Arrhythmogenic potential of myocardial disarray in hypertrophic cardiomyopathy: Genetic basis, functional consequences and relation

Arrhythmogenic Potential of Myocardial Disarray in Hypertrophic Cardiomyopathy: Genetic Basis, Functional Consequences and Relation to Sudden Cardiac Death

Gherardo Finocchiaro[a,b] MD, PhD, Nabeel Sheikh[a,b] MD, PhD, Ornella Leone[c] MD, Joe Westaby[d] MRCP, Francesco Mazzarotto[e,f,g,h] PhD, Antonis Pantazis[h] MD, Cecilia Ferrantini[i,l] MD, PhD, Leonardo Sacconi[i,m] PhD, Michael Papadakis[d] MRCP, MD, Sanjay Sharma[d] MRCP, MD, Mary N Sheppard[d] MD, BCh, BAO, FRCPATH, Iacopo Olivotto[e] MD

Institutions:

[a] Cardiothoracic Centre, Guy's and St Thomas' Hospital, London, United Kingdom

[b] King's College London

[c] Cardiovascular and Cardiac Transplant Pathology Unit, Department of Pathology, Sant'Orsola-Malpighi University Hospital, Bologna, Italy

[d] Cardiovascular Pathology Unit and Cardiology clinical and academic group. St George's, University of London, London and St George's University Hospital NHS Foundation Trust. United Kingdom

[e] Cardiomyopathy Unit, Careggi University Hospital, Florence, Italy

[f] Department of Experimental and Clinical Medicine, University of Florence, Italy

[g] National Heart and Lung Institute, Imperial College London, United Kingdom

[h] Cardiovascular Research Centre, Royal Brompton and Harefield National Health Service Foundation Trust, London, United Kingdom

[i] University of Florence, Florence, Italy

[l] European Laboratory for Non-Linear Spectroscopy, Florence, Italy

[m] Institute for Experimental Cardiovascular Medicine, University of Freiburg, Freiburg, Germany.

Word count: 5100

Author for correspondence:

Gherardo Finocchiaro MD, PhD Consultant Cardiologist,

Guy's and St. Thomas's Hospital, London, United Kingdom, Westminster Bridge Rd, Lambeth, London SE1 7EH. E-mail: gherardo.finocchiaro@nhs.net

Conflict of interest: none

Abstract

Myocardial disarray is defined as disorganized cardiomyocyte spatial distribution, with loss of physiological fiber alignment and orientation. Since the first pathological descriptions of hypertrophic cardiomyopathy (HCM), disarray appeared as a typical feature of this condition and sparked vivid debate regarding its specificity to the disease and clinical significance as diagnostic marker and risk factor for sudden death. Although much of the controversy surrounding its diagnostic value in HCM persists, it is increasingly recognized that myocardial disarray may be found in physiological contexts and in cardiac conditions different from HCM, raising the possibility that central focus should be placed on its quantity and distribution, rather than mere presence. While further studies are needed to establish what amount of disarray should be considered as hallmark of disease, novel experimental approaches and emerging imaging techniques for the first time allow ex-vivo and in-vivo characterization of the myocardium to a molecular level. Such advances hold promise of filling major gaps in our understanding of the functional consequences of myocardial disarray in HCM and specifically on arrhythmogenic propensity and as risk factor for sudden death. Ultimately, these studies will clarify whether disarray represents a major determinant of the HCM clinical profile, and a potential therapeutic target, as opposed to an intriguing but largely innocent bystander.

Keywords: Myocardial disarray, Hypertrophic cardiomyopathy, Sudden death.

Funding. *IO was supported by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no. 777204: "SILICOFCM - In Silico trials for drug tracing the effects of sarcomeric protein mutations leading to familial cardiomyopathy"; by the Italian Ministry of Health (Left ventricular hypertrophy in aortic valve disease and hypertrophic cardiomyopathy: genetic basis, biophysical correlates and viral therapy models" (RF-2013-02356787), and NET-2011-02347173 (Mechanisms and treatment of coronary microvascular dysfunction in patients with genetic or secondary left ventricular hypertrophy) and by the Ente Cassa di Risparmio di Firenze (bando 2016) "juvenile sudden cardiac death: just know and treat".*

MNS and JW are funded by Cardiac Risk in the Young UK

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a genetically determined heart muscle disease characterized by left ventricular (LV) hypertrophy occurring in the absence of a cardiac or systemic cause[1]. Myocardial disarray, which may be defined as disorganized cardiomyocyte spatial distribution with loss of physiological fiber alignment and orientation, represents a classic histopathological hallmark of HCM. However, more than 60 years after its initial description by Teare in 1957[2] (Figure 1), major uncertainties remain regarding its diagnostic value, functional consequences and clinical impact in HCM patients. Myocardial disarray is now known to occur in other cardiac conditions and may to some extent represent a physiological trait in certain areas of heart. To date, the jury is still out on what matters in terms of its amount and localization, and on whether it represents a mere histological attribute or an actual driver of disease manifestations. Novel experimental approaches and emerging imaging techniques using cardiovascular magnetic resonance (CMR) hold the promise for deeper characterization of myocardial disarray, offering for the first time the opportunity to address these issues. We here provide an overview of current knowledge on the role of myocardial disarray in HCM, confront the main open questions and discuss recent advances in the field, in a context where technology leads us to an increasingly precise visualization of the microstructural aspects of human heart and to a deeper understanding of genotype-phenotype correlations.

METHODS

The authors approached the topic formulating the research questions: what is the role of myocardial disarray in the diagnosis and prognostic stratification of HCM, and what is the link between myocardial disarray and ventricular arrhythmias? Therefore a systematic search through the web-based engine Pubmed was conducted in order to identify all studies meeting the eligibility criteria. Most relevant studies answering the main research question were selected. Finally, results were presented systematically, providing an historical perspective and detailing some of the new frontiers in this field, taking in account the complexity of the topic.

Historical perspective

The first histopathological description of HCM in the modern era is attributed to Robert Donald Teare, an English pathologist at St. George's Hospital in London. Teare published a series of eight autopsy cases with "asymmetrical hypertrophy or muscular hamartoma of the heart"[2]. Notably, seven of these eight individuals had died suddenly, while one had died few hours after a mitral valvotomy. The hypertrophic myocardium was characterized by a "pathological picture of bizarre and disorganized arrangement of muscle bundles associated with hypertrophy of individual muscle fibres and their nuclei", i.e. myocardial disarray. As disarray was a consistent feature in the cases described, its mere presence was initially described as pathognomonic of HCM. However, later studies showed that disarray is not confined to HCM, but can be found in a variety of conditions and even in certain regions of the normal heart, such as the junctions between left and right ventricle, creating a debate between experts around this histological feature and its pathological significance[3]. These controversies ultimately led investigators to adopt quantitative or semi-quantitative

approaches to characterize disarray[4]. In the early 1980's Maron et al.[5] comparing histological sections of decedents with HCM and of individuals with normal hearts or with other cardiac diseases, found that septal disorganization involving at least 5% of the relevant areas of the tissue section was a highly sensitive (86%) and specific (92%) marker for HCM. Other investigators suggested that myocardial disarray is specific for obstructive HCM when confined to the thickened subaortic septal area, while non-obstructive HCM likely presents disarray in a diffuse manner throughout the ventricular free wall[6]. More recently the observation that young decedents of SCD exhibit extensive myocardial disarray led to the hypothesis that this histopathological feature may constitute the substrate for potentially fatal arrhythmias, especially in young individuals[7].

The last 3 decades have witnessed a gradual permeation of genetics in cardiovascular medicine with the first discoveries of HCM-causing gene variants in the late 1980s[8,9]. Certain variants have been associated with greater extent of myocardial disarray, leading to speculations on a possible genetic basis of this histopathological feature[10]. Interest in myocardial disarray has been recently re-ignited by the advances in cardiac imaging and specifically by the increasingly widespread use of CMR which holds the promise for an in-vivo imaging of myocardial tissue to a microscopic level[11].

Myocardial disarray: definition and pathological features

Is there a universal definition?

