DISSERTATIONES SCHOLAE DOCTORALIS AD SANITATEM INVESTIGANDAM
UNIVERSITATIS HELSINKIENSIS

84/2016

HUGH CHAPMAN

The hERG1 (K_V11.1) Potassium Channel: Its Modulation and the Functional Characterisation of Genetic Variants

DIVISON OF PHYSIOLOGY AND NEUROSCIENCE
DEPARTMENT OF BIOSCIENCES
FACULTY OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES
DOCTORAL PROGRAMME IN INTEGRATIVE LIFE SCIENCE
UNIVERSITY OF HELSINKI

THE hERG1 (K_V11.1) POTASSIUM CHANNEL: ITS MODULATION AND THE FUNCTIONAL CHARACTERISATION OF GENETIC VARIANTS

Hugh Chapman

DIVISON OF PHYSIOLOGY AND NEUROSCIENCE
DEPARTMENT OF BIOSCIENCES
FACULTY OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES
UNIVERSITY OF HELSINKI

AND

INTEGRATIVE LIFE SCIENCE DOCTORAL PROGRAM

Academic Dissertation

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki in Walter room, EE Building (Agnes Sjöbergin katu 2) on December 9th 2016 at 12 noon.

Helsinki 2016

Supervisor: Docent Michael Pasternack PhD
MSD Finland Oy,
Espoo, Finland

Pre-examiners: Professor Fredrik Elinder PhD
Department of Clinical and Experimental Medicine,
Faculty of Medicine and Health Sciences,
University of Linköping,
Linköping, Sweden

Professor Eero Mervaala MD, PhD
Department of Pharmacology,
Faculty of Medicine,
University of Helsinki,
Finland

Opponent: Professor Pasi Tavi PhD
Department of Biotechnology and Molecular Medicine,
A.I. Virtanen Institute for Molecular Sciences,
University of Eastern Finland,
Kuopio, Finland

Custos: Professor Juha Voipio PhD
Department of Biosciences,
Faculty of Biological and Environmental Sciences,
University of Helsinki,
Finland

ISSN 2342-3161 (print)
ISSN 2342-317X (online)
ISBN 978-951-51-2736-5 (paperback)
ISBN 978-951-51-2737-2 (PDF)
Painosalama Oy, Turku 2016

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals:

I. **Chapman H** and Pasternack M (2007) The action of the novel gastrointestinal prokinetic prucalopride on the HERG K^+ channel and the common T897 polymorph. Eur J Pharmacol 554: 98 – 105.

II. Paavonen KJ, **Chapman H**, Laitinen PJ, Fodstad H, Piippo K, Swan H, Toivonen L, Viitasalo M, Kontula K and Pasternack M (2003) Functional characterization of the common amino acid 897 polymorphism of the cardiac potassium channel KCNH2 (HERG). Cardiovasc Res 59: 603 – 611.

III. **Chapman H***, Ramström C*, Korhonen L, Laine M, Wann KT, Lindholm D, Pasternack M and Törnquist K (2005) Down-regulation of the HERG (KCNH2) K^+ channel by ceramide: evidence for ubiquitin-mediated lysosomal degradation. J Cell Sci 118: 5325 – 5334.

IV. Lahti AL, Kujala VJ, **Chapman H**, Koivisto A, Pekkanen-Mattila M, Kerkelä E, Hyttinen J, Kontula K, Swan H, Conklin BR, Yamanaka S, Silvennoinen O and Aalto-Setälä K (2012) Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. Dis Model Mech 5: 220 – 230.

* Equal contribution

Author's contribution to the original publications included in the thesis:

I: The author designed, conducted all the experiments and the analysis of the data. Also wrote the text and produced the figures for the manuscript.

II: The author participated in designing and conducting the electrophysiological experiments, and in the analysis of that data.

III: The author participated in designing the experiments, conducted the electrophysiological experiments and the analysis of that data, and wrote the manuscript.

IV: The author designed and performed the patch-clamp experiments and their analysis. Also participating in writing of and producing figures for the manuscript

ABBREVIATIONS

5-HT	5-hydroxytryptamine (serotonin)
Å	Ångström (10^{-10}m)
AKAP	A-kinase anchoring protein
APD	action potential duration
BMAL1	brain muscle arnt-like1
CaMKII	Ca^{2+}-calmodulin dependent protein kinase II
Cav	caveolin
CFTR	cystic fibrosis transmembrane conductance regulator
CHIP	C-terminal of Hsc70-interacting protein
CHO	chinese hamster ovary cells
CLOCK	circadian locomotor output control kaput
cNBD	cyclic nucleotide binding homology domain
CNS	central nervous system
COPII	coat-associated protein complex II
COS-7	*Cercopithecus aethiops* kidney fibroblasts
DAG	diacylglycerol
EAD	early afterdepolarisation
ECG	electrocardiogram
EEG	electroencephalogram
E_K	potassium equilibrium potential
ER	endoplasmic reticulum
ERG	Ether-à-go-go Related Gene
$ERK_{1/2}$	extracellular signal-related kinase $_{1/2}$
FAK	focal adhesion kinase

FDA	Food and Drug Administration
FKBP38	38kDa FK506 binding protein
FTPC	free therapeutic plasma concentration
HCN	hyperpolarisation activated cyclic nucleotide gated
hERG	human Ether-à-go-go Related Gene encoded protein
HEK	human embryonic kidney cells
hESC	human embryonic stem cell
HIF	hypoxia inducible factor
Hsp	heat shock protein
IC_{50}	half-maximal inhibitory concentration
ICH	International conference on harmonization of technical requirements for registration of pharmaceuticals for human use
I_{Kr}	rapid component of the delayed rectifier potassium current
I_{Na-L}	late sodium current
iPSC	induced pluripotent stem cell
$[K^+]_o/[K^+]_i$	extracellular/intracellular potassium ion concentration
LQTS	long QT syndrome
miRNA	microRNA
NCE	new chemical entity
Nedd4-2	neural precursor cell expressed developmentally down-regulated protein 4 subtype 2
NF-κB	nuclear factor-κB
NMD	nonsense mediated decay
PAS	Per-Arnt-Sim
PI3K	phosphatidyl inositol-3-kinase
PIP_2	phosphatidylinositol-4,5-bisphosphate
QTc	rate-corrected duration of the QT interval

QT interval	time of ventricular depolarisation and repolarisation (from the onset of QRS complex to the end of the T wave)
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
SCD	sudden cardiac death
SERCA	sarco/endoplasmic reticulum Ca^{2+}-ATPase
SIDS	sudden infant death syndrome
SNP	single nucleotide polymorphism
SQTS	short QT syndrome
SUDS	sudden unexplained death syndrome
τ	time constant
TdP	torsade de pointes
TK	tyrosine kinase
TQT	thorough QT/QTc
TRH	thyrotropin-releasing hormone
UTR	untranslated region
$V_{½}$	half-maximal voltage
VEGF	vascular endothelial growth factor
V_{rest}	resting membrane potential
VSD	voltage-sensing domain
WT	wild-type

ABSTRACT

The human *ether á-go-go related gene* (h*ERG1* or *KCNH2*) encodes the pore forming subunit of the cardiac delayed rectifier potassium (I_{Kr}) channel. Its unique kinetics result in a resurgent current crucial for the repolarisation of the cardiac action potential and a capability to suppress premature excitation. hERG1 is widely expressed with roles e.g. in neuronal firing, intestinal and uterine contractility, and insulin secretion. Furthermore overexpression and ectopic expression of hERG1 occurs in cancer where it is involved in proliferation, migration, chemotherapy resistance etc.

The long QT syndrome (LQTS) often presents as sudden cardiac death in children and young adults. LQTS is characterised by electrocardiogram abnormalities with arrhythmia that can lead to palpitations, syncope, seizure, cardiac arrest and death. Underlying the congenital form of LQTS are mutations in ion channel proteins (including hERG1, the loss-of-function of which gives rise to LQT2) and their interacting proteins. Carriers of a particular mutation may be symptomatic (to varying extents) or asymptomatic, with the deleterious effects only emerging due to the presence of other factors. This is analogous to drug-induced LQTS where arrhythmia may occur in 1 of 120,000 users of certain non-cardiac drugs. Virtually all drug-induced LQTS is caused by inhibition of hERG1. Consequently in the field of safety pharmacology the hERG1 channel has for the last 20 years and continues to have a huge impact as the primary *in vitro* predictor of the proarrhythmic risk for a drug.

Various aspects of the hERG1 channel are investigated in the studies presented in this thesis. The effect of prucalopride, a gastrointestinal prokinetic drug, on hERG1 was examined. Prucalopride exhibited rapid state and concentration dependent inhibition of hERG1 however, at therapeutic concentrations block is insignificant (hERG safety margin of $\geq$300). This *in vitro* prediction has translated to the clinical studies of this drug and the market.

The heterogeneous phenotype associated with LQTS may arise from genetic modifiers such polymorphisms and mutations. Heterologous expression of the prevalent hERG1 K897T polymorphism identified a reduced hERG1 current density as the primary difference from wild-type, a result of decreased protein expression. Additionally a slowing of deactivation and alteration of inactivation was evident. Also studied but using induced pluripotent stem cell (iPSC) derived cardiomyocytes was hERG1 R176W. Unlike previous LQT2 iPSC models the origin here was a relatively asymptomatic individual. The phenotypic characteristics of LQT2 were however still reproduced *in vitro* (i.e. a decrease in I_{Kr} and action potential prolongation) though as a milder version.

Finally the effect of ceramide, a sphingolipid which accumulates in heart failure and is involved in lipotoxicity, on hERG1 was investigated. Ceramide was found to reduce hERG1 current in a time dependent manner through tagging (ubiquitination) of the cell surface protein for internalisation and targeting to lysosomes.

1. INTRODUCTION

Plasma membranes of the cell and its organelles, e.g. sarcoplasmic reticulum (SR) and mitochondria, with their hydrophobic core of phospholipid fatty acyl chains and cholesterol form an impermeable barrier to ion movement. The passage of ions between the aqueous compartments on either side is enabled by the incorporation in plasma membranes of specific proteins: ion channels, transporters and pumps. Ion channels are gated pores through which ions flow by passive diffusion down their electrochemical gradient (Ashcroft, 2006). The fundamental properties of ion channels, and a basis for classification, are selectivity and gating (Ashcroft, 2006; Grant, 2009). Gating is the mechanism of transition between the closed and open states (Fig. 1), and is random (stochastic) with the occupancy of a state determined by probability which can be influenced by e.g. transmembrane voltage (as in Fig. 1), ligand-binding etc. When channels are opened the selectivity exhibited by some for one type of ion over others biases the membrane potential of the cell towards the equilibrium potential of that conducting ion (Marbán, 2002).

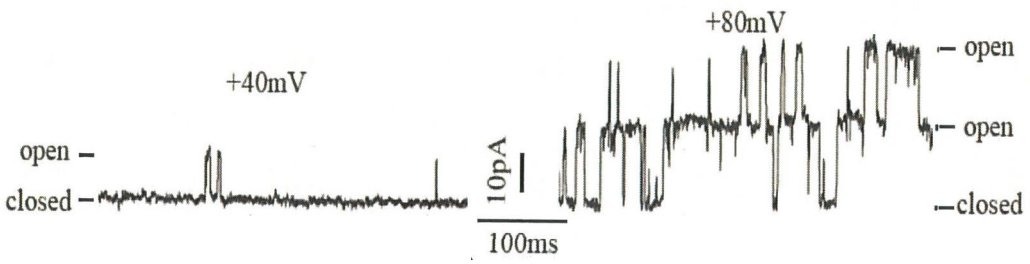

Figure 1. The movement of ions through a channel pore is dependent on its permeability and the electrochemical gradient across the membrane. Single potassium channel currents at different membrane potentials measured with the patch-clamp technique. From the open state during maintained stimulation Na^+, Ca^{2+} and certain K^+ channels enter another state: inactivated, which is non-conducting and the occupancy of which is the basis of refractoriness (Grant, 2009). The K^+ selectivity is determined by the selectivity filter and under physiological conditions the current through such channels is outward as membrane potentials more negative than the K^+ equilibrium potential are never reached.

2. REVIEW OF THE LITERATURE

2.1. THE CARDIAC ACTION POTENTIAL

The heart functions as a pump maintaining systemic and pulmonary circulation. Each heartbeat, a cycle of relaxation (diastole) and contraction (systole), is initiated by the electrical excitation of a population of pacemaker cells that then spreads throughout the heart as evident on the surface electrocardiogram (ECG; Fig. 2A; Marbán, 2002). The cellular representation of the electrical excitation, i.e. the sequential activation and inactivation of inward and outward current, is seen as a change in the transmembrane potential with time: the action potential (Fig 2; Grant, 2009; Nerbonne and Kass, 2005; Schmitt et al., 2014). The action potential differs in the distinct types of myocardial cells (cardiomyocytes) found in the conduction system (the sinoatrial node, atrioventricular node, His-Purkinje fibres) and the contractile cells of the atria and ventricles (Fig. 2A; Nerbonne and Kass, 2005; Schram et al., 2002). Underlying the action potential is the existence of an electrochemical gradient across the surface membrane of each cardiomyocyte. This differential distribution of ions across the cell membrane is generated and maintained by the action of ion pumps and transporters such as the Na^+/K^+ pump and Na^+/ Ca^{2+} exchanger. At rest the surface transmembrane potential is set by the resting potassium conductance which in non-nodal cells is substantial, through inward rectifier current (I_{K1}) channels, and consequently close to the K^+ equilibrium potential (E_K) of ~-90mV.

The action potential of ventricular (Fig. 2B) and atrial myocytes, as well as Purkinje fibres, begins with a rapid depolarisation (phase 0) due to a large inward sodium current (I_{Na}) through the opening of voltage-gated Na^+ channels (Nerbonne and Kass, 2005). This depolarisation activates a number of currents including the rapidly activating and inactivating transient outward K^+ current (I_{to}) that gives rise to, along with Na^+ channel inactivation, a brief repolarisation phase (phase 1). This phase increases the electrochemical gradient for Ca^{2+}, and influences the height and duration of the plateau phase (phase 2). During phase 2 there is balance between the inward L-type Ca^{2+} current (I_{CaL}) at the myocyte T-tubules and the outward delayed rectifier K^+ current (I_K), which includes rapid and slow components (I_{Kr} and I_{Ks}). This extended phase (lasting >200ms in humans) enables the influx of Ca^{2+} to trigger sufficient Ca^{2+} release from the adjacent internal SR stores through cardiac ryanodine receptors (RyR2). The increase in the cytosolic [Ca^{2+}] activates the contractile machinery i.e. excitation-contraction coupling, the *en masse* occurrence of which causes contraction and ejection of blood. As Ca^{2+} channels undergo voltage- and Ca^{2+}/calmodulin-dependent slow inactivation and I_K becomes predominant repolarisation (phase 3) takes place. Three different K^+ currents (I_{Kr}, I_{Ks} and I_{K1}) are primarily responsible for the repolarisation of human (and other large mammal) ventricular cardiomyocytes (Schmitt et al., 2014). The partial overlap of these currents (Fig. 2B) affords some redundancy in the system; this compensatory safe-guard is known as the repolarisation reserve (Roden, 1998, 2006). Following restoration of the resting membrane potential (phase 4) the intracellular Ca^{2+} level is returned to the basal state, through extrusion by the electrogenic Na^+/Ca^{2+} exchanger (NCX1.1) and SR reuptake via SERCA2a, which at the organ level manifests as diastole enabling the filling of blood.

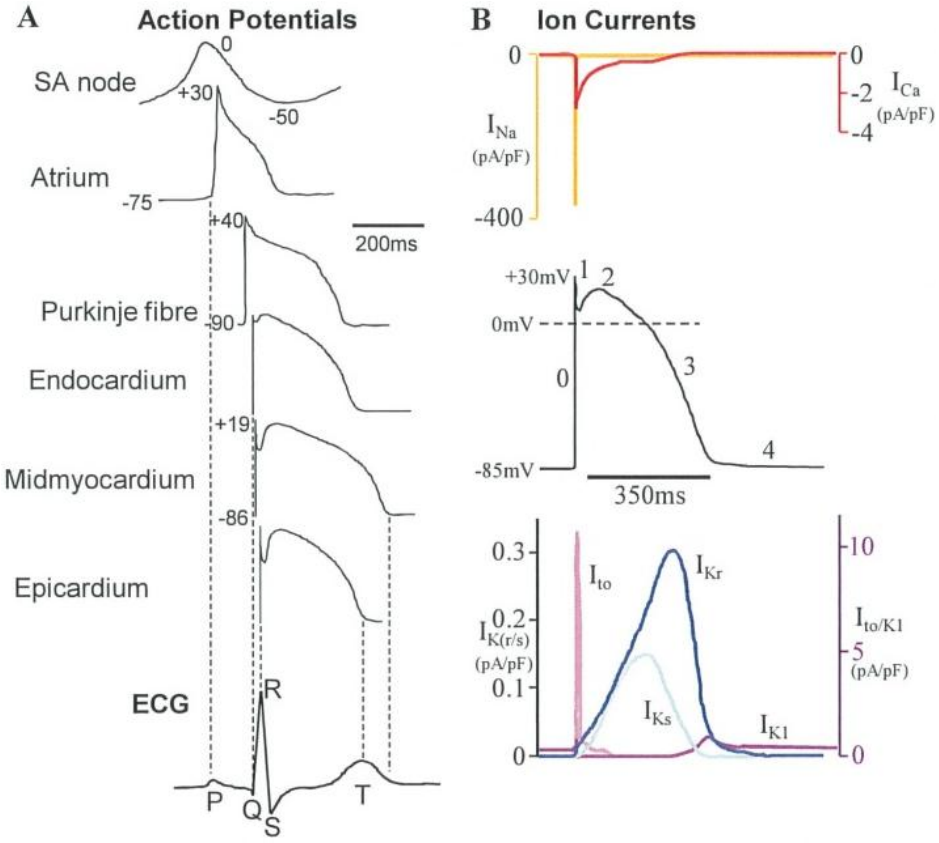

Figure 2. (A) Action potential waveforms from various regions (adapted from Nerbonne and Kass, 2005) and how they correlate with the surface ECG. The numbers refer to membrane potential, in mV (Drouin et al., 1995; Schram et al., 2002). Excitation originates from the spontaneous regular firing of the SA node and spreads to the atria (the P wave of the ECG) resulting in contraction. From the AV node the impulse is conducted through the bundle of His to the Purkinje fibre system resulting in the synchronous excitation of the endocardial surface, ventricular depolarisation (the QRS complex of the ECG) and contraction that generates the systolic blood pressure. Ventricular repolarisation appears as the T wave on the ECG. The propagation of activity is dependent on the electrical coupling between cells, mediated by gap junctional channels formed by connexin proteins (Grant, 2009). Cardiac action potentials are modulated most conspicuously by the autonomic nervous system e.g. parasympathetic stimulation activates, via M_2 receptors, the G-protein coupled inward rectifier I_{KACh} which is prominent in the nodes and atria (but also present in the ventricles) resulting in negative chronotrophy and dromotrophy (Liang et al., 2014; Schmitt et al., 2014). (B) A prototypical human ventricular action potential with its five (0-4) phases (middle) and the associated inward (above) and outward (below) ion channel currents (adapted from Sanguinetti and Tristani-Firouzi, 2006). It should be noted that I_{Na} has a small persistent ("window") component (up to 1% of the peak current termed late I_{Na} (I_{Na-L}); Antzelevitch et al., 2014) present during phase 2 and that other currents, e.g. via Na^+/Ca^{2+} exchanger (I_{NCX}), also underlie the action potential.

The distinct electrophysiological phenotypes of the cardiomyocytes, which contributes to unidirectional excitation propagation and the generation of normal cardiac rhythms, is achieved by quantitative and qualitative differences in ion channel expression (Chandler et al., 2009; Gaborit et al., 2007; Nerbonne and Kass, 2005; Ördög et al., 2006; Schram et al., 2002). While most evident for the pacemaker cells of the sinoatrial (SA; or atrioventricular, AV) node, versus the cardiomyocytes of the atrium or ventricle, differences are also apparent between atrium and ventricle (Fig. 2A). The triangular shape of the atrial action potential and its shorter duration (APD) is derived from additional repolarising K^+ currents including the atrially restricted ultra-rapid delayed rectifier I_{Kur} and the Ca^{2+}-activated I_{KCa} (Schmitt et al., 2014). Futhermore the ventricular myocardium is not a homogenous structure with differences in action potential

configuration and APD between right and left ventricle, between and within the septum and free walls (i.e. transseptal and transmural), and along the apico-basal axis (Drouin et al., 1995; Nerbonne and Kass, 2005; Sicouri et al., 2010; Szentadrassy et al., 2005). Transmurally three myocyte populations (epicardial, endocardial and midmyocardial) have been described with differing densities of I_{NaL}, I_{NCX}, I_{to} and I_{Ks} resulting in APD differences that contribute, together with the degree of electrotonic coupling, to the dispersion of repolarisation (Fig. 2A; Antzelevitch, 2007; Glukhov et al., 2010; Li et al., 2002).

