Familial hypertrophic cardiomyopathy (FHC) is an inherited disorder affecting roughly one in 500 people. Its hallmark is abnormal thickening of the ventricular wall, leading to serious complications that include heart failure and sudden cardiac death. Treatment is complicated by variation in the severity, symptoms and risks for sudden death within the patient population. Nearly all of the genetic lesions associated with FHC occur in genes encoding sarcomeric proteins, indicating that defects in cardiac muscle contraction underlie the condition. Detailed biophysical data are increasingly available for computational analyses that could be used to predict heart phenotypes based on genotype. These models must integrate the dynamic processes occurring in cardiac cells with properties of myocardial tissue, heart geometry and haemodynamic load in order to predict strain and stress in the ventricular walls and overall pump function. Recent advances have increased the biophysical detail in these models at the myofilament level, which will allow properties of FHC-linked mutant proteins to be accurately represented in simulations of whole heart function. The short-term impact of these models will be detailed descriptions of contractile dysfunction and altered myocardial strain patterns at the earliest stages of the disease—predictions that could be validated in genetically modified animals. Long term, these multi-scale models have the potential to improve clinical management of FHC through genotype-based risk stratification and personalized therapy.

**Keywords:** hypertrophic cardiomyopathy; multi-scale; cardiac muscle
population. This phenotypic diversity seems generally explained by the large number (greater than 500) of individual mutations documented in the current medical literature [3], and suggests that predictions of disease phenotypes may be possible for specific genotypes. However, the identification of a substantial number of apparently asymptomatic, gene-positive individuals poses a new challenge to that idea and indicates that phenotypes of advanced FHC are sensitive to multiple factors [7]. In the light of these findings, Tardiff [8, p. 765] has proposed in a recent review that ‘a renewed focus on the most proximal events in both the molecular and clinical pathogenesis of [FHC] will be necessary to achieve the central goal of using genotype information to manage affected patients’.

The implicit hypothesis in current FHC research is that hypertrophy, regardless of its advanced form, is the result of altered acute function at the level of the cardiac sarcomere. Experimental and computational tools exist at present that together could be used to predict this kind of proximal, pre-hypertrophic phenotype based on the properties of mutant proteins (figure 1). When applied to animal models of FHC, bridging genotype and phenotype in this way would enable much more detailed descriptions of the disease process, and the generation of focused, testable hypotheses. Further on, these tools could provide a quantitative means of stratifying risk among patients and assist in clinical decision-making.

In the meantime, many groups continue to pursue FHC research using more traditional approaches (see [6,8,11] for recent reviews). These include genome-wide association studies in humans to identify new mutations, mouse lines engineered to have FHC-linked mutations and \textit{in vitro} studies of altered sarcomeric proteins. A new approach being explored at present is to create induced pluripotent stem cells from human somatic tissues that can then be differentiated into heart cells [12]. Myocytes derived from patients harbouring FHC mutations could then be used for functional cell-scale assays or as a means of obtaining mutant proteins for molecular studies. Genetically engineered mice have the advantage of allowing systematic molecular, structural and functional studies at

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**Figure 1.** Diagram of key experimental methods and potential computational tools for studying familial hypertrophic cardiomyopathy (FHC). Approximate dates show when each experimental approach was first applied to FHC research. Multi-scale computational approaches have not yet been applied to the study of FHC, but many of the necessary tools to do so have emerged in recent years (dates reflect the publication of applicable modelling techniques). [9] P-MRS refers to phosphorus-31 magnetic resonance spectroscopy [10].
The first mutations definitively linked with human FHC were found in the gene MYH7, which encodes β-myosin heavy chain (MHC). β-MHC mutations remain the most common cause of inherited HCM [6]. MHC is the largest of the three subunits that form the myosin molecule, and contains domains responsible for actin binding and nucleotide hydrolysis. The other two subunits of myosin, essential light chain (ELC) and regulatory light chain (RLC), associate with and stabilize an α-helix of MHC known as the lever arm. Motion of the lever arm is responsible for force production by myosin [14]. Relatively rare but well-documented FHC mutations are found in the human genes MYL2 and MYL3 encoding ELC and RLC, respectively, suggesting that these proteins play an important functional role [11]. Mutations to the gene MYBPC3, which encodes the thick filament protein myosin binding protein C (MyBP-C), are the second most frequent among individuals with FHC [11]. While the ability of MyBP-C to modulate sarcomere function is well established, the structural and functional details of its regulatory activity have not been fully determined.