The term myocardial disarray generally refers to a profound derangement of normal myocyte alignment at histology, as characterized by the seminal studies of Maron et al.[5,12,13]

To an experienced cardiac pathologist the concept of disarray is broader and occurs at three different levels[14–16]. The most typical and common pattern found in HCM specimens at light microscopy is an abnormal myocardial cell to cell spatial arrangement: adjacent myocytes are oriented in random perpendicular or oblique relationship, creating whirls, twirls or tangles in a pinwheel configuration. Additionally, myocytes are typically hypertrophied and abnormal in shape, usually have bizarre, enlarged hyperchromatic nuclei and can be associated with central foci of fine connective tissue (Figure 2). Interpretation of this pattern may be challenging depending on morphology variability, the pathologist's familiarity with myocyte arrangement, and inappropriate sampling sites[5,13,17]. The second level is the herringbone pattern where relatively large bundles of myocytes are aligned in groups perpendicularly or obliquely to each other. This type of disorganization combined with interspersed collagen bands is sometimes visible to the naked eye at macroscopy (Figure 2 - D-F). The third level is myofibril arrangement disorganization within single myocytes, best appreciated using electron-microscopy[18](Figure 3).

Is myocardial disarray specific to HCM?

Although much debated[3,5,13] myocardial disarray remains an archetypal feature of HCM[19–21], distinctively characteristic and consistently present, with rare exceptions[22]. The degree of myocardial disarray varies independently of wall thickness and may be present in both normal and hypertrophied areas[23]. It is typically patchy[24] and does not exhibit significant variations among the various regions of the HCM hearts, although there does appear to be a slight predilection for the interventricular septum[19]. However, it is widely recognized that disarray cannot be considered pathognomonic for HCM, as it is also found in other cardiac conditions including congenital heart disease, myocardial remodelling in ischemic heart

disease, systemic or pulmonary arterial hypertension, genetic or acquired cardiomyopathies (Figure 4)[25,26]. Furthermore, it can be found in normal hearts[27–29], where it is physiological at the junction of the septum and right ventricular walls, especially posteriorly, in trabeculations, around blood vessels and in the right ventricle [17]. For these reasons, assessment of myocardial disarray should be based on sections from the mid wall of the left ventricle remote from the junctions. When whole heart examination is not possible and the diagnosis relies on endomyocardial biopsies[15,30] or small surgical myectomies[31], the pathologist should exercise particular caution and duly combine the evidence of disarray with the broader histological and clinical context. Notably, the specificity of myocardial disarray for HCM greatly increases when quantitative criteria are considered[17] and especially when associated with typical myocyte and nuclear abnormalities. The amount of myocardial disarray proposed for a diagnosis of HCM ranges from 5-20% of the myocardium in the slides examined[15,32]. These thresholds are not based on a robust evidence and at the moment we cannot rely on a quantitative model to differentiate between significant and non-significant myocardial disarray. Moreover, there are still open questions on significance relating to a specific cardiac condition. In other words, as myocardial disarray may be found in a wide range of cardiac conditions, its presence and amount in terms of percentage in the slides examined may not be particularly useful from a diagnostic standpoint, if this feature is taken in consideration outside of the clinical and the macroscopic context.

Finally, HCM is characterized by both interstitial and replacement fibrosis. Whilst myocardial disarray is closely associated with interstitial fibrosis, it does not co-localize with replacement fibrosis[33]: this is plausibly related to the embryological considerations below, as interstitial fibrosis appears to originate at the earliest stages of disease, while replacement fibrosis is acquired later in life. Of note, myocytes around areas of replacement

fibrosis may show disorganisation but this is not specific nor diagnostic for HCM. Of note, HCM is also characterized by muscle hyperplasia of the small vessel walls, a feature that has no correlation with myocardial disarray[34].

How to quantify myocardial disarray?

Within an area identified as a "myocardial disarray" region, defining whether the loss of fibres alignment is mild, moderate or severe is often challenging. From a geometric point of view, the loss of myocyte and myofibril alignment within the tissue can vary continuously from a zero degree (in which all the fibres are perfectly aligned) to a maximum degree (in which there is a total anisotropy of orientation).

Currently there are various methods that allow a quantification of this geometric alteration which can be analysed by studying the arrangement of structures that have a spatial periodicity (e.g. the sarcomeres). The quantification relies on a tensor analysis of the myocyte/myofibril orientation, previously performed both on advanced 3D histological analysis[35] or diffusion tensor CMR (DT-CMR) imaging[11].

Embryological implications

The morphologic singularity of myocardial disarray recalls maladaptive alterations and it is not by chance that a similar myocardial disorganization is found in hamartomas of mature cardiac myocytes[36–38]. Many aspects of HCM (disarray may precede left ventricular hypertrophy and be present in utero, it may be diffuse in small children or particularly extensive in young patients following sudden death) have led to hypothesise a developmental origin of disarray associated with HCM[20,38,39]. A fascinating research line on the molecular pathogenesis of the disease suggests that interference with migration and differentiation of pluripotent epicardium-derived cells, crucial to the development of

myocardial architecture, could account for the striking tri-dimensional disorganization observed in HCM hearts[20,40]. Of note, a role of pluripotent epicardium-derived cells would also account for additional otherwise inexplicable manifestations of HCM such as microvascular remodelling, interstitial fibrosis and mitral valve abnormalities.

Imaging myocardial disarray

Until recently, methods for detecting myocardial disarray in-vivo were lacking and its presence could only be inferred indirectly in living patients. Several studies attempted to correlate the histological detection of myocyte disarray with an array of echocardiographic parameters. Menon et al.[41] examined histological specimens from 45 patients undergoing septal myectomy for obstructive HCM. On retrospective review of these patients' echocardiographic data, multivariate analysis revealed a significant relationship between the degree of myocyte disarray and degree of left ventricular diastolic dysfunction. In a similar study, Kobayashi et al.[42] analysed histopathological samples from 117 patients with obstructive HCM and found an independent inverse relationship between severity of myocyte disarray and systolic and early diastolic septal strain rate. The same group described a significant association between these histopathological characteristics and contractile performance of the resected myocardium *in vitro*[43], using semiquantitative histopathologic grading of myocyte disarray and fibrosis. Myocyte disarray was defined as areas of myocardium in which adjacent myocytes were oriented either obliquely or perpendicularly to each other (type 1a, as described by Maron et al.[14]). Each of the sections derived from myectomy specimens was examined field-by-field and scored as disarray present or absent; the number of fields in which disarray was present was then divided by

the total number of fields examined for each section and a percentage derived for the amount of disarray[34]. Disarray was further classified, based on the percentage obtained, as none, mild (1% to 25%), moderate (26% to 50%), and severe (>50%). Fibrosis was quantified using an automated system after staining and as for disarray, was quantified as normal, mild, moderate, or severe. Such techniques have been shown to have good reproducibility in samples derived from patients undergoing myectomy[44].These studies suggest that diastolic function and strain rate may be markers of amount of disarray. However, it has to be considered that diastolic dysfunction in HCM is likely the result of complex mechanical and architectural abnormalities and of a wide spectrum of histological changes and not exclusively of myocardial disarray.

Similar observations have been made using CMR and the technique of tissue tagging, with regional differences in LV function postulated to reflect the presence and regional variation in the amount of myocardial disarray and fibrosis[45]. While the implications of these studies are intriguing, however, there are important theoretical as well as methodological limitations to be considered. Disarray has a slight predilection for the basal septum of the LV, and its overall extent throughout the chamber may be overestimated by an evaluation based on myectomy samples. In addition, the concept that patchy abnormalities in alignment involving ≤20% of LV cardiomyocytes may impact the performance of the whole chamber (and be captured by echocardiography) remains questionable given the statistical weakness of these associations and the complexity of the HCM phenotype.

With the development and mainstream use of CMR, novel techniques have for the first time enabled in-vivo visualisation of myocyte disarray. Notably, traditional contrast imaging using the technique of delayed gadolinium enhancement is capable of identifying increased

interstitial space and fibrosis, but does not seem to capture myocyte disarray[46]. Over the last decade, however, greater success has been achieved by the novel technique of DT-CMR (Figure 5)[47–50]. By mapping the diffusion of water molecules, DT-CMR can visualise fractional anisotropy, which quantifies 3-dimensional diffusion in a single imaging voxel and is reduced in regions of the heart where differing myocyte orientations – i.e. disarray - are found. Results from DT-CMR have been extensively validated against histology ex-vivo on both animal and human hearts[51–55]. Although the technique is challenging and was previously confined to ex-vivo use, recent advances have now allowed DT-CMR to be performed in-vivo on humans[56–58]. A recent study demonstrated how DT-CMR can provide a reliable differentiation of normal versus HCM myocardial architecture, with abnormalities in HCM patients (specifically, reduced fractional anisotropy values), matching myocardial architecture patterns of disarray reported previously on histology[11]. As discussed below, ongoing studies will clarify if and to what extent DT-CMR, by providing quantification of disarray in HCM patients, may contribute to risk prediction and prognostication.

