutated DNA on a polyacrylamide gel, visualised as additional DNA bands.

The *TNNT2, TPM1, MYL3, ACTC* and *TTR* genes were analysed by denaturing high performance liquid chromatography (DHPLC) (198). This method identifies DNA sequence variations by altered interaction of the DNA to the column matrix, visualised as additional peaks in the chromatogram. Intronic sets of oligonucleotide primers were designed according to the published genomic sequence of the genes.

In the analysis of the *MYH7*, only the 24 first exons where examined, whereas every coding exon of the 8 other genes was amplified by “touch down” polymerase chain reaction (PCR) and then subjected to a SSCP or DHPLC analysis. For SSCP analysis of *MYH7, MYL2* and *TNNI3*, the PCR products were heat denatured at 94°C for 5 minutes in a saccharose buffer, and resolved on a 10% polyacrylamide gel (acrylamide/bisacrylamide ratio of 37.5:1) at 8mA per gel (10 cm) in 0.8X Tris-borate-EDTA (TBE) buffer, run at 7°C and 25°C. After migration, DNA was visualised by silver staining (Pharmacia, Uppsala, Sweden) of the gels (199). SSCP analysis of *MYBPC3* was carried out using an automated laser fluorescence system (ALF, Pharmacia, Uppsala, Sweden). Composition of the gels and running conditions were the same as described above, except that the PCR fragments were denatured in formamide buffer and the gels were run at 38 mA. Both forward and reverse primers were fluoresceine-labelled and sequence variations were identified as abnormal fluorescence peaks. Sometimes, running at 7°C yielded technically unsatisfactory results and those exons were reanalysed on standard gels.

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

Heart rate variability (paper III)

Short-term HRV

Short-term recording of HRV was performed in the following manner: After 10 minutes supine rest, the blood pressure was measured and a continuous recording of ECG and respiration (using a thoracic belt) was started. Free spontaneous breathing was continued for 6 minutes, the subjects were then instructed to perform controlled breathing at a rate of 12 breaths per minute during one minute. After passive tilting to 70 degrees head-up position, the recording was continued during four minutes, after which the blood pressure was measured again.

Spectral analysis was performed on segments without artefacts and arrhythmic beats. Recordings in the supine and upright positions were analysed as 2-min data, and as 1-min data in the sequence with controlled breathing. The R-R interval data was transformed to an evenly sampled (2 Hz) time series by cubic spline interpolation, and both the mean and linear trend was removed. The power spectral density was estimated by auto-regressive modelling (140), consequently using thirty parameters. The mean heart rate, the total spectral power (0.003-0.45 Hz) and the power of three different spectral components were calculated; the very low-frequency (VLF) component (0.003-0.04 Hz), low-frequency (LF) component (0.04-0.15 Hz) and high-frequency (HF) component (0.15-0.45 Hz). The LF/HF-ratio was calculated as an indicator of sympathovagal balance.
**Long-term HRV recording**

Twenty-four hour recording of HRV was performed using continuous ambulatory ECG monitoring, with a two channel tape recorder (Tracker 2, Reynolds Medical Ltd, UK). The ECG data was digitized and transferred for computer analysis. A commercially available software (Danica Holter Replay Unit, Danica Biomedical, Sweden) was used to detect pathological events, which were then manually edited by a technician.

The following time-domain variables were calculated. The average R-R interval value was calculated from accepted beats (mean RR). The overall HRV was determined by the standard deviation of all the NN (normal-to-normal) R-R intervals (SDNN), and the baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of NN intervals (TINN). The long-term HRV was quantified by the standard deviation of all 5-minute NN interval means (SDANN). Finally, the beat-to-beat HRV was estimated by the square root of the mean squared differences of successive NN intervals (RMSSD), and the ratio of NN interval differences of successive NN intervals greater than 50 ms to the total number of NN intervals (pNN50).

Power spectral analysis was performed on the R-R interval data by means of fast Fourier transformation. Spectral components (as defined for the short-term recordings) were calculated as average data over 24 hours, as well as hour by hour.