Nearly all other known FHC-causing mutations occur in thin filament proteins (see [8] for a review). These include cardiac actin (ACTC), which forms the thin filament and its myosin binding sites. Tropomyosin sterically blocks these binding sites during relaxation, and mutations to the gene for its α isoform (TPM1) are linked to FHC. Cardiac troponin T (TNNT2) anchors the other troponin subunits to tropomyosin, and is thought to stabilize end-to-end overlap of adjacent tropomyosins. Cardiac troponin I (TNNI3) participates directly in Ca^{2+}-dependent regulation of contraction by binding to actin in a way that prevents the movement of tropomyosin [13]. When Ca^{2+} binds to troponin C (TNNC1), transfer of the inhibitory domains of troponin I from actin to the N-terminal domain of troponin C allows movement of tropomyosin and the formation of actin–myosin crossbridges.

Studies examining the properties and functional consequences of mutant sarcomeric proteins are numerous, and have added substantially to our understanding of genotype–phenotype connections in FHC (see Tardiff [8] and Harris et al. [11] for detailed reviews). Studies on purified proteins having point mutations seen in human FHC patients have been performed, but FHC genes have also been cloned and used to create transgenic and gene-targeted animal models (primarily mice). The resulting data range from measurements on the activity of single molecules (e.g. [15]) to measurements of cardiac function in vivo [16].

Perhaps the most frequently used experiment is to measure the Ca^{2+} sensitivity of force in skinned myocardial preparations containing mutant proteins (see Bai et al. [17] for a recent example). In these experiments, tissue is treated with a detergent to compromise cell membranes, enabling the Ca^{2+} concentration around the myofilaments to be set directly by the bathing solution. The myocardial sample is attached between a force transducer and a motor-controlled lever, and steady-state force produced by the preparation can be measured at varying Ca^{2+}.

**Figure 2.** Schematic of the cardiac sarcomere and main protein constituents. The sarcomere consists of interdigitated arrays of thick and thin filaments. The expanded view labels nine principal components: myosin heavy chain (MHC), essential light chain (ELC), regulatory light chain (RLC), myosin binding protein C (MyBP-C), troponyosin (Tn), troponin I (TnI), troponin T (TnT), troponin C (TnC) and actin. Cardiomyopathy-linked mutations have been identified in the genes encoding each of these nine proteins, which represent the most common causes of inherited HCM [6]. At rest, force is inhibited by Tm, which blocks the myosin binding site on actin (left most myosin molecule; note that the second myosin head in each case is omitted for clarity). Force production is initiated in the sarcomere when Ca^{2+} binds to a low-affinity site on TnC (labelled), triggering a shift in the Tm position to expose binding sites (myosin in the centre). Once attached, myosin releases energy obtained from ATP hydrolysis to rotate its lever arm, distending its tether to the thick filament and producing force. Evidence suggests that disease-linked mutations alter the way this system produces force in response to Ca^{2+}.

**2. SARCOMERIC PROTEINS**

Approximately 70 per cent of inherited forms of HCM can be linked to genes encoding proteins found in the sarcomere [3]. The sarcomeres of cardiac muscle cells are responsible for producing the contractile force, and are composed of two overlapping arrays of protein filaments: thick filaments, containing the motor protein myosin, and thin filaments, consisting of polymerized actin decorated with the regulatory proteins tropomyosin and tropomyosin (figure 2). Contraction is initiated when Ca^{2+} binds to the troponin complex, triggering a series of allosteric signalling events that move tropomyosin on the surface of the actin filament to expose binding sites for myosin heads [13]. Myosin cyclically interacts with actin in a process that converts energy in the form of adenosine triphosphate (ATP) into mechanical work, sliding thick and thin filaments past each other and causing the muscle to shorten.
concentrations. The force–Ca\textsuperscript{2+} relation obtained in this way is typically parametrized by fitting points with the Hill equation,

