Biophysical modelling of functional impacts of potassium channel mutations on human atrial and ventricular dynamics

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy in the Faculty of Science and Engineering

2016

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Contents

Contents ................................................................................................................................. 2
List of Figures .......................................................................................................................... 7
List of Tables ............................................................................................................................ 13
Nomenclature ......................................................................................................................... 15
Abstract ................................................................................................................................. 17
Declaration ............................................................................................................................... 18
Copyright Statement .............................................................................................................. 19
Acknowledgements ................................................................................................................ 21
The Author .............................................................................................................................. 22
Supporting Work ..................................................................................................................... 23

Chapter 1  Introduction ......................................................................................................... 25
  1.1 The heart and cardiovascular disease .............................................................................. 25
  1.2 Computational modelling ............................................................................................... 26
  1.3 Aims and organisation of the thesis ................................................................................. 28

Chapter 2  Background .......................................................................................................... 32
  2.1 The heart ......................................................................................................................... 32
  2.1.1 The circulatory system ............................................................................................... 32
  2.1.2 Anatomy of the heart ................................................................................................. 32
  2.1.3 Cell types of the heart ............................................................................................... 34
  2.1.4 The cardiac conduction system .................................................................................. 35
  2.2 Cardiac myocytes ........................................................................................................... 37
  2.2.1 The sarcolemma ......................................................................................................... 37
  2.2.2 Ion channels ............................................................................................................... 38
  2.2.3 Ion exchangers and pumps ......................................................................................... 39
  2.2.4 Intercalated discs ....................................................................................................... 39
  2.3 Cardiac electrophysiology .............................................................................................. 41
  2.3.1 Action potential ......................................................................................................... 41
  2.3.2 Biomarkers of action potential ................................................................................... 43
  2.3.3 S1 and S1-S2 protocol .............................................................................................. 44
  2.3.4 Effective refractory period ....................................................................................... 45
  2.3.5 Intracellular calcium cycle ....................................................................................... 45
2.4 Mechanical contraction of the heart ................................................. 47
  2.4.1 Cardiac output ........................................................................ 47
  2.4.2 Active force generation mechanism ...................................... 47
  2.4.3 Passive force generation mechanism ...................................... 51
2.5 The electrocardiogram ................................................................. 51
  2.5.1 Normal ECG and intervals .................................................... 51
  2.5.2 12-lead ECG ........................................................................ 52
2.6 Heart diseases ................................................................................ 54
  2.6.1 Cardiac arrhythmias ............................................................. 55
  2.6.2 Ischaemic heart disease ......................................................... 57
  2.6.3 Bundle branch block ............................................................. 57
2.7 Computational cardiology ............................................................. 57
  2.7.1 Nernst equilibrium potential .................................................. 57
  2.7.2 Electric circuit model ............................................................. 58
  2.7.3 Hodgkin-Huxley Models .......................................................... 60
  2.7.4 Markov chain Models ............................................................ 61
  2.7.5 Modelling drug effects on ion channels .................................. 62
  2.7.6 Tissue modelling ..................................................................... 62
  2.7.7 ECG simulation ........................................................................ 64
  2.7.8 Numerical methods ............................................................... 64
  2.7.9 Modelling environment .......................................................... 66

Chapter 3 In silico investigations of KCNA5 mutations-mediated pro-arrhythmic effects in the human atria .................................................................................................................. 68
  3.1 Introduction ................................................................................ 69
  3.2 Methods .................................................................................... 70
    3.2.1 Development of a novel formulation of I_{Kur} ......................... 70
    3.2.2 Single cell modelling ............................................................... 73
    3.2.3 Tissue modelling ..................................................................... 76
  3.3 Results ....................................................................................... 82
    3.3.1 Effects of KCNA5 mutations on single cell electrophysiology ... 82
    3.3.2 Effects of KCNA5 mutations on atrial conduction velocity ....... 94
    3.3.3 Effects of KCNA5 mutations on atrial APD heterogeneity ........ 97
    3.3.4 Effects of KCNA5 mutations on tissue’s vulnerability to uni-directional conduction block (wave break) ................................................................. 100
    3.3.5 Effects of KCNA5 mutations with β-adrenergic stimulation .... 102
    3.3.6 Effects of KCNA5 mutations on spiral wave dynamics ............ 105
  3.4 Discussion ................................................................................... 107
    3.4.1 Proarrhythmic effects of gain-of-function mutations .............. 108
    3.4.2 Arrhythmogenesis in loss-of-function mutations .................... 109
    3.4.3 The role of I_{Kur} in modulating action potential morphology and duration ................................................................. 111
    3.4.4 Use of multiple, independent models of human atrial electrophysiology ................................................................. 111
    3.4.5 Clinical relevance .................................................................... 112
    3.4.6 Limitations ............................................................................ 112
  3.5 Conclusion .................................................................................. 113

Chapter 4 Inotropic effects of KCNA5 mutations on human atria .......... 114
  4.1 Introduction ................................................................................ 114
4.2 Methods ........................................................................................................................................... 115
  4.2.1 Modelling active force development in human atrial myocytes ......................................................... 115
  4.2.2 Inclusion of the stretch-activated channel current ............................................................................... 117
  4.2.3 Modelling the KCNA5 mutations in the atria ...................................................................................... 119
  4.2.4 Parameter analysis of the dependence of atrial contractility on $I_{Kur}$ ............................................. 119
  4.2.5 3D anatomical model of electromechanical coupling ......................................................................... 120
  4.2.6 Numerical methods .......................................................................................................................... 123
4.3 Results .................................................................................................................................................. 124
  4.3.1 Validations to the single cell electro-mechanical model ..................................................................... 124
  4.3.2 The inotropic effects of the KCNA5 mutations on atrial myocytes .................................................. 124
  4.3.3 The mechanisms underlying the inotropic effects of the KCNA5 mutations ..................................... 130
  4.3.4 The effects of $I_{Kur}$ properties on atrial contractile function .............................................................. 135
  4.3.5 The inotropic effects of the KCNA5 mutations at the organ level ...................................................... 137
4.4 Discussion ............................................................................................................................................ 142
  4.4.1 The electromechanical model of the atria ........................................................................................... 142
  4.4.2 The inotropic effects of the KCNA5 mutations .................................................................................. 143
  4.4.3 Parameter analysis on role of $I_{Kur}$ in atrial contractility ................................................................. 145
  4.4.4 Role of $I_{SAC}$ .................................................................................................................................... 145
  4.4.5 Novelty and relevance to previous studies ......................................................................................... 146
  4.4.6 Limitations ....................................................................................................................................... 147
4.5 Summary ............................................................................................................................................... 147

Chapter 5 Antiarrhythmic benefits of combined block of sodium ($I_{Na}$) and ultra-rapid delayed rectifier potassium ($I_{Kur}$) channels in human chronic atrial fibrillation 149

5.1 Introduction ........................................................................................................................................... 150
5.2 Methods ............................................................................................................................................... 152
  5.2.1 Modelling human electrophysiology .................................................................................................. 152
  5.2.2 Modelling effects of $I_{Na}$ block ....................................................................................................... 153
  5.2.3 Modelling $I_{Kur}$ block ....................................................................................................................... 155
  5.2.4 Modelling effects of acacetin on atrial and ventricular electrophysiology ........................................ 157
  5.2.5 1D model of atrial strand .................................................................................................................. 159
  5.2.6 2D tissue models ............................................................................................................................... 159
5.3 Results .................................................................................................................................................. 161
  5.3.1 Simulated atrial electrophysiology under SR and cAF conditions ...................................................... 161
  5.3.2 Effects of individual and combined channel block by acacetin ......................................................... 161
  5.3.3 Effects of combined sodium and potassium channel block under cAF conditions ................................ 165
  5.3.4 Effects of combined Na$^{+}$- and K$^{+}$- block on the AF-selectivity of Na$^{+}$ blockers ......................... 170
  5.3.5 Effects of $I_{Na}$ and $I_{Kur}$ block on AF termination in tissue ............................................................... 174
5.4 Discussion .............................................................................................................................................. 182
  5.4.1 Single cell models ............................................................................................................................ 183
  5.4.2 Effects of $I_{Na}$ block ....................................................................................................................... 183
  5.4.3 Effects of K$^{+}$ channel block ........................................................................................................... 184
  5.4.4 Synergistic effects of Combined Na$^{+}$- and K$^{+}$- block ................................................................. 187
  5.4.5 Relevance to previous modelling studies on $I_{Kur}$ block ............................................................... 189

Haibo Ni
Chapter 6  Developing a human ventricle-torso model for investigating ventricular activities and ECG ................................................................. 191
6.1  Introduction .................................................................................. 192
6.2  Methods ....................................................................................... 194
   6.2.1  Human ventricular cell models .................................................. 194
   6.2.2  Modelling electrical activities in human ventricles .................. 196
   6.2.3  Torso model and ECG ............................................................. 200
   6.2.4  Modelling ventricular ischaemia ............................................ 200
6.3  Results ......................................................................................... 202
   6.3.1  Single cell simulations ............................................................ 202
   6.3.2  Modelling ventricular activation and recovery under normal conditions 204
   6.3.3  Simulated ECGs .................................................................. 209
   6.3.4  Role of electrical heterogeneities .......................................... 211
   6.3.5  Modelling bundle branch block ............................................ 216
   6.3.6  Simulated application of acacetin ......................................... 219
   6.3.7  Modelling ventricular ischaemia ............................................ 219
6.4  Discussion ................................................................................... 223
  6.4.1  Model achievements............................................................... 223
  6.4.2  Role of electrical heterogeneities .......................................... 225
  6.4.3  Novelty and relevance to previous studies .............................. 227
  6.4.4  Limitations ........................................................................... 228
6.5  Summary ..................................................................................... 229

Chapter 7  Modelling functional effects of KCNQ1-G269S mutation on human ventricles and ECG ................................................................. 230
7.1  Introduction .................................................................................. 231
7.2  Methods ....................................................................................... 232
   7.2.1  Modelling I_{Ks} of WT and the KCNQ1-G269S mutation .......... 232
   7.2.2  Modelling ventricular electrophysiology and effects of β-adrenergic stimulation ................................................................. 233
   7.2.3  Modelling vulnerability window in tissue ................................ 234
   7.2.4  Modelling 3D ventricular electrical activity and ECG .......... 234
7.3  Results ......................................................................................... 235
   7.3.1  Effects of KCNQ1-G269S on ventricular APD ...................... 235
   7.3.2  Effects of KCNQ1-G269S on tissue vulnerability .................... 237
   7.3.3  Effects of KCNQ1-G269S in 3D ventricles ............................. 240
   7.3.4  Effects of KCNQ1-G269S on QT intervals ............................. 241
7.4  Discussion ................................................................................... 242
   7.4.1  Summary of major findings .................................................... 242
   7.4.2  Role of I_{Ks} in human ventricles .......................................... 243
   7.4.3  Proarrhythmic effects of the KCNQ1-G269S mutation .......... 243
   7.4.4  Clinical relevance ................................................................. 244
   7.4.5  Limitations ........................................................................... 244
7.5  Summary ..................................................................................... 245

Chapter 8  Discussion and Conclusions ..................................................... 246
8.1 Summary of major findings and significance .............................................247
  8.1.1 Role of $I_{Kur}$ in atrial arrhythmogenesis ...........................................247
  8.1.2 Role of $I_{Kur}$ in modulation of atrial contractility .................................248
  8.1.3 Atrial-selective block of $I_{Kur}$ and $I_{Na}$ ...........................................249
  8.1.4 Ventricle-torso model ........................................................................ 251
  8.1.5 KCNQ1-G269S mutation .....................................................................252
8.2 Clinical relevance .....................................................................................253
8.3 Future work ...............................................................................................254
  8.3.1 Investigating multiple atrial-specific channel block ...............................254
  8.3.2 Population-based modelling ..................................................................255
  8.3.3 Electromechanical model and fluid dynamics model ..............................255
  8.3.4 Precision medicine ................................................................................256
8.4 Closing words ............................................................................................256

References .........................................................................................................257

Appendix A  Supplementary figures ................................................................ 291

Total Word Count = 77,410 Words.
List of Figures

Figure 2.1 Schematic representation of the structure of the heart..................................34
Figure 2.2 Schematic representation of the cardiac conduction system in a mammalian heart..........................................................36
Figure 2.3 Schematic diagram of the structure of cardiac myocytes..........................38
Figure 2.4 Schematic diagram of an ion channel residing in a closed, open or inactivated state..........................................................39
Figure 2.5 Key phases of typical action potentials (AP), and the corresponding primary ion currents during the phases.................................................42
Figure 2.6 Schematic diagram illustrating the key biomarkers of the AP..................43
Figure 2.7 Illustration of S1 and S1-S2 stimulation protocols for eliciting action potentials..........................................................44
Figure 2.8 Schematic diagram of the intracellular Ca\(^{2+}\) cycle. The red and green arrows indicate the direction of Ca\(^{2+}\) flux........................................46
Figure 2.9 Schematic diagram illustrating the microstructure of a cardiac sarcomere...48
Figure 2.10 Schematic diagram showing the detailed structure of actin filament........50
Figure 2.11 Schematic representation of time course of typical ECG traces................52
Figure 2.12 Schematic representation of the configuration of the 12-lead ECG........54
Figure 2.13 Schematic representation of the transmembrane potential and ionic currents using the electric circuit model........................................59
Figure 2.14 Sate diagram representation of Markov chain model for the rapid delayed rectifier K+ current, I\(_{K_r}\)........................................62
Figure 3.1 Model representation describing I\(_{Kur}\) and simulated voltage clamp at room temperature..........................................................72
Figure 3.2 Modelling the electrophysiological properties of I\(_{Kur}\) carried by the mutants at room temperature..........................................................74
Figure 3.3 Simulated regional cell APs using (A) the Colman et al. (B) Courtemanche et al. and (C) the Grandi et al. cell models.................................77
Figure 3.4 Anatomical structure of the 3D atrial model.................................................79
Figure 3.5 A human atrial wedge containing CT and PM used to quantify the temporal vulnerability window to wave breaks.

Figure 3.6 Illustration of the phase distribution method to initiate re-entrant waves in the 3D human atrial model.

Figure 3.7 Effects of the gain-of-function KCNA5 mutations on human atrial AP and the corresponding time courses of current traces elicited by Colman et al., Courtemanche et al. and Grandi et al. models.

Figure 3.8 Effects of the gain-of-function KCNA5 mutations on APD and plateau potential of atrial myocytes.

Figure 3.9 Effects of the loss-of-function KCNA5 mutations on the human atrial AP and the corresponding time courses of current traces elicited by Colman et al., Courtemanche et al. and Grandi et al. models.

Figure 3.10 Effects of the loss-of-function KCNA5 mutations on APD and plateau potential of atrial myocytes.

Figure 3.11 Simulated effects of the KCNA5 mutations on APD restitution of human atrial myocytes elicited by the three electrophysiological models.

Figure 3.12 Analysis of the relationship between the APD$_{90}$ of human atrial myocytes and the parameters related to the kinetics of $I_{Kur}$.

Figure 3.13 Effects of KCNA5 mutations on AP and APD restitution in the presence of cAF remodelling in the three cell models.

Figure 3.14 Simulated effects of gain-of-function KCNA5 mutations on APD, conduction velocity (CV) and excitation wavelength in a 1D model.

Figure 3.15 Simulated effects of loss-of-function KCNA5 mutations on APD, conduction velocity (CV) and excitation wavelength in a 1D model.

Figure 3.16 Simulated atrial activation pattern (A), repolarisation (B) and APD dispersion (C) in a 3D anatomically accurate model under WT conditions.

Figure 3.17 Simulated global APD dispersion and regional APD difference between CT and PM were quantified for isolated cells (using a single cell model) and in tissue (using the 3D anatomical model).

Figure 3.18 Simulated temporal vulnerability window for unidirectional conduction block at the CT/PM junction.

Figure 3.19 Simulated effects of the KCNA5 mutations on human atrial AP and the corresponding time courses of current traces in the presence of β-adrenergic stimulation using the Grandi et al. model.
Figure 3.20 KCNA5 loss-of-function mutations induced EADs following the beta-adrenergic stimulation. ................................................................. 104

Figure 3.21 Simulated spiral waves and APs in a 3D anatomical model of the human atria. ................................................................................. 106

Figure 3.22 Power spectrum analysis of the local electrical activities using (A) the Colman et al. model and (B) the Courtemanche et al. model. ................. 107

Figure 4.1 Simulated force-Ca\textsuperscript{2+} relation for human atrial cells. ............................................................... 118

Figure 4.2 The 3D anatomical atria with segmented regions and traced fibre orientations showing the microstructure of the atria. ................................... 121

Figure 4.3 The simulated electro-mechanical properties of the baseline (RA) model as compared to experimental data.................................................. 125

Figure 4.4 Simulated inotropic effects of KCNA5 mutations on human atrial myocytes using the baseline model. ................................................. 127

Figure 4.5 Simulated inotropic effects of KCNA5 mutations on the human atrial myocytes using the baseline model in the presence of SAC. ................. 128

Figure 4.6 Heterogeneous electro-mechanical activities in the isolated regional atrial cells for the WT (column i), D322H (column ii) and P488S (column iii). .... 129

Figure 4.7 Simulated time courses of L-type Ca\textsuperscript{2+} current, Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger, RyR release flux, SR uptake flux and SR content elicited by AP under the WT and mutations w/o SACs. ........................................... 131

Figure 4.8 Time integral of (A) I_{CaL}, (B) I_{NaCa}, (C) J_{Rel} and (D) J_{SERCA} elicited by APs for WT and the mutations in the absence of I_{SAC}. ......................... 132

Figure 4.9 The individual contribution of I_{CaL} and I_{NaCa} to the systolic [Ca\textsuperscript{2+}]\textsubscript{i} in the atrial cells for the WT and mutations without I_{SAC}. ......................................................... 134

Figure 4.10 The simulated dependence of relative cell shortening on the parameters of I_{Kur} ....................................................................................... 136

Figure 4.11 Snapshots of simulated atrial electromechanical contraction superimposed on the undeformed atrial mesh (in grey) under the WT, D322H and P488S conditions w/o I_{SAC}. .......................................................... 139

Figure 4.12 Simulated activation time sequences of the atria (A) in the absence and (B) in the presence of I_{SAC} under the WT conditions................................. 140

Figure 4.13 Time course of atrial output computed from the emptying volume of both chambers during the electro-mechanical excitation for the WT and mutations... 141

Figure 5.1 A schematic diagram of the guarded-receptor model simulating the state-
dependent Na\(^+\)-channel block. ................................................................. 154
Figure 5.2 Frequency-dependent inhibition of I\(_{Kur}\) by acacetin. .......................... 158
Figure 5.3 An illustration of the cross-shock protocol.............................................. 160
Figure 5.4 Simulated action potentials and calcium transient (CaT) of human atrial
cardiomyocytes under sinus rhythm (SR) and cAF (AF) conditions. ....................... 162
Figure 5.5 Effects of block of individual K\(^+\)-channels by acacetin on the human atrial
AP and APD under SR and cAF conditions...................................................... 163
Figure 5.6 Simulated action potentials and I\(_{Na}\) of cAF-remodelled atrial myocytes and
ventricular cells in response to different combinations of Na\(^+\)- and K\(^+\)- blocks... 166
Figure 5.7 Simulated changes in the APD and peak I\(_{Na}\) following the applications of
Na\(^+\)- and K\(^+\)- block in comparison to the drug-free condition.......................... 167
Figure 5.8 Synergistic effects of combined I\(_{Na,B}\) and I\(_{Kur,B}\), Acacetin/Full at variable
concentrations of the blockers............................................................................. 168
Figure 5.9 Single cell APD restitutions and rate-adaptations of peak I\(_{Na}\) for the control
and Na\(^+\)- and K\(^+\)- block. ................................................................................ 169
Figure 5.10 Simulated activation-recovery interval (ARI, A), Vmax (B) and CV
(conduction velocity, C) measured in 1D atrial strand models as a function of BCL
for control, individual and combined Na\(^+\)- and K\(^+\)- block................................. 171
Figure 5.11 Block strength, rate-, atrial- and AF-selectivity as a function of the open-
and inactivated- state binding rates (K\(_A\), K\(_I\)) for I\(_{Na,B}\), I\(_{Kur,B}\) + I\(_{Na,B}\) and
Acacetin/Full + I\(_{Na,B}\). ..................................................................................... 173
Figure 5.12 Snapshots of simulated re-entrant excitations in a 2D model of an atrial slab
under various conditions...................................................................................... 175
Figure 5.13 The trajectories of spiral wave tips and number of rotor cores plotted as
functions of time under various conditions......................................................... 176
Figure 5.14 Computed pECGs, time courses of membrane potential and total fractional
blocks in I\(_{Na}\) and I\(_{Kur}\) extracted from a local cell located close to the bottom left
corner of the slab in control and I\(_{Na,B}/\)Acacetin/Full........................................... 178
Figure 5.15 Computed pECGs, time courses of membrane potential and total fractional
blocks in I\(_{Na}\) and I\(_{Kur}\) extracted from a local cell located close to the bottom-left
corner of the slab in control and I\(_{Na,B}/I_{Kur,B}\). ................................................. 179
Figure 5.16 Measured lifespan of re-entrant waves in the 2D model under variable
conditions........................................................................................................... 180
Figure 5.17 Dominant frequencies (DF) extracted from simulated pECGs of the re-
entrant waves in the 2D model under variable conditions

Figure 6.1 3D human ventricle geometry reconstructed from DT-MRI images and fibre orientation.

Figure 6.2 Illustration of ventricular stimulation sites.

Figure 6.3 An illustration of the ventricle-torso model.

Figure 6.4 An illustration of ischaemic zones in ventricles.

Figure 6.5 Simulated time courses human ventricular APs at a BCL of 1000 ms.

Figure 6.6 Snapshots of simulated progressive excitation in the ventricles and body surface potential map of the torso.

Figure 6.7 Snapshots of simulated progressive repolarisation in the ventricles and the corresponding body surface potential map of the torso.

Figure 6.8 Activation, repolarisation time patterns and the activation-recovery interval (ARI) in the ventricles under normal conditions.

Figure 6.9 Simulated ECGs under normal conditions.

Figure 6.10 Simulated ventricular RT map and ECG without heterogeneity (HOMO) and with only AB, TM or IV heterogeneity.

Figure 6.11 Simulated RT map and 12-lead ECG for variant electrical heterogeneity configurations by.

Figure 6.12 A comparison of the RT map and corresponding 12-lead ECG obtained with variant TM configurations.

Figure 6.13 Simulated effects of bundle branch block on the AT, RT patterns and ARI distribution of the ventricles.

Figure 6.14 Simulated 12-lead ECG for bundle branch block and comparison to the control simulations.

Figure 6.15 Simulated effects of the administration of acacetin (3 μM) on (A) the ventricular AP and (B) ECG (lead II).

Figure 6.16 Simulated effects of ischaemia on the ventricular AP.

Figure 6.17 Simulated effects of ischaemia on the ECG with respect to variant ischaemic zones in the ventricles.

Figure 7.1 Simulated $I_{Kh}$ as compared to experimental data on the WT and mutation.

Figure 7.2 Simulated effects of WT-G269S and G269S on the AP of endocardium (ENDO), epicardium (EPI), and mid-layer cells (MCELL).

Figure 7.3 Bar plots showing the ventricular APD under the control conditions and with the application of ISO.
Figure 7.4 The relative contribution of the blunted adrenergic activation of the mutated $I_{K_{a}}$ to the total APD-prolongation induced by a genotype. .................................................. 238

Figure 7.5 Effects of mutations on the tissue VW to initiating unidirectional conduction simulated from a 1D strand model. ................................................................. 239

Figure 7.6 Comparison of simulated ventricular ARI map for WT, WT-G269S and G269S under the control conditions and with ISO......................................................... 240

Figure 7.7 Simulated ECGs showing prolongation in QTc by the KCNQ1-G269S mutation as compared to the WT................................................................. 241

Figure A.1 Analysis of the relationship between the APD$_{30}$ of human atrial myocytes and the parameters of $I_{K_{ur}}$ ........................................................................... 291

Figure A.2 Simulated effects of ISO on human atrial AP using the Nygren et al. model. ......................................................................................................................... 292

Figure A.3 Heterogeneous electro-mechanical activities in the isolated regional atrial cells for the WT (column i), D322H (column ii) and P488S (column iii) conditions. ......................................................................................................................... 293

Figure A.4 Simulated time courses of L-type Ca$^{2+}$ current, Na$^{+}$-Ca$^{2+}$ exchanger, RyR release flux, SR uptake flux and SR content elicited by AP under the WT and mutations w/ SACs ......................................................................................................................... 294

Figure A.5 Time integral of (A) $I_{Ca_{L}}$, (B) $I_{NaCa}$, (C) $J_{Rel}$ and (D) $J_{SERCA}$ elicited by APs for WT and the mutations in the presence of $I_{SAC}$ ......................................................................................................................... 295

Figure A.6 The individual contribution of $I_{Ca_{L}}$ and $I_{NaCa}$ to the systolic [Ca$^{2+}$]$_{i}$ in the atrial cells for the WT and mutations with $I_{SAC}$ ......................................................................................................................... 296

Figure A.7 The simulated dependence of relative cell shortening on the parameters of $I_{K_{ur}}$ in the presence of $I_{SAC}$ ......................................................................................................................... 297

Figure A.8 Snapshots of simulated atrial electromechanical contraction superimposed on the undeformed atrial mesh (in grey and indicated with arrows) under the WT, D322H and P488S conditions w/ $I_{SAC}$ ......................................................................................................................... 298
List of Tables

Table 2.1 Primary transmembrane ionic currents in cardiac myocytes. .........................40
Table 3.1 Changes in parameters of steady-state variables and maximum conductance of $I_{Kur}$ carried by the $KCNA5$ mutants relative to WT..................................................75
Table 3.2 Scaling factors for each current of various regions relative to the baseline (right atrium, RA) cell models........................................................................75
Table 3.3 Modifications to the model parameters in simulating the cAF conditions versus SR conditions for the three models. ......................................................78
Table 3.4 Maximum slopes of simulated APD restitution curves for WT and mutant human atrial myocytes.................................................................89
Table 3.5 Simulated global atrial repolarisation dispersion for WT and the $KCNA5$ mutations. .........................................................................................99
Table 4.1 Modifications to the parameters of the Rice et al. myofilament model to simulate the force-Ca$^{2+}$ relation in the human atrial cell. The parameters were defined in [94] and are reiterated below..............................................118
Table 4.2 The correlation coefficients between the relative cell shortening of atrial myocytes and AP biomarkers measured using the Pearson’s correlation and Spearman’s correlation.........................................................137
Table 4.3 Normalised maximum atrial volume emptying rate for WT and the mutations. The rates were normalised to the maximum value of WT w/o $I_{SAC}$.....................................................142
Table 5.1 Parameters for the updated model of human atrial electrophysiology under SR (normal) and cAF conditions. .........................................................................153
Table 5.2 Fitted parameters describing the binding and unbinding kinetics of acacetin on the $K_{v}1.5$ channel. .............................................................................157
Table 5.3 Potency of acacetin inhibiting atrial $K^+$ currents. .........................159
Table 6.1 Scaling factors accounting for the transmural heterogeneity............195
Table 6.2 A summary of difference in ionic currents between apical and basal cells implemented to account for the AB heterogeneity........................................195
Table 6.3 A summary of configurations for electrical heterogeneities used in the present
Chapter. ...................................................................................................................... 198
Table 6.4 Summary of ischaemia-induced alterations to the electrophysiological
parameters of the human ventricles................................................................. 201
Table 6.5 Summary of APD (ms) produced by the regional ventricular models....... 203
Table 6.6 Effect of ischaemia on the APD (ms) of ventricular myocytes. .............. 219
Table 7.1 Modelling effects of adrenergic activation on I_Ks................................. 234
## Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{CaL}$</td>
<td>L-type calcium current</td>
</tr>
<tr>
<td>$I_{CaT}$</td>
<td>T-type calcium current</td>
</tr>
<tr>
<td>$I_{CaP}$</td>
<td>Sarcolemmal calcium pump current</td>
</tr>
<tr>
<td>$I_f$</td>
<td>Funny current</td>
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<td>$I_{K1}$</td>
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<td>Transient outward potassium current</td>
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<td>CaT</td>
<td>Calcium transient</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>Sodium ion</td>
</tr>
<tr>
<td>K$^+$</td>
<td>Potassium ion</td>
</tr>
<tr>
<td>$E_X$</td>
<td>Reversal potential for ion species X</td>
</tr>
<tr>
<td>$V_m$</td>
<td>Membrane potential</td>
</tr>
<tr>
<td>1D</td>
<td>One Dimension</td>
</tr>
<tr>
<td>2D</td>
<td>Two Dimension</td>
</tr>
<tr>
<td>3D</td>
<td>Three Dimension</td>
</tr>
<tr>
<td>AB</td>
<td>Apico-basal</td>
</tr>
<tr>
<td>AF</td>
<td>Atrial fibrillation</td>
</tr>
<tr>
<td>APA</td>
<td>Action potential amplitude</td>
</tr>
<tr>
<td>APD</td>
<td>Action potential duration</td>
</tr>
<tr>
<td>AS</td>
<td>Atrial septum</td>
</tr>
<tr>
<td>AVN</td>
<td>Atrio-ventricular node</td>
</tr>
<tr>
<td>AVR</td>
<td>Atrio-ventricular ring</td>
</tr>
<tr>
<td>BCL</td>
<td>Basic cycle length</td>
</tr>
<tr>
<td>CT</td>
<td>Crista terminalis</td>
</tr>
<tr>
<td>CV</td>
<td>Conduction velocity</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion tensor</td>
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Haibo Ni  

15
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ERP</td>
<td>Effective refractory period</td>
</tr>
<tr>
<td>ENDO</td>
<td>Endocardial</td>
</tr>
<tr>
<td>EPI</td>
<td>Epicardial</td>
</tr>
<tr>
<td>I-V</td>
<td>Current-voltage</td>
</tr>
<tr>
<td>IV</td>
<td>Inter-ventricular</td>
</tr>
<tr>
<td>LA</td>
<td>Left atria</td>
</tr>
<tr>
<td>LBBB</td>
<td>Left bundle branch block</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>M</td>
<td>Mid-myocardial</td>
</tr>
<tr>
<td>MCELL</td>
<td>Mid-myocardial cells</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>Maximum upstroke velocity</td>
</tr>
<tr>
<td>ODE</td>
<td>Ordinary differential equation</td>
</tr>
<tr>
<td>OS</td>
<td>Overshoot</td>
</tr>
<tr>
<td>PDE</td>
<td>Partial differential equation</td>
</tr>
<tr>
<td>PF</td>
<td>Purkinje fibre</td>
</tr>
<tr>
<td>PV</td>
<td>Pulmonary vein</td>
</tr>
<tr>
<td>RA</td>
<td>Right atria</td>
</tr>
<tr>
<td>RBBB</td>
<td>Right bundle branch block</td>
</tr>
<tr>
<td>RMP</td>
<td>Resting membrane potential</td>
</tr>
<tr>
<td>RT</td>
<td>Repolarisation time</td>
</tr>
<tr>
<td>RV</td>
<td>Right ventricle</td>
</tr>
<tr>
<td>RyR</td>
<td>Ryanodine receptor</td>
</tr>
<tr>
<td>SAN</td>
<td>Sino-atrial node</td>
</tr>
<tr>
<td>SERCA</td>
<td>Sarcoplasmic reticulum calcium ATPase</td>
</tr>
<tr>
<td>SK</td>
<td>Small conductance calcium activated potassium channel</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>SVC</td>
<td>Superior vena cava</td>
</tr>
<tr>
<td>TM</td>
<td>Transmural</td>
</tr>
<tr>
<td>TASK</td>
<td>Potassium two pore domain channel subfamily K member 3</td>
</tr>
<tr>
<td>1D</td>
<td>One-dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
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<td>3D</td>
<td>Three-dimensional</td>
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Abstract

ABSTRACT OF THESIS submitted by Haibo Ni
for the degree of Doctor of Philosophy and entitled
Biophysical modelling of functional impacts of potassium channel mutations on human atrial and ventricular dynamics.
December 2016.

Atrial fibrillation (AF) is the most common cardiac arrhythmia causing morbidity and mortality. Despite recent advances, developing effective and safe anti-AF pharmaceutical therapies remains challenging and is prone to adverse effects in the ventricles. Atrial-selective therapies are promising in managing AF. A better understanding of the role of the atrial-specific ion channels in the atrial arrhythmogenesis and contractility, as well as the anti-AF effects of blocking these channels is of interests. Also, a 3D ventricle-torso model capable of modelling ventricular electrical activities and the resulting electrocardiogram (ECG) is a valuable tool in evaluating the selectiveness and safety of an anti-AF pharmaceutical therapy.

In part I, the role of an atrial-specific ion channel, $I_{Kur}$, in atrial electrical and mechanical activities and the potential of the current as a pharmaceutical target for anti-AF therapies were investigated \textit{in silico}. The role of $I_{Kur}$ in atrial arrhythmogenesis and mechanical contraction was revealed by elucidating the functional impacts of the KCNA5 mutations exerting either gain- or loss-in-function, on the atria. First, novel $I_{Kur}$ models were developed and incorporated into multiscale biophysical models of human atrial electrophysiology to assess the effects of mutated $I_{Kur}$ on atrial electrical dynamics. Then, a family of single cell human atrial electromechanical models was developed and incorporated into an updated 3D anatomical electromechanical model of human atria to clarify the effects of mutated $I_{Kur}$ on the atrial contractile function. Finally, the antiarrhythmic effect of $I_{Kur}$ block was assessed together with $I_{Na}$ and other $K^+$-current block. It was shown that the gain-of-function in $I_{Kur}$ impaired atrial contractility and promoted atrial arrhythmogenesis by shortening the APD, whereas the down-regulated $I_{Kur}$ exerted positive inotropic effects and increased the susceptibility of the atria to the genesis of early-afterdepolarisations. Both simulated $I_{Kur}$ and $I_{Na}$ block in human-AF demonstrated antiarrhythmic effects; the multi-channel block exerted synergistic anti-AF effects and enhanced the AF-selectivity of $I_{Na}$ inhibitions.

In Part II, a human ventricle-torso model was developed through proposing a new family of single cell ventricular models accounting for transmural, apicobasal and interventricular electrical heterogeneities and integrating an updated 3D biophysical and anatomical model of human ventricles with a heterogeneous anatomical model of a human torso. First, using the model, the role of heterogeneities in the genesis of T-wave was revealed. Then, ECG manifestations of bundle branch block and ventricular ischaemia were simulated. Finally, the platform was applied to investigate the impact of a long-QT-linked mutation (KCNQ1-G269S) on the ventricles and ECG. Good agreement between simulated and experimental/clinical ECG was reached under both normal and diseased conditions. It was shown that the apicobasal heterogeneity had a more pronounced effect on the T-wave than other heterogeneities. Simulations of the KCNQ1-G269S elucidated the causal link between the mutation and ECG manifestations of the patients.
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路漫漫其修远兮
吾将上下而求索

Qu Yuan, Li Sao

That which we are, we are;
One equal temper of heroic hearts,
Made weak by time and fate, but strong in will
To strive, to seek, to find, and not to yield.

Alfred, Lord Tennyson, Ulysses
Acknowledgements

First and foremost, I would like to thank my supervisor, Prof. Henggui Zhang for his guidance and support. Prof. Zhang has helped tremendously in my PhD funding application and throughout the PhD project, without which this thesis would not be possible. His passion for research has been exceptionally inspiring, which has made the project more enjoyable. I would also like to thank Prof. Mark Boyett, my co-supervisor, for many discussions and help on the project. I wish to express sincere thanks to Dr Michael Colman, for his guidance, help, support and advice in many aspects and stages of the project.

In addition, I would like to express my gratitude to all the members of the Biological Physics Group, in particular, Simon, Dom, Gareth, Tim, Jon, Roy, Le, Kun, Yang, Weijian, Chen, Wei, Petros, Craig, Ismail, for their friendship and help, which has made the life in Manchester much more enjoyable.

I wish to thank my parents, my sister and two brothers, for putting up with me and for their endless love, encouragement, understanding and support. They have been keeping me motivated throughout the doctoral study.

Finally, I would express my sincere gratitude to all people who have helped me over the four years. Also, my project was funded by a Dean’s Award from the Faculty of Engineering and Physical Science, the University of Manchester. I would like to thank the University of Manchester for the financial support.
The Author

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Supporting Work


Whittaker DG, Ni H, Benson AP, Hancox JC, Zhang H. Modelling the Effects of
Disopyramide on Short QT Syndrome Variant 1 in the Human Ventricles. *Computing in Cardiology (CinC)*, 2016. IEEE (Conference proceedings)


Chapter 1

Introduction

1.1 The heart and cardiovascular disease

The heart is located between the lungs and near the anterior chest wall. It is a muscular organ that is responsible for circulating blood through the blood vessels of the circulatory system. Despite its small size of roughly a clenched fist, the heart is a remarkable organ that beats approximately 100,000 times each day, driving nearly 800 litres of blood around the body [1]. The circulation of blood provides the transportation of oxygen, hormones and nutrients, and facilitates a way of metabolic waste removal for the trillions of cells throughout the body [1]. The importance of continuous blood circulation is reflected by the fact that the life of mammals relies heavily on the regularly second to second beating of the heart. It is not surprising that disease related to the organ can lead to catastrophic consequences [2–5].

Cardiovascular disease is a major threat and challenge to the public hearth. It is the leading cause of death in the world, accounting for approximately 17.5 million deaths in 2012, or 31% of all deaths globally in that year [6]. In the USA, heart disease has been the leading cause of death for over six decades [7,8]. In 2013/14 in the UK, cardiovascular disease accounted for 27% of all deaths, only 2% less than that caused by cancer [9]. In the same year, the number of NHS (National Health Service) inpatient episodes related to cardiovascular disease was nearly 1.7 million, accounting for 10% of all inpatient episodes [9]. In line with the magnitude of the mobility and mortality of cardiovascular disease, its economic burden on the public health service is also substantial. In England alone, around £4.3 billion of NHS budget was spent on the cardiovascular disease in 2013/14 [9]. The overall cost to the UK economy due to cardiovascular disease is estimated to be over £15 billion each year [10]. In addition to the economic cost, the aftermath of the disease is usually associated with functional disabilities and reduced quality of life [8].
Cardiovascular disease can happen in various types and forms, such as ischaemic heart disease, cardiac arrhythmia, cardiomyopathy, stroke and congenital heart disease [8,10]. The common risk factors include health factors such as genetics, high blood pressure and cholesterol, diabetes, metabolic syndrome, and lifestyle risk factors such as smoking, physical inactivity and obesity [8,10]. Among these conditions, atrial fibrillation is the most common sustained cardiac arrhythmia [11–13], and ischaemic heart disease is the single leading cause of death in the UK and worldwide [10].

Given the immense health and economic burden cardiovascular disease has imposed globally, great efforts have been made in understanding the pathological mechanisms of the disease as well as developing novel strategies for managing the condition. A wide range of experimental techniques and protocols have been developed and applied to uncover the mechanisms underlying the function of normal and diseased hearts. These techniques facilitate cardiac investigations ranging from uncovering structures and functions of heart-related proteins [14,15], revealing subcellular activities and mechanisms [16], and cellular membrane potentials [17], to noninvasively imaging the activities of the whole heart in vivo [18,18,19]. These experimental techniques and protocols have greatly advanced our understanding of the heart under various related conditions.

However, performing experiments using human tissue has not always been possible due to very limited resources and ethical concerns. Also, an overwhelming fraction of human data is obtained from, as one may expect, diseased hearts. These facts all add to the challenge in understanding the human heart, especially under normal conditions. Consequently, multiple animal experimental models have been introduced to investigate the function and mechanisms of the heart, as well as to discover and test new therapies [20]. These animal models include dogs [21], rats [22], mice [23], rabbits [24], pigs [25], and sheep [26]. Also, ion channel expression in non-cardiac cells (such as Human embryonic kidney cells 293 and Chinese hamster ovary cells) has been a useful tool in understanding the function of single ion channels under normal or mutated conditions [27,28].

1.2 Computational modelling

The heart is an intrinsically complex and coupled nonlinear biological system [29]. Developing computational models has been necessary in order to achieve a quantitative
understanding of the structure and function of the heart in health and pathogenesis [29]. Among the extensively developed models of the physiological systems, cardiac models have been one of the most advanced examples for “virtual organ” [30], with a long cardiac modelling history of more than 50 years [29]. The first biophysically detailed computational model of cardiac myocytes was brought about fifty-six years ago by D. Noble [31]. Since then, the wet research (experimental study) and dry research (modelling, especially computational modelling study) have been conducted and combined in an iterative manner [32,33]. That is, vast observations and data acquired from the experimental and/or clinical research are used first to build and validate the computational models, while the computational studies are performed to test the hypotheses from experiments, make predictions and thereby provide valuable references and targets for the experimental research [32].

Today, cardiac simulations are being performed to uncover activities and the underlying mechanisms of the heart over a diverse range of temporal and spatial scale, such as investigations on the ionic transportation of a single ion channel complex at the atomic scale [34], modelling electrophysiological activities of a single myocytes[35–37], and studying the electrical and mechanical functions at the organ level [35,38].

Compared with experimental research in biological and medical studies, computational modelling has multiple advantages. The first advantage of modelling lies in the ease of fully controlling and monitoring the whole process of a study. For example, in modelling studies one can promptly change conditions like applying pure a single-channel block, altering the size of myocytes or tissue, and clamping a single variable such as intracellular calcium concentration, etc., which may remain difficult or not possible experimentally. The second advantage of modelling is related to the data recording. In modelling studies, it is feasible to record every aspect and the whole process of the study; for example, attaining the gating variables of ion channels in all individual cells in an organ level study can be easily done, in contrast to the presence of experimental challenges in simultaneously recording two variables on a surface [39]. Also, it has not been made available to investigate the variables deep in the tissue. A third advantage of modelling is that it does not rely on the availability of samples, whereas experimental studies are prone to a limited amount of samples which is more prominent in studies related to humans. The fourth advantage for simulation is that modelling is, in general, costing less, both in terms of finance and time. Unlike experiments, modelling studies only need computers, which are increasingly more
powerful while costing less. Simulations of similar conditions could be run repeatedly and continuously without external instructions, whereas experimentally there may be multiple practical limitations.

Computational modelling also has limitations that should be addressed. Models are proposed based on the observations made in experiments, without which it may not be possible to make a valid model. Also, models are designed to reduce the complexity of the system [40] and thus can only take limited variables into account, which may impose a limitation on the accuracy and clinical use of the model. Furthermore, results from models have to be validated by the experiments before they are used to make further predictions. Therefore, it is more desirable to integrate the simulation platform with the experimental framework in an iterative manner to investigate the highly complex biological system as the heart [32].

1.3 Aims and organisation of the thesis

Atrial fibrillation (AF) is the most common cardiac arrhythmia in the world [11–13]. Despite recent advances in understanding the underlying pathological mechanism and developing novel approaches to the management of this arrhythmia [41–44], AF remains one of the major causes of stroke, sudden death, heart failure and high prevalence of cardiovascular diseases globally [13,44,45]. In 2010, the total number of men and women affected by AF was estimated to be 33.5 million in the world [44]. AF imposes substantial public health consequences, including reduced quality of life of the patients, increased hospitalisation rates and medical costs [44–46]. Common risk factors of AF include diabetes, obesity, genetic and family history, and the presence of another heart conditions such as heart failure, ischaemic heart disease and hypertension [44,46]. Additionally, the prevalence rates of AF increase substantially with age, from <0.5%-1% at 50 years to 6%-15% at 80 years old [46], making AF more relevant for countries with aging populations.

Despite recent progress and advances, developing effective and safe antiarrhythmic pharmaceutical therapies remains challenging and unmet [12,13,42,47], and is usually fraught in inducing adverse effects in the ventricles including promoting ventricular arrhythmias [28,48–52]. Developing atrial-selective pharmaceutical therapies is a current strategy for the pharmaceutical management of AF [17–19]. Dissecting the functional role of atrial-specific ion channels in the atrial activities is an essential part of
a wide effort to fulfil this strategy. The ultra-rapid delayed rectifier K\(^+\) current, \(I_{\text{Kur}}\), represents an atrial-selective target in humans. However, the role of \(I_{\text{Kur}}\) in atrial arrhythmogenesis and contractility remains to be fully clarified. Also, given the frequent presence of complications in the ventricles in the anti-AF pharmaceutical therapies, a 3D ventricle-torso model is a valuable tool in assessing the selectiveness and safety of an antiarrhythmic therapy.

The aim of this thesis falls into two areas:

1) To elucidate the role of \(I_{\text{Kur}}\) in atrial arrhythmogenesis and mechanical functions, and its potential in the pharmaceutical management of AF.

2) To develop a ventricle-torso heart that is capable of simulating ventricular electrical activities and electrocardiogram (ECG).

Accordingly, the work of this thesis can be split into two major parts. The part I of this thesis focuses on using multi-scale computational models to thoroughly elucidate the role of the \(I_{\text{Kur}}\) in the functional activities of human atria and the efficacy of \(I_{\text{Kur}}\) block as a therapy for managing AF. In modelling the functional role of \(I_{\text{Kur}}\), the studies concentrate on clarifying the pathological mechanisms of lone-AF linked variants of \(I_{\text{Kur}}\), instead of pure theoretical investigations. Part II presents the development of a biophysically detailed anatomical human ventricle-torso model and its application in the modelling of cardiac abnormalities, such as ventricular ischaemia and long-QT related genetic mutations.

Accordingly, the thesis is structured as follows.

Chapter 2 gives an introduction to the background and the physical and mathematical basis of cardiac computational modelling, such as an introduction to the anatomy and structure of the heart, electrophysiology and ion channels, as well as the mathematical modelling of cardiac physiology and model implementation.

Chapter 3 presents the elucidations of the mechanistic link between variants of \(I_{\text{Kur}}\) and atrial arrhythmogenesis. A novel mathematical model for \(I_{\text{Kur}}\) was developed to describe the WT and newly identified gain- and loss-of-function variants of \(I_{\text{Kur}}\). Three contemporary human atrial single cell models were employed to study the impact of the variants on the atrial electrophysiology. Using a 3D anatomical and heterogeneous model of the atria, the tissue’s vulnerability to the genesis of unidirectional conduction was evaluated; the effect of the altered activities of \(I_{\text{Kur}}\) on the spiral wave dynamics was quantified. Also, the effects of the genetic variants on atrial electrophysiology and conduction were further evaluated in the presence of adrenergic challenge. This work
provides a mechanistic basis to the on-going discussion of both up- and down-regulated repolarisation currents are associated with increased atrial arrhythmogenesis [56,57].

Chapter 4 details a study clarifying the impact of alterations in the activity of I_{Kur} due to genetic variants on the atrial contractility at both the cellular and organ level. An electromechanical single cell model of human atria was first developed and validated. The models of I_{Kur} developed in Chapter 3 were incorporated into the single cell electromechanical model, allowing for investigations of the impact of both gain- and loss-of-function variants of I_{Kur} on the active force and cell shortening. In addition, the mechanisms underlying the inotropic effects modulated by alterations in I_{Kur} were elucidated. Furthermore, the developed single cell electromechanical model was incorporated into an anatomical and anisotropic 3D electromechanical model of human atria to study the impact of the genetic variants on the atrial mechanical function at the organ level. This work adds insights into the role of genetic variations on the mechanics of the atria.

In Chapter 5, the antiarrhythmic effects of pharmaceutical block of multiple channels including I_{Kur} and the Na^{+} current (I_{Na}) in managing AF were investigated. State-dependent models of I_{Na} and I_{Kur} block were employed to describe the actions of channel blockers. The drug actions of acacetin, a representative compound for I_{Kur} blockers that marginally inhibits multiple K^{+}-channels in addition to I_{Kur}, was simulated and compared with pure I_{Kur} block. The antiarrhythmic effects of individual and combined channel-block were investigated at the cellular scale and in tissue. The AF-selectivity of I_{Na} block and combined Na^{+}-and K^{+}-channel block were quantified. The efficacy of different combinations of blockers in terminating re-entries was evaluated in a 2D tissue. This study highlights synergistic anti-AF effects of multi-channel block exerted by combined Na^{+}-and K^{+}-channel block and multiple K^{+}-channel block, which may be a valuable strategy in the pharmaceutical management of AF.

Chapter 6 describes the development and case-study validations of an anatomically accurate human ventricle-torso model. A family of single cell models for the human ventricles was first developed, which considers the apicobasal, transmural and interventricular electrical heterogeneities in the ventricles. The single cell models were subsequently incorporated into a 3D anatomical model of human ventricles with detailed fibre orientation. A modified endo-surface stimulation profile was used to reproduce the heterogeneous activation of the ventricles by the Purkinje fibres. A ventricle-heart model was then constructed by integrating the 3D anatomical model of
the human ventricles and a torso model incorporating internal organs and ribs with heterogeneous conductivities. Through solving the forward problem of electrophysiology, the body surface potential was calculated, from which ECGs were derived. Using the ventricle-torso model, the role of electrical heterogeneities in the genesis of T-wave was assessed. The model was then applied to simulate bundle branch block and acute ventricular ischaemia. A good agreement between simulated and experimental and clinical ECGs from literature was achieved under normal and diseased conditions, demonstrating the validity of the developed model.

Chapter 7 presents an investigation of the functional impact of the KCNQ1-G269S mutation on the ventricular activity and ECG. First, single cell simulations were performed to assess the effect of the mutation on the ventricular electrophysiology under normal conditions and in the presence of adrenergic stimulation. Then, the arrhythmogenesis of the KCNQ1-G269S mutation was assessed using a 1D strand model representing transmural ventricles. Finally, the ventricle-torso model developed in Chapter 6 was employed to evaluate the impact of the mutation on the ECG under both of the physiological conditions. This work uncovers the mechanistic link between a long-QT related genetic mutation and the ECG manifestations of patients under the baseline and during exercise conditions and further demonstrates the usefulness of the ventricle-torso model.

Chapter 8 summarises the major findings and significance of this thesis, as well as their relevance to clinical studies and practices. Possible directions for the future continuation of the thesis are discussed. The thesis closes with a few final remarks on the coming era of precision medicine in cardiology.
Chapter 2
Background

This Chapter gives an introduction to the background the heart and computational cardiology, including general introductions to the anatomy and structure of the heart, and the cardiac electrophysiology and ion channels, as well as the mathematical modelling of cardiac physiology. Some numerical methods and model implementation are also briefed.

2.1 The heart

2.1.1 The circulatory system

In living animals, the body continuously exchanges substances and information with the environment. The circulatory system plays a vital role in carrying and spreading important substances throughout the body, as well as collecting the waste. Consisting of a vast network of organs and vessels, the circulatory system is responsible for the circulation of blood, which transports nutrients, oxygen, hormone and metabolic waste etc. to or from cells all over the body, and hence helps to fight against diseases, maintain body temperature, pH, as well as the ionic homeostasis [1]. In mammals, the circulatory system can be divided into two sub-systems: the pulmonary circuit, whose function is to oxygenate the blood and remove the carbon dioxide from the lung, and the systemic circuit, who is responsible for transporting the oxygenated blood and nutrients to the cells around the body. The heart plays a vital role in the circulatory system providing the essential power to pump the blood throughout the blood vessels of the body.

2.1.2 Anatomy of the heart

The main role of the heart is to pump blood regularly around the body. Its regular
function is vital to life in the sense that a malfunction could lead to a lethal result in a period of minutes [4,5]. The heart is the first organ to develop in the early stages of pregnancy [58] and continues beating till death. In humans, the heart is located in the centre of the chest and between the lungs, with the bulk of the bottom (apex) stretching to the left-hand side of the chest cavity.

As shown in Figure 2.1, the human heart is a hollow muscle consisting of four chambers, namely, two atria and two ventricles. The atria collect blood from the circulatory system and drive it into the respective ventricles located on the same side, whilst the ventricles are responsible for pumping the blood into the lung or the body. The right atrium and right ventricle together pump the blood for the pulmonary circuit, while their left counterparts drive the systemic circuit. The deoxygenated blood is collected from the body (via superior and inferior vena cava) into the right atrium, in which the blood is then driven through the tricuspid valve into the right ventricle, and is finally pumped into the lungs, allowing for removing carbon dioxide from the blood while absorbing oxygen. The oxygenated blood coming out of the lungs is driven through the pulmonary veins into the left atrium and is then forced through the mitral valve into the left ventricle, where the blood is pumped to the rest of the body via the aorta. The heart itself is supplied with oxygenated blood via the coronary system.

