TRANSLATIONAL PHYSIOLOGY

Interpreting genetic effects through models of cardiac electromechanics

S. A. Niederer,¹ S. Land,² S. W. Omholt,³ and N. P. Smith¹,²

¹Department of Biomedical Engineering, King’s College London, King’s Health Partners, Saint Thomas’ Hospital, London, United Kingdom; ²Department of Computer Science, University of Oxford, Oxford, United Kingdom; and ³Centre for Integrative Genetics, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Oslo, Norway

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Niederer SA, Land S, Omholt SW, Smith NP. Interpreting genetic effects through models of cardiac electromechanics. Am J Physiol Heart Circ Physiol 303: H1294–H1303, 2012. First published October 5, 2012; doi:10.1152/ajpheart.00121.2012.—Multiscale models of cardiac electromechanics are being increasingly focused on understanding how genetic variation and environment underpin multiple disease states. In this paper we review the current state of the art in both the development of specific models and the physiological insights they have produced. This growing research body includes the development of models for capturing the effects of changes in function in both single and multiple proteins in both specific expression systems and in vivo contexts. Finally, the potential for using this approach for ultimately predicting phenotypes from genetic sequence information is discussed.

H1294

Review

INTRODUCTION

Molecular-biological technology allow for targeted manipulation of an animal’s genome by substituting, inserting, removing, inhibiting, or overexpressing genes and the subsequent proteins they code for (29). This provides a comprehensive set of genetically based experimental techniques for manipulating biological systems and uncovering physiological insights. However, despite knowledge of the genotype and phenotype in a given system even under controlled conditions (age, temperature, background), the complex network of interactions linking these two data sets often remains obscured (3, 37).

The inherent need to understand these complex links between genotype and phenotype naturally leads to the use of mathematical models to rationally represent and interpret what are typically, otherwise, opaque relations. Computational models provide a systematic method for integrating experimental data to enable this interpretation (22, 36, 46). In particular, using experimental data acquired within studies focused on understanding the effect of genetic manipulations models have provided further analysis and physiological insight through three distinct approaches. First, models have allowed measurements to be translated between experimental assays. With the use of this approach, protein function in the presence or absence of a mutation can be characterized in isolation in expression systems or pharmacologically. This information on physiological function can then be interpreted in situ by use of a computational model (15). Second, models have enabled the confounding effects of changes in multiple proteins to be separated and quantified (47, 48). This means that both the direct and compensatory effects of a genetic manipulation can be simultaneously analyzed and the physiological effects of these changes on an observed phenotype determined. Finally, models allow multiscale interactions and feedback to be analyzed (28). This approach provides a framework for investigating interactions between protein and cell function or between changes in cellular physiology and organ scale phenotypes that are not readily measured experimentally.

Biophysical computational models of cardiac electromechanics have evolved from early models of single myocytes (39) to detailed biophysical whole organ simulations focused on capturing cardiac function in the mouse (44) (see Fig. 1), rat (60), canine (31), and human (40, 57–59). The multiscale frameworks developed through this work have progressively included increasing levels of detail in the form of regulatory pathways (73), metabolism (9, 80), and drug interactions (53), as well as increasing capacity to simulate the interactions between multiple physical systems including electromechanical coupling, fluid-solid interactions, and perfusion-mechanics coupling (61).

Only recently have computational models and experimental techniques evolved to a point where they can be merged to link genetic modifications to physiological function. In particular, the application of modeling to interpret experimental and more recently clinical data has, arguably, been most successfully
applied in the heart. With this context, building upon recent more general surveys (61, 82), we will narrow our focus in this review to work that has focused on providing insight into the genetically controlled regulation of cardiac electromechanics using biophysically based modeling. Within this scope our goal will be to highlight the unique and emerging opportunities offered by computational modeling for mechanistically interpreting experimental observations and providing physiological insights into the effect of genetic manipulations on health and disease.

To demonstrate how this class of electromechanical models, and the modeling approach in general, can be applied to analyze experimental data and provide physiological insight, we review a subset of current state-of-the-art exemplars. These examples demonstrate how physiological studies using genetically modified animal models or assays can be complemented and enhanced by analyzing results by applying the three approaches described above. However, through the covering of this material, our aim is to also demonstrate the need for modeling studies to recognize the importance of genetic background when analyzing results. This requires a move away from modeling a generic animal but to explicitly representing a species and genetic strain, as is routine in experimental studies. We argue that this level of rigor will be required from models if they are to engage in supporting and advancing genetics experimental studies where it is well recognized that genetic background can play a critical role in determining phenotypes (54).