\[ F = F_{\text{max}} \frac{[\text{Ca}\textsuperscript{2+}]}{[\text{Ca}\textsuperscript{2+}]_{50} + [\text{Ca}\textsuperscript{2+}]} \]

where \( F_{\text{max}} \) is the force at saturating Ca\textsuperscript{2+} concentration, \([\text{Ca}\textsuperscript{2+}]_{50} \) is the Ca\textsuperscript{2+} concentration at half-maximal force (often referred to as the ‘Ca\textsuperscript{2+} sensitivity’) and \( n_H \) is the Hill coefficient.

A trend emerging from the many steady-state force–Ca\textsuperscript{2+} relations measured in the presence of mutant sarcomeric proteins is that mutations linked to HCM tend to increase Ca\textsuperscript{2+} sensitivity of the myofilaments, while the small (but significant) number of mutations linked to dilated cardiomyopathy (DCM) tend to decrease it \([8,18]\). This result is significant, but the ability of Ca\textsuperscript{2+} sensitivity to predict phenotype seems limited in that the magnitude of the sensitivity change from baseline does not appear to predict disease severity. Moreover, a single parameter cannot describe the multi-dimensional phenotypic space of FHC, which includes varied patterns of hypertrophy and distinct risks for arrhythmia for different mutations.

Even in a case where Ca\textsuperscript{2+} sensitivity measurements agree with the general HCM/DCM paradigm, the genotype–phenotype connection is only partially achieved. In such a case, the initial challenge to relate a specific mutation to the type of cardiac remodelling becomes one of relating the mutation to Ca\textsuperscript{2+} sensitivity. Knowing the amino acid sequence of normal and mutant proteins should provide important clues, and some progress is being made in this regard through the use of molecular dynamics (MD) simulations (see Ertz-Berger et al. [19] and Lorenz & Holmes [20]). MD uses protein structure data to predict the motion of atoms within molecules on picosecond time scales. In one case, MD has been used to predict motion of a critical region of the troponin T (TnT) molecule in the presence of FHC-linked mutations R92W and R92L [19]. Simulations showed that both mutations tended to destabilize helical structures in the (TnT) molecule in the presence of FHC-linked mutations.

3. INTEGRATIVE MYOFILAMENT FUNCTION

Integrative models of myofilament function predict Ca\textsuperscript{2+} activation on the basis of known structural interactions and conformational states of myofilament proteins (recent examples include [21–23]). While clearly lacking in the molecular detail achievable through MD simulations, these models have the advantage of being able to reproduce functional measurements made in cardiac muscle preparations. In general, these models represent Ca\textsuperscript{2+} binding to troponin C, activation of the troponin–tropomyosin regulatory switch and crossbridge cycling, with interactions among these processes that are suggested by experimental evidence. Integrative myofilament models could be used to analyse force–Ca\textsuperscript{2+} relations and other properties in muscle-containing FHC mutant proteins, pointing towards one or more of the simplified processes they represent as the main functional change(s) induced by the mutation. This has the potential of focusing atomistic simulations towards specific structures and interactions among myofilament proteins.

Improvements in these types of myofilament models in recent years have centred around representation of cooperative activation [24] by Ca\textsuperscript{2+}. Cooperativity describes the steep, sigmoidal relation observed between Ca\textsuperscript{2+} concentration and contractile force under steady-state conditions (represented by the parameter \( n_H \) in the Hill equation). The molecular origin of striated muscle cooperativity is widely believed to be end-to-end interactions arising between adjacent tropomyosin molecules on the actin thin filament [25,26]. According to the steric blocking model of muscle regulation [13], tropomyosin blocks myosin binding to actin under low Ca\textsuperscript{2+} conditions. Interactions among adjacent tropomyosins are thought to couple myosin binding sites such that they tend to be exposed in an ‘all-or-nothing’ fashion consistent with steeply cooperative behaviour.

