I

General Introduction
This chapter will provide a brief and general introduction into the morphological and functional abnormalities of hypertrophic cardiomyopathy (HCM) at different levels; First, an overview of macroscopical changes of the HCM heart will be discussed. Secondly, a section will zoom in from the macroscopical to the microscopical perspective. Then, since this thesis includes both clinical and pre-clinical work, the smallest contractile unit of the heart muscle, the sarcomere, shall be addressed.

The direct translation of hypertrophic cardiomyopathy (HCM) leads to a clear picture of this spectacular disease; pathological thickening ('hypertrophy') of the heart muscle ('cardiomyo'). HCM is a very heterogeneous disease from a genetic, morphological and clinical point of view [1]. Over a 1000 mutations have been linked to HCM, that predominantly encode thick and thin myofilaments of the cardiac sarcomere. Most frequently, myosin binding protein-C (MyBP-C) and β-myosin heavy chain (β-MyHC) mutations are encountered [2]. Since HCM has an autosomal dominant pattern of inheritance, offspring has a 50% chance to carry the mutated gene [3]. The penetration of these mutations is usually approximately 60-70% [2] but differs substantially, even within families. Therefore, at least in part, the phenotypic expression ranges from virtually absent towards extreme left ventricular hypertrophy but other morphological components besides the cardiac muscle may also be involved. Finally, the clinical course of the disease is highly variable and unpredictable; most patients tend to have a benign course, in contrast to others, who develop severe heart failure symptoms at young age or even experience sudden cardiac death (SCD). In this thesis, we aimed to unravel the pathophysiological mechanisms that ultimately lead to the development of HCM. For this purpose, we used advanced, high resolution imaging techniques such as cardiovascular magnetic resonance imaging (CMR).
Hypertrophic cardiomyopathy - the macroscopical level

Unexplained left ventricular hypertrophy

The (morphological) definition of HCM is based upon the presence of unexplained left ventricular (LV) hypertrophy (with a LV wall thickness of ≥ 15mm as an obligatory criterium), in the absence of loading conditions such as hypertension and aortic valve stenosis [1]. The phenotypic manifestation of HCM is very diverse; virtually any segment of the myocardium might be involved. Although single segment involvement is seen in the majority of patients [4], a more extensive pattern of hypertrophy has also been described [5]. Since regional LV hypertrophy predominates HCM phenotype, this presumably explains the observation that approximately 20% of manifest patients have normal LV mass [1]. Most frequently, the anterior portion of the subvalvular interventricular septum is thickened, resulting in the typical appearance of asymmetric septal hypertrophy below the level of the aortic valve. Other phenotypic variants include apical hypertrophy and a more diffuse or concentric pattern of LV hypertrophy [5]. In case of pronounced midventricular thickening of the LV, near obliteration of the ventricle during systole may result in an ‘hourglass’ configuration, which is often accompanied by an intraventricular pressure gradient. It is hypothesized that these midventricular gradients are important contributors to the formation of apical aneurysms in HCM, which are associated with an unfavourable prognosis [6].

Mitral valve abnormalities and papillary muscle changes

Although HCM results from sarcomeric mutations in cardiomyocytes, structural malformations of papillary muscles and mitral leaflets are also associated with this
genetic heart disease. Abnormalities confined to the mitral leaflets predominantly include mitral leaflet elongation and increased leaflet area [7]. Morphological changes of the papillary muscles are a direct insertion into the mitral leaflet [8], anteroapical displacement of the anterolateral papillary muscles and a double bifid morphology [7].

**Scarring**

Autopsy studies pointed out that overt HCM is associated with scarring of the HCM muscle, ranging from single spots to extensive fibrotic tissue deposition. The exact mechanism responsible for the increased collagen synthesis (e.g. the formation of interstitial and/or replacement fibrosis) remains to be clarified, but early intracellular activation of pro-fibrotic pathways as a direct consequence of perturbated sarcomeric function or ischemia-induced apoptosis of cardiac myocytes have been proposed [9]. Ischemia in HCM is most likely caused by several interacting determinants, such as microvascular abnormalities and extravascular compressive forces generated by hypertrophied myocardium surrounding the small arteries [10].