To date, cardiac multiscale and multiphysics models have typically been used in studies where a given phenotype is sought explained by underlying mechanisms. Below we describe how these computational models have been applied to genetic phenotypes with increasing levels of complexity, as shown in Fig. 2. First, we describe early work focused on characterizing changes in the quantity and function of single proteins and how these affect whole cell function. In these studies single protein measurements in expression systems were translated into cellular contexts using computational

<table>
<thead>
<tr>
<th>Modelling Approach</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Translation</td>
<td>Cellular Single Protein</td>
</tr>
<tr>
<td></td>
<td>Expression systems to cellular function</td>
</tr>
<tr>
<td></td>
<td>Cellular Multi-Protein</td>
</tr>
<tr>
<td></td>
<td>Expression systems to cellular function</td>
</tr>
<tr>
<td></td>
<td>Organ/Tissue</td>
</tr>
<tr>
<td></td>
<td>Cellular measurements to organ function</td>
</tr>
<tr>
<td>Deconvolution</td>
<td>Link protein function to cellular physiology</td>
</tr>
<tr>
<td></td>
<td>Isolate effects of individual proteins</td>
</tr>
<tr>
<td></td>
<td>Isolate effects of individual proteins</td>
</tr>
<tr>
<td>Multi-scale</td>
<td>Link protein function to cellular physiology</td>
</tr>
<tr>
<td></td>
<td>Link cellular physiology</td>
</tr>
<tr>
<td></td>
<td>Link cellular physiology to organ function</td>
</tr>
</tbody>
</table>

Fig. 1. Electromechanics Black 6 whole organ mouse model (44). A: raw MRI data, numbered from base to apex, taken 1 mm apart. Manual segmentation of the data by experts is indicated by blue lines. B: fitting of a cubic Hermite finite element mesh (red surfaces) to the segmented data (white surfaces). C: Black 6 mouse heart model at end diastole (0 ms) and midystole (42 ms). At both time points, arrows indicate the direction of the fiber orientation across the heart wall. The color of the arrows and the endocardial surface indicate the fiber strain. The color of the transmural surface is colored at both time points to indicate the intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]), ranging from green (diastolic) to black (peak from single-cell pacing simulations) to white (hyperactivated in tissue). λ, cellular stretch (current length/resting length).

Fig. 2. Schematic showing the how each modeling approach has been applied to investigate the increasing complex single protein, multiple protein, and tissue/organ scale phenotypes.
models, and the effects of changes at the protein scale on cellular function were investigated. We then review the use of models to analyze more complex cellular phenotypes. In these cases, where compensatory mechanisms confound the impact of changes in a single protein, more comprehensive data sets that quantify the multiple protein adaptations were integrated and interpreted within a common computational model. Finally, organ and tissue scale phenotypes resulting from spatial heterogeneities and multiscale feedback and regulatory mechanisms investigated using computational models are discussed. These sections are followed by a review of current work seeking to apply computational models to predict phenotypes from genotypes. Finally, we discuss the challenges in species-specific models and species selection facing computational models.

Single Protein Phenotypes

Many computational models exist for simulating cardiac electrophysiology, calcium dynamics, contraction, and metabolism [for review, see Fink et al. (27)]. With the use of these existing models, it is possible to predict the impact of changes in single protein function or concentration on cellular physiology. These examples demonstrate the use of models for data translation and linking measurements across multiple scales. In these studies protein function has typically been characterized from multiple experimental protocols and the measurements integrated into a single model representation of the protein. The effect of any change in protein function in a whole cell context can then be investigated by translating the model of protein function in an expression system into a cellular modeling context.