Disruption of the finely orchestrated activity of ion channels and transporters, through changes in properties or expression as a result of e.g. mutation, drug block and/or myocardial disease, can alter action potential waveforms and thus orderly synchronisation/propagation predisposing to arrhythmias which destabilise coordinated contraction and lead to insufficient pressure for blood circulation. Arrhythmia induced haemodynamic collapse, primarily in the setting of acute or chronic ischaemia associated with coronary heart disease, results in sudden cardiac death (SCD), ~200,000 cases of which occur (~8% of all deaths) each year in the U.S. (Deo and Albert, 2014; Stecker et al., 2014).

2.2. THE I_{Kr}/hERG1 CHANNEL

The rapid component of the delayed rectifier K^+ current is found in various cardiac cell types (nodal, atrial, Purkinje and ventricular) from a variety of species, including human (Jost et al., 2005; Li et al., 1996; Tseng, 2001; Veldkamp et al., 1995; Wang et al., 1994). I_{Kr} is primarily responsible for the termination of the action potential plateau (phase 2) and the initiation of final repolarisation as I_{K1} activates, and thus influences APD (Li et al., 1996; Gintant, 2000; Jost et al., 2005). In contrast the slow component, I_{Ks}, has a limited role in cardiac repolarisation in the absence of β–adrenergic stimulation under physiological conditions (Banyasz et al., 2014; Jost et al., 2005).

Block of I_{Kr}, e.g. by the methanesulfonanilides E-4031 or dofetilide, results in a delay in repolarisation which on the ECG appears as a prolongation of the QT interval or when corrected for heart rate QTc interval (Katritsis et al., 1997; Vicente et al., 2015). The QTc interval is considered prolonged if >440 ms, though up to 460 ms in females can be normal (with the Bazett correction formula; Schwartz and Crotti, 2014). The delay in action potential repolarisation and concomitant increase in the effective refractory period (i.e. when the tissue is inexcitable, a result of Na^+ channel inactivation) reduces the risk of re-entrant arrhythmias such as ventricular and atrial fibrillation (VF, AF), atrial flutter (Riera et al., 2008). This antiarrhythmic mechanism, a class III drug effect, was actively sought however excessive action potential prolongation, particularly in the setting of hypokalemia (i.e. serum $[K^+]$ <3.5 mM) and bradycardia (i.e. slow heart rate, <60 bpm), is associated with the long QT syndrome (LQTS; Fig. 3).

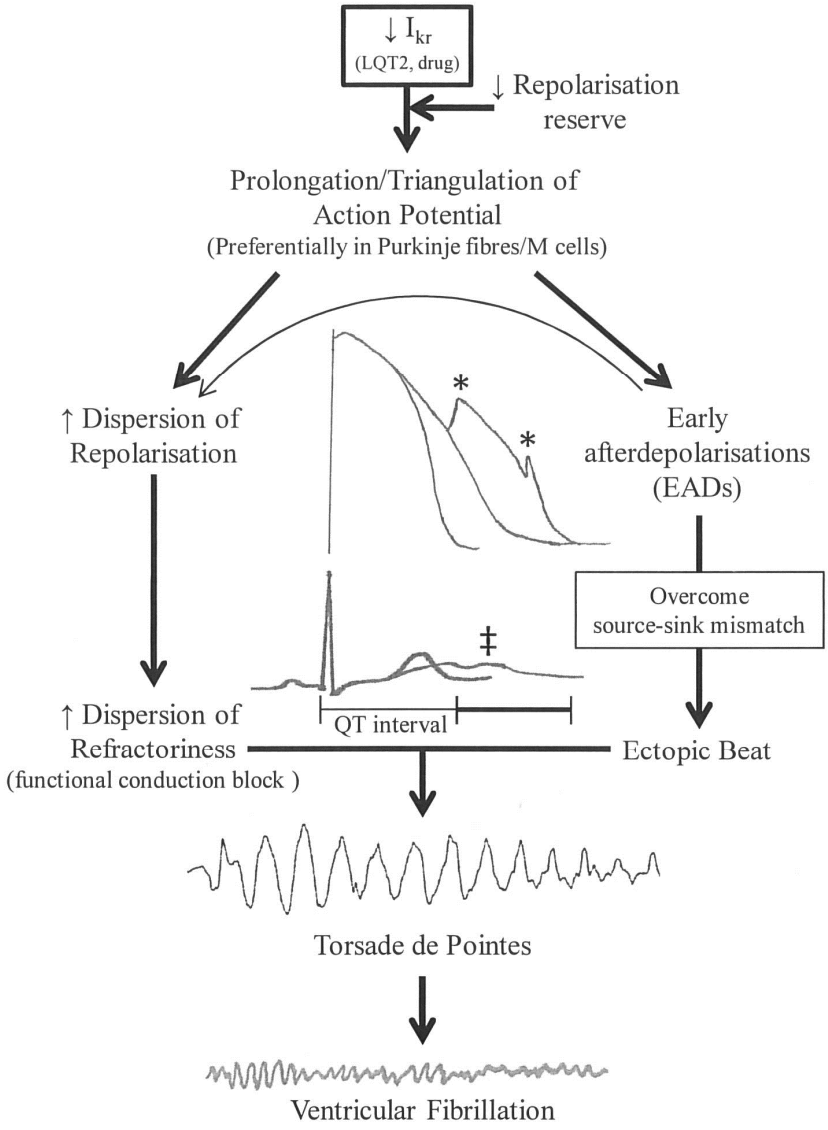

Figure 3. Arrhythmogenesis associated with I_{Kr} deficit (adapted from Antzelevitch and Sicouri, 2012; Yap and Camm, 2003). This is commonly in the setting of a reduced repolarisation reserve such as I_{Ks} loss e.g.due to latent LQT1 (Napolitano et al., 2000; Veerman et al., 2013). Prolongation or more specifically triangulation of the action potential leads to the occurrence of membrane oscillations or EADs (*). On the ECG the QTc interval is prolonged and considered proarrhythmic if >500 ms (Goldenberg et al., 2008, 2011; Migdalovich et al., 2011; Schwartz and Crotti, 2014). A flattening and notching (bifid, ‡) of the T wave may be seen (Dausse et al., 1996; Etheridge et al., 2003; Vicente et al., 2015). EADs are generated by L-type Ca^{2+} channels as the slowing of repolarisation allows their recovery from inactivation and for reactivation (D. Guo et al., 2007; January and Riddle, 1989), with the involvement of I_{NCX} due to SR Ca^{2+} leak (hyperphosphorylation of RyR2) suggested (Terentyev et al., 2014). Alternatively the spontaneous systolic SR release, due to Ca^{2+} overload as a result of a prolonged phase 2, may have a more central role in EAD generation (Kim et al., 2015). If the EAD occurs synchronously in enough myocytes (the source) this will bring the neighbouring repolarised tissue (the sink) to its activation threshold and trigger an action potential (Xie et al., 2010). Propagation of triggered activity appears as ventricular extrasystoles (ectopic beats) on the ECG. In addition to this triggered activity, the other prerequisite for reentrant arrhythmias such as TdP is a substrate (Schmitt et al., 2014). This may be a morphological change, e.g. after infarction, or amplification of the inherent spatial dispersion of repolarisation, particularly transmurally due to preferential prolongation of midmyocardial (M) cell APD, which leads to heterogeneity of refractoriness within the ventricular myocardium (Antzelevitch and Sicouri, 2012; Brunner et al., 2008; Shah and Hondeghem, 2005). This is further augmented, at least transiently, by β-adrenergic stimulation (Antzelevitch and Sicouri, 2012). Recently such heterogeneous APD prolongation giving rise to regions with steep dispersion gradients was shown in the intact human ventricular epicardium of congenital LQTS patients (Vijayakumar et al., 2014). It should be noted that due to the abnormal Ca^{2+}

levels mechanical alterations also occur (Haugaa et al., 2010), e.g. a reduction in systolic function at rest (Leren et al., 2015).

LQTS, whether acquired (i.e. due to an environmental stressor e.g. metabolic abnormality, drug-induced) or congenital, manifests in the setting of a structurally normal heart as syncope and/or seizures due to impairment of cerebral circulation resulting from torsade de pointes (TdP; Schwartz and Crotti, 2014; Shah, 2005). This polymorphic ventricular tachycardia, where the QRS complexes change amplitude appearing to sinusoidally twist around the isoelectric line, can either spontaneously cease or, in ~20 % of cases, degenerate into VF with cardiac arrest and sudden death (Fig. 3; Shah, 2005). An overall mortality of 10-17 % is associated with TdP. Historically two inherited LQTS forms were described: the autosomal dominant Romano-Ward syndrome (i.e. phenotype obtained by alteration of a single allele; Romano et al., 1963; Ward, 1964) and the autosomal recessive Jervell and Lange-Nielsen syndrome, in which deafness is also present (Jervell and Lange-Nielsen, 1957). Later LQTS linkage was found to various loci, including chromosome 7q35-36 (LQT2; Jiang et al., 1994), indicating a heterogeneous molecular basis. Following mapping to the LQT2 locus and screening of the gene, affected members of LQT2 kindreds were discovered to have mutations (including a de novo mutation in a sporadic case) in the then recently found human *ether á-go-go (EAG) related gene* (h*ERG1* or *KCNH2*; Curran et al., 1995).

h*ERG1* was isolated from a human hippocampus cDNA library and identified as a member of a novel family of voltage-gated potassium channel (K_V) genes (Warmke and Ganetzky, 1994). The gene encoded a polypeptide of 1159 amino acids with a molecular mass of 127 kDa. When expressed in *Xenopus* oocytes the h*ERG1* channels (K_V11.1) conducted a voltage activated, potassium selective, inwardly rectifying current (Sanguinetti et al., 1995; Trudeau et al., 1995). As h*ERG1* was already shown to be the LQT2 gene and its mRNA was strongly expressed in the heart (Curran et al., 1995), the identification of the physiological counterpart of its current was simplified. The h*ERG1* current-voltage relationship exhibited similar rectification properties and voltage-dependence of activation to I_{Kr}, in addition to reproducing I_{Kr}'s paradoxical modulation by extracellular [K^+] i.e. lowering K^+_o decreases the outward current despite increasing the electrochemical driving force (Sanguinetti et al., 1995). At the elemental level the conductance and kinetics of single h*ERG1* channels were also comparable to those of I_{Kr} channels (Kiehn et al., 1996). Furthermore like I_{Kr} the h*ERG1* current was sensitive to methanesulfonanilide drugs (Kiehn et al., 1996; Snyders and Chaudhary, 1996; Spector et al., 1996a; Trudeau et al., 1995).

Consequently h*ERG1* encodes the pore forming (α) subunit of the cardiac I_{Kr} channel, an origin for both inherited and drug-induced LQTS/TdP.

2.2.1 EXPRESSION

ERG1 is expressed in the heart, at the mRNA and/or protein level, in various species including human, dog, horse, ferret, rabbit, guinea pig, rat and mouse (Brahmajothi et al., 1997; Finley et al., 2002; Pond et al., 2000; Szabó et al., 2005; X. Wang et al., 2008; Wymore et al., 1997; Zicha et al., 2003). In atrial and ventricular myocytes ERG1 protein was shown to be primarily localised to T-tubules, the site of excitation-contraction coupling, (Jones et al., 2004; Rasmussen et al., 2004) or uniformly distributed in T-tubular and surface sarcolemma (Balijepalli et al., 2007; Ehrlich et al., 2004; Finley et al., 2002; Pond et al., 2000).

In the healthy human heart *ERG1* mRNA is one of the most abundant α-subunit coding transcripts with similar expression in atria, ventricles and Purkinje fibres (Gaborit et al., 2007; Ördög et al., 2006). In another study transcript expression of h*ERG1* isoforms was higher in the right heart chambers (Luo et al., 2008). Though abundantly expressed in human SA node the level of *ERG1* transcript is about half that of the right atrium (Chandler et al., 2009). In the left ventricular free wall *ERG1* mRNA is significantly more expressed in human than either rabbit or guinea pig (Zicha et al., 2003). In human undiseased left ventricular myocardium ERG1 protein is similarly expressed in apical and basal regions, however expression (as in other species except dog) is significantly greater in the epicardium (Brahmajothi et al., 1997; Szabó et al., 2005; Szentadrassy et al., 2005; X. Wang et al., 2008).

I_{Kr}, unlike e.g. I_{Ks}, is present throughout development from embryonic stem cells to cardiomyocytes of the foetus and neonate through to adult (Danielsson et al., 2013; Obreztchikova et al., 2003; Sartiani et al., 2007). However changes in I_{Kr} do occur developmentally e.g. a decline postnatally in dog and most dramatically in mouse ventricular myocytes where it is the sole I_K component of the foetus but absent from the adult (Krishnamurthy et al., 2004; Obreztchikova et al., 2003; Wang et al., 1996; Yang et al., 2014). Underlying this is a lower mERG1 protein level and alteration in the composition of the I_{Kr} channel i.e. a switch in the ratio of the ERG1 variants and lower availability of an accessory subunit protein (X. Wang et al., 2008). The relative importance of I_{Kr} therefore alters, e.g. it is the dominant repolarising current across species for the period from foetal to neonatal development (Cordeiro et al., 2013a; Wang et al., 1996). Thus in teratology studies using rats its inhibition, while little affecting the maternal heart where I_{to} is dominant, can cause embryonic bradycardia and arrhythmia with the resulting episodes of hypoxia/reperfusion and reactive oxygen species (ROS) generation leading to foetal death and malformations (Danielsson et al., 2007).

There are gender differences in potassium channel subunit expression. The mRNA levels of KCNQ1 and hERG1 from human blood samples of healthy females were significantly lower than those of males (Moric-Janiszewska et al., 2011). In human female non-diseased right ventricle there was a reduction in hERG1 transcript (by 30–40 %) and protein in both the epicardium and endocardium compared to male hearts (Gaborit et al., 2010). Unfortunately data on any cellular electrophysiological differences from healthy humans is lacking.

In response to cardiac disease states, e.g. heart failure (HF) and AF, adaptive changes in conduction, repolarisation and Ca^{2+} handling can occur as part of a bid to maintain cardiovascular function (Michael et al., 2009). For instance in HF, where the heart is unable to meet the metabolic demands of the body, the electrophysiological remodelling includes an increase in I_{NaL} and I_{NCX} but a decrease in $I_{to,fast}$ and I_{K1} due to downregulation of respectively $K_V4.3$ and $K_{ir}2.1$ subunits (Li et al., 2004; Michael et al., 2009). The result is a prolongation of the ventricular APD (Glukhov et al., 2010; Holzem et al., 2016; Li et al., 2004); the longer depolarisation during the cardiac cycle increasing the time for excitation-contraction coupling so mitigating the decrease in cardiac output. In the chronic setting such remodelling often gives rise to secondary cardiac dysfunction in particular arrhythmia, a primary cause of death in HF (Michael et al., 2009; Schmitt et al., 2014). In animal models of HF the I_{Kr} amplitude is also significantly, but not always (e.g. Li et al., 2002), reduced (Tsuji et al., 2000; H. Wang et al., 2012). Recently it was shown that though I_{Kr} block induces further APD prolongation in each transmural region of the failing human left ventricle, the extent was attenuated compared to healthy donor (~30 % vs ~70 %; Holzem et al., 2016). Underlying this apparent reduction of I_{Kr} in HF was a decrease in hERG1a protein, but not hERG1b which consequently altered the isoform stoichiometry.

ERG1 is also expressed widely outside of the heart. In humans (and rats or mice) its mRNA is expressed in e.g. the adrenal glands, jejunum and colon, kidney, pancreatic islets, and brain (Farrelly et al., 2003; Rosati et al., 2000; Sarzani et al., 2006; Wymore et al., 1997). The expression of hERG1a mRNA in human prefrontal cortex increases during foetal development, with the level from birth then sustained throughout life (Huffaker et al., 2009). At the protein level for instance contrasting ERG1 expression was shown in the human jejunum and colon, being present in the former in longitudinal and circular muscle as well as enteric neurones while in the latter being mainly in pacemaker (the interstitial cells of Cajal and PDGFRα^+) cells (Farrelly et al., 2003; Tomuschat et al., 2016). The expression and function of extra-cardiac I_{Kr} is detailed later (section 2.4).

2.2.2 STRUCTURE-FUNCTION RELATIONSHIP

BIOPHYSICAL PROPERTIES OF THE hERG1 CHANNEL

The hERG1 channel adopts one of three basic conformations (Fig. 4): closed (non-conducting), open (conducting) or inactivated (non-conducting). In a physiological K^+ gradient at negative voltages (e.g. −80 mV) hERG1 channels are in the closed state. Upon depolarisation of the membrane potential beyond the threshold of ~−50 mV hERG1 channels enter the open state (Fig. 5A; Sanguinetti et al., 1995; Zhou et al., 1998a). The transition involves the opening of an intracellular activation gate so allowing the outward diffusion of K^+ down the electrochemical gradient, with hERG1 being selectively permeable to K^+ over Na^+ by a factor of >120 (Sanguinetti et al., 1995; Torres et al., 2003; Zhou et al., 1998a).

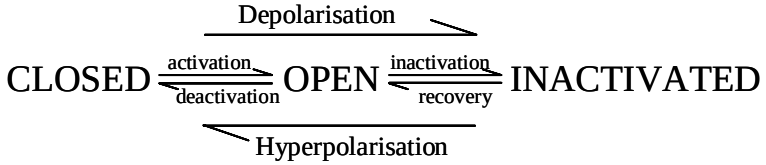

Figure 4. The hERG1 channel exists in three voltage-dependent states. From single hERG channel recordings the dwell time distributions suggest at least two open and closed states (Zou et al., 1997) or one open and three closed states (Kiehn et al., 1999). At the whole-cell level transition through at least three closed states is seen from the sigmoidal shape of the activation time course (Wang et al., 1997a; **II** Fig. 2B). Furthermore single channel data indicates a direct transition from a closed state to the inactivated state that is significant, at least, during strong depolarisation and may contribute to hERG rectification (Kiehn et al., 1999). Two non-K^+ conducting inactivated states are evident in non-physiological solutions with the initial state being Na^+ permeable (Gang and Zhang, 2006). These multiple states and their kinetics have been incorporated into various computational models to simulate or describe different aspects of hERG channel gating and its effects on AP repolarisation (see e.g. Bett et al., 2011 for a comparison of five such models).

At potentials of ≥0 mV inward rectification (i.e. for the same electrochemical potential the magnitude of the outward current is smaller than the inward current) occurs (Fig. 5A; Sanguinetti et al., 1995; Zhou et al., 1998a). This is also evident at the level of the single hERG1 channel, where the conductance of inward and outward currents is respectively 10-12 pS and 4-5 pS in a symmetrical K^+ gradient (Bian et al., 2001; Kiehn et al., 1996, 1999; Zou et al., 1997). Unlike I_{K1} channels where intracellular Mg^{2+} or polyamines block the outward current (Schmitt et al., 2014), the mechanism of rectification is caused by rapid voltage-dependent inactivation, which is ≈100 times faster than activation (Schönherr and Heinemann,

1996; Smith et al., 1996; Snyders and Chaudhary, 1996; Spector et al., 1996b). This rapid transition between the open and inactivated state involves the closure and opening of an extracellular gate and can be revealed by incorporation of a brief hyperpolarisation step, between depolarising pulses, which removes inactivation (Smith et al., 1996; Spector et al., 1996b). A large outward current is evident upon return to the depolarising voltage which then decays as the channels re-inactivate (Fig. 5B).