**Statistical methods**

Statistical analyses were performed with the SPSS software, version 10.0 and 11.5 (SPSS inc., Chicago, IL, USA). Continuous variables are presented as mean ± SD. Group differences for continuous variables were analysed by unpaired Student’s t-test (paper II and III) or by analysis of variance (ANOVA), without correction for multiple comparisons (paper III and V). Age dependence of different HRV variables was analysed by linear regression (paper III). All frequency-domain HRV indices were log-transformed because of skewed distributions (paper III). The chi-square test or Fisher’s exact test was used to compare non-continuous data expressed as proportions (papers II, III, IV and V). Interobserver variability (paper V) was calculated as the mean percentage error, derived as the difference between two sets of measurements, divided by the mean of the observations. The level of statistical significance was defined as a two-tailed p value <0.05.
Ethical aspects
In all studies, informed consent was obtained from each individual and the protocol was approved by the ethics committee of Umeå university.
RESULTS

Cardiac phenotype associated with a mutation in cardiac troponin I (Paper I)
The cardiac troponin I gene (TNNI3) is one of the more recently discovered genes that can cause HCM, at the time of the present study it had only been described in Japanese HCM patients. This study focused on describing the cardiac phenotypes in a family with an unusual mutation in the cardiac troponin I gene. Previous mutations reported in this gene have been missense mutations and one deletion (3 base pairs) not causing a frameshift. In this family, a deletion of 33 base pairs in the last exon (exon 8) of TNNI3 was identified as the cause of HCM. The deletion encompasses the last 24 nucleotides of exon 8, the stop codon and another 6 nucleotides further downstream. Four of nine family members were found to carry this mutation and three of them also fulfilled the criteria for familial HCM according to the criteria by McKenna et al (19). The mother was genetically affected and died of heart failure, 90 years old. A previous echocardiogram at 71 years of age did not show cardiac hypertrophy, but the ECG changes (LVH) were diagnostic for HCM. Her two sons, 61 and 64 years old, were also carriers of the TNNI3 deletion, both showed mild cardiac hypertrophy with a septum of 15 and 16 mm. They had no symptoms of heart failure. One of the grandchildren, a 27-year old asymptomatic woman, with normal ECG and echocardiogram, was found to be a healthy carrier of the same deletion. Thus, the associated disease phenotype in this family was generally mild, with incomplete penetrance and without the presence of severe symptoms or premature sudden death.

Spectrum of disease causing genes in northern Sweden (Paper II)
In this study, 46 unrelated patients with HCM from all parts of northern Sweden were screened for mutations in eight of 10 sarcomeric protein genes known to cause HCM. Eleven cases were probands (first identified case) in families with HCM and 35 were sporadic cases. The family in paper I was also included in this study. The aim of the study was to report the genotypic spectrum and associated phenotypes related to hypertrophic cardiomyopathy in northern Sweden.

In 13 of the 46 individuals in the study, mutations were found in four different genes. A total of 11 different mutations were detected, seven in the cardiac myosin binding protein C gene (MYBPC3) (64%), two in the cardiac β-myosin heavy chain gene (MYH7) (18%)
and one in the cardiac regulatory myosin light chain \((MYL2)\) and \(TNNI3\) genes (9%), respectively (Table 3). Six of the mutations were not previously described.

Mutations were identified in 91% of the familial HCM cases \((10/11)\), but only in 9% of the sporadic cases \((3/35)\). Of the detected mutations, eight were missense mutations, two represent frameshift mutations \((MYBPC3\) and \(TNNI3\)) and one was a nonsense mutation \((MYBPC3)\). Missense mutations lead to the substitution of a single amino acid residue in the final protein product, whereas frameshift and nonsense mutations usually cause truncated protein products, by introducing premature stop codons in the DNA sequence. Not all mutations were definitely characterized as disease causing. Eight of the 11 mutations found were considered to have a strong association with the development of HCM, whereas two mutations were possibly linked to the disease and one mutation was judged to have a weak association with HCM, probably being a benign polymorphism. No disease causing mutation was found in the cardiac troponin T \((TNNT2)\), \(\alpha\)-tropomyosin \((TPM1)\), cardiac essential myosin light chain \((MYL3)\) or cardiac \(\alpha\)-actin \((ACTC)\) genes.

The phenotypes in patients with mutations were those of classical HCM, with asymmetric septal or concentric hypertrophy. The only significant difference in clinical or echocardiographic parameters between sporadic and familial cases was that sporadic cases had more dyspnea than the familial ones \((74\% vs 36\%, p=0.025)\) (Table 4). The study sample was to small to draw any conclusions regarding variations in phenotypic expression between the different mutated genes. Thirty-two individuals were found to carry a mutation in one of the sarcomeric protein genes, and seven of them \((22-59\) years old) carried mutations without showing evidence of hypertrophy in the ECG or at echocardiography (Table 5). Thus, 22% were healthy mutation carriers. The age at diagnosis in the 46 cases ranged from 20 to 78 years. Eleven patients were diagnosed at \(>65\) years of age, showing that HCM can be encountered in patients of all ages.