The early work in modeling single protein phenotypes focused on the congenital long QT (LQT) syndrome caused by a spectrum of mutations across many genes including the human ether-a-go-go-related gene (HERG), mutations in the K$^+$ channel gene (MinK), and cardiac Na$^+$ channel gene (SCN5A). Clancy and Rudy (15) first demonstrated the utility of cardiac cell models for bridging the gap between single channel mutation and clinical data. In this work a model was developed to represent the altered SCN5A channel kinetics, recorded in an expression system, in the presence of the ΔKPQ mutation. This model was inserted into an existing ventricular myocyte electrophysiology model and was able to reproduce changes in the action potential consistent with the changes in ECG observed in the presence of the ΔKPQ mutation. In a second study, Clancy et al. (17) developed a model of the I1768 mutation in SCN5A to reconcile the innocuous changes in steady-state channel properties with the known proarrhythmic phenotype. In a third study, Viswanathan and Rudy (85) altered the channel densities corresponding to HERG, MinK, and SCN5A to demonstrate how these densities affected the cellular action potential that were again interpreted in the context of LQT characteristic ECG wave forms. This work was followed by the investigation of the specific changes in the HERG channel kinetics with mutation R752W, introduced to a preexisting ventricular myocyte model, to quantitatively show the impact of changes in channel kinetics on whole cell function (26). In a fifth study (16), the 1795insD mutation associated with both LQT and Brugada syndrome in the sodium channel was investigated revealing an interesting physiological paradox. Specifically, in LQT this mutation had been shown to increase Na$^+$ channel function and yet in Brugada syndrome it attenuated channel function. To expose the mechanism underpinning these two conflicting observations, a detailed model of the modified sodium channel was developed and introduced into a preexisting cell model to provide physiological boundary conditions. The model demonstrated that the same mutation in the sodium channel could produce both syndromes by having different effects on channel function in different cell types across the heart wall. In this way the model was able to provide a mechanistic link between a known mutation and a complex phenotype. As these methods have gained acceptance, more recent experimental studies concerning how biological systems respond to genetic changes of Na$^+$ current ($I_{Na}$) (77, 86, 91), funny current ($I_{f}$) (83), slow-delayed rectifier K$^+$ current ($I_{Ks}$) (65), inward rectifier K$^+$ current ($I_{Ki}$) (69), and delayed rectifier K$^+$ current ($I_{Kr}$) (14, 51, 71) have all included computational modeling as part of the analysis of the altered ion channel kinetics. Furthermore, computational models have also been applied to the study of single protein alterations in Ca$^{2+}$ regulation. In particular, Metzger and coworkers (20, 21, 24) have published a series of papers combining modeling and experimental studies to characterize the potential for overexpression of parvalbumin and sarco(endo)plasmic reticulum Ca$^{2+}$-ATPase (SERCA) to increase Ca$^{2+}$ relaxation times while sustaining contractile function.

Multiple Protein Phenotypes

In the studies described above, genetic adaptations of a single protein were responsible for the majority of the salient changes in system physiology. In more recent studies computational models have also been applied to investigate complex phenotypes (78, 88). These studies have aimed to simulate changes because of genetic manipulation of specific proteins as well as the compensatory mechanisms altering expression and/or regulation of a multitude of additional proteins (18, 47, 68). While these examples use computational models to combine multiple data sets recorded from a single system, they also have assisted in dissecting out the effects of changes in individual proteins on physiological function. In these more complex phenotypes, the introduction of changes in density or function of a single protein to a system model has not proved sufficient to replicate the observed system level phenotypes. In these cases, to explain phenotypes resulting from changes to multiple proteins has required the integration of comprehensive data sets that quantify both the primary and compensatory changes because of a specific genetic manipulation. These models combine translation of data from expression systems, multi scale analysis linking protein with cellular function and the deconvolution and quantification of the individual effects of the changes to multiple proteins.

An example of the use of models to characterize primary and compensatory responses is demonstrated in the study by Pott et al. (68) who experimentally characterized the changes in single cell electrophysiology resulting from Na$^+$/Ca$^{2+}$ exchanger (NCX) knockout. In this system the changes in electrophysiology could not be explained by alterations in NCX alone. To address this issue, Pott et al. identified upregulation of transient outward current ($I_{to}$) and downregulation and increased inactivation of L-type Ca$^{2+}$ current ($I_{Cai}$) as com-
pensatory effects in addition to the removal of NCX function that could also contribute to changes in cellular electrophysiology. To isolate the impact of each of these changes to the action potential morphology, each adaptation was introduced independently into a mouse cardiac myocyte model allowing the upregulation of $I_{\text{CaL}}$ to be identified as the principal source of the observed shortening of action potential duration present in experiments.