Relation of disarray with genetic background of HCM

It is unknown whether genetics is a driver of the degree of disarray in HCM. None of the major published genotype-phenotype analyses in HCM have assessed whether carrying a pathogenic or likely pathogenic sarcomeric variant is associated with differences in myofibril organization[59–65] in the heart muscle. Such a study would be warranted to expand our current knowledge on the molecular mechanisms underlying HCM and its histopathological features, but was not feasible prior to the development of non-invasive imaging tools such as DT-CMR[11]. The publicly accessible database ClinVar

(https://www.ncbi.nlm.nih.gov/clinvar, accessed on 13/02/2020) comprises only 6 variants unanimously classified as pathogenic/likely pathogenic (in validated HCM genes[66]) for which evidence of disarray is reported in human heart tissue or animal models. These are p.Glu173del, p.Arg104Leu and p.Arg102Gln in the *TNNT2* gene, p.Arg723Gly and p.Gly584Arg in the *MYH7* gene, and p.Glu101Lys in the *ACTC1* gene. Such a limited number of observations is insufficient to draw conclusions, but the predominance of variants in the thin filament genes *TNNT2* and *ACTC1* — estimated to explain approximately 3% of HCM cases in cohort-based analyses[67,68] — may suggest that disarray could be primarily associated with thin filament alterations, or be more pronounced in their presence. While this is speculative at this stage, large-scale genotype-phenotype analyses are warranted to clarify if specific classes of genetic variation can exert a direct impact on cardiomyocyte alignment disturbances in HCM.

Potential role in arrhythmogenesis

It has long been postulated that myocyte disarray plays a central role in the development of ventricular arrhythmias in patients with HCM and therefore represent a substrate for SCD[5,69–73]. McKenna et al.[69] reported on two families with a strong history of premature sudden death in whom severe myocyte disarray was found at post-mortem, without evidence of significant LVH or increased cardiac mass. Genetic testing was subsequently performed on these families, and a novel missense mutation in exon 9 of the troponin T gene discovered[74]. Further work by the same group comparing clinical, genetic and histological findings suggested that troponin T mutations are associated with a greater propensity to juvenile sudden death in the presence of minimal hypertrophy and fibrosis,

but severe myocyte disarray on post-mortem examination, identifying the latter as a potentially malignant phenotype in these individuals[75]. However, subsequent studies have found that specific mutations (including those within the troponin T gene) cannot reliably predict outcome[76–79], except in children[80] .

Varnava et al. assessed the pathological findings and clinical profile of 75 HCM patients at post-mortem or after cardiac transplantation, examining the percentage of fibrosis, disarray and small vessel disease on histology[81]. The presence of myocyte disarray was found to correlate with evidence of ischaemia, a younger age (<21 years) at death or transplantation, a family history of juvenile sudden death and an abnormal blood pressure response to exercise. However, while the degree of myocardial fibrosis appeared to correlate with ventricular arrhythmia and SCD risk profile, disarray did not. Previous work by the same group had also uncovered an inverse relationship between age and disarray, suggesting a more malignant phenotype when large amounts of disarray were present, resulting in a younger age at death[34]. At a purely speculative level, these findings may suggest that disarray causes electrical instability of the myocardium by proxy, e.g. mediated by microvascular ischemia, rather than exert direct arrhythmogenic effects. It is also possible that greater disarray may simply represent an innocent marker of more aggressive disease, not involved *per se* in the genesis of arrhythmias. The results of these mainly post-mortem studies have to be interpreted with caution as the amount of clinical information was often limited and the population included was skewed in order to drive conclusions on the role of myocardial disarray as independent marker of ventricular arrhythmias and sudden death. Notably, a recent study on DT-CMR demonstrated that reduced fractional anisotropy, likely represents myocyte disarray in vivo and was also associated with the occurrence of

ventricular arrythmias[11]. Further evidence is needed to evaluate the link between disarray and potentially lethal arrhythmias, as well as the role of this technique in risk stratification.

Myocardial disarray and sudden death in individuals with idiopathic LVH

A diverse spectrum of diseases are implicated in SCD and the prevalence of specific diseases is generally dependent on the demographics of the victims and the circumstances of death[32,82]. Primary cardiomyopathies, including HCM and channelopathies, are the predominant causes of SCD in the young[83–85]. When ante-mortem diagnosis is not available or autoptic results are inconclusive, physicians are often faced with the challenge of interpreting findings of uncertain significance, whose relationship with SCD is unclear. Indeed, the sole identification of significant LVH in a young individual that died suddenly may often lead to an erroneous diagnosis of HCM. While often the result of comorbid conditions such as long-standing hypertension, in some cases (especially young decedents of SCD) LVH remains unexplained in the absence of the typical histopathological features of HCM, such as myocardial disarray (idiopathic LVH). Recent studies[83,86,87] reveal that in selective cohorts idiopathic LVH may be reported in up to 30% of victims of SCD. Definitions of idiopathic LVH vary and there is no consistency in various series as to the amount of myocardial disarray considered significant. Several studies on decedents of sudden death described idiopathic LVH based on general macroscopic and microscopic findings, such as increased heart weight and wall thickness, and on the absence of histopathological features typical of HCM, without a specific reference to myocardial disarray quantification and thresholds[83,88,89]. Recently, Finocchiaro et al.[90] investigated 125 first-degree relatives of 46 young decedents of SCD with idiopathic LVH at autopsy. HCM was defined by the

macroscopic finding of left ventricular wall thickness >15 mm circumferentially or focally and/or heart weight >500 g (>400 g in females) or increased heart weight as a function of body weight according to established nomograms[91] and by the presence of myocyte hypertrophy and myocyte disarray involving at least 20% of surface in at least two tissue blocks of 4 cm2. Idiopathic LVH shared the same macroscopic criteria of HCM, but differed in terms of microscopic criteria (myocyte disarray < 20% of surface in tissue blocks). This may be considered a high threshold and although myocyte disarray is a recognized histological hallmark of HCM due to pathogenic variants within genes encoding sarcomeric proteins, it is possible that myocyte disarray is minimal in some cases and go undetected, therefore many such cases without overt disarray could still represent HCM. However, family screening did not reveal a single case of HCM despite comprehensive clinical evaluation, including CMR imaging, exercise testing and prolonged ECG monitoring, which would be expected to identify even very mild phenotypes. In this study idiopathic LVH was defined by the combination of LVH and no myocardial disarray, or < 20% in at least two tissue blocks of 4 cm2; the absence of a familiar disease after extensive screening in these cases, confirms that myocardial disarray is indeed a pivotal feature offamilial sarcomeric HCM.

Functional consequences of myocardial disarray: mechano-energetic impairment and arrhythmic susceptibility

Although the pathogenesis of myocardial disarray is unclear, there are several ongoing research lines aimed at discovering its functional consequences. Myocardial disarray affects LV mechanics and energetics by interfering with the homogeneity of contraction and

relaxation[92] and by increasing the relative ATP consumption during tension generation. Dissecting the functional effects of myocardial disarray is challenging and at the moment theoretical, rather than based on direct experimental evidence. Myofilament and myocyte misalignment always occur with other changes in tissue architecture, such as alterations in cell size and fibrosis. In addition, myocyte disarray goes hand in hand with the molecular remodelling of desmosomes and gap junctions[93]. These alterations appear to be distributed rather heterogeneously across the LV wall, creating inhomogeneous mechanical and electrical coupling throughout the ventricles. From the experimental perspective, the functional consequences of myocardial disarray could be studied in human HCM myectomy samples, by employing large multicellular preparations. Animal models, and particularly rodents, only partially reproduce this aspect of the human cardiac phenotype.