**Heart rate variability in hypertrophic cardiomyopathy (Paper III)**

This study sought to evaluate cardiac autonomic function in hypertrophic cardiomyopathy by assessment of heart rate variability. A reduced heart rate variability has been shown to be a risk factor for adverse events in some cardiac diseases, but is not a proven risk indicator in hypertrophic cardiomyopathy. Different methods are currently used to measure HRV, but there is still no general agreement or recommendation when short-term
<table>
<thead>
<tr>
<th>Individual number</th>
<th>Gender</th>
<th>Age at diagnosis (yrs)</th>
<th>Gene</th>
<th>Mutation</th>
<th>Novel/known</th>
<th>Disease association</th>
<th>Familial/ sporadic case</th>
<th>Symptoms</th>
<th>LA (mm)</th>
<th>IVSD (mm)</th>
<th>LVPWD (mm)</th>
<th>IVSD/ LVPWD (mm)</th>
<th>LVEDD (mm)</th>
</tr>
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<tr>
<td>I</td>
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<td>53</td>
<td>MYBPC3</td>
<td>Tyr 237 Ser</td>
<td>Novel</td>
<td>Strong</td>
<td>Familial</td>
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<td>16</td>
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<td>1.1</td>
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<tr>
<td>III</td>
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<td>45</td>
<td>MYBPC3</td>
<td>Arg 668 His</td>
<td>Novel</td>
<td>Strong</td>
<td>Sporadic, D, P</td>
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<td>18</td>
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<td>1.8</td>
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<tr>
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<td>Possible</td>
<td>Familial, D, P</td>
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<td>17</td>
<td>15</td>
<td>1.1</td>
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<td>Female</td>
<td>57</td>
<td>MYBPC3</td>
<td>Lys 565 Stop</td>
<td>Novel</td>
<td>Strong</td>
<td>Familial, P, S</td>
<td>39</td>
<td>16</td>
<td>8</td>
<td>2.0</td>
<td>2.0</td>
<td>48</td>
</tr>
<tr>
<td>VII</td>
<td>Male</td>
<td>26</td>
<td>MYBPC3</td>
<td>(del CG, ins TCT 852)</td>
<td>Novel</td>
<td>Strong</td>
<td>Familial, P, S</td>
<td>39</td>
<td>28</td>
<td>12</td>
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<tr>
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<td>(del CG, ins TCT 852)</td>
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<td>Strong</td>
<td>Familial, P</td>
<td>39</td>
<td>19</td>
<td>17</td>
<td>1.1</td>
<td>1.1</td>
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<tr>
<td>IX</td>
<td>Male</td>
<td>44</td>
<td>MYBPC3</td>
<td>(del CG, ins TCT 852)</td>
<td>Novel</td>
<td>Strong</td>
<td>Familial, AP, P</td>
<td>34</td>
<td>23</td>
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<td>73</td>
<td>MYH7</td>
<td>Ala 430 Glu</td>
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<td>Strong</td>
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<td>14</td>
<td>1.3</td>
<td>1.3</td>
<td>50</td>
</tr>
<tr>
<td>XI</td>
<td>Female</td>
<td>67</td>
<td>MYH7 + MYBPC3</td>
<td>Glu 924 Lys + Val 896 Met</td>
<td>Known + known</td>
<td>Strong + known</td>
<td>Familial, D, P</td>
<td>38</td>
<td>14</td>
<td>9</td>
<td>1.5</td>
<td>1.5</td>
<td>41</td>
</tr>
<tr>
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<td>67</td>
<td>MYL2</td>
<td>Arg 58 Gln</td>
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<td>47</td>
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<tr>
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<td>Male</td>
<td>64</td>
<td>TNNI3</td>
<td>del 33 nt, 202</td>
<td>Known *</td>
<td>Strong</td>
<td>Familial, None</td>
<td>49</td>
<td>16</td>
<td>14</td>
<td>1.1</td>
<td>1.1</td>
<td>48</td>
</tr>
</tbody>
</table>

P – Palpitations, S – Syncope, D – Dyspnea, AP – Angina pectoris, LA – Left atrium, IVSD – Interventricular septum dimension in end diastole, LVPWD – Left ventricular posterior wall dimension in end diastole, LVEDD – Left ventricular end diastolic diameter, *– Maximal wall thickness 20 mm, in lateral free wall. * Previously identified by our group (in paper I).
Table 4. Comparison of phenotypes in familial and sporadic cases of HCM