Similarly, in a study of SERCA2 gene knockout, Li et al. (47) used a computational model to determine the relative roles of $I_{\text{CaL}}$ and NCX compensatory mechanisms. By the comparison of the independent and integrated effects of SERCA knockout, NCX upregulation and $I_{\text{CaL}}$ upregulation, Li et al. were able to identify diastolic Ca$^{2+}$ as a dominant driver of adaptation. To begin to gain some understanding of the regulation of compensatory mechanisms, Li et al. investigated the effects of the alternative compensatory mechanisms that would also maintain diastolic Ca$^{2+}$. Figure 3, from the Li et al. study, shows the effect of upregulation or inhibition of each Na$^+$ or Ca$^{2+}$ transmembrane current on peak and systolic Ca$^2$. The figure shows that upregulation of either NCX or NaK were the only two possible mechanisms for maintaining diastolic Ca$^{2+}$ in the presence of reduced SERCA function. Of these two options NaK required a 50-fold increase in density as opposed to the 3–5 fold increase in NCX. The model was able to demonstrate the impact of protein knockout and the corresponding compensatory mechanisms on excitation and contraction coupling. The conclusion from this analysis is that the compensatory mechanism, observed experimentally, maintained diastolic Ca$^{2+}$ with the minimal energetic cost. More recently, this work on modeling SERCA knockout was extended to investigate the ongoing progression of these animals from a compensated and viable condition, to heart failure and death (48). Tracking the progression of these compensatory adaptations using computational models and proposing mechanisms to explain this progression from a viable state to heart failure provides further understanding of the link between the known genotype and observable phenotypes.

In further studies of cardiac excitation-contraction coupling, Collis et al. (18) experimentally characterized the effects of overexpression of L112-sorcin on Ca$^{2+}$ dynamics. They then introduced these changes into a computational model of mouse electrophysiology (12) by increasing the NCX current and slowing inactivation and reducing the conductance of $I_{\text{CaL}}$. The subsequent simulation showed these changes in the model were insufficient to capture the experimentally measured changes with sorcin overexpression. The model was then used to identify increased SERCA flux as a potential additional alteration, consistent with prior experimental results.

Complex phenotypes, involving multiple cardiac electromechanics proteins, can readily arise from the genetic manipulation of regulatory pathways. Computational models provide a tool to unravel the relative impacts of the altered regulation and potential compensatory mechanisms underpinning an observed phenotype. Applying this methodology, Petkova-Kirova et al. (67) developed a computational model to investigate arrhythmia susceptibility and cardiac electrophysiology in transgenic mice overexpressing tumor necrosis factor-$\alpha$. Increased tumor necrosis factor-$\alpha$ results in altered $I_{\text{K1}}$ kinetics, decreased SERCA pump rate, decreased $I_{\text{Ksuc}}$ conductance, decreased $I_{\text{Kur}}$ conductance, and increase NCX rate/density. By introducing these changes into a cellular model, Petkova-Kirova et al. were able to show that the model could represent the characteristic changes in Ca$^{2+}$ transient and action potential morphology observed in these animals. Similarly, Heijman et al. (32) developed a detailed model of $\beta$-adrenergic regulation in the canine epicardial cell. Using in silico knockouts, they identified phosphodiesterases, adenyl cyclases, protein kinase A, and restricted diffusion as major contributors to the local control of Ca$^2$.Yang and Sautermeier (93) developed a mathematical model of the mouse cardiac myocyte including $\beta$-adrenergic regulation. By comparing simulations of phospholemman and phospholamban knockout mouse models with experimental data, they were able to validate the sodium-calcium dynamics of the model. Using this validated model, they predicted that the effect of phospholemman phosphorylation on sarcoplasmic reticulum loading is the primary mechanism in cytosolic Ca$^{2+}$-adaptation to long term $\beta$-adrenergic stimulation.

Predicting potential mechanisms to explain complex phenotypes has also been applied to mutations in the sarcomere and contraction. Kirk et al. (41) used a model of cross-bridge dynamics and thin filament regulation to explain the change in tension development following the introduction of a troponin-I mutant. The model identified simultaneous changes in the rate of cross-bridge formulation and the persistent Ca$^{2+}$-independent cross bridges as necessary kinetic changes to explain the experimental results. Similarly, Bai et al. (7) were able to fit a model of contraction to determine how these mutations quantitatively affected cross-bridge kinetics. This study identified the elevated and sustained diastolic active tension at the sarcomere level as a potential contributor to diastolic heart failure and impaired relaxation.

