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Hypertrophic Cardiomyopathy and the Troponins: The Enigma Remains

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Hypertrophic cardiomyopathy (HCM) is a heart muscle disorder and is the most common form of Mendelian-inherited heart disease, affecting approximately 0.2% of the global population [1,2]. In adults the disease is often inherited as an autosomal dominant trait caused by mutations, mainly in one of the 23 cardiac sarcomere protein genes [3-5]. HCM is defined as left ventricular wall thickness or mass, in the absence of abnormal loading conditions [3]. Whilst, histologically it is characterised myocardial disarray, fibrosis and small vessel disease [4]. HCM was the first of the cardiomyopathies to be attributed a genetic aetiology, with 50% of HCM cases attributable to a specific disease-causing gene [4,6]. Validation studies through co-segregation and linkage analysis has identified only half of the 23 sarcomeric or sarcomeric associated proteins, but the majority of cases (almost three quarters) arise from 2 genes *MYH7* and MYBPC3 [7]. A number of HCM causing mutations are unique to families known as 'private mutations'. The remainder of cases are found in the Thin filament complexes such as the Troponin T, I and Tropomyosin and rarely in the non sarcomeric proteins or metabolic genes such as PLN (Cardiac Phospholamban) CAV3 (Caveolin-3) and PRKAG2 (5'-AMP-activated protein kinase γ_2). Unfortunately, given the substantial allelic heterogeneity within each disease-causing gene and the large number of distinct mutations (>900) it remains a complex genetic conundrum [8,9]. This is further compounded by the variable disease penetrance, age of onset, and outcome. Hence, this would support the presence of other disease modifiers must exist beit common or intermediate genetic variants across the entire genome, or interactions *via* epigenetic signalling. Molecularly a number of possible molecular pathogenesis for HCM have been postulated, amongst which impaired myofibrillar contractile function, perturbations in calcium sensitivity, increased myocardial fibrosis are the main factors [10-13].

Impaired Myofibrillar Contractile Function

This was initially suggested to be the most-important mechanism, resulting in a 'compensatory' hypertrophy and diastolic dysfunction [14]. However, this generic mechanism of altered contractility caused by various sarcomeric gene mutations is not consistent across many of the common mutations. For example, mutations in *MYH7* have shown to produce either a reduced, or even enhanced. cardiac activity [15,16]. Meanwhile, other mutations in genes encoding thin-filament regulatory proteins, such as the troponins and α-tropomyosin, seem to frequently increase the calcium sensitivity of contractile proteins and so produce an augmented force of contraction within the confines of minimal calcium concentrations [17].

Impaired Calcium Sensitivity

Cardiac contraction is synonymous with calcium flux changes and the ongoing calcium cycling. Therefore, impaired calcium cycling beit from altered expression, phosphorylation or both from proteins such as sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA) has been implicated in both systolic and diastolic heart failure [18]. This same premise has been shown in HCM [19]. Furthermore, experimental data from transgenic mice expressing the mutant form of human cardiac Troponin T with an associated HCM mutation by Knollmann et al. suggest that alterations in calcium cycling and homeostasis might also contribute to ventricular arrhythmias in absence of hypertrophy. Indeed, the cardiomyocytes from these mutant mice showed prolonged calcium transients compared to wild-type control mice,

which went on to trigger delayed after-depolarizations or spontaneous calcium oscillations [20]. This study provided the first *in vivo* evidence in the Troponin mutations that arrhythmogenesis was independent of structural changes in the myocardium; whilst elegantly showing a means to rescue this through Blebbistatin, which decreases calcium sensitivity. However, in contrast to this there has also been reports of reduced calcium sensitivity within the Troponin mutations beit not specific to the *I79N* mutation [21].

Myocardial Fibrosis and HCM

The extent of scar and fibrosis has been shown to be correlated with reduced cardiac output and an increased propensity for heart failure and arhythmogenesis in a HCM model with animals [22]. This is in support of a number of clinical in vivo studies using cardiac MRI and the application of late gadolinium enhancement as a surrogate for scar formation, which have for a while purported the relationship between fibrosis and poor clinical outcomes in HCM [23-25].