Some HCM-related myofilament mutations, such as those in the motor domain of β myosin heavy chain, lower the intrinsic force generating capacity of sarcomeres by modifying cross-bridge turnover kinetics[94,95]. In most cases, however, HCM pathogenesis is associated with a haplo-insufficiency model, meaning that the tension generation capacity of the sarcomere is not compromised by the expression of the mutated protein (e.g. thin filament mutations) but rather is a consequence of the reduction of amount of the native protein incorporated into the sarcomeres (e.g. myosin binding protein C mutations). Importantly, as highlighted in figure 6, when the myocytes and myofilaments are not aligned, the resulting force vector contributing to generate active stress is reduced. Thus, myocardial disarray may act as an independent factor impacting wall stress, even when the force generation capacity of single myocytes is not compromised. This theoretical expectation is confirmed by the observation of a preserved tension level in thin intact HCM multicellular preparations (i.e. trabeculae) from myectomy samples, carefully dissected in order to exclude myocardial disarray[96].

In HCM, the energy balance is often impaired[97]. Indeed, abnormal cardiomyocyte organization and myofibril disarray may *per se* decrease tension while relatively increasing the ATPase of multicellular preparations, thus leading to an artificial increase in tension cost[98]. This consideration holds also for the healthy myocardium, if areas of myocardial disarray are present. In fact, at the single myocyte level, ATP utilization remains constant (or even rises), while the force vector that contributes to generate active stress is reduced, thus the ratio of "effective" tension on ATP consumption is increased.

Arrhythmias susceptibility

In a simple model of action potential (AP) propagation, an axial current flows along a linear cellular structure, or cable, from one depolarized myocyte to its quiescent neighbour via intercellular channels known as gap junctions[99]. If this axial current is sufficient to depolarize the neighboring cell beyond its activation threshold, voltage sensitive Na+ channels will create transmembrane currents capable of propagating the AP. The axial resistance (ra) to such local circuit currents arises from the resistances of the cells' cytosol (the larger is cells' dimeter the lower is ra) and the gap junctions between adjacent cells. Thus, in addition to Na+ channels, gap junctions play a critical role in AP propagation and influence conduction velocity (the higher the number of gup junctions the lower is ra). In the healthy myocardium gup junctions are mode dense in the lungitudinal (end-to-end) junction compared to the transversal myocyte junction, guaranteeing an approximately 3:1 ratio between lungitudinal and transversal conduction velocity.

The following alterations are expected as a consequence of myocardial disarray: (i) the ratio between longitudinal and transversal conduction velocity would decrease and (ii) different

(and potentially longer) pathways for conduction are available. Once again, also from the electrical perspective, it is hard to define the role of myocardial disarray alone. In human HCM, primarily cell width is increased[100] leading to hypertrophy without dilation of the ventricles. This increases in cell volume is paralleled by an increase in cell capacity[96]. Increased cell capacitance per se affects conduction velocity. Computer modelling shows that conduction velocity increases with cell size, and, even further, cell size appears as the dominant factor affecting conduction velocity[101]. Besides that, gap junction are redistributed when myocardial disarray occur and their asymmetrical density (lateral > transversal) is lost. The drop of longitudinal conduction velocity promotes re-entrant conduction. The increase of transversal conduction velocity promotes alternative pathways for the impulse propagation. The presence of myocardial disarray besides reducing conduction velocity, creates additional pathways for the impulse conduction (including shorter pathways due to increased transversal and oblique junctions), thus increases the probability of this condition to occur. Thus, in a context of increased cellular spontaneous activity, with a large increase of local triggers, myocardial disarray constitute a substrate that largely facilitates the occurrence and perpetuation of re-entrant conduction.

Mopho-functional mesoscale imaging and correlative studies

Dissecting the role of myocardial disarray *in vitro* is challenging, mostly due to the inefficacy of standard imaging methods that necessarily imply tissue sectioning and reconstruction. Though sections can be rather thin with current technology (e.g. cryo electron microscopy), they “sample” the tissue randomly and may fail to quantify myocardial disarray that, as mentioned before, can be zonal and non-homogeneusly distributed. The new frontier to

understand the significance of myocardial disarray in HCM (and in cardiac diseases in general) consists of two frameworks, the so called “Mesoscale” imaging and the subsequent “Correlative” studies. With mesoscale imaging, we intend the possibility to perform high resolution imaging (at cellular and sub-cellular level) in massive tissue, i.e. the entire sample/organ. The aim would be to reconstruct the orientation of fibers, collagen and potentially any cellular protein component in very large volumes with sub-cellular resolution. The technique that could allow this type of reconstruction is reported in figure 6 and could be combined with CMR or other in vivo or ex vivo data.

Performing high resolution 3D imaging in tissue is very challenging due to the opaqueness of the tissue itself. The most common approaches in perming 3d reconstruction in massive tissue is to slice the tissue into thin sections, then stain and image the sections with various techniques. However, precise cutting, mounting and imaging take a considerable amount of effort and time. Automated, high-throughput imaging methods based on serial sectioning have been recently developed[102] but tissue deformation introduced by slicing is still a critical point. A different approach that does not require sample cutting is to make the sample transparent. In fact, the main source of tissue opaqueness is not absorption, but scattering and the scattering can be reduced by homogenizing the refractive index inside the sample. During last years, different methods have been developed for clarifying fixed tissue. These span from approaches based on high refractive index organic solvents[103], to approaches involving water-based optical clearing agents[104]. Most of them, however, present several limitations such as tissue shrinkage, structural alteration, fluorescence quenching and incompatibility with immunostaining. The challenge of producing large, transparent and fluorescently labelled volumes has been recently achieved applying a true tissue transformation approach (as CLARITY[105]). This method transforms an intact tissue into a

nanoporous, hydrogel-hybridized, lipid-free form that, by removing membrane lipid bilayers, allows high transparency, immunolabeling and structural and molecular preservation. This method in combination with advanced microscopy, like light-sheet or multi-photon microscopy allows 3d reconstruction of massive heart tissue at sub-cellular resolution[106]. Using this approach, a cytoarchitectonic analysis can be employ to identify cells and to map fibres alignment in three-dimensions.

This methodological framework allow the quantification of cellular disarray in 3d in the same piece of tissue used for electro-mechanical investigations. In this scenario, structural data could be use also used to generate a high-resolution image-based computational model to simulate functional dysfunction in terms the conduction pathway of action potential propagation as well as tensor force across the whole previously characterized tissue. This can elucidate the role of cellular disorganization in the electrical-mechanical dysfunctions in a true correlative manner and pave the way for a unifying model which integrates functional and structural data enabling a comprehensive investigation of the morphological causes that lead to electrical and mechanical alterations after structural remodelling.Correlative studies in the context of myocardial disarray are aimed to correlate tissue reconstruction and functional data (e.g. electrical activity, mechanical function, energy consumption). With a correlative approach, for instance, we can guess that if re-entrant conduction is identified and pointy localized, it can be attributed (with a causative rather than probabilistic link) to a specific structural alteration that the mesoscale reconstruction has identified.

CONCLUSIONS

Myocardial disarray appeared as a core aspect of HCM when first described at post-mortem examination more than 60 years ago. The following decades witnessed a significant controversy regarding this feature, which has been promoted as the central hallmark of the disease by some and discarded as non-specific feature by others. Interestingly, the never-ending debate on myocardial disarray reflects the perpetual quest for the pathognomonic biomarker which is a constant aspect in many fields of cardiovascular medicine and particularly in cardiomyopathies (Table 1 summarizes the main study findings on this topic).

The interpretation of myocardial disarray as a defining attribute should be probably looked at in this spirit, taking in consideration that this feature was recognized in young decedents with “asymmetric hypertrophy of the heart” at the dawn of cardiomyopathies discovery. The exponential increase in diagnosis of HCM even after the 6th decade of life, means that many cases go undetected for many years or die for unrelated issues with no means of pursuing an histological assessment which can potentially demonstrate disarray.

Novel experimental approaches and emerging imaging techniques for the first time allow ex-vivo and in-vivo characterization of the myocardium to a molecular level. Particularly, mesoscopic tissue reconstructions could first answer quantitatively to the question: “what is the real extent of myocardial disarray?”, an unsolvable question with classical techniques that are based on serial random sampling. Such advances hold promise of filling major gaps in our understanding of the functional consequences of myocardial disarray in HCM, including its impact on left ventricular contractile performance and arrhythmogenic propensity. Ultimately, these studies will clarify whether disarray represents a major

determinant of the HCM clinical profile, and a potential therapeutic target, as opposed to an intriguing but largely innocent bystander.