A special additive feature of CMR is the determination of the so-called 'fourth dimension', which includes the characterisation of tissue.

When prominent and macroscopically present, this scarring or interstitial fibrosis can be visualized non invasively using CMR late Gadolinium enhancement (LGE), a widely appreciated imaging technique [11] that enhances myocardial signal intensity when increased extracellular space is focally present (e.g. due to fibrosis). LGE uses Gadolinium-DTPA as a paramagnetic contrast agent, which tends to accumulate in tissue with expansion of the extracellular space, such as oedema or fibrosis (see Figure1). The majority of HCM patients with manifest phenotype show areas of
patchy enhancement. A subset of patients develops ‘end-stage’ disease, which is characterized by extensive scarring and systolic dysfunction [12].

Compared to LGE, a newer technique is T1 mapping, which allows absolute T1 value measurements in any region of the myocardium. As a result, the myocardial extracellular volume fraction (ECV) may be calculated, which represents the amount of interstitial (and not regional) fibrosis [13]. Besides, regional contraction and relaxation can be determined with the use of CMR tissue tagging. Tagging applies a grid of saturated tissue, allowing the visualization of myocardial contraction and relaxation throughout the entire cardiac cycle [14]. The latest imaging techniques rely on hybrid imaging, where two modalities are combined, such as CT and PET, or CMR and PET. This allows the investigation of intra-patient relations between morphology and function for example, often within one acquisition.

Figure 1A. A four-chamber LGE image in a HCM patient, showing extensive intramural fibrosis in the interventricular septum, depicted in white (and indicated by the white arrow). B. This LGE short-axis image, planned perpendicular on image 2A, depicts patchy fibrosis (reflected by the white colourations within the myocardium) in the interventricular septum (again indicated by white arrow).

*Intramyocardial crypts*

The majority of research within this thesis has been based upon the use of cardiovascular magnetic resonance imaging (CMR). This imaging modality is known for it’s...
high spatial resolution, allowing the depiction of subtle abnormalities such as crypts [15]. The first histological description of crypts in HCM was in the late 50’s by Teare [16]. He published a case series of 8 suddenly diseased young individuals showing extensive asymmetric LV hypertrophy. Teare, the first to describe (modern) HCM with this article, noticed ‘clefts’ or ‘fissures’ between myocardial muscle bundles in two of these patients. Decades later, Kuribayshi and Roberts [17] described identical deep tissue invaginations in histological HCM specimens. Besides, the authors showed examples of ‘deep endocardial notches at junctional areas’ in explanted HCM hearts, which were clearly visible for the naked eye. The Kuribayashi group confirmed that these invaginations were lined with simple-layered endothelium and that adjacent myocardial tissue showed marked fascicle disarray.

Not until 2006, Germans et al [15] revealed these subtle disruptions at the inferoseptal myocardium with cardiovascular magnetic resonance imaging (CMR), in HCM mutation carriers (‘carriers’) without hypertrophy. These ‘crypts’ were seen with high prevalence (~70-80%), using a tailored CMR protocol including angulated cine images to optimize the detection rate [15]. After this report, several groups used various imaging techniques, and reported on crypts within the entire spectrum of disease [18,19]. By that time, the clinical implications of crypt formation and the specificity of HCM disease was unknown; these issues will be addressed in this thesis.
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**Hypertrophic cardiomyopathy - the microscopical level**

The histological hallmarks of HCM include the presence of fibrosis, (Figure 3A) myocardial disarray (which represents disruption of laminar myocardial architecture) and thickening of the medial layer of intramural arterioles (Figure 3B) [1,9]. None of these findings are specific for HCM however.