The ventricles and atria differ in size and structure, which is linked to their corresponding functions. Ventricles need to pump the blood into the circulatory system, which necessitates a greater pressure thus more power than collecting blood as seen in the atria. Accordingly, the ventricles are much larger in size (2 - 5 times) and have thicker walls as compared to the atria [1]. Also, the left ventricle is larger than the right counterpart as pumping the blood through the rest of the body needs much more power than pumping it into the lung.

The two atria are separated by the interatrial septum (AS), and ventricles by the interventricular septum. The atria and ventricles are divided by a layer of connective tissue, which plays a vital role in providing electrical insulation between the atrial and ventricular chambers. The cavities of the atria and ventricles are regularly connected and then separated through the tricuspid (right) and mitral (left) valves, respectively (Figure 2.1), which prevents otherwise possible backflow of blood from ventricles into the atria.
2.1.3 Cell types of the heart

The four-chambered heart consists of a number of different cell types, such as the cardiac fibroblasts, endothelial cells, smooth muscle cells, pacemaker cells and cardiomyocytes [60,61]. These types of cells play a role in the structural, biochemical and electrophysiological-mechanical properties of the heart. The cardiac fibroblasts account for more than 50% of the heart cells and are responsible for synthesising the extracellular matrix and collagen [60]. Cardiac fibroblasts are electrically ‘non-excitable’ but can be electrically coupled with cardiomyocytes through connexins, passively affecting electrophysiology and excitation conduction in myocardium [62]. During the healing process of tissue damage, such as myocardial infarction, fibroblasts differentiate into myofibroblasts, which play an important role in the formation of scars [63]. Cardiomyocytes from the atria and ventricles constitute the muscular walls of the heart.
(also called myocardium), the contraction of which directly contributes to the function of the heart as a mechanical pump. Endothelial cells can be found in the interior lining of blood vessels and cardiac valves. Smooth muscle cells are the main constituent in the coronary arteries, as well as the inflow and outflow vasculature. Pacemaker cells and Purkinje fibres are the specialised cardiomyocytes that are capable of spontaneously generating electrical excitation impulses [60]. They constitute the cardiac conduction system, which will be introduced in the following section.

2.1.4 The cardiac conduction system

The cardiac conduction system (CCS) is composed of a number of specialised myocytes including the SAN, AVN, Bachmann’s bundle, atrioventricular node, bundle of His (atrioventricular bundle), the left and right bundle branches and the Purkinje fibre network (Figure 2.2). The main role of CCS is to initiate and conduct the electrical excitation throughout the rest of the heart.

The sinoatrial node (SAN) comprises a group of specialised pacemaker cells which reside in the right atrium and close to the superior vena cava. Under normal conditions, the SAN is responsible for spontaneously generating regular electrical impulses at rates between 80 and 100 BPM in humans [1].

The atrioventricular node (AVN) is located at the junction between the atria and ventricles (Figure 2.2), and functions as a secondary pacemaker of the heart. The spontaneous pacemaking activity of isolated AVN is much slower than that of the SAN, represented by 40 to 60 BPM [1]. Also, the conduction of electrical excitation in the AVN is rather slow: it takes nearly 100 ms for an impulse to pass through the AVN [1], adding a delay in the activation of the ventricles, which allows atria to contract first to force the blood into the ventricles before they contracts. The AVN may act as the main pacemaker in the presence of SAN block or failure [64].

The His-Purkinje system consists of the bundle of His, bundle branches and Purkinje fibre network. Connected with AVN, the bundle of His penetrates the central fibrosis body, acting as the only conduction pathway allowing propagation of impulses from the atria to the ventricles [1,65]. The bundle of His then splits into two branches, namely, the right and left bundle branches, at the crest of the interventricular septum [66]. The right and left bundle branches are notably asymmetric: the right bundle branch continues to descend in a thin cable-like structure, whereas the right bundle branch
splits into a multivesicular fibre network, which is important in achieving the synchronous activation of the left ventricle [67]. At their distal ends, the bundle branches separate into the Purkinje fibre network, which was originally discovered by the Czech scientist Jan Purkinje in 1839 [65,68], although its functional role was not understood until the study by Tawara in 1906 [68]. The Purkinje fibre network covers most of the ventricular endocardium so that the impulses conducted from the bundle branches are distributed rapidly and synchronously to the ventricular myocardium [68,69]. The penetration depth of the Purkinje fibre network into the myocardium is species-dependent: in humans and dogs, the Purkinje fibre network is predominantly subendocardial, whereas it covers throughout the transmural depth in ungulates [67]. Also, the Purkinje fibre network allows for much faster conduction of excitation impulses than the working myocardium [67].

Figure 2.2 Schematic representation of the cardiac conduction system in a mammalian heart. SVC – superior vena cava; SAN – sinoatrial node; CFB – central fibrous body; TV – tricuspid valve; AVN – atrioventricular node; BH – Bundle of His; RBB – right bundle branch; LBB – left bundle branch; IVC – inferior vena cava; PFN – Purkinje fibre network. Adapted from [64].
Under normal conditions, the electrical excitation of the heart is initiated from the SAN, and then rapidly conducted along the crista terminalis (CT) to the right atrium. The left atrium is then activated via the Bachmann’s bundle. The excitation signal is conducted into the slow pathways of the AVN, through which the excitation impulses are then via the His-Purkinje system to the ventricles. The sophisticated nature of the CCS makes it possible for the four chambers of the heart to contract regularly with a predefined temporal sequence.

2.2 Cardiac myocytes

The myocytes, or cardiac working cells, are cylindrically shaped in structure and roughly 10-20 μm in diameter and 50-100 μm in length, respectively [70]. The radial surface of myocytes is covered by the sarcolemma, which separates the cytosol within the cell from the extracellular space (Figure 2.3). The transverse tubules (T-tubules) extend from the plasma membrane, penetrate deep into the myocyte. They play a key role in excitation-contraction coupling [71,72]. A large fraction of the cell volume (36% in rat ventricular myocytes [70]) is possessed by the mitochondria, which are responsible for supplying most of the chemical energy to be used in contraction [70]. Some more fraction of the volume (around 47% [70]) is occupied by the contractile proteins called myofibrils, which is composed by adjoining sarcomeres, the functional unit of contractile apparatus. Other important components include the sarcoplasmic reticulum, which plays a vital role in the Ca^{2+} handling [23,73,74].

2.2.1 The sarcolemma

The sarcolemma, or the cell membrane, is a thin phospholipid bilayer embedded with a number of different proteins, through which charged ions are allowed to move actively or passively, in or out of the myocytes [70].

The difference between ion concentrations of the intracellular and extracellular space results in a potential difference across the membrane, which is called the transmembrane potential, and denoted by $V_m$. By convention, the extracellular potential is used as the reference in the definition of the transmembrane potential:

$$V_m = \phi_i - \phi_e$$

(2.1)

where $\phi_i$ is the potential measured at the intracellular space while $\phi_e$ represents the
extracellular potential. Multiple proteins embedded in the sarcolemma are selectively permeable to certain ion/ions. The main ions involved are the sodium (Na\(^+\)), potassium (K\(^+\)), calcium (Ca\(^{2+}\)) and chloride ions (Cl\(^-\)); the contribution of Cl\(^-\) is less prominent than the rest. Generally, three types of proteins contribute to the movement of these ions: ion channels, ion exchangers and ionic pumps, which will be introduced below.

![Figure 2.3](image)

Figure 2.3 Schematic diagram of the structure of cardiac myocytes. The myocyte is covered by the sarcolemma, where the transverse tubules extend and penetrate into the cell. The mitochondria and contractile proteins occupy a large proportion of the cell space. The sarcoplasmic reticulum plays a crucial role in the complex Ca\(^{2+}\) handling. Adapted from [70].

### 2.2.2 Ion channels

The ion channels are most abundant in the myocytes. They selectively permit a passive movement of certain types of ions down the electrochemical gradient created from the difference in ionic concentrations between the extracellular and intracellular spaces. Ion channels can exist in open or closed state, the transition of which represents the conformational changes of the channel proteins (Figure 2.4).

Ion channels may have both activation and inactivation processes, which can be described as two gates. The channel only allows the flux of ions on the condition when
the activation gate is open while the inactivation gate is not inactivated. A number of factors may contribute to the opening and closing processes of these two gates. These factors include the transmembrane potential (voltage-dependent channels), the presence/absence of bound ligand (ligand-dependent channels), or the mechanical state of the membrane (stretch-activated channels) [75]. Most of the primary ion channels in cardiac myocytes exhibit dependence on the transmembrane voltage [75].

![Schematic diagram of an ion channel residing in a closed, open or inactivated state. Modified from [76].](image)

### 2.2.3 Ion exchangers and pumps

In contrast to the ion channels, the ionic pumps allow for active movement of the ions: they transport ions up to the electrochemical gradient at the cost of consuming ATP. Ion exchangers, on the other hand, exchanges two types of ions without energy consumption: they use the energy provided by one ion that is moving down its electrochemical gradient to force the other ion to move against the respective electrochemical gradient [77]. Ionic pumps and exchangers are important in preserving the ionic homoeostasis in the intracellular space. Apparently, in the absence of ion exchangers and pumps, the passive ion diffusion through the ion channels will inevitably lead to either ion building up or depletion within the cell.

A summary of the primary ion channels/exchanger/pumps in myocytes is listed in Table 2.1.

### 2.2.4 Intercalated discs

Intercalated discs are specialised myocyte-to-myocyte junctions that are responsible for electrically and mechanically connecting the adjacent myocytes [70]. The mechanical
The connection between the adjacent myocytes is important in the transmission of mechanical forces and deformation in tissue [70].

Table 2.1 Primary transmembrane ionic currents in cardiac myocytes.

<table>
<thead>
<tr>
<th>Current</th>
<th>Name</th>
<th>Ion</th>
<th>α-Subunit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Na}$</td>
<td>Fast Na$^+$ current</td>
<td>Na$^+$</td>
<td>Nav1.5</td>
</tr>
<tr>
<td>$I_{Na,L}$</td>
<td>Late Na$^+$ current</td>
<td>Na$^+$</td>
<td>Nav1.5</td>
</tr>
<tr>
<td>$I_{Ca,L}$</td>
<td>L-type Ca$^{2+}$ current</td>
<td>Ca$^{2+}$</td>
<td>Cav1.2</td>
</tr>
<tr>
<td>$I_{Ca,T}$</td>
<td>T-type Ca$^{2+}$ current</td>
<td>Ca$^{2+}$</td>
<td>Cav3.1</td>
</tr>
<tr>
<td>$I_{to}$</td>
<td>Transient outward K$^+$ current</td>
<td>K$^+$</td>
<td>Kv4.3/4.2</td>
</tr>
<tr>
<td>$I_{Kur}$</td>
<td>Ultra-rapid delayed rectifier K$^+$ current</td>
<td>K$^+$</td>
<td>Kv1.5</td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td>Rapid delayed rectifier K$^+$ current</td>
<td>K$^+$</td>
<td>Kv11.1</td>
</tr>
<tr>
<td>$I_{Ks}$</td>
<td>Slow delayed rectifier K$^+$ current</td>
<td>K$^+$</td>
<td>Kv7.1</td>
</tr>
<tr>
<td>$I_{K1}$</td>
<td>Inward rectifier K$^+$ current</td>
<td>K$^+$</td>
<td>Kir2.1/2.3</td>
</tr>
<tr>
<td>$I_{SK}$</td>
<td>Small-conductance Ca$^{2+}$-activated K$^+$ current</td>
<td>K$^+$</td>
<td>KCa2.2</td>
</tr>
<tr>
<td>$I_{TASK-1}$</td>
<td>K2P-mediated K$^+$ current</td>
<td>K$^+$</td>
<td>K2P3.1/TASK-1</td>
</tr>
<tr>
<td>$I_{Kv1.1}$</td>
<td>Kv1.1-mediated K$^+$ current</td>
<td>K$^+$</td>
<td>Kv1.1</td>
</tr>
<tr>
<td>$I_{KATP}$</td>
<td>ATP-sensitive inward rectifier K$^+$ current</td>
<td>K$^+$</td>
<td>Kir6.2</td>
</tr>
<tr>
<td>$I_{K,ACH}$</td>
<td>Acetylcholine activated K$^+$ current</td>
<td>K$^+$</td>
<td>Kir3.1</td>
</tr>
<tr>
<td>$I_{f}$</td>
<td>Hyperpolarisation-activated funny current</td>
<td>Na$^+$/K$^+$</td>
<td>HCN1/4</td>
</tr>
<tr>
<td>$I_{NaCa}$</td>
<td>Na$^+$-Ca$^{2+}$ exchanger current</td>
<td>Na$^+$/Ca$^{2+}$</td>
<td>NCX1</td>
</tr>
<tr>
<td>$I_{NaK}$</td>
<td>Na$^+$-K$^+$ pump</td>
<td>Na$^+$/K$^+$</td>
<td>NaKα1/2/3</td>
</tr>
<tr>
<td>$I_{CaP}$</td>
<td>Ca$^{2+}$ pump</td>
<td>Ca$^{2+}$</td>
<td></td>
</tr>
</tbody>
</table>

Gap junctions are located in the intercalated discs, forming specialised clusters of closely packed intercellular channels that directly connect the cytoplasmic compartment of adjacent cells [78,79]. These intercellular channels enable passage of molecules that smaller than 1 kDa and more importantly, ions [78,79]. The passage of ions through the gap junctions facilitates an electrical coupling of myocytes in tissue, which is vital for the propagation of excitation impulse in the heart [78,79]. Three connexins have been shown to contribute to the function of gap junctions, namely connexin40, connexin43 and connexin45; they differ in the molecular mass and conductance [78,79]. In the ventricular myocardium of adult mammals, gap junctions are mainly found in the
intercalated discs, whereas in atrial tissue, gap junctions may also exist along the lateral membranes of the myocytes [80].

2.3 Cardiac electrophysiology

In quiescent myocytes, relatively static negative potentials (-70 to -85 mV) are sustained across the cell membrane [81,82]. In opposed to the resting potential, in cardiology, the action potential (AP) is use to describe a cyclically changing event in which the transmembrane potential rapidly rises and then recovers to the resting level within a short period.

2.3.1 Action potential

Though large degrees of heterogeneities exist in AP morphologies among different typical cardiac cell types such as the pacemakers, atrial and ventricular cells [69,81,82], they can be described within a general framework. As shown in Figure 2.5, APs of both atrial and ventricular cells can be described using five phases, as introduced bellow.

Phase 4, or the resting phase, during which the transmembrane potential of a majority of working cardiomyocytes is stable resting membrane potential (RMP) in the region of -75 to -85 mV [81,82]. The inward-rectifier K+ current $I_{K1}$ has been suggested to play a dominant role in maintaining the RMP [83,84].

During phase 0, the membrane potential rapidly depolarises to a positive voltage range, primarily fulfilled by the rapid inward flux of the Na+, primarily through $I_{Na}$ in most working cardiac myocytes. During this phase, $I_{Na}$ is rapidly activated and then promptly inactivated. Phase 0 is central to the excitability of myocytes and the wave propagation of the cardiac impulse [75].

Closely following the phase 0 comes the phase 1, during which the transmembrane potential rapidly repolarises, resulting in a pronounced spike in the AP. The fast activation of the transient outward current, $I_{to}$, is mainly responsible for the rapid repolarisation in phase 1.

Phase 2 is characterised by a relatively slow repolarisation rate, exhibiting a "plateau" in the AP. During phase 2, and the outward currents is balanced by the inward currents (primarily the inward L-type Ca$^{2+}$ current, $I_{CaL}$).
Figure 2.5 Key phases of typical action potentials (AP), and the corresponding primary ion currents during the phases. The top panels: atrial and ventricular AP; the five phases are labelled accordingly. Bottom panels: schematic diagrams representing the contribution of depolarisation and repolarisation currents during the AP. The atrial-selective channels are highlighted. The role of $I_{KV1.1}$ in the ventricles is yet to be confirmed [48]. Adapted from [48].

Phase 3 is a phase of rapid repolarization that restores the transmembrane potential to its resting value, which is mainly accomplished by the $I_{Ks}$ and the rapid delayed rectifying $K^+$ current $I_{Kr}$, while $I_{CaL}$ progressively inactivates. At the end of phase 3, the transmembrane potential is recovered to the RMP, and a second phase 4 follows.

It has been well demonstrated that APs of ventricular cells differ from those from atrial cells. Figure 2.5 illustrates the contributions of ionic currents during the AP.
While the atrial and ventricular myocytes do share many common ionic currents, there are multiple $K^+$ currents that are believed to be only present in the atria such as $I_{Kur}$, $I_{K,ACH}$, $I_{SK}$, and $I_{TASK-1}$ [48,53]. Accordingly, these ionic currents have been named as atrial-selective channels. The presence of atrial-selective currents contributes to the atrial-ventricular difference in AP and provides a potential target for atrial-selective anti-AF therapy [48,53].

2.3.2 Biomarkers of action potential

The action potential is one of the most important concepts in electrophysiology. For the favour of quantifying and describing an action potential, multiple key parameters, or biomarkers of the action potential have been proposed and widely used. Here, the most commonly used biomarkers are introduced (Figure 2.6).

**Figure 2.6 Schematic diagram illustrating the key biomarkers of the AP.**

- **Overshoot (OS):** the maximum transmembrane voltage a myocyte achieves during an AP.

- **Action potential amplitude (APA):** the difference between the resting membrane potential and the overshoot. APA is associated with excitation impulse propagation in tissue [85].

- **Maximum upstroke velocity ($V_{\text{max}}$):** the maximum change in voltage per unit time during phase 0 of an AP ($V_{\text{max}} = \frac{dV_m}{dt}|_{\text{max}}$). $V_{\text{max}}$ has been used as a measure of the excitability [85] as well as $I_{Na}$ [12] of a cell. The time instant a cell reaching $V_{\text{max}}$ has also been used to mark the start of an action potential.
**Action potential duration (APD):** the temporal interval between the time instant of Vmax is reached and the moment the transmembrane potential repolarises to a certain level, *i.e.* 90% (APD\(_{90}\)), or 75% (APD\(_{75}\)). APD\(_{90}\) has been widely used as an indication of the AP reaching the terminal phase of repolarisation.

### 2.3.3 S1 and S1-S2 protocol

The S1 and S1-S2 stimulation protocols have been widely used for elucidating the dynamic properties of the cardiac cells. Accordingly, a diagram of S1 and S1-S2 stimulation protocols is illustrated in Figure 2.7.

![Diagram of S1 and S1-S2 stimulation protocols](image)

Figure 2.7 Illustration of S1 and S1-S2 stimulation protocols for eliciting action potentials. (A) S1 stimulation protocol. (B) S1-S2 stimulation protocol. DI - diastolic interval; BCL - basic cycle length.

The S1 stimulation protocol (or steady-state pacing) describes applying external stimulation to a myocyte or myocardium at a fixed rate (Figure 2.7 A). The basic cycle length (BCL) then is defined as the time interval between the time points reaching the maximum upstroke velocity of two consecutive action potentials. Diastolic interval (DI)
is measured as the temporal interval between the time instant of a 90% repolarisation of the previous action potential is reached, and the time the membrane voltage reaching $V_{\text{max}}$ of the second AP (Figure 2.7 A).

In the S1-S2 stimulation, the tissue/cell is paced at chosen basic cycle length until the steady state is acquired. Then, after a certain time delay, a premature stimulus (S2) is applied (Figure 2.7 B). This protocol has been introduced to quantify the effective refractory period and excitability.

### 2.3.4 Effective refractory period

After an AP is elicited, the cardiomyocytes undergo a recovery period during which a smaller or even no AP can be generated regardless of the strength of the stimuli; this period is known as the refractory period. The refractory period is composed of two phases: absolute refractory period during which no second AP can be evoked by an S2 stimulus of any magnitude, and relative refractory period during which a smaller AP can be evoked. Generally, the effective refractory period (ERP) refers to the longest time interval after which the S2 will just fail to capture in tissue. ERP is an important measure for the electrical memory of cardiomyocytes.

### 2.3.5 Intracellular calcium cycle

Similar to the change in the transmembrane potential, the intracellular Ca$^{2+}$ concentration also undergoes cyclical changes following electrical excitations. While Ca$^{2+}$ has been deeply involved in shaping the action potential, it also acts as a second messenger and direct activator of myofilaments [86], a group of proteins that execute the contraction of the cardiomyocytes. Cardiac excitation-contraction coupling (ECC) is a term used to describe the process translating the electrical excitation of the cardiomyocytes to active contraction of the cells [86]. The intracellular Ca$^{2+}$ cycle plays a vital role in the ECC. In this section, the process of the intracellular Ca$^{2+}$ cycle is introduced.

Figure 2.8 illustrates major structures and processes involved in a Ca$^{2+}$ cycle. In the cardiomyocytes, the sarcoplasmic reticulum (SR) maintains a great level of Ca$^{2+}$ (approximately 5000-fold larger than the cytosol Ca$^{2+}$ concentration [87]). On the membrane of SR reside the ryanodine receptor (RyR) and the sarcoplasmic reticulum Ca$^{2+}$ ATPase (SERCA), two protein complexes responsible for releasing Ca$^{2+}$ from and
collecting Ca\(^{2+}\) to the SR, respectively.

Figure 2.8 Schematic diagram of the intracellular Ca\(^{2+}\) cycle. The red and green arrows indicate the direction of Ca\(^{2+}\) flux. Insert: time courses of intracellular Ca\(^{2+}\) concentration and contraction elicited by an AP. NCX – Na\(^+\)/Ca\(^{2+}\) exchanger; ATP – ATPase; PLB – phospholamban; SR – sarcoplasmic reticulum; RyR – ryanodine receptor. Adapted from [86].

The Ca\(^{2+}\) cycle starts following the upstroke of action potentials. Depolarised transmembrane potential activates I\(_{\text{Cal}}\), resulting in a Ca\(^{2+}\) flux entering the cell. The Ca\(^{2+}\) influx causes a small elevation in the Ca\(^{2+}\) concentration of the surrounding cytosol, which activates the RyR located in proximity to the L-type Ca\(^{2+}\) channel complexes, inducing a great Ca\(^{2+}\) release from the SR and markedly raising the intracellular Ca\(^{2+}\) concentration [86]. This process has been termed as Ca\(^{2+}\) induced Ca\(^{2+}\) release (CICR). The free Ca\(^{2+}\) in the cytosol then binds to the myofilaments, after which contraction is initiated.

Several processes are underway in response to the increased amount of free Ca\(^{2+}\). The SERCA actively collects Ca\(^{2+}\) back into the SR at the cost of ATP. In the
meanwhile, the Ca$^{2+}$ is also extruded out of the cell by the ATPase and the Na$^+$/Ca$^{2+}$ exchanger located in the membrane of the myocytes. At the end of the Ca$^{2+}$ cycle, the intracellular Ca$^{2+}$ concentration recovers to the resting level, as does the transmembrane potential.

2.4 Mechanical contraction of the heart

The ultimate function of the heart is to pump the blood strictly complying with certain regulations, which is accomplished by the mechanical contraction of the myocytes, triggered by the electric excitation. Electro-mechanical coupling (ECC) plays a crucial role in this trigger mechanism. In this section, the molecular mechanisms underlying the contraction of cardiomyocytes are introduced.

2.4.1 Cardiac output

At the end of the contraction cycle, the volume of blood in the ventricles is represented by the end-systolic volume (ESV). The heart relaxes after contraction and refills with blood, which is fulfilled by the passive force. The end-diastolic volume (EDV) is used to describe the volume of blood in ventricles just before the contraction. Stroke volume (SV) is a measure of blood ejected per beat and thus can be calculated by finding the difference between the EDV and the ESV.

Cardiac output has been employed to quantitatively describe the function of the heart by measuring the amount of the blood pumped out of the ventricles over a period of time. The calculation of the cardiac output is straightforwardly measured as the sum of SV over heart beats.

2.4.2 Active force generation mechanism

2.4.2.1 The ultra-structure of the sarcomere

Sarcomere is the basic contraction unit in myocytes. The repeated arrangement of the sarcomeres composed the tubular myofibrils, the rod-like units of muscles. The contraction of the heart is fulfilled with numerous functioning sarcomeres.

Figure 2.9 shows a schematic diagram of a cardiac sarcomere. The sarcomere is a segment of a myofibril between two neighbouring Z-lines (or Z-discs). There are two major protein complexes that contribute to the contraction of the sarcomere, namely, the
myosin filament and the actin filament. The myosin filaments protrude from the M-line, whereas the actin filaments are embedded in the Z-line, where they extend toward to the centre of the sarcomere.

The thick filament consists of multiple myosin molecules. The heads protrude from the thick filament through rope-like arms. The arms together with the protruding heads are called the cross-bridges [88,89]. The thin filament consists of actin, tropomyosin and troponin complex.

The two filaments are lying in parallel and close to each other within the A-band. Their overlapping length has a direct effect on the length of the sarcomere and hence the length of the myofibrils.

The M line and Z line in a sarcomere is connected by titin, a giant protein complex that functions as a molecular spring [90,91] (Figure 2.9). Titin is mainly responsible for the passive relaxation and recovery of the sarcomere following a contraction.

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Figure 2.9 Schematic diagram illustrating the microstructure of a cardiac sarcomere. Adapted from [92].

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2.4.2.2 Sliding filament mechanism

In 1954 Huxley A. F. et al. [93] and Huxley H. E. et al. [94] published two groundbreaking papers in Nature, which revealed that the muscle shortens as a result of the sliding between the thick and thin filaments. During contraction, the actin filaments
protruding from the successive Z-lines are pulled towards the centre of the sarcomere, “sliding” along the myosin filaments. This results in shortening of the distance between the corresponding Z-lines and thus the muscle contracts. In this way, the muscle contraction is fulfilled by the sliding filament mechanism. The force causing the crucial, active sliding can be described using the cross-bridge cycling and cooperativity mechanisms.

2.4.2.3 Cross-bridge cycle
Actins possess the sites where bindings occur with the heads from the thick filaments and leading to contraction (Figure 2.10). The tropomyosin (Tm) wraps around the actin array. The troponin complex is attached at intermittent intervals of the Tm protein. It consists of three loosely bound protein subunits: troponin I (TnI) which can strongly attach to the actin, troponin T (TnT) with a high affinity for tropomyosin and troponin C (TnC) which can strongly bind with Ca\(^{2+}\) [88,89].

At the beginning of the cross-bridge cycle, the myosin filament head is not attached to the binding sites on the actin since these sites are covered by the tropomyosin. As shown in Figure 2.10 C, in the absence of Ca\(^{2+}\), the troponin attaches to two actins through TnI, and to the Tm via TnT. Ca\(^{2+}\) binding to the TnC will strengthen the TnC-TnI binding while weakening the TnI-actin binding, allowing TnI to detach from the actin. This allows the Tm to relocate to the surface of the actin filament and thereby uncovers the binding site on the actin, through which the myosin head is allowed to bind with the actin.

Figure 2.10 D illustrates eight steps involved in a cross-bridge cycle. ATP has been served as the source of energy during the cross-bridge cycle [88,89]. At the end of the contraction, myosin heads are strongly attached with the actin. At physiological ATP concentrations (3-5 mM), ATP rapidly and irreversibly binds to myosin (step 1), leading to the detachment of actin from the actin-myosin and ATP complex (step 2). The tension subsequently drops, and the myocytes enter a relaxing period. In the following step, the ATP bound to myosin head is cleaved while the products still bind with the head. In step 4, the bindings between the myosin head and actin are weak in the absence of high-level Ca\(^{2+}\). With increased Ca\(^{2+}\) concentrations following electrical excitation of the cells, the Ca\(^{2+}\) binds to the TnC, allowing the myosin head to strongly bind with actin (step 5). The strong binding causes a conformational change in the head, prompting it to tilt towards the rod-like arm of the cross-bridge, and thus produces the
Figure 2.10 Schematic diagram showing the detailed structure of actin filament. (A) Overlapping thin and thick filaments and the cross-bridges in the sarcomere. (B) Detailed view of the thin filament showing the molecular arrangement of troponin and the overlapping tropomyosins (Tm) along the helical actin. (C) Changes in troponin interactions due to Ca$^{2+}$ binding to TnC, and unbinding of TnI from the actin (labelled as “A”). (D) A schematic diagram of the cross-bridge cycle. The abbreviations are actin (A), myosin (M), inorganic phosphate (Pi), adenosine triphosphate (ATP), adenosine diphosphate (ADP). Figure adapted from [88].
power stroke. Subsequently, in step 7, the cross-bridge undergoes isomerisation, the rate of which is limited. In step 8, the ADP and phosphate ion are allowed to detach from the myosin head, making way to new ATP to bind, which leads to a new cycle.

2.4.2.4 Cooperativity mechanism

The Ca$^{2+}$-force relationship can be fitted using hill functions with a coefficient much greater than 1, suggesting the cross-bridge cycle is a highly cooperative process [88,89,95]. It is believed that the cooperativity of cross-bridge cycles can be attributed to the cooperativity between the Ca$^{2+}$-TnC binding and cross bridge bindings [88,89,95].

2.4.3 Passive force generation mechanism

The text above has been focused on the generation of active force, which accounts for the contraction of the heart. However, the relaxation and recovery following the contraction are also important for the normal function of the heart. This is driven by the passive force, which is generated in order to oppose the change in length, which is mainly contributed by titin [90,91,96–98], although collagen also plays a role in the passive force generation [99].

2.5 The electrocardiogram

The electrocardiogram (ECG) has been implemented as a standard bedside evaluation procedure in the diagnosis of heart diseases for many decades [100–102]. During heart beats, a measurable body surface potential (BSP) is induced by the electrical depolarisations and repolarisations of the cardiac myocardium [103]. The ECG provides a non-invasive diagnostic tool for inspecting the highly dynamic electrical activities in the heart through measurement of the BSP [103].

2.5.1 Normal ECG and intervals

Figure 2.12 illustrates time course of a typical ECG. The very first electrical deflection on the ECG is named as the P wave, which represents the activation of the atria. The P wave is followed by the QRS complex, a dynamic segment characterised by the high amplitude of the signal. The QRS complex reflects the activation of the whole ventricles. The final defection seen in an ECG is normally the T-wave, which reflects the repolarisation of the ventricles. Between these waves two segments have been defined,
namely the PR segment and ST segment (Figure 2.11).

In clinical settings, the ECG has usually been described through interpretations of multiple intervals such as the PR interval and QT interval, as well as the ORS complex duration. The PR interval is a measure of the time period starting from the onset of P wave to the onset of the ORS complex. It represents the total conduction time of excitation wave propagating through the atria, AV node and the His-Purkinje system. The QRS complex duration can be interpreted as the total activation time of the ventricles. Finally, the QT interval is measured from the onset of a QRS complex to the terminal time of the T-wave. It provides an indication of the total ventricular electrical activities during a cardiac cycle.

![Figure 2.11 Schematic representation of time course of typical ECG traces. Adapted from [104].](image)

### 2.5.2 12-lead ECG

The 12-lead ECG is the most widely used configuration of ECG in clinical settings [100–102] (Figure 2.12). It consists of three limb leads (I, II, III), three augmented limb leads (aVR, aVL, aVF) and six chest leads (V1-V6).

The placement of the electrodes of the standard 12-lead ECG is illustrated in Figure
The electrodes on the right arm (RA), left arm (LA) and left leg (LL) compose the Einthoven’s Triangle [105]. The limb leads are obtained from the calculating the difference in the signals of these electrodes.

\[ I = \phi_{LA} - \phi_{RA} \]  
\[ II = \phi_{LL} - \phi_{RA} \]  
\[ III = \phi_{LL} - \phi_{LA} \]  

To find a reference for the body surface potential, the average of these electrodes has been defined as the Wilson’s Central Terminal:

\[ \phi_{WCT} = \frac{\phi_{LA} + \phi_{LL} + \phi_{RA}}{3} \]  

The chest leads are then obtained through:

\[ V1 = \phi_{v1} - \phi_{WCT} \]  
\[ V2 = \phi_{v2} - \phi_{WCT} \]  
\[ V3 = \phi_{v3} - \phi_{WCT} \]  
\[ V4 = \phi_{v4} - \phi_{WCT} \]  
\[ V5 = \phi_{v5} - \phi_{WCT} \]  
\[ V6 = \phi_{v6} - \phi_{WCT} \]  

The augmented limb leads are defined as:

\[ aVL = \phi_{LA} - \frac{\phi_{RA} + \phi_{LL}}{2} \]  
\[ aVR = \phi_{RA} - \frac{\phi_{LA} + \phi_{LL}}{2} \]  
\[ aVF = \phi_{LL} - \frac{\phi_{RA} + \phi_{LA}}{2} \]
Figure 2.12 Schematic representation of the configuration of the 12-lead ECG. The axes of the limb leads are labelled in the hexaxial system. The positions of electrodes for the limb and chest leads are also indicated. Modified from [106].

2.6 Heart diseases

Heart diseases exist in diverse types and forms, such as ischaemic heart disease, myocardial infarction, cardiac arrhythmia, cardiomyopathy and heart failure, stroke, cardiac arrest, congenital heart disease, and inherited (genetic) conditions [8,10]. Many factors have been demonstrated to contribute to the prevalence of these conditions. These factors include health factors such as genetics, high blood pressure and cholesterol, diabetes, metabolic syndrome, and lifestyle risk factors such as smoking, physical inactivity and obesity [8,10]. This section introduces the conditions that are directly related to this thesis.
2.6.1 Cardiac arrhythmias

Cardiac arrhythmias refer to the condition which manifests as a non-physiologically justified rhythm of the heart [107], which may lead to irregular contraction and thus reduced cardiac output and in some cases result in sudden death. The most common arrhythmias are listed below.

2.6.1.1 Bradycardia and tachycardia

Bradycardia is used to describe the condition where the heart rate is abnormally slow (less than 60 BPM). While bradycardia can be symptomatic when the rate drops below 50 BPM for adults, it sometimes causes fatigue, weakness, dizziness and even fainting at very low rates. It is interesting that the trained athletes often exhibit bradycardias. Severe bradycardia may result in a reduction of cardiac output, leading to a lack of oxygen and nutrients supply for the organ, which can be dangerous. Bradycardia can be effectively managed with artificial pacemakers [108].

Tachycardia refers to the condition exhibiting abnormally fast heart rates (higher than 100 BPM). Tachycardia may result in low blood pressure due to reduced cardiac output, which may lead to sudden death. The ventricular tachycardia is considered to be more dangerous than the atrial tachycardia and sometimes presents as *torsades de pointes*, an electrocardiographic form of polymorphic ventricular tachycardia. The *torsades de pointes* is associated with prolonged or abnormal repolarisation that is often caused by congenital or acquired (drug-induced, for example) long QT syndrome [52]. Rapid and prolonged *torsades de pointes* can develop into ventricular fibrillation and sudden death [52].

2.6.1.2 Fibrillation

Fibrillation is characterised by irregular and fast electrical and mechanical activities of the heart. Often multiple re-entrant spiral waves occur in the presence of fibrillation, leading to more irregular conduction patterns and ECG waveforms compared with tachycardia. These persistent excitation waves may lead to substantial malfunction in the coordinated heat contraction and thus a severe reduction in the cardiac output. Also, persistent fibrillation may result in structural remodelling in the heart.

Atrial fibrillation is the most common form of sustained cardiac arrhythmia observed in clinical settings [11–13] and has been associated with stroke, sudden death, heart failure and high prevalence of cardiovascular diseases globally [13,44,45]. Atrial
fibrillation may arise from diabetes, obesity, genetic mutations, and the presence of another heart conditions such as heart failure, ischaemic heart disease and hypertension [44,46]. Ventricular fibrillation can immediately lead to cardiac arrest and further to death, which is much more dangerous.

It is believed that re-entry and ectopic activity underlie the initiation of maintenance of atrial fibrillation [109]. Re-entry often exists as self-perpetuating spiral waves that dominate the activation of the heart instead of the pacemakers. Several factors have been demonstrated to contribute to the maintenance of re-entries, such as shorter refractory period, slower conductions and larger substrates [109]. The known criteria for initiating the classical re-entry are: 1) presence of paralleled and connected conduction pathways; 2) induction of unidirectional conduction block in one pathway; 3) the refractory period of the tissue is shorter than the global conduction time of the circuit [110]. Functional re-entry is a more recent concept describing a type of re-entry that requires no anatomical obstacles and presents no fully excitable gap within the circle [110,111]. Similarly, rotor is another functional re-entrant excitation that is characterised by a singularity joined by curved wavefront and wavetail and non-refractory tissue at the centre [111].

The ectopic activity refers to spontaneous activation of the working myocytes, which may result from enhanced automaticity, and triggered activity of the myocytes [109]. Enhanced automaticity can occur in the presence of accelerated phase 4 depolarisation that is often driven by increased expression of the hyperpolarisation-activated nonselective currents (I_{h}) [109]. In working myocytes of Purkinje fibres, enhanced automaticity can give rise to ectopic activity, resulting in extra beats or tachycardia [110]. Early after-depolarisations (EAD) and delayed after- depolarisations (DAD) underlie the triggered activity of cardiomyocytes [109,110]. EADs can be induced in the presence of abnormally prolonged AP that gives rise to reactivation of I_{Ca,L}, whilst DADs occur with abnormalities in the intracellular Ca^{2+} handling and Ca^{2+} overloading [109].

2.6.1.3 *Inherited cardiac arrhythmias*

Cardiac arrhythmias can be inheritable. Examples include long-QT, short-QT, Brugada syndrome, the catecholaminergic polymorphic ventricular tachycardia and progressive cardiac conduction defect. These conditions may develop into ventricular arrhythmias and even sudden death. The Long QT syndrome (LQTS) is represented by abnormally
long QT intervals in the ECG and has been associated with an increased risk of induction of the *torsade de pointes* and ventricular fibrillation [112,113].

### 2.6.2 Ischaemic heart disease

Ischaemic heart disease, or the coronary heart disease, is used to describe the condition inducing a restriction in blood supply to one or multiple areas of tissue, which may induce cell deaths. Ischaemic heart disease is one of the world’s leading causes of death [100,114,115]. Ischaemia can cause hyperkalemia, acidosis and hypoxia [116,117], which markedly impair the APD and excitability of myocardium. Ischaemic heart disease has been associated with ventricular fibrillation and sudden death [5,118].

### 2.6.3 Bundle branch block

Bundle branch block refers to, as the name suggests, the failure in conducting the excitation impulse in one of the bundle branches. Right bundle branch block is generally considered benign [119]. Left bundle branch block has been associated with reduced ejection fraction [120] and may be a manifestation of heart failure and ischaemia [119].

### 2.7 Computational cardiology

In 1952, Alan Hodgkin and Andrew Huxley published series of papers in the Journal of Physiology, which revolutionised our understanding of the function of ion channels [121–125]. Their model (Hodgkin-Huxley, or H-H model) is still being used today in a great portion of the cardiac and neural modelling studies. Their discovery demonstrated that cell membrane and ionic currents could be represented by different electrical components within a simple circuit. Using the H-H model, Denis Noble [31] proposed the first biophysically detailed computational model for the canine Purkinje fibre in 1960. Together with Hodgkin and Huxley, their work established the basis for cardiac modelling.

#### 2.7.1 Nernst equilibrium potential

The ions located at either side of the cell membrane are affected by two forces: the chemical force due to the concentration gradient and the electrical force due to the non-
zero transmembrane potential. The Nernst equilibrium potential \( (E_r) \), or the reversal potential, is the potential at which the electrical force balances the chemical force. At this potential, the equilibrium is reached, and hence there will be no net charge flow through the passive ion channels, i.e., the currents for this channel is zero.

The Nernst equilibrium potential can be calculated using the following equation:

\[
E_{r_{\text{ion}}} = \frac{RT}{zF} \ln \left( \frac{[\text{ion}]_e}{[\text{ion}]_i} \right)
\]  

(2.15)

where \( E_{r_{\text{ion}}} \) is the Nernst equilibrium potential, \( R \) is the universal gas constant, \( T \) the absolute temperature (in Kelvin), \( F \) is the Faraday’s constant, \( z \) is the valence number of the ion, and \([\text{ion}]_e\) refers to the extracellular concentration of the ion, while \([\text{ion}]_i\) is the intracellular concentration.

### 2.7.2 Electric circuit model

In electric circuit model, the membrane with the lipid bilayer is considered equivalent to a capacitor. The ion channels/exchangers/pumps allow for the ions passing through the membrane either actively or passively. They are treated in the same way as resistors. Furthermore, since the channels/pumps/exchangers function independently, they should be aligned in parallel in the model. Therefore the electric circuit model of the cell membrane can be illustrated in the manner as shown in Figure 2.13. The complex nature of the transmembrane potential and the ionic currents can be considered and investigated using the electric circuit principle.
According to Kirchhoff’s Law, the total current of the circuit (the sum of the ionic currents $I_{\text{ion}}$, and the capacitive current $I_{\text{cm}}$) should be zero, which is:

$$I_{\text{ion}} + I_{\text{cm}} = 0 \tag{2.16}$$

Hence,

$$I_{\text{ion}} = -I_{\text{cm}} \tag{2.17}$$

The $I_{\text{cm}}$ can be calculated using the transmembrane potential $V_m$,

$$Q = C_m V_m \tag{2.18}$$

$$I_{\text{cm}} = \frac{dQ}{dt} \tag{2.19}$$

Therefore,

$$I_{\text{cm}} = C_m \frac{dV_m}{dt} \tag{2.20}$$

Thus the transmembrane potential dependence over time can be determined by the following equation by combining Equation (2.17) and Equation (2.20):

$$\frac{dV_m}{dt} = -\frac{I_{\text{ion}}}{C_m} \tag{2.21}$$

Therefore, in order to calculate $V_m$, the $I_{\text{ion}}$ should be worked out first, which necessitates the accurate computations of individual ion currents.
Here the ion channel currents are taken as an example. The current for an individual ion channel can be derived using:

\[ I_{\text{ion}} = g(V_m - E_{r\text{ion}}) \]  \hspace{1cm} (2.22)

where \( g \) is the conductance, which is highly nonlinearly dependent on time and transmembrane voltage.

### 2.7.3 Hodgkin-Huxley Models

The conductance of a passive ion channel cannot be worked out straightforwardly as the driving voltage. Hodgkin and Huxley proposed time- and voltage-dependent formulations for Na\(^+\) and K\(^+\) channels [121], which are still prevalent in computational cardiology and neuroscience. In the Hodgkin-Huxley formulation, the conductance of Equation (2.22) changes dynamically, which are modeled incorporating both activation and inactivation gates. They made the basic assumptions that the activation and inactivation process are mutually independent, while they both contribute to the kinetics of the conductance. Note that in a single cell there are a number of ion channels which are of the same type. At certain time \( t \), the proportion of activated channels is \( m \), and the \( h \) refers to the fraction of channels that are not inactivated. Therefore, the total channel current of this type can be given by:

\[ I_{\text{ion}} = g_{\text{max}} m^a h^b (V_m - E_{r\text{ion}}) \]  \hspace{1cm} (2.23)

where \( g_{\text{max}} \) is the maximum conductance of the channel, \( m \) and \( h \) are activation and inactivation variables respectively, while \( a \) and \( b \) are the powers of the gating variables which correspond to the structural formation of the channel.

It is worthy of noting that some ion channels may not have an inactivation process, in which case only the activation variable may be included in the formulation.

The Hodgkin-Huxley model also allows for incorporating both time and voltage dependence of the activation/inactivation variables. Here the formulations are briefly introduced. For a gating variable \( y \) representing the fraction of channels in the activated state, the corresponding fraction of channels that are not in the activated state must be \( (1-y) \). Since some of the activated gates are closed and vice versa, the transition of the two states can be illustrated the following form:
\[(1 - y) \xleftarrow{\alpha} \xrightarrow{\beta} y\]  \hspace{1cm} (2.24)

where \(\alpha\) and \(\beta\) correspond to the rate of one state transforms to the other. Therefore changing of \(y\) over time can be described using the following differential equation:

\[\frac{dy}{dt} = \alpha (1 - y) - \beta y\]  \hspace{1cm} (2.25)

\[\frac{dy}{dt} = \frac{y_\infty - y}{\tau_y}\]  \hspace{1cm} (2.26)

Then:

\[y_\infty = \frac{\alpha}{\alpha + \beta}\]  \hspace{1cm} (2.27)

\[\tau_y = \frac{1}{\alpha + \beta}\]  \hspace{1cm} (2.28)

### 2.7.4 Markov chain Models

The Markov chain model is an alternative method for describing the conductance \(g\) in Equation (2.22). Similar to Hodgkin-Huxley model, it also utilises probabilities to describe the actual conductance of the channel. The difference lies in that, instead of considering only the two gates (activation/inactivation), Markov chain model is capable of taking conformational changes of the ion channel protein into account by assuming that the transition from one state to another only depends on the current state. Hence the Markov chain model is considered to be able to describe the channel in a much more sophisticated and biophysically detailed manner. Therefore, Markov chain model is considered to be more accurate [126], although H-H model can be regarded as a simplification or subset of Markov chain model. The disadvantage of the Markov chain model is also apparent: it is much more challenging to develop, and the implementation consumes more computational power. Figure 2.14 illustrates an example of Markov chain model, which has been used for modelling \(I_{kr}\).
2.7.5 Modelling drug effects on ion channels

To model the drug-ion channel interactions in the a simulated application of pharmaceutical ion channel block, both the simple pore block model and the state-dependent block model have been proposed [127].

The simple pore block model assumes the affinity of the drug molecules binding to the ion channel receptors is time and voltage independent, i.e., in a simple pore block, the fractional block is only dependent on the concentration of the drug applied [127].

In contrast, in a state-dependent block scheme, the binding and unbinding kinetics of the drug to the ion channel complex are both considered to be time and voltage dependent [127]. Two models have been proposed to describe the state-dependent block. The modulated receptor theory assumes that the binding and unbinding can happen regardless of whether the channel resides in an open or closed state. On the contrary, the guarded receptor model believes that the drug-channel interaction kinetics are channel-conformation dependent. For example, in a guarded receptor model, a drug may not interact or disassociate with the channel if it is staying in an inactivated state.

2.7.6 Tissue modelling

The electrical excitation can propagate from myocytes to another, which is primarily achieved through the gap junctions (see 2.2.4).

A continuum approach for modelling the excitation propagation in tissue has been used in simulating the electrical activities of the heart in tissue, regardless of the fact the gap junctions and myocytes are intrinsically discreet. The bidomain model considers the extracellular and intracellular spaces as two separate and overlapping domains. The
transmembrane potential is found through finding the difference between the potential of these two spaces. Assuming the intracellular and extracellular conductivities are proportional, the bidomain model is then simplified to the monodomain model [128], which can be described as:

$$\frac{∂V_m}{∂t} = \nabla \cdot D(\nabla V_m) - \frac{I_{ion}}{C_m} \tag{2.29}$$

where $D$ is the diffusion tensor, $V_m$ is transmembrane potential, $I_{ion}$ is the total ionic current and $C_m$ is the membrane capacitance.

In modelling cardiac excitations in tissue, the no-flux boundary conditions are applied [128]:

$$\mathbf{n} \cdot D \nabla V_m = 0 \text{ on } \Gamma \tag{2.30}$$

where $\mathbf{n}$ is the vector outward normal to the physical boundary $\Gamma$.

It is well established that the excitation propagation in the myocardium is anisotropic [35,129]: the conduction is faster along the fibrous structure than transverse to it, which may be explained by the fact that the gap junctions are mainly found at the two ends of the cylindrical cell. In the local coordinate system the diffusion tensor (denoted as $\tilde{D}$) can be represented by a diagonal matrix:

$$\tilde{D} = \begin{bmatrix} d_f & 0 & 0 \\ 0 & d_x & 0 \\ 0 & 0 & d_n \end{bmatrix} \tag{2.31}$$

where $d_f$, $d_x$ and $d_n$ are the diffusion coefficients parallel to the fibre, transverse to the fibre, and across the sheet, respectively. $\tilde{D}$ has to be transformed into a global coordinate system when the cell is incorporated into the tissue model, which is accomplished by:

$$D = A \tilde{D} A^T \tag{2.32}$$

where $A$ is the matrix representing the local fibre orientations, and $A^T$ is the transpose matrix of $A$, which is represented by:

$$A = \begin{bmatrix} e_f & 0 & 0 \\ 0 & e_x & 0 \\ 0 & 0 & e_n \end{bmatrix} \tag{2.33}$$

where $e_f$, $e_x$ and $e_n$ are the vectors parallel to the fibre, transverse to the fibre or orthogonal to the sheet, respectively.
2.7.7 ECG simulation

The body surface potential represents the change in the surface potential that is generated in response to the electrical activity in the heart. Considering a torso with homogeneous conductivity ($\sigma$) with a negligible tissue capacitance, the body surface potential ($\Phi$) and the current density within the heart ($J^s$) can be linked through [130]:

$$\nabla^2 \Phi = \frac{\nabla \cdot J^s}{\sigma} \quad (2.34)$$

The process of simulating the body surface potential within known $J^s$ is termed as the forward problem of electrophysiology [130]. Using the body surface potential, ECGs can be derived by finding the potential at the specialised positions.

In a 1D or 2D simulations where a torso may not be available, a pseudo-ECG may be calculated. This is done through [131,132]:

$$\phi(x', y', z') = k \int [-\nabla V_m \cdot \left(\nabla \frac{1}{r} \right)] d\Omega \quad (2.35)$$

$$r = (x - x')^2 + (y - y')^2 + (z - z')^2 \quad (2.36)$$

where $\nabla V_m$ is the spatial gradient of the transmembrane potential and $r$ is the distance measured from a point source $(x', y', z')$ to the coordinate of an virtual electrode $(x', y', z')$; $\Omega$ indicates integrating across the domain of the tissue, $k$ is a constant accounting for the ratio between the extracellular and intracellular conductivities.

2.7.8 Numerical methods

This section mainly introduces the method used in the implementation of the models, including the numerical methods and the modelling environment. For the deterministic cardiac models, the governing mathematical equations are either ordinary differential equations (ODEs) or partial differential equations (PDEs). The numerical methods for solving these problems are introduced separately.

2.7.8.1 Numerical methods for solving ODEs

The single cell models are usually represented by a group of ODEs: all the state variables are only first-order differentiated over time. For non-stiff systems, the ODEs can be solved using several simple schemes including the forward Euler method, the Runge-Kutta method and the Rush-Larson method.
The forward Euler method is a first-order, one step scheme for solving ODEs. It is one of the most prevalent solvers in cardiac modelling. Consider an initial value problem:

\[
\frac{dy}{dt} = f(t, y)
\]

\[y(t_0) = y_0\]  (2.37)

Given a time step \(\Delta t\), the solution to the next time step is obtained:

\[y_{n+1} = y_n + \frac{dy_n}{dt} \Delta t\]  (2.39)

Apparently, the forward Euler method is very efficient in terms of implementation and computing. However, it necessitates the control of the time steps as large time step may incur stability concerns.

Runge-Kutta method is a multi-step ODE solver. The fourth-order Runge-Kutta scheme is given by following equations:

\[y_{n+1} = y_n + \frac{1}{6} (k_1 + 2k_2 + 2k_3 + k_4)\]  (2.40)

\[k_1 = h f(t_n, y_n)\]  (2.41)

\[k_2 = h f(t_n + \frac{1}{2} h, y_n + \frac{1}{2} k_1)\]  (2.42)

\[k_3 = h f(t_n + \frac{1}{2} h, y_n + \frac{1}{2} k_2)\]  (2.43)

\[k_4 = h f(t_n + h, y_n + k_3)\]  (2.44)

where \(h\) is the time step.

The fourth-order Runge-Kutta scheme is the weighted average of the \(k_1\)-\(k_4\). The fourth order Rung-Kutta is more computationally intensive than the Euler method but does provide a more accurate solution. Because of that, it is used in single cell models but not as common in models of high complexity.