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Familial, n=11</th>
<th>Sporadic, n=35</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>7M/4F</td>
<td>18M/17F</td>
<td>0.49</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>58.6 ± 11.3</td>
<td>62.5 ± 13.5</td>
<td>0.38</td>
</tr>
<tr>
<td>Age at diagnose (yrs)</td>
<td>46.7 ± 13.6</td>
<td>54.1 ± 15.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>36%</td>
<td>74%</td>
<td>0.025</td>
</tr>
<tr>
<td>Angina</td>
<td>18%</td>
<td>29%</td>
<td>0.48</td>
</tr>
<tr>
<td>Palpitations</td>
<td>73%</td>
<td>59%</td>
<td>0.42</td>
</tr>
<tr>
<td>Syncope</td>
<td>18%</td>
<td>21%</td>
<td>0.87</td>
</tr>
<tr>
<td>Previous myectomy</td>
<td>18%</td>
<td>14%</td>
<td>0.76</td>
</tr>
</tbody>
</table>

| Echocardiography    |                |                |         |
| LA (mm)             | 41.3 ± 5.7     | 45.9 ± 8.2     | 0.10    |
| IVSD (mm)           | 17.1 ± 2.4     | 19.1 ± 4.1     | 0.13    |
| LVPWD (mm)          | 12.7 ± 2.9     | 14.0 ± 3.6     | 0.28    |
| IVSD/LVPWD >1,3     | 36%            | 58%            | 0.23    |
| LVEDD (mm)          | 43.8 ± 4.7     | 44.5 ± 6.3     | 0.72    |
| FS (%)              | 41.8 ± 11.8    | 41.6 ± 8.8     | 0.95    |
| LVOT-obstruction    | 36%            | 34%            | 0.67    |
| SAM                 | 18%            | 34%            | 0.32    |

| ECG                 |                |                |         |
| LVH*                | 36%            | 50%            | 0.44    |

LA – Left atrium, IVSD – Interventricular septum dimension in end diastole, LVPWD – Left ventricular posterior wall dimension in end diastole, LVEDD – Left ventricular end diastolic diameter, FS – Fractional shortening, LVOT-obstruction – Left ventricular outflow tract obstruction >30 mmHg at rest or > 50 mmHg under stress, SAM – Systolic anterior motion, LVH* – Left ventricular hypertrophy, defined as Romhilt-Estes score ≥ 4.

Data given as mean ± SD or n (%).

or long-term HRV registrations should be used (140). Because of lack of comparative studies, we evaluated the results from a short-term laboratory method with an ambulatory (24-hour) method in the same patients with HCM. Patients with HCM are frequently being treated with beta-blockade and another objective was to study the effects of beta-blockade on different HRV parameters.
Table 5. Maximal wall thickness in genotype positive individuals

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Maximal wall thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
</tr>
<tr>
<td>8</td>
<td>4.0</td>
</tr>
<tr>
<td>9</td>
<td>4.5</td>
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<tr>
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<tr>
<td>29</td>
<td>14.5</td>
</tr>
<tr>
<td>30</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Seven of the 32 individuals carrying a sarcomeric protein gene mutation had a maximal wall thickness of <13mm and did not fulfil the diagnostic criteria for HCM.

**Short-term method**

Young HCM patients (<40 yrs) without beta-blockade had statistically significantly lower total spectral power, lower HF power and increased LF/HF-ratio during one or more of the three procedures (supine rest, controlled breathing and upright tilt) compared to controls. Young HCM patients treated with beta-blockade did not show any significant differences in total spectral power, HF power or LF/HF-ratio compared to controls. Nor did the middle-aged and old HCM patients (with or without beta-blockade) show any significant differences in the same HRV parameters, compared to controls.
**Long-term method**

Spectral analysis of both the hourly averages and the 24-hour total average showed that young HCM patients without beta-blockade had statistically significantly lower total-, HF- and LF-power compared to controls, whereas the middle-aged and old subjects did not show any significant differences in these parameters.

Analysis of time-domain indices over 24 hours showed statistically significantly lower values for NNmean, SDNN, TINN, RMSSD and pNN50 only in the young HCM patients without beta-blockade compared to controls.

Patients treated with beta-blockade showed no significant differences in time-domain indices in young or middle-aged patients, compared to controls. In the spectral analysis, only LF- and HF-power remained significantly lower in young patients, both in the hourly averages and the average over 24 hours. Spectral power and time-domain indices did not correlate in a systematic way with either echocardiographic findings or symptoms.

The findings indicate a decreased parasympathetic activity in the young patients, the signs of which were attenuated in the group treated with beta-blockade. The findings were similar in the short- and long-term registrations.