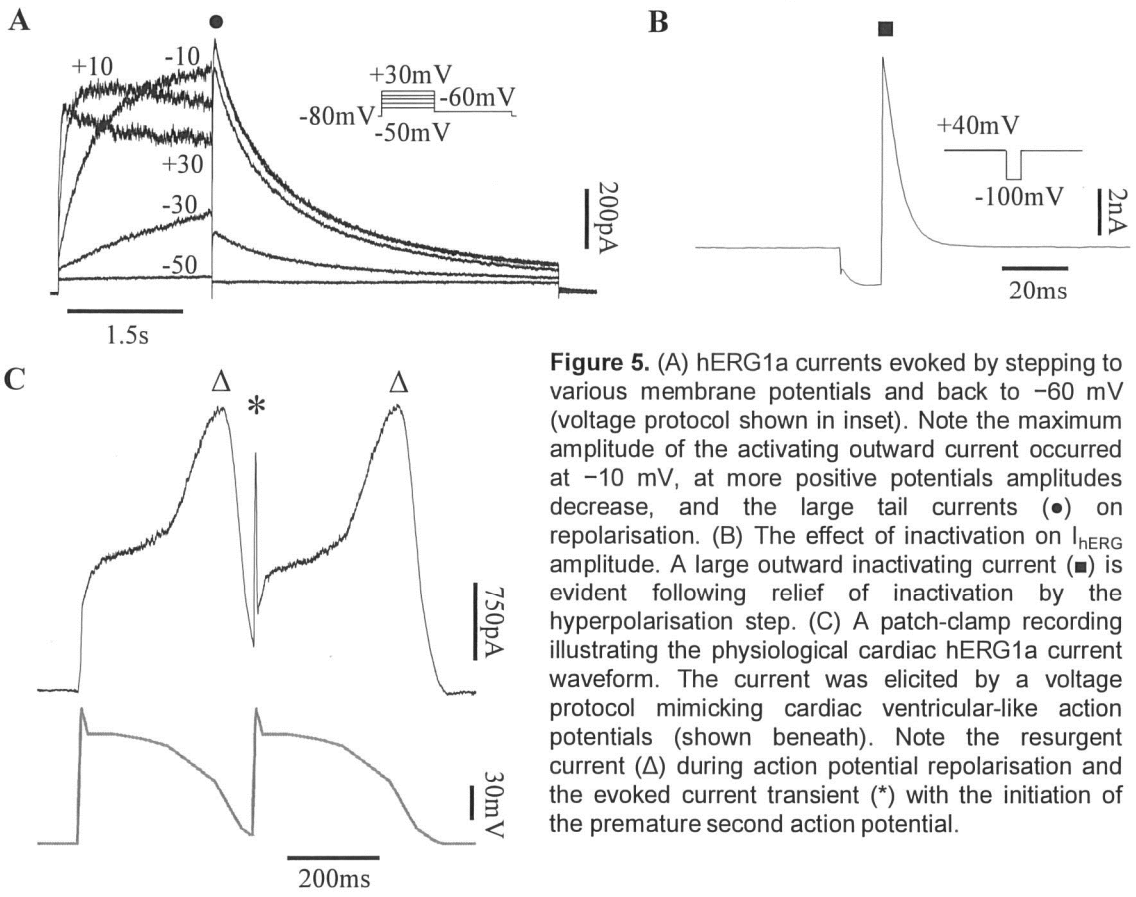

Figure 5. (A) hERG1a currents evoked by stepping to various membrane potentials and back to −60 mV (voltage protocol shown in inset). Note the maximum amplitude of the activating outward current occurred at −10 mV, at more positive potentials amplitudes decrease, and the large tail currents (●) on repolarisation. (B) The effect of inactivation on I_{hERG} amplitude. A large outward inactivating current (■) is evident following relief of inactivation by the hyperpolarisation step. (C) A patch-clamp recording illustrating the physiological cardiac hERG1a current waveform. The current was elicited by a voltage protocol mimicking cardiac ventricular-like action potentials (shown beneath). Note the resurgent current (Δ) during action potential repolarisation and the evoked current transient (*) with the initiation of the premature second action potential.

The activation and inactivation gating properties of hERG1 are unique compared to the other K_V channels. Activation gating is relatively slow (with time constants (τ) ranging from hundreds of milliseconds to seconds) while inactivation gating is very fast (τ of milliseconds to tens of milliseconds) and both are voltage sensitive (Snyders and Chaudhary, 1996; Spector et al., 1996b; Zhou et al., 1998a). The unusual kinetics of hERG1 underlie the I_{Kr} current profile during the ventricular action potential and are thus crucial to normal and aberrant cardiac function:

- Following I_{Kr} activation during the cardiac action potential upstroke and early part of the plateau (phase 2), inactivation results in channels rapidly accumulating in the inactivated state. This limits the outward current during depolarisation thus contributing to the low conductance and maintenance of the plateau phase of the action potential ensuring adequate time for Ca^{2+} entry as well as preventing premature excitation by rendering the cell refractory (Banyasz et al., 2014; Hancox et al., 1998; Jost et al., 2005; Spector et al., 1996b; Vandenberg et al., 2012). Inhibition of I_{Kr} has little effect on these phases of the action potential (Gintant, 2000; Jost et al., 2005; Li et al., 1996; Sanguinetti and Jurkiewicz, 1990).

- As phase 2 proceeds with gradual repolarisation hERG channels recover from the inactivated state increasing the outward K^+ current, despite the diminished electromotive force as E_m is closer to E_K, so sustaining further repolarisation as the channels only slowly deactivate. This resurgent current peaks during the transition to phase 3, at potentials negative to -30 mV, after which it rapidly declines as the membrane voltage returns to resting potential (Fig. 5C; Banyasz et al., 2014; Gintant, 2000; Hancox et al., 1998; Jost et al., 2005). Of note in terms of arrhythmia is that the voltage of peak hERG current occurs in the window current voltage range of the L-type calcium channel and thus opposes its reactivation. The current profile and peak potential depend on the action potential waveform, so that for an epicardial waveform hERG conductance is ~8 times greater than for atrial as full activation can occur during the more depolarised plateau (Lu et al., 2001). Such differences may be offset by a higher atrial I_{Kr} density (Gintant, 2000).
- Furthermore the slow deactivation ensures channels are available to counteract premature stimuli that can initiate arrhythmias and to potentially modulate conduction and the early phase of the AP (Larsen et al., 2010a; Lu et al., 2001; Verkerk et al., 2005). A premature stimulus evokes an instantaneous opposing large transient outward current through the open/slowly deactivating hERG channels, due to the increase in the electrochemical gradient for K^+ and the channels rapidly inactivating (Fig. 5C; Lu et al., 2001).
- In the post-repolarisation interval of SA and AV node cells I_{Kr} is a major determinant of the maximum diastolic potential, which since being positive to E_K results in residual deactivating outward current. The deactivation of I_{Kr} contributes to phase 4 depolarisation and pacemaking, thus its inhibition slows spontaneous SA node firing (Couette et al., 2006; Vandenberg et al., 2012; Zhou et al., 1998a).

STRUCTURAL BASIS OF hERG1 CHANNEL FUNCTION

The first atomic resolution K^+ channel structure, KcsA of *Streptomyces lividans* (Doyle et al., 1998), and subsequent X-ray crystallographic structures including the rat $K_V1.2$ and the so called $K_V1.2/K_V2.1$ paddle chimera (X. Chen et al., 2010; Long et al., 2005a, 2007) have revolutionized the understanding of the mechanisms and dynamics of conduction, selective K^+ permeability and channel gating. The use of these structures as templates for homology models, as no complete crystal structure for hERG1 is currently available, and molecular dynamics simulations (e.g. Dempsey et al., 2014; Stansfeld et al., 2008) as well as the electrophysiological investigation of numerous site-specific mutations have provided insights into the structural basis of hERG1 channel function. This will be furthered by the very recently reporting of a rat EAG1 channel structure (Whicher and MacKinnon, 2016).

Like other K_V subunits the hERG1 subunit has six α-helical transmembrane segments (S1-S6), the first four (S1-S4) of which constitute the voltage-sensing domain (VSD) while the last two together with the intervening pore loop (S5-P-S6) form the pore domain, with cytoplasmic amino and carboxy temini (Fig. 6). Functional K_V, including hERG1, channels are composed of four subunits, their pore domains line a central conduction pathway while their VSDs lie at the periphery spanning the membrane with an hourglass appearance (X. Chen et al., 2010; Long et al., 2005a; L. Wang et al., 2009; Whicher and MacKinnon, 2016).

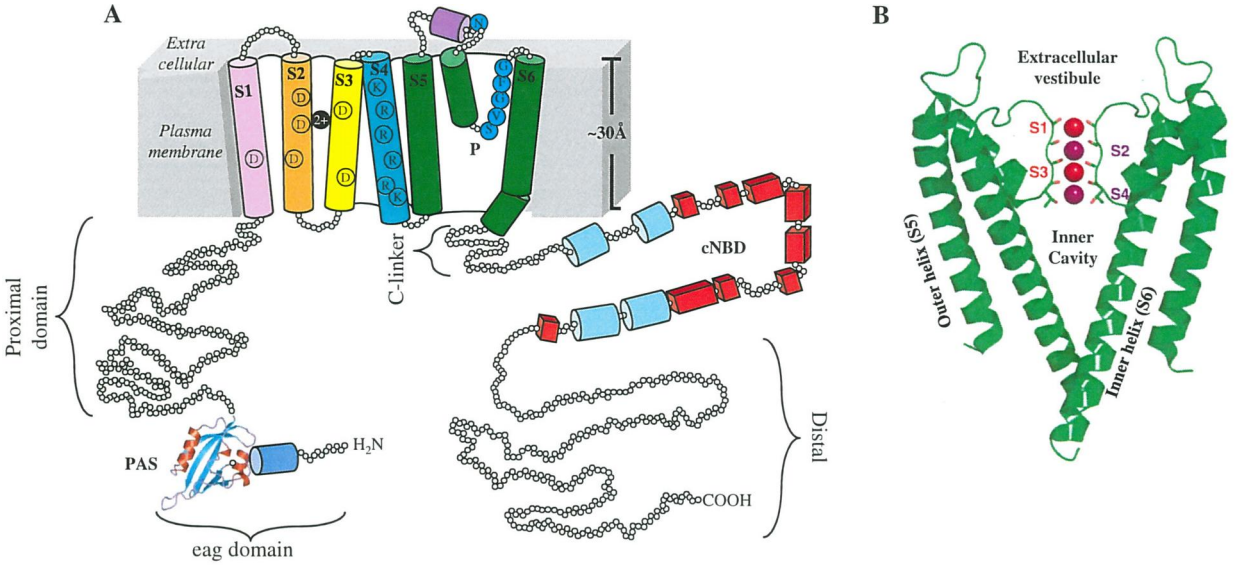

Figure 6. (A) Structural features of the hERG1 subunit (K_V11.1 splice variant 1a; the transmembrane segment residues are designated as in the sequence alignment of Whicher and MacKinnon, 2016). In the pore domain (S5-P-S6) the residues that form the selectivity filter of the pore (S624-G628) and the glycosylation site (N598) are shown. In the VSD (S1-S4) the basic Lys (K) and Arg (R) residues of S4 and the negatively charged acidic Asp (D) residues of S1-3 are indicated. During gating these negatively charged residues, as well as the phospholipid head groups and hydration waters in the vestibules (Islas, 2016), stabilise the positively charged S4 residues within the membrane's hydrophobic environment by forming transient salt bridges (Liu et al., 2003; Zhang et al., 2005). Possibly such interactions preferentially form in the closed states, slowing S4 outward movement. A common binding site for divalent cations and protons is shown (Fernandez et al., 2005; Shi et al., 2014). In the intracellular amino terminal the structure of the PAS domain is shown as deduced by X-ray crystallography (Morais-Cabral et al., 1998). The secondary structure of the hERG cNBD, with its 9 β strands and 4 α helices, is shown (Li et al., 2014). Beneath the pore domain of EAG1 the C-linkers form a ring under which the cNBDs are docked with the PAS domains at their periphery able to interact with the VSDs (Whicher and MacKinnon, 2016) (B) Structure of KcsA channel, with only two of the four subunits that form these channels shown, crystallized in the closed (Doyle et al., 1998; Figure by Boghog2, https://commons.wikimedia.org/w/index.php?curid=3471420). In the selectivity filter two potassium ions are shown occupying S2 and S4 (purple spheres) while two water molecules occupy the other sites (their oxygen atoms depicted as red spheres).

The pore domain and permeation

Potassium channels are both efficient and highly selective. The throughput is in part optimised by limiting the interaction between ions and the channel to the extracellular end of the pore, the selectivity filter (Fig. 6B; Doyle et al., 1998). Beneath the selectivity filter is a water-filled cavity in the middle of the channel protein that is lined by the S6 helices. Typically from the electrochemical gradient, hydrated K^+ ions enter from the cytoplasm and are held due to the nature of the lining, both via hydrogen bonding interactions and its hydrophobicity, at the centre of the cavity for passage through the selectivity filter to the extracellular entryway (Zhou et al., 2001).

The selectivity filter (of 12 Å in length) is formed by a highly conserved stretch of amino acids (Thr/Ser-X-Gly-Tyr/Phe-Gly) contained in each pore loop (Fig. 6). The selectivity filter is a highly electronegative environment with the backbone carbonyl oxygen atoms of the signature sequences together with the side chain hydroxyl oxygen atom of the threonines/serines directed towards the pore (Fig. 6B; Doyle et al., 1998; Long et al., 2005a, 2007; Morais-Cabral et al., 2001). This structure underlies selective ion conduction. The oxygen atoms of the filter act as surrogate waters coordinating the dehydrated K^+ ions creating

four K^+ binding sites in a row (S1-S4), occupied alternately by K^+ ions and water molecules (Fig. 6B; Doyle et al., 1998; Morais-Cabral et al., 2001; Zhou et al., 2001). The translocation of K^+ ions bound within the selectivity filter from S4,S2 to S3,S1 and finally S2,S0 (at the extracellular entryway) is driven by an incoming third ion from the cavity (Bernèche and Roux, 2001; Jensen et al., 2010; Morais-Cabral et al., 2001)[1]. Permeation through the hERG1 selectivity filter appears less energetically favourable and particularly by this knock-on mechanism, which could account for the low conductance (2 pS in a physiological K^+ gradient; Kiehn et al., 1996) of the channel (Ceccarini et al., 2012). Also likely contributing is the few negative charges at the intracellular vestibule which is formed by the cyclic nucleotide binding homology domain (cNBD, Fig. 6) of the C-terminus (Whicher and MacKinnon, 2016).

From the KcsA crystal structure it was posited that the carbonyl groups, while perfectly positioned for K^+ ions, are unable to move to coordinate the Na^+ ion so compensation for the loss of hydration is less favourable for the Na^+ ion than for the K^+ ion (Doyle et al., 1998). However this snug-fit view does not take into account the inherent structural flexibility of the channel (Noskov and Roux, 2006). Alternative determinants, such as the magnitude of the repulsive interactions of the partial negative charge of the binding site carbonyl oxygen atoms being lessened by the larger cation (Noskov and Roux, 2006), are proposed but the primary mechanism of the inherent preference for K^+ is unresolved (Lockless, 2015). However as this binding site equilibrium selectivity can also be exhibited by non-selective channels it is insufficient to explain the selectivity of K^+ ion permeation (Lockless, 2015). It seems, as with permeation, that multiple K^+ ions simultaneously bound in the selectivity filter is critical with the queue of ion-binding sites functioning as a unit. The mechanism underlying how a multi-ion queue determines selective ion conduction in K^+ channels is unclear.

Activation & deactivation

In K^+ channels the activation gate is formed by the criss-crossing of the four S6 helices near the cytoplasmic interface constricting the ion conduction pathway (Fig. 6B; Doyle et al., 1998). For hERG1 the gate is proposed to be the side chain of Gln664, located at the intracellular extreme of S6 more than one helical turn distal to that of the archetypal K_V channel Shaker (Thouta et al., 2014; Wynia-Smith et al., 2008). In the open state the S6 helices display a bend which splays the carboxy terminal ends outwards from the central axis of the pore thus removing the physical barrier to ion passage between the intracellular solution and the selectivity filter (Jiang et al., 2002; Long et al., 2005a). Comparison of the presumed closed and open crystallographic structures gives a picture of the nature of activation-deactivation gating as a swinging motion of the S6 helices about a hinge, which for the bacterial channels is proposed to be a highly conserved glycine and for K_V1-K_V4 channels a Pro-X-Pro motif (Jiang et al., 2002; Long et al., 2005a; Yellen, 2002). The Pro-X-Pro motif is absent from hERG1, while substitution of Gly648 and/or Gly657, which is in the analogous position to the motif's second Pro, has relatively minor effects or shifts the gating equilibrium to the open state (Hardman et al., 2007; Mitcheson et al., 2000a). In contrast to Shaker, the hERG1 pore is more stable in the open than closed conformation with the inherent flexibility of the S6 helices sufficient for activation gating (Hardman et al., 2007; Whicher and MacKinnon, 2016).

Activation of hERG1 involves transitions between several closed states prior to the final step where a concerted interaction of all four S6 helices opens the activation gate (Wang et al.,

[1] This commonly accepted view of permeation, intervening water molecules and therefore weakened Coulomb repulsion between the cations, has been challenged (Köpfer et al., 2014).

1997a). These early transitions, as in other K_V channels, are thought to correspond to the independent serial movements of each subunit's S4 helix leading to its outward displacement while the other VSD segments remain largely static (Jensen et al., 2012; Tao et al., 2010; Wang et al., 2013). This S4 movement must be completed by all four subunits for the pore to open. In hERG1 the S4 residues L523 to L529 move from the membrane into the extracellular solution during depolarisation (Elliott et al., 2009). The S4 of K_V channels is the primary sensor of the transmembrane electric field, with its positively charged Arg or Lys residues at every third position (Fig. 6; Sanguinetti and Tristani-Firouzi, 2006). The change in polarity of the transmembrane voltage with depolarisation exerts an electrostatic force on those positive charges that are within the focused electric field at the waist of the VSD's hourglass structure resulting in a change in their position (Islas, 2016; Long et al., 2005b; M. Zhang et al., 2004). This region of the VSD separates aqueous inner and outer vestibules and contains an aromatic residue from S2 (Phe463 in hERG1) which together with two negatively charged residues form the charge transfer centre that facilitates the sequential movement the S4 gating charges (Cheng et al., 2013; Islas, 2016; Tao et al., 2010). For the K_V S4 this equates to a ~15Å movement across the membrane, though for EAG family members the magnitude may be less (Jensen et al., 2012; Tao et al., 2010; Whicher and MacKinnon, 2016). In hERG1 F463 and D466 interact with the outer S4 charge (K525) in the resting configuration of the voltage sensor i.e. the closed state (Cheng et al., 2013). The first three S4 positive charges (K525, R528 and R531) were initially identified as the gating charges, with a wider scanning mutagenesis study adding further residues such that a spiral thread around the S4 was formed (Piper et al., 2005; M. Zhang et al., 2004) which equates with the notion of its movement being a helical twist or rotation (Pathak et al., 2007).

From where does the unusually slow activation gating of hERG1 originate? Slow S4 movement was the general view (Piper et al., 2003; Smith and Yellen, 2002), however more recent gating current, S4 accessibility, and voltage-clamp fluorometry measurements suggest that ionic current activation is preceded by S4 translocation, which occurs at a more hyperpolarised potential and is ~4-fold faster at 0mV than channel opening (Es-Salah-Lamoureux et al., 2010; Goodchild et al., 2015; Wang et al., 2013). Events subsequent to S4 movement are then suggested to harbour the rate limiting step.

In K_V channels the intrasubunit transmission of S4 motions to the activation gate of S6 (electromechanical coupling) occurs via the S4-S5 linker. The intracellular S4-S5 linker forms an amphipathic α-helix that runs parallel to the membrane and acts as a rigid lever on the carboxy portion of the S6 helix which it passes over (Long et al., 2005b). However, this is not the case with hERG1 since physical continuity between the voltage sensing and pore domains is not completely necessary for voltage-dependent gating as shown by the functional coupling of co-expressed independent hERG1 modules split at Y545 of the S4-S5 linker (Lörinczi et al., 2015). Moreover the S4-S5 linker of the EAG family members is much shorter and forms a loop resulting in a fundamentally different voltage dependent gating mechanism to K_V channels (Whicher and MacKinnon, 2016). It is proposed that in its resting state S4 would interact directly with the C-linker to induce a bend in S6 after Gln664 closing the channel, while the outward movement of S4 would enable the rotation of the C-linker and S6 relieving the high-energy bend and opening the channel. This mechanism consequently allows the cytoplasmic domains of EAG family members to modulate both the activation gate and VSDs.