**Histology - fibrosis**

Already in the early eighties, biopsy studies evaluated the topography and specificity of fibrosis in HCM [20]. Using picrosirius staining, St John Sutton et al. demonstrated four distinct subtypes of intramyocardial fibrosis. The first subtype was the ‘microscopical scar’, which represents an area with replacement fibrosis visible for the naked eye. Secondly, ‘interstitial fibrosis’ was discerned, based upon small bundles of collagen surrounding the myofibers. The third subgroup consisted of perivascular fibrosis, which is collagenous connective tissue surrounding intramural small arteries, and finally, plexiform fibrosis was described, referring to a unique type of collagen situated in strands of myocardial disarray. Independent assessment of all these forms of fibrosis revealed that none was specific for HCM, but increased concentrations of connective tissue were identified in both the left and right
ventricle [21]. After the introduction of surgical myectomy (e.g. 'Morrow-procedure'), the opportunity arose to evaluate the subaortic myocardium in clinical HCM patients. Histology revealed small discrete foci of replacement fibrosis throughout the specimens, but only minor increments of interstitial connective tissue [22]. Routinely, endocardial fibrosis of the resected septal tissue (e.g. 'fibrous cap') is not qualitatively assessed, as it is the presumed consequence of alterations in outflow tract physiology. Attempts have been made to correlate fibrosis of myectomy samples to several parameters including maximum wall thickness and heart weight, but only mildly positive correlations were discovered [23].

![Figure 3](image)

Figure 3 (left panel). Elastic von Gieson (EVG) staining of HCM tissue, displaying pink strands of interstitial fibrosis (indicated by black arrows). (right panel). The asterisk (*) marks an area with myocardial disarray, characterized by malalignment of myocytes. The black arrows indicate thickening of the medial layer of small intramyocardial arterioles.

**Histology – myocardial disarray and arteriolar changes**

Microscopically, autopsy studies demonstrated besides fibrosis also evidence of myocardial disarray, hypertrophied cells with dismorphic nuclei and intramural thickening of small arterioles (see Figure 2B). Disarray, which is the unorganized and chaotic architecture of myofibers, has been detected in patients with several diseases, such as aortic stenosis and congestive cardiomyopathy [20]. In HCM,
disarray has been detected in all regions of the heart, but especially the septum was involved and severity of disarray seemed greater than in other parts of the heart [22].

**Hypertrophic cardiomyopathy - the level of the sarcomere**

The HCM muscle is based upon striated muscle cells, consisting of bundles of myofibrils. Advanced imaging techniques, such as electromicroscopy (EM), showed that myofibrils in turn were composed of serially aligned sarcomeres, which are the smallest contractile units of the heart. Experimental studies demonstrated that both myosin heavy and light chains form the backbone of the sarcomere apparatus, consisting of myosin and actin respectively. Since there is overlap of these chains at certain areas of the sarcomere, bands and lines with various intensity appear at EM pictures (see Figure 3). Protein analysis showed that the sarcomere is a complex structure, with various proteins interacting during contraction and relaxation, such as myosin binding protein C (cMyBP-C), myosin heavy chain (MYH7) and troponins (C, T and I). Besides, the largest protein found in humans, titin, plays an important role by stiffening the sarcomere, ensuring background strain during systole and diastole [24].

The function of the interacting sarcomeric proteins is regulated by post-translational modifications, such as phosphorylation. Various pathways, such as the β-adrenergic signalling route, modify and control the complex interplay of proteins during LV ejection [25]. The incorporation of mutant proteins into the sarcomere in HCM has been shown to alter kinetic properties of myofibrils [26,27]. The increased ATP consumption by dysfunctional sarcomeres is thought to play an important role in the pathogenesis of HCM [28]. A deficit in ATP would compromise the re-uptake of
calcium by the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase pump. As a result, increased diastolic calcium concentrations and prolonged cytosolic Ca\(^{2+}\) transients stimulate Ca\(^{2+}\)-dependent hypertrophic signaling pathways in HCM.

Figure 4. Electro-microscopical (EM) image of myofibrils in a genetically engineered HCM mouse. Notice the disturbance of normal alignment of fibers, resulting in a chaotic architecture.