REFERENCES:

1. Elliott PM, Anastasakis A, Borger MA, et al.: 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy. Eur Heart J 2014; 35:2733–2779.

2. TEARE D: Asymmetrical hypertrophy of the heart in young adults. Br Heart J [Internet] 1958; 20:1–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13499764

3. Becker AE, Caruso G: Myocardial disarray. A critical review. Br Heart J [Internet] 1982; 47:527–538. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7044398

4. Van Noorden S, Olsen EG, Pearse AG: Hypertrophic obstructive cardiomyopathy, a histological, histochemical, and ultrastructural study of biopsy material. Cardiovasc Res [Internet] 1971; 5:118–131. Available from: http://www.ncbi.nlm.nih.gov/pubmed/5544950

5. Maron BJ, Anan TJ, Roberts WC: Quantitative analysis of the distribution of cardiac muscle cell disorganization in the left ventricular wall of patients with hypertrophic cardiomyopathy. Circulation [Internet] 1981; 63:882–894. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7193536

6. St John Sutton MG, Lie JT, Anderson KR, O'Brien PC, Frye RL: Histopathological specificity of hypertrophic obstructive cardiomyopathy. Myocardial fibre disarray and myocardial fibrosis. Br Heart J [Internet] 1980; 44:433–443. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7191711

7. Varnava AM, Elliott PM, Mahon N, Davies MJ, McKenna WJ: Relation between

myocyte disarray and outcome in hypertrophic cardiomyopathy. Am J Cardiol [Internet] 2001; 88:275–279. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11472707

8. Jarcho JA, McKenna W, Pare JA, Solomon SD, Holcombe RF, Dickie S, Levi T, Donis-Keller H, Seidman JG, Seidman CE: Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. N Engl J Med [Internet] 1989; 321:1372–1378. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2811944

9. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, Seidman JG: A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell [Internet] 1990; 62:999–1006. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1975517

10. Varnava AM, Elliott PM, Baboonian C, Davison F, Davies MJ, McKenna WJ: Hypertrophic cardiomyopathy: histopathological features of sudden death in cardiac troponin T disease. Circulation [Internet] 2001; 104:1380–1384. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11560853

11. Ariga R, Tunnicliffe EM, Manohar SG, Mahmod M, Raman B, Piechnik SK, Francis JM, Robson MD, Neubauer S, Watkins H: Identification of Myocardial Disarray in Patients With Hypertrophic Cardiomyopathy and Ventricular Arrhythmias. J Am Coll Cardiol 2019; 73:2493–2502.

12. Maron BJ, Roberts WC: Quantitative analysis of cardiac muscle cell disorganization in the ventricular septum of patients with hypertrophic cardiomyopathy. Circulation [Internet] 1979; 59:689–706. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/570464

13. Maron BJ, Sato N, Roberts WC, Edwards JE, Chandra RS: Quantitative analysis of cardiac muscle cell disorganization in the ventricular septum. Comparison of fetuses and infants with and without congenital heart disease and patients with hypertrophic cardiomyopathy. Circulation [Internet] 1979; 60:685–696. Available from: http://www.ncbi.nlm.nih.gov/pubmed/572271

14. Maron BJ, Wolfson JK, Roberts WC: Relation between extent of cardiac muscle cell disorganization and left ventricular wall thickness in hypertrophic cardiomyopathy. Am J Cardiol [Internet] 1992; 70:785–790. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1519531

15. Davies MJ: The current status of myocardial disarray in hypertrophic cardiomyopathy. Br. Heart J. 1984, pp. 361–363.

16. Davies MJ: Hypertrophic cardiomyopathy: One disease or several? Heart. 1990, pp. 263–264.

17. Hughes SE: The pathology of hypertrophic cardiomyopathy. Histopathology [Internet] 2004; 44:412–427. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15139989

18. Kanzaki Y, Yamauchi Y, Okabe M, Terasaki F, Ishizaka N: Three-dimensional architecture of cardiomyocytes and connective tissues in hypertrophic cardiomyopathy: a scanning electron microscopic observation. Circulation [Internet] 2012; 125:738–739. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22311886

19. Marian AJ, Braunwald E: Hypertrophic cardiomyopathy: Genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. Circ Res 2017; 121:749–770.

20. Olivotto I, Cecchi F, Poggesi C, Yacoub MH: Developmental origins of hypertrophic cardiomyopathy phenotypes: a unifying hypothesis. Nat Rev Cardiol [Internet] 2009; 6:317–321. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19352336

21. Maron BJ, Maron MS: Hypertrophic cardiomyopathy. Lancet (London, England) [Internet] 2013; 381:242–255. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22874472

22. Davies MJ, McKenna WJ: Hypertrophic cardiomyopathy--pathology and pathogenesis. Histopathology [Internet] 1995; 26:493–500. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7665141

23. Maron BJ, Wolfson JK, Roberts WC: Relation between extent of cardiac muscle cell disorganization and left ventricular wall thickness in hypertrophic cardiomyopathy. Am J Cardiol 1992; 70:785–790.

24. Maron BJ: Hypertrophic cardiomyopathy: a systematic review. JAMA [Internet] 2002; 287:1308–1320. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11886323

25. Maron BJ, Edwards JE, Moller JH, Epstein SE: Prevalence and characteristics of disporportionate ventricular septal thickening in infants with congenital heart disease. Circulation [Internet] 1979; 59:126–133. Available from: https://www.ahajournals.org/doi/10.1161/01.CIR.59.1.126

26. Van Der Bel-Kahn J: Muscle fiber disarray in common heart diseases. Am J Cardiol [Internet] 1977; 40:355–364. Available from: https://linkinghub.elsevier.com/retrieve/pii/0002914977901576

27. Jones M, Ferrans VJ, Morrow AG, Roberts WC: Ultrastructure of crista

supraventricularis muscle in patients with congenital heart diseases associated with right ventricular outflow tract obstruction. Circulation [Internet] 1975; 51:39–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/122789

28. Bulkley BH, D'Amico B, Taylor AL: Extensive myocardial fiber disarray in aortic and pulmonary atresia. Relevance to hypertrophic cardiomyopathy. Circulation [Internet] 1983; 67:191–198. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6681520

29. Maron BJ, Roberts WC, Arad M, Haas TS, Spirito P, Wright GB, Almquist AK, Baffa JM, Saul JP, Ho CY, Seidman J, Seidman CE: Clinical outcome and phenotypic expression in LAMP2 cardiomyopathy. JAMA [Internet] 2009; 301:1253–1259. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19318653

30. Fujiwara H, Hoshino T, Fujiwara T, Kawai C, Hamashima Y: Classification and distribution of myocardial fascicle and fiber disarray in 14 hearts with hypertrophic cardiomyopathy in 25 mu thick sections. Jpn Circ J [Internet] 1982; 46:225–234. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7199592

31. Lamke GT, Allen RD, Edwards WD, Tazelaar HD, Danielson GK: Surgical pathology of subaortic septal myectomy associated with hypertrophic cardiomyopathy. A study of 204 cases (1996-2000). Cardiovasc Pathol [Internet] 12:149–158. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12763554

32. Finocchiaro G, Papadakis M, Robertus J-L, Dhutia H, Steriotis AK, Tome M, Mellor G, Merghani A, Malhotra A, Behr E, Sharma S, Sheppard MN: Etiology of Sudden Death in Sports: Insights From a United Kingdom Regional Registry. J Am Coll Cardiol [Internet] 2016; 67:2108–2115. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/27151341

33. St John Sutton MG, Lie JT, Anderson KR, O'Brien PC, Frye RL: Histopathological specificity of hypertrophic obstructive cardiomyopathy. Myocardial fibre disarray and myocardial fibrosis. Br Heart J 1980; 44:433–443.

34. Varnava AM, Elliott PM, Sharma S, McKenna WJ, Davies MJ: Hypertrophic cardiomyopathy: The interrelation of disarray, fibrosis and small vessel disease. Heart 2000; 84:476–482.