A number of theoretical and computational models have been developed to describe these and other putative mechanisms of myofilament cooperativity under steady-state conditions [27–32]. Others have been formulated for the purpose of predicting myofilament activation during the transient changes in [Ca\textsuperscript{2+}] that occur in the beating heart [9,21,33]. Ideally, these models would be capable of translating measurements of myofilament Ca\textsuperscript{2+} sensitivity and cooperativity from skinned muscle preparations into their true effects under physiological conditions. In spite of the variety and the number of published myofilament models, this exciting prospect remains largely unrealized.

Our own recent work has led to the formulation of a Markov model of myofilament activation that can simultaneously reproduce the steady-state and dynamic force–Ca\textsuperscript{2+} relations [23]. The model is based on the three states of the cardiac thin filament regulatory unit originally proposed by McKillop & Geeves [28] and later verified by structural data [34]. When transitions between the three states are considered as dependent on the states of neighbouring regulatory units (through tropomyosin interactions), the system exhibits both steady-state and dynamic aspects of cooperative activation. The model was validated against
data collected in skinned cardiac muscle under a variety of conditions, including the addition of NEM-S1 (a soluble myosin sub-fragment that binds actin with high affinity), elevated inorganic phosphate concentration and shortened thin filament length, among others.

The ability of this model to reproduce experiments that use molecular-scale perturbations supports its use in analysing steady-state force–Ca$^{2+}$ relations in the presence of FHC-linked mutations. A simplified example of this is shown in figure 3a, where the model is used to reproduce a leftward shift and loss of cooperativity in the force–Ca$^{2+}$ relation seen in the FHC-linked tropomyosin mutation E180G [17]. Both changes were achieved simultaneously by lowering a single parameter, the free energy change associated with nearest-neighbour Tm coupling (control and E180G Tm are solid and dashed lines, respectively). This parameter change is indicative of either increased Tm flexibility or destabilization of end-to-end binding. Each curve has been normalized by maximum tension to emphasize the difference in Ca$^{2+}$ sensitivity. (b) The model can also be used to predict the effects of the E180G Tm mutation on twitch dynamics. Using the same parameters as in (b), twitches were elicited in response to an idealized Ca$^{2+}$ transient (not shown). The simulation suggests that the mutation increases diastolic tension and slows rates of both contraction and relaxation, consistent with known phenotypes [17].

4. VENTRICULAR MYOCYTES

During each heartbeat, the signal to contract reaches cardiac muscle cells in the form of an electrical impulse. At the level of the cell, this electrical excitation causes rapid release of Ca$^{2+}$ ions into the cytosol from intracellular stores to activate contraction in the sarcomeres. This process from electrical stimulation to force production is known as cardiac excitation–contraction coupling or simply EC coupling [35].

Several cellular structures and a host of related proteins are involved in EC coupling in ventricular myocytes [35]. Ion channels and their accessory proteins at the cell membrane are responsible for detecting and propagating the transient changes in membrane potential that initiate contraction. Changes in membrane potential open L-type Ca$^{2+}$ channels, which triggers Ca$^{2+}$ release from the sarcoplasmic reticulum (SR) in a process known as Ca$^{2+}$-induced Ca$^{2+}$ release (CICR). Membrane-bound proteins in the SR are responsible for Ca$^{2+}$ re-uptake from the cytosol, which lowers Ca$^{2+}$ and causes contraction to end.

It could be argued that the entire process of force generation by the myofilaments forms part of EC coupling. Traditionally, EC coupling has been considered complete at the point Ca$^{2+}$ binds to troponin C (TnC), but two observations suggest that this view neglects potentially important mechanisms in the behaviour of cardiac muscle. The first is that the Ca$^{2+}$ affinity of TnC is increased nearly 10-fold by myosin
Figure 4. Myofilament models are capable of reproducing contraction events in living myocytes. (a) A measured Ca\(^{2+}\) transient was used as input to a myofilament activation model coupled with equations representing an internal elastic load to simulated cell shortening. (b) Free parameters were adjusted in the model until predicted shortening (dotted trace) matched the experimentally measured response (solid trace, 0.2% relative error). Rat ventricular myocyte at 25°C.