**Transthyretin gene mutations in hypertrophic cardiomyopathy (Paper IV)**

Familial amyloid polyneuropathy (FAP), Portuguese type, is caused by a mutation in the transthyretin gene (ATTR Val30Met), with symptoms of peripheral polyneuropathy and frequent gastrointestinal, kidney and heart involvement. Conduction defects are the typical heart manifestations, but cardiac hypertrophy may also be present. In a minority of patients with FAP, severe cardiac hypertrophy has been described. This study was initiated by the finding of an interesting index case with hypertrophic cardiomyopathy, without symptoms of polyneuropathy, where the cardiac hypertrophy was shown to be caused by cardiac amyloidosis. The aim of the study was to investigate if the hypertrophy in some patients primarily diagnosed with HCM, in fact can be explained by an unusual phenotypic expression of familial amyloid polyneuropathy, characterized by predominating cardiac hypertrophy. The patient group was the same as in paper II.

In three of the 46 individuals (7%) and the index case, one missense mutation in the transthyretin gene was detected, identical with the mutation found in Swedish patients with FAP. A G→A transition in exon 2 of the transthyretin gene substitutes valine for
methionine at residue 30 (ATTR Val30Met). The three mutation carriers were all elderly sporadic HCM cases and were all deceased within eight years from HCM diagnosis. None of them had symptoms of polyneuropathy. The index case and two of the other ATTRVal30Met positive cases had conduction disturbances and received pacemaker, while none of the 43 HCM patients without this mutation had severe conduction disturbances (P=0.001, Fisher’s exact test). The patients did not carry a mutation in any of the eight HCM genes previously studied in paper II. Initial biopsies from abdominal fat and rectum in the index case did not show amyloid deposits. Still, cardiac amyloidosis was verified in the index case by a myocardial biopsy that showed small myocytes and rich deposits of amyloid that reacted with an anti-TTR antibody.

Right ventricular function in hypertrophic cardiomyopathy studied by myocardial performance index (Paper V)

Abnormalities in systolic and diastolic cardiac function have previously been described in patients with hypertrophic cardiomyopathy. The clinical interest has mainly been focused on the LV function. More recently, the importance of right ventricular function in heart failure has been recognized. However, echocardiographic evaluation of the RV function has been limited by the complex geometry of the RV. Therefore, non-geometric echocardiographic methods, such as the myocardial performance index (MPI), tricuspid annular plane systolic excursion (TAPSE) and tricuspid annular peak systolic velocity have been developed. The MPI combines systolic and diastolic cardiac performance by expressing the isovolumic timings in the cardiac cycle in relation to the ejection time. This index increases in the presence of cardiac dysfunction and has shown prognostic value in other cardiac conditions, but has not been evaluated in patients with hypertrophic cardiomyopathy. In this study, global and regional ventricular function in HCM was studied by conventional Doppler and tissue Doppler imaging, using the MPI and two other non-geometric methods.

From conventional Doppler measurements, global RV and LV myocardial performance indices were significantly higher in the patient group, than in the controls. Both isovolumic contraction and relaxation time intervals were prolonged in the patient group, whereas ejection time was decreased. Patients being symptomatic with dyspnoea had a tendency towards an increased global RV MPI, compared to patients without dyspnoea,
(MPI 0.54 vs 0.42, $p=0.059$). TAPSE and tricuspid annular peak systolic velocity, both measures of global systolic RV function, were reduced in the patients. Regional myocardial function was studied by tissue Doppler imaging. A regional MPI was measured at the basal segment of the LV lateral, septal and RV free wall. In patients, this index was elevated at the septal and RV free wall sites, compared to controls. Apart from the expected LV dysfunction, this study shows the presence of global and regional RV dysfunction in HCM, both in systolic and diastolic function.
DISCUSSION

Genotype-phenotype correlations (Papers I, II and IV)

Cardiac phenotype associated with a mutation in cardiac troponin I (Paper I)

Troponin I is a constituent protein (with inhibitory function) of the troponin complex located on the thin filament of striated muscle that provides a calcium-sensitive switch for contraction. Most of previously described mutations in troponin I in HCM are located in the C-terminal part of the protein, which shows a high degree of conservation between different isoforms, whereas the N-terminal part of the protein is more divergent. The molecular mechanisms of cardiac hypertrophy caused by troponin I mutations remains unclear, but mutations in the C-terminal part of the protein might affect the inhibitory function and lead to an increased contractility of myocytes. This could lead to cardiac hypertrophy as a compensatory phenomenon.