Considering the nature of the Hodgkin-Huxley models, the Rush-Larsen scheme [133,134] has been introduced for solving the specific problems associated with the gating variables. Equation (2.25) can be solved using the Rush-Larsen scheme:
\[
    y(t + \Delta t) = y_\infty - [y_\infty - y(t)] \exp\left(-\frac{\Delta t}{\tau_y}\right)
\]  

(2.45)

This method is considered to be more accurate than the forward Euler, and thus it has been universally applied to solve the gating variables in the non-stiff cardiac systems.

For a system with stiff ODEs, the methods introduced above may not be favourable as these methods could necessitate extremely small integration steps. In this case, some robust, usually implicit schemes should be considered. There are many open source ODE solvers available to use, including the Intel ODE [135] and CVODE [136].

2.7.8.2 Numerical methods for solving PDEs

Tissue level modelling can be generalised use PDEs. In cardiac modelling, the excitation wave propagation described by Equation (2.29) is a good example. Also, the mechanics equations describing the beating heart are also PDEs. Currently, PDEs in cardiac modelling are being solved either using the finite difference method (FDM) or the finite element method (FEM).

FDM is a simple and commonly used method discretising the monodomain equations. In a 1D problem, for a given cell at location \(x\), time step \(\Delta t\) and spatial interval \(\Delta x\), the voltage in Equation (2.29) is solved using the FDM:

\[
    V_{x+\Delta t} = V_x^t + \Delta t \frac{D}{(\Delta x)^2}(V_{x-\Delta x}^t + V_{x+\Delta x}^t - 2V_x^t)
\]  

(2.46)

Here the forward Euler is used for the discretisation over time. The term \(\Delta t D / (\Delta x)^2\) is related to the stability of the solution, and must be smaller than 0.5 in order to achieve convergences.

Compared with FDM, FEM has certain advantages such as the natural boundary conditions, and the capabilities for solving problems with arbitrary geometries. The implementation of FEM is nontrivial. Similar to the stiff ODE solvers, there are many open source FEM libraries available online, such as deal.II [137], and FENICS [138].

2.7.9 Modelling environment

2.7.9.1 Programming languages

Due to the computationally demanding nature of the cardiac modelling, the C++/C language is widely used by many researchers in this field. MATLAB is also used by
some researchers, especially for developing single cell models. For example, the Grandi et al. atrial model [139] was implemented originally using MATLAB. Some other languages, such as Python, are used for special purposes including data analysis and model optimisation [140] and figure plotting [141].

2.7.9.2 Parallelisation

3D cardiac modelling necessitates large scale computing and can be implemented in parallel. The parallelisation can be achieved using the OpenMP [142], MPI [143] or CUDA [144].

OpenMP or open multi-processing is an API (application programming interface) that supports multi-platform parallelisation programming in C/C++ and Fortran. OpenMP is implemented by sharing a global memory, and may not be used on distributed computing clusters. OpenMP can be implemented using a general syntax consisting of pre-processor directives which can be placed within existing code, making it rather friendly for all level users.

MPI (Message Passing Interface) is a library enables passing messages between workstations via the network, which MPI makes it possible to run a large model using a large number of cores as seen in the high-performance computing centres.

CUDA is short for Compute Unified Device Architecture, which is a graphics processing unit (GPU) programming language released by NVIDIA®. It can be implemented as a general extension of the standard C and C++ programming. CUDA enables programs to execute on the GPUs, with even thousands of parallel cores, making it distinct from the former two parallelisation methods. Generally, CUDA allows for more speed-ups than the former two, given the same amount of cost in purchasing computers. However, CUDA is considered to be more programming consuming, and special care is needed on the optimisation of the performance.
Chapter 3  \textit{In silico} investigations of KCNA5 mutations-mediated pro-arrhythmic effects in the human atria

\textit{Addendum}  The work presented in this Chapter was based on a collaborative project between the author and Dr Michael A. Colman. Dr Colman provided the source code of single cell and 3D models from Colman et al. [35] and contributed to aspects of simulations, data analysis and interpretation of the results. Aspects of the work have been presented in a conference proceeding [145]:


The ultra-rapid delayed rectifier current, $I_{\text{Kur}}$, contributes to atrial repolarisation but is absent in ventricular myocytes. In a recent study with six mutations of KCNA5, three of which resulting in gain-of-function and the rest loss-of-function in $I_{\text{Kur}}$, were identified to be associated with lone-AF. However, the causative link between the mutant $I_{\text{Kur}}$ (either gain- or loss-of-function) and arrhythmogenesis, and the difference and similarity between the two mutant groups, has not been elucidated. In this Chapter, multiscale computational models of the atria were used to investigate the mechanism of arrhythmogenesis mediated by the two groups of mutations. It was shown that these two groups of mutants carrying $I_{\text{Kur}}$ produced multiple mechanisms of atrial arrhythmogenesis, with substantial differences between the two groups. The results suggest that the gain-of-function mutations shortened atrial action potential duration, and stabilised and accelerated re-entrant excitation waves in tissue, whereas the loss-of-function mutations promoted early-after-depolarisations following beta-adrenergic stimulation and thus wave breaks in tissue. This study provides new insights into
understanding the mechanisms by which mutant $I_{K_{ur}}$ contributes to atrial arrhythmias. In addition, as $I_{K_{ur}}$ is an atrial-specific channel and a number of $I_{K_{ur}}$-selective blockers have been developed as anti-AF agents, this study also helps to understand the mechanisms underlying some contradictory results on both pro- and anti-arrhythmic effects of blocking $I_{K_{ur}}$.

3.1 Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia in clinical settings [11,146]. Whilst AF can arise secondary to other heart conditions such as hypertension [147], and is generally associated with electrical and structural remodelling in the atria [148–150], it may also emerge in the absence of overt cardiovascular diseases [147,151] (referred to as ‘lone-AF’). The heritability of AF has been associated with genetic variations in the ion channels, transcription factors and a wide range of other genes [147,151,152]. Dissecting the role of the genetic variations in the arrhythmogenesis of the phenotypes in the atria is an essential component in understanding the mechanisms as well as developing treatment strategies of AF.

It is well received that the spontaneous ectopic activity, re-entry or an interplay between both, underlie the initiation and maintenance of AF [11,43,109,153]. AP duration (APD) is a critical factor determining the maintenance of re-entries. APD shortening is associated with a reduction of the effective refractory period (ERP), which may result in a decreased wavelength (defined as the product of ERP/APD and conduction velocity), a determinant of the likelihood of anatomic re-entry [11,153]. The ectopic activities can arise from an enhanced automaticity, and incidences of both early and delayed afterdepolarisations (EADs and DADs, respectively) [109]. Moreover, large regional gradients in the atrial APD can enhance tissue vulnerabilities to developing into self-perpetuating re-entrant excitation by promoting the susceptibility to wave-breaks and unidirectional conduction block [35,154,155].

$I_{K_{ur}}$ plays an important role in the repolarisation of the atrial AP in humans and other mammals [156,157]. Previous studies [27,149,156] have demonstrated that impaired activities of $I_{K_{ur}}$ are associated with proarrhythmic effects. In a recent study, Christophersen et al. [156] identified six novel variants in KCNA5 encoding the Kv1.5 channel carrying $I_{K_{ur}}$ in patients with early-onset lone-AF. Of the six mutations, three resulted in a gain-of-function (D322H, A305T, E48G) and three a loss-of-function
(P488S, Y155C, D469E) [156]. The causal link between the mutations and arrhythmogenesis of the phenotypes remains to be elucidated.

$\text{I}_{\text{Kur}}$ is increased following adrenergic stimulation [158], which plays a crucial role in counteracting the increased $\text{I}_{\text{CaL}}$ [159]. In previous experimental and modelling study in isolated cells [27,139], pharmaceutical block of $\text{I}_{\text{Kur}}$ accompanying adrenergic stimulation was associated with an increased incidence of EADs. It is likely that the loss-of-function mutations impairing the activity of $\text{I}_{\text{Kur}}$ promote the susceptibility of atrial myocytes to the induction of EADs. The presence of EADs is associated with triggered activity that can give rise to ectopic activity predisposing AF.

In this Chapter, the diversity in $\text{I}_{\text{Kur}}$ properties associated with the genetic variations of $\text{KCNA5}$ therefore was hypothesised to facilitate atrial arrhythmogenesis through different mechanisms: gain-of-function mutations abbreviate the APD, whereas the loss-of-function mutations promote the genesis of EADs. To assess the hypothesis, the experimental data of $\text{I}_{\text{Kur}}$ carried by the wild type (WT) and mutated $\text{Kv1.5}$ were integrated into a multi-scale computational model of the human atria to elucidate the functional impact of the six identified mutations on the atrial electrical activity at both the cellular and tissue scale.

Over the years a number of mathematical models of cardiac cells have been developed with different formulations for ionic currents, as well as intracellular calcium handling systems based on variant experimental datasets [35,37,139,160]. Consequently, these models produce variable electrophysiological properties which represent the substantial variations observed experimentally, including 3 distinct AP profiles in human atrial myocytes [161]. Investigating the effects of $\text{I}_{\text{Kur}}$ mutations using multiple models is necessary as these models take into account of biological variability, thus enabling the extrapolation of implications of the simulations towards model-independent conclusion. In this study, three different mathematical models of human atrial electrophysiology [35,37,139] were used.

### 3.2 Methods

#### 3.2.1 Development of a novel formulation of $\text{I}_{\text{Kur}}$

In modelling the kinetics of $\text{I}_{\text{Kur}}$, temperature correction was implemented to extrapolate the gating variables from room temperature to physiological conditions with a Q10
coefficient of 3 in accordance with a previous study [37]. The new formulation of $I_{Kur}$ is presented as follows.

$$K_{Q10} = 3.52 \quad (3.1)$$

Activation gate:

$$a_{inf} = \frac{1.0}{1.0 + \exp\left(\frac{V + 17.67}{-5.75}\right)} \times \frac{1.0}{1.0 + \exp\left(\frac{V + 8.45}{-11.51}\right)}$$

$$\tau_a = \left(\frac{45.67}{1.0 + \exp\left(\frac{V + 11.23}{11.53}\right)} + 4.27\right) \times \left(\frac{0.26}{1.0 + \exp\left(\frac{V + 35.87}{-3.88}\right)} + 0.29\right) \quad (3.3)$$

$$\tau_a = \frac{\tau_a}{K_{Q10}} \quad (3.4)$$

$$\frac{da}{dt} = \frac{a_{inf} - a}{\tau_a} \quad (3.5)$$

Inactivation gate:

$$i_{inf} = \frac{0.52}{1.0 + \exp\left(\frac{V - 15.11}{7.57}\right)} + 0.46$$

$$\tau_i = \frac{2328}{1.0 + \exp\left(\frac{V - 9.44}{3.58}\right)} + 1739.14 \quad (3.7)$$

$$\tau_i = \frac{\tau_i}{K_{Q10}} \quad (3.8)$$

$$\frac{di}{dt} = \frac{i_{inf} - i}{\tau_i} \quad (3.9)$$

$I_{Kur}$ current:

$$I_{Kur} = 0.64 \left(4.51 + \frac{1.90}{1.0 + \exp\left(\frac{V - 20.52}{-8.27}\right)}\right) a \cdot i(V - E_K) \quad (3.10)$$

The new model mediates an explicit dependence of channel conductance on the membrane voltage. The maximum channel conductance was tuned so that the ratio of maximum current densities of $I_o$ (the transient outward potassium current) and $I_{Kur}$ is...
within the range of experimental observations [162]. The new formulation of $I_{Kur}$ closely reproduces the kinetics of the current in experimental conditions (Figure 3.1). Note that the experimental voltage clamp traces for $I_{Kur}$ typically show two types of activation – fast and slow (Figure 3.1B) – for different individual isolated single cells. In the new $I_{Kur}$ model, the time constants of the activation gate were modelled by the weighted mean of the fast and slow kinetics observed in the raw data set from the study [156], representing the homogenising effects of electrical coupling in tissue.

Figure 3.1 Model representation describing $I_{Kur}$ and simulated voltage clamp at room temperature. A Steady-state activation and inactivation; B Time constants; C Current trace obtained from a simulated voltage clamp. Inserts: top right – voltage protocol; bottom right – experimental current traces; D I-V relationship. In the figure the simulation data were shown using lines, and experimental data represented by points. Experimental data were taken from Christophersen et al. [156]; specifically, in panel B the time constants were derived from current traces from [156] by fitting the action phase of the current trace to a mono-exponential equation.

To obtain models of the six $KCNA5$-mutant currents, parameters of channel conductance, steady-state activation and inactivation variables were fitted to individual
mutation data from Christophersen et al. [156], whereas the time constants were kept the same as the WT model. The resultant activation, inactivation gates and I-V relationship computed from simulated voltage clamp are presented in Figure 3.2. It was shown that the gain-of-function mutations was associated with left-ward shifts (3~5 mV) of the steady-state activation curve; D322H also presented a slightly steeper slope in the activation curve. The steady-state activations of the loss-of-function mutations were not markedly different from the WT. All mutations resulted in alterations to the steady-state availability of \( I_{\text{Kur}} \): the gain-of-function mutations and Y155C demonstrated increased availability at positive voltages by increasing non-inactivation fraction or shifting the curve to the right; D469E and P488S reduced the availability for voltages above -20 mV, primarily by reducing the non-inactivation fraction of the channel.

### 3.2.2 Single cell modelling

#### 3.2.2.1 Modelling effects of KCNA5 mutations in the cellular models

Multiple contemporary models of human atrial electrophysiology (Courtemanche et al. [37]; Grandi et al. [139]; and Colman et al. [35]) were used in this Chapter. These models exert variant human atrial action potential properties and morphologies. The novel formulation of \( I_{\text{Kur}} \) was incorporated into the Colman et al. model. In Courtemanche et al. and Grandi et al. models, effects of mutations on \( I_{\text{Kur}} \) were simulated by incorporating the relative changes in steady-state variables and conductance relative to the WT \( I_{\text{Kur}} \), which is summarised in Table 3.1.

#### 3.2.2.2 Families of regional cell models

Regional cell models, accounting for the intrinsic variation in APD and AP morphology of different regions within the atria [35,38,154,163], were derived for the Colman et al. and Courtemanche et al. models from previous modelling studies for the purpose of assessing the impact of KCNA5 mutations on the electrical heterogeneities in the atria. This was achieved by scaling the major ionic currents in the models. The scaling parameters (Table 3.2) were derived from Colman et al. [35], and were applied in simulations using Colman et al. and Courtemanche et al. cell models.
Figure 3.2 Modelling the electrophysiological properties of $I_{Kur}$ carried by the mutants at room temperature. A Steady-state activation; B Steady-state availability; C I-V relationship of $I_{Kur}$ elicited by voltage clamp step protocol for the (i) gain- and (ii) loss-of-function mutations, compared to the WT (black). In the plots points indicate the experimental data [156] and lines represent simulated results.
Table 3.1 Changes in parameters of steady-state variables and maximum conductance of \(I_{Kur}\) carried by the \(KCNA5\) mutants relative to WT. Steady-state activation and inactivation were described using the Boltzmann equation. (For activation: \(I/I_{\text{max}} = 1/[1 + \exp((V-V_{1/2})/k)]\), and inactivation \(I/I_{\text{max}} = (1-MA)/[1 + \exp((V-V_{1/2})/k) + MA]\). Parameters were fitted from the experimental data digitalised from [156].

<table>
<thead>
<tr>
<th></th>
<th>D332H</th>
<th>E48G</th>
<th>A305T</th>
<th>Y155C</th>
<th>D469E</th>
<th>P488S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{1/2})</td>
<td>- 3.26</td>
<td>- 3.02</td>
<td>- 4.07</td>
<td>- 0.89</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(K)</td>
<td>(\times 0.81)</td>
<td>(\times 0.96)</td>
<td>(\times 1.10)</td>
<td>(\times 0.75)</td>
<td>(\times 1)</td>
<td>(\times 0.83)</td>
</tr>
<tr>
<td><strong>Inactivation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{1/2})</td>
<td>+ 9.62</td>
<td>+ 4.03</td>
<td>+ 6.03</td>
<td>+ 5.01</td>
<td>+ 4.55</td>
<td>- 1.41</td>
</tr>
<tr>
<td>(K)</td>
<td>(\times 0.80)</td>
<td>(\times 0.94)</td>
<td>(\times 0.74)</td>
<td>(\times 0.78)</td>
<td>(\times 0.84)</td>
<td>(\times 0.68)</td>
</tr>
<tr>
<td><strong>Minimum Availability (MA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ 0.07</td>
<td>+ 0.04</td>
<td>+ 0.07</td>
<td>+ 0.04</td>
<td>- 0.09</td>
<td>- 0.11</td>
</tr>
<tr>
<td><strong>Maximum Conductance</strong></td>
<td>(\times 1.80)</td>
<td>(\times 1.32)</td>
<td>(\times 1.44)</td>
<td>(\times 0.47)</td>
<td>(\times 0.55)</td>
<td>(\times 0.04)</td>
</tr>
</tbody>
</table>

Table 3.2 Scaling factors for each current of various regions relative to the baseline (right atrium, RA) cell models. The abbreviations: CT - crista terminalis, BB - Bachmann’s bundle, PM - pectinate muscles, AVR - atrio-ventricular ring, RAA - right atrial appendage, AS - atrial septum, LA - left atrium, LAA - left atrial appendage, PV - pulmonary veins.

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>BB</th>
<th>PM</th>
<th>AVR</th>
<th>RAA</th>
<th>AS</th>
<th>LA</th>
<th>LAA</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_{CaL})</td>
<td>1.68</td>
<td>1.72</td>
<td>0.94</td>
<td>0.67</td>
<td>1</td>
<td>0.4</td>
<td>1</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>(I_{so})</td>
<td>1.35</td>
<td>1.35</td>
<td>1.0</td>
<td>0.6</td>
<td>0.53</td>
<td>0.21</td>
<td>1</td>
<td>0.53</td>
<td>0.75</td>
</tr>
<tr>
<td>(I_{Kur})</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td>1</td>
<td>0.67</td>
<td>1</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td>(I_{Na})</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td>1</td>
<td>1.3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(I_{Kr})</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.63</td>
<td>1</td>
<td>1</td>
<td>1.6</td>
<td>1.6</td>
<td>2.4</td>
</tr>
<tr>
<td>(I_{K1})</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td>1</td>
<td>(V_{1/2}) -6</td>
<td>1</td>
<td>1</td>
<td>0.62</td>
</tr>
</tbody>
</table>

For the Grandi et al. model, the scaling factors for right atrium (RA) and pectinate muscles (PM) were kept the same as those in Table 3.2, while the RA-LA difference native to the model was used here. For the CT cell model, the scaling to the
conductance of $I_{CaL}$ (Table 3.2) in the CT was changed from 1.68 to a factor of 1.2 to avoid the generation of EADs in normal conditions, which was observed at the full 1.68 scaling of $I_{CaL}$ (Table 3.2). The APs produced using the regional cell models are shown in Figure 3.3.

3.2.2.3 Modelling effects of β-adrenergic stimulations

To study the impact of the mutations on EAD production, the effect of isoprotenerol (ISO, a β-agonist reproducing sympathetic activity) was incorporated into the Grandi et al. model according to their original study [139] and into the Courtemanche et al. and Colman et al. models based on the same parameter modifications.

3.2.2.4 Modelling the effects of chronic AF-induced electrical remodelling

In order to provide insights into the functional impacts of these mutations on the atria in the presence of chronic AF-induced remodelling (cAF), models of the $KCNA5$ mutants were also combined with models of cAF effects (as used in previous studies [35,139]). The cAF models involve modifications to the conductance and kinetics of multiple ion channels, as illustrated in Table 3.3.

3.2.3 Tissue modelling

3.2.3.1 Mathematical model of electrical excitation wave propagation

In accordance with previous studies [35,154], the well-known monodomain equation [35,128,154] was used to describe the electrical excitation propagation in the tissue. Details to the equation are given in Chapter 2 (Equation (2.29)).

3.2.3.2 1D strand model

Simplified tissue models were developed in which a string of single cells are coupled into a 1D strand. Specifically, the RA models from the Colman et al. and Courtemanche et al. models were used to obtain the conduction velocity restitutions. A 1D strand model consisting of CT and PM cells based on the Grandi et al. model was also constructed for the purpose of studying the effects of the application of β-adrenergic in the tissue level. Half of the model is the CT and half the PM, representing this region of the right atrium. The present study did not attempt to use the Grandi et al. model to assess effects of the mutations on conduction velocity restitution due to the failure in
conduction for high pacing rates under normal conditions as revealed in a previous study [160]. The diffusion coefficient was set to give a conduction velocity close to the experimentally observed values: along the fibre direction of 1.3 m/s for CT strand, and around 0.75 m/s for RA strand [35].

Figure 3.3 Simulated regional cell APs using (A) the Colman et al. (B) Courtemanche et al. and (C) the Grandi et al. cell models. The cells were paced at 1Hz and the APs evoked by the 100th stimulation were plotted here. Abbreviations are the same as those in the legend for Table 3.2.
Table 3.3 Modifications to the model parameters in simulating the cAF conditions versus SR conditions for the three models; parameters are taken from [35,139].

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Colman et al. model</th>
<th>Courtemanche et al. model</th>
<th>Grandi et al. model</th>
</tr>
</thead>
<tbody>
<tr>
<td>I\textsubscript{Na}</td>
<td>No change</td>
<td></td>
<td>-10%</td>
</tr>
<tr>
<td>I\textsubscript{CaL}</td>
<td>-70%</td>
<td></td>
<td>-50%</td>
</tr>
<tr>
<td>I\textsubscript{Kur}</td>
<td>-50%</td>
<td></td>
<td>-55%</td>
</tr>
<tr>
<td>I\textsubscript{to}</td>
<td>-65%</td>
<td></td>
<td>-80%</td>
</tr>
<tr>
<td>I\textsubscript{K1}</td>
<td>+100%</td>
<td></td>
<td>+100%</td>
</tr>
<tr>
<td>I\textsubscript{Ks}</td>
<td>+100%</td>
<td></td>
<td>+100%</td>
</tr>
<tr>
<td>I\textsubscript{NaCa}</td>
<td>+55%</td>
<td></td>
<td>+40%</td>
</tr>
<tr>
<td>I\textsubscript{Kr}</td>
<td>No change</td>
<td></td>
<td>No change</td>
</tr>
<tr>
<td>SERCA</td>
<td>+50%</td>
<td></td>
<td>Reduced maximum pump rate</td>
</tr>
<tr>
<td>RyR</td>
<td>+300%</td>
<td></td>
<td>Ca\textsuperscript{2+} sensitivity increased by 2-fold</td>
</tr>
<tr>
<td>SR Ca\textsuperscript{2+} leak</td>
<td>+25%</td>
<td></td>
<td>+25%</td>
</tr>
</tbody>
</table>

3.2.3.3 3D anatomical human atrial model

The previously developed 3D virtual human atria (Figure 3.4) from this research group [35,154,164] was applied to evaluate the functional impact of the mutations on electrical wave behaviour in the organ. The anatomical atrial geometry was derived from the visible human dataset [165] and segmented into the major differentiated anatomical regions [165]. Details of the model have been previously published [35,154].

3.2.3.4 3D Modelling of atrial vulnerability to unidirectional conduction block

It is well established that a period of vulnerability in coupled stimuli exists whereby the second electrical stimulation can initiate self-perpetuating spiral waves through induction of unidirectional conduction block [166]. The PM ridge in the RA could facilitate excitation wave break and contribute to initiation and maintenance of re-entry [167]. The APD of CT cells has been demonstrated to be markedly larger compared with PM cells. The APD gradient in the CT/PM may result in a markedly larger vulnerability window than that of homogeneous tissue [35]. Therefore, the CT/PM junction could form a substrate for initiation of wave breaks.
Therefore, the atrial vulnerability to unidirectional conduction block was quantified by using an S1S2 pacing protocol applied to the heterogeneous junction [35,154,168] between the CT and PM within a 3D atrial wedge containing RA, CT and PM that are close to the SAN region (Figure 3.5). A train of 7 S1 stimuli (S1 = 500ms) was applied to a spherical tissue region of 3.3 mm in radius, which was followed by a further stimulus at variant intervals (S2) applied to the same site. A similar protocol has been used in previous studies [35]. The temporal vulnerability window was defined as the temporal range of S2 giving rise to wave break in either direction (propagation exclusively though CT or PM cells) but not both.

Figure 3.4 Anatomical structure of the 3D atrial model. (A) Segmented anatomical reconstruction of the human atria; the major regions were labelled. (B) Stream traces representing fibre orientations in the CT, PM and BB regions. The abbreviations of anatomical regions were defined in Table 3.2. A more detailed description of the geometry can be found in [35,154].
Figure 3.5 A human atrial wedge containing CT and PM used to quantify the temporal vulnerability window to wave breaks. Three different views are given: left – inside-out view showing endocardium of RA, CT and PM; the arrows indicate the positions of SVC and IVC, respectively; middle – transmural view of epicardial to endocardial RA; right – epicardial RA. S1-S2 were applied to the junction of CT/PM, which is marked with red dot in the left panel of the figure; the labels are: SVC/IVC – superior/inferior vena cava, RA – right atrium (in brown), PM – pectinate muscle (in green), CT – crista terminalis (in blue), RAA – right atrial appendage (in red).

3.2.3.5 Simulating spiral waves in the human atria

To study the effect of the mutation induced alterations to parameters of AP on re-entrant wave dynamics, a phase-distribution method [169,170] was implemented in order to initiate a re-entrant excitation in the atria. Though this initiation method does not account for the initiation of re-entry, it allows for the investigation of the long term dynamics of re-entry.

The phase distribution method [169,170] employs an asymmetric phase map to create desirable distribution of all state variables of the cellular models. This was achieved by (a) exporting the state variables from the resultant AP of the final stimulus applied to a single cell at discrete intervals across the first 400 ms following current stimuli, creating 205 individual state variable vectors (Figure 3.6A); (b) mapping these
state variable vectors into the nodes of 3D model according to the phase values of the
nodes in the 3D model (Figure 3.6B).

Figure 3.6 Illustration of the phase distribution method to initiate re-entrant waves in the
3D human atrial model. (A) The distributed phase map used on the anatomical model
(i), with numbered indications of the phases of the AP (ii) which is mapped onto each
location. (B) The asymmetric distribution of initial conditions facilitated spontaneously
developed sustained re-entries.

Note that in simulations the diffusion coefficient ($D$, diffusion tensor in the
monodomain equation) in the model, functional describing the intercellular electrical
coupling, was varied between 100% (control, lone AF) and 40% (severe remodelling) to
simulate possible remodelling in the connexin and atrial structures (e.g. 30% increase in
the atrial volume) associated with AF [171–173], which resulted in up to a 40% reduction in the conduction velocity as seen in previous studies [172,174,175].

In the simulations with the Colman et al. model, the diffusion coefficient was reduced to 60% of the control values to sustain re-entrant excitations. For the Courtemanche et al. model, it was necessary to use a smaller value (40%) to sustain a reasonable life-span of re-entrant waves and thus facilitating comparisons between the mutations and WT.

For each mutation and WT with Colman et al. and Courtemanche et al. models, a time period of 5000 ms was simulated and recorded for analysis. The dominant frequencies of sustained re-entrant excitation (where re-entry is sustained) were then computed through Fourier analysis of the electrical activity in multiple single cells throughout the tissue [35].

3.3 Results

3.3.1 Effects of KCNA5 mutations on single cell electrophysiology

3.3.1.1 Gain-of-function mutations

To reveal the effects of gain-of-function KCNA5 mutations on human atrial electrophysiology, atrial single cell models were paced at 1 Hz, and the AP, calcium transient (CaT) and time courses of ionic currents are shown in Figure 3.7. The APD and plateau potential were quantified and are illustrated in Figure 3.8. It was demonstrated that the mutations markedly altered the profile of the human atrial AP in all three models.

Under the WT conditions, the Colman et al. and Courtemanche et al. models produced baseline APs exhibiting a spike-and-dome morphology and pronounced phase 2 plateau that is similar to the experimentally recorded type-2 and type-1 APs in human atrial myocytes [161]. The Grandi et al. model elicited APs that are triangular in shape, which is similar to the type-3 human atrial AP observed in [148,161].

In comparison to the WT, the gain-of-function mutations abbreviated atrial APD and hyperpolarised the AP plateau towards negative voltages (Figure 3.7 A and Figure 3.8 A-B). With the mutations, the activities of \( I_{Kur} \) during phase 1 repolarisation of AP were markedly augmented, presenting substantially increased peak current (Figure 3.7 C), which contributed to accelerated phase-1 repolarisation of AP and thus a less
prominent plateau and decreased plateau potential. Due to the change in the AP profile, \( I_{\text{Kur}} \) was reduced during late phase 2 and 3 of the AP, though in the presence of the gain-of-function mutations. Current densities of \( I_{\text{Cal}} \) during phases 2 and 3 of the AP were also reduced by the secondary effect of the changed AP profile caused by the mutations as compared with the WT, although the peaks of \( I_{\text{Cal}} \) were slightly increased following upstroke of the AP current (Figure 3.7 D). The simulated calcium transient elicited by the mutant cell models demonstrated a pronounced reduction in systolic calcium concentrations compared with WT; the diastolic calcium levels were also decreased, but to a much lesser extent. Collectively, the amplitudes of CaT in the mutant myocytes were reduced, which can be attributed to the decreased \( I_{\text{Cal}} \) (thus a smaller calcium flux from the sarcoplasmic reticulum (SR) through less active ryanodine receptors (RyR)). The reduced \( I_{\text{Cal}} \) during phase 2 and 3 also led to a more repolarised AP; the resultant APDs measured at 30% repolarisation (APD\(_{30}\)) were markedly reduced in the mutant cells vs. WT (Figure 3.8 B). The more negative AP at phase 2 and 3 of AP rendered less activation and smaller slow delayed rectifier potassium currents (\( I_{\text{Kr}} \) and \( I_{\text{Ks}} \), Figure 3.7 E and F), contributed to the slower rate of phase-3 repolarisation. The resting membrane potential and overshooting potential of AP were not substantially affected by the mutations.

The alterations to atrial APD\(_{90}\) by the individual mutations were slightly different in the models (Figure 3.8 A). In the Courtemanche et al. model, D322H, the mutant exhibiting most substantial increase in the channel conductance of \( I_{\text{Kur}} \), resulted in the most pronounced reduction in APD\(_{90}\) of human atrial myocytes (245 ms for D322H vs. 296 ms for WT, 17.3% reduction); The mutant E48G and A305T shortened APD\(_{90}\) by a smaller amount (21 ms by E48G, 25 ms by A305T). In both the Colman et al. and the Grandi et al. models, A305T induced the most marked reduction in APD\(_{90}\), whereas the mutants E48G and D322H produced a similar amount of abbreviation.

Nevertheless, the alterations to APD\(_{30}\) and plateau potentials of the human atrial myocytes by the mutations were consistently demonstrated in the three models, and are closely linked to the relative increase in the \( I_{\text{Kur}} \) conductance by the mutations. In all three models, D322H induced the most substantial shortening to atrial APD\(_{30}\) and plateau potential, whereas the mutant E48G mediating the least increase in \( I_{\text{Kur}} \) current density, resulting in smaller changes to the profile of atrial AP (Figure 3.8 B and C). These effects can be attributed to the important role \( I_{\text{Kur}} \) plays in phase 1 and phase 2 of human atrial AP, during which the ADP\(_{30}\) and plateau potential are measured.
Collectively, the gain-of-function mutations abbreviated APD, decreased plateau potential and reduced CaT in the human atrial myocytes.

Figure 3.7 Effects of the gain-of-function KCNA5 mutations on human atrial AP and the corresponding time courses of current traces elicited by Colman et al., Courtemanche et al. and Grandi et al. models. (A) Human atrial AP with $I_{Kur}$ carried by WT/mutant Kv1.5. (B) Time courses of calcium transient (CaT) elicited by the AP. (C - F) Time courses of $I_{Kur}$, $I_{CaL}$, $I_{Kr}$, $I_{Ks}$ during AP. Results related to the three models being used are indicated at the top of each column.
3.3.1.2 Loss-of-function mutations

In order to characterise the functional effects of loss-of-function KCNA5 mutations on human atrial APs, the descriptions of the loss-of-function mutations (Table 3.1) were incorporated into the three models of atrial electrophysiology. Figure 3.9 shows the resultant human atrial APs, CaT and corresponding time courses of ion channels during the AP. The APD and plateau potentials of the AP from simulated mutant cells were quantified and are shown in Figure 3.10.

The time courses of several major ionic currents elicited by atrial APs were substantially affected by the mutations. In comparison to WT conditions, the mutations
markedly reduced the activities of $I_{Kur}$ throughout phase 2 and 3 of the AP (Figure 3.9 C), leading to markedly slowing down in phase 1 repolarisation (Figure 3.9 A). The decrease in $I_{Kur}$ during phase 1 of the AP contributed to elevations in plateau potential and APD$_{30}$ (Figure 3.9 A, Figure 3.10 B and C). $I_{CaL}$ during phase 3 of the AP was also increased in mutant cells vs. WT, though the peaks were reduced during phase 1 of the AP. Furthermore, the two slow delayed rectifier currents were markedly increased under the mutations conditions vs. WT (Figure 3.9 E and F). For simulations with P488S, the mutation almost abolishing $I_{Kur}$, the current densities of $I_{Kr}$ and $I_{Ks}$ were nearly doubled. The increased $I_{Ks}$ and $I_{Kr}$ contributed to an accelerated rate of the phase 3 repolarisation of human atrial AP (Figure 3.9 A). The systolic intracellular calcium concentrations were increased under the mutant conditions vs. WT; the diastolic calcium content was also elevated (Figure 3.9 B). Collectively, the mutations induced augmentations to the CaT amplitude in the human atrial myocytes. These effects were consistently demonstrated across the three models, and closely associated with the relative changes in the conductance of $I_{Kur}$ by the mutations.

The models did produce different changes to APD$_{90}$ of human atrial AP (Figure 3.10 A). In the Courtemanche et al. model, the increased $I_{CaL}$ during phase 3 repolarisation of AP were counterbalanced by augmented $I_{Ks}$ and $I_{Kr}$, resulting in shortening of APD$_{90}$ in the mutant cells vs. WT. In both the Colman et al. and Grandi et al. models, the increases in the slow delayed rectifier currents were not able to compensate increased inward currents, leading to a marked increase in APD$_{90}$ by the mutations vs. WT.

### 3.3.1.3 Alterations to action potential duration restitution

To investigate how the mutations affect APD restitution properties of human atrial myocytes, the S1-S2 protocol was applied to simulate the response of myocytes to various S2 stimulation delayed ranging from 250 to 1000 ms following 100 S1 stimuli paced at 1Hz. The results are shown in Figure 3.11. The maximum slopes of APD restitution curves for WT and mutant conditions are quantified in Table 3.4.
Figure 3.9 Effects of the loss-of-function KCNA5 mutations on the human atrial AP and the corresponding time courses of current traces elicited by Colman et al., Courtemanche et al. and Grandi et al. models. (A) Human atrial AP with $I_{Kur}$ carried by WT/mutant Kv1.5. (B) Time courses of calcium transient (CaT) elicited by the AP. (C - F) Time courses of $I_{Kur}$, $I_{Cal}$, $I_{Kr}$, $I_{Ks}$ during AP. The three models being used are indicated at the top of each column.
Figure 3.10 Effects of the loss-of-function KCNA5 mutations on APD and plateau potential of atrial myocytes. (A) APD measured at 90% repolarisation (APD_{90}). (B) APD measured at 30% repolarisation. (C) Plateau potential, measured as the mean of membrane potentials between 10 ms and 50 ms following application of a current stimulus. The three models being used are indicated at the top of each column.

In comparison with the WT conditions, the gain-of-function mutations flattened the APD restitution curve, showing reduced rate-dependent adaptations of myocytes (the rate-dependent adaptations were quantified by the change in APD over the whole range of S2 applied). The maximum slopes of APD restitution curves were in general reduced (Table 3.4). The flattening of APD restitution curves due to the mutations was similar to the experimental observations on atrial myocytes isolated from chronic AF patients [148,176]. The three models consistently exhibited reduced APD for S2 larger than 400 ms for these mutations vs. WT. In both the Colman et al. and Courtemanche et al. models, the APD restitution curves computed from mutant cell models show an increased ability of the mutant atrial cells to support higher pacing rate of electrical
excitations. The simulations with Courtemanche et al. models also demonstrated left-shift in the APD restitution curve by the gain-of-function mutations.

The loss-of-function mutations enhanced the rate-dependent adaptation of APD as well as steepened the slopes of APD restitution curve in the human atrial myocytes in the simulations using the Colman et al. model compared with WT conditions (Figure 3.11, Table 3.4). The APD restitution curves were also right-shifted by the mutations compared with WT using the same model. In the Courtemanche et al. model, the maximal APD restitution slope was steepened for Y155C, unchanged for D469E and reduced for P488S; the overall rate-dependent adaptations of APD (defined as the maximal variation of APD within the range studied) were enhanced by the mutations vs. WT. The mutations did not shift the APD restitution curve in the simulations using the Courtemanche et al. model, primarily due to the slight abbreviations to APD by the mutations vs. WT produced by the model.

Specifically, for the Grandi et al. model, although APD restitution curves were obtained, the model exhibited very small upstroke velocity of AP for S2 lower than 400 ms (typically less than 20 V/s, which seems unphysiological; see discussion in 3.4.6), which is consistent with a previous benchmark study on human atrial models [160]. Nevertheless, the simulated behaviours of the mutations using the Grandi et al. model were comparable to those obtained from simulations with the Colman et al. model.

Table 3.4 Maximum slopes of simulated APD restitution curves for WT and mutant human atrial myocytes.

<table>
<thead>
<tr>
<th></th>
<th>Colman et al. model</th>
<th>Courtemanche et al. model</th>
<th>Grandi et al. model</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.696</td>
<td>2.686</td>
<td>0.805</td>
</tr>
<tr>
<td>D322H</td>
<td>0.376</td>
<td>1.924</td>
<td>0.843</td>
</tr>
<tr>
<td>E48G</td>
<td>0.474</td>
<td>2.107</td>
<td>0.83</td>
</tr>
<tr>
<td>A305T</td>
<td>0.380</td>
<td>2.290</td>
<td>0.755</td>
</tr>
<tr>
<td>Y155C</td>
<td>0.932</td>
<td>2.887</td>
<td>0.828</td>
</tr>
<tr>
<td>D469E</td>
<td>0.894</td>
<td>2.680</td>
<td>0.803</td>
</tr>
<tr>
<td>P488S</td>
<td>1.096</td>
<td>2.340</td>
<td>0.838</td>
</tr>
</tbody>
</table>
Figure 3.11 Simulated effects of the KCNA5 mutations on APD restitution of human atrial myocytes elicited by the three electrophysiological models. (A-C) are results obtained using Colman et al. model, Courtemanche et al. model and Grandi et al. model, respectively. Effects of the gain-of-function group of mutations are shown in the left panels, and the loss-o-function group in the right.

### 3.3.1.4 Effects of the mutation-induced changes in $I_{Kur}$ channel properties (i.e. parameters) on action potential duration

The KCNA5 mutations exhibited alterations to channel properties (i.e. the maximal channel conductance and the steady-state activation of $I_{Kur}$). Moreover, in fitting the kinetics of experimental $I_{Kur}$ current traces elicited by voltage clamp, both fast and slow
activation kinetics were observed among the different data-sets. Here, the impact of these changes in channel properties (reflected by altered parameters of \( I_{Kur} \) channel kinetics) on human atrial AP was studied by varying the conductance and activation time constants over parameter spaces using the *Colman et al.* model: the conductance of \( I_{Kur} \) was varied from full inhibition to 2-fold increase vs. the control value, and the activation time constant was changed in a range of 0.5-fold reduction to 2.5-fold increase vs. the control value. The effects of steady-state activation of \( I_{Kur} \) were also considered by shifting the half-activation value up to \( \pm 5 \) mV. The simulated APD of the cells with respect to the parameter spaces are shown in Figure 3.12. It was demonstrated that increased time-constant (slowing down of activation and deactivation) generally lead to shortened \( APD_{90} \) (Figure 3.12 Ai-v), whereas the varying conductance rendered more complex behaviour. For varying the steady-state activation \( V_{1/2} \) alone, a negative shift to the \( V_{1/2} \) resulted in a reduction in atrial \( APD_{90} \), whereas a positive shift to the \( V_{1/2} \) prolonged APD (Figure 3.12 B). For models with positively shifted \( V_{1/2} \) of \( I_{Kur} \) activation, \( APD_{90} \) was prolonged with either an increase or decrease in the channel conductance of \( I_{Kur} \) vs. control. Similar effects were observed with negatively shifted \( V_{1/2} \) of \( I_{Kur} \) activation in the presence of a reduced time-constant. In the conditions of negatively shifted \( V_{1/2} \) of \( I_{Kur} \) activation and slow activation and deactivation kinetics, the increased \( I_{Kur} \) conductance led to a monophasic decrease in the atrial \( APD_{90} \) (Figure 3.12 Ai). Furthermore, the dependence of \( APD_{90} \) in conductance and activation kinetics of \( I_{Kur} \) was more pronounced with negatively shifted \( V_{1/2} \).

Similarly, the dependence of atrial \( APD_{30} \) on these parameters of \( I_{Kur} \) was also quantified (Figure A.1 of Appendix A). While varying \( V_{1/2} \) and activation time constants demonstrated similar effects on \( APD_{90} \) and \( APD_{30} \), the atrial \( APD_{30} \) was consistently reduced following an increase in the conductance of \( I_{Kur} \). These results are in agreement with simulated effects of the mutations D322H and A305T on APD presented in Figure 3.7 and Figure 3.8.
Figure 3.12 Analysis of the relationship between the APD$_{90}$ of human atrial myocytes and the parameters related to the kinetics of I$_{Kur}$. In (A), activation time constants were scaled by 0.5 to 2.5 vs. control values; the conductance of I$_{Kur}$ was varied from 0 to 2-fold increase vs. control. (i-v) The $V_{1/2}$ of steady-state activation was shifted by (i) -5 mV, (ii) -2.5 mV, (iii) 0 mV (iv) 2.5 mV and (v) 5 mV, respectively. The APD$_{90}$ of AP elicited from the control time constant and conductance was marked with a red triangle in each panel. (B) The relationship between APD$_{90}$ and the $V_{1/2}$ of steady-state activation.
3.3.1.5 Effects of the KCNA5 mutations in the presence of cAF-remodelling

The functional impacts of the KCNA5 mutations on atrial electrophysiology were further evaluated in the presence of cAF induced electrical remodelling (excluding the electrical remodelling to \( I_{Kur} \)) to the atrial myocytes, to reveal the behaviours of AF-myocytes in response to mutated \( I_{Kur} \) currents. The results are shown in Figure 3.13.

The functional impact of the mutations on single-cell AP morphology in cAF conditions was consistent between cell models. Compared with WT-cAF cells, gain-of-function mutations in \( I_{Kur} \) further shortened the APD and flattened the APD restitution curve (Figure 3.13 A-C), indicating the role of these mutations in arrhythmogenesis. The loss-of-function mutations, on the other hand, prolonged the atrial APD and enhanced rate-dependent adaptation of APD vs. WT cAF conditions (Figure 3.13 D-F).

![Figure 3.13](image-url)

Figure 3.13 Effects of KCNA5 mutations on AP and APD restitution in the presence of cAF remodelling in the three cell models. (Ai-ii, Di-ii) Simulated AP and APD restitution from steady-state pacing using the Colman et al. model. (Bi-ii, Ei-ii) Simulated AP and APD restitution from steady-state pacing using the Courtemanche et al. model. (Ci-ii, Fi-ii) Simulated AP and APD restitution from steady-state pacing using the Grandi et al. model.
3.3.2 Effects of KCNA5 mutations on atrial conduction velocity

The functional impacts of the mutated $I_{Kur}$ on atrial excitation wave conduction were assessed in a 1D model of a human atrial strand. The S1-S2 protocol was implemented. Results for gain-of-function and loss-of-function mutations are shown in Figure 3.14 and Figure 3.15, respectively.

The effects of mutations on APD measured in tissue are in accordance with previous single cell simulations: gain-of-function mutations reduced atrial APD for $S2 > 400$ ms, induced left-shift to the APD restitution curve as well as weakened rate-dependent adaptation (Figure 3.14 A). The CV was not affected by the gain-of-function mutations for $S2 > 500$ ms, while the CV restitution curve was left shifted (Figure 3.14 B). Collectively, the gain-of-function mutations resulted in decreased tissue excitation wavelength for $S2 > 400$ms; the wavelength vs. $S2$ relationship was also left-shifted (Figure 3.14 C). The tissue with gain-of-function mutations is associated with a smaller $S1-S2$ interval allowing excitation conduction as compared with the WT conditions, indicating a reduced effective refractory period of the tissue by the mutations. These effects were more pronounced in simulations with the Courtemanche et al. model than those with other models.

Similarly, the effects of loss-of-function mutations of atrial APD measured in tissue are consistent with single cell simulations (Figure 3.15). In the Colman et al. model, the loss-of-function in $I_{Kur}$ resulted in an increased APD for $S2 > 420$ ms, and reduction in APD for APs evoked from smaller $S1-S2$ intervals; the APD restitution curve was shifted to the right while the overall rate-adaptation was enhanced. The CV was not affected by the mutations at greater $S2$ interval while the CV restitution curve was right shifted. Consequently, the tissue excitation wavelength was increased at greater $S2$ intervals, while its restitution curve was shifted to the right. The minimum $S2$ interval inducing atrial capture was increased by the mutations vs. WT, demonstrating anti-arrhythmic effects.

In simulations using the Courtemanche et al. model, the mutations increased the rate-adaptation of atrial AP vs. WT. Whereas the APD of APs evoked by $S2>500$ ms was in general moderately increased (decreased in P488S for $S2>700$ms) vs. WT, small $S1-S2$ intervals produced marked reduction in APD. The CV restitution curve was not markedly affected by the mutations, with a slight increase in the CV in response to $S2>500$ms for the mutations vs. WT. Collectively, the tissue excitation wavelength was
moderately increased by the mutations compared with WT at S2>500 ms, and markedly reduced for smaller S2 interval. The minimum S2 interval inducing atrial capture was not affected by the mutations.

Figure 3.14 Simulated effects of gain-of-function KCNA5 mutations on APD, conduction velocity (CV) and excitation wavelength in a 1D strand model. (A) APD$_{90}$ measured in tissue; to avoid boundary effects, an atrial cell close to the middle and far away from the stimulation site and both ends of the strand was selected to quantify in tissue APD. (B) Atrial conduction velocity, computed from the time delay between APs of two cells reaching -20mV and the distance between the two cells. (C) Atrial excitation wavelength plotted against S1-S2 interval. Wavelength was computed as the product of APD and CV [177]. The models used are indicated on top of the panels.
Figure 3.15 Simulated effects of loss-of-function KCNA5 mutations on APD, conduction velocity (CV) and excitation wavelength in a 1D model. (A) APD$_{90}$ measured in tissue. To avoid boundary effects, an atrial cell close to the middle and far away from the stimulation site and both ends of the strand was selected to quantify in tissue APD. (B) Atrial conduction velocity, computed from the time delay between APs of two cells reaching -20 mV and the distance between the two cells. (C) Atrial excitation wavelength plotted against S1-S2 interval. Wavelength was computed as the product of APD and CV [177].
3.3.3 Effects of KCNA5 mutations on atrial APD heterogeneity

The atria are intrinsically heterogeneous in cellular electrophysiological properties and characterised by a large degree of dispersion in APDs of cells from different regions [35,154,165,178–180], including a large APD difference in cells in CT and PM regions [35,178]. The electrical heterogeneity is believed to be an important determinant in arrhythmogenesis [180]. Previous modelling studies have demonstrated the role of atrial regional APD dispersion in the initiation and development of arrhythmic excitations [35,163].

Another important factor in determining atrial vulnerability of arrhythmogenesis is the repolarisation time dispersion across the whole atria [181]. Previous studies have shown that increased atrial repolarisation dispersion is associated with paroxysmal AF [182,183] and aging [184], and may contribute to the development [182] and maintenance of AF [184]. However, it is unclear in the condition of the identified KCNA5 mutation condition, how atrial heterogeneity is altered, which may contribute to arrhythmogenesis. Here the heterogeneous and anisotropic model of the human atria [35] was used to evaluate the effect of the mutated $I_{Kur}$ current on atrial APD and repolarisation dispersions. In simulations, the Colman et al. models of regional atrial cells were used to represent the regional differences in electrophysiology.

Figure 3.16 shows the simulated atrial activation sequence, heterogeneous APD distribution and dispersion in atrial repolarisation under WT conditions. The atria were paced at the sinoatrial node (SAN) region with a pacing cycle length of 1000 ms (i.e., mimicking the normal heart rate) and reached steady-state before the results were recorded for analysis (Figure 3.16 A). The whole atria were activated within 126 ms following application of a current stimulus (Figure 3.16 A), in agreement with previous experimental observations [185]. The AS and PV were the regions first to recover from activation; the global repolarisation of atria spanned from 224 ms to 320 ms (Figure 3.16 B). The in-tissue APD was shown to be greater in the regions closer to the SAN, and smaller in the distal regions, which is in accord with previous experimental [179] and modelling studies [163].

The global APD dispersion and regional APD difference between CT and PM were quantified for isolated cells (using single cell model) and in intact tissue (using 3D anatomical model). The results are shown in Figure 3.17. The atrial repolarisation dispersion under the mutation conditions is shown in Table 3.5. It was demonstrated
Figure 3.16 Simulated atrial activation pattern (A), repolarisation (B) and APD dispersion (C) in a 3D anatomically accurate model under WT conditions. The atria were stimulated from the sinus node region and labelled in (A). The repolarisation time was measured at the moment when the AP was recovered to -60 mV from a depolarisation. The in tissue APD was computed as the temporal interval between the activation and repolarisation. The labelled CT and PM cells were selected to quantify the electrical heterogeneity between the two regions.
Figure 3.17 Simulated global APD dispersion and regional APD difference between CT and PM were quantified for isolated cells (using a single cell model) and in tissue (using the 3D anatomical model). The APD differences are labelled above each box plot. The pacing interval was 1000 ms in the simulations.

Table 3.5 Simulated global atrial repolarisation dispersion for WT and the KCNA5 mutations.

<table>
<thead>
<tr>
<th></th>
<th>Repolarisation Upper Bound (ms)</th>
<th>Repolarisation Lower Bound (ms)</th>
<th>Repolarisation Dispersion (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>320</td>
<td>224</td>
<td>96</td>
</tr>
<tr>
<td>D322H</td>
<td>313</td>
<td>216</td>
<td>97</td>
</tr>
<tr>
<td>E48G</td>
<td>303</td>
<td>221</td>
<td>82</td>
</tr>
<tr>
<td>A305T</td>
<td>289</td>
<td>212</td>
<td>77</td>
</tr>
<tr>
<td>Y155C</td>
<td>334</td>
<td>220</td>
<td>114</td>
</tr>
<tr>
<td>D469E</td>
<td>329</td>
<td>218</td>
<td>111</td>
</tr>
<tr>
<td>P488S</td>
<td>363</td>
<td>231</td>
<td>132</td>
</tr>
</tbody>
</table>
that the gain-of-function mutations smoothed out the global heterogeneity both for the isolated cells and in coupled tissue compared with WT conditions, whereas the global gradient in APD was augmented by the loss-of-function mutations. In comparison with WT conditions, the APD gradient at the CT/PM junction was reduced by the mutations E48G and A305T, and not affected by D322H; the loss-of-function mutations enhanced the APD heterogeneity at the junction.

The atrial activation time was not affected by the mutations, which can be explained by the negligible alteration to CV at slow pacing rate by the mutations (Figure 3.14 and Figure 3.15). The global atrial repolarisation dispersion was either not markedly affected (for D322H) or decreased (for E48G and A305T) by the gain-of-function mutations, and markedly increased by the loss-of-function mutations vs. WT (Table 3.5).