Integrative models of myocyte electromechanics have great potential in the realm of FHC research for several reasons. Many FHC mutations are associated with high risk for lethal arrhythmias [8], and the myocyte is the simplest system in which sarcomeric mutations could exert an effect on electrical activity in the heart. Myocytes are also the simplest experimental preparation in which the effects of FHC mutations on twitch characteristics can be observed. Typically in these experiments, Ca\(^{2+}\) transients and unloaded cell shortening are measured in myocytes isolated from engineered mice expressing FHC-linked mutant proteins. Twitch characteristics gleaned from unloaded shortening measurements are frequently different from wild-type controls, but interpretation of twitch phenotypes based on genotype is complicated by the fact that the Ca\(^{2+}\) transients driving twitch can also be altered in these animals (e.g. the E22K mutation in MYL3 [45]). By combining experiments with quantitative model analysis, the effects of a mutated sarcomeric protein on twitch itself can be separated from those exerted by the Ca\(^{2+}\) transient. For example, our recent model of myofilament activation [23], when coupled with appropriate equations for length and velocity dependence of contraction [41], can be used to quantitatively link measured Ca\(^{2+}\) transients with cell shortening (figure 4; see the electronic supplementary material for additional details). Ca\(^{2+}\)-handling differences could be accounted for by fitting model parameters to reproduce measured shortening in response to measured Ca\(^{2+}\) transients. Differences in the fitted contraction model parameters would reveal the nature of functional changes to myofilament activation caused by the mutant protein.

A biophysically detailed electromechanical myocyte model would allow even more integrative analysis to examine not only altered twitch, but also the basis for alterations to the Ca\(^{2+}\) transient. Ca\(^{2+}\) handling undergoes certain well-known adaptive changes in hypertrophy and heart failure that are not directly dependent on the myofilaments. However, acute changes in Ca\(^{2+}\) handling early in the disease process could be mediated by changes to Ca\(^{2+}\) buffering by TnC caused by mutant binding to actin [36]. This means that, during a twitch, the capacity of TnC as a buffer of cytosolic Ca\(^{2+}\) cannot be considered constant. A second general observation is that, in numerous cases, modification of myofilament proteins alters the overall Ca\(^{2+}\) sensitivity and presumably Ca\(^{2+}\) buffering of the sarcomeres, whether through post-translational modification [37] or cardiomyopathy-linked mutations [17]. The abundance of TnC in the cytosol means that factors modifying myofilament Ca\(^{2+}\) sensitivity have the potential to influence EC coupling in the whole cell, one form of a phenomenon known as mechanoelectric feedback [38]. Hence, an understanding of the relationship between excitation and the force produced by contraction depends critically on the properties of myofilaments.

Integrative ventricular myocyte models that combine representations of electrophysiology, Ca\(^{2+}\) handling and myofilament contraction allow quantitative study of the complex interplay among these processes [39–41]. Computational models of myocyte electrophysiology have evolved over several decades and now include mechanistic descriptions of many ion channels and transporters. The most recent of these are being used to predict the effects of mutated channel proteins on cardiac action potentials [42]. Innovative mathematical approaches developed in the last decade have simultaneously improved the biophysical accuracy and the computational efficiency of Ca\(^{2+}\) handling and CICR models [43]. We recently used a canine EC coupling model containing this improved CICR representation coupled with the contraction model of Rice et al. [22] to investigate sources of electromechanical heterogeneity in endocardial, mid-myocardial and epicardial myocytes [39]. One prediction from that work was that differences in EC coupling alone could not explain more rapid contraction and relaxation rates in epicardial cells. Instead, an increase in the rate of crossbridge cycling consistent with elevated expression of the faster α-MHC isofrom was needed to explain experimental measurements. This result was subsequently supported by the discovery of increased α-MHC expression in porcine ventricular epicardium that correlated with faster crossbridge kinetics in skinned epicardial cells [44].
Since then, differences in ion channel current density of the heart [49], and this raises the possibility that FHC behaviour critically affect mechanics and pump function. The hypothesis is that faster contraction in epicardial cells would allow them to ‘catch up’ to endocardial cells, which are activated earlier during the cardiac cycle. However, a computational model predicts that heterogeneities affect ventricular deformation throughout most of systole, not just in its opening moments (figure 5) [49].