At the protein level, the mutation found in this study will lead to a deletion in the C-terminal part of the protein, encompassing the last 8 amino acids, the termination codon, and generate 19 abnormal amino acids before the next UGA stop codon. Previously, mutations in this gene have been described only in the Japanese population (160), five missense mutations and a 3 base pair deletion, which does not disrupt the DNA reading frame. In this study, we present the first non-Japanese family with a mutation in the troponin I gene, which is also a new type of genetic defect in this gene as it is a 33 base pair deletion. The family described here showed HCM with a mild phenotype, without severe hypertrophy or premature death. The mother in the family did not show hypertrophy on echocardiography at the age of 71 years, but HCM was diagnosed by the ECG, showing signs of LVH. The mild phenotype is further exemplified by the presence of a 27-year old grandchild, who was found to be a healthy mutation carrier. This suggests that the deletion has either less influence on the function of the protein, or others factors, such as environmental or genetic factors (modifier genes) may influence the severity of the phenotype.
Almost all familial HCM in northern Sweden is caused by four of the known HCM genes, the MYBPC3, MYH7, MYL2 and TNNI3 genes. A mutation was identified in 10 of 11 cases that had a definite family history of HCM. The MYBPC3 is the major gene for familial HCM in northern Sweden, accounting for 64% of the mutations. Two of the MYBPC3 mutations detected in the familial cases were also found in individuals with sporadic HCM. The population in northern Sweden is genetically homogenous and the high frequency of mutations in MYBPC3 might partly be explained by founder effects. One mutation, the delCG/insTCT 852, was found in three unrelated individuals and a subsequent haplotype analysis suggested the presence of a common founder. Furthermore, one missense MYBPC3 mutation (Arg668His) was found in two unrelated individuals from different geographic regions. These results challenge the present generally accepted view that the MYH7 gene is the most common cause of familial HCM, while in this study it only represented 9% of the mutations found in familial cases. This is in concordance with a recent study from Finland, which also showed that the MYBPC3 gene was predominant in eastern Finland (200). The results in this study are based on observations in 46 individuals, constituting the majority of correctly diagnosed HCM patients seen by cardiologists in northern Sweden. Patients only seen by non-cardiologists would not be identified by the search system used and is a limitation of the study.

Sporadic cases

The proportion of sporadic cases in this study was higher than expected (35 of 46 individuals) and only three of them were found to have a mutation in one of the HCM genes studied (Table 3). The reason for the high proportion of sporadic cases is not clear. When including patients in the study, an effort was made to include all available cases, both sporadic and familial, regardless of age. It is possible that some of these sporadic cases represent the subset of elderly-onset HCM, which usually occurs as a sporadic disease (122). At the time of diagnosis, 11 patients (24%) were > 65 years old.

Clinical evaluation of the mutations

Not all mutations found can automatically be regarded as disease causing. The clinical significance of a missense mutation should be interpreted with some caution, especially in the MYBPC3 gene, since the distinction between a rare (but benign) polymorphism and
disease causing mutation is not always evident. When interpreting the possible disease association for each mutation, the following factors were therefore taken into account: A, if the mutation cosegregates with the disease in the family. B, if the mutation has been found in the healthy controls. C, the degree of conservation in the amino acid sequence. In this study, all but three mutations were considered to have a strong disease association. These three mutations (Arg326Gln, Ala833Thr and Val896Met), where there was a doubt about their correlation to the disease, were all missense mutations in the MYBPC3 gene. The Arg326Gln mutation was considered to have a possible disease association in this study, since it had been found in healthy controls in other studies (122) (200) (but not in our controls). The novel Ala833Thr mutation was also considered to have a possible disease association in this study, since the mutation was found in one of our healthy controls and the phenotype assessment in the family was obscured by hypertension in two subjects.

The Val896Met mutation was considered to have a weak disease association in this study. It was previously reported as a disease causing mutation in the South African population (201). In our study, it was found in a 67-year old woman with obstructive, familial HCM, who was found to be double heterozygous, with a missense mutation in MYH7 gene (more likely disease causing) as well. The Val896Met mutation was found in the healthy controls in this study and another study (200). This amino acid residue was also less conserved than in the other mutations found. Altogether, the evidence suggests that it represents a non-disease related polymorphism in the population of northern Sweden.