3.3.4 Effects of KCNA5 mutations on tissue’s vulnerability to uni-directional conduction block (wave break)

The Colman et al. and Courtemanche et al. models were used to assess the impact of the mutations on the tissue’s vulnerability to uni-directional conduction block at the junction using the S1S2 pacing. The results are shown in Figure 3.18. Compared to the WT, the gain-of-function mutations produced a small increase to the extent of tissue vulnerability window (except that it was not affected by the mutation E48G in the simulations with the Colman et al. model). The window size was reduced by the loss-of-function mutations in the simulations with the Courtemanche et al. model, whereas It was either slightly increased (Y155C and D469E) or not affected (P488S) in the simulations using the Colman et al. model. The mutations caused a marked shift to the excitation intervals of S2 over which the vulnerability windows were observed: the gain-of-function mutations shifted the excitation intervals towards shorter coupling intervals whereas the loss-of-function mutations prolonged the coupling excitation intervals.
Figure 3.18 Simulated temporal vulnerability window for unidirectional conduction block at the CT/PM junction. (A) Snapshots of electrical wave propagation in an RA wedge following an S2 stimulus showing (i) a capture failure, (ii) a wave break - the electrical wave propagates along the PM/RA but not CT, and (iii) normal conduction. (B) Measured temporal vulnerability window at the CT/PM junction for the two models. The extent of the window is labelled above each box plot. In the simulations, three S1 at 500 ms was applied, which is followed an S2 stimulus.
3.3.5 Effects of KCNA5 mutations with β-adrenergic stimulation

The effects of the mutations in the presence of β-adrenergic challenge were demonstrated by simulating the application of Isoprenaline (ISO, 1μM) using the Grandi et al. model. The results are shown in Figure 3.19. The gain-of-function mutations produced consistent results on AP, CaT and ionic currents with/without application of ISO (Figure 3.19 left panels). The increased I_{Kur} resulted in a reduced APD, more negative plateau potential, and decreased CaT, whereas the loss-of-function mutations induced pronounced EADs following application of ISO (Figure 3.19 right panels), accompanied by markedly augmented CaT and reactivation of I_{CaL}. I_{Kr} and I_{Ks} were increased under the loss-of-function mutation conditions vs. WT, although they could not counter balance against the increased inward currents.

In simulations using regional cell models, the CT was found to be more susceptible to the development of EADs than the PM and RA due to its larger expression of I_{CaL} [35,154]. More substantial EADs were observed in the CT for Y155C and P488S, whereas D469E led to EADs in the CT and RA but not in the PM (Figure 3.20 A).

Implications of observed EADs on tissue excitation patterns were evaluated in a 1D strand of human atrial cells. In these simulations, substantial EADs in tissue were also observed (Figure 3.20 B-C), showing an increased susceptibility to conduction abnormalities. Figure 3.20 B shows that following the application of ISO, 2:1 conduction block was induced by P488S in a 1D strand model of RA paced at 700 ms, but not in WT.

In a 1D heterogeneous strand model consisting CT and PM cells, tissue vulnerability windows to initiate uni-directional conduction block were measured using S1S2 protocol. The results demonstrated an increase in the extent of vulnerability window by gain-of-function mutations vs. WT in the absence of ISO, which is consistent with results obtained in Figure 3.18. The application of ISO markedly diminished the size of tissue vulnerability windows for WT and gain-of-function mutations; however, the vulnerability window size was markedly widened under the loss-of-function mutations, resulting in a substantial increase in tissue vulnerability for loss-of-function mutations vs. WT (Figure 3.20 C-D). Pronounced EADs were produced by the models of CT cells under the mutation P488S both with and without ISO, leading to conduction block in the strand model. Therefore, the quantification of vulnerability for P488S was not attempted.
Figure 3.19 Simulated effects of the KCNA5 mutations on human atrial AP and the corresponding time courses of current traces in the presence of β-adrenergic stimulation using the Grandi et al. model. (A) human atrial AP; (B) Time courses of CaT elicited by the AP; (C - F) Time courses of $I_{Kur}$, $I_{CaL}$, $I_{Kr}$, $I_{Ks}$ during AP.
Figure 3.20 KCNA5 loss-of-function mutations induced EADs following the beta-adrenergic stimulation. (A) A comparison of the response of AP to the application of ISO for CT, PM and RA cells under the mutation D469E conditions. (B) Simulated wave propagations in 1D models consisting of RA cells following application of ISO under (i) WT and (ii) P488S conditions. (C) Wave propagations in a 1D model consisting of CT and PM cells showing (i) unidirectional propagation for S2 = 430 ms.
and (ii) bilateral conduction for $S2 = 431 \text{ ms}$ in the presence of ISO. (C) The measured extent of vulnerability windows (i) without and (ii) with the application of ISO. In (B-C), the stimulation sites were marked with red bars while the stimulation time was labelled with black bars; black arrows indicate occurrences of EADs.

### 3.3.6 Effects of KCNA5 mutations on spiral wave dynamics

The previously developed 3D anatomical atrial model [35] was used to assess the impact of the KCNA5 mutations on the dynamics of spiral excitation waves. Both the Colman et al. and Courtemanche et al. single cell models were used in the simulations. Spiral waves were initiated using the phase distribution method. Figure 3.21 shows representative snapshots of the re-entrant waves and representative local APs of atrial myocytes during the re-entrant excitations. The APs were subsequently analysed to extract the power spectrum of the re-entrant excitation Figure 3.22.

In the simulations using the Colman et al. model, spiral excitations persisted in all conditions (Figure 3.21 Bi). In contrast, for the Courtemanche et al. model only D322H and A305T were able to sustain a re-entry throughout the 5 s episode of simulation (Figure 3.21 Bii), although a lower diffusion coefficient was used in the model.

In comparison to the WT, the simulations demonstrated that the gain-of-function mutants in general accelerated the spiral excitations in the atria (except no change for E48G using the Colman et al. model) (Figure 3.21, Figure 3.22). The acceleration was much more pronounced in the Courtemanche et al. model accompanied by prolonged life span. For the loss-of-function mutations, the simulations using the Colman et al. models exhibited either a slight acceleration (Y155C and D469E) or deceleration (P488S) compared with WT. In the simulations using the Courtemanche et al. model, D469E produced a higher dominant frequency than WT, whereas the re-entrant waves were terminated within 1 s of stimulation under the mutations Y155C and P488S, much shorter than the WT, which could be attributed to the pronounced alternans seen in the AP traces during the re-entrant episodes (Figure 3.21).

Further incorporation of cAF conditions to the models consistently revealed accelerated re-entrant excitations by the gain-of-function mutations and slowing down
Figure 3.21 Simulated spiral waves and APs in a 3D anatomical model of the human atria. (A) Representative snapshots of spiral waves in the atria using (i) the Colman et al. and (ii) Courtemanche et al. models. (B) Simulated atrial APs during the re-entrant excitation using the (i) Colman et al. model and (ii) Courtemanche et al. models. The APs were taken from a right atrial myocyte located close to the IVC.
in the spiral waves under the conditions of loss-of-function mutations compared with the WT, which is in accordance with the effects of these mutations on the AP and APD restitutions (Figure 3.13).

Figure 3.22 Power spectrum analysis of the local electrical activities using (A) the Colman et al. model and (B) the Courtemanche et al. model. (i) The normalised power spectrum of local electrical activities; the power density was normalised to the maximum values of WT. (ii) The dominant frequency of local electrical activities for the WT and mutations under Lone AF and cAF conditions.

3.4 Discussion

In this Chapter, the impacts of six mutations in KCNA5 on human atrial electrical function were assessed in silico. Using the multiscale models of the human atria, the arrhythmogenesis of the six mutations in KCNA5 were elucidated. It was demonstrated the mechanistic link between the mutations and the AF incidence was distinct for the two groups of mutations: the proarrhythmia of gain-of-function mutations was associated with APD shortening whereas the arrhythmogenicity of loss-of-function mutations manifested as a promoted susceptibility to EADs. The presented results have implications that can be extrapolated beyond the specific mutations, providing general
insights into the role of I_{Kur} in atrial arrhythmogenesis further to previous modelling [155,186–188] or experimental [189–191] studies, which have primarily focused on anti-arrhythmic properties of I_{Kur} block/down-regulation alone.

3.4.1 Proarrhythmic effects of gain-of-function mutations

Gain-of-function mutations promoted the initiation and maintenance of arrhythmic re-entrant excitation through reducing APD across wide range of pacing cycle length via shortening of the cellular APD (Figure 3.9 and Figure 3.10) and flattening of the rate-dependent adaptation of APD (Figure 3.11), and facilitating atrial capture towards shorter coupling intervals (Figure 3.14), as well as accelerating re-entrant excitations in tissue (Figure 3.22).

In single cells, the increased activity of I_{Kur} during phase-2 of the AP led to a substantial reduction of the plateau potential and thus APD_{30} in comparison to WT, directly contributing to a shortened APD_{90}. In tissue, the gain-of-function in I_{Kur} allowed for shorter coupling intervals facilitating atrial capture by stimuli in tissue, while reducing excitation wavelength in response to slow coupling intervals. The reduced APD and wavelength are proarrhythmic. It is well received that a shortened wavelength promotes maintenance of re-entries by reducing the size of functional re-entry circuits and thereby increasing the potential number of simultaneous circuits [11,35,109] as well as promoting the maintenance of re-entries [192].

Moreover, the simulated vulnerability windows exhibited increases in the size of the temporal window by the gain-of-function mutations in 8 of 9 cases across simulations using the three models (Figure 3.18, Figure 3.20), mediating an increased susceptibility of atrial tissue to induction of wave breaks by the gain-of-function mutations; these effects have been demonstrated to be associated with increased susceptibility to the initiation of re-entries [35].

In addition, for the simulated spiral waves using the 3D atrial model, the mutations exerted more rapid spiral wave excitations (5 out of 6 cases, Figure 3.22). Specifically, in the simulations using Courtemanche et al. model, these mutations prolonged the life span of re-entrant waves. These effects added to the promoted maintenance of re-entries by the mutations.

Therefore, the gain-of-function mutations promoted the initiation and maintenance of re-entries through abbreviations in the APD and hence wavelength, promotions of
unidirectional conduction blocks, and accelerations in the spiral wave dynamics.

3.4.2 Arrhythmogenesis in loss-of-function mutations

At the cellular level, the decreased $I_{Kur}$ during phase 2 of the AP resulted in an elevation in the AP-plateau. This could lead directly to a prolongation of the $APD_{90}$, but through secondary effects on other currents including $I_{Kr}$ and $I_{Ks}$, the $APD_{90}$ could be unchanged or slightly shortened compared to the WT (Figure 3.9, Figure 3.10). While these inconsistencies could be attributed to the different intrinsic properties of the models, they could also indicate a link with the baseline AP types the models represent individually. It is important to note that both prolongation and attenuation in APD have been demonstrated in previous experimental studies on effects of $I_{Kur}$ block. For example, in an experimental study an application of 50 μM 4-aminopyridine (4-AP) markedly prolonged human atrial APD by 66% [161]. In another experimental study where the AP exhibited typical triangular-shaped AP, the application of 4-AP also led to a marked elevation of plateau potential and prolongation of APD in human atrial myocytes [148]. In contrast, other experimental studies where myocytes showing typical type 2 atrial AP demonstrated that blocking $I_{Kur}$ with 4AP resulted in shortening of APD in both human and canine atria [189,193]. These experiments suggest the substantial dependence of effects of $I_{Kur}$ block on baseline profile of atrial AP.

The inconsistency in the alterations to APD by the mutations led to variable effects on the APD restitution and atrial conduction as well as tissue vulnerability. In the simulations using the Colman et al. model in which an APD prolongation by the mutations was observed, the wavelength was increased for slow coupling stimulation intervals while the threshold of minimum S2 facilitating atrial capture was increased (Figure 3.15), demonstrating anti-arrhythmic effects. However, these effects were not extrapolated into the modulations of the mutations on the dynamics of spiral waves, in which only P488S showed slowing down in the re-entrant excitation, whereas it was slightly accelerated by D46E and Y155C (Figure 3.22 Aii).

In the simulations using the Courtemanche et al. model, the wavelength was markedly reduced by the mutations for fast coupling intervals, whereas the threshold of coupling intervals that is necessary for atrial capture was not affected by the mutations (Figure 3.15), which collectively demonstrated pro-arrhythmic effects. In spiral wave simulations, however, only the mutation D469E was able to maintain spiral excitations,
and the dominant frequency was faster than WT, accompanied by a greater lifespan (Figure 3.21 B right panel). These effects agreed with a previous experimental study in which Burashnikov and Antzelevitch [189] reported an increased susceptibility to AF in the presence of low concentration of 4-AP (exhibiting I_{Kur} block) in healthy canine atrial preparations. The earlier termination of the re-entrant waves by Y155C and P488S may be attributable to the presence of alternans in AP. In summary, both models demonstrated loss-of-function mutations could present pro-arrhythmic and antiarrhythmic effects at the cellular level or in tissue.

The extent of the vulnerability window was slightly increased by loss-of-function mutations in the simulations using the Colman et al. model, whereas it was reduced in the simulations using the Courtemanche et al. model by the mutations vs. WT (Figure 3.18). Both models show a positive shift in the coupling intervals over which the VWs were observed, which could promote the susceptibility of atrial tissue to the induction of arrhythmia.

Simulated effects of these mutations in the presence of ISO did reveal another mechanism by which the loss-of-function mutations promote the occurrence of EADs both in single cells and in tissue (Figure 3.19, Figure 3.20). The loss of the repolarising current and promoted I_{CaL} re-activation underlie the enhanced susceptibility to EADs in the presence of ISO: the reduced activity in I_{Kur} by mutations was not able to counteract the increased activity of I_{CaL}, leading to reduced repolarisation reserve and an increased incidence of I_{CaL} re-activation and giving rise to EADs (Figure 3.19 Dii). These effects have also been observed previously in both experimental and modelling studies in which I_{Kur} was pharmaceutically inhibited [27,139]. In tissue, the EADs markedly increased the tissue vulnerability to unidirectional conduction (Figure 3.20 C-D). Enhanced vulnerability to the induction of wave-breaks (unidirectional block) has been mechanistically linked to the development and subsequent breakdown of re-entry [109,153].

EADs have been associated with proarrhythmic effects [194–196] and contribute to the incidences of ectopic activity [109]. In tissue simulations of the present study, EADs were observed to promote conduction block in the atria. In a 1D strand model of RA, it was demonstrated that EADs could cause 2:1 conduction block at a cycle length of 700 ms with the mutation P488S, but not for WT or in the absence of ISO, suggesting a potential mechanism for the development of re-entry independent of ectopic pacing. Therefore, the loss-of-function mutations could promote the development of
unidirectional conduction patterns at junctions of two regions or through heterogeneous application/distribution of ISO and hence promote both the initiation and recurrence of AF.

3.4.3 The role of \( I_{Kur} \) in modulating action potential morphology and duration

The present study suggests a non-linear role of \( I_{Kur} \) kinetics and conductance in shaping the morphology and duration of atrial AP, which is associated with the baseline AP morphology and duration. Analysis of the parameters of \( I_{Kur} \) on modulation of APD shows that both an increase and decrease in \( I_{Kur} \) conductance can lead to a prolongation of the APD (Figure 3.12 Aiii-v), which is attributable to the interplay between \( I_{Ks} \), \( I_{Kr} \) and \( I_{Kur} \) (Figure 3.7 E-F and Figure 3.9 E-F). Due to the presence of inter-subject variations and inter-cellular variations from a same patient, this non-linear behaviour may be clinically relevant, especially in an effort of developing pharmaceutically inhibiting \( I_{Kur} \) as a strategy of AF management. Interestingly, the simulated results did not demonstrate a nonlinear relationship between the conductance of \( I_{Kur} \) and APD and AP-plateau (Figure A.1, Figure 3.8 B and Figure 3.10 B), suggesting that the dependence of these AP biomarkers on the activity of \( I_{Kur} \) is monotonic.

3.4.4 Use of multiple, independent models of human atrial electrophysiology

In this chapter, three independent atrial models were employed to assess the functional impacts of the mutations in the atria. This was done in order to extrapolate the implications of the simulations towards model-independent conclusions.

The Grandi et al. model was not employed to assess the impact of genetic variations on the CV restitution and re-entry dynamics due to a limitation of the model, which will be discussed in 3.4.6.

Additionally, the induction of EADs and their role in atrial conduction were only assessed using the Grandi et al. model. In preliminary simulating the effects of ISO using the Courtemanche and Colman et al. models, the loss-of-function mutation did not promote the susceptibility to the production of EADs. Further simulations were performed using the Nygren et al. model [197], and results did show a promoted incidence of EADs with impaired \( I_{Kur} \) activities (Appendix A Figure A.2 ). It is noteworthy that the promoted incidence of EADs due to ISO and a loss of \( I_{Kur} \) has been observed experimentally [27] and demonstrated in stimulations using the Grandi et al.
model [139]. It is likely that the Courtemanche et al. and Colman et al. models possess limitations in simulating EADs, and that the induction of EADs by loss-of-function mutations and the application of ISO may be observed experimentally and not an artefact of the models.

3.4.5 Clinical relevance

Developing atrial selective ion channel blockade is a current strategy for the treatment of AF [53,54]. \( I_{Kur} \) is atrial specific and thus manifest a desirable potential for pharmacological interventions on the atria without inducing adverse effects on the ventricles. In this regard, \( I_{Kur} \) has been of general interest as a target for AF management [53,54] and multiple compounds inhibiting \( I_{Kur} \) have been developed [54]. However, the effects of \( I_{Kur} \) block on the atrial electrophysiology have been shown to be controversial both in modelling [155,187] and experimental [148,157,189,193] studies.

The present study adds insights to the effects of \( I_{Kur} \) inhibition in the atria. Under cAF conditions, impaired functions of \( I_{Kur} \) were shown to prolong APD (Figure 3.13 D-F), which is antiarrhythmic. Whilst under normal conditions the reduced activities of \( I_{Kur} \) led to either an increase or decrease in the APD, the impaired \( I_{Kur} \) was also associated with an increased susceptibility to EADs following \( \beta \)-adrenergic stimulation challenge. This may have implications: for paroxysmal AF in which AP properties may remain unaltered [17], inhibiting \( I_{Kur} \) may result in pro-arrhythmic consequences such as EADs. Therefore, \( I_{Kur} \) block could produce anti-arrhythmic effects in cAF-remodelled atria, but may not be in favour for the treatment of paroxysmal AF.

3.4.6 Limitations

Limitations associated with the original cellular and 3D models have been discussed in detail elsewhere [35,37,139,154] and hence are not reiterated here. This section discusses the limitations that are native to the present Chapter.

In the simulations modelling effects of the genetic variants on the biophysical properties of \( I_{Kur} \), multiple assumptions were made due to the absence of relevant experimental data. It was necessary to assume that the relative regional difference in the biophysical properties of \( I_{Kur} \) throughout the atria was not affected by the mutations; the response of \( I_{Kur} \) to \( \beta \)-adrenergic stimulation was not affected by the mutants; and the relative difference between mutant variants of \( I_{Kur} \) and the WT were identical for lone
and chronic AF. Additionally, it was assumed that these mutations did not directly induce structural remodelling including fibrosis and atrial enlargement. These assumptions may warrant further experimental justifications.

The Grandi et al. model exhibited a limitation in simulating APD restitutions (3.3.1.3). The model produced small upstroke velocity of AP (typically less than 20 V/s), which appeared unphysiological. Whilst the limitation has been implied in a benchmark study by [160], it prevented performing further simulations evaluating CV restitutions in a 1D strand using the model, which may be explained by the $I_{Na}$ formulation in the model. Therefore, in this Chapter, the Grandi et al. model was not used to quantify CV and wave propagation in tissue. The limitation, however, is expected to have little effect on simulated EADs, which was primarily driven by reactivation of $I_{CaL}$ instead of $I_{Na}$.

In simulations of stable re-entrant excitations in the atria, the electrical coupling was assumed to be homogeneous between the regions. Further simulations incorporating such heterogeneities may be necessary to investigate the effects of KCNA5 mutations in the presence of anisotropy in electrical coupling.

### 3.5 Conclusion

Genetic variation in KCNA5 promoted multiple mechanisms of arrhythmogenesis for both gain- and loss-of-function mutations, highlighted by the promotion in developing re-entrant excitation and the increased susceptibility of EADs, respectively.
Chapter 4

Inotropic effects of KCNA5 mutations on human atria

In Chapter 3, it was shown that mutations in Kv1.5 carrying the ultra-rapid delayed rectifier current (IKur) dynamically modulated the atrial action potential (AP) and intracellular calcium transient (CaT), which might have implications for modulating atrial active force production and inotropic effects, which remains to be elucidated. In this Chapter, multi-scale electromechanical models of the human atria were used to assess the impact of altered IKur by six KCNA5 mutations on the contractile function of the atria both at cellular and organ levels. The results demonstrated that the gain-of-function mutations reduced active contractile force primarily through decreasing the CaT via a reduction in ICaL due to shortened APD, whereas the loss-of-function mutations mediated positive inotropic effects by increased CaT via enhancing the reverse mode of the Na+/Ca2+ exchanger. The 3D atrial electromechanical coupled model predicted different functional impacts of the KCN5A mutation variant on atrial mechanical contraction by either reducing or increasing atrial output, which is associated with the gain-of-function mutations or loss-of-function mutations in KCNA5 respectively. This study adds insights into the functional impact of KCNA5 mutations in modulating atrial contractile functions.

4.1 Introduction

The ultra-rapid rectifier delayed potassium current, IKur, plays an important role in modulating the shape of the human atrial AP as well as atrial contractility [157,193]. Previous studies have demonstrated that blocking IKur in the human atrial myocardium led to an elevation of the plateau potential of the action potential and an increase in the active force [157,193]. In Chapter 3, the six genetic variations [56] in KCNA5 encoding Kv1.5 channels in the human atria were demonstrated to have marked impacts on the
AP and CaT of atrial myocytes, which may consequentially lead to inotropic implications. This has not yet been revealed. In this Chapter, electro-mechanical models of the human atria at cellular and organ levels were used to investigate such implications.

Mechanoelectrical feedback (MEF) is mechanisms by which mechanical load imposed on myocytes or membrane induces alterations to the electrophysiological properties of such myocytes [198]. Previous studies have shown that the MEF contributes to the arrhythmogenesis of the heart [198–200], and stretch induced dilation in the atrial wall increases tissue’s vulnerability to initiation of AF [201–204]. Several mechanisms have been shown to underlie the arrhythmogenesis of MEF, including stretch-induced changes to the intracellular Ca$^{2+}$ handling system [205], modulation of the AP through alterations to the coupled cardiac fibroblasts [206,207], and more markedly via activations of the stretch-activated channels (SACs) [199,208]. In accord with previous studies [209–211], SACs were considered in the electromechanical model. The effects of the mutations on atrial contractile function were modelled and compared both in the presence and absence of the SACs.

4.2 Methods

4.2.1 Modelling active force development in human atrial myocytes

The Colman et al. electrophysiological model of the human atrial cell [35] was coupled to the Rice et al. myofilament model [95], forming a set of ordinary differential equations describing the dynamical time course of the AP and active force development of atrial myocytes. This Rice et al. model was selected as it is built on a cross-bridge cycling model of force generation in cardiac myofilament and is capable of reproducing key experimental observations and findings including steady-state force-sarcomere length (F-SL) relations and sarcomere length-dependent steady-state force-calcium relations under various physiological conditions [95]. Since the Rice et al. model was developed for rat and rabbit myocytes, it was necessary to modify several parameters to better reproduce the force-Ca$^{2+}$ relationship observed in human atrial cells [212].

The isometric protocol has been used to measure the steady-state active force-Ca$^{2+}$ relationship in cardiac myocytes/myocardium at fixed cell/trabeculae length [95,212,213]. To account for the human atrial specific force-Ca$^{2+}$ observed
experimentally, a number of parameters governing the myofilament dynamics were optimised to reproduce the experimental steady-state force-Ca$^{2+}$ relationship observed at room temperature using the isometric protocols [95,212]. In the parameter optimisation, the difference between the simulated and experimental force-Ca$^{2+}$ relations [212] using the isometric protocol was defined as the objective function, and was subsequently minimised using the Nelder-Mead Simplex algorithm [214] built in the *scipy-optimize* package, an open source optimisation library as part of the SciPy ecosystem [140]. The resulting parameters are listed in Table 4.1, in which they are compared to the original parameters of the model [95] representing myofilaments of ventricular cells in the rat. The alterations to the parameters are introduced as follows.

Firstly, parameters regulating the binding and unbinding kinetics of Ca$^{2+}$ to troponin were modified. In the Rice et al. model [95], both binding sites of high and low affinities (defined as $CaTrop_H$ and $CaTrop_L$, respectively) were considered, and the kinetics are given by:

$$\frac{d}{dt} CaTrop_H = k_{on}[Ca^{2+}]_l(1 - CaTrop_H) - k_{off,H} CaTrop_H \quad (4.1)$$

$$\frac{d}{dt} CaTrop_L = k_{on}[Ca^{2+}]_l(1 - CaTrop_L) - k_{off,L} CaTrop_L \quad (4.2)$$

where $[Ca^{2+}]_l$ is the intracellular concentration of Ca$^{2+}$, $k_{on}$ the rate constant for binding of both sites, $k_{off,H}$ the rate constant for unbinding from high-affinity sites, and $k_{off,L}$ the rate constant for unbinding from low affinity sites. Furthermore, Rice et al. defined a nonlinear function $permtot$, presenting the shift of a regulatory unit to permissive state through the nearest neighbour cooperativity [95]:

$$permtot = \left(1/(1 + (perm_{50}/Trop_R)^{nPerm})\right)^{0.5} \quad (4.3)$$

where $Trop_R$ is the fraction of thin filament regulatory units bound to Ca$^{2+}$, which is a function of sarcomere length; $perm_{50}$ a half-activation constant; $n_{perm}$ the Hill coefficient.

Parameters of rate constants related to cross-bridge cycling dynamics were also modified. These include the constant $f_{app}$, which determines the cross-bridge attachment state to the first strongly bound state $XB_{PreR}$; the reverse rate constant $g_{app}$ as well as the forward and backward transition rates $h_f$ and $h_b$, and the rate modifier $g_{xb}$. Detailed definitions to these parameters can be found in [95].

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Haibo Ni 116
Figure 4.1 illustrates the simulated force-Ca\(^{2+}\) relation using the myofilament model with the fitted parameters, which is compared to the experimental force-Ca\(^{2+}\) relation [212]. Figure 4.1 (B) shows the active force plotted against Ca\(^{2+}\) at various sarcomere lengths (SL): the force-Ca\(^{2+}\) relation was shifted to the left for higher SLs, indicating the pronounced dependence of active force on the SL. The temperature dependent rate constants were corrected to the physiological temperature using the temperature correction functions native to the Rice et al. model [95].

The electro-mechanical coupling was achieved by coupling the intracellular calcium transient (CaT) produced by the single cell model of electrophysiology into the updated Rice et al. myofilament model. To model the active force development of myocytes during action potentials, the isotonic stretch protocol was adopted: an external constant force was applied so that the cell reaches an equilibrium state at a sarcomere length of 2.2 μm. The electrical stimuli were then applied to evoke action potentials.

### 4.2.2 Inclusion of the stretch-activated channel current

A model of the stretch-activated channel (SAC) current was incorporated into the Colman et al. model [35], which represents the effect of mechano-electric feedback (MEF). SACs are mechanically activated ionic channels, exhibiting direct modulations on action potentials of myocytes in a strain-dependent manner. In accord with previous studies [210,215,216], SACs were modelled using the following formulation:

\[
I_{SAC} = G_{stretch}P_m(V_m - E_{stretch})
\] (4.4)

where \(I_{SAC}\) is the stretch activated current, \(G_{stretch}\) and \(E_{stretch}\) are the maximum conductance and reversal potential of the channel, respectively, and \(P_m\) is the normalised open probability of \(I_{SAC}\), given by:

\[
P_m = \frac{1}{1 + \exp\left(-\left(\varepsilon - \varepsilon_{half}\right)/K_\varepsilon\right)}
\] (4.5)

where \(\varepsilon\) is the engineering strain (linearly dependent on the stretch), \(\varepsilon_{half}\) is the half activation strain, and \(K_\varepsilon\) is the activation slope. In this study, \(G_{stretch} = 0.0061 \text{ mS/\mu F}\); \(E_{stretch}\) is the reversal potential, and was set to -1 mV based on previous studies [209,215,217], \(\varepsilon_{half} = 0.16\) [216], \(K_\varepsilon = 0.027\). The channel was assumed to be equally permeable to the three major ions: sodium (Na\(^+\)), potassium (K\(^+\)) and calcium (Ca\(^{2+}\)).
Table 4.1 Modifications to the parameters of the Rice et al. myofilament model to simulate the force-Ca\(^{2+}\) relation in the human atrial cell. The parameters were defined in [95] and are reiterated below.

<table>
<thead>
<tr>
<th>Rate Constant</th>
<th>Original value [95]</th>
<th>Optimised value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{on}) (ms(^{-1})µm(^{-1}))</td>
<td>50</td>
<td>87.7</td>
</tr>
<tr>
<td>(k_{offL}) (ms(^{-1}))</td>
<td>250 × 10(^{-3})</td>
<td>397.2 × 10(^{-3})</td>
</tr>
<tr>
<td>(k_{offH}) (ms(^{-1}))</td>
<td>25 × 10(^{-3})</td>
<td>39.7 × 10(^{-3})</td>
</tr>
<tr>
<td>(\text{perm}_{50})</td>
<td>0.5</td>
<td>0.99</td>
</tr>
<tr>
<td>(n_{\text{perm}})</td>
<td>15</td>
<td>7.67</td>
</tr>
<tr>
<td>(f_{app}) (ms(^{-1}))</td>
<td>500 × 10(^{-3})</td>
<td>249.7 × 10(^{-3})</td>
</tr>
<tr>
<td>(g_{app}) (ms(^{-1}))</td>
<td>70 × 10(^{-3})</td>
<td>28 × 10(^{-3})</td>
</tr>
<tr>
<td>(h_{f}) (ms(^{-1}))</td>
<td>2000 × 10(^{-3})</td>
<td>998.9 × 10(^{-3})</td>
</tr>
<tr>
<td>(h_{b}) (ms(^{-1}))</td>
<td>400 × 10(^{-3})</td>
<td>160.1 × 10(^{-3})</td>
</tr>
<tr>
<td>(g_{xb}) (ms(^{-1}))</td>
<td>70 × 10(^{-3})</td>
<td>28 × 10(^{-3})</td>
</tr>
</tbody>
</table>

Figure 4.1 Simulated force-Ca\(^{2+}\) relation for human atrial cells. (A) A comparison between the simulated and experimental force-Ca\(^{2+}\) relations in human atrial cells at room temperature; experimental data was digitalised from Fig. 3 (a) of [212]. The active forces were normalised to the value produced at a saturated concentration of Ca\(^{2+}\) (\(-\log_{10}\left[Ca^{2+}\right]\_i = 4.5\)). (B) Simulated active forces at various sarcomere lengths at physiological temperature. The forces were normalised to the maximum force of the cell produced at SL = 2.3 µm. SL – sarcomere length.
4.2.3 Modelling the KCNA5 mutations in the atria

The models of I_{Kur} under WT and mutation conditions used in Chapter 3 were adopted in the present study. Briefly, three mutations (D322H, E48G, A305T) mediated gain-of-function, whereas another three (Y155C, D469E, P488S) resulted in a loss-of-function in I_{Kur}. The models successfully reproduced the steady-state activation, inactivation and I-V relationship elicited by an identical voltage clamp used in the experimental study identifying the mutations [56].

4.2.4 Parameter analysis of the dependence of atrial contractility on I_{Kur}

To further elucidate the role of I_{Kur} in modulating the contractility of atrial myocytes, the key parameters of I_{Kur} channel properties including the conductance, the slope of the steady-state activation curve (K_{Activation}), half-activation voltage (V_{1/2}) and time constants of activation and deactivation (τ_{Activation}) were varied over parameter spaces that are within physiologically relevant ranges. For simplicity, the steady-state activation model for I_{Kur} was reduced to:

\[
a_{inf} = \frac{1.0}{1.0 + \exp\left(\frac{V_m - V_{1/2}}{K_{Activation}}\right)}
\]  

(4.6)

where the variables have their usual meanings. The control values of V_{1/2} and K_{Activation} were set to -6.0 mV and 8.6 mV, respectively, based on previous experimental and modelling studies [37,56].

The parameters analysis in Chapter 3 suggested a nonlinear dependence of APD_{90} on I_{Kur} conductance, which was not observed for APD_{30}. The correlation between APD and relative cell shortenings was investigated through generated atrial models with random parameters of I_{Kur}. The ranges of parameter variation were {0, 2}, {0.2, 2.2}, {0.2, 2.2} and {-5, 5} for the scaling of conductance, activation gate time constant, K_{Activation}, and the shift in V_{1/2}, respectively. The random parameters were distributed equally within the parameter ranges. Latin hypercube sampling [218,219] was adopted to generate 10,000 sets of random parameters. The sampling method has been used in a number of studies [220–222] on analysing the dependence of electrophysiological properties on parameters.
4.2.5 3D anatomical model of electromechanical coupling

4.2.5.1 Regional single cell models
The Colman et al. family of regional single cell models used was used to simulate the regional electrical heterogeneities in the human atria, which was achieved by scaling the conductance of major ionic currents [35]. The scaling parameters were kept the same as those in Chapter 3, except that the current densities of $I_{CaL}$ and $I_{to}$ were scaled by 2.0 and 1.17 respectively for CT.

4.2.5.2 3D anatomical atrial model of electro-mechanical coupling
A recently developed 3D atrial anatomically accurate model of electromechanical coupling for the human atria [38] was adapted to evaluate the implications of inotropic effects of these mutations at the organ level. The 3D electro-mechanical model of human atria was described in detail in [38] and briefly introduced here.

The anatomical geometry of the human atria in [38] was based on the reconstructed geometry from the Visible Female dataset, and segmented into major anatomical regions [223] (Figure 4.2 A). In the absence of realistic fibre orientations native to the geometry, the fibre orientations generated using a semi-automatic rule-based approach [224,225] were used in this study (Figure 4.2 B).

To simulate the electrophysiology in the 3D tissue, the widely used monodomain equation [35,128] representing the excitation wave propagation was modified to incorporate the Right Cauchy-Green deformation tensor, $\mathbf{C}$ [38,215], accounting for the feedback to electrical propagation from the mechanical deformation in tissue. The updated monodomain equation is given as follows:

$$\frac{\partial V_m}{\partial t} = \nabla \cdot (\mathbf{D} \mathbf{C}^{-1} \nabla V_m) - \frac{I_{stim} + I_{ion}}{C_m}$$ (4.7)

where $C_m$ is the cell capacitance per unit surface area, $V_m$ is the transmembrane voltage, $I_{ion}$ is the total ionic currents, and $I_{stim}$ represents an externally applied stimulus current, $\mathbf{D}$ is the diffusion tensor governing the intercellular electrical coupling. The diffusion parameters were selected so that the activation time and conduction velocities within the atria matched previous studies [35,154]. The conductivity ratio in parallel vs. transverse to the fibre direction was set to 3:1 [38]. $\mathbf{D}$ values in BB and CT regions were set to 2.5-fold larger than that of RA to account for a faster propagation in these regions [35,154] as compared to RA.
Figure 4.2 The 3D anatomical atria with segmented regions and traced fibre orientations showing the microstructure of the atria. (A) Segmented regions in the human atria from two different views; the major regions are marked with labels. (B) Traced fibre orientations of the atria from two different views. The colour map indicates the Z-component (into-paper direction of the left panel) of the longitudinal vector of the fibres. The abbreviations of the regions are kept the same to Chapter 3, and are reiterated here: RA – right atrium, CT - crista terminalis, BB - Bachmann’s bundle, PM - pectinate muscles, AVR - atrio-ventricular ring, RAA - right atrial appendage, AS - atrial septum, LA - left atrium, LAA - left atrial appendage, PV - pulmonary veins, SAN – sino-atrial node.

The human atrial tissue was modelled as an inhomogeneous, anisotropic, nearly incompressible material [38], which has also been used in other previous studies.
An active strain approach [228,229] was adopted in the present study as the approach does not require tunings of the active forces produced by single cell models to drive the observed deformations in the cardiac tissue [38].

The total potential energy function was formulated based on a two-field variational principle, with the deformation vector $\mathbf{u}$ and hydrostatic pressure $p$ as the two fields [38,215]. The total potential energy was formulated as:

$$\Pi(\mathbf{u}, p) = \Pi_{\text{int}}(\mathbf{u}, p) + \Pi_{\text{ext}}(\mathbf{u})$$

(4.8)

where $\Pi_{\text{int}}(\mathbf{u}, p)$ is the total strain energy of the body and $\Pi_{\text{ext}}(\mathbf{u})$ external potential energy representing the potential energy arising from the external loading of the body.

Within the active strain framework, the deformation tensor $\mathbf{F}$ was decomposed into a microscopic (active) component and a macroscopic (passive) component [38,228]:

$$\mathbf{F} = \mathbf{F}_e \mathbf{F}_o$$

(4.9)

where the active component $\mathbf{F}_o$ accounts for the length change in the micro-scale due to the active contraction, whereas the passive component $\mathbf{F}_e$ measures the passive mechanical deformation in response to both external loads and internal microscopic deformation. The active component $\mathbf{F}_o$ was linked with the microscopic active strain through:

$$\mathbf{F}_o = \mathbf{I} + \eta \mathbf{f} \otimes \mathbf{f}$$

(4.10)

where $\mathbf{I}$ is the identity tensor, $\eta$ is the active strain field that is dependent on the sarcomere length of the cells; $\mathbf{f}$ is the fibre orientations.

$$\eta = (SL - SL_0)/SL_0$$

(4.11)

where $SL_0$ is the sarcomere length of a cell at rest.

Therefore, the passive (macroscopic) component $\mathbf{F}_e$ can be obtained from:

$$\mathbf{F}_e = \mathbf{F} \mathbf{F}_o^{-1}$$

(4.12)

and thus the resultant Right Cauchy-Green strain tensor and Green-Lagrange strain tensor are given by:

$$\mathbf{C}_e = \mathbf{F}_e^T \mathbf{F}_e$$

(4.13)

$$\mathbf{E}_e = \frac{1}{2}(\mathbf{C}_e - \mathbf{I})$$

(4.14)

The constitutive law of the cardiac tissue was defined using the Guccione constitutive
law [230]:

$$W = W(F_e) = C_1 e^Q$$  \hspace{1cm} \text{(4.15)}

where:

$$Q = C_2 E_{11}^2 + C_3 (E_{22}^2 + E_{33}^2 + E_{23}^2) + 2C_4 (E_{12} E_{21} + E_{13} E_{31})$$  \hspace{1cm} \text{(4.16)}

where $C_1 = 0.831 \text{kPa}, C_2 = 14.31, C_3 = 4.49$ and $C_4 = 10.0$ [38].

In the simulations, a subset of the epicardial surface in the left atria was mechanically clamped (by setting the displacement $u = 0$ for the mechanical boundary condition) to avoid possible rigid body motions.

In this chapter, the geometry used in [38] was further updated to remove holes and sharp edges, resulting in smoother surfaces in the sinoatrial node, right atrial appendage and pulmonary vein regions, and an improved mesh quality. The updated meshed geometry consisted of 13889 vertices and 45580 tetrahedra. To obtain the electrical mesh, the tetrahedra in the mechanical mesh was refined twice if the volume of the element was greater than 0.1 mm$^3$, giving rise to 361128 vertices and 1798677 tetrahedra.

### 4.2.6 Numerical methods

The single cell models were solved using the same method as Chapter 3.

The 3D electro-mechanics problem was solved using finite element method, which was implemented using the automated scientific computing library, FEniCS [138]. The electrophysiological problem (Eq. (4.7)) was solved using an operator splitting method [231]: the single cell electrophysiology and diffusion parts were solved in multiple steps. The diffusion part was discretised in time using the Crank-Nicholson method [232]. The scalar strain field obtained in the electrophysiological domain was interpolated to the mechanical mesh. The mechanics problem (Eq. (4.8)) was solved using the Newton-Raphson iteration method. To facilitate a faster convergence, single cell models were paced for 100 s to reach an equilibrium state, and the state variables were saved to serve as the initial conditions for the single cells in the 3D simulations. A more detailed description of the implementation procedures is given in [38].

The electro-mechanical problem was updated to be fully parallelised using MPI, resulting in an approximately 13-fold speed up (vs. a single threaded implementation) on a cluster consisting of 2 Intel Xeon CPU E5-2680 V2 @ 2.80 GHz with 40 hyper
threads in total. A simulation of electromechanical activities spanning 700 ms necessitated around 3 hours computing time.

4.3 Results

4.3.1 Validations to the single cell electro-mechanical model

The electromechanical model was first validated by comparing the baseline (RA) model with experimental data (Figure 4.3). It was shown that the electro-mechanical model successfully reproduced the characteristics of the time courses of active force development and cell shortening. The simulated active force profile (Figure 4.3 Ai) elicited by the CaT was qualitatively agreeable with an experimental force trace of a human atrial cell [157]. In the absence of data on the relative cell shortening of human atrial cells, the simulated cell shortening was compared with experimental data from canine atria. Note that such an approach of incorporating and comparing data from alternative species has been adopted in a number of previous studies, including [35,37,154,223]. It was shown that the time courses of simulated active force and cell shortening during AP were also qualitatively comparable to the results reported in a number of experimental studies [157,233–235] in canine atrial cells (Figure 4.3 Aiv, Bii). The model produced a relative cell shortening of around 7-10% depending on conditions and region, which agreed well with previous experimental studies in canine atria showing the cell shortening ranged from approximately 4% to 10% (4% in [157], 7.8% in [236] and 10% in [234]).

It was also revealed that the non-selective SAC plays a role in modulating the AP and CaT. As shown in Figure 4.3A, the inclusion of \( I_{\text{SAC}} \) depolarised resting potential (from -75.5 mV to -69.4 mV), reduced the overshoot of the action potential and slightly increased the diastolic and systolic levels of the intracellular calcium transient, leading to an increased active force and relative cell shortening. The impact of \( I_{\text{SAC}} \) on the AP is concordant with previous studies in atria [209,217] and ventricles [215].

4.3.2 The inotropic effects of the KCNA5 mutations on atrial myocytes

The inotropic effects of KCNA5 mutations were investigated here using the baseline single cell electromechanical model in the absence/presence of SAC (Figure 4.4, Figure 4.5). The alterations to the CaT of atrial myocytes by the mutations as demonstrated in
Chapter 3 exerted substantial changes to the active force production and SL shortening in the atria.

![Simulations](image)

![Experiments](image)

Figure 4.3 The simulated electro-mechanical properties of the baseline (RA) model as compared to experimental data. (A) Simulated (i) AP, (ii) CaT, (iii) active force and (iv) relative cell shortening in the absence/presence of SAC. (Bi) An experimental trace showing the active force in a human atrial cell. The force was normalised to the peak value. (Bii) An experimental time course of relative cell shortening elicited by an action potential in canine atrial myocytes. The experimental traces were digitalised from [157,234] and were shifted to align with the bottom panels in (A).

In the simulations without SAC, the reduction to the CaT by the gain-of-function
mutations led to pronounced decreases in the active force of the myocytes: 29.8%, 16.2% and 27.9% by D322H, E48G and A305T vs. the WT conditions, respectively (Figure 4.4 Ci, Ei). The impaired contractile function induced more substantial changes to the SL shortening; the relative SL shortening was markedly decreased (by 48.2% for D322H, 30.0% for E48G and 46.0% for A305T, Figure 4.4 Di).

The augmented CaT by the loss-of-function mutations resulted in a 12.4%, 11.0% and 21.4% increase in the active force of atrial myocytes for Y155C, D469E and P488S, respectively as compared to that of WT (Figure 4.4 Cii), which was translated into a profound 31.5%, 27.8% and 57.1% augmentation in maximum SL shortening by the corresponding mutants, respectively (Figure 4.4 Dii).

These effects were further consistently demonstrated in simulations considering the contributions of SACs (Figure 4.5): the gain-of-function mutations exerted impaired contractility to the human atrial myocytes, whereas the loss-of-function mutations mediated positive inotropic effects.

In addition, the inotropic consequences of the mutations were also demonstrated in other types of atrial cells as shown in Figure 4.6. In the figure, the electro-mechanical activities of various types of atrial cells for mutations D322H (the most pronounced gain-of-function KCNA5 mutation) and P488S (the most severe loss-of-function mutation) were compared with those in the WT conditions. It was revealed that the APs from the family of single cell models under both WT and mutant conditions exhibited pronounced heterogeneities in both profiles and durations of AP (Figure 4.6 A) accompanied by noticeable differences in CaT (Figure 4.6 B), active force (Figure 4.6 C) and cell shortening (Figure 4.6 D). The inter-regional heterogeneities in AP and contractilities were in accord with the previous study [38], in which the regional cell models were built on a different baseline model (the Courtemanche et al. model of atrial electrophysiology [37]). It was shown that CT and BB exhibited greater contractility than the cells from the rest of the regions, due to a markedly higher systolic $[\text{Ca}^{2+}]_{\text{i}}$, and CaT. PV and AS cells elicited more depolarised resting potential compared with other cell types, contributing to a smaller channel availability in the fast sodium current and hence reduced excitability. A comparison of the three phenotypes revealed that as compared to the WT, P488S prolonged AP and promoted contractility in all regions, which was accompanied by enhanced electrical and mechanical heterogeneities among them; and that D322H exerted reversed effects in all regions.
Figure 4.4 Simulated inotropic effects of KCNA5 mutations on human atrial myocytes using the baseline model. (A) AP. (B) CaT. (C) Time courses of active force; the data were normalised to the maximum value under the WT conditions. (D) SL shortening. (E) Bar chart plots showing the peak active force relative to the WT case in various mutation conditions. In the simulations, SACs were not incorporated.
Figure 4.5 Simulated inotropic effects of KCNA5 mutations on the human atrial myocytes using the baseline model in the presence of SAC. (A) AP. (B) CaT. (C) Time courses of active force; the data were normalised to the maximum value under the WT conditions. (D) SL shortening. (E) Bar chart plots showing the relative peak active force exerted by the mutants as compared to that by WT.
Figure 4.6 Heterogeneous electro-mechanical activities in the isolated regional atrial cells for the WT (column i), D322H (column ii) and P488S (column iii). (A) AP. (B) CaT. (C) Normalised active force. (D) Sarcomere length shortening. The SACs were not incorporated in these simulations.
Similar effects were also seen in the simulations incorporating SAC (Appendix A Figure A.3). The resting potentials of PV were more markedly increased by the inclusion of $I_{\text{SAC}}$, showing a marked reduction in the overshoot and upstroke velocity of the AP, which could cause excitation conduction failures of the region in tissue.

4.3.3 The mechanisms underlying the inotropic effects of the KCNA5 mutations

In order to investigate the mechanisms underlying inotropic effects of the KCNA5 mutations, a side-by-side comparison was made between effects of mutations on the key ionic currents and the intracellular system regulating CaT, including the L-type calcium current ($I_{\text{CaL}}$), Na$^+$-Ca$^{2+}$ exchanger ($I_{\text{NaCa}}$) RyR release flux, SR uptake flux and SR content, in the absence/presence of SAC. The results are shown in Figure 4.7 (w/o $I_{\text{SAC}}$) and Figure A.4 (w/ $I_{\text{SAC}}$).

In comparison to the WT, it was shown that although the peak amplitude of $I_{\text{CaL}}$ was slightly increased by the secondary effect of the gain-of-function mutations (as has been demonstrated in Chapter 3), the current was substantially smaller during later phases of AP-repolarisation (Figure 4.7 Ai) due to shortened APD; the inward component of $I_{\text{NaCa}}$ was increased during phase-2 and -3 of the AP (Figure 4.7 Bi), exhibiting an enhanced forward mode of the channel to extrude Ca$^{2+}$ out of the cell; consequently the uptake of Ca$^{2+}$ to the SR was reduced, leading to a decreased Ca$^{2+}$ release from the SR in the gain-of-function mutation condition ((Figure 4.7 Ci, Di); a reduction was also seen in the resting SR level, despite the fact that the minimum SR content during APs was not markedly affected by the mutations (Figure 4.7 Ei).

For the condition of loss-of-function mutations, altered AP profile produced a slight decrease in the $I_{\text{CaL}}$ current during the plateau phase of AP (as seen in Chapter 3, and more substantial for P488S; these data are consistent with a previous study of blocking $I_{\text{Kur}}$ in canine atrial cells [157]), but an increased $I_{\text{CaL}}$ during later phases of the AP (Figure 4.7 Aii). These mutations also led to an increased outward component of the $I_{\text{NaCa}}$ during the plateau and early phase-3 of AP (Figure 4.7 Bii), suggesting an enhanced reversed mode of the exchanger which was in favour of Ca$^{2+}$ import. The resting SR levels were markedly increased by the mutations (Figure 4.7 Eii), and so were the RyR and SERCA Ca$^{2+}$ fluxes (Figure 4.7 C-Dii).

Similar alterations were also observed in the presence of $I_{\text{SAC}}$ (Figure A.4).

The time integral of $I_{\text{CaL}}$, $I_{\text{NaCa}}$, $J_{\text{Rel}}$ and $J_{\text{SERCA}}$ during the time course of an AP
Figure 4.7 Simulated time courses of L-type $\text{Ca}^{2+}$ current, $\text{Na}^+\text{Ca}^{2+}$ exchanger, RyR release flux, SR uptake flux and SR content elicited by AP under the WT and mutations w/o SACs. (A) L-type $\text{Ca}^{2+}$ current ($I_{\text{CaL}}$). (B) $\text{Na}^+\text{Ca}^{2+}$ exchanger ($I_{\text{NaCa}}$). (C) RyR release flux ($J_{\text{rel}}$) of the subspace compartment. (D) SR uptake flux ($J_{\text{SERCA}}$) of the bulk cytosol. (E) SR content ($[\text{Ca}^{2+}]_{\text{SR}}$).
under various conditions were obtained as shown in Figure 4.8 and Figure A.5. It was demonstrated that the time integral of $I_{\text{CaL}}$ elicited by the AP was markedly reduced for the gain-of-function mutations vs. WT, indicating smaller $\text{Ca}^{2+}$ entries through the channel. In addition, the integral of $I_{\text{NaCa}}$ manifested slightly increased activities of the exchanger working in the forward mode; the integrals of RyR and SERCA activities were both reduced by the gain-of-function mutations. For the loss-of-function mutations,
the integral of $I_{\text{Cal}}$ was slightly (more noticeably for P488S) reduced for the first 100 ms following the upstroke of the AP, and was markedly increased in the later phases of AP; the time integrals of the Ca$^{2+}$ fluxes released from and uptake into the SR were both augmented; the integral of $I_{\text{NaCa}}$ shows a consistently and markedly enhanced reversed mode dominated the exchanger through all the repolarisation phases of the AP, which could directly contribute to the increased systolic $[\text{Ca}^{2+}]_i$.

To reveal the individual contribution of $I_{\text{Cal}}$ and $I_{\text{NaCa}}$ to the $[\text{Ca}^{2+}]_i$ for the different genotypes, the time integrals of the two currents at the peaking time of the $[\text{Ca}^{2+}]_i$ were plotted in Figure 4.9 (w/o $I_{\text{SAC}}$) and Figure A.6 (w/ $I_{\text{SAC}}$).

A marked reduction in the time integral of $I_{\text{Cal}}$ until the peaking time of $[\text{Ca}^{2+}]_i$ was observed for the gain-of-function mutations: by 56.4 A/F·ms or 18.2% for D322H, 29.5 A/F·ms or 9.5% for E48G and 47.1 A/F·ms or 15.2% for A305T, as shown in Figure 4.9 Ai). Accordingly, the time integral of $I_{\text{NaCa}}$ until peaking of $[\text{Ca}^{2+}]_i$ was 7.9, -2.6, 1.9, 0.32 A/F·ms for WT, D322H, E48G and A305T, respectively, which was substantial but less prominent compared with the reductions seen in the integrals of $I_{\text{Cal}}$ under the same conditions.