Yet, the molecular mechanism and trigger for this inherent process remains still an enigma. Some have identified the role of fibrosis to be attributed to apoptosis of myocytes, with the subsequent replacement by an expansion of the interstitial matrix individually leading onto cardiomyocyte hypertrophy, which may or may not be mutually exclusive [26]. In addition, further reports have shown fibrosis to be a mixture of pro-fibrotic molecules including collagens, elastin, with the main signalling pathway being through transforming growth factor β (TGF-β) [22]. This was confirmed when fibrosis was reduced following the administration of TGF-β-neutralizing antibody and Losartan, an angiotensin-II-receptor antagonist, given the role played by angiotensin in promoting TGF-β expression. Furthermore, the cardiac MRI data on fibrosis also highlighted areas of increased wall thickness, which implicated a relationship between cell turnover and growth of cardiomyocytes and fibrosis [27]. This was coupled with the imbalanced collagen turnover which was a result of the mismatch between extracellular-matrix synthesis and degradation hence leading onto the early histopathological remodelling that define HCM [28,29].

Sudden Death and HCM

The clinical phenotype for HCM is variable ranging from lifelong asymptomatic forms, dyspnea on exertion to early Sudden Cardiac Death (SCD). It is the most common cause of sudden cardiac death in individuals younger than 35 years of age and frequently those who are asymptomatic; importantly there appears to be an overall cumulating mortality of 1-2% per year for such patients [5,7]. The defining paradigm in SCD is the generation of dangerous if not fatal arrhythmias, the trigger for which is unclear, but likely multifactorial including the development of non atherosclerotic induced hypoxic stress within the heart. The primary causative arrhythmia being either sustained Ventricular Tachycardia (VT) or Ventricular Fibrillation (VF), which is often initiated by premature ventricular complexes on a background of normal sinus rhythm [12]. Although there are many putative mechanisms for the induction of ventricular arrhythmias ranging from fibrosis to inefficient energy utilisation; a popular hypothesis for the induction of such arrhythmias has been increased alterations in calcium cycling homeostasis at a cardiomyocyte level and increased calcium sensitivity at the myofilament causing the generation of VT [12,13,27].

These effects were seen in the absence of hypertrophy, implying that arrhythmogenesis was not only due to macroscopic physical changes of the heart. Yet, the underlying molecular mechanisms as to how calcium induces ventricular arrhythmias and the signalling pathways remain unclear. Furthermore, the majority of such studies have taken place in the mice, which has distinct electrophysiological differences to humans; namely the high basal heart rate (300bpm), the very negative action potential (AP) plateau phase and the shorter duration of the AP (Figure 1).

Genetic Testing in HCM

Meanwhile, the prospect of genetic testing for risk prediction remains a rather mute topic. The initial analyses within large families of affected patients in single center's attempted to implicate the possibility of some mutations being associated with a more "malignant" course with a higher risk of sudden cardiac death and progressive heart failure [30]. However, in other large cohort and family studies, this was not always recapitulated [6]. Nevertheless, besides such conflicting data, some paradigms have been confirmed, namely involving the variable penetrance and clinical phenotype of particular mutations. One such gene is *TNNT2* , which is known to cause HCM but with little or even no hypertrophy. However, Individuals with these mutations seem to still possess a high risk of malignant ventricular arrhythmias and sudden cardiac death [8-10]. Given this intriguing disparity we decided to further investigate the Troponin and thin filament complexes and their interactions with each other in the context of clinical outcomes. This is particularly challenging given that sometimes it has been reported that certain mutations related to a HCM phenotype could also cause DCM and restrictive cardiomyopathies. This underlies the longstanding complexity between genotype-phenotype correlations. Yet, a full holistic and unbiased representation of the data, especially one which is able to integrate structural, genomic and clinical phenotypic data is lacking. In addition, the dynamic nature of cardiac contraction namely its effect with calcium and this interplay