**Multicellular Phenotypes**

In the subcellular and cellular computational models described above, there has often been an extrapolation from...
changes at the cellular level to analogous changes at the whole organ scale. For example, delayed calcium recoveries suggest diastolic heart failure (20) and changes in action potential duration are linked to the QT segment on the ECG (15). However, the heart is not homogenous (6, 13, 30), and different regions both function (11, 43) and interact (10, 90) differently. Hence, changes in protein expression or function can, as demonstrated above (16), have different regional effects that in turn regulate the resulting, whole organ, emergent response. To quantitatively link changes in protein function with the cell to whole organ function requires the use of multiscale computational models that explicitly represent the interactions and feedback mechanisms between tissue, cellular, and protein function. These models also make use of data integration and data translation, which enable combination of multiple experimental data sets into a common framework and interpretation of data recorded in expression systems in cell, tissue, and organ scale contexts.

To investigate the impact of single gene mutations on whole organ function in the presence of heterogeneous cell types and cell to cell coupling, Flaim et al. (28) introduced gene mutations into a model of cardiac tissue containing known spatial variations in cellular properties. Specifically, variants of a canine cell model were developed to represent the transmural gradient in cell types across the heart wall. These models were then combined into a model of the cardiac myocardium using the monodomain equations and by letting the original sodium channel model be replaced with the I1768V mutation sodium channel model developed by Clancy et al. (17). Activation and repolarization waves were then simulated, and the resulting pseudo-ECGs were evaluated. The analysis from this study was able to link the genetic mutation in a single channel with whole organ measurements by reproducing Himalayan T waves observed in patients with this mutation (25). In a parallel study, Sauccerman et al. (74) investigated proarrythmic effects of the KCNQ1-G589D gene mutation, associated with LQT that prevents β-adrenergic regulation of the Iks channel in single cell and tissue models. This cell model demonstrated that the mutation was only proarrythmic with β-adrenergic stimulation and the tissue models identified larger hearts as being more susceptible to arrhythmias resulting from this mutation. Similarly, Ahrens-Nicklas et al. (1) introduced a model of β-adrenergic stimulation (73) into a strand of cells containing the ΔKPQ Na+ channel LQT mutation (15) to investigate the effect of β-adrenergic stimulation and blockade on the action potential morphology in the presence and absence of the ΔKPQ mutation. The model showed that LQT resulting from Na+ channel mutation is mediated by β-activation, and the use of β-blockers to treat these patients may need to be reevaluated. In further tissue modeling studies of LQT, Weiss et al. (92) investigated the impact of mutations in the HERG channel in LQT syndrome in a human heart model containing transmural heterogeneities in cell types. Pseudo-ECGs predicted decreased T-wave amplitude and cell-specific reduction in action potential duration that provide a set of testable predictions from the model.

These multicellular and tissue level LQT studies have investigated the role of mutations in initiating arrhythmias, demonstrating the potential of tissue scale models to facilitate the investigation of mechanisms that underpin sustaining arrhythmias by combining spatial and temporal information. Noujaim et al. (62) have used the same approach to confirm the importance of Ik1 in sustaining arrhythmias in a transgenic mouse that overexpresses Kir2.1 (Ik1). Specifically by combining the fluorescence imaging of arrhythmias in Langendorff-perfused hearts with tissue models, Noujaim et al. showed that increased Ik1 both decreased action potential duration and increased fast Na+ recovery, facilitating faster and more stable arrhythmias.