The time integral of $I_{\text{Cal}}$ until peaking of $[\text{Ca}^{2+}]_i$ was also altered by the loss-of-function mutations: Y155C and D469E slightly increased the integral (by 11.2 and 13.4 A/F·ms, and 3.6% and 4.3%, respectively), whereas it was reduced by the mutant P488S (decreased by 30.6 A/F·ms or 9.9%), by which $I_{\text{Kur}}$ was almost abolished. With these mutations, the relative changes in the time integral of $I_{\text{NaCa}}$ until peaking of $[\text{Ca}^{2+}]_i$ compared to WT were 10.3, 9.4 and 23.8 A/F·ms for Y155C, D469E and P488S, respectively.

The total Ca$^{2+}$ entry through the two currents following the evoking of an AP and peaking time of CaT were compared in the bottom panels of Figure 4.9 and Figure A.6. The relative total Ca$^{2+}$ entry contributing to the systolic $[\text{Ca}^{2+}]_i$ was calculated as $\int -I_{\text{Cal}}dt + 2\int I_{\text{NaCa}}dt$ and normalised to the value of WT. Additionally, the percentage-wise contribution of $I_{\text{NaCa}}$ towards the total Ca$^{2+}$ entry preceding the peaking of $[\text{Ca}^{2+}]_i$ was shown in hatched shadings. In comparison to the WT, the total Ca$^{2+}$ entry until the peaking time of $[\text{Ca}^{2+}]_i$ was profoundly reduced by the gain-of-function mutations (by 23.7%, 12.7% and 19.1% for D322H, E48G and A305T, respectively), while it was increased by the loss-of-function mutations (by 9.7%, 9.8% and 5.2% for Y155C, D469E and P488S, respectively). Furthermore, the relative contribution to the
Figure 4.9 The individual contribution of $I_{CaL}$ and $I_{NaCa}$ to the systolic $[Ca^{2+}]_i$ in the atrial cells for the WT and mutations without $I_{SAC}$. (A) The time integral of $I_{CaL}$ at the peaking time of $[Ca^{2+}]_i$. (B) The time integral of $I_{NaCa}$ at the peaking time of $[Ca^{2+}]_i$. (C) Relative total $Ca^{2+}$ entry contributing to the systolic $[Ca^{2+}]_i$, which was calculated as $\int -I_{CaL} \, dt + 2 \cdot \int I_{NaCa} \, dt$ and normalised to the value of WT; specially, the contribution of $I_{NaCa}$ to the $Ca^{2+}$ entry preceding the peaking of $[Ca^{2+}]_i$ was shown in hatched shadings.

total $Ca^{2+}$ was also affected by the mutations: for WT 4.8% of $Ca^{2+}$ entered the cytosol through the exchanger preceding the peaking of $[Ca^{2+}]_i$; whereas the values were -1.6% (a negative value indicates the forward mode dominates the exchanger), 1.2% and less than 0.1% for D322H, E48G and A305T, respectively. For the loss-of-function mutations, the relative contributions were 11.2%, 10.7% and 19.5% for Y155C, D469E and P488S, respectively. As a result, of the reductions in $Ca^{2+}$ entry preceding the peaking of $[Ca^{2+}]_i$ observed in the gain-of-function mutations, a fraction of 27.2%, 29.0% and 24.5% could be attributed to the increased forward mode of $I_{NaCa}$ for D322H, E48G and A305T; whereas the same metrics were measured to be 64.9%, 58.4% and 280.9% for Y155C, D469E and P488S, respectively. Therefore, the reduction of $Ca^{2+}$ entry seen
in the gain-of-function mutations were dominated by the decreases in $I_{\text{CaL}}$, whereas the enhanced reverse modes of $I_{\text{NaCa}}$ were more prominent in the increased Ca$^{2+}$ entry by the loss-of-function mutations. These results provide insights into the mechanisms of inotropic effects exerted by the KCNA5 mutations.

4.3.4 The effects of $I_{\text{Kur}}$ properties on atrial contractile function

In order to have a more general understanding of the dependence of atrial contractility on the properties of $I_{\text{Kur}}$, simulations were performed to quantify the relative cell shortenings with respect to varying parameters in $I_{\text{Kur}}$. The results are shown in Figure 4.10 (w/o SAC) and Figure A.7 (w/ SAC).

Simulations varying the activation curve of $I_{\text{Kur}}$ alone demonstrated that a positive shift in the $V_{1/2}$ resulted in an increased relative cell shortening (6.8% vs. 8.4% vs. 9.8% w/o $I_{\text{SAC}}$ and 8.1% vs. 9.6% vs. 10.9% w/ $I_{\text{SAC}}$ for $V_{1/2} = -11.0$, -6.0 and -1.0 mV, respectively).

In panel A and B of Figure 4.10 and Figure A.7, a reduction in the conductance of $I_{\text{Kur}}$ led to a substantial increase (adding to a further 7% in the relative cell shortening) in the cell contractility, whereas the augmented current densities exerted markedly blunted cell shortenings; A steeper steady-state activation curve ($K_{\text{Activation}}$ scaled by 0.2-0.8 fold) in $I_{\text{Kur}}$ contributed to an increased cell shortening, whilst shallower steady-state activations exhibited negative inotropic effects. Slightly decreased cell shortenings were also observed for the simulations with slower kinetics in the activation gate of $I_{\text{Kur}}$. In panel C, $K_{\text{Activation}}$ and $r_{\text{Activation}}$ were varied while the conductance was kept the same as control. Consistent negative inotropic effects were demonstrated for slower kinetics in the activation/deactivation of $I_{\text{Kur}}$. The effects of varying $K_{\text{Activation}}$ within the parameter space were shown to be dependent on the $V_{1/2}$: for positively shifted $V_{1/2}$ shallower $K_{\text{Activation}}$ reduced the contractility of atrial cells, while both an increase and decrease in $K_{\text{Activation}}$ from its control value could lead to increased cell shortenings for the $V_{1/2}$ at the control or left-shifted values. Nevertheless, it should be noted that these effects were shown to be relatively small in comparison to the results of varying the conductance.

Further simulations incorporating random parameters generated using the Latin hypercube sampling method were performed to examine the effects of these parameters on cell contractility over the complete parameter space. Both Pearson’s correlation and
Figure 4.10 The simulated dependence of relative cell shortening on the parameters of $I_{Kur}$. The responses of the relative cell shortening to (A) the varying conductance and $K_{Activation}$; (B) the varying conductance and $\tau_{Activation}$; (C) the varying $K_{Activation}$ and $\tau_{Activation}$. The ranges of scaling are shown as labelled. In columns (i-iii), the $V_{1/2}$ of steady-state activation was shifted as labelled. In each panel the difference in cell contractility was quantified as the change in relative cell shortening with respect to the relative cell shortening under the control parameters (both not scaled, labelled with *).

Spearman’s correlation, two primary measures of correlation widely used in the analysis of biological data [237], were used as an indication of the correlation between two
variables of interest. The correlations between multiple biomarkers of atrial APs and peak $I_{Kur}$, and cell contractility are illustrated in Table 4.2.

It was demonstrated that both APD and plateau potential were positively correlated with the relative cell shortening ($P < 0.001$), and the peak $I_{Kur}$ was strongly negatively correlated with the contractility of atrial myocytes ($P < 0.001$). Furthermore, the cell shortening was demonstrated to have the highest strength of association with APD$_{30}$ as compared to other biomarkers of AP.

Table 4.2 The correlation coefficients between the relative cell shortening of atrial myocytes and AP biomarkers measured using the Pearson’s correlation and Spearman’s correlation.

<table>
<thead>
<tr>
<th></th>
<th>APD$_{90}$</th>
<th>APD$_{50}$</th>
<th>APD$_{30}$</th>
<th>Plateau Potential</th>
<th>Peak $I_{Kur}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>w/o I$_{SAC}$</td>
<td>Pearson $r$</td>
<td>0.7696</td>
<td>0.9168</td>
<td>0.9283</td>
<td>0.8848</td>
</tr>
<tr>
<td></td>
<td>Spearman $\rho$</td>
<td>0.7357</td>
<td>0.9006</td>
<td>0.9387</td>
<td>0.8952</td>
</tr>
<tr>
<td>w/ I$_{SAC}$</td>
<td>Pearson $r$</td>
<td>0.7620</td>
<td>0.9100</td>
<td>0.9750</td>
<td>0.8880</td>
</tr>
<tr>
<td></td>
<td>Spearman $\rho$</td>
<td>0.7312</td>
<td>0.8901</td>
<td>0.9852</td>
<td>0.9003</td>
</tr>
</tbody>
</table>

4.3.5 The inotropic effects of the KCNA5 mutations at the organ level

The implications of the inotropic effects exhibited by the KCNA5 mutations at the cellular level were demonstrated using a 3D anatomically accurate electro-mechanical model of human atria incorporating heterogeneous regional single cell models. In the simulations, the atria were paced at 1Hz from the SAN region. Both scenarios of excluding or incorporating I$_{SAC}$ were modelled.

Figure 4.11 and Figure A.8 illustrate the simulated atrial electromechanical contraction at the whole organ level in the absence/presence of I$_{SAC}$ for WT, D322H and P488S. For the sake of appreciating the deformations in the tissue, the undeformed atrial mesh was superimposed and shown as a reference.

Without considering I$_{SAC}$ (Figure 4.11), at 100 ms the whole atria were almost entirely electrically activated, which was not noticeably affected by the mutations; at 200 ms, a substantial atrial contraction was observed for WT, which was more and less
pronounced for P488S and D322H, respectively; the atrial deformations peaked between 200 and 250 ms; at 300 ms the atria relaxation was underway for WT but was nearly completed for D322H whilst the relaxation just took place for P488S; These results demonstrated that the atrial repolarisation and mechanical relaxation were accelerated by the gain-of-function mutation, whereas they were delayed by the loss-of-function, which is concordant with the respective alterations to the APD and CaT by the mutations observed at single cell level; at 600 ms fully electrically and mechanically recovered atria were seen for all phenotypes.

Following the inclusion of ISAC (Figure A.8), the atrial electrical activation was slowed down compared with the conditions without ISAC, which is attributed to a reduced INa availability caused by elevations in the resting potentials of atrial myocytes (Figure 4.5, Figure A.3). At 100ms, a majority of RA and part of LA tissue were depolarised. A substantial slowing of conduction and propagation failure was seen in the PV regions, resulting from a substantial reduction in excitability by the incorporation of ISAC (Figure A.3). Similar time courses of atrial contraction as the simulations w/o ISAC were observed, whilst a noticeable augmentation in atrial deformations was consistently observed across the WT and mutations for the simulations w/ ISAC than w/o.

The time sequences of atrial electrical activation w/ and w/o ISAC are shown in Figure 4.12. The activation sequence was not markedly altered by the mutations, and therefore only the results for WT are shown. W/o ISAC, the atria were fully activated at 132 ms, and concordant with previous simulations (Chapter 3, and [35]). The inclusion of ISAC induced a 20-ms delay in the activation and abnormal conduction in the PV regions.

The time course of atrial output was computed from the emptying volume during the electro-mechanical activities and is shown in Figure 4.13. These results show that the atrial output was markedly impaired by the gain-of-function mutations in KCNA5 whereas it was markedly increased by the group of variants impairing the activities of Kv1.5. In the simulations w/o ISAC, the relative decrease in emptying volume compared with the WT conditions were 46.3%, 27.7% and 42.4% for D322H, E48G and A305T, respectively, whereas a 25.8%, 22.8% and 48.9% increase in the atrial emptying volume were observed in the simulations with the loss-of-function mutations (Y155C, D469E and P488S). Similar changes were demonstrated in the simulations w/ ISAC. These changes to the atrial contraction volume were attributable to the alterations
Figure 4.11 Snapshots of simulated atrial electromechanical contraction superimposed on the undeformed atrial mesh (in grey and indicated with arrows) under the WT, D322H and P488S conditions w/o $I_{\text{SAC}}$. The simulated time following the application of an external stimulation from the SAN region is labelled in the left of the panels. Insert: time course of computed atrial volume.
to the active force and cell shortening by the mutations observed at the cellular level (Figure 4.4 and Figure 4.5). In addition, the time spans of contraction for the gain-of-function mutations were attenuated whereas they were extended by the loss-of-function mutations in comparison to the WT conditions, which further enhanced the inotropic effects brought about by the respective mutations.

Figure 4.12 Simulated activation time sequences of the atria (A) in the absence and (B) in the presence of I_{SAC} under the WT conditions. In (B), the PV regions failed to initiate normal excitations (coloured in grey).

The rate of volume emptying was computed as an indicator for the atrial contraction velocity, as shown in Table 4.3. The emptying rate was defined as $dV_{emptying}/dt|_{max}$, where $V_{emptying}$ is the atrial emptying volume shown in Figure 4.13. It was shown that complementary to the changes to the emptying volume, the volume emptying rate was also altered by the mutations. In comparison to the WT, the gain-of-function mutations induced a pronounced slowing down in atrial contraction (by 41% for D322H and A305T, and 23% for E48G, respectively in the absence of $I_{SAC}$). The metric was increased by the loss-of-function mutations and the percentages were 32%, 19% and 51% for Y155C, D469E and P488S, respectively. The rate in atrial
emptying was consistently accelerated by the incorporation of $I_{SAc}$ (Table 4.3).

Collectively, the gain-of-function mutations impaired the atrial contractile functions by reducing atrial emptying volume, attenuating the time span of contraction and slowing the emptying rate. In contrast, an increased atrial volume accompanied by an accelerated emptying rate and prolonged atrial contraction duration were exhibited by the loss-of-function mutations.

Figure 4.13 Time course of atrial output computed from the emptying volume of both chambers during the electro-mechanical excitation for the WT and mutations. (A) w/o and (B) w/ $I_{SAc}$. In all panels, the curves were normalised to the maximum volume change of WT in (A).
Table 4.3 Normalised maximum atrial volume emptying rate for WT and the mutations. The rates were normalised to the maximum value of WT w/o $I_{\text{SAC}}$.

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Gain-of-function mutations</th>
<th>Loss-of-function mutations</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>D322H</td>
<td>E48G</td>
<td>A305T</td>
</tr>
<tr>
<td>w/o $I_{\text{SAC}}$</td>
<td>1.00</td>
<td>0.59</td>
<td>0.77</td>
</tr>
<tr>
<td>w/ $I_{\text{SAC}}$</td>
<td>1.16</td>
<td>0.62</td>
<td>0.82</td>
</tr>
</tbody>
</table>

### 4.4 Discussion

In this Chapter, multi-scale biophysically detailed models of atrial electrophysiology and mechanical functions were used to assess the impact of KCNA5 mutations on the contractile function of the atria. This involves demonstrations of inotropic effects brought about by the mutations at both the cellular and whole organ level, as well as investigations on the mechanisms underlying these effects.

#### 4.4.1 The electromechanical model of the atria

The present Chapter started with constructing an electromechanical model of human atrial myocytes. The Rice et al. myofilament model [95] describing cross-bridge dynamics and $\text{Ca}^{2+}$ binding/unbinding kinetics was updated to reproduce the force-$\text{Ca}^{2+}$ relation observed in native human atrial myocytes (Figure 4.1) and was subsequently coupled to the Colman et al. model of atrial electrophysiology [35]. Mechanoelectrical feedback was introduced to the model by the inclusion of $I_{\text{SAC}}$, enabling a more comprehensive assessment of the inotropic effects with/without mechanoelectrical feedback. The electromechanical model produced qualitatively comparable results in the time course of the active force development seen in human atrial cells reported in a previous experimental study. The simulated relative cell shortening was validated against a number of experimental observations in canine atria in the absence of human atrial data and was shown to be both qualitatively and quantitatively comparable to the experimental data.

A family of electromechanical models incorporating regional electrical heterogeneities in the atria was derived from the Colman et al. family of human atrial
models [35]. The recently published 3D anatomically accurate model of atrial electro-mechanics was updated with the new family of single cell models and improved geometry. The 3D model was fully parallelised with MPI, achieving a speedup of more than 13-fold over a single threaded implementation on a 40-hyperthreaded computing cluster.

4.4.2 The inotropic effects of the KCNA5 mutations

4.4.2.1 Gain-of-function mutations

Simulations with gain-of-function mutations of $I_{Kur}$ demonstrated negative inotropic effects of the group of genetic variants both at the cellular and organ level.

At the single cell level, the gain-of-function mutations led to marked reductions in the CaT resulting from modulations to the AP morphology by increased current densities during phase-1 and 2 repolarisations of AP (Figure 4.4, Figure 4.5). The impairments in the CaT due to mutations led to markedly reduced active force productions and hence markedly decreased relative cell shortenings (by 48.2%, 30.0% and 46.0% in comparison to WT for D322H, E48G and A305T, respectively; Figure 4.4).

At the organ level, the negative inotropic effects brought about by the mutations manifested markedly smaller deformations in the atrial myocardium (Figure 4.11), notable reductions in the atrial emptying volume (by 46.3%, 27.7% and 42.4% for D322H, E48G and A305T, respectively, Figure 4.13) accompanied by decelerated atrial emptying rates (Table 4.3), and attenuated time spans of atrial contraction, demonstrating impaired atrial contractile functions. The slowing down in atrial volume emptying rates could be linked with decelerated atrial wall contraction and hence slower blood flow in the upper chambers of the heart, which might be favourable to blood stasis and thus formation of new thrombi as occurs in AF [238]. At rest, the contraction of the atria contributes approximately 20% of stroke volume in the left ventricle [239]. A reduction in atrial contraction could also induce decreased ventricle outputs. Note that impaired atrial contraction has also been associated with AF [240].

The mechanism underlying the impairment to atrial contractility by the gain-of-function mutations was revealed by analysing the amount of Ca$^{2+}$ entry and export. The modulations by these mutations to the atrial electrophysiology highlighted a decrease in Ca$^{2+}$ entry through $I_{CaL}$ resulting from the modified AP shape and an enhanced Ca$^{2+}$
export via increased $I_{\text{NaCa}}$ working in the forward mode. Consequently, the amount of Ca$^{2+}$ contributing to the activation of RyR was lessened. The alterations to these two major currents facilitating the Ca$^{2+}$ entry and export also resulted in a reduction in the SR content as a secondary effect, weakening the Ca$^{2+}$ release flux from the SR through the RyR to the cytosol. Collectively, these mutations resulted in reduced Ca$^{2+}$ induced Ca$^{2+}$ release (CICR) in atrial myocytes. The simulated time integral of $I_{\text{CaL}}$ and $I_{\text{NaCa}}$ indicated that the reduction in $I_{\text{CaL}}$ dominated the reduction of the net Ca$^{2+}$ entry.

4.4.2.2 Loss-of-function mutations

In contrast, the loss-of-function mutations in KCNA5 mediated positive inotropic effects in the human atria.

At the cellular level, the augmented CaT by the mutations resulted in a 12.4%, 11.0% and 21.4% increase in the active force for Y155C, D469E and P488S, respectively (Figure 4.4 Cii), which was responsible for a profound 27.8% to 57.1% augmentation in the relative cell shortening for the mutations vs. WT (Figure 4.4 Dii).

3D simulations demonstrated stronger atrial contraction for the loss-of-function mutations, which was supported by noticeably greater deformations in the myocardium, a 22.8% to 48.9% increase in the atrial emptying volume as well as a similar increase in the atrial emptying rate (Table 4.3), and an extension of the time span of atrial contraction. Collectively, these effects are expected to contribute to an increase in atrial output and accelerated blood flow in the atria. Furthermore, the increased atrial contractile function could also enhance ventricular output via increasing the filling of ventricles.

The investigations into the underlying mechanisms of inotropic effects mediated by these mutations highlighted an important role of $I_{\text{NaCa}}$ in atrial contractility. In these simulations, the net Ca$^{2+}$ entry until peaking of CaT contributing to the systolic Ca$^{2+}$ was increased, which was accompanied by an elevated SR content, leading to enhanced CICR activities. The increased net Ca$^{2+}$ entry was primarily attributed to the increased $I_{\text{NaCa}}$ working in the reverse mode which is favourable for Ca$^{2+}$ entry. Specifically for P488S, the time integral of $I_{\text{CaL}}$ that could directly contribute to the systolic Ca$^{2+}$ was in fact reduced and was compensated by the increased Ca$^{2+}$ entry through $I_{\text{NaCa}}$.

It is noteworthy that the simulated positive inotropic effects are concordant with previous experimental studies on effects of $I_{\text{Kur}}$ block in the atria. Following applications of AVE0118, an $I_{\text{le}}/I_{\text{Kur}}$ blocker, profound increases in the atrial
contractility were observed in the atria of goats [241], dogs, and humans [157]. In the study using atrial myocytes from human and canine hearts, Schotten et al. [157] revealed the enhanced atrial contractility seen following $I_{Kur}$ block was attributable to a promoted $Ca^{2+}$ entry via the reverse mode of $I_{NaCa}$. In the present study, the simulations suggest that loss-of-function mutations in $I_{Kur}$ induced positive inotropic effects through a similar mechanism. Aligned with the experimental data [157,241], the modelling data support that $I_{Kur}$ block is a potentially valuable strategy in the effort to enhance atrial contractility, which is desirable for the treatment of AF – especially following cardioversion of AF.

4.4.3 Parameter analysis on role of $I_{Kur}$ in atrial contractility

The key parameters of $I_{Kur}$ were varied over ranges of parameter spaces in order to expand the effects demonstrated by the mutations to general scenarios, and thereby to better understand the role of electrophysiological properties of $I_{Kur}$ on atrial electromechanical activities. It was shown that the conductance, slope and $V_{1/2}$ of steady-state activation and kinetics of the activation gate of $I_{Kur}$ play important roles in atrial contractility: increased conductance, flatter steady-state activation, left shifted activation-voltage relation and slower activation/deactivation kinetics result in compromised atrial contractility. Among these parameters, the conductance dominated the modulations of the current on the active contraction of atrial myocytes. Furthermore, the simulations incorporating random parameters showed that APD$_{30}$ correlated more closely with the relative cell shortening than did APD$_{90}$, indicating that APD$_{30}$ is a better indicator for atrial contractility.

4.4.4 Role of $I_{SAC}$

A non-selective stretch-activated current, $I_{SAC}$, was incorporated to form a feedback to the electrical activities in response to mechanical deformations. Both simulations w/o and w/ $I_{SAC}$ were performed to investigate the inotropic effects of the mutations under both conditions.

A similar stretch-activated current was incorporated in previous modelling studies modelling the electromechanical activities of the heart [209,211,215]. Concordant with these simulations, the inclusion of $I_{SAC}$ depolarised the resting potential of AP which contributed to reduced excitabilities of myocytes. At the organ level, this reflected as a
substantial conduction slowing down and longer activation time within the whole atria due to a reduction in the conduction velocity. Furthermore, it was observed that the elevation of the resting potential of PV cells following the inclusion of $I_{\text{SAC}}$ was the most substantial, which could be explained by a smaller current density of $I_{K1}$ in the region [242]. In 3D, the effects of incorporating $I_{\text{SAC}}$ in the PV regions manifested as a substantial conduction delay and abnormal activation in the region, suggesting a possible mechanism of arrhythmogenesis in the PV regions under stretch.

A slight increase in the cell contractility was consistently seen in the simulations w/ $I_{\text{SAC}}$ than w/o, which is in agreement with previous simulations [215].

4.4.5 Novelty and relevance to previous studies

The present study highlights the role of genetic mutations in potassium channels in modulations of atrial contractility and represents, to the best of the author’s knowledge, the first attempt to investigate such modulations using biophysically detailed and anatomically accurate multi-scale models. The simulations expand our understanding on the role of $I_{\text{Kur}}$ and relevant mutations in atrial contractility.

Although extensive simulations on the functional role of $I_{\text{Kur}}$ in the atria have been performed [186,188,243,244], these studies focused on the impact of modulations of the current on electrical activities of the atria without considering atrial contractile function. Effects of $I_{\text{Kur}}$ block on atrial contraction have been investigated experimentally both at cellular and organ level [157,241], providing mechanistic insights into the positive inotropic effects of $I_{\text{Kur}}$ block. However, the impact of increased $I_{\text{Kur}}$ by mutations on atrial mechanical functions is not yet known.

Although electromechanical models have been extensively developed and used for investigating electromechanical activities of the heart [210,211,215,245–248], these simulations have mainly focused on the ventricles, and multi-scale simulations of atrial electromechanics are relatively rare [38]. The recently published 3D atrial model [38] incorporating an anatomically accurate structure and heterogeneous electrical properties was inherited and updated in the present study. The updated model shows substantial improvement regarding computational efficiency. Using the multi-scale electromechanical models, the inotropic effects of the KCNA5 mutations were investigated, expanding understanding of the functional impacts of these mutations on atrial mechanical activities in addition to their effects on the electrical activities.
4.4.6 Limitations

The limitations of the single cell model of electrophysiology [35], myofilament model [95], and 3D atrial model [38] have been discussed in detail. Hence, only limitations specific to the present study are discussed here.

In the present study, the inotropic effects of KCNA5 mutations were compared with WT. It was assumed that these mutations do not mediate any changes other than alterations to $I_{Kur}$ alone, i.e. the contraction apparatus of the myocytes, the electrical coupling in tissue, mechanical properties and atrial structure were not affected by the mutations. The validity of such assumptions necessitates further experimental investigations.

The simulated diastolic force profile in single cell simulations did not perfectly match experimental curves (Figure 4.3), which might be a limitation produced by the profile of the CaT.

Another limitation lies in the validations to the single cell relative shortening. In the absence of experimental data from human atrial cells, the model was validated against multiple studies in canine atria. However, such an approach of incorporating and comparing data from alternative species has been adopted in a number of previous studies [35,37,154,223]. Furthermore, the main objective of this chapter was to compare the atrial contractility between phenotypes of KCNA5. The results are not compromised by the baseline model.

Mechanoelectrical feedback was incorporated by the inclusion of a non-selective stretch-activated channel following previous simulations [209,211,215]. Other effects introduced by mechanoelectrical feedback such as stretch-induced changes to the intracellular $Ca^{2+}$ handling system [205] and modulation of the AP through alterations to the coupled cardiac fibroblasts [206,207] were not considered. The impact of incorporating such effects together with the role of the interplay between the mutations and $I_{SAC}$ in the arrhythmogenicity of these mutations may warrant further investigations and is beyond the scope of the present study.

4.5 Summary

In this chapter, a single cell model of human atrial electro-mechanics was developed and integrated into a 3D anatomical model of atrial electromechanical coupling. The
models allowed for assessments of the functional impact of KCNA5 mutations on the contractile functions of human atria at both cellular and organ level.

The present study highlights an important role of $I_{Kur}$ in modulating human atrial contractility. The gain-of-function KCNA5 mutations resulted in a profound impairment to atrial contractility, primarily through reducing the $Ca^{2+}$ entry via $I_{CaL}$. The loss-of-function KCNA5 mutations mediated positive inotropic effects though promoting the $Ca^{2+}$ entry via increased reverse mode of $I_{NaCa}$. These findings add insights into the functional impacts of genetic variations in KCNA5 on human atrial contractility further to the modulations of the mutations on atrial electrical activities.
Chapter 5

Antiarrhythmic benefits of combined block of sodium ($I_{Na}$) and ultra-rapid delayed rectifier potassium ($I_{Kur}$) channels in human chronic atrial fibrillation

**Addendum** This Chapter has been presented in the form of a conference proceeding [249]:


The text has been reworded where possible.

Atrial fibrillation (AF) is the most common cardiac arrhythmia. Developing effective and safe anti-AF drugs remains an unmet challenge. Atrial-selective block of the ultra-rapid delayed rectifier potassium current ($I_{Kur}$) and Na$^+$ channel ($I_{Na}$) has been reported to possess antiarrhythmic effects. However, the impact of blocking these channels in atrial fibrillation remains to be elucidated.

In this Chapter, the antiarrhythmic effect of $I_{Kur}$ and $I_{Na}$ block on human atria with chronic atrial fibrillation conditions was assessed individually first in silico. The effect of $I_{Na}$ block was also simulated in the ventricles to facilitate a quantification of atrial-selectivity of such a block. The inhibitive effects on the atria exerted by acacetin, a multichannel blocker primarily inhibiting $I_{Kur}$ while less potently blocking $I_{to}$, $I_{Kr}$ and $I_{Ks}$, was simulated, enabling investigations on multi K$^+$-channel block in addition to pure blockade of $I_{Kur}$. The results show that either of the blockers exhibited antiarrhythmic effects under AF conditions. The application of $I_{Kur}$ blocker rendered substantial APD
prolongation. Rate- and atrial-selective inhibitions of $I_{Na}$ were observed in the simulated $I_{Na}$ block. The combined multi $K^+$-channel block mimicking effect of acacetin exhibited synergistic APD prolongations that were greater than pure $I_{Kur}$ block. Synergistic effects of $I_{Na}$ inhibition were also observed in combined block of $I_{Na}$ and $I_{Kur}/K^+$-channels. These effects were also demonstrated in 2D simulations, where combined blockers showed an enhanced efficiency in terminating re-entrant excitation waves, exerting improved antiarrhythmic effects in the human atria as compared to a single-channel block. This Chapter highlights synergistic antiarrhythmic benefits of combined block of $I_{Kur}$ and $I_{Na}$ as well as combined multi $K^+$-channel block, which may be a valuable strategy for the treatment of AF.

5.1 Introduction

Despite recent advances in the management of AF, the world’s most common cardiac arrhythmia [11,12], developing effective and safe antiarrhythmic drugs remains challenging [12,47], which is usually fraught with potential complications including promoting ventricular arrhythmias [48–50] by prolonging action potential durations (APD) and QT intervals measured from ECGs, which may lead to life-threatening consequences. Developing atrial-selective drugs is a current strategy for the treatment of AF [250]. The atrial and ventricular fast Na$^+$ currents ($I_{Na}$) exhibit different properties in the voltage-dependent inactivation of the channel, mediating the possibility for atrial-selective Na$^+$ channel block [250–252]. Previous simulation studies demonstrated that by optimising the state-dependent Na$^+$-channel blocking parameters, the atrial-selective block of $I_{Na}$ could be achieved to maximise the pharmaceutical effects on the atria while minimising proarrhythmic actions in the ventricles [12,47].

The ultra-rapid delayed rectifier potassium current ($I_{Kur}$) carried by the $K_v1.5$ channel contributes to repolarization in the atria but not in the ventricles [186,253]. Recent studies suggest that the atrial-selective blockade of $I_{Kur}$ is a potentially effective strategy for the pharmacological treatment of AF [254–257]. However, the efficacy of $I_{Kur}$ block in the treatment of AF remains controversial [189]. Nevertheless, multiple $I_{Kur}$ blockers have been developed over the decades [255,256,258,259]. In addition, $I_{Kur}$ blockers may also inhibit other potassium channels including $I_{Kur}$, $I_{to}$ and $I_{K,Ach}$ [189]. Examples include AVE0118 [260], AVE1231 [261], AZD7009 [262], and acacetin
Among these channel blockers, acacetin, a natural flavone initially isolated from a traditional Chinese medicine *Xuelianhua*, potently blocks $I_{Kr}$, $I_o$ and $I_{K,ACH}$, while it has a smaller potency in inhibiting $I_{Kr}$ and $I_{Ks}$ [254], similar to AVE0118 [241,260]. Acacetin has been demonstrated to be a promising atrial-selective agent for the treatment of AF [254]. The actions of acacetin on human atrial electrophysiology, especially in the presence of AF-induced electrical remodelling, remain to be elucidated. Furthermore, as most $I_{Kr}$ blockers inhibit other potassium channels, whether the “extra” inhibitive actions present favourable antiarrhythmic effects has not been investigated thoroughly. A better understanding of the role of these “extra” inhibitive effects could provide insights into evaluating and developing antiarrhythmic drugs.

The multiple-channel blockade is receiving a growing interest as empirical observations suggest that multi-channel blockers generally mediate more effective antiarrhythmic effects [12,263,264]. A recent study with mathematical simulations and experiments on canine hearts suggest that adding potassium channel block enhances the AF-selective anti-AF effects obtainable from optimised $I_{Na}$ blockade. However, the effects of combined administrations of $Na^+$ and $I_{Kr}$ blocks in the human atria, especially in the presence of chronic atrial fibrillation remodelling, remain to be elucidated. In the present study, it is hypothesised that combined block of $I_{Na}$ and potassium channels (predominantly $I_{Kr}$) produce antiarrhythmic benefits over the applications of either individual blocker.

There are two aims of the present study: (i) to dissect the effects of $I_{Kr}$ blockers on atrial electrophysiology under cAF conditions; and (ii) to assess the antiarrhythmic effects of combined applications of $I_{Na}$ and $I_{Kr}$ blockers.

Acacetin was considered to be a representative $I_{Kr}$ blocker in the present study. To dissect the functional effects of pure $I_{Kr}$ block and effects of combined potassium channel block by acacetin on human atrial electrophysiology, the drug actions of acacetin were modelled by considering the effects of acacetin on (a) $I_{Kr}$ only, and (b) all the respective potassium currents. This approach allows for modelling the effects of selective $I_{Kr}$ block as well as uncovering the role of “extra” inhibitive effects of acacetin on potassium channels other than $I_{Kr}$.

Following previous studies [12,47], $I_{Na}$ block was simulated using a state-dependent model with varying kinetic parameters. This approach allows for investigations into the role of the kinetic parameters of $I_{Na}$ blockers in the drug actions, and effects of combined $I_{Kr}$ block on the atrial selectivity of $Na^+$-channel block.

*Haibo Ni*
5.2 Methods

5.2.1 Modelling human electrophysiology

First, the Colman et al. model of human atrial electrophysiology [35] was updated. A list of modifications to the model is given in Table 5.1. The steady-state activation of \( I_{Kur} \) was updated based on recently published data [56]:

\[
a_{inf} = \frac{1.0}{1.0 + \exp\left(\frac{V + 5.52}{8.81}\right)}
\]

(5.1)

where \( a_{inf} \) is the steady-state activation of \( I_{Kur} \), and \( V \) is the membrane voltage. The rest of the \( I_{Kur} \) formulation was identical to that described in Chapter 3.

To simulate the effects of cAF-induced electrical remodelling, a cAF model was built based on the parameters from [35] was incorporated into the updated atrial single cell model (Table 5.1).

In order to assess the atrial-selectivity of \( \text{Na}^+ \)-block, the effects of \( \text{Na}^+ \) block were also evaluated in human ventricles. In simulating the effects of \( \text{Na}^+ \) blockers on human ventricular AP, the mathematical model published by O’Hara et al. [36] was adopted. A recent benchmark study on the behaviours of human ventricular models in tissue demonstrated that O’Hara et al. model produced a much smaller upstroke velocity of AP and a slower conduction velocity in tissue, presumably due to the \( I_{Na} \) formulation used in the model. Therefore in the present study, the \( I_{Na} \) formulation in the ventricular model was replaced with that employed by Luo and Rudy [265], which has also been implemented in [35].

In the single cell simulations presented in the Chapter, the virtual single cells were paced for 1,500 s to ensure an equilibrium state was reached, after which the AP, current traces, and state variables were recorded for analysis.
Table 5.1 Parameters for the updated model of human atrial electrophysiology under SR (normal) and cAF conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SR conditions Relative to the Colman et al. model [168].</th>
<th>cAF conditions Relative to the SR model parameters based on [35].</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_{bNa})</td>
<td>+ 70%</td>
<td>No change</td>
</tr>
<tr>
<td>(I_{Cap})</td>
<td>+ 26%</td>
<td>No change</td>
</tr>
<tr>
<td>(I_{NaCa})</td>
<td>+ 40%</td>
<td>+ 40%</td>
</tr>
<tr>
<td>(I_{NaK})</td>
<td>+ 28%</td>
<td>No change</td>
</tr>
<tr>
<td>(I_{CaL})</td>
<td>- 24%</td>
<td>- 65%</td>
</tr>
<tr>
<td>(I_o)</td>
<td>+ 5%</td>
<td>- 65%</td>
</tr>
<tr>
<td>(I_{Kur})</td>
<td>- 10%</td>
<td>- 50%</td>
</tr>
<tr>
<td>(K_{SR, leak})</td>
<td>- 50%</td>
<td>+ 50% [266]</td>
</tr>
<tr>
<td>(D_{Ca^{2+}})</td>
<td>+ 150%</td>
<td>No change</td>
</tr>
<tr>
<td>(I_{K1})</td>
<td>No change</td>
<td>+ 110%</td>
</tr>
<tr>
<td>(I_{Ks})</td>
<td>No change</td>
<td>+ 100%</td>
</tr>
<tr>
<td>SERCA</td>
<td>- 25%</td>
<td>- 40% [266]</td>
</tr>
<tr>
<td>RyR</td>
<td>No change</td>
<td>+ 200%</td>
</tr>
<tr>
<td>(\tau_{RyRadapt})</td>
<td>250 ms</td>
<td>+ 170%</td>
</tr>
<tr>
<td>(\tau_{RyRact, cytosol})</td>
<td>5 ms</td>
<td>No change</td>
</tr>
<tr>
<td>(\tau_{RyRact, subspace})</td>
<td>5 ms</td>
<td>No change</td>
</tr>
<tr>
<td>(\tau_{RyRinact, cytosol})</td>
<td>30 ms</td>
<td>+ 170%</td>
</tr>
<tr>
<td>(\tau_{RyRinact, subspace})</td>
<td>15 ms</td>
<td>+ 170%</td>
</tr>
</tbody>
</table>

5.2.2 Modelling effects of \(I_{Na}\) block

Following previous studies [12,47], a guarded receptor model was employed to simulate the binding and unbinding kinetics of the drug to the \(Na^+\) channel in a drug concentration-dependent manner. Figure 5.1 gives a schematic illustration of the guarded receptor model. An \(I_{Na}\) channel could occupy an open, inactivated or a closed state. It was assumed that the drug predominantly binds to the activated and inactivated states of the \(Na^+\) channel. The binding and unbinding kinetics of a drug were described by first-order transitions. The blockade of \(I_{Na}\) is given by [12,47]:
\[ I_{Na} = g_{Na}(1 - B_A - B_I)m^3 h j (V - E_{Na}) \] (5.2)

\[ \frac{d B_A}{dt} = K_A[D_{Na^+}]m^3 h j (1 - B_A - B_I) - L_A B_A \] (5.3)

\[ \frac{d B_I}{dt} = K_I[D_{Na^+}](1 - h)(1 - B_A - B_I) - L_I B_I \] (5.4)

where \( g_{Na} \) is the channel conductance of I_{Na}; \( B_A \) and \( B_I \) are the fractional activated and inactivated blocks; \( m \) is the activation gate, \( h \) and \( j \) are the inactivation variables of the Na\(^+\) channel; \( V \) is the transmembrane potential; \( E_{Na} \) is the reversal potential of Na\(^+\); \( K_A \), \( K_I \) are the binding constants and \( L_A \), \( L_I \) are the unbinding constants; \([D_{Na^+}]\) is the concentration of a Na\(^+\)-blocker. In the present study, a drug concentration of 60 μM was studied unless otherwise stated. Similar to previous studies [12,47], a parameter set \((K_A = 100\text{ms}^{-1} \cdot \text{M}^{-1}, K_I = 100\text{ms}^{-1} \cdot \text{M}^{-1}, L_A = 1 \text{ ms}^{-1}, L_I = 0.01 \text{ ms}^{-1})\) was first used to represent the kinetics of a theoretical I_{Na}-selective blocker.

Figure 5.1 A schematic diagram of the guarded-receptor model simulating the state-dependent Na\(^+\)-channel block. (A) Transitions between the closed, open and inactivated states can be described using the Hodgkin-Huxley model. (Bi) The transitions between the drug-free open states and the drug-bound open states. (Bii) The transitions between the drug-free inactivated states and the drug-bound inactivated states.
Similar to previous studies [12,47] investigating the AF-selectivity of $I_{Na}$ block under normal conditions, the binding and unbinding kinetics of the theoretical $I_{Na}$ blockers were varied to assess how these parameters affect the anti-AF effects of the theoretical drugs, and whether an atrial-selective anti-AF action could be achieved under cAF conditions. This method also allows for a more general evaluation of the pharmaceutical benefits of combined applications of $I_{Na}$ and $I_{Kur}$ blockers.

The AF-selectivity of Na$^+$-channel blockade was defined as the product of atrial-selectivity, rate-selectivity and block strength. Given a fractional block ($B_f$) by Na$^+$-channel blockers measured as the relative reduction in the peak $I_{Na}$, the rate-selectivity was defined as the ratio of $B_f$ measured in an atrial myocyte paced at 6 Hz to that paced at 1 Hz [12,47]. Atrial-selectivity was used to determine the extent of atrial-ventricular difference in response to the drug, which was represented by the ratio of $B_f$ observed from an atrial cell to that of a ventricular cell both paced at 1Hz. The block strength ($E$) was defined as:

$$E = \frac{1.0}{1.0 + \left(\frac{0.5}{B_{f,6Hz}}\right)^4}$$  \hspace{1cm} (5.5)

where $B_{f,6Hz}$ is the fractional block of peak $I_{Na}$ measured in an atrial cell paced at 6 Hz. The block strength was introduced to balance the measure of AF-selectivity when the fractional block observed in a ventricular cell paced was minimal, which otherwise could give a great value in AF-selectivity regardless of a small $B_{f,6Hz}$.

In order to optimise the AF-selectivity, the unbinding constants $L_A$ and $L_I$ were first varied over a parameter space from $10^{-5}$ to $10^0$ ms$^{-1}$ (index varied from -5 to 0 in increments of 0.5) while $K_A$ and $K_I$ were fixed ($K_A = K_I = 100$ ms$^{-1}$ · M$^{-1}$). The resulting unbinding constants were used in subsequent optimisations varying $K_A$ and $K_I$. The parameter space was {1, 10, 100, 500, 2500, 10000} for $K_A$ and {1, 10, 100, 200, 500, 2500} for $K_I$.

5.2.3 Modelling $I_{Kur}$ block

Previous modelling studies demonstrated the important role of the kinetic properties of drug actions in $I_{Kur}$ block [186,188,243]. In addition, the pharmaceutical effects of acacetin on $I_{Kur}$ are characterised by use- and rate-dependencies [253], which have also been observed in other $I_{Kur}$ blockers [255,257]. Therefore it was necessary to adopt a
state-dependent block model [267] to simulate the blockade of I\textsubscript{Kur} by acacetin. Hence, in the present study, the inhibition of I\textsubscript{Kur} by acacetin was also simulated using the guarded receptor formalism. Similar to the modelling of the I\textsubscript{Na} block, the binding and unbinding kinetics of a drug are described by first-order transitions. Also, experimental studies revealed that acacetin binds to the open and closed gates of K\textsubscript{V}1.5 [253]. Following the guarded-receptor formulas given in equations (5.2)-(5.4), the inactivation-state binding and unbinding kinetics in I\textsubscript{Na} block were introduced to simulate the closed-state block of I\textsubscript{Kur} by acacetin. The drug action kinetics are functions of the binding constants, given by:

\[ I_{\text{Kur}} = g_{\text{Kur},v}(1 - B_O - B_C)ai(V - E_K) \]  

(5.6)

\[ \frac{dB_O}{dt} = K_O \exp \left( Z_{K_O} \frac{VF}{RT} \right) [D_{K^+}]ai(1 - B_O - B_C) - L_O \exp \left( -Z_{L_O} \frac{VF}{RT} \right) B_O \]  

(5.7)

\[ \frac{dB_C}{dt} = K_c \exp \left( Z_{K_c} \frac{VF}{RT} \right) [D_{K^+}](1 - a)i(1 - B_O - B_C) \]

\[ - L_c \exp \left( -Z_{L_c} \frac{VF}{RT} \right) B_C \]  

(5.8)

where \(g_{\text{Kur},v}\) is the conductance of I\textsubscript{Kur}; \(B_O\) and \(B_C\) are the fractional open and closed state blocks, respectively; \(a\) and \(i\) are the activation and inactivation gate variables; \(V\) is the membrane voltage; \(E_K\) is the reversal potential of potassium; \(F\), \(R\) and \(T\) are the Faraday’s constant, universal gas constant and temperature respectively. \(K_O\) and \(K_c\) are the binding constants; \(L_O\) and \(L_c\) are the unbinding constants; \(Z_{K_O}, Z_{L_O}, Z_{K_c}, L_c\) are the drug charge parameters for the corresponding binding and unbinding processes; \([D_{K^+}]\) is the concentration of acacetin applied. In the present study, a concentration of 3 \(\mu\)M was considered unless otherwise stated. Applying 3 \(\mu\)M of acacetin exerted a 50% block in I\textsubscript{Kur} using the voltage clamps repeated at 0.5 Hz [253].

The binding and unbinding parameters were obtained by fitting the model to the experimental data on the rate-dependent blockade of I\textsubscript{Kur} by acacetin (3\(\mu\)M) [253]. In fitting the parameters, a cost function was defined as the difference between the simulated and experimental relative-current against the pulse number (as shown in Figure 5.2 C). The parameters were attained by minimising the cost function using the Nelder-Mead Simplex algorithm [214] built in the scipy-optimize package, an open source optimisation library as part of the SciPy ecosystem [140]. The fitted parameters
are listed in Table 5.2.

Figure 5.2 shows a simulated frequency dependent blockade of $I_{Kur}$ by acacetin (3μM), which was compared to the experimental data. It was demonstrated that repeating the voltage command (Figure 5.2 B, insert) at 0.5 Hz, an approximately 50% blockade in the current was attained by applying acacetin of 3 μM. Increasing the pacing rate to 4 Hz markedly increased the relative fractional block to around 63% (Figure 5.2 C). Note that a similar effect cannot be simulated using the simple pore block model.

Table 5.2 Fitted parameters describing the binding and unbinding kinetics of acacetin on the $K_{V1.5}$ channel.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_O$</td>
<td>0.000194</td>
<td>μM$^{-1}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$L_O$</td>
<td>0.000291</td>
<td>ms$^{-1}$</td>
</tr>
<tr>
<td>$Z_{KO}$</td>
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<td></td>
</tr>
<tr>
<td>$Z_{LO}$</td>
<td>-0.0128</td>
<td></td>
</tr>
<tr>
<td>$K_C$</td>
<td>0.00249</td>
<td>μM$^{-1}$ ms$^{-1}$</td>
</tr>
<tr>
<td>$L_C$</td>
<td>0.000286</td>
<td>ms$^{-1}$</td>
</tr>
<tr>
<td>$Z_{KC}$</td>
<td>0.327</td>
<td></td>
</tr>
<tr>
<td>$Z_{LC}$</td>
<td>0.837</td>
<td></td>
</tr>
</tbody>
</table>

5.2.4 Modelling effects of acacetin on atrial and ventricular electrophysiology

In addition to inhibiting $I_{Kur}$ in the atria, acacetin potently blocks $I_{to}$ and $I_{K,Ach}$, and affects $I_{K}$ and $I_{Ks}$, exhibiting a multiple potassium channel block. The parameters of Hill equations fitting use-dependent inhibitions of these channels by acacetin are shown in Table 5.3. In the simulations, the effects of acacetin on these channels were modelled using a simple pore block model [127]. The present study did not simulate the effects of $I_{K,Ach}$ inhibitions.
Figure 5.2 Frequency-dependent inhibition of $I_{Kur}$ by acacetin. (A) Experimental traces of $K_V1.5$ current elicited from the 20th voltage step repeating at 0.5 Hz and 4 Hz in control (left panel) and after exposure to 3 μM of acacetin (right panel). (B) Simulated traces of $I_{Kur}$ evoked by the 20th voltage step pacing at 0.5 Hz and 4 Hz in control (left panel) and after exposure to 3 μM of acacetin (right panel). (C) Relative remaining $I_{Kur}$ following the inhibition by acacetin at various frequencies plotted against the pulse number of the voltage step. The simulated data (lines) were compared with experimental values (squares). The relative fraction was obtained by normalising the end step current measured from each pulse after application of acacetin to that of control. The experimental data were manually digitalised from Figures 5 and 6 of Wu et al. [253].
The effects of acacetin on human ventricular APs are unknown, although experimental data demonstrated that acacetin of 30μM did not affect the heart rate and QT interval in isolated rabbit hearts [254]. In the present study, it was assumed that similar effects on the potassium channels (I_{to}, I_{Kr}, I_{Ks}) could be extrapolated onto the ventricular myocytes, and therefore the same blockade effects were modelled in ventricular simulations. I_{Kur} is believed to be absent in ventricles, therefore in simulations of modelling I_{Kur} block alone, the ventricular electrophysiology was not affected by the pure I_{Kur} block.

Table 5.3 Potency of acacetin inhibiting atrial K^+ currents. Data extracted from [254].

<table>
<thead>
<tr>
<th>IC_{50} (μM)</th>
<th>I_{Kur}</th>
<th>I_{to}</th>
<th>I_{Kr}</th>
<th>I_{Ks}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.2</td>
<td>9.3</td>
<td>32.4</td>
<td>81.4</td>
</tr>
<tr>
<td>Hill coefficient</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Fractional inhibition at 3.2 μM</td>
<td>50%</td>
<td>28%</td>
<td>11%</td>
<td>7%</td>
</tr>
</tbody>
</table>

5.2.5 1D model of atrial strand

1D models were used to quantify the effects of channel blockers on the atrial conduction velocity and its restitution properties. A detailed description of the 1D model has been given in Chapter 3. The values of interest were measured from cells located in the middle of the strand to avoid boundary effects.

5.2.6 2D tissue models

2D simulations were performed on a 7.5×7.5 cm^2 sheet of human atrial myocytes with 250 nodes along each direction. Re-entrant excitations were initiated within the 2D tissue to facilitate studies on the antiarrhythmic effects of the channel blockades. To solve the excitation wave propagation problem, the well-known mono-domain equation [128] (Chapter 2 Equation (2.29)) was used. The diffusion coefficient was chosen so that the model gives a conduction velocity of 0.7 m/s measured using plane wave for the tissue with normal human atrial cells. In simulations of atrial tissue under AF conditions, cAF induced remodelling to the coupling was considered by reducing the diffusion tensor coefficient D to 80% of its normal value under SR conditions to account for the cAF-induced remodelling in electrical coupling and atrial structure.
[35,268]. The numerical method used in previous studies ([35] and Chapter 3) was adopted here. The monodomain equation was integrated at a time step of 0.02 ms.

To initiate re-entrant waves in tissue, the S1-S2 cross-shock protocol [12] was used (Figure 5.3): a train of S1-stimuli were first applied to the left lateral of the 2D slab tissue to allow for propagation of a planar wave; a further stimuli of the same strength and duration as the S1 was applied to the bottom half of the tissue with a temporal delay of duration S2, representing a cross-shock. A successfully initiated re-entry is shown in Figure 5.3C by implementing the protocol.

Figure 5.3 An illustration of the cross-shock protocol. (A) A train of S1 stimuli were applied to one end of the 2D tissue. (B) After a delay of S2, a further stimulus was applied to the bottom-half of the 2D tissue. (C) A screen shot showing a simulated re-entrant wave initiated using the cross-shock protocol.

To quantify the frequency of the rotor in the simulated tissue, pseudo-ECGs (pECG) were computed using the equation as follows [131,132]:

\[
\phi(x', y', z') = D \int \left[ -\nabla V_m \cdot \left( \frac{1}{r^2} \right) \right] d\Omega
\]  

(5.9)

\[
r = (x - x')^2 + (y - y')^2 + (z - z')^2
\]  

(5.10)

where \( \nabla V_m \) is the spatial gradient of the transmembrane potential and \( r \) is the distance measured from a point source \( (x', y', z') \) to the coordinate of an virtual electrode \( (x', y', z') \); \( \Omega \) indicates integrating across the domain of the tissue. In the present chapter, \( \phi \) was computed for a virtual electrode located 25 mm to the right of the 2D tissue. pECG traces were analysed using the Fast Fourier Transform (FFT) to obtain the
dominant frequencies of the simulated re-entrant excitations. The phase singularity
detection method proposed by Bary and Wikswo [269] was adopted to trace the tip
trajectories of the spiral waves.

The initial conditions of the 2D atrial model were imported from the state variables
of single cell simulations paced for 1,500 s at an interval of 160 ms.

5.3 Results

5.3.1 Simulated atrial electrophysiology under SR and cAF conditions

The updated model of atrial electrophysiology was first used to simulate APs and
calcium transients (CaTs) under both SR and cAF conditions (Figure 5.4). The AP
attained from the updated model shows a pronounced spike-and-dome morphology.