For hERG1 the slow rate dependent on the interaction between cytoplasmic domains. Deletion of the N-terminus (Δ2-354/373) substantially accelerates deactivation (τ decreased ~10-fold), an effect that can be attenuated or mimicked by, respectively, intracellular application of an exogenous peptide corresponding to the channel's first 16 amino acid residues or deletion of residues 2-12 (Schönherr and Heinemann, 1996; Spector et al., 1996b; Wang et

al., 1998, 2000). These initial residues act as a dynamic flexible tail and together with the subsequent amphipathic α-helix (residues 13-23) comprise the cap region of the N-terminus eag domain (Li et al., 2010; Muskett et al., 2011; Ng et al., 2011). The remainder of this domain is a Per-Arnt-Sim (PAS) domain, a helix-loop-helix motif, formed by residues 26-135 (Fig. 6; Morais-Cabral et al., 1998). The N-terminus eag domain interacts with the cNBD of the adjacent subunit (Gianulis et al., 2013; Gustina and Trudeau, 2011; Li et al., 2014; Whicher and MacKinnon, 2016). The deletion of the cNBD has an effect analogous to that of N-terminus deletion and negates the normalising effect to the latter construct of recombinant eag domain expression (Gianulis et al., 2013; Gustina and Trudeau, 2011). Stable interactions form between the PAS and cNBD which position and allow the tail of the N-terminus cap to dynamically interact with the C-linker, via charge-charge interactions of its arginines 4 and 5, and/or the S4-S5 linker (de la Peña et al., 2015; Ng et al., 2014). This more transient interaction would then modulate the closure of the activation gate and result in the stabilisation of the open state (as evident from the single-channel open times) and allosterically of the activated S4 limiting its rate of return (Goodchild et al., 2015; Ng et al., 2014; Wang et al., 2000). Slow deactivation seems to be a concerted process requiring all four hERG1a subunits (Thomson et al., 2014).

Inactivation

hERG1 is devoid of N-type inactivation, with its inactivation resembling C-type (Schönherr and Heinemann, 1996; Smith et al., 1996; Spector et al., 1996b). C-type inactivation results from structural changes in the selectivity filter, which fluctuates between conductive and non-conductive conformations[2] (Cordero-Morales et al., 2006, 2007; Cuello et al., 2010; Zhou et al., 2001). Determining the stability of the selectivity filter's conductive conformation and its transitions to a non-conductive state as well as collapse into a more stable inactivated state are hydrogen bond networks (Cordero-Morales et al., 2006, 2007). Though none of the residues taking part in these networks within KcsA are conserved in hERG1 (Cordero-Morales et al., 2007; Doyle et al., 1998; Vandenberg et al., 2012), molecular dynamic simulations do show an intra-subunit network involving a water molecule behind the selectivity filter, F627 and G628 of the selectivity filter, N629 and from the pore helix S620 (Stansfeld et al., 2008). This hydrogen bond network and/or inter-subunit hydrogen bonds between N629 and G628 stabilise the backbone of the selectivity filter, however these are unstable (Köpfer et al., 2012; Stansfeld et al., 2008). Their breakdown and S620-N629 interaction, within all four subunits (Wu et al., 2014), is suggested to allow the flipping of backbone carbonyls of the selectivity filter away from the conduction path thus disrupting the K^+ coordination sites resulting in a collapsed conformation (Köpfer et al., 2012; Stansfeld et al., 2008).

Often omitted from these models is the S5-P linker, this plays a crucial role in hERG1 inactivation (Liu et al., 2002). The S5-P linker is much longer in the EAG family than other K_V channels (~40 amino acids in hERG1 compared with typically 12-15; Torres et al., 2003). Some of the residues here, as well as in the pore helix and outer mouth e.g. S620 and S631, are though not conserved in the related reduced or non-inactivating EAG1 and ELK2 (Clarke et al., 2006; Köpfer et al., 2012; McPate et al., 2008; Perrin et al., 2008). A segment of S5-P linker forms an amphipathic α-helix that is hypothesised to be involved in inactivation voltage sensing, the N588/Q592 face of the helix interacting with a charged residue in the outer pore (Clarke et al., 2006; Torres et al., 2003). Inactivation voltage sensing appears distinct from that

[2] The correspondence of these non-conductive conformations of the K^+ channel selectivity filter to the inactivated state structure is disputed (Devaraneni et al., 2013).

of activation as they are differentially affected by mutation (e.g. McPate et al., 2008) and external cations (e.g. Fernandez et al., 2005). Moreover S4 movement does not report inactivation and disruption of inactivation has little impact according to gating currents or fluorescence signals (Es-Salah-Lamoureux et al., 2010; Piper et al., 2003). However inactivation was suggested, by alanine substitution induced perturbations in ionic and gating currents, to localise to a helical face of S4 which is distinct from areas involved in activation gating (Piper et al., 2005).

Prior to the collapse of the selectivity filter and stabilisation of the final inactivated state there are widespread rearrangements of the hERG1 channel. The trigger is the loss of K^+ from the selectivity filter which leads to conformational rearrangements in the pore helix, then S5, S5P linker, S4, S4-S5 linker, S6 and again in the pore helix (Perry et al., 2013a, b; Wang et al., 2011). The pore helix acts as the selectivity filter interface, initially through coupling (e.g. T618) with S5 residues of the same subunit and later, via intra- and inter-subunit interactions with S6, when to signal the final step (Perry et al., 2013b). The intervening motions in the sequence are coordinated by other domain-domain interactions e.g. between hydrophobic S4 and S5 residues of neighbouring subunits (Perry et al., 2013a).

2.2.3 ERGs AND ERG1 VARIANTS

To customise the properties of the hERG channel to the requirements and various roles in the multitude of hERG1 expressing cell types, the channel is both spatially and temporally heterogeneous. Thus the cardiac I_{Kr}, though this is itself heterogeneous, has e.g. much faster activation and deactivation kinetics than the hERG1 current (Sanguinetti et al., 1995; Weerapura et al., 2002). Native I_{Kr}/hERG channels are dynamic heteromeric macromolecular complexes. The diversity originates from various mechanisms (compositional and regulatory):

- Heteromultimeric assembly of α-subunits, which are encoded by related genes and/or splice variants of h*ERG1*
- Association of β-subunits and interaction with other proteins
- Post-translational modifications

The EAG family

The EAG family (*KCNH1-8*) consists of three subfamilies: EAG, ERG, and ELK, each with multiple members in mammals (Ganetzky et al., 1999; Gutman et al., 2005). Although the members encode voltage-activated potassium channel subunits in which structural elements (GFG selectivity filter, eag and cNBD domains) are conserved, heteromultimers can only be formed within a subfamily due to recognition domains in the N- and C-termini (Lin et al., 2014; Schönherr et al., 2002; Wimmers et al., 2001; Zou et al., 2003).

Originally ERG2 (K_V11.2, *KCNH6*) and ERG3 (K_V11.3, *KCNH7*) were thought to be found exclusively in the nervous system (Shi et al., 1997). Moreover h*ERG3* mRNA expression appeared confined to the human brain (Kang et al., 2001a). However more recently it was shown that transcripts of all three ERG members were expressed by mouse and human pancreatic islets (Hardy et al., 2009) and that ERG3 was functionally expressed by the GI tract interstitial cells of Cajal influencing their spontaneous pacemaker activity which drives the slow waves in membrane potential of the smooth muscle determining phasic contraction (White et al., 2008). There is also transient expression of both *ERG2* and *ERG3* mRNA in the developing

mouse heart (Polvani et al., 2003). In the adult mouse CNS ERG1 and 3 proteins are widely coexpressed although in some areas (e.g. superior colliculus and substantia nigra) ERG3 protein is more expressed while in other areas (e.g. hippocampal dentate gyrus) ERG1 is more expressed (Guasti et al., 2005). ERG2 protein expression is low and restricted.

As with *hERG1* the other ERG channels display at positive potentials inward rectification of the steady-state current and elicit upon repolarisation large tail currents (Einarsen et al., 2009; Kang et al., 2001a; Shi et al., 1997; Wimmers et al., 2002). Consistent with the conservation of residues in the P and S6 domains ERG2 and ERG3 are also sensitive to the small molecule blockers of ERG1 (Elmedyb et al., 2007; Kang et al., 2001a; Shi et al., 1997). Peptide toxins, e.g. APETx1 (from the sea anemone *Anthopleura elegantissima*) and various ones from *Grammostola rosea* tarantula, and the agonist RPR260243 exhibit some selectivity between ERG subtypes (Kang et al., 2005; Redaelli et al., 2010; Restano-Cassulini et al., 2006).

The formation of heteromeric K_V11 channels has not been conclusively shown *in vivo* yet, though individual rat lactotrophs express either *ERG* mRNA transcripts alone or in various combinations (Schäfer et al., 1999). In brain regions where co-expression occurs there is evidence for the formation of heteromeric channels (ERG1/ERG3) as the functional properties appear distinct from those of the homomers (Hardman and Forsythe, 2009; Hirdes et al., 2005). Using a concatemer approach to define the subunit composition the biophysical properties of heteromeric rat ERG channels were investigated (Wimmers et al., 2002). Whereas the activation rate was dominated by the quicker activating subunit, the rates of recovery from inactivation and deactivation were dominated by the $K_V11.1$ subunit.

ERG1 variants

The human *KCNH2* gene spans approximately 33 kb of the long arm (q) of chromosome 7 at position 36.1 (Farrelly et al., 2003; Splawski et al., 1998; Vandenberg et al., 2012). A number of variants are generated from it:

- hERG1a, the full-length transcript from 15 exons encodes a protein of 1159 amino acid residues.

- hERG1b, an independent transcript with its own transcription start sites and promoter within intron 5 (Luo et al., 2008). This isoform starts from an alternative exon (1b) prior to exon 6 so it has a 56 amino acid long N-terminus, of which the initial 36 residues are unique and the others identical to hERG1a from residue 377 onwards, and a total length of 819 residues (Phartiyal et al., 2007; Sale et al., 2008; Splawski et al., 1998).

- hERG1-3.1, a primate specific N-terminal variant of hERG1a with a transcription start site in intron 2 (Huffaker et al., 2009; Vandenberg et al., 2012). The first 102 residues, which are encoded by the missing first two exons, are replaced with 6 unique amino acids.

- hERG-USO is a C-terminal variant, generated by polyadenylation of intron 9 instead of splicing, that results in the last 359 amino acids of hERG1a/b (i.e. from within the cNBD) being substituted by 88 residues encoded by the USO exon (Gong et al., 2010; Guasti et al., 2008; Kupershmidt et al., 1998).

The ERG1b transcript and protein is expressed within the cardiac tissue of various mammals including humans (Jones et al., 2004; London et al., 1997; X. Wang et al., 2008). In

human right atrium and left ventricle hERG1b mRNA constitutes 19 % and 12 % respectively of the total hERG1a/1b mRNA (Larsen et al., 2008). However there is a shift in the relative expression of the transcripts with human developmental age, in foetal heart hERG1b is 2-fold more abundant than hERG1a (Crotti et al., 2013). Whereas hERG1a transcript expression was far greater in the heart than other human tissues, for the hERG1b transcript its expression in the pancreas and colon was over double the level in the heart (Luo et al., 2008). In mouse hepatic portal vein the expression of ERG1b mRNA is nearly 4-fold greater than ERG1a mRNA (Ohya et al., 2002a). Both of these ERG1 transcripts are expressed in adult mouse brain with the expression level of the ERG1b transcript, in contrast to the ERG1a transcript, being greater in the brain than the heart (Guasti et al., 2005). Furthermore both proteins are highly expressed in the mouse brain with similar expression patterns and intensities. *In vivo* coassembly of these variants was found in human neuroblastoma cells (Crociani et al., 2003), in mouse brain and heart (Guasti et al., 2005) and in canine and human ventricular myocytes (Jones et al., 2004). Thus the composition of endogenous ERG channels includes both ERG1a and ERG1b subunits.

The expression of mouse and human orthologs of ERG1b results most noticeably in currents with accelerated deactivation kinetics (Larsen et al., 2008; Lees-Miller et al., 1997; London et al., 1997; Trudeau et al, 2011). Homomeric ERG1b channels though are poorly expressed (London et al., 1997; Sale et al., 2008), due to retention within the endoplasmic reticulum (ER; Phartiyal et al., 2008). Coexpression with ERG1a rescues the ERG1b subunits and produces tail currents with novel intermediate deactivation kinetics even with RNA ratios equivalent to those found in the human heart (Larsen et al., 2008; London et al., 1997). hERG1a/1b currents exhibit less rectification, due to a faster activation rate but an unaltered inactivation rate, and approximately three-fold faster deactivation kinetics than hERG1a currents, properties emanating from the presence of fewer eag domains (Larsen et al., 2008; Sale et al., 2008; Trudeau et al, 2011). Thus during a ventricular action potential hERG1a/1b currents are larger and peak earlier (i.e. at a more depolarised potential) resulting in 80 % more charge transferred (Sale et al., 2008). The current of these heteromeric channels bears closer resemblance to I_{Kr} and indeed hERG1b was shown to functionally contribute to human cardiomyocyte I_{Kr} (Jones et al., 2014). The disruption of hERG1b, as expected from the above, reduced the magnitude of native I_{Kr} and slowed its deactivation consequently prolonging APD and triggering EADs. Differences in the relative abundance of ERG1b in cardiomyocytes may underlie the variability in I_{Kr}, present at least in the canine heart (Gintant, 2000; Szabó et al., 2005), and contribute to APD dispersion (Larsen and Olesen, 2010). Finally the importance of hERG1b is underlined by the identification of hERG1b specific mutations (A8V and R25W) in a LQTS patient and an intrauterine foetal death which *in vitro* result in loss-of-function (Crotti et al., 2013; Jones et al., 2016; Sale et al., 2008).

hERG1-3.1 mRNA is found in various brain regions at comparable levels to hERG1a but within the heart there is a difference of over three orders of magnitude (Huffaker et al., 2009). Of note is that hERG1-3.1 mRNA is most abundant prenatally, at least in the human prefrontal cortex. Homomeric hERG1-3.1 channels also poorly express and exhibit impaired trafficking (Calcaterra et al., 2016). The most obvious functional difference between hERG1-3.1 and hERG1a is in deactivation which is substantially faster for hERG1-3.1 and becomes intermediate with co-expression (Heide et al., 2012; Huffaker et al., 2009).

hERG-USO fails to form functional channels when expressed by itself in mammalian systems but when coexpressed with either hERG1a or hERG1b there is coassembly and a reduction in the current density (Guasti et al., 2008; Kupershmidt et al., 1998). The hERG-USO containing heteromeric channels are retained within the ER and undergo ubiquitin-dependent degradation (Guasti et al., 2008). However more recently minimal association of hERG-USO with hERG1a was reported and that hERG-USO had little effect on hERG1a trafficking or

function (Stump et al., 2012a). Anyway the nonfunctional hERG-USO variant at least regulates the post-transcriptional expression of hERG1a/b through competition between alternate processing of a single pre-mRNA precursor (Gong et al., 2010).

hERG-USO mRNA is found in human left ventricle, atrium and midmyocardium as well as uterus, lung, lymphocytes, jejunum, colon, pancreas and brain (Farrelly et al., 2003; Gong et al., 2010; Kupershmidt et al., 1998). Moreover expression varies in different tissues, e.g. about two-thirds of hERG1 pre-mRNA is processed to hERG-USO in the heart compared to equal amounts in the brain, suggesting regulation of alternative processing possibly through tissue-specific variability in the relative strengths of the splice site and poly (A) signal (Gong et al., 2010). The importance of the relative expression of these variants is highlighted by a LQT2 mutation IVS9-2delA which disrupts the splicing of intron 9 thus resulting solely in polyadenylation and so a switch from hERG1a to hERG1a-USO (Gong et al., 2014a).

2.2.4 BIOGENESIS AND TRAFFICKING

The start site for h*ERG1a* transcription is upstream of the ATG translation start codon by 79 base pairs (bp), and the immediate upstream 5´-untranslated region (UTR) to 487 bp forms the core promoter (Luo et al., 2008). As there is differential spatial and temporal expression of the hERG transcript variants, transcription is clearly regulated though until recently this had received little attention. The expression of *hERG1* is regulated in the embryonic development of, at least, mouse by the homeobox transcription factor Nkx2-5, which is essential for cardiac specification during development and homeostasis thereafter (Furtado et al., 2016). Both mERG1a and particularly mERG1b mRNA was down-regulated in Nkx2-5 knockout mice embryo hearts, with Nkx2-5 and its partners (Gata4 and Tbx5) shown to bind to the m*ERG1*b promoter region and transactivate transcription. The h*ERG1* promoter was also shown to be transactivated by transcription factors BMAL1 and CLOCK which form the positive limb of the loop that coordinates circadian expression (Schroder et al., 2015). Furthermore, due to the cardiomyocyte molecular clock, *hERG1* transcripts were shown to exhibit a circadian pattern of expression in the rodent heart (the peak at the light to dark transition and the trough at the reverse transition 12 hrs later).

Various sequence and structure-specific elements, i.e. noncoding information, within the hERG1 mRNA affect translation and the protein trafficking efficiency (Sroubek et al., 2013). The hERG pre-mRNA undergoes alternative polyadenylation, with polyadenylation at a poly(A) signal in exon 15 or within intron 9 generating the hERG1a and hERG-USO variants respectively (Gong et al., 2010). Moreover LQT2 mutations have highlighted the importance of pre-mRNA splicing to mRNA as well as the stability of those mRNA templates (Fig. 7). The spliceosome deposits onto the mRNA 20-24 nucleotides upstream of each splice junction an exon junction protein complex, which plays a key role in mRNA quality control. During the pioneer round of translation these complexes are displaced by the ribosome. However for LQT2 mutations which result in premature termination codons that occur >54-60 nucleotides upstream of the most 3´ splice junction, i.e. the junction of exons 14-15 in hERG1a, the downstream exon junction complex serves as a marker and platform for the recruitment of factors that trigger the nonsense mediated decay (NMD) of the mRNA transcript (Gong et al., 2014b).

MicroRNAs (miRNAs), endogenous non-coding RNAs of ~22 nucleotides, can through Watson-Crick base pairing to the 3´-UTR of mRNA inhibit the expression of proteins. Of the >50 miRNAs predicted to target hERG (Wang et al., 2015), miRNA-133 which is specifically

expressed in adult cardiac and skeletal muscle as well as miRNA-34a, miRNA-96 and miRNA-362-3p were shown to reduce the expression of ERG protein and therefore I_{Kr} (Feng et al., 2014; Shan et al., 2013; Shao et al., 2013; Wang et al., 2015). Additionally miRNA-365-3p also targets and suppresses ERG1, in the spinal cord this interaction contributes to mouse nociception (Pan et al., 2016).

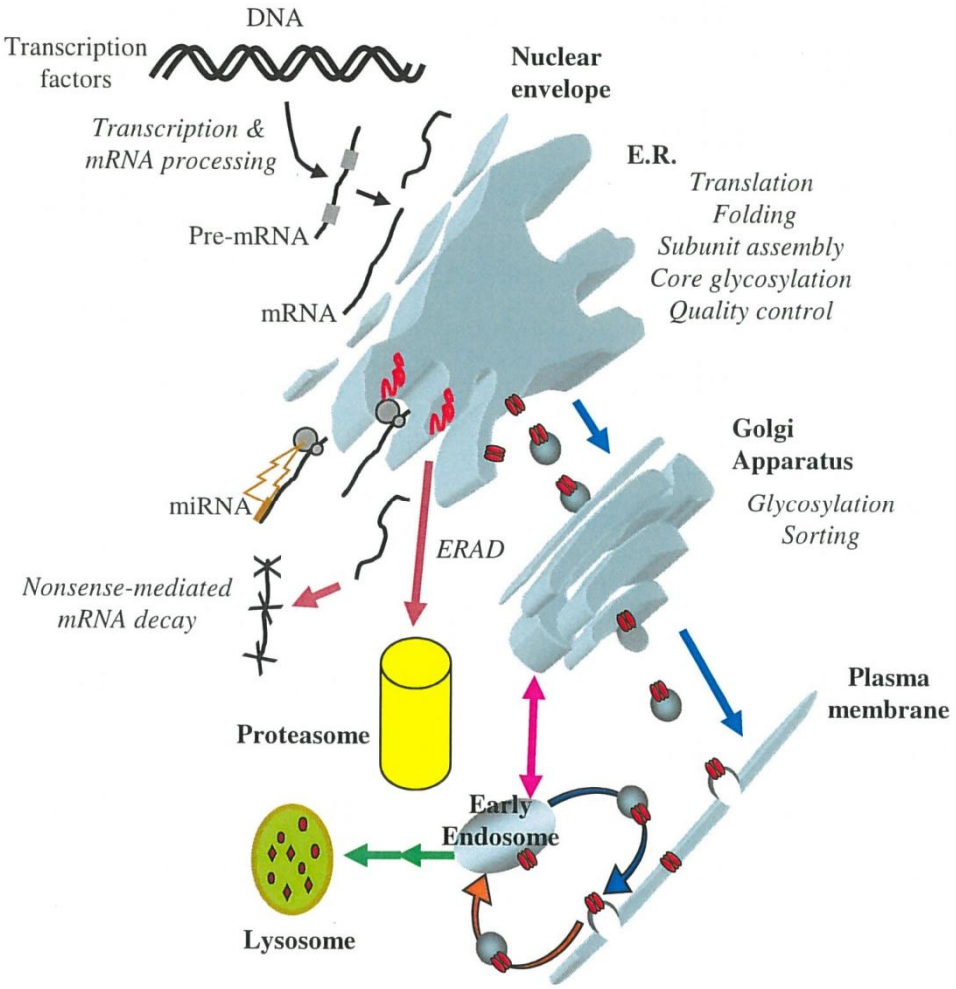

Figure 7. The biogenesis and trafficking of hERG (adapted from Vandenberg et al., 2012). Anterograde (biosynthetic delivery and recycling of endocytic channels; blue arrows) and retrograde (endocytosis; orange arrow) trafficking determine the density of hERG channels at the plasma membrane. About 60 % of the core-glycosylated hERG1 in the ER is processed to the mature form (Gong et al., 2005).