There is strong evidence that in some cases FHC mutations interfere directly with transmurally heterogeneous properties, which could be an intriguing source of phenotypic variation. For example, RLC contains a phosphorylation site that modulates contractile function in the heart [48,60]. The hypothesis is that faster contraction in epicardial cells would allow them to ‘catch up’ to endocardial cells, which are activated earlier during the cardiac cycle. However, a computational model predicts that heterogeneities affect ventricular deformation throughout most of systole, not just in its opening moments (figure 5) [49].

Any influence that FHC mutations have on cardiac function takes place against the backdrop of naturally occurring spatial variation in expression and composition of proteins in the sarcomere (and elsewhere) that modify EC coupling properties of individual cells [46–48]. Simulations suggest that regional differences in contractile behaviour critically affect mechanics and pump function of the heart [49], and this raises the possibility that FHC mutations could alter or otherwise interact with natural heterogeneities to produce disease pathology. Regional patterns in the morphology and duration of myocyte action potentials were among the first of many heterogeneous properties described in the past 20 years [50]. Since then, differences in ion channel current density [51], Ca$^{2+}$ transients [48,52,53], myosin isoform expression [44,54–56], myofilament protein phosphorylation [57,58] and unloaded cell shortening [48,52] have been noted. In some cases, it has been possible to correlate variations at the molecular level to functional differences among myocytes isolated from the respective myocardial regions [44,46,59]; however, much remains to be learned about the practical implications of observed heterogeneities, even at the level of individual cells.

The same may be said of the role these heterogeneities play in the function of the whole heart. For instance, cells in the outer, epicardial region of the left ventricle tend to have shorter action potentials and Ca$^{2+}$ transients, and contract more rapidly than those in the inner or endocardial region [48,52]. It has been proposed that this type of heterogeneity coordinates contraction in the heart [48,60].

5. TRANSMURAL HETEROGENEITY

Cardiac hypertrophy is observed clinically in response to hypertension or any other condition that places increased load on the heart. HCM is the default diagnosis for patients presenting with increased left ventricular wall thickness, but without obvious causative factors such as hypertension [6]. When mutant sarcomeric proteins were identified as the main cause of inherited HCM, it made logical sense to many as alterations to the contractile apparatus of the heart would be expected to result in changes in mechanical loading and therefore trigger hypertrophy. In recent years, non-invasive imaging studies have shown that

Figure 5. A three-dimensional model can be used to predict the effects of cell-type distribution on ventricular mechanics and function. The simulation data rendered in this figure were generated during a previous study (see [49] for complete details). (a) This view of the model shows the ventricular geometry and orientation of cardiac fibres. (b) The model was used to predict left ventricular (LV) pressure waveforms during systole. Here, a physiological distribution of cell types (baseline) was compared with a hypothetical case in which the ventricle is composed entirely of mid-myocardial cells (all mid). Circles mark the opening and closing of the aortic valve. (c) The two cases also show differences in their patterns of ventricular wall strain, shown here on cross sections through the ventricular wall. These results suggest that accounting for normally occurring heterogeneity as a back-drop for the effects of sarcomeric mutations will be critical in correctly predicting early myocardial strain phenotypes. Blue, endocardial region; red, mid-myocardial region; green, epicardial region.
alterations to normal patterns of myocardial strain coincide with or even precede remodelling of heart tissue in various pathologies [62,63]. These results offer a possible explanation for the phenotypic diversity in patterns of ventricular hypertrophy seen among FHC patients. Each mutation, acting on a background of spatially heterogeneous sarcomeric and cellular properties, has the potential of affecting myocardial strain in the left ventricle in different ways. Subtle differences in strain and loading could give rise to diverse remodelling phenotypes.