Tissue simulations also allow phenotypes characterized by spatial variation to be modeled. Zhu and Clancy (94) used a computational model to investigate Timothy syndrome by introducing a mutated ICaL channel model into a cardiac electrophysiology model. Simulations were performed for strands of cells allowing a pseudo-ECG to be calculated. The simulated ECGs generated characteristic T-wave morphologies of Timothy syndrome from this work have suggested that the ICaL mutation is sufficient to cause the clinical phenotype. In another example of this type of tissue simulation, Thomas et al. (79) investigated the impact of a genetic reduction in connexin 43 on conduction velocity in synthetic strands of neonatal cardiac myocytes. Despite having decreased connexin 43, the strands showed no change in conduction velocity but did show an increase in ISna conduction. By introducing these changes into a model, these authors were able to demonstrate that the combination of decreased cell-cell coupling and increased ISna resulted in no change in the overall conduction velocity. Hu et al. (34) also used a strand of cell models to study a family that experienced a SCN5A mutation. Mutations in the SCN5A gene can cause cardiac conduction disease and Brugada syndrome that can manifest concurrently or individually. This particular family only experienced cardiac conduction disease, and the reason for the absence of Brugada syndrome was unclear. The multicellular modeling work of Hu et al. was central to identifying the decreased ISna and increased ICaL function in these patients. Specifically, the model showed that decreased ISna function alone would lead to a decrease in the action potential overshoot, decreased conduction velocity, and would cause the loss of the action potential dome, consistent with Brugada syndrome. However, when increased ICaL function was introduced into the model, the action potential dome morphology returned, removing the substrate for Brugada syndrome.

Finally, multicellular mechanics phenotypes have also been studied, although less extensively, at the organ scale using nonlinear finite element models. In particular, Omens et al. (64) and Costandi et al. (19) studied the effect of muscle LIM protein knockout on cardiac mechanics through alterations in tissue microstructure and passive stiffness. Applying these changes within mathematical models, the relative effects of geometry and material stiffness on chamber stiffness and the effect of changes in microstructure on deformation were quantified in the muscle LIM protein knockout mouse.

Predicting Phenotypes from Genotypes

The studies described above have investigated the mechanisms that underpin observed phenotypes from a known genotype classified under the headings of the three different approaches outlined in the introduction. In all of these problems, the phenotype is known and the model provides a method for working backward to infer the mechanism. Alternately, in
cases where the genome is known, computational models have the potential to assist in predicting the resulting phenotype. While perhaps more conceptually straightforward, this mapping of genes to function represents, in most respects, fundamentally a much bigger scientific challenge (45). However, the ability to solve this class of inverse problems remains an essential step in our understanding of the genome and the ability to infer function from both single and multiple gene mutations has the potential to revolutionize the translation of physiology to clinical medicine. The first steps in this direction have been made by combining molecular dynamics models of individual ion channels with channel Markov models that can then be introduced into single cell models and from there into whole organ simulations (75). This strategy links changes in channel amino acids to whole organ phenotypes. This represents a significant epistemic achievement and provides a rigorous framework for linking genetic variation with whole organ function. However, at present this approach requires a channel structure, which is not at present universally available for all channels, exchangers, or transports. Furthermore, with currently available methods, our capacity to predict protein structure from sequence information remains limited, meaning this approach is not likely to be generally applicable in the near future. However, an alternative approach is to use the types of computational frameworks reviewed above to integrate statistical inference with deterministic simulation to identify genotype-phenotype relationships that are otherwise beyond reach.

This approach is based on the hypothesis that if a mathematical model is capable of describing the observed phenotypic variation in a population, then much of the genetic variation underlying this phenotypic variation must be expressed through variation in the parameters of the model, as these parameters are themselves lower-level phenotypes. Although a particular value of a given parameter may be due to numerous low-level processes in terms of controlling the observed phenotypes, the parameter is a key quantity that effectively compresses genetic information into a set of biophysically based control points of physiological function. Figure 4A shows the phenotype covariation observed by generating a population of simulation results for every permutation of parameter values perturbed by 150, 100, or 50% from their initial values. Visualizing this covariation for the simulation results is used to highlight phenotype clustering, indicating that some phenotype pairs may be regulated by a limited number of these control points, which in the context of this approach directly map to simulated genes. This is a rationale behind a strategy for linking genetic variation to phenotypes through modeling that does not demand detailed molecular information about the mapping from genotypes to parameters. However, it still makes it possible to address and better understand a whole range of