Conclusion

In conclusion, MYBPC3 is the most common gene for familial HCM in northern Sweden, a gene associated with a mild, late onset disease (low penetrance). The reduced penetrance associated with this gene emphasizes the importance of adequate family screening when evaluating new cases with HCM, since the familial nature of the disease might easily be overlooked. Information about the genotype in a family could therefore be useful, especially when dealing with healthy family members, in doubt about their risk of developing the disease. As a result of this study, a genetic counselling program for HCM families has been initiated at our hospital.
Transthyretin gene mutations in hypertrophic cardiomyopathy (Paper IV)
In the present study we demonstrate that amyloidosis can present itself as HCM. The classical cardiac manifestation in amyloidosis is considered to be that of a restrictive cardiomyopathy. Therefore, patients presenting with HCM are most probably not investigated for cardiac amyloidosis. However, the distinction between hypertrophic and restrictive cardiomyopathy is not always obvious from non-invasive investigations, since different ventricular filling patterns may occur in HCM and cardiac hypertrophy may be present in restrictive cardiomyopathy. Cardiac amyloidosis is progressive and potentially fatal, but can be treated with liver and heart transplantation.
Three of the 46 HCM patients (7%) and the index case were identified with the ATTR Val30Met mutation, associated with FAP. They did not have any signs of polyneuropathy and had not been diagnosed with FAP. Two of the study patients and the index case had conduction disturbances, necessitating pacemaker implantation. The three study cases were elderly sporadic cases and were all deceased, within eight years from HCM diagnosis.
It is interesting to note that amyloid in peripheral tissues in the index case was detected only after repeated biopsies. Still, cardiac amyloidosis could be confirmed by myocardial biopsy. Thus, neither a negative skin/subcutaneous fat biopsy nor a negative rectal biopsy excludes systemic amyloidosis, contrary to the current opinion. As a correct diagnosis is mandatory for a potentially life saving treatment, transthyretin mutations should be considered in cases of hypertrophic cardiomyopathy not explained by mutations in sarcomeric protein genes. Elderly, sporadic cases of HCM with conduction disturbances should be especially suspected of having cardiac amyloidosis.

Heart rate variability in hypertrophic cardiomyopathy (Paper III)
Impairment of vagal autonomic regulation was found to be a prominent feature in young HCM patients without beta-blockade. When short- and long-term HRV registrations were compared, the frequency-domain indices yielded similar information, most importantly a decrease in total and HF power, as well as an increase in the LF/HF-ratio in young patients.
**Effect of beta-blockade**

Patients treated with beta-blockade showed higher HRV in parameters reflecting vagal tone, compared to non-treated patients. Several mechanisms may contribute to the effects of beta-blockade, including a direct effect on beta-adrenoreceptors with inhibition of sympathetic over activity. It has been proposed that the relative increase in vagal tone contributes to the beneficial effect of beta-blockade treatment on prognosis in heart failure and after myocardial infarction (202-204).

**Effect of age**

HRV decreases with age in normal individuals (205), which was not the case for the HCM patients, who showed a more constant level, or even increase in many of the HRV parameters with age. It is possible that arrhythmias, such as supraventricular ectopic beats late in the cardiac cycle, which may not be classified as premature beats, could contribute to an increase in HRV and partly explain why the differences in HRV between patients and controls were not significant in middle-aged and old patients.

**Comparison with previous studies**

Previous studies on HRV in HCM have shown wide discrepancies and even contradictory results (Table 2). A decreased global and/or parasympathetic activity is the most frequent finding in previous studies, as in the present one. Apparent problems in comparisons between HRV studies are the lack of standardisation of the methods (140), the varying severity of the disease in the patients from different studies and the possible residual effect of cardioactive drugs such as beta-blockade.

It has been hypothesized that the autonomic nervous system (ANS) may play a role in the development of HCM, with an increased sympathetic activity in the heart, evidenced by a downregulation of myocardial beta adrenoreceptors (80) and locally increased levels of noradrenaline (81). This could result in reduced efferent vagal-cardiac firing, with a subsequent reduction in HRV, which might also be a rationale for treating HCM patients with beta-blockade.

**Conclusion**

In conclusion, reduced HRV, is a risk factor for adverse events in some cardiac diseases, but is so far not a proven risk indicator in HCM. There is evidence that parasympathetic activity is decreased in HCM, especially in young patients, which may be of clinical relevance as a substrate for lethal arrhythmias, most often encountered in younger
patients. The reduction in HRV was attenuated by beta-blockade, suggesting the need for further studies to assess HRV as a risk factor in HCM and the possible protective effect of beta-blockade. The different HRV methods used in this study, short- and long-term registrations, as well as time- and frequency-domain indices, showed similar results, with signs of reduced overall and beat-to-beat variability in young patients, both largely dependent on vagal modulation. Furthermore, the reduced HRV found in the short-term registrations was significant already during supine rest, suggesting that short-term HRV might be sufficient to assess cardiac autonomic function in patients with HCM.