The incorporation of cAF-induced remodelling to the model markedly abbreviated
APD (Figure 5.4 A, 247 ms in SR vs. 157 ms in AF model), whilst the AP exhibited a
typical triangular morphology. The amplitude of CaT was also markedly reduced with
cAF remodelling (Figure 5.4 B). These results are well aligned with previous
restitutions attained by steady-state pacing are shown in Figure 5.4 C. The updated
model under SR conditions showed increased rate-adaptation of the APD in comparison
to the original Colman et al. model [35]. The inclusion of cAF remodelling effects
markedly flattened the APD restitution and decreased APD across the whole range of
simulated BCLs, which is in good agreement with previous studies [176,270,272]. At
BCLs of 150 to 200 ms, alternans in AP were observed in the single cell simulations
under cAF conditions (Figure 5.4 C).

5.3.2 Effects of individual and combined channel block by acacetin

To dissect the roles of inhibition of individual channels by acacetin in the modulation of
AP by the compound, the individual and combined blockades of I_{to}, I_{Kr}, I_{Kur} and I_{Ks} by
acacetin (3 μM) under both SR and cAF conditions were simulated. Figure 5.5
illustrates the effects of individual and combined K⁺-channel inhibitions by the drug on
AP. The alterations to APD relative to the control were quantified and are shown in the
panels C and D of Figure 5.5.
Figure 5.4 Simulated action potentials and calcium transient (CaT) of human atrial cardiomyocytes under sinus rhythm (SR) and cAF (AF) conditions. (A-B) The action potential duration measured at 90% repolarization (APD\(_{90}\)) and the amplitude of CaT are quantified and shown in the right panels. The cells were paced at 1 Hz. (C) Simulated steady-state APD restitution curve of atrial myocytes under SR and cAF conditions.
Figure 5.5 Effects of block of individual K⁺-channels by acacetin on the human atrial AP and APD under SR and cAF conditions. (A) Effects on the atrial AP under SR conditions. (B) Effects on the atrial AP in the presence of cAF remodelling. In both panels, a zoomed-in view for the traces of AP during phase 3 is plotted to the right. (C) Alterations to the (i) APD₉₀ and (ii) APD₃₀ by the channel blockades obtained from atrial cells under normal conditions. (D) Changes in atrial (i) APD₉₀ and (ii) APD₃₀ due to the blockades in the presence of cAF remodelling. The cells were paced at 1 Hz.
Under SR conditions, the simulated \( I_{Ks} \) and \( I_{Kr} \) block by acacetin presented insubstantial alterations to the atrial AP: the atrial repolarisation was delayed by 1.3 and 5.9 ms, respectively, whilst no changes were observed in the plateau phase. These effects were expected due to the small potency of acacetin on the two channels (Table 5.3). Similar effects were also demonstrated in the simulated blocks \( I_{Kr} \) and \( I_{Ks} \) by the compound in the cAF-remodelled atrial cells.

Blocking \( I_{0} \) alone by acacetin elevated the atrial plateau potential under both SR and cAF conditions, which led to minor prolongations in \( APD_{30} \) (by 6.4 and 3.6 ms under SR and cAF conditions, respectively). The changes in \( APD_{90} \) due to \( I_{0} \) block varied between the two conditions: under SR conditions the atrial \( APD_{90} \) was shortened by 2.4 ms, whereas it was prolonged by 3.7 ms in the presence of cAF remodelling.

Blocking \( I_{Kur} \) alone exhibited pronounced alterations to the shape and duration of the atrial AP under both conditions. The inhibition in \( I_{Kur} \) markedly elevated the plateau potential of atrial AP (by 7.1 and 5.7 mV under SR and cAF conditions, respectively), which was accompanied by marked prolongations in \( APD_{30} \) (by 105.9 and 23.6 ms under SR and cAF conditions, respectively). The prolongation in \( APD_{90} \) induced by the \( I_{Kur} \) block was 9.8 ms for normal atrial cells, and was more pronounced (23.6 ms) in cAF remodelled atrial myocytes, despite the fact that \( I_{Kur} \) was down-regulated in AF conditions (Table 5.1). These effects indicate anti-arrhythmic potentials of \( I_{Kur} \) block under cAF conditions.

Combined effects of acacetin (3 μM) of human atrial cells were simulated by applying the corresponding inhibitive effects on the \( K^+ \)-channels simultaneously. It was demonstrated that the combined effects of acacetin produced more substantial alterations to the AP than any simulated individual blocks. These effects produced by the combined blockade were compared with the sum of the effects seen in individual blocks. Synergistic effects were observed in the changes in \( APD_{90} \), represented by a further prolongation of 9.3 and 1.1 ms in \( APD_{30} \) under SR and cAF remodelled conditions, respectively. Additionally, it was shown that the effects of \( I_{Kur} \) block dominated the changes in AP by the application of the compound, which is consistent with the high potency of acacetin on the channel (Table 5.3).
5.3.3 Effects of combined sodium and potassium channel block under cAF conditions

5.3.3.1 Effects on single cell AP and $I_{\text{Na}}$

Individual and combined effects of $\text{Na}^+$-block (represented by $I_{\text{Na}B}$) and $\text{K}^+$-block (represented by the application of acacetin) on human atrial electrophysiology under cAF conditions were simulated to assess the anti-AF actions of these blockades. Effects of acacetin ($\text{K}^+$-block) were simulated in two scenarios: $I_{\text{Kur}}$ block alone (denoted by $I_{\text{Kur}B}$) and the combined block of all $\text{K}^+$ currents in Table 5.3 (represented by Acacetin/Full). In addition, the effects of $\text{Na}^+$- and $\text{K}^+$- block on human ventricular cells were also modelled. The results are shown in Figure 5.6. Figure 5.7 illustrates the changes in APD and relative inhibitions in peak $I_{\text{Na}}$.

In the atrial cells paced at 1 Hz, $I_{\text{Kur}B}$ and Acacetin/Full prolonged atrial APD (as discussed in 5.3.2) whilst the effects on $I_{\text{Na}}$ were minimal (reducing peak $I_{\text{Na}}$ by less than 0.3%). The peak $I_{\text{Na}}$ was slightly reduced by $I_{\text{Na}B}$ alone (1.63%). The fractional inhibition was slightly increased by a further introduction of $I_{\text{Kur}B}$ or Acacetin/Full (Figure 5.7 Aii). In the ventricles, the simulated application of acacetin presented a prolongation of 19.5 ms in $\text{APD}_{90}$ compared with that in control (drug-free) condition. $I_{\text{Na}B}$ alone showed a negligible inhibitive effect on the ventricular $I_{\text{Na}}$ (by 0.42%), which was not affected by the combined block of $I_{\text{Na}B}$ and Acacetin/Full (Figure 5.7 Cii).

In the atrial cells paced at 6 Hz, AP alternans were observed under the control conditions: the APD was 100.1 and 88.1 ms for the big and small APs, respectively (Figure 5.6 Ci). Considering the presence of AP alternans, the changes in APD by the $\text{Na}^+$- and $\text{K}^+$- blocks were quantified by comparing the corresponding big APs, while the fractional reductions in peak $I_{\text{Na}}$ were calculated from the $I_{\text{Na}}$ giving rise to the corresponding small APs. The results showed that applying $I_{\text{Kur}B}$ or Acacetin/Full alone both abolished the AP alternans while prolonging the APD to 116.3 and 126.3 ms and reducing the peak $I_{\text{Na}}$ by 5.9% and 20.1%, respectively. The application of $I_{\text{Na}B}$ alone manifested itself as a minor prolongation in APD (by 3.5 ms) and a reduction of 16.2% in peak $I_{\text{Na}}$. Combining block of $I_{\text{Na}}$ with $I_{\text{Kur}B}$ or Acacetin/Full markedly promoted the AP alternans, resulting in substantial prolongations in the APD of the big AP (by 35.4 ms for $I_{\text{Kur}B} + I_{\text{Na}B}$, and 55.6 ms for Acacetin/Full + $I_{\text{Na}B}$) and dramatic decreases in the peak $I_{\text{Na}}$ (by 57.5% for $I_{\text{Kur}B} + I_{\text{Na}B}$ and 88.2% for Acacetin/Full + $I_{\text{Na}B}$). These
Figure 5.6 Simulated action potentials and $I_{Na}$ of cAF-remodelled atrial myocytes and ventricular cells in response to different combinations of Na$^+$- and K$^+$-blocks. (Ai) APs from atrial cells paced at 1 Hz; (Aii) Time courses of corresponding $I_{Na}$ during the upstroke phase. (B) Simulated time courses of AP and $I_{Na}$ of a ventricular cell paced at 1 Hz. (C) Illustration of (i) APs and (ii) the corresponding time courses of $I_{Na}$ of a human atrial cell paced at 6 Hz. In the simulations, rate constants for $I_{Na,B}$ were: $K_A = 100 \text{ ms}^{-1} \cdot \text{M}^{-1}$, $K_I = 100 \text{ ms}^{-1} \cdot \text{M}^{-1}$, $I_A = 1 \text{ ms}^{-1}$, $I_I = 0.01 \text{ ms}^{-1}$. 
Figure 5.7 Simulated changes in the APD and peak $I_{Na}$ following the applications of Na$^{+}$- and K$^{+}$- block in comparison to the drug-free condition. (A) Changes in (i) APD and (ii) peak $I_{Na}$ measured in an atrial cell paced at 1 Hz. (B) Changes in (i) APD and (ii) peak $I_{Na}$ measured in an atrial cell paced at 6 Hz. In the presence of alternans, the changes in APD were quantified by comparing the corresponding big APs, and the fractional reductions in peak $I_{Na}$ were calculated from the $I_{Na}$ giving rise to the corresponding small APs. (C) Changes in (i) APD and (ii) peak $I_{Na}$ measured in a ventricular cell paced at 1 Hz.
values suggest that the combined block of $I_{\text{Na}}$B and Acacetin/Full /$I_{\text{Kur}}$B exhibited pronounced synergistic antiarrhythmic effects represented by prolongations in APD and reductions in $I_{\text{Na}}$, mainly through the increased susceptibility to AP alternans.

To further demonstrate the synergistic effects attained in combined $I_{\text{Na}}$B and $K^+$-block, simulations were performed to model the response of atrial AP and $I_{\text{Na}}$ to the channel blockers at lower doses. The concentrations of Na$^+$- and K$^+$-blocks were simultaneously varied from 0 to 100% of the dose. Figure 5.8 illustrates the changes in APD and fractional reductions in $I_{\text{Na}}$. It was demonstrated that $I_{\text{Kur}}$B and Acacetin/Full both consistently prolonged APD, which was in general enhanced at higher doses. As expected, applying $I_{\text{Na}}$B increased the fractional $I_{\text{Na}}$ block in a dose-dependent manner. These effects were promoted by combined blocks at high concentrations. The synergistic effects of APD prolongation and $I_{\text{Na}}$ reduction by the combined blocks were quantified and illustrated in Figure 5.8 B. These results suggest that synergistic antiarrhythmic benefits can be consistently obtained for combined Na$^+$- and K$^+$-block,

![Figure 5.8](image)

Figure 5.8 Synergistic effects of combined $I_{\text{Na}}$B and $I_{\text{Kur}}$B, Acacetin/Full at variable concentrations of the blockers. (Ai) Changes in APD and (ii) fractional peak $I_{\text{Na}}$ reductions plotted against the concentration of blockers. (B) Synergistic effects in the APD prolongation and $I_{\text{Na}}$ reduction. The zigzags in the line plots are attributable to the presence of AP alternans.
5.3.3.2 Effects on steady-state restitutions

Figure 5.9 shows the effects of different combinations of Na\(^+\)- and K\(^+\)- block on the electrical restitution properties in an atrial cell. As expected, APD prolongation was observed consistently over the range of simulated BCLs for $I_{Kur}$ and Acacetin/Full as compared to the control conditions; the rate-dependent adaptation of APD was also promoted. Whilst $I_{Na}$ alone had little effect for slow pacing rates, it induced a slight prolongation at short BCLs (Figure 5.9 A). The reduction in peak $I_{Na}$ in $I_{Na}$ alone was rate-dependent and markedly greater at fast pacing rates. K\(^+\)- block alone ($I_{Kur}$ or Acacetin/Full) slightly shifted the rate-dependency to larger BCLs via prolonging the APD without affecting peak $I_{Na}$ at slow pacing rates. Synergistic rate-dependent reduction in peak $I_{Na}$ was observed in combined blocks of $I_{Na}$ with $I_{Kur}$ or Acacetin/Full as compared to single channel blocks. AP alternans were observed in control and became more pronounced with K\(^+\)-blocks, and further enhanced by combined $I_{Na}$ and K\(^+\)-blocks. In addition, the threshold of BCL allowing alternans was increased in these conditions as compared to control (Figure 5.9 B).

![Figure 5.9](image)

Figure 5.9 Single cell APD restitutions and rate-adaptations of peak $I_{Na}$ for the control and Na\(^+\)- and K\(^+\)- block. (A) Steady-state APD restitutions. (B) Rate-adaptations of peak $I_{Na}$ elicited during APs. For the ease of comparison with control and $I_{Na}$, results of $I_{Kur}$ and Acacetin/Full are plotted on the left and right panels, respectively.
The antiarrhythmic effects of Na\(^+\)- and K\(^+\) block were further demonstrated in tissue using 1D models of an atrial strand. Figure 5.10 shows the atrial APD and conduction velocity (CV) restitutions as well as the rate-adaptation of V\(_{\text{max}}\) measured in tissue. Consistent with single cell simulations, the atrial activation-recovery interval (ARI) was not affected by I\(_{\text{Na}}\)B alone, whereas it was substantially lengthened by K\(^+\)-block as compared to control. Applying K\(^+\)-block alone also shifted the CV and V\(_{\text{max}}\) restitutions to higher BCLs. The rate-adaptations of V\(_{\text{max}}\) and CV were progressively strengthened by I\(_{\text{Na}}\)B and the combined blocks over fast pacing rates. Synergistically enhanced rate-dependent reductions in V\(_{\text{max}}\) and CV were observed in the combined blocks. Furthermore, K\(^+\)-block also increased the susceptibility of conduction block by elevating the threshold of BCL allowing 1:1 atrial capture in the 1D strand as compared with the control conditions (Figure 5.10 C).

### 5.3.4 Effects of combined Na\(^+\)- and K\(^+\)- block on the AF-selectivity of Na\(^+\) blockers

The AF-selectivity of Na\(^+\) blockers were examined by varying the drug binding and unbinding constants over the parameters spaces. This was done through independently changing \(L_A\) and \(L_I\) for fixed \(\{K_A, K_I\}\) and then varying \(K_A\) and \(K_I\) for fixed \(\{L_A, L_I\}\) to obtain the maximum AF-selectivity over the parameter space of drug binding and unbinding kinetics. The same procedure was repeated for simulations for I\(_{\text{Na}}\)B + I\(_{\text{Kur}}\)B and I\(_{\text{Na}}\)B + Acacetin/Full, which allows for assessing the pharmaceutical benefits in AF-selectivity by the combined blocks over the pure I\(_{\text{Na}}\)B.

Figure 5.11 illustrates the block strength, rate-selectivity, atrial-selectivity and the resulting AF-selectivity for I\(_{\text{Na}}\)B alone and the combined blocks as a function of \(K_A\) and \(K_I\). Clearly, for an identical Na\(^+\) blocker, the combined blocks achieved markedly greater AF-selectivity than I\(_{\text{Na}}\)B alone. The maximum attainable AF-selectivity was increased nearly 6-fold for I\(_{\text{Kur}}\)B + I\(_{\text{Na}}\)B and more than 14-fold for Acacetin/Full + I\(_{\text{Na}}\)B as compared to I\(_{\text{Na}}\)B alone (Figure 5.11 C). These dramatic increases were attributed to the markedly greater values in all metrics contributing to the AF-selectivity. The maximal block strength (defined in Equation (5.5)) achieved by I\(_{\text{Na}}\)B alone was 0.77, and was increased to 0.81 and 0.94 for I\(_{\text{Kur}}\)B + I\(_{\text{Na}}\)B and Acacetin/Full + I\(_{\text{Na}}\)B, respectively. A more appreciable increase in the maximal rate-selectivity was observed by the combined blocks as compared to I\(_{\text{Na}}\)B alone (8-fold for I\(_{\text{Kur}}\) + I\(_{\text{Na}}\)B and nearly 10-
Figure 5.10 Simulated activation-recovery interval (ARI, A), $V_{\text{max}}$ (B) and CV (conduction velocity, C) measured in 1D atrial strand models as a function of BCL for control, individual and combined Na$^+$- and K$^+$- block. ARI was measured as the temporal interval between the time at which the AP depolarises to $–20$ mV and the time it reaches 90% repolarisation.
fold for Acacetin/Full + I_{Na}B). Additionally, the atrial-selectivity was also increased by the combined blocks, although to a lesser extent. I_{Kur}B + I_{Na}B exhibited a greater atrial-selectivity than that of Acacetin/Full + I_{Na}B as I_{Kur}B was assumed to have no effect on the ventricles.

Furthermore, these simulations revealed that the block strength, rate-selectivity and atrial-selectivity were strongly dependent on the inactivation-state binding rate $K_f$, and the open-state binding kinetics $K_A$ to a much lesser extent. The block strength was mainly determined by $K_f$: an increase in $K_f$ led to substantial increase in the block strength. In combined blocks, the maximal rate- and atrial-selectivity were observed for $K_A = K_f = 1 \text{ms}^{-1} \cdot M^{-1}$ and an increase in $K_f$ resulted in substantial reductions in the rate- and atrial-selectivity. In I_{Na}B alone, the parameter set $K_A = 1 \text{ms}^{-1} \cdot M^{-1}, K_f = 200 \text{ms}^{-1} \cdot M^{-1}$ produced a maximal value in atrial-selectivity. Collectively, the optimal $K_f$ maximising AF-selectivity was $200 \text{ms}^{-1} \cdot M^{-1}$ for I_{Na}B and I_{Kur}B + I_{Na}B, and smaller ($100 \text{ms}^{-1} \cdot M^{-1}$) for Acacetin/Full + I_{Na}B. The optimal $K_A = 1 \text{ms}^{-1} \cdot M^{-1}$ was seen for all conditions. Increasing $K_A$ consistently resulted in a smaller rate- and atrial-selectivity and therefore reduced AF-selectivity. These results suggest that the inactivation-state binding rate might be a more favourable targeting parameter than the open-state binding kinetics in the optimisations of AF-selectivity.
Figure 5.11 Block strength, rate-, atrial- and AF-selectivity as a function of the open- and inactivated- state binding rates ($K_A, K_I$) for $I_{NaB}$, $I_{KurB} + I_{NaB}$ and Acacetin/Full + $I_{NaB}$. In calculating the block strength, the mean value in $B_{f,6Hz}$ (Equation (5.5)) of two successive APs was used in the presence of alternans. $L_A = 1ms^{-1}, L_I = 0.01 ms^{-1}$. 

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5.3.5 Effects of $I_{Na}$ and $I_{Kur}$ block on AF termination in tissue

Using the cross-shock protocol, re-entrant activities were initiated in a 2D tissue representing an atrial slab. For each condition, a 10 s episode of electrical activities was simulated. The sequential screenshots of the re-entrant excitations in control and following application of drugs are presented in Figure 5.12. The trajectories of the tips of re-entrant rotors under these conditions were traced and are shown in Figure 5.13 A. The number of rotors was also measured (Figure 5.13 B). In these simulations, the parameter set representing Na$^{+}$-block was $K_A = 1\, m/s^{-1}$, $M^{-1}$, $K_I = 100\, m/s^{-1}$, $M^{-1}$, $L_A = 1\, m/s^{-1}$, $L_I = 0.01\, m/s^{-1}$.

In control (drug-free conditions), a single rotor was formed at $t = 630$ ms, which broke into two spiral waves at $t = 830$ ms. The two rotors were stably anchored with star-shaped trajectories at the bottom half of the slab and persisted throughout the rest of the simulated 10-s episode (Figure 5.12 A and Figure 5.13 Ai & B).

In simulations of drug blocks, a drug was applied at $t = 2500$ ms. For $I_{Na}B$ the dual rotors progressively became unstable, and the tips of the spiral waves meandered out of the tissue at around $t = 6000$ ms, leading to self-termination of the re-entries (Figure 5.12 B, Figure 5.13 Aiv & B). The double spiral waves persisted throughout the period of the simulation applying $I_{Kur}B$ alone (Figure 5.12 C and Figure 5.13 Aii). For $I_{Na}B + I_{Kur}B$, the rotor close to the bottom left corner of the slab turned unstable and meandered out of the tissue at around $t = 3800$ ms, whereas the trajectory of the second rotor was confined to a small area until $t = 7000$ ms and then gradually became chaotic with up to 3 tips of the spiral waves were transiently observed before being terminated at $t = 8334$ ms (Figure 5.12 D and Figure 5.13 Av and B). A similar but more marked effect was seen in the simulations with the full actions of acetatin: the bottom left rotor quickly meandered out of the tissue at $t = 3510$ ms whilst the trajectory of the second rotor became chaotic and terminated at $t = 5439$ ms. The combined actions of $I_{Na}B$ and Acacetin/Full demonstrated the strongest potency in terminating re-entrant excitations in these simulations: the two rotors transiently turned unstable and chaotic, and died out at $t = 3555$ ms, exerting up to 5 tips during the excitation in the slab (Figure 5.12 F and Figure 5.13 Avi and B). These simulations clearly demonstrated beneficial anti-AF effects of the combined blocks over that of applying K$^{+}$-block alone.
Figure 5.12 Snapshots of simulated re-entrant excitations in a 2D model of an atrial slab under various conditions. (A) Control (drug-free conditions). (B) $I_{Na,B}$ alone. (C) $I_{Kur,B}$ alone. (D) Combined $I_{Na,B}$ and $I_{Kur,B}$. (E) Applying Acacetin/Full alone. (F) Combined effects of $I_{Na,B}$ and Acacetin/Full. The time sequence (ms) is indicated at the top left corner of each screenshot.
Figure 5.13 The trajectories of spiral wave tips and number of rotor cores plotted as functions of time under various conditions. (A) The trajectories of spiral wave tips for (i) CTL, (ii) \( I_{Kur} \), (iii) Acacetin/Full, (iv) \( I_{Na} \), (v) \( I_{Kur} + I_{Na} \) and (vi) Acacetin/Full + \( I_{Na} \). (B) The number of core of spiral waves with respect to time.
The electrical activities of a local cell close to the bottom left of the slab were recorded and analysed. Figure 5.14 and Figure 5.15 illustrate the simulated pECGs, membrane voltage traces extracted from a local cell and the corresponding total fractional blocks (sum of open- and inactivated-/closed- state blocks) of \( \text{I}_{\text{Na}} \) and \( \text{I}_{\text{Kur}} \) measured from the same cell under various conditions. Consistent with the results in the re-entrant wave dynamics, the AP and pECG in control exhibited an alternating but organised pattern, whereas those observed in \( \text{I}_{\text{Na}} \)B and combined blocks progressively became irregular and diminished within the 10 s simulated episode. In the simulations incorporating Na\(^+\)-block, the total fraction of blocked \( \text{I}_{\text{Na}} \) transiently increased to up to 33\% and was not affected by the further introduction of K\(^+\)-blocks. In simulations, with administration of K\(^+\)-blocks, the maximal total fractional block in \( \text{I}_{\text{Kur}} \) was 72\% for Acacetin/Full or \( \text{I}_{\text{Kur}} \)B and was slightly smaller with combined actions of \( \text{I}_{\text{Na}} \)B (69\% for \( \text{I}_{\text{Kur}} \)B + \( \text{I}_{\text{Na}} \)B and 68\% for Acacetin/Full + \( \text{I}_{\text{Na}} \)B). Note these fractional blocks in \( \text{I}_{\text{Kur}} \) were substantially greater than 50\% suggested by the inhibition-dose relation at 3 μM, which was attributed to the characteristics of rate-dependent block of \( \text{I}_{\text{Kur}} \) by acacetin.
Figure 5.14 Computed pECGs, time courses of membrane potential and total fractional blocks in $I_{Na}$ and $I_{Kur}$ extracted from a local cell located close to the bottom left corner of the slab in control and $I_{Na}B$/Acacetin/Full. (A) pECGs. (B) Membrane voltage. (C) Fractional block of $I_{Na}$ computed from the sum of open- and inactivation-state blocks. (D) Fractional block of $I_{Kur}$ computed from the sum of open- and close-state blocks.
Figure 5.15 Computed pECGs, time courses of membrane potential and total fractional blocks in $I_{Na}$ and $I_{Kur}$ extracted from a local cell located close to the bottom-left corner of the slab in control and $I_{Na}B/I_{Kur}B$. (A) pECGs. (B) Membrane voltage. (C) Fractional block of $I_{Na}$ computed from the sum of open- and inactivation-state blocks. (D) Fractional block of $I_{Kur}$ computed from the sum of open- and close-state blocks.
The anti-arrhythmic benefits of combined Na\(^{+}\) and K\(^{+}\) block were demonstrated in further simulations with a different Na\(^{+}\)-blocker (\(K_A = 1 \text{ ms}^{-1} \cdot M^{-1}, K_I = 200 \text{ ms}^{-1} \cdot M^{-1}, L_A = 1 \text{ ms}^{-1}, L_I = 0.01 \text{ ms}^{-1}\)) and at a reduced (75%) dose of both blockers. The life span of re-entrant excitations was measured at the time interval starting from \(T_{\text{Drug}}\) (2500 ms) until the time point when the re-entrant tips extinguished, as shown in Figure 5.16. For each case, the pECC was also computed and the segment from \(t = 3000 \text{ ms}\) to \(500 \text{ ms}\) before the termination of re-entries (or the end of the simulation if the rotor sustained) was analysed using FFT to obtain the dominant frequency of the re-entrant excitations. The dominant frequency information is illustrated in Figure 5.17.

![Image](image.png)

Figure 5.16 Measured lifespan of re-entrant waves in the 2D model under variable conditions. (A) Lifespan under control conditions and application of drugs at the control dose (\([D_{K^+}] = 3 \mu M, [D_{Na^+}] = 60 \mu M\)). (B) Lifespan under the control condition and application of drugs at a reduced dose (\([D_{K^+}] = 2.25 \mu M, [D_{Na^+}] = 45 \mu M\)).
It was shown that at the simulated doses of K\textsuperscript{+}-block, I\textsubscript{Kur}B alone did not lead to termination of re-entrant waves in the entire duration of simulations (7500 ms) after T\textsubscript{Drug}, whereas the rotors became terminated in the simulations for Acacetin/Full at both doses (Figure 5.16), indicating synergistic anti-AF benefits of combined K\textsuperscript{+}-channel block. For the simulated I\textsubscript{Na}B alone, the Na\textsuperscript{+}-blocker with $K_I = 100 \text{ms}\textsuperscript{-1}\cdot \text{M}\textsuperscript{-1}$ led to termination of AF at the control dose (lifespan of 4102 ms) but not at the smaller dose; increasing $K_I$ of the Na\textsuperscript{+}-blocker to 200 ms\textsuperscript{-1}\cdot \text{M}\textsuperscript{-1} resulted in a substantially reduced lifespan (1305 ms for $[D_{Na^+}] = 60 \mu\text{M}$, and 1459 for $[D_{Na^+}] = 45 \mu\text{M}$). The lifespan for combined blocks of I\textsubscript{Na}B + Acacetin/Full were consistently shorter than that of any individual applications of I\textsubscript{Na}B or Acacetin/Full alone regardless of the drug concentrations and values for $K_I$. A similar synergistic effect was also observed for the combined I\textsubscript{Na}B and I\textsubscript{Kur}B at lower concentrations of blockers and $K_I = 200 \text{ms}\textsuperscript{-1}\cdot \text{M}\textsuperscript{-1}$ (Figure 5.16 Bii).

Consistent decrease in the dominant frequency (DF) was observed in the drug-modulated re-entrant excitations as compared to those in the drug-free conditions (Figure 5.17). Under the control conditions, the DF extracted from the pECG was 8.63 Hz, which is within the range of clinical observations during AF [273]. Applying Na\textsuperscript{+}- or K\textsuperscript{+}- block individually resulted in slowing down in the rate of the rotors, which was dependent on the concentrations and parameter of the blockers. For I\textsubscript{Kur}B the DF was 8.25 Hz with the control dose and 8.30 Hz for the reduced dose of acacetin. In the simulations with full actions of acacetin, the DF was 6.63 Hz and was not affected by the 25% reduction in the dose of the compound. For I\textsubscript{Na}B alone the DF was 7.81 Hz for Na\textsuperscript{+}-blocker of $K_I = 100 \text{ms}\textsuperscript{-1}\cdot \text{M}\textsuperscript{-1}$, and was substantially smaller (6.24 Hz) for the Na\textsuperscript{+}-blocker of $K_I = 200 \text{ms}\textsuperscript{-1}\cdot \text{M}\textsuperscript{-1}$. A synergistic deceleration of the rotors was observed for I\textsubscript{Na}B + I\textsubscript{Kur}B. The short lifespan of re-entry for Acacetin/Full + I\textsubscript{Na}B prevented calculations of reliable DFs.
Figure 5.17 Dominant frequencies (DF) extracted from simulated pECCs of the re-entrant waves in the 2D model under variable conditions. (A) DF under the control conditions and applications of drugs at the control dose ($K^+$-blocker – 3 μM, $Na^+$-blocker 60 μM). (B) DF under the control conditions and applications of drugs at a reduced dose ($[D_{K^+}] = 3 \mu M, [D_{Na^+}] = 60 \mu M$). The DF of three simulations for combined Acacetin/Full + $I_{NaB}$ was not calculated due to a small lifespan of the re-entries following the application of the drugs. ($[D_{K^+}] = 2.25 \mu M, [D_{Na^+}] = 45 \mu M$).

5.4 Discussion

Despite decades of advances, developing effective and safe antiarrhythmic drugs for the treatment of AF remains an unmet need. Developing atrial-selective antiarrhythmic drugs is a current favourable strategy for the pharmaceutical management of AF. In this
Chapter, the effects of $I_{K_{ur}}$ and $I_{Na}$ block, two potentially effective atrial-selective blocks, on human atrial electrophysiology were investigated in silico both at the cellular and tissue level. The simulation results demonstrated that both Na$^+$-block and K$^+$-block exhibited anti-arrhythmic effects in the atria with cAF-remodelling. The present study highlighted that multi-channel blocks (multi-K$^+$ channels, and combined Na$^+$ and K$^+$ block) exerted beneficial synergistic antiarrhythmic effects over that of a single channel block, suggesting that multi-channel block may be a favourable strategy for the development of novel pharmaceutical therapies for AF.

5.4.1 Single cell models

The mathematical model of human atrial electrophysiology by Colman et al. [35] was updated and used in the present study. The updated model exhibited an increased rate-adaptability of APD under normal (SR) conditions as compared to its predecessor (Figure 5.4). The model describing cAF-induced remodelling in human atria by Colman et al. [35] was incorporated in the updated atrial model to simulate atrial fibrillation. The resultant changes in APD, APD restitution and CaT by the cAF-induced remodelling showed good agreement with previous experimental [162,176,266,270,272] and simulation studies [35,139,160,271].

5.4.2 Effects of $I_{Na}$ block

An atrial-ventricular difference in the parameters of $I_{Na}$, especially in the voltage dependence of steady-state inactivation, has been demonstrated by a number of studies in the literature [250,274–276]. In these studies, the atrial voltage dependent steady-state inactivation curves of $I_{Na}$ were found to be negatively shifted (by 5 to 16 mV) as compared to the ventricular parameters, which gave rise to an on-going interest in developing an atrial-selective blocker of $I_{Na}$ as a strategy in terminating AF [250–252,277]. Similar to previous studies [12,47], in this Chapter the kinetic parameters in drug actions of Na$^+$-blockers were varied over parameter spaces to simulate the effects of Na$^+$-block in ventricular cells and fibrillating atria. The results revealed that in the atrial-fibrillated hearts of humans, atrial-selective block of $I_{Na}$ could be achieved (Figure 5.6 and Figure 5.7) and the AF-selectivity could be maximised by optimising the binding and unbinding rates of the Na$^+$-blocker (Figure 5.11). Also, the fractional inhibition of $I_{Na}$ by the Na$^+$-blocker was characterised by a substantial dependence on
the rate of pacing (Figure 5.6, Figure 5.7 and Figure 5.9), which was quantified using the rate-selectivity (Figure 5.11).

At the cellular level, Na\(^+\)-block exerted a substantial inhibition in \(I_{\text{Na}}\) at fast pacing rates and minimal effects within the range of normal heart rates (Figure 5.10, Figure 5.11). The antiarrhythmic implications of these effects were demonstrated in simulations of coupled tissue. In a 1D model representing an atrial strand, applying Na\(^+\)-block progressively enhanced the rate-adaptations of \(V_{\text{max}}\) and CV over a larger range of BCLs whereas the atrial APD was not affected; at fast pacing rates \(V_{\text{max}}\) and CV were markedly reduced suggesting diminished excitabilities of atrial myocytes (Figure 5.10). These results are concordant with the recent study [12] where similar effects of Na\(^+\)-block on the canine atria were demonstrated \textit{in silico} and experimentally in undiseased coronary perfused hearts.

In the simulated re-entrant excitations of 2D tissue representing an atrial slab, Na\(^+\)-block shortened the lifespan and caused slowing down in the excitation rate of the initiated spiral waves (Figure 5.12, Figure 5.16 and Figure 5.17). These effects clearly demonstrated that Na\(^+\)-block was effective in terminating re-entrant activities in the remodelled atria.

5.4.3 Effects of K\(^+\) channel block

K\(^+\) channel blockers represented by class III agents delay the repolarisation phase of the AP and thus prolong atrial APD and refractory period, which can cause disruptions and eventually termination of the re-entrant circuits [53]. However, K\(^+\)-channel blockers such as dofetilide and sotalol which inhibit \(I_{\text{Kr}}\) can potentially promote ventricular arrhythmias, prolong QT interval, and increase the risk of \textit{Torsades de pointes} arrhythmias [52,278]. Pharmacologically blocking atrial-specific K\(^+\)-channels may overcome the potential risks of adverse effects in the ventricles while exerting antiarrhythmic effects in the atria. \(I_{\text{Kur}}\) is believed to be such an atrial-selective substrate for drug interventions, and effects of \(I_{\text{Kur}}\) block have been extensively studied [186,188,189,243,254–257]. Interestingly, many existing \(I_{\text{Kur}}\) blockers potently block other K\(^+\)-channels including \(I_{\text{to}}\) and \(I_{\text{K, Ach}}\) [189,254,260,261]. The additional blockades of these channels may contribute to the antiarrhythmic effects of those drugs, which is not well understood.

In this Chapter, acacetin, a compound initially isolated from the traditional Chinese
medicine *Xuelianhua*, was considered as a representative $I_{Kur}$ blocker. The effects of acacetin on atrial electrophysiology were revealed in two ways: simulating a) the full actions of acacetin on its targeting channels ($I_{f0}$, $I_{Kur}$, $I_{Kr}$ and $I_{Ks}$) [254] and b) the effects of acacetin blocking $I_{Kur}$ only. This method allowed for investigations into the effects of $I_{Kur}$ block alone as well as revealing the potential benefits of additional inhibitive effects of $I_{Kur}$ blockers on other $K^+$ channels.

5.4.3.1 **Selective $I_{Kur}$ block**

Blocking $I_{Kur}$ with 3 μM acacetin exerted APD prolongation under normal conditions (9.8 ms) (Figure 5.5). Experimentally, the effect of $I_{Kur}$ block on human atrial APD$_{70-90}$ under normal (SR) conditions can exert prolongations or shortenings in the APD, which is dependent on the baseline AP morphology [157,189,193,256,270]. Additionally, the extension in APD by $I_{Kur}$ block observed in the present study is aligned with the previous study presented in Chapter 3 simulating the effects of genetic variations down-regulating $I_{Kur}$ also presented both APD prolongations and abbreviations in different baseline models. Moreover, inhibiting $I_{Kur}$ under normal conditions elevated the AP plateau potential and APD$_{30}$ (Figure 5.5), which matched well with experimental studies [157,189,193,256,270] and the simulations on the genetically down-regulated $I_{Kur}$ in Chapter 3.

Under the cAF conditions, a more pronounced prolongation in APD (by 23.6 ms for 1 Hz and 16.2 ms at 6 Hz, Figure 5.7) was exerted by $I_{Kur}$ block simulating effects of 3 μM acacetin, despite the fact that the current was down-regulated in cAF remodelling [279–281] (Table 5.1). These results are in accord with previous experimental studies blocking $I_{Kur}$ with [281], XEN-D0101 [282], AVE-0118 [193], 4-AP [193] and MK-0448 [256]. In addition, $I_{Kur}$ block exhibited enhanced rate-dependent adaptations in APD both at cellular (Figure 5.9) and 1D strand (Figure 5.10) models. The CV restitution curve was shifted towards higher BCLs whilst the CV at slow pacing rates was not affected (Figure 5.10). In tissue simulations, applying $I_{Kur}$ block alone (3 μM acacetin) destabilised the cores of rotors and caused a slight slowing down of the spiral waves without terminating the re-entries within the simulated time period (Figure 5.12, Figure 5.13), suggesting a limited efficacy of terminating re-entries of $I_{Kur}$ block alone. Similarly, in an experimental study Burashnikov *et al.* [189] showed that block of $I_{Kur}$ using 4-AP had limited efficacy in suppressing AF in an experimental model of AF in canine atria. This limited efficacy could be attributed to the fact that $I_{Kur}$ density was
reduced at a high frequency of activation (as suggested in [189,283] and shown in Figure 5.2) as well as by cAF-induced remodelling [279–281]. In addition, $I_{Kur}$ is primarily active during phase 2 of AP, and hence the pure $I_{Kur}$ block exerted a relatively greater prolongation in APD$_{30}$ than APD$_{90}$ (Figure 5.6), in contrast to other $K^+$-blocks including dofetilide mediating greater anti-AF effects through the prolongation of the terminal phase of the AP [12,189].

Furthermore, in this study $I_{Kur}$ block was simulated using a state-dependent block model, which successfully reproduced the use- and rate-dependent inhibition of acacetin (Figure 5.2). The rate-dependent block of $I_{Kur}$ exerted a higher fractional inhibition in the current at faster pacing rates, which is likely favourable for producing greater anti-AF effects in the presence of high-frequency excitations including re-entrant waves. Along with the previous modelling studies on investigating effects of $I_{Kur}$ block [186,243], this study demonstrated the importance and advantage of explicitly considering the kinetic properties of the blockade in computational efforts revealing the consequences and underlying mechanisms of $I_{Kur}$ block.

### 5.4.3.2 Effects of combined $K^+$-blocks

The combined block of $K^+$ channels (as exhibited by acacetin and many other $I_{Kur}$ blockers) exerted synergistic effects in APD prolongation and an increased efficacy in terminating re-entries in tissue as compared to the pure $I_{Kur}$ block.

At the cellular level, the combined actions of acacetin produced greater prolongations in atrial APD than the sum of changes by individual channel blocks under both normal and cAF remodelled conditions (Figure 5.5). In the pure $I_{Kur}$ block, the elevated and prolonged plateau phase of the AP could promote the activation of $I_{Kr}$, which in return accelerate the repolarisation of AP-phase 3 (As observed in Chapter 3). In this case, additional inhibition in $I_{Kr}$ by an identical fraction is expected to result in a greater APD prolongation than a pure $I_{Kur}$ block. The combined $K^+$-blocks also increased the rate-adaptively of APD as compared to the pure $I_{Kur}$ block (Figure 5.9), which was consistently observed in the 1D simulations (Figure 5.10).

In 2D simulations, the combined $K^+$-blocks exerted a greater efficacy in suppressing AF than the pure $I_{Kur}$ block: the meandering of spiral rotors was promoted (Figure 5.13) and the lifespan of re-entries was markedly shortened(Figure 5.16) accompanied by a more pronounced slowing down in the spiral wave activations (Figure 5.17).
The synergistic effects demonstrated by the combined K⁺-blocks have implications on developing novel pharmaceutical anti-AF therapies. Given that Iᵦ, I_Kr and I_Ks contribute to the repolarisations of ventricular APs, inhibitions in these channels may possess risks of promoting side effects in the ventricles. In this regard, combined block of atrial-specific K⁺ channels may be favourable. Recently, another two families of K⁺-channels dominantly expressed in the atria have been identified: the small-conductance Ca²⁺-activated K⁺ (SK) channels (I_{SK}) [284–290], and the two-pore K⁺ (K2P3.1) channel (I_{TASK-1}) [291–296], further to the well-known constitutively active acetylcholine (Ach)-activated K⁺ current (I_{K,Ach}). Seeking and utilising various combinations of the combined blocks of these atrial-specific channels may exert greater and safer antiarrhythmic effects in the atria, warranting future investigations.

5.4.4 Synergistic effects of Combined Na⁺- and K⁺- block

The present study highlights synergistic effects of combined Na⁺ and K⁺-block (I_{Na} and pure-I_{Kum}/multi-K⁺-channel block), in addition to the synergistic benefits demonstrated by the multi-K⁺ channel block.

In cAF-remodelled atria in comparison to a single channel block, combined block of Na⁺- and K⁺-channels markedly increased the fractional I_{Na} inhibition and APD prolongation (Figure 5.6) and promoted pronounced AP alternans at a rapid pacing rate (6 Hz), mediating synergistic antiarrhythmic effects. These effects were also consistently demonstrated in the simulations modelling the actions of blockers at low concentrations (Figure 5.8). In the simulations varying the blockade kinetics of I_{Na} block, the combined blocks dramatically augmented the attainable maximal AF-selectivity as a result of promoted atrial-selectivity and rate-selectivity as compared to the pure Na⁺-block (Figure 5.11).

In the 1D model of an atrial strand, combined Na⁺ and K⁺-block produced synergistic reductions in V_{max} and CV; the threshold of BCL allowing a 1:1 conduction was elevated as compared to the control conditions (Figure 5.10). In simulated re-entries, the combined I_{Na}B + Acacetin/Full exhibited a greater efficacy in suppressing AF with a decreased lifespan of rotors as compared to that by either individual block (Figure 5.12, Figure 5.13, Figure 5.16). The combined I_{Na}B + I_{Kum}B did not further reduce the lifespan of spiral waves as compared to the I_{Na}B alone; however, the combination did lead to the extinction of one of the two rotors (Figure 5.13, Figure
5.16) and deceleration in re-entrant activations (Figure 5.17). Additionally, the combined Na\(^+\)- and K\(^+\)-block consistently produced greater anti-AF effects compared to K\(^+\)-block alone. Moreover, further simulations reducing concentrations of blockers demonstrated consistent synergistic antiarrhythmic effects, clearly demonstrating the favourable antiarrhythmic benefits of combined Na\(^+\)- and K\(^+\)- block.

The multi-channel blockade is increasingly recognised as a strategy for pharmaceutical therapy of AF both experimentally [12,263,264,297] and clinically [298,299]. In the previous study by Aguilar et al. [12], synergistic AF-suppressing effects were demonstrated both in silico and experimentally in healthy canine hearts. Additionally, the favourable synergistic antiarrhythmic effects have also been reported in combined blocks of I\(_{SK}\) and I\(_{Na}\) in an experimental atrial-fibrillated guinea pig model [263]. The present study advanced the previous studies by revealing the synergistic effects of combined Na\(^+\)- and K\(^+\)- block in cAF-remodelled human atria, providing insight to the on-going interest in developing multi-channel blockade for the treatment of AF.

Although the multi-channel block is being regarded as a promising pharmaceutical therapy for treating cardiac arrhythmias, it has also been reported to cause lethal consequences. Dronedarone, a multi-channel blocker, was originally shown to be beneficial in restoring sinus rhythm while decreasing hospitalisation and lethal cardiac events for patients with intermittent (paroxysmal and persistent) atrial fibrillation [300]. In the HARMONY trial, the combined administration of ranolazine and dronedarone in paroxysmal AF patients exhibited synergistic reduction in AF burden [299]. In another clinical trial, PALLAS (Permanent Atrial Fibrillation Outcome Study Using Dronedarone on Top of Standard Therapy), administration of dronedarone in patients with permanent atrial fibrillation increased incidences of stroke, heart failure, and lethal events of cardiovascular causes [301]. Similarly, while it has been demonstrated that combined ranolazine and amiodarone administration increased efficacy in the management of AF [297], it may also be responsible for incidences of Torsade de points [302]. Therefore, the effects, efficacy and safety of these drugs can be dependent on the stage of the arrhythmia, and the current and history conditions of the patient. Care must be taken in interpreting the anti-arrhythmic effects of the multi-channel blocks presented in the present Chapter, and further investigations are warranted to evaluate the safety of such combined channel block in the wide effort of pharmaceutical therapy of AF.
5.4.5 Relevance to previous modelling studies on $I_{Kur}$ block

There have been extensive efforts in modelling the actions and mechanisms of K+-block, specifically the blockade of $I_{Kur}$ in the atria [186,188,243]. Using variant models describing $I_{Kur}$ block, these studies highlight the important role of the blockade kinetics in the antiarrhythmic effects exerted by $I_{Kur}$ block. The present study utilised a state-dependent block model to simulate the drug actions of acacetin, an experimentally justified compound that was initially isolated from a Chinese traditional medicine, which is an advance compared to previous studies simulating effects of theoretical blockers. Furthermore, a major focus of the present study was to assess the synergistic effects of multichannel block, differing from these studies focusing on pure $I_{Kur}$ block.

5.4.6 Limitations

The single cell model was inherited from the Colman et al. human atrial model [35]. Therefore, the limitations native to the Colman et al. model [35] apply in the present study.

Parameters of atrial $I_{io}$ were demonstrated to be different to those of ventricular $I_{io}$ in human [303]. The potency of acacetin on ventricular $I_{io}$ was assumed to be identical to that on atrial $I_{io}$. Previous studies suggest the IC$_{50}$ of 4-AP block of atrial $I_{io}$ is one-third of that of ventricular $I_{io}$ [303,304]. Therefore this assumption may not hold and its validity warrants further studies. However, if a similar inter-atrial-ventricular difference in the IC50 of $I_{io}$ block could exist for acacetin, the effects of acacetin on the ventricular electrophysiology would be less substantial, which might contribute to a smaller change in ventricular $I_{Na}$ for the combined blocks of $I_{Na}$B and Acacetin/Full and thus enhance the computed atrial-selectivity and AF-selectivity of the combined blockades. A similar limitation lies in modelling the effects of acacetin on ventricular $I_{Kr}$ and $I_{Ks}$. Given that applying acacetin in vivo did not prolong QT intervals in dogs [254], the realistic prolongation in ventricular APD may not be greater than the simulated results (Figure 5.6 B and Figure 5.7 C). Therefore, the assumption of no atrial-ventricular difference in the potency of acacetin on K+-channels may not compromise the conclusions on the atrial-selectivity of combined Na$^+$ and K$^+$-block.

Additionally, in the absence of detailed experimental data for state-dependent blocks of $I_{io}$, $I_{Kr}$ and $I_{Ks}$ by acacetin, the block of these channels was modelled using a single pore block model. Also, the present work did not attempt to model the effects of
acacetin on $I_{K,Ach}$, although the study shows the current is potently blocked by the compound.

Furthermore, clinically-used administrations of class Ic agents representing Na$^+$-block in the cardiac arrhythmia suppression trial were associated with increased mortality [305]. Further investigations are warranted to assess the safety of the proposed Na$^+$-block in the heart, especially in the ventricles.

5.5 Summary

In the present chapter using state-dependent block models, the antiarrhythmic effects of Na$^+$-block and K$^+$-block on the cAF remodelled atria were demonstrated. The kinetic parameters in K$^+$-block were chosen to describe the actions of acacetin, a compound initially isolated from a traditional Chinese medicine inhibiting $I_{Kur}$, $I_{io}$ and $I_{Kr}$. The present study demonstrated that combined block of multiple K$^+$-channels as well as combined blockade of Na$^+$- and K$^+$-channels produced synergistic anti-AF effects, suggesting that developing multi-channel (multiple K$^+$ channels and/or combined Na$^+$- and K$^+$-channel) blockade is a potentially valuable strategy for the treatment of AF.
Chapter 6
Developing a human ventricle-torso model for investigating ventricular activities and ECG

Addendum In this Chapter, the torso model for simulating the body surface potential was provided by Dr Erick A. Perez Alday. Aspects of work in this Chapter has been presented in [306]:
in which the author (HN) contributed to all ventricular simulations. The corresponding text has been reworded where possible in this Chapter.
The experimental 36-channel ECG (Figure 6.9 A) was kindly provided by collaborators (Zhang C, Gan Z) from Peking University, China. The data have also been used in [306].

The last three chapters have been mainly focused on investigations of the K⁺-channel meditated arrhythmogenesis and functional impacts in the atria. Whilst the most common arrhythmias in clinical settings are associated with the upper chambers of the heart [11,307], ventricular arrhythmias are more prominent and lethal, which can lead to sudden death [4]. In the diagnosis of these cardiac conditions, the electrocardiogram (ECG) has been implemented as a standard bedside evaluation procedure for many decades [100–102]. A virtual heart-torso model that simultaneously simulates the dynamic activities of the ventricles and the respective ECGs may be valuable to help advance understandings to the heart conditions and provide insights into novel therapies.
In this chapter, a biophysically detailed 3D model of human ventricles with accurate anatomical geometry and fibre orientations was developed to simulate the ventricular electrical activation and recovery. The model was then integrated with a human torso model to simulate the body surface potential arising from the electrical activity of the heart. The ECG was derived from the computed body surface potential.
The virtual human ventricle-torso platform was applied to study the impact of bundle branch block and ventricular ischaemia on the ECG.

6.1 Introduction

Although the most common arrhythmias are associated with the atria in clinical settings [11,307], ventricular arrhythmias are more substantial and lethal, which can lead to sudden death [4]. It is estimated that sudden death from cardiac causes accounts for approximately 50% of all deaths from cardiovascular causes [4]. This is expected due to that the ventricles, especially the left ventricle, are the major contributors driving the blood through the circulatory system. The lethal ventricular arrhythmias are linked with variant conditions including myocardium ischaemia [308], hypertrophy, heart failure, congenital heart disease, neurological disorders and cardiomyopathies [309]. Pharmaceutical therapies may also promote ventricular arrhythmia through interactions with cardiac ion channels [51,310–312], which has to be taken into considerations in developing novel antiarrhythmic drugs and therapy for the management of AF.

The ECG has been employed in clinical settings for many decades [313]. ECG monitoring has been adopted in the diagnosis of complex cardiac arrhythmias, myocardial ischaemia and QT prolongations [314]. Clinically, alterations to ECGs are generally associated with cardiac abnormalities. For example, the torsades de points indicate a presence of ventricular arrhythmias [52]; myocardium ischaemia induces changes in the ST-segment of ECGs [5,100,306,315,316]; prolonged QT intervals have implications of pro-arrhythmic consequences [3,52,312].

The waveform of the ECG is closely related to the electrical activity of the heart. The QRS complex represents the activation phase of ventricles whilst the T-wave corresponds to the repolarisation phase. Although the two major deflections of the ECG represent two opposite processes of the electrical activity of the myocardium, the QRS complex and T-wave are both positive in the majority of ECG leads [313,317]. A generally accepted hypothesis underlying this phenomenon was given by Wilson in 1931: the activation and repolarisation waves should propagate in opposite directions in order to produce concordant T- and R-waves [313,317]. In this regard, the dispersion of repolarisation is important for the genesis of the T-wave [313,317–319], although the underlying spatial electrophysiological heterogeneities of the genesis of T-wave remain controversial [313,317]. The spatially heterogeneous APD of ventricular cells and the
Haibo Ni

activation sequence determine the dispersion of repolarisation pattern.

Large gradients in the APD of ventricular cells along multiple axes have been reported, suggesting that the ventricular electrical heterogeneity is intrinsically complex. Previous experimental studies have demonstrated the existence of APD gradients between the apical and basal ventricular cells [317,320–324], between the two ventricles [325–328] and transmurally across the endocardium and epicardium [313,329–334]. The heterogeneous distribution of ion channels underlies, at least in part, the electrical heterogeneities in the ventricles [328].