**Fig. 4.** 
A: bivariate phenotypic distributions (scatter plots) and univariate distributions (histograms) for action potential and calcium transient morphology (separated by the dark black lines) for the Black 6 mouse cardiac myocyte electrophysiology model (84). B: schematically, the creation of synthetic genomes and the correspond parameter set for a cardiac cell model. These synthetic genomes where generated from single nucleotide polymorphisms (SNPs) extracted for individuals from the HapMap3 data base (37a), which were then expanded using the simuPOP program (66) that maintains allele frequencies and linkage disequilibrium patterns [for further details, see Wang et al. (87)]. C and D: comparison of the variation explained by causal SNPs identified by phenotype genome-wide association studies (GWAS) and parameter GWAS, respectively, showing a significant increase in the level of variation explained by causal SNPs identified using parameter, as opposed to phenotype-based GWAS. This variation is quantified by constructing a multiple regression analysis relating each phenotype or parameter value of a training set to the causal SNPs detected by GWAS. The y-axis of C and D report the $R^2$ values from these regressions. Abbreviations are consistent across all panels: apd25, apd50, apd75, and apd90 correspond to time till 25, 50, 75, and 90% depolarization of the action potential, respectively. Similarly, ctd25, ctd50, ctd75, and ctd90 correspond to time till 25, 50, 75, and 90% relaxation time of the calcium transient, respectively, apamp, appeak, apttp, and apbase correspond to action potential amplitude, peak, time to peak, and baseline value, respectively. Similarly, ctamp, ctpeak, ctttp, and cbase correspond to calcium transient amplitude, peak, time to peak, and baseline value, respectively.
genetic phenomena associated with complex traits (84). These include an understanding of how phenotypic penetrance of genetic variation vary as a function of regulatory architecture and genetic background and how the genicity of a trait may change from monogenic to oligogenic to polygenic as a function of which low-level phenotypes are influenced by genetic variation. As it is evident that model parameters can be considered state variables in a lower-level dynamic system richer in biological detail, one may foresee layers of models that finally bring us to a resolution level where we can make a causally cohesive link between genetic variation and high-level phenotypes.

Recently, Wang et al. (87) have demonstrated the potential of this type of methodology for identifying the low-level phenotypes (i.e., model parameters sets) that can be used in genome-wide association studies (GWAS) to reveal significantly more genetic variation compared with using standard statistical-genetics approaches on higher-level phenotypes. By combining a genome sequence generator with an a priori statistical map assumed to define the relationship between genes and model parameters, a set of cardiac cell models, each linked to a generated genome, was created, as shown in Fig. 4B. With the use of GWAS, the correlations between the generated single nucleotide polymorphisms (SNPs) and cell model parameters and phenotypes were calculated. The phenotypic variation explained by causal SNPs derived from the phenotype GWAS and the parameter value GWAS, which resulted from this study, are replotted in Fig. 4, C and D, respectively. The higher $R^2$ values for causal SNPs identified from the model parameters as opposed to phenotypes show a significant improvement in the identification of SNPs that explain phenotype variability. This suggests that enabling statistical-genetic studies to be guided by computational physiology may not just help us to reveal more of the variation underlying a complex trait but also facilitate an understanding of how this variation affects the phenotype variation of that trait in terms of regulatory mechanisms. Computational physiology may thus become a very important tool for guiding the development of phenomics technology (33).

Discussion

The modeling methods presented above demonstrate the utility of computational models in informing mechanistic links between phenotypes and genes and how new methods are developed that have the potential to inform protein function and phenotypes from genomic information. A major challenge in the use of mathematical models to interpret genetics studies is the importance of species and genetic background. These two factors are routinely considered in experimental studies but are often absent in modeling studies (56, 76).

For the most part, the studies summarized above have investigated the effects of genetic change by adjusting a subset of model parameters or equations in a preexisting model without necessarily considering species or genetic background consistency. However, in experimental physiology the importance of the genetic background of a mutation is well known (54). For complex traits it will thus become important for computational models to not only represent the genetic adaptation but also provide a realistic representation of the specific genetic background of the adaptation. To address this issue in our own work, recent models for the mouse, arguably the most commonly used genetically manipulated mammal, have been proposed. Specifically, Li et al. (49) developed a single cell model representing the Ca$^{2+}$ dynamics and electrophysiology of the common experimental C57BL/6 (Black 6) strain that has recently been extended by Land et al. (44) to simulate whole organ electromechanics, as shown in Fig. 1. Li et al. used the single cell framework to differentiate between the effect of overexpression of native murine NCX and canine NCX, where murine NCX does not have an additional cytosolic calcium regulatory site. This model showed that overexpressing canine NCX with a regulatory site causes an increase of NCX into the cell, overexpressing murine NCX causes a depletion of calcium. It is important to note that these results may depend on the binding affinity of the regulatory calcium site, which remains controversial (70, 89). However, independent of this debate, these simulation results demonstrate the importance of species consistency also in mathematical models.