35. Garcia-Canadilla P, Cook AC, Mohun TJ, Oji O, Schlossarek S, Carrier L, McKenna WJ, Moon JC, Captur G: Myoarchitectural disarray of hypertrophic cardiomyopathy begins pre-birth. J Anat [Internet] 2019; 235:962–976. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/joa.13058

36. Fealey ME, Edwards WD, Miller D V, Menon SC, Dearani JA: Hamartomas of mature cardiac myocytes: report of 7 new cases and review of literature. Hum Pathol [Internet] 2008; 39:1064–1071. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18508110

37. Miller D V, Tazelaar HD: Cardiovascular pseudoneoplasms. Arch Pathol Lab Med [Internet] 2010; 134:362–368. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20196664

38. Bradshaw SH, Hendry P, Boodhwani M, Dennie C, Veinot JP: Left ventricular mesenchymal hamartoma, a new hamartoma of the heart. Cardiovasc Pathol [Internet] 20:307–314. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20850353

39. Garcia-Canadilla P, Cook AC, Mohun TJ, Oji O, Schlossarek S, Carrier L, McKenna WJ, Moon JC, Captur G: Myoarchitectural disarray of hypertrophic cardiomyopathy begins pre-birth. J Anat [Internet] 2019; 235:962–976. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31347708

40. Lie-Venema H, van den Akker NMS, Bax NAM, Winter EM, Maas S, Kekarainen T, Hoeben RC, DeRuiter MC, Poelmann RE, Gittenberger-de Groot AC: Origin, fate, and function of epicardium-derived cells (EPDCs) in normal and abnormal cardiac development. ScientificWorldJournal [Internet] 2007; 7:1777–1798. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18040540

41. Menon SC, Eidem BW, Dearani JA, Ommen SR, Ackerman MJ, Miller D: Diastolic Dysfunction and Its Histopathological Correlation in Obstructive Hypertrophic Cardiomyopathy in Children and Adolescents. J Am Soc Echocardiogr Elsevier Inc, 2009; 22:1327–1334.

42. Kobayashi T, Popovic Z, Bhonsale A, Smedira NG, Tan C, Rodriguez ER, Thamilarasan M, Lytle BW, Lever HM, Desai MY: Association between septal strain rate and histopathology in symptomatic hypertrophic cardiomyopathy patients undergoing septal myectomy. Am Heart J Mosby, 2013; 166:503–511.

43. Dhillon A, Sweet W, Popovic ZB, Smedira NG, Thamilarasan M, Lytle BW, Tan C, Starling RC, Lever HM, Moravec CS, Desai MY: Association of noninvasively measured left ventricular mechanics with in vitro muscle contractile performance: A prospective study in hypertrophic cardiomyopathy patients. J Am Heart Assoc 2014; 3:1–11.

44. Kobayashi T, Popovic Z, Bhonsale A, Smedira NG, Tan C, Rodriguez ER, Thamilarasan

M, Lytle BW, Lever HM, Desai MY: Association between septal strain rate and histopathology in symptomatic hypertrophic cardiomyopathy patients undergoing septal myectomy. Am Heart J [Internet] 2013; 166:503–511. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24016500

45. Kramer CM, Reichek N, Ferrari VA, Theobald T, Dawson J, Axel L: Regional heterogeneity of function in hypertrophic cardiomyopathy. Circulation 1994; 90:186–194.

46. Moon JCC, Reed E, Sheppard MN, Elkington AG, Ho SY, Burke M, Petrou M, Pennell DJ: The histologic basis of late gadolinium enhancement cardiovascular magnetic resonance in hypertrophic cardiomyopathy. J Am Coll Cardiol [Internet] 2004; 43:2260–2264. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15193690

47. Nielles-Vallespin S, Khalique Z, Ferreira PF, et al.: Assessment of Myocardial Microstructural Dynamics by In Vivo Diffusion Tensor Cardiac Magnetic Resonance. J Am Coll Cardiol 2017; 69:661–676.

48. MacGowan GA, Parikh JD, Hollingsworth KG: Diffusion Tensor Magnetic Resonance Imaging of the Heart: Looking Into the Layers of the Myocardium. J Am Coll Cardiol 2017; 69:677–678.

49. Khalique Z, Ferreira PF, Scott AD, Nielles-Vallespin S, Firmin DN, Pennell DJ: Diffusion Tensor Cardiovascular Magnetic Resonance Imaging: A Clinical Perspective. JACC Cardiovasc Imaging 2019; .

50. Khalique Z, Pennell D: Diffusion tensor cardiovascular magnetic resonance. Postgrad Med J 2019; 95:433–438.

51. Hsu EW, Muzikant AL, Matulevicius SA, Penland RC, Henriquez CS: Magnetic resonance myocardial fiber-orientation mapping with direct histological correlation. Am J Physiol - Hear Circ Physiol 1998; 274:1627–1634.

52. Holmes AA, Scollan DF, Winslow RL: Direct histological validation of diffusion tensor MRI in formaldehyde- fixed myocardium. Magn Reson Med 2000; 44:157–161.

53. Scollan DF, Holmes A, Winslow R, Forder J: Histological validation of myocardial microstructure obtained from diffusion tensor magnetic resonance imaging. Am J Physiol - Hear Circ Physiol 1998; 275:2308–2318.

54. Teh I, McClymont D, Zdora MC, Whittington HJ, Davidoiu V, Lee J, Lygate CA, Rau C, Zanette I, Schneider JE: Validation of diffusion tensor MRI measurements of cardiac microstructure with structure tensor synchrotron radiation imaging. J Cardiovasc Magn Reson Journal of Cardiovascular Magnetic Resonance, 2017; 19:1–14.

55. Tseng WYI, Wedeen VJ, Reese TG, Neal Smith R, Halpern EF: Diffusion tensor MRI of myocardial fibers and sheets: Correspondence with visible cut-face texture. J Magn Reson Imaging 2003; 17:31–42.

56. Ferreira PF, Kilner PJ, Mcgill LA, et al.: In vivo cardiovascular magnetic resonance diffusion tensor imaging shows evidence of abnormal myocardial laminar orientations and mobility in hypertrophic cardiomyopathy. J Cardiovasc Magn Reson 2014; 16:1–16.

57. Tunnicliffe EM, Scott AD, Ferreira P, Ariga R, McGill LA, Nielles-Vallespin S, Neubauer S, Pennell DJ, Robson MD, Firmin DN: Intercentre reproducibility of cardiac apparent diffusion coefficient and fractional anisotropy in healthy volunteers. J Cardiovasc

Magn Reson 2014; 16:1–12.

58. McGill LA, Ismail TF, Nielles-Vallespin S, et al.: Reproducibility of in-vivo diffusion tensor cardiovascular magnetic resonance in hypertrophic cardiomyopathy. J Cardiovasc Magn Reson 2012; 14:1–15.

59. Ho CY, Day SM, Ashley EA, et al.: Genotype and Lifetime Burden of Disease in Hypertrophic Cardiomyopathy. Circulation 2018; 138:1387–1398.

60. Li Q, Gruner C, Chan RH, Care M, Siminovitch K, Williams L, Woo A, Rakowski H: Genotype-positive status in patients with hypertrophic cardiomyopathy is associated with higher rates of heart failure events. Circ Cardiovasc Genet 2014; 7:416–422.

61. Bos JM, Will ML, Gersh BJ, Kruisselbrink TM, Ommen SR, Ackerman MJ: Characterization of a phenotype-based genetic test prediction score for unrelated patients with hypertrophic cardiomyopathy. Mayo Clin Proc [Internet] 2014; 89:727–737. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24793961

62. Lopes LR, Syrris P, Guttmann OP, O'Mahony C, Tang HC, Dalageorgou C, Jenkins S, Hubank M, Monserrat L, McKenna WJ, Plagnol V, Elliott PM: Novel genotype–phenotype associations demonstrated by high-throughput sequencing in patients with hypertrophic cardiomyopathy. Heart 2014; :heartjnl-2014-306387.

63. van Velzen HG, Schinkel AFL, Oldenburg RA, van Slegtenhorst MA, Frohn-Mulder IME, van der Velden J, Michels M: Clinical Characteristics and Long-Term Outcome of Hypertrophic Cardiomyopathy in Individuals With a MYBPC3 (Myosin-Binding Protein C) Founder Mutation. Circ Cardiovasc Genet 2017; 10.

64. Ingles J, Sarina T, Yeates L, Hunt L, Macciocca I, Mccormack L, Winship I, Mcgaughran

J, Atherton J, Semsarian C: Clinical predictors of genetic testing outcomes in hypertrophic cardiomyopathy. Genet Med 2013; .