A virtual ventricle-torso model integrates multiscale electrophysiological components from ion channels to a human torso [245,335]. Such a model enables investigations on the impact of alterations in the ion channels on the organ as a whole, which manifests as modifications to the body surface potential (BSP). The BSP is determined by the electrical potentials within the heart and can be obtained by solving the forward problem of electrophysiology [335–337]. The ventricle-torso model, therefore, is valuable in elucidating mechanisms of cardiac functions and diseases [306] as well as developing novel treatment strategies for cardiac conditions such as anti-AF drug safety assessment [335]. The integrated ventricle-torso model has also been applied to advance our understandings in the role of electrical heterogeneities in ventricles along variant axes in the genesis and polarity of T-wave [337,338].

In this Chapter, an integrated ventricle-torso model was developed based on biophysically detailed models of human ventricular electrophysiology and anatomically accurate geometry of human ventricles and torso. The ventricles were heterogeneously stimulated from the surface of the endocardium, accounting for the initiation of electrical activations from the Purkinje fibres (PF). In order to account for the repolarisation gradients in the ventricles [328], transmural (TM), interventricular (IV) and apicobasal (AB) gradients in the ventricular APDs were incorporated into the 3D ventricular model. ECGs were derived from selective positions of BSP attained by solving the forward problem of electrophysiology. The usefulness of the ventricle-torso model was demonstrated by simulated effects of bundle branch block (BBB), drug administration and acute ventricular ischaemia on ECGs.
6.2 Methods

6.2.1 Human ventricular cell models

6.2.1.1 Single cell model of human ventricles
To simulate the APs of the human ventricular myocytes, the updated version of the human ventricular single cell model used by Adeniran et al. [126] was employed. This single cell model was based on the updated version [339] of the human ventricular model proposed by Tusscher, Noble, Noble and Panfilov [340]. In the recent study, Adeniran et al. [126] incorporated a Markovian formula for the rapid delayed rectifier potassium current (I_{Kr}) to replace the original model of I_{Kr}, and demonstrated an advantage in reproducing the biophysical properties of the current. Following O'Hara et al. [36], a late component of the Na^+ current (I_{NaL}) was incorporated through adapting the model of I_{NaL} from the model developed used in O'Hara et al. [36].

6.2.1.2 Transmural heterogeneity
It is well received that a transmural gradient exists across the ventricular myocardium. Previous studies have demonstrated that the Endo myocardium (ENDO) has a longer APD than the cells from the epicardium layer (EPI) in rabbit [313], canine [331] and human [329,334,341]. Although the M cells (a regional type of cells located within the deep sub-endocardium) have been observed in multiple experiments and species [82,331,332,342], many studies failed to detect the presence or demonstrate the functional role of M cells in vivo or in vitro mappings [317,329,343–345].

In this Chapter, multiple configurations of TM segmentations both with and without M cells were implemented in order to investigate the role of TM heterogeneity in the genesis and deflection of T-wave (more details given in 6.2.2.2). A model of TM heterogeneity was developed based on that by Adeniran et al. [126]. In the new TM model, an EPI-ENDO gradient in I_{Kr} and I_{Ks} was incorporated to account for the ENDO-EPI gradient in APD as seen in [329,334,341], in contrast to the original TM model [126]. A TM gradient in I_{NaL} was incorporated as suggested in [346]. The parameters for the TM model are listed in Table 6.1.

6.2.1.3 Apico-basal heterogeneity
The experimental data on the apicobasal gradients of ionic currents presented in Szentadrassy et al. [321] were incorporated to account for the electrical heterogeneities
along the AB axis. Table 6.2 illustrates the model parameters for the AB heterogeneity.

Table 6.1 Scaling factors accounting for the transmural heterogeneity. The factors were defined as the ratio of the conductance of EPI/M to that of ENDO cells. Parameters were compiled from the source code of Adeniran et al. [126]. These scaling factors applied to both right and left ventricles.

<table>
<thead>
<tr>
<th>Ionic current</th>
<th>EPI/ENDO</th>
<th>M/ENDO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{K1}$</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>$I_o$</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>$I_{Ks}$</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>$I_{NaL}$</td>
<td>1.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 6.2 A summary of difference in ionic currents between apical and basal cells implemented to account for the AB heterogeneity. Parameters were adapted from [321].

<table>
<thead>
<tr>
<th>Ionic currents</th>
<th>Difference in parameters for basal cells relative to apical cells</th>
</tr>
</thead>
</table>
| $I_o$         | Conductance: $\times 0.56$  
                | $V_{1/2}$ of steady-state inactivation variable: shifted by $-4$ mV  
                | Slope $K$ of steady-state inactivation variable: $\times 0.76$.  |
| $I_{Ks}$      | Conductance: $\times 0.56$  
                | $V_{1/2}$ of steady-state activation variable: shifted by $-4.9$ mV  |

6.2.1.4 *Inter-ventricular heterogeneity*

The IV heterogeneity in the ventricular APD is attributable to the greater activities of $I_o$ and $I_{Ks}$ in the RV as compared to the LV [347]. In the present study, a family model of RV cells was constructed by a 1.8-fold increase in the conductance of both $I_{Ks}$ and $I_o$ of the LV cell models while keeping the TM heterogeneity model identical to the LV models (Table 6.1). The IV gradient in $I_o$ and $I_{Ks}$ was selected based on the IV difference in the current density of these channels presented in Volders et al. [347], which is concordant with the expression levels reported in Opthof et al. [327].


6.2.2 Modelling electrical activities in human ventricles

6.2.2.1 3D model of human ventricles

In 3D, the electrical activity in the ventricles was simulated using an anatomically accurate human ventricular geometry that was reconstructed from DT-MRI scanned ventricles [126,348]. In order to reduce computational cost, the geometry was down-sampled so that the spatial resolution was 0.3 mm along all axes with approximately 7.2 million nodes in total, in contrast to around 24 million in the original model [126]. Fibre orientations were incorporated to account for the intrinsically anisotropic properties of the media [126,348]. The ventricular geometry and fibre orientations are illustrated in Figure 6.1.

The excitation and wave propagation in the tissue was abstracted to be a diffusion-reaction problem and modelled with the monodomain equation [126,128]. The diffusion tensor \( \mathbf{D} \) describing the conductivities of the tissue along different directions was set to 0.18 mm\(^2\)/ms along the fibre direction and 0.06 mm\(^2\)/ms across the fibre direction, respectively, assuming a 3:1 ratio in the conductivities along the two directions. These values in \( \mathbf{D} \) corresponded to a planar conduction velocity (CV) of 71.9 cm/s along fibre direction and 42.5 cm/s across fibre direction, respectively, computed using a 2D slab model of ventricular tissue with a same spatial resolution. The CV along the fibre orientation is close to the 70 cm/s conduction velocity along the fibre direction found in human ventricles [349]. The simulated cross-fibre CV is also concordant with the observed intramural CV of 46.4 cm/s by Durrer et al. [350].

Following previous studies [126,351], in the present study, the ventricles were paced at the empirically determined activation sites across the endo-surface of ventricular walls. The timing of applying a stimulus for individual sites was heterogeneous. Such a method allows for simulations of a complex ventricular excitation pattern in the absence of a detailed Purkinje conduction network. The placement of the stimulation sites on the endo-surface of the ventricular walls is valid, considering that the Purkinje fibres penetrate less than one-third of the endocardium [352].

The parameters of the endocardium stimulation profile used in previous studies [126,351] were utilised and updated in this study. Durrer et al. [350] suggest the presence of a small right-to-left contribution to the activation of the interventricular septum, which was modelled by Ten Tusscher and Panfilov [353]. Therefore, in the
present Chapter, further simulation sites were added to represent the Purkinje-ventricle junctions on the right side of the interventricular septum. The time instant of the stimulation sites from [351] were semi-automatically modified. This was done through multiple iterations until good agreement was reached between the calculated QRS complexes and the corresponding clinical recordings of the Einthoven lead II. In this study, instead of matching the QRS complex of a single lead, the profile of time instant of the stimulation sites was further optimised in order to reach a good agreement in the deflections of QRS complexes for all leads of a 36-lead ECG. The optimised endocardium stimulation profile is illustrated in Figure 6.2.

### 6.2.2.2 Implementation of electrical heterogeneities

In contrast to previous simulation studies [126,337,338,348] where M cells were simulated as a distinctive layer of the myocardium, in the present study, the M cells were first assumed to exist as isolated small islands in the endocardium of the ventricles. This was done based on the observations that M cells does not present or have no functional role in the ventricles in vivo or in vitro mapping [317,329,343–345], and the hypothesis that the small islands of myocytes exhibiting longer APDs than the neighbouring cells as seen in [334,341] was due to the electrotonic coupling at the Purkinje-ventricle junctions (PVJ) [329,354]. In these simulations, the endocardium stimulation sites were segmented as M cells; the ENDO cells accounted for approximately 50% of the myocardium, as illustrated in Figure 6.1 A.

In 3D simulations, the AB and IV gradients in the electrophysiological properties of ventricular cells were incorporated, assuming that the parameters vary linearly with respect to the location of each cell, which appears likely in recent experimental observations [327].

The parameters governing the AB gradient of an arbitrary cell is given by:

\[
s_x = s_{\text{Base}} + (s_{\text{Apex}} - s_{\text{Base}}) \cdot f_{\text{AB}}
\]

\[
h_x = (h_{\text{Apex}} - h_{\text{Base}}) \cdot f_{\text{AB}}
\]

where \(s_x\) is a scaling factor representing an ion channel conductance or the slope of a steady state variable (as used in Table 6.2) of the arbitrary cell, \(s_{\text{Apex}}\) and \(s_{\text{Base}}\) are the corresponding values for the cells at the apex and base, respectively; \(h_x\), \(h_{\text{Apex}}\) and \(h_{\text{Base}}\) represent the \(V_{1/2}\) of steady-state variables of the arbitrary, apical and basal cells,
respectively; $f_{AB}$ is the gradient factor, which is linearly dependent on the location $z$ along the AB axis:

$$f_{AB} = \frac{z - z_{Base}}{z_{Apex} - z_{Base}}$$

(6.3)

The IV gradient was implemented using a linear equation similar to Equation (6.1):

$$g_x = g_{LV} + (g_{RV} - g_{LV}) \cdot f_{IV}$$

$$f_{IV} = \frac{l - l_{LV}}{l_{RV} - l_{LV}}$$

(6.4)

(6.5)

where $g$ represents the conductance of $I_{to}$ and $I_{Ks}$; $f_{IV}$ is the IV gradient factor and $l$ is the location measured along the IV direction.

Further simulations incorporating different settings of TM heterogeneity both with and without M cells were performed, in order to evaluate the functional role of TM heterogeneity in the genesis and polarity of T-wave. The effects of AB and IV heterogeneities on T-wave were also assessed. A list of these configurations is illustrated in Table 6.3.

Table 6.3 A summary of configurations for electrical heterogeneities used in the present Chapter. The ratio of TM heterogeneity indicates ENDO: M : EPI or otherwise labelled.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>TM het.</th>
<th>IV het.</th>
<th>AB het.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMO</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Cells were simulated using the model of ENDO cells.</td>
</tr>
<tr>
<td>HET/Control</td>
<td>5:0:5</td>
<td>Yes</td>
<td>Yes</td>
<td>The stimulation sites were segmented as M cells.</td>
</tr>
<tr>
<td>AB</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Cells were simulated using the model of ENDO cells.</td>
</tr>
<tr>
<td>IV</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Cells were simulated using the model of ENDO cells.</td>
</tr>
<tr>
<td>TM</td>
<td>5:0:5</td>
<td>No</td>
<td>No</td>
<td>The stimulation sites were segmented as M cells.</td>
</tr>
<tr>
<td>w/o AB</td>
<td>5:0:5</td>
<td>Yes</td>
<td>No</td>
<td>The stimulation sites were segmented as M cells.</td>
</tr>
<tr>
<td>w/o IV</td>
<td>5:0:5</td>
<td>No</td>
<td>Yes</td>
<td>The stimulation sites were segmented as M cells.</td>
</tr>
<tr>
<td>w/o TM</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Cells were simulated using the model of ENDO cells.</td>
</tr>
<tr>
<td>TM-433</td>
<td>4:3:3</td>
<td>Yes</td>
<td>Yes</td>
<td>The stimulation sites were segmented as ENDO cells.</td>
</tr>
<tr>
<td>TM-343</td>
<td>5:0:5</td>
<td>Yes</td>
<td>Yes</td>
<td>The stimulation sites were segmented as ENDO cells.</td>
</tr>
<tr>
<td>TM-235</td>
<td>2:3:5</td>
<td>Yes</td>
<td>Yes</td>
<td>The stimulation sites were segmented as ENDO cells.</td>
</tr>
<tr>
<td>TM-NEG55</td>
<td>5:0:5</td>
<td>Yes</td>
<td>Yes</td>
<td>The stimulation sites were segmented as ENDO cells. The ENDO cells were modelled using the model of EPI cells and vice versa, creating a negative transmural gradient in APD.</td>
</tr>
</tbody>
</table>
Figure 6.1 3D human ventricle geometry reconstructed from DT-MRI images and fibre orientation. (A) Anterior and cross-section views of the human ventricular geometry and segmentation. The ventricles were segmented into LV and RV; transmurally the ventricles were segmented into EPI, ENDO, and M cells were represented by the stimulation sites. (B) Traced fibre orientation of the ventricles.

Figure 6.2 Illustration of ventricular stimulation sites. The top panels show an anterior view, whilst a cross-section view is given in the bottom panels. The colour map of the simulation sites indicates the timing of stimulation.
6.2.3 Torso model and ECG

The 3D ventricular model was integrated with a previously developed anatomical model of human torso [306,355,356] (Figure 6.3). Heterogeneous conductivities of lungs, liver, stomach, kidneys, blood masses, spinal cord and ribs were incorporated into the torso model (Figure 6.3 A). The forward problem of electrophysiology was solved using the boundary element method (BEM, [357]) to obtain the body surface potential of the torso resulting from the electrical activity of the ventricles. The standard 12-lead ECG was derived from the calculated body surface potential. A 36-channel ECG was also computed for the purpose of validation. An illustration of the positions of virtual electrodes for the 12- and 36-lead configurations is given in Figure 6.3 B-C.

![Figure 6.3 An illustration of the ventricle-torso model. (A) Geometries of the ventricle-torso model, internal organs, spinal cord and ribs. (B) Positions for virtual electrodes for a 12-lead ECG setting. (C) Positions for virtual electrodes for a 36-lead ECG setting.](image)

6.2.4 Modelling ventricular ischaemia

Cardiovascular ischaemia remains one of the world’s leading causes of death [100,114,115]. In the present study, effects of acute ventricular ischaemia on the ECGs were simulated using the proposed ventricle-torso model. Acute ischaemia occur in 2 phases: phase 1A (first 2 – 10 minutes) and 1B (15 – 45 minutes after coronary occlusion) [358,359]. Here only the simulations concerning the first 10-minute post
occlusion were performed. Three pathological mechanisms of ischaemia were considered: (i) hyperkalemia: an increase in extracellular potassium concentration; (ii) acidosis: decreases in the maximum conductivity of Na\(^+\) and L-type Ca\(^{2+}\) currents (\(I_{\text{Na}}\) and \(I_{\text{CaL}}\)); (iii) hypoxia: activation of ATP-dependent potassium current, \(I_{\text{KATP}}\) [116,117]. A detailed summary of these changes is given in Table 6.4.

\(I_{\text{KATP}}\) was modelled using the formula from Kazbanov et al. [116], given by:

\[
I_{\text{KATP}} = G_{\text{KATP}} \cdot f_{\text{ATP}} \cdot \left( \frac{[K^+]_o}{5.4} \right)^{0.3} \cdot \frac{1.0}{40 + 3.5e^{0.025V}} \cdot (V - E_K)
\]  

(6.6)

where \(G_{\text{KATP}}\) is the maximum channel conductance, \(f_{\text{ATP}}\) the fraction of open, \([K^+]_o\) the extracellular \(K^+\) concentration, \(V\) the membrane potential, \(E_K\) the Nernst reversal potential for \(K^+\). The parameters of \(I_{\text{KATP}}\) were kept the same as Kazbanov et al. 2014 [116].

Table 6.4 Summary of ischaemia-induced alterations to the electrophysiological parameters of the human ventricles. \([K^+]_o\) – extracellular \(K^+\) concentration; \(S_{\text{GNa}}\) and \(S_{\text{GCaL}}\) – scaling factors in the conductance of \(I_{\text{Na}}\) and \(I_{\text{CaL}}\), respectively; \(G_{\text{NaL}}\) – the conductance of \(I_{\text{NaL}}\). Parameters derived from [116,117].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Acute ischaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>([K^+]_o)</td>
<td>5.4</td>
<td>10.0</td>
</tr>
<tr>
<td>(f_{\text{ATP}})</td>
<td>0</td>
<td>0.55%</td>
</tr>
<tr>
<td>(S_{\text{GNa}})</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>(S_{\text{GCaL}})</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>(G_{\text{NaL}})</td>
<td>0.0065</td>
<td>0.065</td>
</tr>
</tbody>
</table>

To perform a comparison of ischaemia-induced changes in the ECG, multiple ischaemic lesion models of various locations were created. To simplify the problem, circular zones of 27 mm in radius representing transmural ischaemic regions were used. Five representative ischaemic lesions were modelled: ischaemic lesions in the RV, SEP and LV walls, as shown in Figure 6.4. Similar to previous studies [247,360], the lesions consisted of central zones (CZ) and border zones (BZ), with CZ accounting for the myocardium lesion that is within 80\% of radius to the centre. The parameters describing the ischaemic effects were varied linearly from CZ to the normal myocardium (Figure
6.3 Results

6.3.1 Single cell simulations

Ventricular cells were stimulated at 1 Hz, and the APs are shown in Figure 6.5. In simulating RV cells, $f_{IV}$ was set to 1.0 representing maximum gradient of IV.

The cell models of various regions are compared, and the regional APDs are summarised in Table 6.5. As compared to the ENDO cells, the phase 1 repolarisation of M and EPI cells were shown to be more pronounced and further enhanced in RV cells, which was attributable to a greater activity of $I_{io}$ in the two latter cell types (Table 6.1). Large gradients in APD were observed along all axes. In all regions, the M cells exerted longest APD, which was followed by ENDO and then EPI cells. The TM heterogeneities in APD (M-EPI difference) measured at the centre (along the long axis of the heart) of the ventricles were 89 ms and 78 ms for LV and RV, respectively. The
IV gradient in APD was also observed: for the central ventricles the APD difference between the corresponding EPI, M and ENDO cells of the two ventricles was 38, 49 and 47 ms, respectively. The AB gradient APD was measured to 72, 85 and 81 ms for EPI, M and ENDO cells of LV, respectively. Similar values were also found in the RV-AB gradient. Furthermore, the IV and TM gradients were greater at the base as compared to those measured at the apex of ventricles: (by 13-15 ms for TM, and 2-5 ms for IV).

Figure 6.5 Simulated time courses human ventricular APs at a BCL of 1000 ms. (A) APs of LV cells: (i) ENDO cells from LV (LVENDO), (ii) M cells from LV (LVM) and (iii) EPI cells from LV (LVEPI). (B) APs of RV cells: (i) ENDO cells from RV (RVENDO), (ii) M cells from RV (RVM) and (iii) EPI cells from RV (RVEPI). In each panel, APs of cells at the apex (Apex), central (Centre) and base (Base) of the ventricles are plotted.

Table 6.5 Summary of APD (ms) produced by the regional ventricular models.

<table>
<thead>
<tr>
<th></th>
<th>Apex</th>
<th>Centre</th>
<th>Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEPI</td>
<td>285</td>
<td>312</td>
<td>357</td>
</tr>
<tr>
<td>LVM</td>
<td>368</td>
<td>401</td>
<td>453</td>
</tr>
<tr>
<td>LVENDO</td>
<td>309</td>
<td>339</td>
<td>390</td>
</tr>
<tr>
<td>RVEPI</td>
<td>248</td>
<td>274</td>
<td>318</td>
</tr>
<tr>
<td>RVM</td>
<td>319</td>
<td>352</td>
<td>404</td>
</tr>
<tr>
<td>RVENDO</td>
<td>265</td>
<td>292</td>
<td>341</td>
</tr>
</tbody>
</table>
6.3.2 Modelling ventricular activation and recovery under normal conditions

Simulations were performed to model the activation and recovery of human ventricles under normal conditions. To achieve a faster convergence, the state variables of the cellular models in 3D were obtained from single cell simulations: single cell models incorporating variant gradient parameters were stimulated at 1 Hz to reach steady-state, and state variables were then used as initial conditions for the cellular models in 3D.

Figure 6.6 shows the progression of the simulated excitation in the ventricles and snapshots of body surface potentials. In the simulations, the first site activated was the lower part of the anterior RV wall and close to the interventricular septum (paraseptum). At t = 10 ms, three sites in the left ventricular endocardium wall began to activate: one in the lateral wall close to the base and another two in the anterior and posterior wall close to the apex. At t = 20 ms these activation sites expanded to larger areas in the endo-surface and the intramural conduction was much slower. The RV excitation spread towards the posterior and basal directions. At t = 30 ms, in the LV the lower activation sites spread towards the base and septum, and joined the wavefront from the RV excitations; the basal stimulation site expanded towards the apex whilst epicardium breakthrough was about to happen. At t = 40 ms, the majority of LV endocardium were activated except for the anterior wall and interventricular septum that were close to the base. At t = 60 ms, LV myocardium activation was almost completed, while the wavefront just reached the basal posterior wall of the RV. The total excitation of the ventricles was accomplished at t = 80 ms.

The calculated body surface potential showed a progressively changing pattern of positive-to-negative potential distribution (Figure 6.6 B): the positive-to-negative vector was aligned to approximately 135 ° (lower right of the torso) at t = 10 ms, and a progressive anti-clock rotation was observed for following snapshots (rotated to approximately 45 ° and – 45 ° at t = 40 and 70 ms, respectively).

Figure 6.7 illustrates a simulated repolarisation process of the ventricles and snapshots of the body surface potential map in the torso. At t = 260 ms, the apical RV myocardium began to recover, which was followed by repolarisations in the apical myocardium of the LV at t = 280 ms. Repolarisation of the ventricles spread vertically along the apex-to-base axis and transmurally from the EPI to ENDO. At t = 340 ms, recovery was almost completed in the apex of both ventricles. At t = 360 ms, the anterior walls of the RV and LVEPI have repolarised, whereas the recovery was still
Figure 6.6 Snapshots of simulated progressive excitation in the ventricles and body surface potential map of the torso. (A) Snapshots showing ventricular excitation in two views. (B) Body surface potential map during the period of ventricular activation; the white arrows indicate the vector of positive to negative potentials.
Figure 6.7 Snapshots of simulated progressive repolarisation in the ventricles and the corresponding body surface potential map of the torso. (A) Snapshots showing ventricular excitation in two views. (B) Body surface potential map during the period of ventricular activation.
underway in the endocardium of the LV. The posterior RV and LV endocardium close to the base were the last to recover.

Figure 6.7 B shows snapshots of body surface potential map during the period of ventricular repolarisation. A large proportion of the front of the torso maintained a positive potential during the process, whilst a negative potential was observed in the surface of the right side of the torso.

The activation, repolarisation pattern and activation-recovery interval (ARI) of the ventricles were quantified, as illustrated in Figure 6.8. The activation time (AT) of a cell was measured as the time instant the membrane potential of the cell depolarised to -20 mV. The repolarisation time (RT) was defined as the time when the AP of a cell reached -70 mV following a depolarisation.

Consistent with the snapshots in Figure 6.6 A, in the simulations the early activation sites were: i) the paraseptal-anterior RV endocardium wall close to the apex, ii) the LV lateral endocardium close to base, and iii) the anterior, septal and posterior endocardium of lower fourth of the LV (Figure 6.8 Ai). The first breakthrough was observed in the anterior paraseptal region of the RV, which is in good agreement with an experimental study using a non-invasive electrocardiographic imaging in vivo [361] and an in vitro study [350]. In the interventricular septum, the activation of the lower half was spread from the RV, whereas the rest was activated following the excitation of the left side. The basal posterior wall of RV was the last region to be activated, which has also been demonstrated in the in vitro study [350] (Figure 6.8 Aii). The global excitation of the ventricles spanned 83 ms, which is close to experimental studies in vitro (62 to 90 ms in [327], and 62 to 74 ms in [350]).

The recovery progressively started from the lower paraseptal region of RV (Figure 6.7 A, Figure 6.8 B). The repolarisation map manifested as a large gradient in the RT along the long axis and transmurally in the LV. The global ventricular repolarisation dispersion was 113 ms, which is well within the range of 99 to 145 ms observed in [327].

The simulated ARI map was obtained by subtracting the activation time from the repolarisation time (Figure 6.8 C). The distribution of ARI in the ventricles was characterised by large gradients in TM, AB and IV axes, which was attributable to the implementation of electrical gradients in the single cell models (Figure 6.5).
Figure 6.8 Activation, repolarisation time patterns and the activation-recovery interval (ARI) in the ventricles under normal conditions. (A) (i) Simulated activation time in the ventricles as compared to (ii) experimental observations; in (ii) figure was modified from [350]; the insert indicates where the heart was opened in the isochronic representation. (B) Simulated ventricular repolarisation pattern. (C) Simulated ARI distribution in the ventricles. In (Ai, B and C), a cross-section view is given in the top panels and anterior and posterior views are plotted in the left and right of bottom panels, respectively; a light grey mask was superimposed on the contour plots to indicate the profile of the ventricles.
6.3.3 Simulated ECGs

ECGs were derived from the calculated body surface potentials. A 36-lead ECG was first obtained to facilitate experimental validations against a measured 36-channel ECG using a similar configuration of electrode placement (Figure 6.3 C). A good agreement in the spatial distribution of the signals was demonstrated between the experimental data and simulated traces (Figure 6.9 A). The QRS complex in the right superior part of the torso was shown to be negative, and positive in the left inferior part; identical polarities were observed in a substantial majority of the QRS complexes of the 36-lead ECG. A positive T-wave was seen in most channels other than those in the right superior part of the torso. The simulated deflections of T-wave well matched the experimental data.

A 12-lead ECG was also derived from the calculated body surface potential (Figure 6.9 B). The precordial leads manifested a negative to positive transition from lead V1 to V6. For the limb and augmented limb leads, the QRS complexes of lead I and aVL were found to be positively deflected, aVR and lead III were negative in the QRS complex, and a biphasic QRS complex was observed in lead II and aVF. T-wave was found to be positive in all leads but aVR. These waveforms were qualitatively similar to the normal ECGs found in humans [362,363]. The clinical metrics of standard ECG were quantified: the simulated QRS complex duration was 82 ms; QT interval was 395 ms. These values were within the range of clinical observations [362–364].
Figure 6.9 Simulated ECGs under normal conditions. (A) Comparison of 36-lead ECG between the simulated data and experimental measurement. The numbers (top) and letters (left) indicate the position of a virtual electrode, as illustrated in Figure 6.3 C. (B) Simulated 12-lead ECG showing QRS complex and T-wave. In both panels, the ECG traces were normalised to the amplitude of each channel/lead. The normalisation of the ECGs was carried out in this Chapter otherwise mentioned.
6.3.4 Role of electrical heterogeneities

In order to investigate the role of electrical heterogeneities in the ventricular electrical activity and the resulting ECG, especially the T-wave morphology, simulations were performed with variable configurations of electrical heterogeneity (Table 6.3). Figure 6.10 illustrates the repolarisation map and corresponding 12-lead ECG without heterogeneity (HOMO) or with only AB, TM or IV heterogeneity.

In the HOMO setting, the RT map manifested a spatial distribution pattern similar to the activation time map (Figure 6.8 Ai and Figure 6.10 Ai): a transmural delay in the RT from ENDO to EPI was observed, and the basal posterior RV was the last region to recover. The overall RT dispersion was 79 ms, which is close to the extent of the global AT dispersion (Figure 6.10 Ai). The resulting ECG showed a substantial reduction in the amplitude of T-wave in all leads as compared to the HET; the polarity of T-wave remained positive in most leads other than aVR and aVL (Figure 6.10 B).

Introducing the AB heterogeneity to the HOMO setting resulted in a pronounced increase in the RT dispersion (by 37 ms as compared to that of the HOMO), which was mainly contributed by the increased RT gradient along the long axis of the ventricles (Figure 6.10 Aii). The T-wave from the AB setting manifested itself as an improved amplitude, duration and morphology in comparison to that of the HOMO configuration, despite the fact that aVL exerted inverted T-wave (Figure 6.10 B).

Compared to the HOMO setting, the incorporation of TM heterogeneity introduced a TM gradient to the RT map (Figure 6.8 Aiii), with an increased global dispersion (92 ms); the resulting T-wave exhibited an increase in the amplitude in most leads other than lead III, V5 and V6, in which the T-wave amplitude and morphology was compromised (Figure 6.10 B).

The simulations with the IV heterogeneity alone demonstrated a reduction in the RT dispersion compared with that of the HOMO configuration (Figure 6.8 Aiv). The corresponding T-wave showed a slightly reduced amplitude and duration in most leads as compared to the HOMO setting. Leads V5, V6 exerted biphasic T-wave, whilst it was inverted in aVL (Figure 6.10 B).

As compared to the simulations under individual heterogeneity and the homogeneous conditions, the HET setting exhibited a greater amplitude of T-wave in most leads.
Figure 6.10 Simulated ventricular RT map and ECG without heterogeneity (HOMO) and with only AB, TM or IV heterogeneity. (A) RT maps of (i) HOMO, (ii) AB, (iii) TM and (iv) IV. (B) Simulated ECGs with variant configurations. The configurations are detailed in Table 6.3.
Simulations with the AB, IV or TM heterogeneity removed from the HET configuration were also performed (Figure 6.11). It was shown that excluding the AB heterogeneity resulted in a reduced RT dispersion (by 32 ms, Figure 6.11 Ai) as compared to that of HET. The resulting T-wave was less positive in all leads, and notched T-wave was observed in leads III, V5 and V6 (Figure 6.11 B). In simulations removing the TM heterogeneity from the HET configuration, the peaking time of the T-wave was delayed accompanying a prolongation of the QT interval as compared to the HET setting. Also, in general, the amplitude of T-wave was reduced. Furthermore, an inverted T-wave was seen in aVL. These results are similar to that of simulations with the AB heterogeneity only (Figure 6.10 B). In simulations excluding the IV heterogeneity, the RT was delayed in the RV in comparison to the HET setting; the amplitude of T-wave was slightly increased; a delay in the terminal phase of T-wave was seen in leads II, III and aVF; the trace of T-wave was slightly less positive preceding its peaking time (T_peak) in these leads. This was attributable to the fact that the incorporation of IV heterogeneity contributed to an earlier recovery in the RV.

In order to further assess the role of TM in the genesis of T-wave, simulations with variant TM configurations with M cells were performed (Figure 6.12). In simulations with TM-433 and TM-343, the inclusion of M cell resulted in a delayed RT as compared to the HET configuration, which was more pronounced in the superior half of the endocardium (Figure 6.12 Ai-ii); the effects on the T-wave manifested as a delay in the T_peak of T-wave in these two settings. Additionally, the amplitude of T-wave in leads V1-V4 and the limb leads was less negative in comparison to that of the HET setting (Figure 6.12 B). For simulations with TM-235 (replacing sub-endocardium with M cells), an increase in the amplitude of T-wave relative to the HET configuration was seen in most leads. Also, notched T-wave was observed in lead III, and chest leads V3 to V6 (Figure 6.12 B).

To reveal the effects of a negative TM gradient on the T-wave morphology, simulations with TM-NEG55 (endocardium modelled using the EPI cell model, and vice versa) were also carried out. Consequently, the repolarisation of endocardium preceded that of epicardium (Figure 6.12 Aiv). Compared to that of the HET configuration, the T-wave was reduced in amplitude for leads I, II, aVR, V1-V4, and became notched in leads V1-V3 (Figure 6.12 B).
Figure 6.11 Simulated RT map and 12-lead ECG for variant electrical heterogeneity configurations by excluding the AB, TM or IV heterogeneity from the HET setting. (A) Simulated RT map. (B) Comparison of the ECG traces for variant electrical heterogeneities.
Figure 6.12 A comparison of the RT map and corresponding 12-lead ECG obtained with variant TM configurations. (A) The RT map for (i) TM-433, (ii) TM-343, (iii) TM-235 and (iv) TM-NEG55. (B) Comparison of the simulated ECGs with these configurations and the HET setting.
6.3.5 Modelling bundle branch block

The developed ventricle-torso model was then utilised to simulate the effect of bundle branch block on the ventricular activation and its manifestations on the 12-lead ECG. Both left and right bundle block (LBBB and RBBB) was modelled through disabling the corresponding stimulation sites on the endocardium of LV and RV, respectively. Figure 6.13 illustrates the AT and RT patterns as well as the ARI distribution in the ventricles under the LBBB and RBBB conditions. The corresponding ECG was compared with that of control, as shown in Figure 6.14.

In simulations with RBBB, the activation in the LV progressed as normal, whereas the activation of the RV started following the penetration of the septum, resulting in a conduction pattern along the septum to the lateral RV-wall direction (Figure 6.13 Ai). Consequently, a similar pattern was seen in the RT map with RBBB (Figure 6.13 Bi). In contrast, in simulations with LBBB the RV was activated as normal, whereas the LV was activated from the apical septum region. This resulted in a diagonal conduction pattern from the apical septum area of the LV and ended in the basal-lateral region of the LV (Figure 6.13 Aii). As a result, the RT map exhibited a similar pattern for the LBBB (Figure 6.13 Bii). The degree of AT dispersion was markedly enhanced for both RBBB and LBBB as compared to the control conditions: the global AT spanned 156 ms and 139 ms for RBBB and LBBB, respectively, which is markedly longer than the AT dispersion of 83 ms seen under the control conditions. This contributed to a substantial increase in the RT dispersion (159 ms and 199 ms for RBBB and LBBB, respectively, and 113 ms for the control). The spatial distribution and dispersion of ARI were not affected by either block of the bundle branches (Figure 6.13 C).

These simulated effects of bundle branch block on the AT and RT maps of ventricles are in good agreement with experimental and clinical observations in patients with LBBB [19,365] and RBBB [366].
Figure 6.13 Simulated effects of bundle branch block on the AT, RT patterns and ARI distribution of the ventricles. (A) AT map. (B) RT map. (C) ARI distribution. Results for RBBB are shown in column (i) and LBBB in (ii).

A 12-lead ECG was calculated from the 3D ventricular simulations under the LBBB and RBBB (Figure 6.14). The ECG for simulated RBBB showed a substantial increase in the duration of the QRS complex (158 ms measured from V5 vs. 82 ms for control conditions), which was mainly caused by the flattened terminal phase of the S wave. The QRS complex in V1 and V2 were most markedly affected by RBBB: they were upright in these leads, highlighted by ‘M’ shaped QRS in V2. The Q and R waves in other leads were altered to a lesser extent. These changes are in good agreement with documented observations of clinical settings [366–369]. The terminal phase of T-wave was prolonged in the limb leads, whilst the amplitude of T-wave was increased in V4-V6.
The simulated LBBB exerted more substantial alterations to the profile of ECG. The QRS complex was markedly broadened in all leads, showing a duration of 140 ms. Note that clinically LBBB has been associated with QRS complex duration exceeding 120 to 140 ms [119,365,370]. Leads II, III, aVF and V3 exhibited a pronounced S wave in the QRS complex while V1-V2 showed a predominant S wave. An ‘M’-shaped notch was seen in the QRS complex of leads V5 and V6, and V4 manifested a ‘W’ shaped notch. The T-wave became biphasic in leads aVL, V5 and V6. The QT interval was also prolonged as compared to that of the control conditions (480 ms for LBBB vs. 395 ms under the control conditions). These changes in the duration and profile of QRS complex well matched clinical data [119,365,366,369,370]. The observed mid-QRS ‘M’ notches in the lateral leads (V5 and V6) have also been considered as a classical mark of LBBB [119,365].
6.3.6 Simulated application of acacetin

One motivation driving the development of a ventricle-torso model is its potential in the assessment of drugs and its manifestations on ECGs. Here, the effect of acacetin (3 μM), a K⁺-channel blocker that has been broadly investigated in the atria in Chapter 5, on the ventricles and ECG was simulated. The alterations to the AP at the single cell level are shown in Figure 6.15 A. The developed ventricle-torso model was used to assess how the administration of acacetin affects the ECG. It was demonstrated that by applying acacetin, the ventricular APD was slightly prolonged in all regions of the ventricles (by up to 12 ms as seen in the LVM, Figure 6.15 A). The simulated ECGs showed a negligible prolongation in the QT interval by the application of acacetin (prolonged by 7, 5, and 3 ms with the full action of acacetin, I_{Kr} block only and I_{Ks} block only, respectively; QT interval was not affected by simulated I_{Io} block only. Figure 6.15 B). These results are concordant with the experimental study [254] showing that applying acacetin to canine hearts did not prolong the QT intervals.

6.3.7 Modelling ventricular ischaemia

Effects of ventricular ischaemia were first investigated at the cellular level (Figure 6.16, Table 6.6). As compared to the normal ventricular AP, the ischaemia-induced changes (Table 6.4) manifested as a substantial abbreviated APD of the ventricular cells (by approximately 50% in the central ischaemic zone), a markedly depolarised resting potential (by 10 mV and 18 mV in the border and central ischaemic zone, respectively), and a dramatic reduction in the upstroke velocity of the AP (116.2 and 25.7 V/s for the cells in the border and central ischaemic zone, respectively, vs. 369.7 V/s for the normal conditions). These results are concordant with previous studies [116,247,360].

<table>
<thead>
<tr>
<th>Ischaemic condition</th>
<th>LVEPI</th>
<th>LVM</th>
<th>LVENDO</th>
<th>RVEPI</th>
<th>RVM</th>
<th>RVENDO</th>
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<tr>
<td>Normal</td>
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<td>401</td>
<td>339</td>
<td>274</td>
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<td>301</td>
<td>269</td>
<td>228</td>
<td>276</td>
<td>242</td>
</tr>
<tr>
<td>Central ischaemic zone</td>
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<td>181</td>
<td>169</td>
<td>150</td>
<td>172</td>
<td>158</td>
</tr>
</tbody>
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Figure 6.15 Simulated effects of the administration of acacetin (3 μM) on (A) the ventricular AP and (B) ECG (lead II). In (B) $I_{to}$-, $I_{Kr}$- or $I_{Ks}$- Block represents the simulated effect of acacetin considering blocking $I_{to}$, $I_{Kr}$ or $I_{Ks}$ alone.

Figure 6.16 Simulated effects of ischaemia on the ventricular AP. Both ischaemic effects at the border and central ischaemic zone are shown in each panel.
The effect of acute ventricular ischaemia was then elucidated using the developed ventricle-torso model. Five Ischaemic zones at different locations were created in the walls of central ventricles (Figure 6.4). The standard 12-lead ECG was calculated and shown in Figure 6.17.

It was shown that the ischaemia-induced alterations to the ECG mainly manifested as a change in the ST-segment, T-wave and QRS complex amplitude. In simulated ischaemia in the lateral wall of the RV (RV-LAT), a small ST-segment elevation was seen in the limb leads, whilst the chest and augmented limb leads exerted a slight depression in the ST-segment (Figure 6.17 B). These changes were, in general, less substantial than those induced by the ischaemic zones in other positions. The incorporation of an ischaemic zone in the septum (SEP) resulted in a more pronounced ST-segment depression in aVL and the chest leads V1-V5 as compared to that of ischaemic RV-LAT. Adding an ischaemic zone in the central-lateral wall of LV (LV-LAT), substantial ST-elevations were seen in V4-V6, leads I-II, aVL and aVF compared with the normal ECG, with the lateral leads (V5 and V6) demonstrating the most substantial changes; the R wave in leads I, II, V4-V6 was also less positive. The ischaemia in the posterior wall of the LV (LV-POS) induced a dramatic ST-elevation in leads II, III and aVF; a substantial ST-depression was also detected in aVR, aVL and V1-V4; a taller T-wave was seen in leads II, III and aVF. In simulations with an ischaemic zone in the anterior LV (LV-ANT), leads II, III, aVF exerted an ST-depression whereas V2-V3 exhibited ST-elevations, in contrast to changes of ECGs from simulations for other ischaemic zones; lead aVL showed the most substantial change in the ST-segment in this ischaemic setting.

Collectively, these simulations demonstrated a strong dependence of the pattern of ischaemia-induced changes in the ST-segment on the position of the ischaemic zone in the ventricles. The results presented here are generally in good agreement with clinical documentations and observations [118,371–373].
Figure 6.17 Simulated effects of ischaemia on the ECG with respect to variant ischaemic zones in the ventricles. (A) Comparison of ECG traces with variant ventricular ischaemic zone or normal ventricles. (B) Relative change in the ST-segment (quantified as the normalised potential at $t = 120$ ms following ventricular activation) with respect to variant ventricular ischaemic zone vs. that of normal conditions.
6.4 Discussion

6.4.1 Model achievements

In this Chapter, a biophysically detailed and anatomically accurate human ventricle-torso model has been developed. A family of single cell models for the human ventricles was first developed to account for the electrical heterogeneities. These single cell models were subsequently incorporated into a 3D anatomical model of the human ventricles with fibre orientation. The ventricle-torso model was constructed through integrating the 3D ventricular model and an anatomical human torso model incorporating internal organs and heterogeneous conductivities. The integrated model was used to simulate body surface potential and ECGs, which was in good agreement with an experimental 36-channel ECG. The role of electrical heterogeneities in the genesis of T-wave was assessed, showing that the AB, TM and IV all contributed to the profile and amplitude of the T-wave.

Further simulations were performed to model the ECGs with bundle branch blocks and ventricular ischaemia, both demonstrating a good agreement with clinical observations. These simulations support the validity of the developed ventricle-heart model. Additionally, as a preliminary study, the ventricle-torso model was also employed to assess the effect of an anti-AF drug administration on the ECG.

6.4.1.1 Single cell models

A family of single cell models of human ventricular electrophysiology accounting for AB, IV and TM heterogeneities were developed based on the ventricular model used in Adeniran et al. [126]. A model of \( I_{NaL} \) was also adapted into the model and contributed to the TM heterogeneity in ventricular APD (Table 6.1, [346]). The AB heterogeneity model was developed based on the experimental data presented by Szentadrassy et al. [321]. The IV heterogeneous model was constructed through the incorporation of IV difference in the activities of \( I_{to} \) and \( I_{Ks} \) [327,347].

6.4.1.2 3D model of human ventricles

The 3D ventricular model was constructed upon an anatomical geometry and detailed fibre orientation of the ventricles [126,348]. In the absence of a Purkinje network native to the ventricular geometry, in simulations of ventricular activation, the empirically determined activation sites used in [126,351] was adapted and further optimised in order
to match the QRS-profile of the simulated 36-channel to experimental measurements. The simulated global ventricular AT dispersion was 83 ms, which was within the range of experimental observations (62 to 90 ms in [327,350]). The simulated global RT dispersion was 113 ms, which is well within an experimental range of 99 to 145 ms in [327,361]. In the LV the recovery spread from apex to base, which matched the RT map presented in [361]. The resultant global ARI dispersion was 101 ms, which is close to the measured value of 98 ms in [327]. Also, similar to [361] the developed 3D ventricular model exhibited an apex-to-base gradient in the ARI map (Figure 6.8 C).

6.4.1.3 Simulated ECG
The validity of the ventricle-torso model was assessed by comparing the calculated ECG with experimental and clinical data. The calculated ECG resulting from the simulated electrical activities of the ventricles was comparable to the experimental and typical clinical traces. A 36-lead ECG was first obtained and compared with an experimental measurement, demonstrating a good agreement in the spatial distribution pattern of the QRS complex and T-wave (Figure 6.9 A). Also, a 12-lead ECG was derived (Figure 6.9 B), in which the QRS complex exhibited a typical pattern seen in clinical ECGs [362,363]. The waveforms of T-wave were qualitatively similar to the normal ECGs found in humans [362,363]. Furthermore, the measured QRS complex duration and QT interval were both well within the range of clinical observations [362–364]. These results support the validity of the developed ventricle-torso model.

6.4.1.4 Simulated bundle branch block
While RBBB is generally considered benign [119], LBBB is associated with reduced ejection fraction [120] and may be a first manifestation of another myocardial disease such as heart failure and ischaemia [119]. Both RBBB and LBBB were simulated in the present Chapter. These simulations demonstrated a substantial prolongation in the global AT of the ventricles (156 and 139 ms for RBBB and LBBB, in contrast to 83 ms for control), leading to a substantially increased RT dispersion. The simulated patterns of the ventricular AT and RT map are in good agreement with experimental and clinical observations in ventricles with LBBB [19,365] and RBBB [366].

The simulated 12-lead ECG with RBBB was characterised by a prolonged QRS complex duration with a delayed S wave, upright QRS in V1 and V2, and a ‘M’ shaped notch in the R wave of V2. These changes are in good agreement with documented diagnostic markers of RBBB in the clinical ECGs [366–369]. In simulations with LBBB,
the QRS complex was markedly broadened in all leads, showing a duration of 140 ms. A notched QRS complex was observed in the lateral leads and a predominant S wave was seen in leads V1 and V2. These changes well matched clinical diagnostic markers of LBBB [119,365,366,369,370].

**6.4.1.5 Simulated ventricular ischaemia**

At the single cell level, the ischaemia-induced effects on the AP manifested as an approximately 50% reduction in the APD, a marked depolarisation in the resting potential and a dramatic decrease in the upstroke velocity of the AP, which is in accordance with previous studies [116,247,360]. Manifestations of ventricular ischaemia were assessed using the 3D ventricle-torso model through incorporating transmural ischaemic zones in various positions of the ventricles. The simulated ischaemia ECGs were characterised by a markedly altered ST-segment (in the form of elevation/depression) in the leads that correspond to the position of the ischaemic zone, which is in good agreement with clinical observations [118,371–373].

**6.4.2 Role of electrical heterogeneities**

The AB, IV and TM heterogeneities have been incorporated into the 3D ventricular model. The role of individual heterogeneity in the genesis of T-wave was assessed.

In the absence of electrical heterogeneity, an upright T-wave was observed in most leads, although the amplitude was relatively small (Figure 6.10 B), suggesting a role of activation sequence in the genesis of T-wave since RT was determined by AT in the absence of spatial heterogeneity.

**6.4.2.1 TM heterogeneity**

At the single cell level, the TM heterogeneity model accounted for a TM APD difference of 78 to 89 ms in the central LV (Table 6.5); these values are within the experimental range of TM gradient in APD [329,333,374]. The simulated ENDO-EPI difference in APD was also close to the reported range of 25 to 48 ms [322].

The presence and physiological role of M cells in the ventricles are subject to debate: despite the fact that they have been observed in multiple experiments and species [82,331,332,342], the presence or the functional role of M cells has not been demonstrated in tissue [317,329,343–345]. In line with the hypothesis that the small island of myocytes exhibiting longer APDs [334,341] was due to the electrotonic
coupling [329, 354], in this Chapter the stimulation sites were first modelled using the model of M cells instead of modelling the M cells as a distinctive layer.

Compared with the HOMO configuration, the incorporation of TM resulted in a marked increase in the T-wave amplitude in most leads other than lead III and V5-V6 (Figure 6.10 B). Additionally, removing the TM heterogeneity from the HET configuration, the amplitude of T-wave was generally less positive (Figure 6.11 B). Incorporating a negative TM heterogeneity (TM-NEG55) into the HET configuration, leads I, V1-V3 were less positive, and aVL was inverted. These results suggest that the TM heterogeneity contributes to the genesis of T-wave, and that the T-wave of aVL was most sensitive to the TM heterogeneity as compared to the other leads.

Further simulations with classical TM configurations [126, 337, 338, 348] incorporating the M-cell as a distinctive layer of myocardium exerted changes in the T-wave amplitude but not polarity (Figure 6.12 B). Collectively, these results suggest that M cells may not be required in the genesis of upright T-wave.

6.4.2.2 AB heterogeneity

Discrepant data on the AB heterogeneity have been presented in multiple studies: APD of an apical cell was found to be shorter than that of a basal cell [22, 321, 361, 375, 376], whilst other studies observed a greater APD in the apical cells vs. basal cells [313, 322, 377, 378]. It is noteworthy that these discrepancies were not due to a difference in species, as both opposing scenarios have been reported in canine: the APD of apical cells were greater in [377, 378], and shorter in [321] as compared to that of basal cells. A similar discrepancy was also demonstrated in human [322, 361, 377].

The AB heterogeneity model was developed based on the experimental study on canine and human hearts [321], producing an apicobasal APD gradient of 72 to 85 ms at the cellular level. Using the none-invasive electrocardiographic imaging, the LV AB-dispersion in APD was found to be 30 to 52 ms in vivo [361]. The simulations demonstrated a qualitative agreement with the experimental findings.

Using the ventricle-torso model, it was revealed that the AB heterogeneity plays an important role in the genesis of T-wave. The incorporation of AB heterogeneity alone produced T-waves comparable to those of HET configuration; the amplitude in T-wave was greater than that of HOMO, TM or IV configuration (Figure 6.10). Removing the AB heterogeneity from the HET configuration, the T-wave amplitude was reduced, accompanied by a notched T-wave in V5 and V6 (Figure 6.11). These results suggest
that AB heterogeneity plays an important role in shaping T-wave, which was more pronounced for V5-V6. Also, given that AB and TM heterogeneity produced a similar gradient of APD, these results suggest that the role of the AB heterogeneity may be more prominent than that of the TM heterogeneity.

6.4.2.3 **IV heterogeneity**

The APD of RV cells has been shown to be shorter than that of LV cells in humans [318,326,327,361] and many other species [347,379,380]. In the present Chapter, at the cellular level, the IV heterogeneity model contributed to an abbreviation of 37 to 49 ms in the APD of RV vs. LV, which is close to an experimentally observed IV difference in ARI using the electrocardiographic imaging (32 ms in [361]). Inspired by the presence of a progressive spatial gradient in the distribution of ion channel expressions [318], in 3D the IV heterogeneity was incorporated as a linear function dependent on the position of an RV-cell, similar to that of AB heterogeneity. The results suggest that the IV heterogeneity plays a less substantial role in the genesis of T-wave as compared to the AB and TM heterogeneities.

6.4.3 **Novelty and relevance to previous studies**

While this work was built upon a previously developed ventricular model [126] and torso model [245] from the same research group, the present study advances the previous work in the following ways: 1) the single cell model was updated and extended to a family of ventricular models accounting for the AB, IV and TM heterogeneities; 2) In 3D, these electrical heterogeneities were incorporated, allowing for an assessment of the individual role of the heterogeneities as well as the presence of M cells in the genesis of T-wave; 3) the endocardium simulation profile was updated to better match the QRS complex of the calculated ECGs to the experimental traces; 4) the torso model was replaced with an anatomical model with detailed internal organs and structure; 5) the simulated ECG was validated against an experimental 36-channel ECG; 6) bundle branch block and ventricular ischaemia and their manifestations on the ECGs were simulated, both demonstrating a good agreement with experimental and clinical data.

Over the years, multiple ventricle-torso models have also been developed from other research groups [335,337,338,351,381]. Still, the present Chapter advances these studies with 1) a detailed model of electrical heterogeneities at both the cellular and organ level; 2) validations of ECG against an experimental 36-channel ECG; 3)
simulated bundle branch block and ventricular ischaemia demonstrating a good agreement with experimental and clinical data.

6.4.4 Limitations

Limitations in the single cell model have been detailed in [126,339] and hence are not reiterated here.

A first limitation of the present study is related to the endocardium stimulation profile used in the 3D model. While this endocardium stimulation method provides an alternative in the absence of Purkinje network, its limitations may be apparent: the description of the activation delay of the ventricular using such a stimulation profile may warrant further experimental validations; the current simulation applied to the stimulation sites may produce an AP upstroke that differs to that resulting from electrotonic coupling; the endocardium stimulation profile does not allow for investigations of role of the Purkinje network in the arrhythmogenesis of the ventricles as seen in [382].