After the hERG mRNA is processed in the nucleus it reaches the cytosol whereupon translation is initiated by ribosomes (Vandenberg et al., 2012). In the first few codons is a signal which when recognised results in halting of translation and targeting of the RNA/ribosome/polypeptide complex to the ER. Here translation is resumed with the nascent hERG1 polypeptide translocated across the ER membrane via the translocon and the N-termini emerging into the luminal space of the ER where they interact (Phartiyal et al., 2007). In addition to the cotranslational interaction of the nascent polypeptides, the hERG1a and 1b transcripts are associated facilitating the synthesis and assembly of the heteromeric channels

within the ER (Liu et al., 2016). The interaction with hERG1a subunit masks the Arg-X-Arg (RXR) ER retention motif in the hERG1b N-terminus enabling ER export and surface expression of the subunit (Phartiyal et al., 2008). As the nascent oligopeptide exits the ribosome there is a risk for aggregation, especially for hydrophobic residues exposed to the aqueous environment, until the proper secondary structure is assumed (Chen et al., 2009). The binding of chaperones such as the cytosolic heat shock proteins (Hsp70 and Hsp90α) and their co-chaperones (e.g. FKBP38 in the ER membrane) to at risk sites in hERG1 prevents this misfolding (Ficker et al., 2003; Li et al., 2011; Peterson et al., 2012; Walker et al., 2007). The detection of local misfolding is probably aided by the presence of multiple RXR motifs in hERG1, such as at residues 1005-1007 (Kupershmidt et al., 2002), which would be hidden in the correctly folded protein (Vandenberg et al., 2012). Quality control is also likely similarly ensured by the numerous diacidic motifs, which are a classic ER export signal.

Folding and tetrameric assembly appears to be a difficult process with the majority of hERG1 protein as a consequence residing in the ER (Sroubek et al., 2013). Furthermore perturbation of this process underlies the deleterious nature of most LQT2 mutations (C. Anderson et al., 2006, 2014; Gong et al., 2006; Jones et al., 2016). With misfolding the association of hERG protein with Hsp70 and Hsp90 is increased and the chaperones remain bound in a sustained effort to direct the folding intermediates into the native conformation, thus facilitating the maturation of mutant as well as wild type hERG protein (Ficker et al., 2003; Iwai et al., 2013; Li et al., 2011). Moreover they inhibit the binding to hERG of heat shock cognate 70 (Hsc70), which has the opposite action to Hsp70, and the ubiquitin ligase CHIP, which ubiquitinates hERG i.e. the covalent binding of one or a chain of ubiquitin proteins (Iwai et al., 2013; Li et al., 2011). The outcome of such competitive interactions in the chaperone network determines the fate of the hERG protein with the hERG-DJA-Hsc70-CHIP complex leading to polyubiquitination of hERG and therefore active removal from the ER to the cytosol for degradation by the proteasome (Ficker et al., 2003; Gong et al., 2005; Walker et al., 2010). In addition to this ER-associated degradation (ERAD), trafficking-deficient hERG mutants can activate ER stress pathways, which have a general negative impact on cellular function, such as the unfolded protein response that leads to increased synthesis of ER chaperones (Keller et al., 2005; Mehta et al., 2014; Y. Wang et al., 2012).

hERG1 contains two consensus extracellular *N*-glycosylation sites (Petrecca et al., 1999), but only Asn598 is glycosylated within the ER lumen (Gong et al., 2002). The core glycosylated hERG protein is then exported in COPII vesicles along microtubules, unconventionally via a Rab11B GTPase dependent pathway, to the Golgi apparatus with the interaction between hERG1 and the Golgi protein GM130 tethering the vesicles to the *cis* face (Fig. 7; Delisle et al., 2009; Roti Roti et al., 2002; Smith et al., 2013). In the Golgi apparatus hERG1 undergoes further glycosylation to render the fully mature glycosylated form. The glycosylation process is readily traceable in Western blots of hERG expressing cell lines as two bands, weighing ~135 and ~155 kDa in the case of hERG1a or ~85 and ~95 kDa in the case of hERG1b, representing the immature, core-glycosylated and the maturely glycosylated cell surface expressed species (Crociani et al., 2003; Jones et al., 2004; Zhou et al., 1998a, b). While glycosylation is not obligatory for trafficking to the cell surface, it is required for the interaction with the ER chaperone calnexin and plays a role in the stability of hERG1 protein at the cell surface i.e. nonglycosylated hERG has a faster turnover rate (Chen et al., 2015; Gong et al., 2002, 2006).

The mature hERG channels are exported from the Golgi apparatus to the plasma membrane, where in heterologous systems they reside with a half-life of ~11 hrs (Ficker et al., 2003; Ke et al., 2013). In both native and heterologous systems hERG1 channels are localised in cholesterol and sphingolipid enriched membrane microdomains (Balijepalli et al., 2007), specifically

caveolae. This is a form of lipid raft with a flask-shaped morphology attained by the insertation of caveolins (Cav) into the cytoplasmic leaflet of the bilayer. Cav3 in muscle and Cav1 in other cell types physically interacts with and negatively regulates hERG1 (Guo et al., 2012; Lin et al., 2008; Massaeli et al., 2010a; but see also Balijepalli et al., 2007). Underlying the inhibitory effect of Cav3 on I_{hERG} is the recruitment from the cytosol to the membrane by Cav3 of the ubiquitin-protein ligase Nedd4-2 (Guo et al., 2012). Nedd4-2 binds to a PY motif in the C-terminus of hERG1 mediating its ubiquitination, tagging the target protein for endocytosis and degradation (Albesa et al., 2011; Guo et al., 2012). The interaction of hERG1 with Nedd4-2 mediates the homeostatic regulation of hERG1 channel expression at the cell membrane and therefore I_{hERG}/I_{Kr} density (Kang et al., 2015).

The internalisation of hERG1 is clathrin-independent, though the exact mechanism may depend on the circumstance. The rapid constitutive endocytosis of hERG1 was found to involve the small GTPase Arf6 and not dynamin (Karnik et al., 2013). This excludes a dynamin-dependent Cav mechanism, which was earlier shown to underlie hERG1 internalisation in response hypokalaemia and the cholesterol lowering agent probucol (Guo et al., 2011a; Massaeli et al., 2010a). The endocytic vesicles then deliver the internalised protein to early endosomes for recycling or sorting to intraluminal vesicles and eventually lysosomal degradation. The recycling of hERG1 to the plasma membrane is Rab11-mediated and is constitutive in hERG-HEK cells (Chen et al., 2015). This process is also enhanced, possibly via phosphatidylinositol-3-phosphate-5-kinase PIKfyve (Pakladok et al., 2013), by serum and glucocorticoid inducible kinases (SGK1 and SGK3), which additionally inactivate Nedd4-2, increasing hERG1 channel expression (Lamothe and Zhang, 2013).

2.2.5 hERG1-PROTEIN INTERACTIONS

In addition to the interactions already mentioned, e.g. with Hsp70 and 90, the hERG1 protein associates with various other proteins including the tumour necrosis factor-α (TNF- α) receptor 1 (Wang et al., 2002), sigma-1 receptor (Crottès et al., 2011), K^+ channel regulator 1 (KCR1; Kupershmidt et al., 2003), kinases (e.g. various protein kinases, Src tyrosine kinase) and viral proteins (HIV Tat and coxsackie). hERG1 also co-immunoprecipitates with epidermal growth factor receptor in pancreatic ductal adenocarcinoma and regulates the receptor's signaling (Lastraioli et al., 2015). Moreover in cancer cells but not in the heart hERG1 associates with β-integrin to form signalling hubs (see section 2.4.3).

A reduction in I_{Kr} by drugs results in functional as well as expressional, through post-transcriptional upregulation, compensatory increases in I_{Ks} (Jost et al., 2005; Xiao et al., 2008). Furthermore a physical interaction between the hERG1 and KCNQ1 proteins, which involves their C-termini, of the distinct channels occurs (Ehrlich et al., 2004; Guo et al., 2011b; Organ-Darling et al., 2013; Ren et al., 2010; cf. Finley et al., 2002). This interaction affects the surface expression of hERG1 (cf. Guo et al., 2011b), however the consequences are unclear with both increased and decreased I_{hERG} reported (Biliczki et al., 2009; Ehrlich et al., 2004; Hayashi et al., 2010; Ren et al., 2010).

Another significant interaction is with the single transmembrane domain KCNE family, in particular with KCNE1 (or minK, which partners with KCNQ1 to form I_{Ks} channels) and KCNE2 (or MiRP1, minK-related peptide 1). The latter, a 123 amino acid protein, was initially suggested to be the hERG1 β-subunit (Abbott et al., 1999). Upon co-expression the current density (along with the single-channel conductance) of hERG1 was decreased and deactivation was accelerated at hyperpolarised potentials but in the physiologically relevant voltage range

deactivation was unaltered or slowed (Abbott et al., 1999; Gordon et al., 2008; Lu et al., 2003; Mazhari et al., 2001; Scherer et al., 2002; Weerapura et al., 2002). In contrast co-expression of hERG1 with KCNE1 resulted in an increase in the current density, while the single-channel conductance remained unchanged, and a small negative shift in the activation curve (Du et al., 2013; McDonald et al., 1997). Also differentially KCNE1 primarily associates with hERG in the ER, whereas KCNE2 trafficks independently to the cell membrane (Mounguey et al., 2008; Um and McDonald, 2007). The inclusion of KCNE1 and KCNE2 in the cardiac I_{Kr} channel complex of various species is evident from their co-immunoprecipitation with ERG1 (Balijepalli et al., 2007; Finley et al., 2002; Jiang et al., 2004; X. Wang et al., 2008). Furthermore knockdown of KCNE1 expression by antisense oligonucleotides eradicated I_{Kr} from cultured neonatal mouse ventricular myocytes (Ohyama et al., 2001). KCNE2, like KCNE1, is promiscuous and exhibits differential interactions with its α-subunit partners such as KCNQ1 and HCN4 (Nawathe et al., 2013; Roepke et al., 2008; Y. Yang et al., 2004). Alterations in those complexes as well as the hERG1-KCNE2 channel complex will contribute to KCNE2 linked inherited and drug-induced LQTS (Abbott et al., 1999; Gordon et al., 2008; Sesti et al., 2000; Y. Yang et al., 2004). Key is probably KCNE2's localisation to Purkinje fibers with their important role in cardiac electrophysiology and arrhythmias (Pourrier et al., 2003). The significance of the hERG1-KCNE1 interaction is evident clinically. While *KCNE1* variants mostly affect I_{Ks}, some such as the single nucleotide polymorphism (SNP) D85N and the A8V mutation also or only cause loss-of-function through their interaction with hERG1 resulting in a LQT5 phenotype that mimicks LQT2 (Du et al., 2013; Nishio et al., 2009; Nof et al., 2011; Ohno et al., 2007). Though the underlying pathogenic mechanisms in regards to hERG1 have yet to be elucidated, the KCNE1 D85N and KCNQ1 interaction appears to result in enhanced degradation of the protein via the ubiquitin-proteasome system (Sakata et al., 2014).

2.2.6 REGULATION

I_{hERG} can be modulated not only by protein interactions but by a multitude of other factors e.g. acidosis, alkali and transition metal cations, ATP, lipids (e.g. long chain fatty acids and acylcarnitines, phospholipids), oxidative stress. In regards to LQTS and particularly LQT2 three modulators standout: sex hormones extracellular K^+ and adrenergic stimulation (Roden, 1998, 2006). The latter is discussed below as it has most relevance to this thesis.

Adrenergic

To increase cardiac performance for the flight-or-fight response the sympathetic nervous system accelerates heart rate and increases contractility amongst other effects (Lymperopoulos et al., 2013). The positive chronotropy and inotropy upon adrenergic stimulation occurs through mainly β_1–adrenergic receptor (β_1AR) mediated adenosine 3´,5´-monophosphate (cAMP) and cAMP-dependent protein kinase (PKA) modulation of HCN channels, L-type calcium channels and Ca^{2+} handling proteins e.g. RyR, SERCA and CaMKII. Similar modulation of I_{Ks} brings about a concomitant shortening of the cardiac APD, i.e. rate-dependent adaptation, to ensure diastolic filling time (Harmati et al., 2011; Terrenoire et al., 2005). The role of I_{Kr} is not so clear, e.g. following βAR activation I_{Kr} either increases (Balijepalli et al., 2012; Harmati et al., 2011; Heath and Terrar, 2000) or decreases (Karle et al., 2002). Most recently I_{Kr} evoked in AP clamp from guinea pig ventricular myocytes was shown to be largely insensitive to

isoproterenol, while the augmentation of I_{Ks} resulted in it becoming the dominant repolarising current (Banyasz et al., 2014).

While it was controversial (Cui et al., 2000, 2001; Sanguinetti et al., 1995), cAMP (or cGMP) does not directly modulate hERG1 as the site in the cNBD, the β-roll cavity, is negatively charged and occupied by a unique β9-strand (Brelidze et al., 2009, 2013; Li et al., 2016). However a number of components within the β–adrenergic signalling pathway have a direct action on the hERG1 channel complex. The hERG1 subunit contains four PKA phosphorylation sites with phosphorylation of the channel resulting in a reduction of current amplitude, a depolarising shift in the voltage-dependence of activation and an acceleration of deactivation (Cui et al., 2000; Thomas et al., 1999). This regulation is mediated by A-kinase anchoring proteins (AKAP) that target PKA to hERG, though which AKAP and how remains unknown (Li et al., 2008). Furthermore PKA phosphorylated hERG1 channels can interact with 14-3-3 proteins (Kagan et al., 2002). These proteins exist as dimers and bind to a phosphorylated PKA site in both the N- and C-termini of hERG1 resulting in inter- and intrasubunit cross-linking. Consequently there is a hyperpolarising shift in the voltage-dependence of activation, an acceleration of the activation rate and a prolongation of the phosphorylation state (Choe et al., 2006; Kagan et al., 2002). However a determinant of this association and thus whether the I_{hERG} has a 14-3-3 or PKA phenotype is the local concentration of free 14-3-3 with hERG1 competing against other targets including the β_1–adrenoceptor itself (Tutor et al., 2006).

Adrenergic activity also stimulates the cardiac α_{1A}–adrenoceptor which, like other Gαq-coupled receptors such as metabotropic glutamate, angiotensin II, endothelin and muscarinic receptors (Cockerill et al., 2007; Hirdes et al., 2009; Kruse and Hille, 2013; Magyar et al., 2000; Niculescu et al., 2013; Selyanko et al., 1999; Y. Wang et al., 2008), leads to inhibition of I_{Kr}/I_{hERG} (Bian et al., 2001, 2004; Thomas et al., 2004; Wang et al., 2009; Zankov et al., 2009). The subsequent $G_{\alpha q}$-protein activation of phospholipase C results in hydrolysis of PIP_2 into the second messengers inositol 1,4,5-phosphate and diacylglycerol (DAG) (Lymperopoulos et al., 2013). The consumption of PIP_2 decreases hERG1 current density, though in the heart this may have little effect as PIP_2 resynthesis can keep up with its breakdown (Bian et al., 2001; Kruse and Hille, 2013; Rodriguez et al., 2010). PIP_2 binds to a polycationic region in the C-terminus (Bian et al., 2004) and appears to act on a late voltage-independent transition stabilising the hERG1 open state (Rodriguez et al., 2010). DAG and/or the rise in $Ca^{2+}{}_i$ activates protein kinase C (PKC), which can directly phosphorylate sites on the hERG1 N-terminus (Cockerill et al., 2007; Gómez-Varela et al., 2003). Though different to the PKA sites, PKC causes similar effects on hERG1 functioning (Barros et al., 1998; Cockerill et al., 2007; Thomas et al., 2003). The effect of PKC activation on ventricular myocyte I_{Kr} however varies in different reports (Bian et al., 2004; Heath and Terrar, 2000; Wang et al., 2009).

In e.g. HF there is a chronic elevation in sympathetic activity as well as other neurohormonal mechanisms (e.g. renin-angiotensin system) in an effort to compensate for the decreased cardiac function (Lymperopoulos et al., 2013; Michael et al., 2009). Over time as cardiac function cannot be maintained it becomes counterproductive with β_1AR selectively down-regulated, β_2AR signalling altered and the presynaptic α_2AR autoinhibitory feedback lost (Lymperopoulos et al., 2013). In chronic HF β–blockers are the therapeutic mainstay substantially reducing mortality. The inhibitory effect of β_2–adrenergic stimulation on I_{Kr} was shown to be enhanced in a guinea-pig HF model (H. Wang et al., 2012). However, though evidence in disease states is lacking, chronic β– and α-adrenergic stimulation results in augmentation of native and heterologously expressed ERG1 protein, but not other cardiac K^+ channel proteins (Chen et al., 2009, 2010). The increase in hERG1 protein is due to enhanced translation, as a consequence of kinase phosphorylation of the protein, and produces an increase

in I_{hERG}. There is partial overlap of the PKC and PKA phosphorylation sites involved and cross-talk between the pathways (Chen et al., 2010), though the identity of the mechanisms and the co-factor(s) remains unknown (Krishnan et al., 2012). However PKC, possibly a different isoform, is involved in the loss of surface hERG protein, due to enhanced proteasomal degradation, that occurs with chronic angiotensin II exposure (Cai et al., 2014).

2.3 hERG1 AND THE HEART: PATHOPHYSIOLOGY

The importance of hERG1 to cardiac function is underscored by the association of its mutation, both loss- and gain-of-function, and inhibition by drugs with QT interval changes, tachycardia and sudden cardiac death (Bhuiyan et al., 2008; Brugada et al., 2004; De Bruin et al., 2005; van Noord et al., 2011).

2.3.1 CONGENITAL LQTS

Congenital LQTS often presents in an otherwise healthy young individual (termed the proband) as unexplained syncope or seizures, though for a significant portion (13%) the first manifestion is cardiac arrest and sudden death (Priori et al., 2003; Tester and Ackerman, 2007). Approximately 10-15 % of sudden infant death syndrome (SIDS) and ~20 % of sudden unexplained death (SUD) in the young may be caused by LQTS (Arnestad et al., 2007; Gladding et al., 2010; Tester and Ackerman, 2007; Tester et al., 2012; D. Wang et al., 2014). The prevalence of congenital LQTS was estimated to be at least 1 in 2534 apparently healthy live births (Schwartz et al., 2009), though in some populations it is higher (Lahtinen et al., 2013). In line with such prevalence is the relatively common occurrence (5-11 % of carriers) of multiple (≥2) mutations, i.e. compound, in the same or different LQTS genes, and rarely homozygous mutations (Itoh et al., 2010; Kapplinger et al., 2009; Lieve et al., 2013; Napolitano et al., 2005; Stattin et al., 2012; Tester et al., 2005). While inheritance of the mutation predominates, sporadic (or *de novo*) mutations occurs in ≤12 % of cases (Napolitano et al., 2005)

Mutations, predominantly missense and family specific (Christiansen et al., 2014; Kapplinger et al., 2009; Lieve et al., 2013; Splawski et al., 2000; Tester et al., 2005), in 15 different LQTS-associated genes (Romano-Ward syndrome) have been identified (Table 1; Schwartz et al., 2013). However loss-of-function mutations in *KCNQ1* and *KCNH2*, the LQT1 and LQT2 genotypes respectively (Hedley et al., 2009), accounts for about two thirds of patients with clinically definite LQTS (Napolitano et al., 2005; Tester et al., 2006). Homozygous or compound heterozygous mutations of *KCNQ1* (or *KCNE1* in ~10 %) are also the basis of Jervell and Lange-Nielsen (J-LN) syndrome, the most severe LQTS variant in which 86 % exhibit cardiac events with 50 % occurring before 3 years of age (Schwartz et al., 2006). LQT3, the other major LQTS susceptibility genotype, results from gain-of-function mutations in *SCN5A* which encodes $Na_V1.5$, the predominant cardiac Na_V α-subunit, leading to increased inward Na^+ current that prolongs the APD (Hedley et al., 2009).