65. Lopes LR, Rahman MS, Elliott PM: A systematic review and meta-analysis of genotype–phenotype associations in patients with hypertrophic cardiomyopathy caused by sarcomeric protein mutations. Heart 2013; 99:1800–1811.

66. Ingles J, Goldstein J, Thaxton C, et al.: Evaluating the Clinical Validity of Hypertrophic Cardiomyopathy Genes. Circ Genomic Precis Med 2019; 12:e002460.

67. Mazzarotto F, Girolami F, Boschi B, et al.: Defining the diagnostic effectiveness of genes for inclusion in panels: the experience of two decades of genetic testing for hypertrophic cardiomyopathy at a single center. Genet Med [Internet] 2019; 21:284–292. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29875424

68. Walsh R, Buchan R, Wilk A, et al.: Defining the genetic architecture of hypertrophic cardiomyopathy: re-evaluating the role of non-sarcomeric genes. Eur Heart J [Internet] 2017; 38:3461–3468. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28082330

69. McKenna WJ, Stewart JT, Nihoyannopoulos P, McGinty F, Davies MJ: Hypertrophic cardiomyopathy without hypertrophy: two families with myocardial disarray in the absence of increased myocardial mass. Br Heart J [Internet] 1990; 63:287–290. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1024478&tool=pmcentrez&rendertype=abstract

70. Davies MJ, McKenna WJ: Hypertrophic cardiomyopathy — pathology and

pathogenesis. Histopathology 1995; 26:493–500.

71. Janse MJ, De Bakker JMT: Arrhythmia substrate and management in hypertrophic cardiomyopathy: From molecules to implantable cardioverter-defibrillators. Eur Hear Journal, Suppl 2001; 3:15–20.

72. Sepp R, Severs NJ, Gourdie RG: Altered patterns of cardiac intercellular junction distribution in hypertrophic cardiomyopathy. Heart 1996; 76:412–417.

73. Saumarez RC, Slade AKB, Grace AA, Sadoul N, Camm AJ, McKenna WJ: The significance of paced electrogram fractionation in hypertrophic cardiomyopathy: A prospective study. Circulation 1995; 91:2762–2768.

74. Varnava A, Baboonian C, Davison F, De Cruz L, Elliott PM, Davies MJ, McKenna WJ: A new mutation of the cardiac troponin T gene causing familial hypertrophic cardiomyopathy without left ventricular hypertrophy. Heart 1999; 82:621–624.

75. Varnava AM, Elliott PM, Baboonian C, Davison F, Davies MJ, McKenna WJ: Hypertrophic Cardiomyopathy. Histopathological Features of Sudden Death in Cardiac Troponin T Disease. Circulation 2001; 104:1380–1384.

76. Pasquale F, Syrris P, Kaski JP, Mogensen J, McKenna WJ, Elliott P: Long-term outcomes in hypertrophic cardiomyopathy caused by mutations in the cardiac troponin T gene. Circ Cardiovasc Genet 2012; 5:10–17.

77. Van Driest SL, Ackerman MJ, Ommen SR, Shakur R, Will ML, Nishimura RA, Tajik AJ, Gersh BJ: Prevalence and severity of “benign” mutations in the β-myosin heavy chain, cardiac troponin T, and α-tropomyosin genes in hypertrophic cardiomyopathy. Circulation 2002; 106:3085–3090.

78. Landstrom AP, Ackerman MJ: Mutation type is not clinically useful in predicting prognosis in hypertrophic cardiomyopathy. Circulation 2010; 122:2441–2449.

79. Semsarian C, Yu B, Ryce C, Lawrence C, Washington H, Trent R: Sudden cardiac death in familial hypertrophic cardiomyopathy: Are “benign” mutations really benign? Pathology 1997; 29:305–308.

80. Maurizi N, Passantino S, Spaziani G, et al.: Long-term Outcomes of Pediatric-Onset Hypertrophic Cardiomyopathy and Age-Specific Risk Factors for Lethal Arrhythmic Events. JAMA Cardiol [Internet] 2018; 3:520–525. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29710196

81. Varnava AM, Elliott PM, Mahon N, Davies MJ, McKenna WJ: Relation Between Myocyte Disarray and Outcome in Hypertrophic Cardiomyopathy. Am J Cardiol 2001; 88:275–279.

82. Bagnall RD, Weintraub RG, Ingles J, et al.: A Prospective Study of Sudden Cardiac Death among Children and Young Adults. N Engl J Med [Internet] 2016; 374:2441–2452. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27332903

83. Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO: Sudden Deaths in Young Competitive Athletes: Analysis of 1866 Deaths in the United States, 1980-2006. Circulation [Internet] 2009; 119:1085–1092. Available from: http://circ.ahajournals.org/cgi/doi/10.1161/CIRCULATIONAHA.108.804617

84. Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G: Does sports activity enhance the risk of sudden death in adolescents and young adults? J Am Coll Cardiol [Internet] 2003; 42:1959–1963. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/14662259

85. Harmon KG, Drezner JA, Maleszewski JJ, Lopez-Anderson M, Owens D, Prutkin JM, Asif IM, Klossner D, Ackerman MJ: Pathogeneses of Sudden Cardiac Death in National Collegiate Athletic Association Athletes. Circ Arrhythmia Electrophysiol [Internet] 2014; 7:198–204. Available from: http://circep.ahajournals.org/cgi/doi/10.1161/CIRCEP.113.001376

86. Harmon KG, Asif IM, Maleszewski JJ, Owens DS, Prutkin JM, Salerno JC, Zigman ML, Ellenbogen R, Rao AL, Ackerman MJ, Drezner JA: Incidence, Cause, and Comparative Frequency of Sudden Cardiac Death in National Collegiate Athletic Association AthletesCLINICAL PERSPECTIVE. Circulation [Internet] 2015; 132:10–19. Available from: http://circ.ahajournals.org/lookup/doi/10.1161/CIRCULATIONAHA.115.015431

87. Risgaard B, Winkel BG, Jabbari R, Behr ER, Ingemann-Hansen O, Thomsen JL, Ottesen GL, Gislason GH, Bundgaard H, Haunsoø S, Holst AG, Tfelt-Hansen J: Burden of sudden cardiac death in persons aged 1 to 49 years nationwide study in denmark. Circ Arrhythmia Electrophysiol 2014; 7:205–211.

88. Harmon KG, Drezner JA, Maleszewski JJ, Lopez-Anderson M, Owens D, Prutkin JM, Asif IM, Klossner D, Ackerman MJ: Pathogeneses of sudden cardiac death in national collegiate athletic association athletes. Circ Arrhythm Electrophysiol [Internet] 2014; 7:198–204. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24585715

89. Harmon KG, Asif IM, Maleszewski JJ, Owens DS, Prutkin JM, Salerno JC, Zigman ML, Ellenbogen R, Rao AL, Ackerman MJ, Drezner JA: Incidence, Cause, and Comparative Frequency of Sudden Cardiac Death in National Collegiate Athletic Association

Athletes: A Decade in Review. Circulation [Internet] 2015; 132:10–19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25977310

90. Finocchiaro G, Dhutia H, Gray B, et al.: Diagnostic yield of hypertrophic cardiomyopathy in first-degree relatives of decedents with idiopathic left ventricular hypertrophy. Europace [Internet] 2020; . Available from: http://www.ncbi.nlm.nih.gov/pubmed/32011662

91. Kitzman DW, Scholz DG, Hagen PT, Ilstrup DM, Edwards WD: Age-related changes in normal human hearts during the first 10 decades of life. Part II (Maturity): A quantitative anatomic study of 765 specimens from subjects 20 to 99 years old. Mayo Clin Proc 1988; 63:137–146.

92. Ho CY, Sweitzer NK, McDonough B, Maron BJ, Casey SA, Seidman JG, Seidman CE, Solomon SD: Assessment of diastolic function with Doppler tissue imaging to predict genotype in preclinical hypertrophic cardiomyopathy. Circulation [Internet] 2002; 105:2992–2997. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12081993

93. Kessler EL, Boulaksil M, van Rijen HVM, Vos MA, van Veen TAB: Passive ventricular remodeling in cardiac disease: focus on heterogeneity. Front Physiol [Internet] 2014; 5:482. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25566084

94. Ferrantini C, Belus A, Piroddi N, Scellini B, Tesi C, Poggesi C: Mechanical and energetic consequences of HCM-causing mutations. J Cardiovasc Transl Res [Internet] 2009; 2:441–451. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20560002

95. Witjas-Paalberends ER, Piroddi N, Stam K, et al.: Mutations in MYH7 reduce the force generating capacity of sarcomeres in human familial hypertrophic cardiomyopathy.