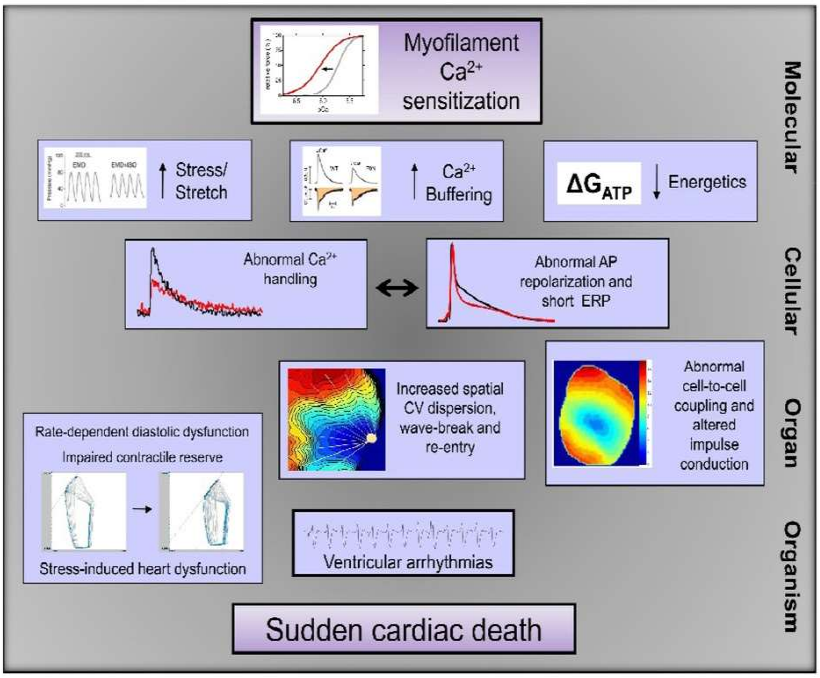

Figure 1: Above: Mechanism of arrhythmogenesis caused by myofilament Ca^{2+} sensitisation. The illustration highlights components likely to be involved in the signalling pathway from myofilament Ca^{2+} sensitisation to sudden cardiac death (As adapted from [5]- all rights remain with original author).

within such a model is also of importance to provide a more physiologically relevant model. The development of such a model will not only enhance our understanding of the genomic landscape in regards to disease 'hot spots' it may also provide a biological context prior to further experimental studies on mutations and their underlying mechanisms.

The Troponins

Since their discovery in 1963 the Troponin (Tn) proteins have been identified as part of a thin filament regulatory unit of the sarcomere. Troponin mutations are clinically relevant given they are thought to contribute approximately 15% of all the known sarcomeric protein cardiomyopathies. Unlike other such mutations it has been noted that the Troponin mutations they are however, show a more defined clinical course Troponin is a complex of three subunits. Troponin-I (Tn-I) inhibits Actomyosin ATPase; troponin-C (Tn-C) binds Calcium and troponin-T (Tn-T) links the complex to Tropomyosin (Tm) and is believed to be responsible for movement of Tm on the thin filament, modulating binding of the myosin head to Actin. The subunits are arranged in a 1:1:1 stoichiometric ratio along the thin filament with one Tn:Tm complex bound to every seven actin monomers. Troponin functions to couple calcium concentration changes to azimuthal movement of Tropomyosin on the thin filament. The position of Tropomyosin on actin controls the access of cross-bridges to the thin filaments and thus regulates the cross-bridge cycling that drives contraction. At low calcium levels, Tropomyosin is held by troponin at a location that sterically blocks myosin binding sites on Actin, thus producing relaxation; this is the blocked or B state of the thin filament [26]. Thin filaments are switched on when Calcium binds to Troponin, which moves Tropomyosin to the closed or C-state position, where the myosin binding sites are partly uncovered. Myosin binding to the thin filament also alters the position of Tropomyosin, and full activation of the thin filament requires binding of both calcium and myosin. However, due to a lack of crystal structure data for the proteins namely Troponin T it has not been possible to infer the functional consequence of mutants in a robust manner and how a particular mutation may confer binding changes in relation to other members of the troponin complex such as to Troponin T and Troponin I.

Although by no means the most common causal genetic culprit for HCM, the prospect of further work in the field of mechanisms induced through the troponin complex mutations remain active. It is therefore foreseeable that the ongoing research in this area will have direct implications for therapeutic targets, clinical application and moreover supplement our more detailed molecular pathological understanding of this clinically devastating disease and one for which within cardiovascular disease remains the most promising for translational outcomes.

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