Multi-scale models of ventricular mechanics could be used to explore the connection between altered sarcomere behaviour and patterns of myocardial strain. A general approach would be to use data from mice expressing a mutant FHC protein to create a finite-element mesh of the left ventricle with realistic three-dimensional geometry and contractile behaviour. Properties of isolated myocardium from these mice could be studied and used to construct mechanistic myofilament and cell-level parameter sets. These in turn would be embedded in fully coupled electromechanical simulations of the left ventricle to predict global and regional function of the myocardium, which could be validated against in vivo strain measurements.

It may be particularly instructive to build and compare multi-scale models of hearts having either hypertrophic or DCM-linked sarcomeric mutations. Modelling hearts at an early age prior to anatomical changes could reveal patterns of myocardial strain unique to each type of ventricular remodelling. If validated, these models would offer a powerful tool for relating mutations to the mechanical cues that drive myocardial remodelling at the level of individual cells. Simulations of this kind could also suggest new imaging-based strategies that would allow clinicians to non-invasively detect the existence of underlying molecular pathologies.

Recent advances in magnetic resonance (MR) imaging and software have made the process of creating realistic meshes of left ventricular geometry more rapid and accessible, even in mice [64]. The orientation of cardiac fibres in the left ventricle is a critical determinant of ventricular remodelling. If validated, these models would offer a powerful tool for relating mutations to the mechanical cues that drive myocardial remodelling at the level of individual cells. Simulations of this kind could also suggest new imaging-based strategies that would allow clinicians to non-invasively detect the existence of underlying molecular pathologies.

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Regional strain in the myocardium for model validation can be acquired using either MR tagging with harmonic phase (HARP) tracking or echocardiographic strain imaging (speckle-tracking). It is critical to note that both MR tagging and speckle-tracking have recently been shown to be accurate enough to detect changes in regional strain in mice at time points that precede remodelling and changes in heart function [63,64]. This means that FHC mutant mice could be studied at a young age (less than eight weeks), prior to the occurrence of hypertrophy and the emergence of heart failure, in line with the paradigm proposed by Tardif [8].

New methods for coupling cell-level models with models of action potential propagation and ventricular biomechanics have emerged in recent years. These advances are important as they allow experimental data obtained in reduced systems such as skinned fibres or isolated cardiac myocytes to be scaled up for predicting function at the level of the intact heart. An initial challenge in this area was numerical instability in simulations arising from coupling length-dependent contraction models with iterative solvers for the finite-element mechanics problem [67,68]. Naive coupling is accomplished through an operator splitting approach, wherein the system of differential and algebraic equations (DAEs) representing cellular electromechanics is solved separately from the partial differential equations (PDEs) that govern mechanical deformation of the three-dimensional ventricular mesh. Sarcomere length and velocity are computed from the mechanics PDEs and supplied to the cell-level DAEs at discrete time intervals throughout the simulation. Values of active tension computed by cell-level DAEs are passed to the mechanics PDEs at the same time. Instability results in this case because, as the iteration scheme deforms nodes in the mesh to balance forces, a constant value for active tension is assumed even though length dependence in the contractile model means that it should change for each iteration [67]. Taking extremely small time steps or re-solving the entire cell model, DAEs with each mechanics iteration can mitigate instability but are both computationally prohibitive. Instead, the so-called ‘update’ schemes have been employed in which simplified forms of the cell model are used to update or re-compute an approximate value for active tension with each iteration [49,67,68].

Physiological loads can be applied to ventricular models by coupling left ventricular volume and boundary conditions on the endocardial surface to lumped-parameter systems models of the circulation [69]. This has enabled simulations of regional cardiac function that are providing clinically relevant insights [70]. These simulations that mimic in vivo loading will allow accurate validation of regional myocardial strains against those measured in animal models. Haemodynamic parameters, which are frequently assessed in engineered mouse models of FHC [71], are also produced in these simulations and would provide an additional point of validation.