Cardiovasc Res [Internet] 2013; 99:432–441. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23674513

96. Coppini R, Ferrantini C, Yao L, et al.: Late Sodium Current Inhibition Reverses Electromechanical Dysfunction in Human Hypertrophic Cardiomyopathy. Circulation [Internet] 2013; 127:575–584. Available from: http://circ.ahajournals.org/cgi/doi/10.1161/CIRCULATIONAHA.112.134932

97. Piroddi N, Witjas-Paalberends ER, Ferrara C, et al.: The homozygous K280N troponin T mutation alters cross-bridge kinetics and energetics in human HCM. J Gen Physiol [Internet] 2019; 151:18–29. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30578328

98. Wijnker PJM, Sequeira V, Kuster DWD, Velden J van der: Hypertrophic Cardiomyopathy: A Vicious Cycle Triggered by Sarcomere Mutations and Secondary Disease Hits. Antioxid Redox Signal [Internet] 2019; 31:318–358. Available from: https://www.liebertpub.com/doi/10.1089/ars.2017.7236

99. Rohr S: Role of gap junctions in the propagation of the cardiac action potential. Cardiovasc Res [Internet] 2004; 62:309–322. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15094351

100. Formigli L, Ibba-Manneschi L, Perna AM, et al.: Altered Cx43 expression during myocardial adaptation to acute and chronic volume overloading. Histol Histopathol [Internet] 2003; 18:359–369. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12647785

101. Spach MS, Heidlage JF, Dolber PC, Barr RC: Electrophysiological effects of remodeling

cardiac gap junctions and cell size: experimental and model studies of normal cardiac growth. Circ Res [Internet] 2000; 86:302–311. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10679482

102. Li A, Gong H, Zhang B, Wang Q, Yan C, Wu J, Liu Q, Zeng S, Luo Q: Micro-optical sectioning tomography to obtain a high-resolution atlas of the mouse brain. Science [Internet] 2010; 330:1404–1408. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21051596

103. Dodt H-U, Leischner U, Schierloh A, Jährling N, Mauch CP, Deininger K, Deussing JM, Eder M, Zieglgänsberger W, Becker K: Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. Nat Methods [Internet] 2007; 4:331–336. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17384643

104. Ke M-T, Fujimoto S, Imai T: SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. Nat Neurosci [Internet] 2013; 16:1154–1161. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23792946

105. Chung K, Wallace J, Kim S-Y, et al.: Structural and molecular interrogation of intact biological systems. Nature [Internet] 2013; 497:332–337. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23575631

106. Olianti C, Costantini I, Giardini F, Lazzeri E, Crocini C, Ferrantini C, Pavone FS, Camici PG, Sacconi L: 3D imaging and morphometry of the heart capillary system in spontaneously hypertensive rats and normotensive controls. Sci Rep [Internet] 2020; 10:14276. Available from: http://www.nature.com/articles/s41598-020-71174-9

107. Pianca N, Di Bona A, Lazzeri E, et al.: Cardiac sympathetic innervation network shapes

the myocardium by locally controlling cardiomyocyte size through the cellular proteolytic machinery. J Physiol [Internet] 2019; 597:3639–3656. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31116413

Figure Legends

Figure 1. The original description of myocardial disarray in 8 young decedents exhibiting asymmetric LVH by cardiac pathologist Donald Teare[2].

Figure 2. Figure 2. Myocardial disarray in three patients with recurrent ventricular arrhythmias who underwent heart transplantation for HCM: male of 51 years; female of 14 years; male infant of 8 months. A-C: Pinwheel pattern. Misaligned hypertrophic myocytes create whirls (A: Azan Mallory trichrome, 200x), twirls (B: Haematoxylin-Eosin, 400x) or tangles (C: Azan Mallory trichrome, 200x) with a background of some loose collagen. D-F. Herringbone pattern: groups of parallel myocytes lying obliquely (D: Haematoxylin-Eosin, 50x; E: Azan Mallory trichrome, 100x) or in a more chaotic pattern (F: Azan Mallory trichrome, 50x).

Figure 3. Backscattered electron image of a heart sample from a patient with hypertrophic cardiomyopathy. A, Lower-magnification image of cardiomyocytes and myocardial fibers. B and C, The higher-magnification image shows A bands as bright zones and I bands as dark zones. Myofibrillar disarray (arrows) and sarcomere disarray (arrowheads) can be observed. Magnifications are ×300 (A), ×1000 (B), and ×1500 (C). Bar indicates 20 μm. Reproduced with permission from Kanzaki et al[18] .

Figure 4. Examples of myocardial disarray in other cardiac diseases. A: EMB of a male of 13 years suffering from Danon disease (Azan Mallory trichrome, 200x). B: EMB of a female of 25 years with Fabry disease (Azan Mallory trichrome, 100x). C: EMB of a male of 48 years with hereditary transthyretin-related amyloidosis (arrows indicate bundle disarray) (Congo red under polarized light, 50x). D: Native heart of a 17 year-old male transplanted for non-

compaction cardiomyopathy (Haematoxylin-Eosin, 400x). E: Native heart of a 3 year-old female transplanted for mitochondrial cardiomyopathy (Haematoxylin-Eosin, 400x). F: EMB performed in a 67 year-old male with concentric left ventricular hypertrophy and severe pulmonary hypertension diagnosed one year before (Azan Mallory trichrome, 400x).

Figure 5. Multi-modal CMR assessment of myocardial diasarray using using diffusion tensor-cardiac magnetic resonance (DT-CMR), late gadolinium enhancement (LGE), and extracellular volume (ECV) mapping. The helix angle (HA) is the average myocyte orientation, and fractional anisotropy (FA) is a surrogate measure of underlying cell organization.

Figure 6. Drawing representing the functional consequences of myocardial disarray: Force as well as longitudinal and transmural conduction velocity vectors are represented in the absence (A) and in the presence (B) of myocardial disarray. Panels C and D show 3D imaging in the murine myocardium: The challenge of producing large, transparent and fluorescently labelled volumes has been recently achieved applying a true tissue transformation approach: CLARITY. This method transforms an intact tissue into a nanoporous, hydrogel-hybridized, lipid-free form that, by removing membrane lipid bilayers, allows high transparency, immunolabeling and structural and molecular preservation. This method in combination with advanced microscopy, allows 3d reconstruction of massive heart tissue at sub-cellular resolution. An image of whole left ventricle before (top) and after tissue clearing (bottom) is shown in panel C. In panel D an example of 3D rendering of the sympathetic neurons network imaged upon whole-mount. Figure modified from Pianca et al.[107]

Table 1. Summary of main studies on myocardial disarray in HCM.

Key points	**Studies**
Myocardial disarray appears a consistent feature in 8 young individuals who died suddenly exhibiting asymmetric hypertrophy of the heart	Teare[2] (1958)
Myocardial disorganization involving > 5% of the relevant areas of the tissue section was a highly sensitive (86%) and specific (92%) marker for HCM	Maron et al[5] (1981)
Myocardial disarray is a distinctive aspect of HCM even without significant LVH.	McKenna et al[69] (1990)
Myocyte disarray correlate with evidence of ischaemia, a younger age (<21 years) at death, family history of young sudden deaths in decedents of sudden death	Varnava et al[81] (2001)
Patients with HCM harbouring troponin T mutations have severe disarray, with only mild hypertrophy and fibrosis. These patients tend to die suddenly at a young early age	Varnava et al[10] (2001)
In decedents of SCD, HCM is differentiated from idiopathic LVH by the presence of myocardial disarray >20% in at least 2 tissue blocks of 4 cm2	Davies et al[70] (1995) Finocchiaro et al[32] (2016) Finocchiaro et al[90] (2020)
Diffusion tensor CMR can provide in vivo assessment of normal versus HCM myocardial architecture, with abnormalities in HCM patients (specifically, reduced fractional anisotropy values), matching myocardial architecture patterns of disarray	Ariga et al[11] (2019)

Abbreviations: CMR: cardiovascular magnetic resonance; HCM: hypertrophic cardiomyopathy; LVH: left ventricular hypertrophy.