Right ventricular function in hypertrophic cardiomyopathy studied by myocardial performance index (Paper V)

Hypertrophic cardiomyopathy has mainly been regarded as a disease of the LV. It was therefore not surprising to find signs of impaired LV function in this study. However, RV function has gained increasing attention in evaluating patients with heart failure and has been shown to be of clinical importance both in terms of morbidity and mortality. This study demonstrates the presence of both global and regional RV dysfunction in HCM. Previous echocardiographic studies have not demonstrated systolic RV dysfunction in HCM, but we find evidence of systolic dysfunction expressed as an increased MPI, decreased TAPSE and decreased tricuspid annular peak systolic velocity. Right ventricular diastolic abnormalities in our study are in concordance with previous findings. The RV dysfunction seen is suggested mainly to be a primary phenomenon. Right ventricular hypertrophy in HCM has previously been demonstrated by echocardiography and myocardial disarray has been shown to be present in all parts of the LV, as well as in the RV. Therefore, RV dysfunction may be caused by the direct involvement of the myopathic process in the RV wall. In cases of severe LV dysfunction, RV function might also be affected by an interdependence of the hypertrophied LV, in particular the interventricular septum, which is shared by both ventricles.

Interestingly, there was a tendency for patients with exertional dyspnoea to have an increased global RV myocardial performance index compared to patients without dyspnoea. This was not seen in the LV, which emphasizes the importance of functional studies of both ventricles in patients with HCM.
Contrary to previous studies, we show the presence of both global and regional RV systolic dysfunction in HCM. This study shows that HCM is not just a disease confined to the LV, but should be regarded as a biventricular disease.
CONCLUSIONS

The myosin binding protein C gene (MYBPC3) is the most common gene causing familial HCM in northern Sweden. This gene constitutes 64% of the mutations found and is known to be associated with a mild, late onset disease (only ≈50% penetrance at 30 years of age). The reduced penetrance emphasizes the importance of adequate family screening when evaluating patients with HCM, since the familial nature of the disease might easily be overlooked. Most previous reports from other countries have reported another gene, the cardiac β-myosin heavy chain gene as the most common cause of HCM. That gene is in general, associated with much higher penetrance and more severe disease, compared to MYBPC3.

A disease causing mutation is identified in 91% of familial HCM cases. Of the 11 cases with a definite family history of HCM, a mutation was found in 10 cases, in four different sarcomeric protein genes.

Knowledge about the genotype has clinical applications. Genetic counselling is now available at Umeå University hospital for the HCM families where the disease causing mutation is known. In clinical practice, one must be aware of the low penetrance associated with mutations in the most common gene, the MYBPC3.

A disease causing mutation is identified in only 9% of sporadic HCM cases. Only three of 35 sporadic HCM cases carried a mutation in a sarcomeric protein gene.

Sporadic HCM cases are more common than familial ones. In our study, 24% of the cases were familial and 76% were sporadic. Previous reports have suggested that HCM is familial in 55% of the cases. The reason for this distribution in northern Sweden is not known. Perhaps we have recruited a larger proportion of elderly patients (“elderly-onset HCM”), which is most often a sporadic form of HCM.
Sporadic and familial HCM cases have similar phenotypes. ECG and echocardiographic characteristics did not differ in a significant way between sporadic and familial cases.

Amyloid heart disease can mimic HCM. Three of the 46 HCM cases (7%) were found to carry the ATTR Val30Met mutation, associated with familial amyloidotic polyneuropathy. Cardiac amyloidosis was verified by a myocardial biopsy in one index case.

There is evidence of autonomic dysfunction in HCM. Young patients with HCM had signs of autonomic dysfunction, expressed as reduced heart rate variability. Treatment with beta-blockade attenuated these effects, suggesting a possible protective effect of beta-blockade, remaining to be studied further.

Right ventricular function is disturbed in HCM. Patients with hypertrophic cardiomyopathy have evidence of global and regional dysfunction in the left and right ventricles, expressed as an increased myocardial performance index. The results show that hypertrophic cardiomyopathy should be regarded as a biventricular disease. Attention must also be paid to the right ventricle when evaluating cardiac function in HCM.

A glimpse into the future
The studies so far have been descriptive in nature. A cohort of patients with HCM has been recruited from all parts of northern Sweden and their genotypes and phenotypes have been presented as cross-sectional studies. Genetic counselling in HCM is a reality, but can be further developed in the clinical management of HCM. By following the patients prospectively, we hope to learn more about the clinical consequences of carrying different mutations, as well as the consequences of alterations in heart rate variability and right ventricular function.
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