7. LIMITATIONS

Using a multi-scale modelling approach to study FHC mutations entails several challenges that will have to be addressed in implementation. Some limitations are common to the broader field of multi-scale heart modelling (see Clayton et al. [72] for a detailed review). For instance, many questions remain about the ability of cell models to reproduce behaviour of myocardial tissue. In most cases, functionally coupled cells are represented using continuum approximations, which neglect complex aspects of tissue microstructure such as voids and the presence of fibroblasts or other non-myocyte cells [72]. More work is needed to understand the implications of tissue heterogeneity at this scale. There is also evidence that models constructed from single cell data do not always extend well to conditions present in tissue. Cherry & Fenton [73] demonstrated that two published models of canine ventricular myocyte electrophysiology displayed substantially different
spiral wave dynamics in two-dimensional tissue simulations, in spite of the fact that the models are based on data from the same species. Hence, cell-level models that are useful for studying one question may not be suitable for addressing others, particularly where crossing scales are concerned.

Other obstacles to multi-scale modelling in FHC are more specific to the disease. As in the human population, there can be large phenotypic variation in murine strains engineered to have human FHC mutations (e.g. [74]). Without a consistent pattern of ventricular hypertrophy, it would be harder to link changes in regional mechanics to localized areas of tissue remodelling. It may be necessary to construct mouse-specific models for strains with highly variable hypertrophic phenotypes, which would greatly increase the cost and effort required.

Studying the proximal events in FHC has been advocated in large part because later stages involve secondary responses that complicate interpretation of data and could be major sources of phenotypic variation [8]. While many confounding influences can be avoided by studying young animals, some compensatory pathways, such as those regulating blood pressure, are likely to be altered from the earliest stages of FHC. For instance, evidence of altered β-adrenergic signalling at the level of the myofilaments can be seen in transgenic mice expressing non-phosphorylatable myosin RLC [75]. If an FHC mutation indirectly alters phosphorylation or the isoform expression profile of other myofilament proteins, these changes will have to be considered in order to properly account for their influence on Ca$^{2+}$-contraction dynamics [76].

Another potentially critical component of acute responses to mutant sarcomeric proteins is their impact on cell metabolism. The tendency of FHC-linked mutations to cause gains in function such as increased myofibrillar Ca$^{2+}$ sensitivity and shortening velocity implicates inefficient use of ATP as a disease mechanism. While we have focused on mechanical stimuli as drivers of FHC in this review, other authors have suggested that altered energy metabolism is the central cause. This hypothesis originated in a study demonstrating lower phosphocreatine to ATP ratios in FHC patients that were independent of the specific gene mutation [10], and similar findings have been noted in engineered mouse lines (see [77] and references therein). Integrative models of ventricular myocytes that include mitochondrial bioenergetics have been developed (e.g. [78]), and could be used to investigate the metabolic consequences of altered myofilament function in multi-scale models.

These points reinforce the necessity for careful validation of modelling results at all scales and particularly in vitro if multi-scale models of FHC are to be useful. It is likely that the models, their scope, the volume and the type of data included and even experimental protocols used for validation will have to be optimized before multi-scale models can accurately predict time-varying patterns of myocardial strain or other properties that could be correlated with hypertrophy. Iteration between computational models and experiments may constitute a limitation of the approach, but it also represents a significant advantage since integrative analysis of this kind enables the completeness of hypotheses to be quantitatively assessed.

8. CONCLUSION

Multi-scale models of the heart have the potential to facilitate the next advances in understanding and treating FHC by providing a quantitative link between sarcomeric mutations and the conditions that precede maladaptive hypertrophy. The acute effects of mutant contractile proteins on function of the whole organ cannot be predicted in a useful way by intuition alone, considering the volume and complexity of information at intervening biological scales. On the other hand, multi-scale heart models are advancing to the point that they will soon be capable of predicting pre-hypertrophy strain patterns on the basis of genotype. These hypothesized ‘pre-hypertrophy phenotypes’ will allow those studying FHC mutations in mice to shift focus from the endpoint of hypertrophy to the more subtle mechanical changes that might be present at much earlier stages.

We thank Dr Kenneth S. Campbell for the use of laboratory equipment to measure myocyte function (figure 4), secured through NIH grant HL090749. This research was also supported in part by NIH grants 5P01HL63445 (Knowlton), P41RR08605 (Arzberger) and 1R01HL96444 (McCulloch).

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