Gene	***Protein***	***Frequency***	***Pathophysiological mechanism***
Major LQTS genes			
KCNQ1 (LQT1/J-LN)	$K_V7.1$	47.3	$\downarrow I_{Ks} \rightarrow \downarrow$ adaptive QT shortening
KCNH2 (LQT2)	$K_V11.1$	33.6	$\downarrow I_{Kr}$
SCN5A (LQT3)	$Na_V1.5$	11.6	$\uparrow I_{Na\text{-}L}$

Minor LQTS genes			
KCNE1	KCNE1	2.5	↓ I_{Ks}
KCNE2	KCNE2	1.2	↓ I_{Kr}
CACNA1C	$Ca_V1.2\alpha_1$	0.8	↑ I_{CaL}
CAV3	Caveolin-3		↑ $Na_V1.5$ nitrosylation → ↑ $I_{Na\text{-}L}$
SCN4B	$Na_V\beta4$		↑ $I_{Na\text{-}L}$
AKAP9	Yotiao		↓ adrenergic (PKA) regulation of I_{Ks}
SNTA1	α1-syntrophin		↑ $Na_V1.5$ nitrosylation → ↑ $I_{Na\text{-}L}$
KCNJ5	$K_{ir}3.4$	-	↓ I_{KACh}
CALM1	Calmodulin	-	↓ Ca^{2+}/CaM mediated
CALM2	Calmodulin	-	inactivation of $Ca_V1.2$
Atypical LQTS			
Ankyrin-B syndrome			Loss of anchor ↓ Na^+/K^+ pump & NCX
ANKB	Ankyrin B	0.4	at T-tubule → ↑ Na^+_i & SR Ca^{2+} load
Andersen-Tawil			
KCNJ2	$K_{ir}2.1$	2.5	↓ I_{K1}
Timothy syndrome			
CACNA1C	$Ca_V1.2\alpha_1$		↑ I_{CaL} (impaired inactivation)

Table 1. LQTS a cardiac channelopathy: the associated genes, the protein encoded, frequency of mutation (% for each LQT gene in the 241 single mutation positive individuals of the 855 referred for LQT1-12 genetic testing; Lieve et al., 2013) and the proposed underlying pathopathological mechanism (Adsit et al., 2013; Curran and Mohler, 2011; Limpitikul et al., 2014; Tester and Ackerman, 2014). Many of the minor susceptibility genes encode interacting proteins of Nav1.5, Kv7.1 &/or Kv11.1 thus mutations in e.g. *AKAP9* and *SNTA1* result in impairment of I_{Ks} and I_{Na} and so a clinical phenotype analogous to LQT1 and 3. The three atypical LQTS are multisystem disorders, in ankyrin-B syndrome and Andersen-Tawil syndrome (formerly LQT4 and LQT7) the QT prolongation is modest (Schwartz and Crotti, 2014). Mutations in *CACNA1C* yield Timothy Syndrome as well as nonsyndromic LQTS (Boczek et al., 2013). In addition to supporting the diagnosis, the identification of the genotype enables gene-specific management and finding of carriers through cascade screening of the proband's relatives. Furthermore this enables risk stratification of LQTS patients, within LQT2 the risk of life-threatening cardiac events is 17-fold greater for those who have experienced prior syncope and have a QTc ≥ 500 ms and/or are female > 13 years than for those with no such risk factors (Migdalovich et al., 2011). It should be noted that for ~20 % of patients with clinically unequivocal LQTS the genetic basis is elusive, explanations for this include large deletions/duplications in *KCNH2* and *KCNQ1* (Eddy at al., 2008; Koopmann et al., 2006), mutations in noncoding regions (e.g. in *KCNH2* as Crotti et al., 2009a), mutation detection misses (Medlock et al., 2012), mosaicism (Priest et al., 2016) and novel LQTS genes (Altmann et al., 2015; Reed et al., 2015).

The genotype significantly influences the clinical course of LQTS including the incidence of life-threatening (i.e. aborted cardiac arrest and SCD) or any (inclusion of syncope) cardiac events (Priori et al., 2003). Together with other variables like QTc and gender the risk in LQTS is modulated. Thus for instance, in LQT2 the risk of life-threatening cardiac events for females after adolescence is more than double that of males (Migdalovich et al., 2011). Furthermore while the risk for any cardiac event is reduced in pregnancy, it as well as the risk of life-threatening events is increased (3 and 4-fold) during the 9-month postpartum period (Seth et al., 2007). This is primarily among LQT2 women, the rate being ~4-fold greater than that for LQT1.

The majority of LQT1 and approximately half of LQT2 and LQT3 individuals experience no cardiac events (Priori et al., 2003). Furthermore 25 % of LQT1-3 mutation carriers exhibit a normal QTc (silent or subclinical mutation carriers) and though their risk for life-threatening cardiac events is significantly less (4 % vs 15 % over the first 40 years of life) it is still 10-fold greater than wild-type (WT) family members (Goldenberg et al., 2011). Thus LQTS displays incomplete penetrance i.e. expression of the clinical phenotype can vary between and within

families carrying the same pathogenic mutation so carriers may either be symptomatic (with variable expressivity) or asymptomatic (Priori et al., 1999). This variable penetrance and expressivity is exemplified by KCNQ1 A341V which, due to a common ancestral origin (founder effect), occurs in over 25 South African LQTS families (Brink and Schwartz, 2009). Underlying this phenomenon is the heterogeneity of the repolarisation reserve. In addition to established repolarisation reserve modifiers (e.g. gender, hypokalaemia and use of QTc prolonging drugs) are the increasing explored common genetic variants. Preeminent in this regard are SNPs in noncoding regions of *NOS1AP*, the gene encoding nitric oxide synthase 1 adaptor protein, which are shown to modulate the QT interval and risk of life-threatening events in e.g. the KCNQ1 A341V founder population and LQT2 (Crotti et al., 2009b; Earle et al., 2014; Kolder et al., 2015; Tomás et al., 2010). Another genetic modifier mechanism is compound mutations; these LQTS patients exhibit a longer QTc and more severe phenotype (Itoh et al., 2010; Mullally et al., 2013). Analogously a severe LQTS phenotype associated with a KCNQ1 mutation may arise from a disruption of the hERG chaperone effect, *in vivo* transfection of KCNQ1 T587M into adult guinea pig myocardium results in a large reduction in I_{Kr} (Biliczki et al., 2009).

The triggering of cardiac events exhibits genotype specificity so in LQT1 (and J-LN) the majority occur during exercise (e.g. swimming) while in LQT3 it is during sleep/rest (Schwartz et al., 2001, 2006), which correlates with the high prevalence of *SCN5A* mutations in SIDS (Arnestad et al., 2007; D. Wang et al., 2014). In LQT2 the most common trigger is emotional stress, such as anger and sudden arousal from sleep by an alarm clock or ringing of telephone, where there is an abrupt neural release of noradrenaline while the vagal tone is high (heart rate is low) which impairs the required QT shortening (Schwartz et al., 2001; Wilde et al., 1999). This trigger likely contributes to the predominance of LQT2 in postpartum cardiac events (Seth et al., 2007). Furthermore in LQT2 the risk for arousal-triggered cardiac events is higher in women likely a consequence of gender differences in the repolarisation reserve and the baseline autonomic tone (Kim et al., 2010). While the adrenergic regulation of LQT2 mutant channels may be unaltered, the α_{1A}–adrenoceptor mediated reduction in I_{Kr} may underlie this link (Zankov et al., 2009). Another potential mechanism is hyperexcitability in the sympathetic outflow to the heart and/or increase in circulating catecholamines as ERG1 is present in peripheral sympathetic ganglia and the adrenal glands (Gullo et al., 2003; Shi et al., 1997). The rapid onset, within 10s, would preclude a systemic effect though evidence for hERG affecting cardiac autonomic control was recently reported. Inhibition of hERG was shown to enhance the endogenous parasympathetic rhythms (i.e. high frequency oscillations) of heart rate which increased the short-term variability in QT interval and led to triggering of TdP in cynomolgus monkeys (Champeroux et al., 2015).

As sudden sympathetic activity is the predominant trigger the first line and mainstay treatment for prevention of cardiac events in LQT2 (as with the other forms, whether the patient is symptomatic or not) is β-blocker pharmacotherapy e.g. propranolol (Schwartz and Crotti, 2014). Though beneficial in LQT2 (Seth et al., 2007), β-blockers are less efficacious than in LQT1 due to associated bradycardia (Migdalovich et al., 2011; Priori et al., 2004). For those who remain symptomatic despite β-blocker therapy, another antiadrenergic strategy left cardiac sympathetic denervation (where the lower ⅔ of the left stellate ganglion and the thoracic ganglia T2-T4 are ablated) is adjunctly implemented and/or an implantable cardioverter defibrillator used (Mizusawa et al., 2014). The latter alternative, which does not prevent arrhythmias but acts as a safety net, is immediately implanted in cases of cardiac arrest (Schwartz and Crotti, 2014). Additional LQT2 management approaches include K^+ supplements, avoidance of QT prolonging drugs and the removal of alarm clocks and telephones from bedrooms.

LQT2 mutations

Loss-of-function mutations in *hERG1* underlie ~37 % of LQTS (Kapplinger et al., 2009; Lieve et al., 2013; Napolitano et al., 2005; Tester et al., 2005) and are an etiology of intrauterine foetal death (Bhuiyan et al., 2008; Crotti et al., 2013), SIDS (accounting for ~1 %; Arnestad et al., 2007) and SUD (where it accounts for ~4 %; Tester et al., 2012). Approximately 600 LQT2-associated mutations are reported (Human Genetic Mutation Database, http://www.hgmd.cf.ac.uk/ e.g. C. Anderson et al., 2014; Kapplinger et al., 2009; Lieve et al., 2013; Napolitano et al., 2005; Splawski et al., 2000; Tester et al., 2005), though many may be classified as variants of uncertain significance as their pathogenicity awaits verification or are questionable/innocuous (Ackerman et al., 2003; Refsgaard et al., 2012). Missense mutations are the most frequent (~62 %) followed by frameshift (~25 %) with the remaining ~13 % consisting of nonsense, splice site, and inframe insertions/deletions (Kapplinger et al., 2009; Shimizu et al., 2009). Mutations are located throughout the hERG1 subunit, with ~29 % in the N-terminus, ≈36 % in its transmembrane spanning region (≈73 % of which are located within the pore domain and these are predominantly missense), and ≈35 % in the C-terminus (Kapplinger et al., 2009; Shimizu et al., 2009). Unlike for radical mutations, the site of the missense mutation is an important determinant of pathogenicity with subjects in general having significantly higher cardiac event rates for mutations in the pore domain than the termini (Kapa et al., 2009; Migdalovich et al., 2011; Shimizu et al., 2009).

Frameshift mutations, deletions and insertions likely result in premature termination codons which, if detected and are not so early within the mRNA to enable reinitiation of translation at in-frame downstream methionine codons (Gong et al., 2014b; Stump et al., 2012b, 2013), lead to NMD of the mRNA (Gao et al., 2013; Gong et al., 2007; Sun et al., 2009; Zarraga et al., 2011). NMD is predicted to occur in 161 of the 196 reported LQT2 mutations that introduce premature termination codons (Gong et al., 2014b). Thus the production of truncated proteins which may be nonfunctional and often have dominant negative effects is minimised resulting in a milder heterozygous phenotype by conversion to haploinsufficiency i.e. the WT subunits are able to form homotetrameric channels consequently there is a 50 % or less loss-of-function. A dominant negative phenotype arises from the multimeric nature of the channel so when e.g. N470D subunits, which are trapped in an intermediate folding state (Gong et al., 2006), or G572S subunits assemble with WT subunits the entire channel may be retained by quality control in the transitional ER and degraded by ERAD resulting in suppression of hERG channel surface expression and a loss of current of up to ~90 % (Gong et al., 2004a, 2005; Smith et al., 2011, 2013; Zhao et al., 2009). Such behaviour is common for pore domain mutations, unlike other regions, which may explain the more severe clinical phenotype associated with this location (C. Anderson et al., 2014).

Trafficking deficiency can arise from mutations throughout hERG1 and underlies the loss-of-function for ~90 % of LQT2 missense mutations (C. Anderson et al., 2006, 2014), the extent of the deficit can vary between mutations (Gianulis and Trudeau, 2011; Ke et al., 2013; Perry et al., 2016). A subset of these mutants can be rescued by low temperature, chemical chaperones (e.g. glycerol) and/or I_{Kr} inhibitors (C. Anderson et al., 2006, 2014; Ficker et al., 2002; Gong et al., 2004a; Harley et al., 2012; Jones et al., 2016; Lin et al., 2010; Zhou et al., 1999). The inhibitors bind to and stabilise the inner cavity soon after the assembly of the tetramer promoting native-like folding, hence restoring protein trafficking and functional expression of the channel (Ficker et al., 2002; Gong et al., 2006). As a therapy for mutants which also function normally at the cell membrane, e.g. G601S (Furutani et al., 1999), it is inherently limited, but as a prolonged corrective effect can be obtained without continual channel inhibition a drug with a short half-life may have potential (Smith et al., 2013). Furthermore

pharmacological rescue can occur without significant or in the absence of I_{hERG} inhibition (Delisle et al., 2003; Rajamani, et al., 2002). However rescued mutant channels have increased turnover at the plasma membrane (Apaja et al., 2013; Ke et al., 2013), likely due to their innate conformational instability e.g. G601S channels are >5-fold more sensitive to trypsinolysis (Apaja et al., 2013).

The loss-of-function in LQT2 can arise from other mechanisms (Zhou et al., 1998b). Mutations in splice sites can affect pre-mRNA processing resulting in intron retention and/or use of a cryptic splice site with insertion of intronic sequence/deletion of exonic sequence (Crotti et al., 2009a; Gong et al., 2008; Stump et al., 2011; Zhang et al., 2004). The consequence can be a frame-shift that introduces a premature termination codon into the transcript leading to NMD or if in-frame an approximate full length trafficking impaired protein which has a dominant negative effect on WT subunits. Other mutations may alter channel gating, e.g. the LQT2 mutants G584S and R56Q exhibit primarily enhanced inactivation and accelerated deactivation respectively (Berecki et al., 2005; Gianulis and Trudeau, 2011; Zhao et al., 2009), or permeation as with G628S where conduction is blocked by physiological $[K]_i$ (Es-Salah-Lamoureux et al., 2011). These loss-of-function mechanisms may coexist e.g. T421M exhibits both trafficking deficiency and closed-open state gating abnormalities (Balijepalli et al., 2012).

2.3.2 DRUG-INDUCED LQTS

First recognised, as syncope associated, in the 1920s with the introduction of quinidine (Roden, 2006), the potential of not only class I but class III antiarrhythmic and antianginal drugs to induce LQTS is clear with TdP incidences of 0.5-8 % and 0-10.5 % during quinidine and dofetilide therapy (Roden, 1998; Yap and Camm, 2003). A TdP/QTc liability is also carried by numerous noncardiovascular drugs from a wide range of structural and therapeutic (e.g. antipsychotics, antibiotics, antidepressants) classes (Poluzzi et al., 2009; Redfern et al., 2003; Yap and Camm, 2003). Due to reported adverse cardiac events certain noncardiac drugs have had prescribing information revised, been refused approval or been withdrawn from the market (Roden, 2004; Shah, 2005; Stockbridge et al., 2013**)**. Among the thirteen latter drugs deemed to have an unacceptable risk/benefit ratio (i.e. prescribed for benign conditions and/or safer alternatives available) was cisapride, a gastrointestinal prokinetic popular for the treatment of nocturnal heartburn due to gastro-oesophageal reflux disease. In the period from its marketing (1993) till the end of 1999 the US Food and Drug Administration (FDA) received reports of serious cardiac adverse drug reactions (including 107 TdP and 18 VF reports) in 341 patients, 80 of whom died (Wysowski et al., 2001). The incidence of arrhythmia with cisapride was estimated to be 1 in 120,000 patients (Ahmad and Wolfe, 1995).

Drug-induced LQTS and TdP predominantly, though not exclusively as with antimony-based leishmaniasis treatments (Kuryshev et al., 2006) and the benign prostatic hyperplasia medication alfuzosin (Lacerda et al., 2008), occurs through selective inhibition of the hERG1 current at relevant plasma concentrations (Table 2; Crumb et al., 2016; Lacerda et al., 2001; Mohammad et al., 1997; Redfern et al., 2003; Roden et al., 1996). However not all drugs that block hERG1 are associated with TdP, as exemplified by the angina drugs verapamil and ranolazine, due to concurrent equipotent, at least, I_{CaL} and/or I_{NaL} inhibition which prevents EAD formation (Table 2; Crumb et al., 2016; Kramer et al., 2013). Block of hERG1 is also present in a large proportion of new chemical entities (NCEs i.e. a drug with no active moiety that has regulatory approval; Lu et al., 2008; Shah, 2005) so the question arises why is the

hERG1 channel so susceptible to direct drug blockade? The basis for this may be attributed to various unique structural features of the hERG1 central inner cavity, the occupancy of which obstructs ion conduction through the pore (Mitcheson et al., 2000a). Firstly the hERG1 inner cavity is large, though the underlying explanation is unclear (Mitcheson, 2008). hERG1, like other members of the EAG family, lacks the S6 Pro-X-Pro motif of most other K_V channels which may kink the domain reducing the volume of and restricting access to the inner cavity (Fernandez et al., 2004). Access to the inner cavity and the drug binding site is gained from the cytoplasmic aspect (e.g. Rajamani et al., 2008; Zhang et al., 1999) by the opening of the activation gate resulting in state dependence of block (Kamiya et al., 2006; Kiehn et al., 1996; Snyders and Chaudhary, 1996; Spector et al., 1996a). The size of the hERG inner cavity is such as to accomodate diverse structures without impediment to activation gate movement thus drugs can be trapped upon repolarisation by closure of the gate prohibiting recovery from block (Kamiya et al., 2006; Mitcheson et al., 2000b; Stork et al., 2007).

	hERG1	KCNQ1	K_V4.3	Ca_v1.2	Na_v1.5	FTPC (nM)	↑QTc (ms)
Dofetilide*[1,2,3]	0.002-0.005	416	>0.006	>>0.006	>>0.006	2.2	74
Quinidine*[1,2]	0.3	4.9	3.5	>5.4	>5.4	829	79
Ranolazine*[1,2]	6.5	>>23	>69	>>23	7.9	1727	12
Terfenadine*[1]	0.019	>>0.8	>0.8	0.7	>0.8	0.3/9[§]	
Cisapride*[1,4]	0.012	>>0.13	>0.13	>>0.13	>>0.13	1.5/4.8[#]	6/25[#]
Thioridazine[5,6]	0.19-0.45	7.5		2.3	4.1	21	30
l-acetylmethadol[7,8]	2.2-3.0	>>10	>10			200	

Table 2. Comparison of the inhibitory potencies of cardiac drugs (above the line) and noncardiac drugs withdrawn from the market at cardiac ion channels (underlying I_{Kr}, I_{Ks} (KCNQ1/KCNE1), I_{to}, I_{CaL} and I_{Na}), expressed in mammalian cells. The IC_{50} values are given or denoted as >> insensitivity to (i.e. <10 % block)/ > maximal tested concentration, (in µM) and were obtained using the manual patch-clamp technique. $Na_V1.5$ refers to block of late and/or peak current. Chronic exposure to dofetilide increases the late (and peak) I_{Na} due to PI3K pathway inhibition affecting gating (Yang et al., 2014). * For these drugs $K_{ir}2.1$ (I_{K1}) was also measured, it was insensitive to the maximal tested concentration and in the case of dofetilide IC_{50} was 98.8 µM[3]. [§] In the presence of a CYP3A4 inhibitor, otherwise FTPC is 0.3 nM. For each drug the maximal estimated effective free therapeutic plasma concentration, FTPC, in humans is shown and the increase in QTc at FTPC is given for some drugs. [#] average steady-state concentration as monotherapy and when clarithromycin coadministered since average QTc over 24 hr were given. References: [1]Crumb et al., 2016; [2]Vicente et al., 2015; [3] Obejero-Paz et al., 2015; [4]van Haarst et al., 1998; [5]Champeroux et al., 2011; [6]Kongsamut et al., 2002; [7]Katchman et al., 2002; [8]Kang et al., 2003.