The importance of species consistency is, however, in tension with the ability of models to translate results between assays. This is particularly an important issue when we try to interpret and assess the importance of animal data in a human setting. Unlike experimental physiology, in computational modeling the only cost to performing simulations in humans as opposed to mice or other animal models is computational, and even then this cost is only incurred at the whole organ scale (44, 55). This introduces a conflict in the priorities for model development concerning whether one should focus on exploiting the large amount of data and experimental possibilities provided by animal systems or directly simulate human physiology. As has been demonstrated above, significant insight can be gained by introducing a channel mutation, characterized in an expression system, into an electrophysiological cell model (14–16, 26, 71, 77, 83, 85, 86, 91). For studies where there are limited compensatory mechanisms, this strategy is likely to continue to provide important insight, even for multiple concurrent mutations (34). This type of study is ideally suited for interpretation using models by introducing submodels of the relevant genetically mutated ion channels into a given model of a human myocyte. However, at present there are no models that describe the evolution of compensatory mechanisms that can occur in the presence of a genetic change. As these compensatory mechanisms can obfuscate the link between a genetic change and a phenotype (68), this currently presents a fundamental limitation. Thus, without the ability to predict compensatory responses, it will remain challenging to link genetic variation to function in the presence of secondary effects in human models.

To gain a better understanding of complex phenotypes in the presence of compensatory mechanisms requires informing computational models from comprehensive experimental data sets, characterizing both the altered proteins function and any secondary compensatory effects. This requirement of extensive experimental data necessitates a compromise between ethics, economics, and practicalities. The trade off often realized in experimental genetics has been to use the mouse as the preferred animal model for genetic studies (50, 52, 54a, 63, 72). However, more recently, techniques developed in the mouse have been translated to the rat (2, 38) and have the potential to be applied to other species in the future. For understanding human cardiac electromechanics, studying the mouse has some
clear and significant limitations (50). When compared with the human heart, the mouse heart beats at significantly higher rates, does not have a positive force frequency response, has high sodium concentrations, and has no action potential plateau, and the calcium dynamics are shifted for more intracellular cycling as opposed to moving calcium ions across the membrane. There are also the obvious differences in the life span, size, and disease etiology between the species [for a review of the similarities and differences between human and mouse cardiac electromechanics models, see Tranquillo and Sunkara (81)]. Despite these inherent limitations for human comparison, the mouse has a number of strengths (25, 50). The mouse is a mammal, is readily bred, has a well-characterized physiology, and is affordable. The mouse cardiac physiology, although different from humans, still relies on many of the same underlying physiological mechanisms for electrically activating cardiac myocytes, regulating calcium dynamics, generating tension, and pumping blood around the body.

The first model of mouse electrophysiology captured many of the salient features of mouse cardiac electrophysiology and has been used for many subsequent studies (12); however, this model simulated physiological function at room temperature, did not conserve charge (8, 35), and had stability issues (42). The next generation of models addressed many of these issues (47, 49), although many have still sought to characterize function at room temperature (67, 93), which limits the frequency of simulations and the ability to compare model results with in vivo physiological data. It will thus be important to continue to develop computational models to complement and inform mouse genetic experiments; at the same time it is important to recognize the limitations of extrapolating conclusions from mouse, or indeed any other animal study, to human physiology and pathology.

Conclusions

Computational modeling is increasingly providing an essential tool for interpreting and integrating experimental results from genetic studies. The examples provided in this review from the field of cardiac electromechanics demonstrate the significant progress that has already been made in this area. This body of work provides a foundation from which the potential of modeling to link changes in protein function with multiscale phenotypes to provide additional novel physiological insights is likely to be further developed in the near future. Ultimately, this approach provides real promise for underpinning a strategy for addressing a significant postgenomic challenge: the prediction of phenotypes from genetic data alone.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.A.N. and N.P.S. analyzed data; S.A.N., S.L., S.W.O., and N.P.S. interpreted results of experiments; S.A.N., S.L., S.W.O., and N.P.S. prepared figures; S.A.N., S.W.O., and N.P.S. drafted manuscript; S.A.N., S.L., S.W.O., and N.P.S. edited and revised manuscript; S.A.N. and N.P.S. approved final version of manuscript.

REFERENCES


GENETICS AND MODELS OF CARDIAC ELECTROMECHANICS