**Clinical findings in HCM**

In general, the incidence of disease related adverse events seems to correlate with the extent of disease. The ongoing process of LV wall thickening often leads to the formation of regional hypertrophy of the interventricular septum below the aortic valve, generating obstruction of LV outflow [29]. As a result, heart failure symptoms may occur (especially during exercise), due to diminished blood flow during systolic ejection. Another substrate for the occurrence of heart failure is impaired relaxation of the heart muscle (e.g. diastolic dysfunction), which is often the combined result of thickening of the heart, formation of fibrosis, myocardial disarray, and ischemia due to arteriolar changes. Importantly, the presence of focal fibrosis has been related to supraventricular and ventricular arrhythmias such as atrial fibrillation and (non)-
sustained ventricular tachycardia, which is an established risk factors for SCD [30,31].

**Insights into the development of HCM and screening**

Cross-sectional studies have shown that HCM patients with advanced age generally display a more severe phenotype than younger individuals. This observation suggests an age-dependant process of pathological LV wall thickening in HCM [31], but most longitudinal studies failed to demonstrate significant increases in LV mass [32,33]. Nevertheless, early diagnosis and identification of patients at risk for developing manifest HCM is needed to ultimately prevent adverse events. Nowadays, advances in genetic screening yield an increasing amount of asymptomatic HCM mutation carriers. Guidelines regarding the optimal time-interval to monitor these patients differ substantially, and often carriers are lost to follow-up [34]. In daily clinical practice, the evaluation of progression of disease is regularly performed with echocardiography and/or electrocardiography. In few specialized centres, monitoring is performed with serial CMR acquisitions. Evaluation of carriers by state-of-the-art techniques such as CMR, may provide valuable insights into the natural development of disease. And maybe more important, it may elucidate risk factors for the hypertrophic response in these asymptomatic individuals, which are currently still unknown. This poses a large clinical challenge, necessitating studies with long-term follow-up.
Aim and outline of the thesis

The aim of this thesis was to provide insights into the development of LV hypertrophy in HCM, using CMR as a clinical assessment tool. For this purpose, both asymptomatic HCM mutation carriers and patients with manifest HCM were subjected to CMR investigations. Besides, genetically engineered mice underwent serial acquisitions and were ultimately sacrificed to increase our understanding of the complex pathophysiology of HCM.

The list below summarizes the content of the various chapters of this work.

The second chapter provides a review of HCM with a translational approach. Perturbations at the molecular and protein level are linked to in-vivo alterations in both mutation carriers and manifest HCM patients. Besides, future directions for research and therapy are given.

In the third chapter, the torsional deformation of the heart in mutation carriers is evaluated with CMR tissue tagging, and compared with controls. For the determination of torsion, study subjects under CMR tissue tagging examinations.

The fourth chapter focuses on the characterization of crypts in mutation carriers. Crypts are compared with those found in other patient groups and controls. Besides, the optimal imaging plane for the detection of these disruptions of normal cardiac architecture is evaluated. Subsequently, the importance of crypt detection in preclinical HCM is discussed in an editorial (Chapter 4.1).

The fifth chapter provides the outcome of a long-term follow-up study in mutation carriers. There is a special emphasis is on the elucidation of risk factors for the hypertrophic response in these patients.
The sixth chapter describes the use of T1 mapping in various regions of the cardiac muscle in manifest HCM. The study is designed to investigate whether regions devoid of LGE have different ECV (which represents interstitial fibrosis), compared to controls.

In chapter seven, results of an animal experimental study are presented. In this pre-clinical investigation, the properties of the sarcomere in septum and left ventricular free wall are compared in pre-hypertrophic heterozygous MyBPC mice. To ensure that mice show no signs of ultimate HCM phenotype, CMR is used to evaluate myocardial LV wall thickness at 6 months of age. The extent of protein expression, maximal active and passive force and calcium sensitivity of myofilaments are compared between septum and lateral LV free wall.

Chapter eight, the summary, gives a general overview of all chapters of this thesis. Subsequently, the work is evaluated in the context of current literature and future directions of research are provided.
References


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