Secondly facing the central cavity is the S6 aromatic residues Tyr652 and Phe656, which are absent from homologous positions in non-EAG family K_V channels and when mutated in hERG1 substantially reduce the potency of high (nM) and low (µM) affinity blockers (e.g. Dong et al., 2013; Guo et al., 2006; Jehle et al, 2013; Kamiya et al., 2006; Lees-Miller et al., 2000; Mitcheson et al., 2000a; Perry et al., 2004; Sánchez-Chapula et al., 2002; Takemasa et al., 2007). A compound binds in a number of different configurations as the eight aromatic residues of the hERG1 inner cavity offer multiple diverse potential interactions (Dempsey et al., 2014). These are thought to include π-stacking and hydrophobic interactions, in addition for those drugs with a protonated basic nitrogen a cation-π interaction with Y652 may occur (Aronov, 2008; Fernandez et al., 2004). For most drugs the interaction with F656 appears the more important (Melgari et al., 2015; Mitcheson, 2008). Interestingly F656 also interacts with the aromatic ring of 17β-oestradiol (E2), the resulting gating modification acutely reducing I_{hERG} by 21 % at 3 nM (Kurokawa et al., 2008). Other less unique residues at the base of the pore helix (the polar Thr623 and Ser624) are also differentially implicated in the binding of

blockers (Guo et al., 2006; Kamiya et al., 2006, 2008; Mitcheson et al., 2000a; Perry et al., 2004), though this may not be a direct interaction (Dempsey et al., 2014).

These pore features are conserved within the EAG and ERG subfamilies, though hELK2 (K_V12.2) lacks an aromatic residue and is >1000-fold less sensitive than hERG1 to dofetilide and astemizole (Becchetti et al., 2002). While the difference from hEAG1 (K_V10.1), which is e.g. 57-fold less sensitive to E-4031 (Gessner and Heinemann, 2003), is suggested to arise from the orientation of the aromatic residues with respect to the cavity. In hERG1 these may be optimally positioned for interaction through the rotation of S6 that precedes inactivation state entry (Chen et al., 2002; Lin et al., 2005; Stansfeld et al., 2007). Inactivation is absent from EAG, but linked to the binding affinity of some drugs (Ficker et al., 1998, 2001; Gerlach et al., 2010; Gomez-Varela et al., 2006; Guo et al., 2006; McPate et al., 2008; Perrin et al., 2008; Ulens and Tytgat, 2000; Wang et al., 1997b; Yang et al., 2004).

Other binding determinants for hERG blockers (e.g. F557 in S5; Saxena et al., 2016) and secondary drug binding sites may exist as neither S6 aromatic residue is critical for block by the antidepressant fluvoxamine, the high affinity antiarrhythmic dronedarone or the macrolide antibiotic erythromycin (Duncan et al., 2006; Milnes et al., 2003a; Ridley et al., 2004). Erythromycin was proposed to bind externally, with occupancy of the site allosterically affecting the inner cavity binding site (Crumb, 2014). A similar negative allosteric effect on the affinity of classical inhibitors is also seen with recent novel compounds (Yu et al., 2016). The most prominent external binders are peptide toxins e.g. BeKm-1, from the scorpion *Buthus eupeus*, which binds to the outer vestibule of the hERG1 channel (Tseng et al., 2007). Furthermore the S5-P linker was also proposed as the binding site for anti-Ro/SSA antibodies, which can arise in the course of autoimmune diseases such as Sjögren syndrome and systemic lupus erythematosus but are also present in the general population (Lazzerini et al., 2016; Yue et al., 2015). The resulting off-target direct inhibition of hERG1 current by these antibodies underlies a novel acquired autoimmune-associated LQTS in adults and may act as risk factor.

Another mechanism underlying drug-induced LQTS is disruption of hERG1 channel trafficking (Ficker et al., 2004), emulating the congenital LQT2 mechanism. Pentamidine, an antiprotozoal drug, is associated with LQTS/TdP as prolonged exposure selectively reduces hERG1 channel surface expression due to occupancy of the conventional drug binding site in a folding intermediate which arrests its maturation (Cordes et al., 2005; Dennis et al., 2012; Kuryshev et al., 2005). Pentamidine, like cardiac glycosides e.g. digoxin (Wang et al., 2007) which are used in the treatment of congestive HF and also disrupt hERG1 trafficking, has little acute effect on hERG1 with its IC_{50} being >450-fold the therapeutic free concentration (Cordes et al., 2005). For cardiac glycosides, which inhibit the Na^+/K^+ pump, it is proposed that the depletion of $[K^+]_i$ induces conformational changes in hERG originating from the normally K^+ stabilised selectivity filter (L. Wang et al., 2009). The structural destabilisation of hERG induced by low $[K^+]_i$ also accelerates turnover at the plasma membrane by the peripheral quality control machinery (Apaja et al., 2013). Though appearing to be a trafficking blocker probucol is different (J. Guo et al., 2007, 2011a). Instead of blocking the forward trafficking of hERG1 the disruption of the cholesterol level by the drug destabilises and accelerates surface membrane caveolin turnover and concomitantly, due to their physical association, hERG1 degradation (Guo et al., 2011a). Importantly a number of direct hERG1 blockers (e.g. the antidepressant fluoxetine and the antifungal ketoconazole) also exhibit, in a similar concentration range, trafficking hERG1 inhibition (Rajamani et al., 2006; Takemasa et al., 2007).

The incidence of drug-induced LQTS/TdP has been estimated at 3.2 per million per year (Sarganas et al., 2014). Thus drug-induced TdP is a rare event arising in only a subset of patients e.g. with methadone maintenance 16 % and 4 % were reported to have respectively a

QTc of ≥500ms and TdP (Ehret et al., 2006). Prior to and after washout of drug the QTc interval may appear normal (Itoh et al., 2009a; Napolitano et al., 2000; Paulussen et al., 2004), furthermore TdP may occur after weeks or months of drug treatment (Pratt et al., 2006; Zeltser et al., 2003). This reinforces the supposition that evolving/existing subclinical decrements to the repolarisation reserve, due to e.g. hypokalaemia, HF, and female gender, underlies the predisposition of an individual (Roden, 1998, 2006; Sarganas et al., 2014; Zeltser et al., 2003). In addition to decreasing I_{Kr} and I_{K1}, hypokalaemia potentiates the block of hERG1 by some, but not all (Guo et al., 2006; Pareja et al., 2013), drugs (Wang et al., 1997b; Yang et al., 2004). The mechanisms underlying the differential sensitivity of drugs, e.g. due to trapping by closure of the activation gate, or the potassium dependency of block, e.g. via enhancement of inactivation, are not clear. Similarly, as well as their genomic and non-genomic effects on cardiac electrophysiology, sex hormones may influence hERG1 drug sensitivity, e.g. E2 enhances E-4031 and erythromycin inhibition (Ando et al., 2011; Kurokawa et al., 2008), and contribute to the gender-related difference in drug-induced LQTS/TdP (van Noord et al., 2011). A common feature in terfenadine and cisapride induced LQTS cases was the concomitant administration of other substrates, such as erythromycin, of their metabolizing cytochrome P450 isoenzyme (CYP3A4), this pharmacokinetic interaction led to an increase in the drug plasma concentration (Roden, 2006; Wysowski et al., 2001). Pharmacogenetic variables in drug-induced LQTS exist (Kannankeril et al., 2005) including an increased burden of K^+ channel LQTS gene rare variants (Ramirez et al., 2013; Weeke et al., 2014), subclinical or latent LQTS mutations (e.g. Donger et al., 1997; Itoh et al., 2009a; Napolitano et al., 2000; Yang et al., 2002) and SNPs e.g. in *NOS1AP* (Jamshidi et al., 2012), the metabolising CYP isozymes (Eap et al., 2007; Roden, 2006) and *KCNE*s (Paulussen et al., 2004; Weeke et al., 2014). While the sensitivity of hERG1 towards a culprit drug is unaltered for those cases involving its mutation (Bellocq et al., 2004; Itoh et al., 2009a), this is not necessarily so when coexpressed with variants of KCNE1 and KCNE2 (Abbott et al., 1999; Du et al., 2013; Friederich et al., 2004; Sesti et al., 2000). For example the I_{hERG} inhibitory effect of cisapride was enhanced (the IC_{50} halved) by hERG-KCNE1 D85N, whereas quinidine potency was unaffected (Du et al., 2013). It is unknown how these variants can affect the drug binding site and why drugs are differentially affected. So in addition to possibly reducing I_{Ks} and the repolarisation reserve, KCNE1 D85N reduces I_{Kr} (Du et al., 2013; Nishio et al., 2009; Nof et al., 2011) and for some drugs may directly increase susceptibility to inhibition (Du et al., 2013). KCNE1 D85N was associated with a 9-fold risk for drug-induced TdP (Kääb et al., 2012).

2.3.3 hERG1 GAIN-OF-FUNCTION

Congenital short QT syndrome (SQTS) was first described as an inherited clinical entity by Gussak et al. (2000). It is characterised by a constantly shortened QTc (QTc ≤340 ms or ≤360 ms with symptomaticity or family history) and poor rate adaptation of the QT interval as well as risk of syncope, AF, cardiac arrest and sudden death (Giustetto et al., 2011; Mazzanti et al., 2014). Underlying the symptoms is an abbreviation of myocardial repolarisation, both in the atria and ventricles, and consequently the effective refractory period which then provides a substrate for re-entry resulting in atrial and ventricular tachyarrhythmias including VF which is invariably fatal. The probability of cardiac arrest by 40 years of age is 40-50 % with recurrence in ⅔ of the survivors and since e.g. the degree of QTc shortening does not predict risk an implantable cardioverter defibrillator is the primary therapy (Mazzanti et al., 2014).

A gain-of-function mutation (N588K, located in the α-helix of the S5-P linker) of hERG1 was the first identified in SQTS (Brugada et al., 2004). The primary functional effect of the mutation is to abolish inactivation within the physiological range of membrane potentials, as the $V_{½inact}$ shifts by >+100 mV (Cordeiro et al., 2005; Grunnet et al., 2008; Perrin et al., 2008), leading to greater I_{hERG} in the plateau phase and overall a dome-shaped current profile during a ventricular AP (Brugada et al., 2004; McPate et al., 2005). The potency of blockers, e.g. sotalol, dofetilide and cisapride, is also decreased, though those with less affinity for the inactivated state, such as disopyramide and quinidine, may have therapeutic value (McPate et al., 2006, 2008; Perrin et al., 2008; Schimpf et al., 2007; Wolpert et al., 2005). Other hERG1 (SQT1) mutations (E50D, I560T and T618I) were subsequently identified (El Harchi et al., 2012; Harrell et al., 2015; Hu et al., 2011; Martinez et al., 2011; Y. Sun et al., 2011). These later mutations also augment the current density on depolarisation and shift, though to a lesser extent (from +12 to +49 mV), steady-state inactivation.

So far gain-of-function mutations in two other K^+ channel genes (*KCNQ1* and *KCNJ2*) have also been associated with SQTS (SQT2 and 3; Schwartz and Crotti, 2014). Additionally a mixed SQT/Brugada phenotype results from loss-of-function mutations to the L-type Ca^{2+} channel subunit genes *CACNA1C* and *CACNB2b* (SQT4 and 5; Antzelevitch et al., 2007). However, none of the SQT genotypes accounts for >5 % of the clinically affected probands (Mazzanti et al., 2014). A genotype-phenotype correlation is becoming apparent e.g. manifestation occurs later in SQT1 than the other genotypes and penetrance appears to be higher for SQT1-3 (Harrell et al., 2015). Though as with LQTS, the clinical phenotype is variable even with the same mutation (e.g. N588K in KCNH2) between patients and in families ranging from asymptomatic through to only AF or AF and VT to sudden death/SIDS (Brugada et al., 2004; Hong et al., 2005; Suzuki et al., 2014). Recently mutation of *SLC22A5*, an organic cation transporter, which results in reduced cellular uptake of carnitine was also shown to induce SQTS (Roussel et al., 2016).

Several hERG1 agonists have been identified which exhibit distinct mechanisms of action (Sanguinetti, 2014): slowing of deactivation (e.g. RPR260243; Kang et al., 2005), depolarising shift in the voltage dependence of inactivation (e.g. ICA-105574; Gerlach et al., 2010), hyperpolarising shift in the voltage dependence of activation (e.g. mallotoxin) and increased channel open probability (e.g. PD-118057). Most agonists including those listed affect multiple gating properties, typically at μM concentrations. Though their primary gating effects differ, the hERG1 agonists so far investigated interact with a similar region of the pore domain at overlapping but not identical residues (Sanguinetti, 2014). The putative binding site is a hydrophobic pocket between two adjacent subunits, i.e. distinct from the antagonist inner cavity binding site, and occupancy of all four sites in the channel is required for maximal achievement of the primary effect (Garg et al., 2011; Sanguinetti, 2014; Wu et al., 2015). Also the conformational changes induced by agonist binding appear to be restricted by N-terminal interactions with the pore domain since the effects of agonists are more pronounced with ERG1b channels (Larsen et al., 2010b; Schuster et al., 2011).

hERG1 agonists were proposed as a novel pharmacotherapy for LQTS (Bentzen et al., 2011; H. Zhang et al., 2012). However excessive shortening of APD and refractoriness is a real safety concern as some of these agonists, at least in *ex vivo* preparations, can produce AF or VT and VF (Bentzen et al., 2011; Lu et al., 2008; Nof et al., 2010a; Patel and Antzelevitch, 2008). Other reported hERG agonist effects include an increase in heart rate and slowing of conduction which also shortens the cardiac wavelength increasing the risk for reentry arrhythmia (Bentzen et al., 2011; Larsen et al., 2010a). Furthermore it is interesting to note that the tail I_{hERG} was enhanced when exposed to the serum of HF patients with a history of VT/VF, but not with serum of HF patients without VT/VF, compared to control (Sugiyama et al., 2011). The

emergence of hERG1 agonists and other APD/QT shortening compounds in preclinical pharmaceutical development has led to debate of the potential significance for NCEs and clinically used drugs (Holbrook et al., 2009; Lu et al., 2008; Shah, 2010). The incidence of drug-induced SQT is unknown, possibly in part due its malignancy, but is occasionally associated with digitalis toxicity (Garberoglio et al., 2007). In the FDA Adverse Event Reporting System (2004-2010) there were 42 cases of QT-interval shortening (cf 5323 QT prolongation reports) of which 83 % were associated with arrhythmia related events (Raschi et al., 2011). The most suspected drugs were paracetamol, digoxin and ziprasidone but there are inherent data issues (e.g. evaluation of causality). The present deficit in understanding along with the generally heightened concerns of a drug's proarrhythmic potential and risk adverse environment has made for a cautious approach to drug-induced SQT by both the companies and regulatory authorities (Himmel and Hoffmann, 2010; Holbrook et al., 2009; Shah, 2010). A noteable exception is rufinamide, which at therapeutic dose shortens QTc by a mean of up to 20 ms, approved in 2007/2008 as an antiepileptic add-on drug in Lennox-Gastaut syndrome (Schimpf et al., 2012; Shah, 2010).

2.3.4 ATRIAL FIBRILLATION AND BRUGADA SYNDROME

AF is the most common clinical arrhythmia with a prevalence of 1 – 2 % in the general population and is associated with significant morbidity and mortality due to risk of stroke and worsening HF (Andrade et al., 2014). AF and particularly the subgroup of lone AF, i.e. AF without any apparent cardiovascular disease, have a significant genetic basis. Nine common non-coding SNPs and many monogenic mutations, including both loss-of-function and particularly gain-of-function in a number of K^+ channel subunits e.g. *KCNQ1*, *KCNA5* and *KCNEs*, have been identified (Hayashi et al., 2015; Olesen et al., 2014a). In LQTS, where atrial APD can also be prolonged, the prevalence of early onset AF is 1.7 % which is ~20 times the expected background, the susceptibility mediated by an atrial torsade mechanism (Johnson et al., 2008; Kirchhof et al., 2003). LQT2 mutations, e.g. E939X, and hERG1 gain-of-function mutations, e.g. T895M and the SQTS mutation N588K, can cause paroxysmal or persistant AF and atrial tachycardia (Hayashi et al., 2015; Hong et al., 2005; Kirchhof et al., 2000, 2003). However such monogenic causes of AF are scarce (Andreasen et al., 2013) and other sources must contribute to the complex heritability. One source may be the presence and interaction (epistatic effects) of rare variants in K^+ channel and other AF candidate genes, their frequency being significantly higher in AF (Mann et al., 2012; Olesen et al., 2014b). While individually the variants may have a modest effect (e.g. E444K in hERG1), in combination with common variants the net effect though could be substantial but this total variant burden is difficult to predict/show pathogenicity (Mann et al., 2012; Weeke et al., 2015).

Gain-of-function *hERG1* mutations are also associated with Brugada syndrome (BrS), a heritable arrhythmia disorder characterised by ST-segment elevation and male predominance (Schwartz et al., 2013). While loss-of-function *SCN5A* mutations are the major (20-25 %) cause, *hERG1* mutations were found in 1.7 % of a population of BrS probands (Q. Wang et al., 2014). These patients also, mostly, exhibit a shortened QTc interval. The six *hERG1* variants thus far identified all reside in either the N- or C-termini and increase I_{hERG} density without the SQT1 depolarising shift in $V_{½inact}$ (Itoh et al., 2009b; Verkerk et al., 2005; Q. Wang et al., 2014). While not causative, these variants likely act as modifiers of BrS with simulation studies showing a larger transient I_{hERG} elicited by the upstroke resulting in a loss of the epicardial AP

dome in the right ventricle, a key mechanism in BrS, at faster stimulation rates (Verkerk et al., 2005; Wilders and Verkerk, 2010).

2.4 EXTRA-CARDIAC hERG: PHYSIOLOGY & PATHOPHYSIOLOGY

2.4.1 THE NERVOUS SYSTEM

I_{Kr} is found in mouse cerebellar Purkinje neurones, mouse embryonic GABAergic spinal ventral interneurones, rat embryonic serotonergic neurones, mouse mitral/tufted olfactory bulb neurones, mouse vestibular nucleus neurones, mouse vomeronasal neurones and mouse (but not rat) auditory brainstem neurones (Furlan et al., 2005; Hagendorf et al., 2009; Hardman and Forsythe, 2009; Hirdes et al., 2005, 2009; Niculescu et al., 2013; Pessia et al., 2008; Sacco et al., 2003). The neuronal I_{Kr} displays both functional and temporal diversity to tailor to the needs thus it may activate and deactivate faster, the latter by 5-fold, than or be similar to I_{ERG1a} (Hirdes et al., 2005, 2009; Sacco et al. 2003). Moreover in basal vomeronasal neurones expression of ERG1 (mRNA and protein) and I_{Kr} is increased with pheromone exposure, along with a concomitant alteration of the slopes of both activation and availability curves and slower deactivation as the relative composition of subunits (ERG1a/b:ERG3:KCNE2) changes (Hagendorf et al., 2009). Similar changes in I_{Kr}, which results from heteromeric ERG1/ERG3 channels, occur in auditory brainstem neurones as I_{Kr} increases with age (Hardman and Forsythe, 2009).

Due to the short duration (~1 ms) of the action potential and its slow activation, I_{Kr} has little effect on the resting membrane potential, shape or duration of individual neuronal action potentials (Hardman and Forsythe, 2009; Pessia et al., 2008; Sacco et al., 2003). However with repetitive or prolonged depolarisation, such as the postsynaptic complex spike of the adult Purkinje neurone, channels begin to accumulate in the open state due to the slow deactivation kinetics of I_{Kr} at the resting membrane potential resulting in mounting, sustained current (Sacco et al., 2003; Schönherr et al., 1999). This can then contribute to repolarisation and to the gradual termination of the burst of evoked action potentials, spike frequency adaptation (Chiesa et al., 1997; Sacco et al., 2003; Schönherr et al., 1999). Thus for many of the neuronal cell types described I_{Kr} inhibition increases firing frequency and the firing pattern becomes repetitive, non-adapting (Furlan et al., 2007; Hardman and Forsythe, 2009; Pessia et al., 2008; Sacco et al., 2003). In neonatal Purkinje and rat midbrain dopaminergic neurones I_{Kr} appears though not to play a part in spike frequency adaptation but in respectively setting the action potential threshold, due to the temporary abundance of ERG3, and in the slow afterhyperpolarisation following a long lasting depolarising stimulus (Nedergaard, 2004; Niculescu et al., 2013). Thus in these dopaminergic neurones its major role *in vivo* is to control the incidence and mean frequency of bursting activity (Ji et al., 2012; Nedergaard, 2004). Furthermore during spontaneous firing in these cells (which is ~3 Hz) and others I_{Kr} is active, due to the pacemaker induced depolarisation traversing the activation threshold and only partial deactivation occurring during the hyperpolarised state between the action potentials, thus its block removes inhibitory drive on the pacemaker activity increasing the resting firing frequency (Ji et al., 2012; Nedergaard, 2004; Pessia et al. 2008).