A second limitation lies in the simulated AT pattern. The simulated activation sequence only showed a small degree of similarity with the experimental activation pattern presented by Durrer et al. [350] (Figure 6.8 A): a close global activation time; the posterior basal wall of the RV is the last to be activated; the anterior paraseptal surface or the RV was the first site of epicardial breakthrough, which has also been reported in [361]. Durrer et al. [350] demonstrated that the first site of ventricular activation was related to the LV, in contrast to the modelling here. Although studies have demonstrated the presence of inter-subject variations in the activation pattern of human ventricles [327,361], considering the dependence of RT map on that of AT, and that T-wave morphology is determined by the RT map, conclusions related to the T-wave may be affected if the AT pattern is compromised. Further experimental data on the AT of human ventricles are expected to improve the model here.

A third limitation is associated with the IV heterogeneity implemented in the 3D model. Experimental data on IV heterogeneity are scarce [326]. Similar to the pattern of ion channel expression levels in the ventricles [318], a gradient model of the IV heterogeneity was implemented. The implemented IV heterogeneity demonstrated a minor role in shaping T-wave, which may be dependent on the implementation of the IV heterogeneity. Further experimental studies are warranted to confirm these results.
A fourth limitation is associated with the simulated T-wave morphology under the RBBB condition. Clinically, RBBB normally exhibits a negative T-wave in V1 [367,369], which is absent in the simulations. This suggests a possible limitation in the endocardium stimulation profile used in the model, or due to the fact that tissue deformation from systolic contraction was not included in the model. Future investigations are warranted in order to improve the model.

6.5 Summary

In this Chapter, a biophysically detailed and anatomically accurate human ventricle model accounting for the AB, TM and IV heterogeneities was developed and integrated with an anatomical human torso model with detailed internal organs and structure. The activation and recovery of the ventricles were simulated. The body surface potential arising from the ventricular electrical activities was calculated. The derived ECGs demonstrated a good agreement with experimental and clinical data. The manifestations of bundle branch block and ventricular ischaemia in the ECG were assessed using the ventricle-torso model, both showing good agreement with clinical data and demonstrating the validity of the model.
Chapter 7
Modelling functional effects of KCNQ1-G269S mutation on human ventricles and ECG

Addendum  This Chapter has been presented in the form of a conference proceeding [383]:
The text has been reworded where possible.

In a recent study [384], a loss-of-function mutation (G269S) in the KCNQ1 gene encoding the α subunit of I_Ks was identified in patients with the long-QT syndrome. These patients normally remained asymptomatic without manifesting a substantial alteration to the corrected QT (QTc) interval. However, a markedly prolonged QTc was found in the patients during exercise. The underlying mechanistic link remains yet to be elucidated. In this preliminary study, the torso-heart model developed in Chapter 6 was applied to assess the functional impacts of the KCNQ1-G269S mutation on human ventricular electrophysiology and ECG. It was shown that the mutation exerted moderate prolongations in the ventricular action potential duration (APD) in the absence of adrenergic stimulation, and slightly increased the tissue vulnerability to produce unidirectional conduction block. These effects were much more pronounced after adrenergic stimulation. Simulated ECGs revealed moderate and severe QT prolongations for at rest and after exercise conditions respectively, which matched the clinical data. The simulations in the present Chapter add insights into the pathological mechanisms of the KCNQ1-G269S mutation.
7.1 Introduction

Long QT syndrome (LQTS) is characterised by abnormal prolongations of QT interval in the ECG and has been associated with an increased risk of induction of the torsade de pointes and ventricular fibrillation [112, 113]. Numerous genetic mutations have been found to be responsible for multiple subtypes of LQTS, among which LQT1 is the most prevalent [384, 385]. Mutations in KCNQ1 encoding the alpha subunit of $I_{Ks}$ are the most common cause of LQT1 [384–386].

$I_{Ks}$ contributes to the repolarisation of AP after the plateau phase of action potentials in ventricular myocytes [384, 387, 388]. Upon a β-adrenergic stimulation, the increased $I_{Ks}$ is vital in counterbalancing an increased activity in the L-type Ca$^{2+}$ current ($I_{CaL}$) [384]. A recent study identified a moderate loss-of-function mutation KCNQ1-G269S in patients with LQTS. The patients remained asymptomatic during rest, but exhibited markedly prolonged QTc intervals after exercise [384]. Through electrophysiological studies, it was found that the KCNQ1-G269S mutation exerted a blunted response of $I_{Ks}$ to the adrenergic stimulation, in addition to the loss-of-function in the current. The compromised adrenergic activation of $I_{Ks}$ due to the G269S mutation was conceived to underlie the symptom [384]. However, the exact mechanistic link remains unclear.

The aim of the present Chapter was to utilise computational models to investigate the functional impact of the KCNQ1-G269S mutation on human ventricular electrophysiology, tissue’s vulnerability to the induction of unidirectional conduction block of excitation waves and the impact of the mutation on the QT interval. To achieve this, the O’Hara-Rudy model of human ventricular cells was modified to incorporate an updated model of $I_{Ks}$ and an adrenergic activation model. The single cell models were then incorporated into a 1D strand model to quantify the effects of the mutation on tissue vulnerability in the genesis of uni-directional conduction block. The ventricle-torso model developed in Chapter 6 was used to assess the effect of the G269S mutation on the ventricular electrical activity at the organ level and the resulting ECGs. In these simulations, both the control conditions and the application of β-adrenergic stimulation accounting for the action of the sympathetic nervous system during exercise were considered.
7.2 Methods

7.2.1 Modelling $I_{Ks}$ of WT and the KCNQ1-G269S mutation

The WT and KCNQ1-G269S $I_{Ks}$ was modelled based on the original $I_{Ks}$ formulation of O’Hara et al. [36], which was updated to better describe the experimental data on steady-state activation and kinetics [384] (Figure 7.1). The formula of $I_{Ks}$ for the WT and mutation was obtained by updating the steady-state variable $x_{s1\infty}$ and the deactivation time constant $\tau_{xs2}$, which is given as follows.

**WT $I_{Ks}$ model:**

$$x_{s1\infty} = \frac{1.0}{1.0 + \exp\left(\frac{V + 17.8}{-15.1}\right)}$$  \hspace{1cm} (7.1)

$$\tau_{xs2} = \frac{1.0}{0.01 \exp\left(\frac{V - 50}{20.0}\right) + 0.0071 \exp\left(-\frac{V + 90.5}{69.08}\right)}$$  \hspace{1cm} (7.2)

**WT-G269S $I_{Ks}$ model:**

$$x_{s1\infty} = \frac{1.0}{1.0 + \exp\left(\frac{V + 0.88}{-16.55}\right)}$$  \hspace{1cm} (7.3)

$$\tau_{xs2} = \frac{1.0}{0.01 \exp\left(\frac{V - 50}{20.0}\right) + 0.01767 \exp\left(-\frac{V + 93.9}{43.89}\right)}$$  \hspace{1cm} (7.4)

**G269S $I_{Ks}$ model:**

$$x_{s1\infty} = \frac{1.0}{1.0 + \exp\left(\frac{V - 31.296}{-20.11}\right)}$$  \hspace{1cm} (7.5)

$$\tau_{xs2} = \frac{1.0}{0.01 \exp\left(\frac{V - 50}{20.0}\right) + 0.02906 \exp\left(-\frac{V + 106.26}{48.74}\right)}$$  \hspace{1cm} (7.6)

The rest of the formula was identical to that used in the original ORd model [36].

The $I_{Ks}$ model of WT slightly differed from the original ORd formula of $I_{Ks}$ in the slope of the tail current (Figure 7.1 A) and the deactivation time constants (Figure 7.1 B). As compared to WT condition, both the heterozygous WT-G269S and G269S variants exhibit right-shifted steady-state activation and faster deactivation, showing a
loss-of-function in $I_{Ks}$.

![Figure 7.1](image.png)

Figure 7.1 Simulated $I_{Ks}$ as compared to experimental data on the WT and mutation. (A) Normalised tail current. (B) Deactivation time constant. (C) Current traces elicited from a voltage clamp shown to the right; the insert represents the experimental traces of WT $I_{Ks}$. (D) A comparison of the I-V relationship of $I_{Ks}$ for the WT and mutations. The experimental data were digitalised from Wu et al. [384].

### 7.2.2 Modelling ventricular electrophysiology and effects of β-adrenergic stimulation

The ionic models of $I_{Ks}$ were introduced into the O’Hara-Rudy model (ORd) [36] to simulate human ventricular action potentials. The model of $I_{Na}$ in ORd model was replaced with that of the ten Tusscher, Noble, Noble, Panfilov model of human ventricular electrophysiology [340]. The replacement was necessary due to that the model produced a much smaller upstroke velocity of AP and a slower conduction velocity in tissue [389], presumably due to the $I_{Na}$ formulation used in the model.
To model the adrenergic stimulation effects on the ventricular myocytes, the models used in O’Hara et al. [386] and Lee et al. [390] were adopted and then modified to consider the effects of the adrenergic stimulation on both the WT and mutated IKs. In the present Chapter, a saturating concentration of isoprenaline (ISO, 1 μM) was applied to represent the adrenergic stimulation. The effects of ISO on IKs were modelled as shown in Table 7.1, where blunted adrenergic activation of IKs was considered.

Table 7.1 Modelling effects of adrenergic activation on IKs

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>WT-G269S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steady-state activation</strong></td>
<td>7 mV [385]</td>
<td>2.8 mV</td>
</tr>
<tr>
<td><strong>Activation time constant</strong></td>
<td>$\tau_{x_{51}} \times 0.6$ [391]</td>
<td>$\tau_{x_{51}} \times 0.84$</td>
</tr>
<tr>
<td><strong>Conductance of IKs</strong></td>
<td>$\times 3.2$ [386]</td>
<td>$\times 1.88$</td>
</tr>
</tbody>
</table>

7.2.3 Modelling vulnerability window in tissue

A 1D strand model consisting of transmural human ventricular cells was used to investigate the tissue vulnerability to induction of abnormal conduction [126]. The 1D strand model consisted of 100 ventricular cells with a spatial interval of 0.15 mm. By using S1-S2 stimulus protocol, the vulnerability window was quantified as the temporal window of S2 leading to unidirectional conduction.

7.2.4 Modelling 3D ventricular electrical activity and ECG

The ventricle-heart model developed in Chapter 6 was employed here to assess the impact of the KCNQ1-G269S mutation on the ventricular electrical activity and ECG. In 3D, both AB and TM heterogeneities were considered. The AB heterogeneity was based on an identical implementation of that in Chapter 6. The TM heterogeneity was simulated using the TM model native to the ORd model [36]. Following the classical segmentation of the ventricles in the previous simulations studies [126,337,338,348], the ventricles were segmented to the endocardium (ENDO), epicardium (EPI) and M cells (MCELL). The IV heterogeneity was not considered in the present Chapter.
7.3 Results

7.3.1 Effects of KCNQ1-G269S on ventricular APD

Under the control conditions (ISO-free), APD (measured at a 90% repolarisation of the AP) was moderately prolonged by both mutants as compared to that of the WT (Figure 7.2 A, Figure 7.3 A-B). At a BCL of 1000 ms, the APD was prolonged by 14 ms in ENDO, 10 ms in EPI and 26 ms in MCELL by G269S. The heterozygous genotype WT-G269S produced only a slightly smaller APD-prolongation despite a markedly higher $I_{Ks}$ current density as compared to that of G269S (Figure 7.1 D). At a higher pacing rate (BCL = 500 ms), the APD was lengthened by approximately a same amount (Figure 7.3 B). Moreover, the mutants also increased the APD heterogeneity among the transmural cells, as the MCELL-EPI APD difference was 108, 121 and 134 ms for WT, WT-G269S and G269S, respectively.

In the presence of ISO, the prolongations in APD by the mutations were much more pronounced (Figure 7.2 B, Figure 7.3 C-D). At a BCL of 1000 ms, APD was lengthened by 24 ms in ENDO, 21 ms in EPI, and 84 ms in MCELL for WT-G269S. For G269S, the prolongations were 27 and 24 ms in ENDO and EPI, respectively. Stable and pronounced early afterdepolarisations (EADs) were observed in the MCELL for mutant G269S, resulting in a profound prolongation of 274 ms in the APD. Furthermore, at a BCL of 500 ms, APD was prolonged by 24 ms in ENDO, 23 ms in EPI, and 51 ms in MCELL for the mutant WT-G269S and by 28 ms in ENDO, 26 ms in EPI, and 61 ms in MCELL for G269S. EADs were absent at this pacing rate. Similarly, the regional APD difference was also augmented by the mutants in the presence of ISO (BCL = 1000 ms: 101 vs. 164 vs. 350 ms; BCL = 500 ms: 76 vs. 104 vs. 111 ms for WT, WT-G269S and G269S, respectively).

To reveal the role of the blunted response of the mutant $I_{Ks}$ to the adrenergic stimulation in the modulation of the APD, further simulations were performed to assess the APD-prolongation (relative to the WT) by the mutant $I_{Ks}$ with a normal response to the adrenergic stimulation (Figure 7.2 C). The APD-prolongation by the mutations with and without a normal adrenergic activation was compared. In these simulations, the mutation-induced APD-prolongation in the ENDO and EPI cells was slightly reduced by the introduction of the normal adrenergic activation (by 6 ms for WT-G269S, 3 ms for G269S). In MCELL, restoring normal adrenergic activation function to the G269S-
$I_{\text{Ks}}$ did not abolish the EADs for $BCL = 1000\,\text{ms}$, while it exhibited a $9\,\text{ms}$ decrease in the APD-prolongation at $BCL = 500\,\text{ms}$ (Figure 7.2 C). Restoring normal adrenergic activation to the WT-G269S, the reduction in the mutation-mediated APD-prolongation in MCELL was $32\,\text{ms}$ for $BCL = 1000\,\text{ms}$, and $13\,\text{ms}$ for $BCL = 500\,\text{ms}$.

Figure 7.2 Simulated effects of WT-G269S and G269S on the AP of endocardium (ENDO), epicardium (EPI), and mid-layer cells (MCELL). (A) Comparison in the AP traces produced under the control conditions for the transmural cells. (B) Comparison of the simulated AP traces produced with ISO for the transmural cells. (C) Comparison of the simulated AP traces produced with ISO, assuming a normal response of mutated $I_{\text{Ks}}$ to the adrenergic stimulation.
Figure 7.3 Bar plots showing the ventricular APD under the control conditions and with the application of ISO. (A-B) Comparison of ventricular APD under the control conditions with a BCL of (A) 1000 ms and (B) 500 ms. (C-D) Comparison of ventricular APD with the application of ISO for a BCL of (C) 1000 ms and (D) 500 ms.

The relative contribution of the blunted adrenergic activation of the mutated $I_{\text{Ks}}$ to the total APD-prolongation effect by the mutations was also quantified (Figure 7.4). Overall, the impaired adrenergic activation of $I_{\text{Ks}}$ by the mutations accounts for more than 20% for WT-G269S and 10% for G269S.

7.3.2 Effects of KCNQ1-G269S on tissue vulnerability

Using a 1D strand model, the effect of mutations on the tissue’s vulnerability to induction of unidirectional conduction in response to a premature stimulus applied to the refractory tail of a conditioning excitation was evaluated (Figure 7.5). An illustration of conduction pattern for variant S2 is given in Figure 7.5 A.
Figure 7.4 The relative contribution of the blunted adrenergic activation of the mutated \( I_{Ks} \) to the total APD-prolongation induced by a genotype. (A) BCL = 1000 ms. (B) BCL = 500 ms. The relative contribution was defined as the relative difference in the APD-prolongation by the mutations with and without a normal adrenergic activation. * indicates no difference was observed.

Under the control conditions, both mutations delayed the occurrence of the temporal vulnerable window (VW) in S2; the measured width of VW at the MCELL/EPI junction was much wider than that at ENDO/MCELL junction primarily owing to a more profound APD heterogeneity between MCELL and EPI under all conditions. Moreover, the widths of VW were slightly increased at the MCELL/EPI junction but not at the ENDO/MCELL junction. Following the application of ISO, the VWs at ENDO/MCELL junction were almost diminished (VW < 0.5 ms) for WT and WT-G269S, whereas it was increased to 11 ms for G269S; at the MCELL-EPI junction, the S2 giving rise to VWs were delayed, accompanied by an increase in the width of VW (by 4-5 ms) by the mutants.
Figure 7.5 Effects of mutations on the tissue VW to initiating unidirectional conduction simulated from a 1D strand model. (A) Demonstration of conduction pattern in response to variant S1-S2 interval: (i) bilateral conduction; (ii) unidirectional conduction; (iii) conduction block. (B) Tissue vulnerability window under (i) the control conditions and (ii) with ISO. In these simulations, a train of 10 S1 at 500 ms was first applied. S2 stimuli were applied to the ENDO/MCELL and MCELL/EPI junctions. * indicates where a VW was almost diminished.
7.3.3 Effects of KCNQ1-G269S in 3D ventricles

The realistic 3D human ventricle model was used to assess the effect of mutations on the ventricular electrical activity at the organ scale (Figure 7.6). In simulating the control conditions, a BCL of 850 ms was applied to represent a normal heart rate (70 bpm) at rest. In simulations with the application of ISO, a BCL of 500 ms was selected to account for a faster heart rate during exercise.

![Figure 7.6 Comparison of simulated ventricular ARI map for WT, WT-G269S and G269S under the control conditions and with ISO. (A) The ARI map for the WT and mutations under the control conditions. (B) The ARI map for the WT and mutations in the presence of ISO (1 μM). In each panel, both anterior and posterior views of the ARI map were given.](image)

It was shown that RT was delayed by the mutants in the absence of noticeable alterations to AT under both conditions. Consequently, the activation repolarisation interval (ARI) was prolonged by the mutants. In these simulations, a substantial TM gradient in ARI was seen in all cases. Also, the dispersion in the global ARI was increased by the mutations under the control conditions (87, 96 and 98 ms for WT, WT-
G269S and G269S, respectively), whereas it was decreased in the presence of ISO (87, 81, 81 ms for WT, WT-G269S and G269S, respectively).

### 7.3.4 Effects of KCNQ1-G269S on QT intervals

Using the ventricle-torso model, the manifestations of the mutations on the QT interval was simulated. Lead II from the simulated ECG was analysed. The QT interval was corrected using Bazett’s formula [392]. The traces of lead II and quantified QTc are shown in Figure 7.7.

**Figure 7.7** Simulated ECGs showing prolongation in QTc by the KCNQ1-G269S mutation as compared to the WT. A(i) and B(i): control conditions, BCL = 850 ms. A(ii) and B(ii): ISO = 1 μM, BCL = 500 ms. Corrected QT interval (QTc) was evaluated using Bazett’s formula [392].
At rest, the WT model produced a QTc of 396 ms. Both WT-G269S and G269S models exhibited moderately prolonged QTc (412 ms for WT-G269S, and 417 ms for G269S), extended T-wave duration and slightly elevated T-wave amplitude. Notice these values fall into the normal QTc range [392]. In contrast, after exercise QTc was markedly prolonged to 478 ms for WT-G269S and 486 ms for G269S, compared with 434 ms in WT. Additionally, the amplitude of T-wave was not markedly altered by the mutations as compared to the WT.

Similar to APD analysis, the contribution of blunted adrenergic activation of I\(_{KS}\) was quantified. As a result, the contribution of blunted adrenergic activation of I\(_{KS}\) accounted for 24% of QTc prolongation for WT-G269S, and 14% for G269S, which is in concordance with the contributions in the APD prolongations.

### 7.4 Discussion

#### 7.4.1 Summary of major findings

In the present Chapter, multi-scale computational models have been used to assess the functional impact of the WT-G269S mutation on the ventricular electrophysiology and QT interval. At the cellular level, the simulations demonstrated that both variants produced a small to moderate prolongation in the ventricular APD (10 ms in EPI and 26 ms in MCELL, Figure 7.2 A, Figure 7.3 A-B) under the control conditions (free from adrenergic stimulation). In contrast, in the simulations with ISO, a more pronounced APD prolongation was observed; pronounced EADs were observed in the MCELL for the mutation G269S (Figure 7.2 B, Figure 7.3 C-D). In a 1D strand model of ventricular cells, the mutations slightly increased the size of the VW of the tissue (Figure 7.5 B). In 3D, the effects of the mutated genotypes on the ventricles manifested as a markedly delayed RT map and markedly prolonged ARI (Figure 7.6). ECG was calculated from the body surface potential, showing that the QTc was moderately affected by the mutations under the control conditions (QTc prolongation was 16 and 21 ms for WT-G269S and G269S as compared to the WT). In contrast, in the simulations with ISO representing the conditions during exercise, a substantial prolongation in the QTc was observed for the mutations (by 44 and 52 ms for WT-G269S and G269S as compared to the WT). These findings are consistent with the clinical records [384].

Additionally, the role of the blunted response of the mutated I\(_{KS}\) to adrenergic
activation in the APD-prolongation was assessed. The results showed that the impaired response accounted for 20% to 40% in the total APD-prolongation induced by WT-G269S, and was markedly smaller for G269S. A similar value was found in the simulated QTc prolongation. These results suggest that the effects of the mutations on the APD following adrenergic stimulation may be primarily due to loss-in-function in $I_{Ks}$, and that blunted adrenergic activation also contributes to the profound APD prolongation but to a lesser extent.

### 7.4.2 Role of $I_{Ks}$ in human ventricles

Under control conditions with a slow pacing rate, the APD of human ventricular AP is mainly determined by $I_{CaL}$ and the rapid component of delayed rectifier $K^+$ current, $I_{Kr}$ [36,384,393]. In accordance with this notion, the simulation data from this Chapter revealed that APD was only moderately prolonged by the WT-G269S and G269S mutation under the control conditions, despite the profound positive shift in steady-state activation of $I_{Ks}$ in these mutants. These results are concordant with a previous experimental study [394] showing that blocking $I_{Ks}$ did not induce substantial alterations to the human ventricular APD.

In contrast, the regulatory role of $I_{Ks}$ to APD becomes pronounced in the presence of the adrenergic stimulation as the increase in $I_{Ks}$ is crucial in offsetting the augmented $I_{CaL}$ to prevent excessive prolongations in APD after adrenergic activation [384]. Here, in simulations following application of ISO, the prolongations in APD by the mutations were roughly doubled in ENDO and EPI cells, and much more pronounced in MCELL. At slow pacing rates, EADs were induced in the MCELL for the mutation G269S, resulting in a dramatic prolongation in APD.

### 7.4.3 Proarrhythmic effects of the KCNQ1-G269S mutation

The LQTS is associated with proarrhythmic implications including the risk of the *torsade de pointes* and ventricular fibrillation [112,113]. In the present study, the proarrhythmic effect of the KCNQ1-G269S was also evaluated. At the cellular level, pronounced EADs were observed in the MCELL with the mutation G269S. It is well established that EADs are associated with the *torsade de pointes* [108,395–398]. Therefore, the KCNQ1-G269S increased the susceptibility of the ventricles to induction of *torsade de pointes*. Additionally, the 1D strand simulations showed that tissue’s VW
to the genesis of unidirectional conduction was slightly extended and delayed in timing, indicating an increased vulnerability to induction of arrhythmia by the KCNQ1-G269S mutation [108,109]. The increase in the VW became more pronounced in the presence of ISO.

7.4.4 Clinical relevance

The LQTS is a life-threatening cardiac condition and a leading cause of sudden death in the young population [112,113]. Genetic variations in KCNQ1 are the most common cause of LQT1 [384–386], the most prevalent subtype of LQTS [384,385]. Recently, other KCNQ1 mutations resulting in loss-of-function of IKs have been identified in asymptomatic patients under the normal conditions [386,399,400]. Also, a majority (62%) of cardiac events LQT1 patients experienced were found to be during exercise [401]. Using multi-scale computational models, the functional impact of the KCNQ1-G269S mutation was assessed. The mechanistic link between the symptoms of the mutation carriers and the genotype was elucidated. While the simulations were performed explicitly based on the data for the KCNQ1-G269S mutation, this study can be extrapolated to other mutations exerting a similar effect on IKs.

7.4.5 Limitations

Limitations related to the ORd model have been discussed in detail in [36] and thus are not discussed here.

In the present study, M cells were demonstrated to have an increased susceptibility to induction of EADs. Additionally, in tissue models, M cells were also incorporated. Given that the presence and/or the physiological role of the M cells remain open to debate [343,402], care should be taken in interpreting the implications of these results.

Furthermore, in the present Chapter, the inter-ventricular electrical gradient in the ventricles was not considered. The presence of IV gradient may interplay with the effect of mutations and thus may modulate the ventricular ARI and RT, thereby affecting the ECG and QT interval. Therefore, further 3D simulations considering the IV heterogeneity may be warranted.

Thirdly, in simulating the effects of sympathetic stimulation, a simplified model was used. The rapid component of the delayed rectifier potassium current, IKr, is known to be modulated by adrenergic stimulation [403], which has not been
considered in the current study and thus warrants future investigations. In the present study, the application of isoprenaline was used to represent the effect of activation of the sympathetic nervous system on the heart, which may incur limitations. In the heart, the adrenergic receptors constitute the interface between the sympathetic fibres and the cardiovascular system. Following the activation of the sympathetic nervous system, the sympathetic transmitters bind to both the α and β adrenergic receptors that are present in the human heart [404,405], whereas isoprenaline exerts little activity on the α adrenergic receptors [405]. Also, the ventricular sympathetic innervation is highly inhomogeneous with a gradient from base to apex [406], the effect of which cannot be represented by homogenous application of isoprenaline in the model.

7.5 Summary

In this Chapter, multi-scale human ventricular computational models were employed to elucidate the mechanistic link between the KNCQ1-G269S mutation and the symptom in the patients. It was demonstrated that the mutation produced a moderate prolongation in the APD under normal conditions, which was translated to an insubstantial prolongation in the QT interval in 3D modelling. In contrast, the mutation-induced APD prolongation became much more pronounced in the presence of adrenergic stimulation, which was mirrored by a markedly prolonged QTc calculated using the ventricle-torso model. Additionally, the functional impact of the mutations was demonstrated to be dominated by the loss-of-function in I\textsubscript{Ks}, while the blunted adrenergic response of I\textsubscript{Ks} also contributed to the APD and QTc prolongation, but to a lesser extent. Furthermore, in the simulations, the proarrhythmic effects of the mutations manifested as a promoted susceptibility to the genesis of EADs and an increased vulnerability to induction of unidirectional conduction block in tissue. The present Chapter adds insights into the pathological effects of the KCNQ1-G269S mutation.
Chapter 8
Discussion and Conclusions

This Chapter summarises the major findings and significance of the studies from this thesis. Their key clinical relevance is outlined, and future directions for the continuation work of this thesis are discussed. The thesis is closed with a few remarks on the coming era of precision medicine.

The major contributions of this thesis are summarised as follows.

Part I of this thesis focused on using multi-scale computational models to elucidate the role of $I_{Kur}$, an atrial-specific $K^+$ channel that contributes to the repolarisation of the atrial AP, in the functional activities of human atria. In Chapter 3, the mechanisms underlying the arrhythmogenesis six novel mutations in the KCNA5 gene encoding the $\alpha$-subunit of $I_{Kur}$ were revealed using multi-scale computational models of human atria. Chapter 4 presented a work using a multi-scale electromechanical model of the atria to elucidate the impact of gain- and loss-of-function in $I_{Kur}$ on the atrial contractile function. In Chapter 5, anti-arrhythmic benefits of a pharmaceutical block of $I_{Kur}$ and that of the combined block of $I_{Kur}$ and other channels including $I_{Na}$ were assessed in silico. This part contributes to a thorough understanding of the role of $I_{Kur}$ in human atrial arrhythmogenesis, contractility and pharmaceutical therapy.

Part II detailed the development of a computational human ventricle-torso model and its application in modelling cardiac conditions. In Chapter 6, a biophysically detailed and anatomically accurate computational model for the human ventricle-torso was developed and then applied to simulated manifestations of bundle branch block and acute ventricular ischaemia. Chapter 7 presented an investigation of the mechanistic link between the genetic mutation KCNQ1-G269S and symptoms in the corresponding patients.
8.1 Summary of major findings and significance

8.1.1 Role of \(I_{\text{Kur}}\) in atrial arrhythmogenesis

A recent study associated six novel mutations in the \(KCNA5\) gene exerting either gain- or loss-in-function of \(I_{\text{Kur}}\) with a prevalence of lone-AF. In Chapter 3, the arrhythmogenesis of six mutations in \(KCNA5\) were elucidated using multiscale computational models of human atria. Three contemporary human atrial single cell models were applied in order to attain model-independent insights. It was demonstrated the causal link between the mutations and the lone-AF incidence was distinct in the two groups of mutations exhibiting either up/down-regulation in \(I_{\text{Kur}}\).

At the cellular level, mutations exerting gain-of-function in \(I_{\text{Kur}}\) markedly hyperpolarised the AP-plateau potential, abbreviated the atrial APD and flattened the APD restitution curve. In tissue, these mutations reduced the excitation wavelength at a slow pacing rate while shifting the wavelength restitution curve to shorter S1-S2 coupling intervals. In 3D, these mutations exhibited a slight increase in the tissue’s temporal vulnerability to induction of wave break and accelerated spiral wave excitations. Collectively, these results suggest that the gain-of-function mutations promote the initiation and maintenance of re-entries through abbreviating the APD and hence shortening the wavelength, increasing the susceptibility of conduction wave break, and accelerating the spiral wave dynamics.

In contrast, the loss-of-function in \(I_{\text{Kur}}\) depolarised the AP-plateau whilst the resultant APD alteration was found to be dependent on the baseline profile of the AP: both APD abbreviation and prolongation were demonstrated in different models. A similar phenomenon has also been demonstrated experimentally with \(I_{\text{Kur}}\) block [148,161,189,193]. These simulations did not highlight the effects of shortening of wavelength or increases in tissue’s temporal vulnerability to induction of unidirectional wave break. The effects of the mutations on the spiral wave dynamics were found to be dependent on the baseline model of the AP and the mutation itself.

In simulations with the presence of \(\beta\)-adrenergic stimulation, the loss-of-function mutations markedly promoted the susceptibility to the genesis of EADs both at the single cell level and in a 1D strand of atrial cells. In addition, the pronounced in-tissue EADs mediated by the loss-of-function mutations also promoted the susceptibility to induction of 2:1 conduction block and markedly increased the tissue’s vulnerability to...
the genesis of unidirectional conduction pattern.

The work is, to the best of the author’s knowledge, the first comprehensive modelling study addressing the distinctive mechanisms of arrhythmogenesis of the opposing up- and down-regulation of $I_{Kur}$ in the atria. Also, the effect of β-adrenergic stimulation in the atria was simulated, highlighting the importance of considering various physiological relevant conditions in the effort of dissecting the functional roles of AF-related genetic variations as well as investigating the anti-arrhythmic effects of ion channel block, which was not considered in the previous modelling [155,186–188] studies.

Furthermore, the mechanistic link between the gain- or loss-of-function in $I_{Kur}$ and the atrial arrhythmogenesis revealed in this study can provide insights into proarrhythmic mechanisms of other repolarisation channels with altered activities. Recent studies have demonstrated that both gain- and loss-of-function of $I_{Ks}$ are associated with an enhanced susceptibility to AF [57,407,408]. Atrial arrhythmogenesis may also be linked with loss-of-function in the TASK-1 channel [293]. It is likely that the arrhythmogenesis associated with these mutations can be explained by similar mechanisms.

8.1.2 Role of $I_{Kur}$ in modulation of atrial contractility

The role of $I_{Kur}$ in the modulation of atrial contractility was investigated through elucidating the effects of the $KCNA5$ mutations on the atrial contractile function using a multiscale electromechanical model of human atria. A single cell model of electromechanical coupling was first developed and expanded to a family of human atrial cell models accounting for regional electrical heterogeneities in the atria. These single cell models were mapped to a 3D anatomical model with fibre orientations representing anisotropies in electrical wave conduction and mechanical deformation.

The increased activity in $I_{Kur}$ exerted gain-of-function mutations demonstrated negative inotropic effects both at the cellular and organ level. At the single cell level, the negative inotropic modulation of these mutations on atrial contractility manifested as a markedly reduced active force and cell shortening. At the organ level, the gain-of-function mutations demonstrated markedly weaker active deformations of the atrial myocardium, a notable reduction in the atrial emptying volume, accompanied by a decelerated atrial emptying rate and an attenuated span of the contraction time course.
In contrast, the down-regulated $I_{Kur}$ by the loss-of-function mutations in $KCNA5$ exhibited positive inotropic effects in human atria. At the single cell level, the enhanced contractility was supported by a profound augmentation in the CaT and hence relative cell shortening by these mutations. In 3D, simulations of the loss-of-function mutations demonstrated a greater deformation of the myocardium and an extended atrial contraction time span, in addition to a substantial increase in the atrial emptying volume and rate. These effects may result in an increase in the atrial output and accelerate blood flow in the chambers.

Mechanistic simulations revealed the mechanisms underlying the inotropic effects of altered $I_{Kur}$ activities. The negative inotropic effect exerted by the gain-of-function in $I_{Kur}$ was attributed to the decreased CaT primarily through a reduction of $I_{CaL}$ secondary to the modulation on the AP. In contrast, the increased contractility seen with the down-regulated $I_{Kur}$ was explained by the increased $I_{NaCa}$ working in the reverse mode, which dominated the increased $Ca^{2+}$ entry.

This wok represents, to the best of the author’s knowledge, a first attempt to investigate modulation of genetic mutations on the atrial contractility using multiscale electromechanical models and demonstrates substantial advances over previous modelling studies [186,188,243,244], in which atrial mechanical function was not considered. Also, the negative inotropic effects of the gain-of-function in $I_{Kur}$ demonstrated in this study markedly expands our understanding of the role of $I_{Kur}$ in atrial contraction, in addition to the previous experimental studies on the effects of down-regulated $I_{Kur}$ on atrial contraction [157,241].

8.1.3 Atrial-selective block of $I_{Kur}$ and $I_{Na}$

Chapter 5 presents an attempt of assessing the anti-AF effects of an atrial-selective block in chronic AF settings. In Chapter 5, the antiarrhythmic effect of $I_{Kur}$ and/or $I_{Na}$ block was assessed in silico. In addition to modelling the effect of pure $I_{Kur}$ block, minor inhibitive effects of typical $I_{Kur}$ blockers on multiple $K^+$-channels as exhibited by acacetin were simulated, allowing the assessments of the anti-AF benefits of multiple $K^+$-channel block. This study highlights synergistic antiarrhythmic benefits of combined $I_{Kur}$ and $I_{Na}$ block as well as combined multi $K^+$-channel block, which may be a valuable strategy in the management of AF.

At the cellular level, $I_{Na}$ block exerted a substantial inhibition in atrial $I_{Na}$ at fast
pacing rates and minor effects at normal heart rates, showing a substantial rate-selectivity. Through varying the kinetics of drug action, an atrial-selective block of $I_{Na}$ can be achieved, and the AF-selectivity can be maximised. In tissue simulations, $I_{Na}$ block enhanced the rate-adaptations of $V_{max}$ and CV over a larger range of BCL, showing markedly reduced excitabilities of atrial myocytes, which was mirrored by a shortened lifespan and deceleration in the dynamics of simulated re-entries.

$I_{Kur}$ block exerted prolongations of APD in chronic AF, which was relatively greater for APD$_{30}$ than APD$_{90}$. In tissue, inhibiting $I_{Kur}$ shifted CV restitution curve towards higher BCLs without affecting the CV at a slow pacing rate. Pure $I_{Kur}$ block showed limited anti-AF efficacy in 2D simulations, as a 50% inhibition of $I_{Kur}$ destabilised and decelerated simulated spiral waves without terminating the re-entries.

The combined multiple K$^+$-channel block exerted greater prolongations in atrial APD than the sum of changes by individual channel blocks, showing synergistic effects in APD prolongation. Compared with the pure $I_{Kur}$ block, the combined multiple K$^+$-channel block further enhanced the APD rate-adaptation in single cells and increased efficacy in terminating re-entries in tissue.

The combined Na$^+$- and K$^+$-channel block ($I_{Na}$ and pure-$I_{Kur}$/multiple K$^+$-channel block) produced synergistic antiarrhythmic effects including 1) markedly increased fractional $I_{Na}$ inhibition and APD prolongation, 2) dramatically augmented the attainable maximal AF-selectivity of $I_{Na}$ block, 3) a greater efficacy in terminating simulated spiral waves.

This study represents a substantial advance over previous modelling studies on the effect of $I_{Kur}$ block [186,188,243]. In contrast to these studies in which various models and theoretical parameters were adopted in simulating $I_{Kur}$ block, the present study utilised a state-dependent block model rigorously reproducing the drug actions of acacetin, an experimentally justified compound. In addition, instead of focusing on $I_{Kur}$ block alone, the present study highlighted synergistic anti-AF beneficial effects of multiple K$^+$-channel block and combined Na$^+$ and K$^+$-block. Furthermore, this study differs from previous studies on combined Na$^+$ and K$^+$-block in undiseased canine atria [12,47] in three ways: 1) modelling human chronic AF, 2) incorporating multiple K$^+$-channel block and 3) using the biophysical detailed state-dependent model of $I_{Kur}$ block.

The anti-AF benefits of combined Na$^+$ and K$^+$ block presented in this study also provides insights into understanding the efficacy of the multichannel blocker vernakalant [259,409,410]. Vernakalant inhibits $I_{Kur}$, $I_{K,Ach}$ and rate-dependently blocks.
I_{Na}, and is the only I_{Kur} blocker that has proceeded to Phase III of a clinical trial.

8.1.4 Ventricle-torso model

In Chapter 6, a biophysically detailed and anatomically accurate human ventricle-torso model has been developed. To achieve this, a family of single cell models was developed for the human ventricles, which considers the electrical heterogeneities in the ventricles. A 3D anatomical model with fibre orientation was modified to optimise the endo-surface stimulation profile. The single cell models were subsequently incorporated into the updated 3D ventricular model. The ventricle-heart model was constructed by integrating the 3D ventricular model and a torso model incorporating internal organs and ribs with heterogeneous conductivities. ECGs were then derived from the body surface potential, which was calculated by solving the forward problem of electrophysiology.

The APD heterogeneities produced by the family of single cell models were close to the ranges of experimental values. The 3D human ventricular model was first validated by verifying the global activation time, repolarisation and activation-recovery interval dispersions against experimental observations. The ventricle-torso model was validated through achieving good agreement in the characteristic patterns as well as the durations of the QRS complex and T-wave of the simulated and experimental ECGs (36- and 12-lead).

RBBB and LBBB were simulated using the ventricle-torso model. The simulated patterns of the ventricular AT and RT map well matched experimental and clinical observations in literature. Additionally, the derived 12-lead ECG from both simulations exhibited diagnostic markers clinically used for the respective conditions, including prolonged QRS durations, upright QRS complex in leads V1 and V2 for RBBB and a ‘M’ notched QRS complex in the lateral leads for LBBB.

The simulated ischaemic ECGs were characterised by alterations in the ST-segment of the leads that correspond to the position of the ischaemic zone, which is in good agreement with clinical observations [118,371–373]. Together with the simulated LBBB and RBBB, these results further demonstrated the validity of the model.

The role of individual heterogeneity in the genesis of T-wave in a 12-lead ECG was assessed. In the absence of electrical heterogeneities, a minor but upright T-wave was observed in most leads. The implemented IV heterogeneity had minor effects on
the T-wave. The incorporation of the TM heterogeneity resulted in a markedly taller T-wave in most leads other than lead III and V5-V6. Also, the M cells were not required to produce normal T-wave profiles. The AB heterogeneity was found to be playing a dominant role in the genesis of T-wave in most leads.

Despite that multiple ventricle-torso models have also been developed previously, the present ventricle-torso model advances these studies in 1) detailed models of electrical heterogeneities at both the cellular and organ level, 2) validated ECGs against an experimental 36-channel ECG, and 3) simulated bundle branch block and ventricular ischaemia demonstrating a good agreement with experimental and clinical data. Furthermore, in the light of controversies over the mechanisms underlying the genesis of T-wave [313,317], the present study adds insights into the individual role of electrical heterogeneities in shaping the T-wave.

8.1.5 KCNQ1-G269S mutation

In Chapter 7, multi-scale human ventricular models and the ventricle-torso model were employed to assess the functional impact of the KCNQ1-G269S mutation on the ventricular electrical activity and its manifestations in ECG.

Under baseline (normal) conditions, the mutation induced a moderate prolongation in the single cell APD, which was reflected by a minor prolongation of the QT interval. However, in the presence of β-adrenergic challenge, the mutation-induced APD-prolongation was markedly increased. Derived ECGs from the ventricle-torso simulations presented a substantial increase in the QTc. These results help to elucidate the mechanistic link between the KCNQ1-G269S mutation and the symptom in the patients.

The relative contribution of the blunted response of the mutated $I_{KS}$ in the pathological effects exerted by the mutation was quantified to 20% to 40% for WT-G269S, and less than 15% for G269S, suggesting that the blunted response of the mutated $I_{KS}$ contributed to the pathological effects secondary to the down-regulation of $I_{KS}$ by the mutation.

The proarrhythmic effects of the mutations manifested as an enhanced susceptibility to the induction of EADs in single cells and an increased vulnerability to induction of the excitation wave break in tissue.

The work further demonstrates the usefulness of the developed ventricle-heart
model in the wide effort to understand the pathological mechanisms of inherited heart diseases. Also, this study echoes the study on the arrhythmogenesis of down-regulated $I_{Kur}$ in highlighting the necessity and importance of considering multiple physiological conditions including the presence of adrenergic stimulations in an effort to clarify arrhythmogenesis of down-regulated repolarisation currents.

8.2 Clinical relevance

The broad aim of the thesis was to contribute to the understanding of the mechanisms and developing novel approaches to the management of cardiac diseases such as AF and ischaemia, which is well aligned with the goal of clinical practices. This section summarises the major clinical relevance and implications presented in this thesis.

In the light that developing atrial-selective pharmaceutical therapies is a currently proposed strategy for the pharmaceutical management of AF [53,250], understanding the functional role of the atrial-specific ion channels in normal and pathological conditions is increasingly important. The part I of this thesis focused on the role of an atrial-specific current, $I_{Kur}$, in the atrial arrhythmogenesis, contractility and anti-AF therapy.

$I_{Kur}$ has been of general interest as a potential target for pharmaceutical intervention of AF [53–55], reflected by extensive investigations of anti-AF effects of $I_{Kur}$ block [12,76,186,189,241,257,281,282] and multiple $I_{Kur}$ blockers have been developed [54]. Chapter 3 has demonstrated that down-regulated $I_{Kur}$ promotes incidence of EADs in the presence of adrenergic stimulation. In this regard, for paroxysmal AF in which AP and $I_{CaL}$ remain unaltered while the CaT is increased [17], blocking $I_{Kur}$ is likely to induce proarrhythmic consequences such as EADs and thus may not be a good option for managing the paroxysmal AF. Also, the effects of $I_{Kur}$ block on the atrial electrophysiology have been shown to be controversial both in modelling [155,187] and experimental [148,157,189,193] studies. In Chapter 5, simulations with pure $I_{Kur}$ block also showed limited efficacy in terminating re-entries, while the study highlighted the synergistic effects of a combined block of multiple channels. Therefore, the anti-AF efficacy of $I_{Kur}$ block may be improved with an additional block of other channels, as seen in the multichannel blocker vernakalant [259,409,410]. Furthermore, simultaneously blocking atrial-selective $K^+$ channels such as $I_{Kur}$, $I_{K,Ach}$, $I_{SK}$, $I_{TASK-1}$ and/or $I_{Na}$ may produce even higher efficacy in terminating AF.
Furthermore, successful cardioversion is often followed by a loss of mechanical contraction and hence has been associated with increased risk of thromboembolism [411,412]. The increased inotropic effects demonstrated by down-regulated $I_{Kur}$ as shown in Chapter 4 suggest that block $I_{Kur}$ may improve the mechanical function of the atria without affecting the ventricles, and therefore contribute to improving the condition following cardioversion.

Whilst the developed ventricle-torso model in Chapter 6 advances our understanding of the electrical activities of the heart and the genesis of ECGs, it can be used to screen anti-AF drugs and their ECG manifestations in silico, and to associate the electrophysiological properties of genetic variants with the ECGs.

Finally, Chapter 7 demonstrated an application of the ventricle-torso model revealing the causal link between the genotype and corresponding phenotype. Given that the genetic variations in KCNQ1 are the most prevalent cause of LQT1 [384–386], the most common subtype of LQTS [384,385], and that a majority (62%) of cardiac events LQT1 patients experienced were found to be during exercise [401], the mechanistic link between the KCNQ1-G269S mutation and the ECG manifestations revealed in Chapter 7 may be extrapolated to the analyses of the mechanisms underlying the manifestations of these patients.

### 8.3 Future work

#### 8.3.1 Investigating multiple atrial-specific channel block

The synergistic anti-AF effects exerted by multiple $K^+$-channel block revealed in Chapter 5 have implications for a new avenue in developing antiarrhythmic pharmaceutical therapies. Blocking an atrial-specific channel together with minor inhibitions in the non-specific $K^+$-channels may produce beneficial anti-AF effects over the single atrial-specific channel block alone, whilst having little adverse consequences in the ventricles. Additionally, recent developments revealed two additional atrial-specific channels, namely, the small-conductance $Ca^{2+}$-activated $K^+$ (SK) channels ($I_{SK}$) [284–290], and the two-pore $K^+$ (K2P3.1) channel ($I_{TASK-1}$) [291–296], further to the well-known constitutively active acetylcholine-activated $K^+$ current ($I_{K,Ach}$) and $I_{Kur}$. Simultaneously blocking these atrial-specific channels and/or $I_{Na}$ may markedly enhance the efficacy of the anti-AF pharmaceutical therapy, which warrants future
investigations.

In simulating the drug effects, the structural remodelling in the atria was not taken into consideration in this thesis. The structural remodelling such as atrial enlargement and tissue fibrosis may result from and contribute to the persistence and therapeutic resistance of AF [42]. It is, therefore, important to assess the efficacy of proposed pharmaceutical therapy in the presence of such structural remodelling, warranting future studies.

8.3.2 Population-based modelling

Chapter 3 suggests that role of ionic currents and their variants in the cardiac electrophysiology are dependent on the baseline electrophysiological properties. In line with the presence of large inter-subject variations, it is important to take these differences into account when deciphering the pathological mechanisms underlying cardiac diseases and developing safe and effective therapies. The population-based modelling [220,413] simultaneously considers an extensive number of variants instead of one single generic model as being used in most simulation studies. Such a method provides a potential to produce model-independent results, which is more substantial when it comes to virtual drug screening, and therefore warrants further investigations.

8.3.3 Electromechanical model and fluid dynamics model

The electromechanical model presented in Chapter 4 can be employed in many other studies. In the management of AF, restoring SR to the atria is not the only goal to achieve: restoring atrial mechanical function is also important since a cardioversion has been associated with a loss of mechanical contraction and increased the risk of thromboembolism [411,412]. The presented electromechanical model can be applied to evaluate how the anti-AF drug therapy affects the atrial contractions.

In the ventricle-torso model developed in Chapter 6, the mechanical function of the ventricles was not taken into considerations. The mechanical deformation in the ventricles may alter the body surface potential distribution and thus warrants incorporating of electromechanics in the future. The updated ventricle-torso model may be a valuable tool in understanding and improving the electromechanical functions of the heart. Examples include the cardiac resynchronisation therapy and treatment of heart failure.
The complexity of understanding the cardiac function and diseases is associated with a multi-physics problem incorporating bioelectricity, tissue mechanics and the dynamics of blood. The latter has not been considered in this thesis. The incorporation of the fluid-tissue interaction into the 3D models will facilitate investigations towards more realistic conditions, including electromechanical feedback posed by the changing blood pressure, and the thromboembolism due to the loss of atrial contractions.

8.3.4 Precision medicine

The dependence of the role of ionic currents in cardiac electrophysiology has further implications for the management of cardiac diseases: it is, therefore, important to consider the patient-specific variability [414]. These patient-specific properties including genotype, phenotype, personal health state and history and structure of the heart, etc., can be incorporated into computational models, and thus warrants future efforts.

8.4 Closing words

This thesis demonstrates how and what computational models can contribute to the wide effort in revealing the underlying mechanisms of cardiac diseases and developing new therapies. In the light of a promising coming era of precision medicine in cardiology [415,416], computational modelling is expected to play an unparalleled role in fulfilling this goal. It is the author’s belief and hope, that sophisticated, personalised cardiac models and platforms that run in real time contribute to the diagnoses, interpretations of cardiac conditions and decision making in clinical settings.
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Haibo Ni 280


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Haibo Ni 288


Appendix A Supplementary figures

Figure A.1 Analysis of the relationship between the APD30 of human atrial myocytes and the parameters of IKur. In (A), activation time constants were scaled by 0.5 to 2.5 vs. control values; the conductance of IKur was varied from 0 to 2-fold increase vs. control. (i-v) The V1/2 of steady-state activation was shifted by (i) -5 mV, (ii) -2.5 mV, (iii) 0 mV (iv) 2.5 mV and (v) 5 mV, respectively; the APD30 of AP elicited from the control time constant and conductance was marked as a red triangle in each panel. (B) The relationship between APD30 and the V1/2 of steady-state activation.

Haibo Ni
Figure A.2 Simulated effects of ISO on human atrial AP using the Nygren *et al.* model. The effects of ISO were simulated by using similar changes as those implemented in the *Grandi et al.* model. To model down-regulation in $I_{Kur}$ by the loss-of-function mutations, the maximum conductance of $I_{sus}$ in the Nygren *et al.* model was reduced to 40% of the original value.
Figure A.3 Heterogeneous electro-mechanical activities in the isolated regional atrial cells for the WT (column i), D322H (column ii) and P488S (column iii) conditions. (A) AP. (B) CaT. (C) Normalised active force. (D) Sarcomere length shortening. The SACs were incorporated in these simulations.
Figure A.4 Simulated time courses of L-type Ca$^{2+}$ current, Na$^+$-Ca$^{2+}$ exchanger, RyR release flux, SR uptake flux and SR content elicited by AP under the WT and mutations w/ SACs. (A) L-type Ca$^{2+}$ current (I$_{CaL}$). (B) Na$^+$-Ca$^{2+}$ exchanger (I$_{NaCa}$). (C) RyR release flux (J$_{Rel}$) of the subspace compartment. (D) SR uptake flux (J$_{SERCA}$) of the bulk cytosol. (E) SR content ([Ca$^{2+}]_{SR}$).
Figure A.5 Time integral of (A) $I_{\text{CaL}}$, (B) $I_{\text{NaCa}}$, (C) $J_{\text{Rel}}$ and (D) $J_{\text{SERCA}}$ elicited by APs for WT and the mutations in the presence of $I_{\text{SAC}}$. 
Figure A.6 The individual contribution of $I_{CaL}$ and $I_{NaCa}$ to the systolic $[Ca^{2+}]_i$ in the atrial cells for the WT and mutations with $I_{SAC}$. (A) The time integral of $I_{CaL}$ at the peaking time of $[Ca^{2+}]_i$. (B) The time integral of $I_{NaCa}$ at the peaking time of $[Ca^{2+}]_i$. (C) Relative total $Ca^{2+}$ entry contributing to the systolic $[Ca^{2+}]_i$, which was calculated as $\int -I_{CaL} \, dt + 2 \cdot \int I_{NaCa} \, dt$ and normalised to the value of WT; specially, the contribution of $I_{NaCa}$ towards the $Ca^{2+}$ entry preceding peaking of $[Ca^{2+}]_i$ was shown in hatched shadings.
Figure A.7 The simulated dependence of relative cell shortening on the parameters of $I_{Kur}$ in the presence of $I_{SAC}$. The responses of the relative cell shortening to (A) the varying conductance and $K_{Activation}$; (B) the varying conductance and $\tau_{Activation}$; (C) varying $K_{Activation}$ and $\tau_{Activation}$. The ranges of scaling are shown as labelled. In columns (i-iii), the $V_{1/2}$ of steady-state activation was shifted as labelled. In each panel the difference in cell contractility was quantified as the change in relative cell shortening with respect to the relative cell shortening under the control parameters (1.0 for both, indicated with * in the figure).
Figure A.8 Snapshots of simulated atrial electromechanical contraction superimposed on the undeformed atrial mesh (in grey and indicated with arrows) under the WT, D322H and P488S conditions w/ $I_{\text{SAC}}$. The simulated time following the application of an external stimulation from the SAN region is labelled in the left of the panels. Insert: time course of computed atrial volume.