I_{ERG1} is found in rat hippocampal astrocytes with its blockade increasing the basal and the neuronal activity induced $[K^+]_o$ so that, together with inward rectifier currents, it appears to play a role in the voltage dependent buffering by glia of the extraneuronal space $[K^+]$ (Emmi et al., 2000). However Fano et al. (2012) reported E-4031 had no effect on $[K^+]_o$. I_{Kr} is also present in rat microglia and the intermediate cells of the stria vascularis with ERG1 protein expressed in these and other cells (e.g. hair cells) of the mouse inner ear (Nie et al., 2005; Zhou et al., 1998). Only ERG1 transcript, according to single-cell RT-PCR, and its protein are expressed and functional in the cell bodies, but not the axon terminals, of horizontal cells of the mouse retina (Feigenspan et al., 2009). In these cells I_{Kr} controls the depolarised (-33 mV) resting membrane potential that occurs in the dark due to the constant presynaptic glutamate release from photoreceptors. However in a subsequent study ERG1 protein was not found in horizontal cells but to be abundant particularly in rod bipolar cells and photoreceptors (Cordeiro et al., 2011).

In the anterior pituitary both gonadotropes and lactotropes exhibit I_{Kr} which contributes to the maintenance of the resting membrane potential (Bauer et al., 1999; Hirdes et al., 2010). Thyrotropin-releasing hormone (TRH) and gonadotropin-releasing hormone, via G protein coupled receptor phospholipase C activation and the former at least by a pathway independent of PKA and PKC, inhibit I_{Kr} by decreasing the maximal current amplitude and a depolarising shift in the activation curve (Hirdes et al., 2010; Schäfer et al., 1999; Schledermann et al., 2001). This leads to depolarisation of the lactotrope and gonadotrope, calcium influx and stimulation of prolactin and luteinizing hormone secretion (Bauer et al., 1999; Hirdes et al., 2010). Furthermore in corticotrophs I_{Kr} underlies the delayed glucocorticoid inhibition of adrenocorticotropin release, which may involve upregulation of ERG1 mRNA (Yamashita et al., 2009).

LQTS when evident as syncope and seizures has often been initially misdiagnosed as epilepsy and retrospectively ascribed in cases of sudden unexplained death in epilepsy[3] (MacCormick et al., 2009; Tu et al., 2011). However, seizure is 3-fold more prevalent in LQTS (even with β-blocker use) with the highest prevalence occurring in LQT2 patients (Auerbach et al., 2016; Johnson et al., 2009). This suggests that rather than seizures being cardiogenic in origin there may be a relationship between *hERG1* mutation and neuronal hyperexcitability, that LQT2 and epilepsy could coexist as highlighted by case reports (Anderson et al., 2012; Partemi et al., 2013; Zamorano-León et al., 2012). The prevalence of concomitant epilepsy (electroencephalogram-documented) and LQT2 was found to be 3.7 %, significantly greater than the other LQT subtypes (J. Anderson et al., 2014). In addition a pilot study showed that abnormal EEG activity, indicative of cortical hyperexcitability, was more frequent in LQT2 (and LQT1) individuals than healthy controls (Haugaa et al., 2013). A mechanism for hERG1 involvement in seizure susceptibility of temporal lobe origin could be through impairment of its role in potassium homeostasis by hippocampal glia which would result in accumulation of $[K^+]_o$, known to be pro-epileptogenic (Emmi et al., 2000). Alternatively ERG channel inhibition was also shown to enhance stimulus-induced and neuronal excitability in the hippocampus (Fano et al., 2012).

Many first and second generation antipsychotics inhibit hERG1 (and when tested hERG3) at concentrations comparable to the affinities of these compounds for dopamine D_2 and 5-HT_{2A} receptors, the antagonism of one or both of which is believed to underlie therapeutic efficacy (Kang et al., 2001a; Kongsamut et al., 2002; Shepard et al., 2007). It raises the question of

[3] Though what role antiepileptic drugs have in sudden unexplained death in epilepsy is subject to debate (Hesdorffer and Tomson, 2013), they can block hERG at clinically relevant concentrations and reduce hippocampal ERG protein expression (Danielsson et al., 2005; Heo and Kang, 2012).

whether block of CNS I_{Kr} contributes to their therapeutic action or neurological side effects. One side effect is hyperprolactinaemia which though attributed to dopamine receptor antagonism, dopamine being an inhibitor of prolactin secretion, may also result from block of native lactotroph I_{Kr} (Bauer et al., 1999, 2003; Cookson et al., 2012).

An increased incidence of schizophrenia and related intermediate phenotypes (cognitive deficits, hippocampal volume reduction and inefficient information processing in healthy controls) was shown to be significantly associated with SNPs within intron 2 of *hERG1* (Huffaker et al., 2009). For the one SNP tested subsequently in other populations these associations were confirmed (Atalar et al., 2010; Hashimoto et al., 2011). The SNPs promote transcription from an alternative start site and the expression of the hERG1-3.1 isoform (Huffaker et al., 2009). As this is a primate brain specific isoform with markedly faster deactivation kinetics a role in normal human higher order cognition is suggested where sustained high-frequency firing patterns, as seen in prefrontal cortex neurones, may be vital. Also hERG1a mRNA, unlike hERG1b, is decreased in individuals with schizophrenia so that the ratio of hERG1-3.1 to hERG1a is 2.5-fold higher. This imbalance towards a more rapid deactivating current and consequent lessening of hERG1 current accumulation would give rise to an increase in spike frequency and conversion of firing patterns to non-adapting resulting in neuronal dysfunction consistent with the pathogenesis of the disorder. Furthermore whether the therapeutic effects of antipsychotics may be related in part to activity at hERG1-3.1 has been studied. The *hERG1* genotype impacts on antipsychotic treatment response, with those genotypes associated with schizophrenia and expression of hERG-3.1 exhibiting greater efficacy in terms of positive syndrome and general psychopathology ratings (Apud et al. 2012). This is particularly in response to risperidone in the setting of slow metabolism (Heide et al., 2016). Moreover unlike other antipsychotic drugs, risperidone inhibits hERG1-3.1 to a greater extent than hERG1a (IC_{50} 220 nM vs 508 nM).

2.4.2 SMOOTH MUSCLE, ENDOCRINE AND OTHER CELLS

I_{Kr} contributes significantly to the resting membrane potential (V_{rest}) of smooth muscle as this potential (from ~-60 to -40 mV) falls within the range of potentials where the channels are open (the overlap of activation and inactivation curves) conducting window current. Thus hERG block has been shown to depolarise the membrane potential and increase contractility of smooth muscle from the opossum oesophagus (Akbarali et al., 1999), rat stomach (Ohya et al., 2002b), human jejunum (Farrelly et al., 2003), guinea pig gallbladder (Parr et al., 2003), mouse hepatic portal vein (Yeung and Greenwood, 2007) and bovine epididymus (Mewe et al. 2008).

Dynamic regulation of I_{Kr} function is clearly illustrated by uterine smooth muscle. In the myometrium of non-pregnant mice or late pregnant humans I_{Kr} block and activation increases and inhibits respectively contractility (Greenwood et al., 2009; Parkington et al., 2014). These effects are absent or blunted in the myometrium of late pregnant mice and in-labour humans, coinciding with a significant reduction in I_{Kr} amplitude and no change in ERG1 but a marked increase in KCNE2 protein expression. Furthermore in mice post partum (within 8 hrs), the myometrial I_{Kr} sensitivity was regained. In humans the loss of I_{Kr} in-labour likely contributes to the change in action potential pattern, from plateau-type to a long duration bursting form, which is necessary for parturition (Tong et al., 2014). The absence of this I_{Kr} dynamism in obese laboring women and resultant suppression of contractile strength may explain the higher prevalence of labour progression failure and consequent need for caesarean delivery (Parkington et al., 2014).

I_{Kr} does not contribute to the V_{rest} of human pancreatic β-cells, which is ~-60 mV, but blockade induces hyperexcitability of β-cells, with an increase in firing frequency, in response to glucose or arginine leading to greater islet insulin secretion (Rosati et al., 2000). Likewise in mouse β-cells selective inhibition of ERG1, by rBeKm1, enhanced the glucose stimulated increase in intracellular calcium and secretion of insulin (Hardy et al., 2009). ERG1 current, though half the density found in β-cells, was also present in mouse α–cells where its inhibition impaired glucagon secretion probably through membrane potential depolarisation leading to inactivation of sodium and calcium channels.

I_{Kr} is present in rat adrenal chromaffin cells in particular, according to the selective expression of ERG1 protein, the adrenaline cell type (Gullo et al., 2003). Inhibition of I_{Kr} resulted in depolarisation and an increase in firing frequency which led to an increase in cytosolic calcium levels and induction of catecholamine release from cells. It is possible that loss-of-function mutations in *hERG1* could therefore give rise to a disproportionate circulating adrenaline levels.

2.4.3 CANCER

Following the initial report of high levels of hERG1 mRNA and I_{Kr} in 17 human and murine cancer cell lines of distinct histogenesis (Bianchi et al., 1998), hERG1 has been found to be ectopically or overexpressed in various human tumour cell lines and primary cancers (Cherubini et al., 2000; Crociani et al., 2003; Feng et al., 2014; Lastraioli et al., 2004; Pillozzi et al., 2002; Shao et al., 2008; Smith et al., 2002). For example, 67 and 82 % of primary human endometrial cancer tissue expressed *hERG1* mRNA and protein respectively giving rise to functional I_{Kr} as compared to 18 % of normal endometrium and an absence in endometrial hyperplasia (Cherubini et al., 2000). Likewise no expression was detected in normal colonic mucosa or adenomas whereas in human colorectal cancers hERG1 protein was expressed in ~60 % of primary tumours with the highest incidence in metastatic cancers (Lastraioli et al., 2004). Here, as in gastric cancer (Crociani et al., 2014), it is the hERG1a transcript that is overexpressed, however in leukaemias and other cancers it is the hERG1b transcript (Crociani et al., 2003; Erdem et al., 2015; Pillozzi et al., 2007). A similar association to aggressiveness was found with glioma, the more malignant the glioma the greater the hERG1 transcript, protein and I_{Kr} expression (Masi et al., 2005), and with other cancers e.g. gastric cancer and melanoma (Arcangeli et al., 2013; Shao et al., 2008). Thus hERG1 expression could serve as a biomarker for diagnostic and prognostic purposes (Ding et al., 2010; Dolderer et al., 2010; Lastraioli et al., 2012, 2015). Furthermore the aberrant expression of hERG1 may also indicate a precancerous phenotype (Dolderer et al., 2010; Lastraioli et al., 2006).

Ectopic expression of hERG1 is sufficient to induce cellular transformation thus transfected mouse fibroblasts lose contact inhibition, change morphology, migrate more rapidly and demonstrate anchorage-independent proliferation (Pier et al., 2014). But how is hERG1 ectopically or overexpressed in cancer? Overexpression in gastric cancer cell lines is suggested to be attributable to the increased stability of hERG1a mRNA (Crociani et al., 2014). Alternatively expression of hERG transcript may be augmented at the protein level due to downregulation of miRNAs as suggested by glioblastoma multiforme (a high-grade astrocytoma) and pancreatic cancer samples (Feng et al., 2014; Wang et al., 2015).

The overexpression of the hERG-USO isoform in human primary samples and cell lines of neuroblastoma and leukaemia provides a modulating mechanism for the optimal expression of hERG1 by tumour cells (Guasti et al., 2008). Overexpression of hERG1a can induce cell

differentiation and promote apoptosis[4] (Guasti et al., 2008; Han et al., 2004; Wang et al., 2002), the hERG-USO isoform by decreasing the hERG1 current density, due to ER retention, maintains an appropriate functional level and the neoplastic phenotype (Guasti et al., 2008). Consistent with this cisplatin was found to increase hERG1 expression in human gastric cancer cells, an action essential for the drug to induce apoptosis (R. Zhang et al., 2012). It is also noteworthy that *hERG* expression can be downregulated in cancer as its promoter becomes methylated, this epigenetic aberration occurs in a significant percentage of patients with various types of non-Hodgkin lymphoma (Bethge et al., 2013).

A functional role of hERG1 in cancer cells was first demonstrated when leukaemia cell lines were exposed to hERG blockers and their proliferation rate was reduced (Pillozzi et al., 2002; Smith et al., 2002). This result was subsequently repeated in various cancer cell lines, but not all (Masi et al., 2005; Roy et al., 2008), and suggests the role of hERG1 in cell cycle progression exhibits a dependency on the tissue origin of the neoplasm. Thus while hERG1 function is important in leukaemia cells as well as pancreatic and gastric cancer cells for G_1/S transition, since cells accumulate in G_1 phase with block, for ovarian cancer cells it is elsewhere in the cycle, as cells accumulate in S and G_2/M phase (Asher et al., 2011; Feng et al., 2014; Li et al., 2007; Shao et al., 2005, 2008).

The proliferative potential of cell types is correlated to the V_{rest}. Cycling cells have a depolarised V_{rest}, however the relationship of membrane potential to cell cycle stages is more complex. Between -30 and -50 mV, the V_{rest} of cancer cells (Bianchi et al., 1998), the hERG1 window current is substantial and so in high resistance cells may contribute significantly to V_{rest}. For neuroblastoma cells that is certainly the case, so during the S phase of the cell cycle there is a depolarisation of membrane potential (from -31 mV in G_1 to -25 mV) due to an oscillation in hERG1a and hERG1b isoform expression (Arcangeli et al., 1995; Crociani et al., 2003). The decrease in the hERG1a:hERG1b ratio in S phase causes a shift of the activation curve and consequently the window current occurs at more depolarised values. The mechanism by which hERG1 modulates mitotic cycle progression of primary myeloid leukaemia cells as well as leukaemia cell lines is though different with hERG1 not, at least always, contributing to the V_{rest} (Crociani et al., 2003; Crottès et al., 2011; Pillozzi et al., 2007). Moreover though hERG1 contributes to the V_{rest} of small cell lung carcinoma cell lines it is not this but the channel protein itself independent of ion flux that influences proliferation (Glassmeier et al., 2012).

As expected from the clinical data regarding acquisition of an invasive/metastatic phenotype, hERG1 plays a role in tumour progression. Inhibition of hERG1 decreases the invasiveness of colon and gastric cancer cells for example, with the amount of hERG protein and I_{Kr} correlating with invasion capacity (Lastraioli et al., 2004; Shao et al., 2008). Interestingly the migration of cells of an anaplastic thyroid cancer and a melanoma cell line were also reduced by hERG inhibition but their hERG channels were non-conducting (Afrasiabi et al., 2010; Asghar et al., 2012). In glioblastoma multiforme hERG1 activity is related to neoangiogenesis rather than proliferation (Masi et al., 2005). Inhibition of hERG1 specifically decreased the secretion of vascular endothelial growth factor (VEGF), via *VEGF* transcription levels. Thus here hERG1 contributes to malignancy by promoting the secretion of angiogenic factors from glial tumour cells.

[4] The hERG1-apoptosis relationship is not simple. Block of hERG1 can also induce apoptosis of HEK-hERG cells and gastric cancer cells but not of leukaemia or ovarian cancer cells (Asher et al., 2011; Li et al., 2007; Pillozzi et al., 2002; Shao et al., 2005, 2008; Thomas et al., 2008).

The pleiotropic effects exerted by hERG1 often result from its recruitment by cell adhesion molecule β_1 integrin into multiprotein membrane complexes, within which hERG1 physically and functionally interacts with the partners (Pillozzi and Arcangeli, 2010). Engagement of β_1 integrin as when a human neuroblastoma cell or colorectal cancer cell makes contact with laminin or fibronectin results in an increase in hERG current, which for the former cell is mediated by a pertussis toxin-sensitive G-protein and induces neurite emission (Crociani et al., 2013; Pillozzi and Arcangeli, 2010). It is believed though that hERG1 channels modulate the partner protein(s) through voltage-dependent conformational coupling rather than through alterations of ion conductance i.e. ion-independent signaling (Pillozzi and Arcangeli, 2010).

In epithelial cells, such as colorectal cancer cells, the β_1/hERG1 complex is translocated into caveolae/lipid rafts and recruits FAK, a cytoplasmic tyrosine kinase (TK), the small GTPase Rac1 and the p85 subunit of PI3K (Crociani et al., 2013; Pillozzi and Arcangeli, 2010). This recruitment is dependent on hERG1 activity as it is impaired by hERG inhibition. The function of the complex is to trigger angiogenesis and modulate cell invasiveness following adhesion.

Two more types of these integrin/hERG1 complexes have been identified. In activated normal haematopoietic precursors and acute myeloid leukaemia cells, where hERG1b is more prevalent than hERG1a, a complex occurs consisting of β_1 integrin, predominantly hERG1b and VEGF receptor 1 (Pillozzi et al., 2007). Both integrin activation and hERG1 activity modulate the activation of VEGFR-1, a TK receptor, which subsequently activates the mitogen-activated protein kinase (MAPK) and PI3K/Akt signalling pathways. This complex regulates VEGF induced leukaemia cell migration, the importance of which was shown in a murine model as exit from bone marrow spaces into the bloodstream and invasion into extramedullary organs. Furthermore angiogenesis was present within the bone marrow attributable to the autocrine production of VEGF by leukemia cells which is hERG1 dependent.

In acute lymphoblastic leukaemia cells the complex incorporates the chemokine receptor CXCR4 (Pillozzi et al., 2011). Leukaemia cells are protected from chemotherapy induced apoptosis by bone marrow mesenchymal cells. These cells give rise to the formation of the complex through both the interaction of their vascular cell adhesion molecule-1 with the β_1 integrin and the release of the CXCR4 ligand stromal-derived factor 1α (SDF-1α)[5]. The assembly activates the $ERK_{1/2}$ and the PI3K/Akt pathways which phosphorylate substrates regulating apoptosis producing antiapoptotic effects. The activation of these kinases and the resulting chemotherapy resistance is dependent on the activity of hERG1 within the complex as evident from hERG block reducing their phosphorylation and potentiating the proapoptotic effects of chemotherapeutics *in vitro* and *in vivo* models.

The tumour microenvironment, due to imperfect angiogenesis and uncontrolled proliferation, is characterised by hypoxia and this is a key element of tumour progression which, through expression of hypoxia-inducible factor (HIF) dependent genes, drives towards a more aggressive phenotype and mediates therapy resistance (Arcangeli, 2011). HIF dependent genes include those triggering angiogenesis such is VEGF, which is functionally linked to hERG1 (Crociani et al., 2014; Masi et al., 2005; Pillozzi et al., 2007). In colorectal cancer cell lines the β_1 integrin-hERG1-PI3K complex was shown to modulate the expression of HIFs, via Akt and NF-κB, and consequently VEGF transcription and secretion (Crociani et al., 2013).

[5] SDF-1α also induces proliferation and migration of leukemia cells with both processes mediated by hERG1 (Li et al., 2009; Zheng et al., 2011). SDF-1α increases hERG1 current when applied acutely but also increases *hERG1* transcription.

Thus the hERG1 conductance via this pathway can determine the growth and angiogenesis within colorectal cancer cell derived tumours *in vivo* as well as their metastatic spread. Hypoxia also effects hERG1, the window current of neuroblastoma cells is markedly increased due to a large hyperpolarising shift in the activation curve (Fontana et al., 2001). This may counterbalance the hypoxia associated depolarisation of V_{rest} that occurs with Na^+/K^+ pump failure from ATP shortage. Likewise an increase in hERG1 conductance resulting from hyperkalaemia in the ischaemic environment may also confer another selective advantage by hERG1 (Bianchi et al., 1998). Into the microenvironment cancer cells shed microvesicles which can influence, amongst other things, infiltration and cancer progression as in the case of leukaemia cells they can induce their chemotaxis and enhance their adhesion to endothelial cells (Zheng et al., 2012a). The shedding of microvesicles from leukaemia cell lines is modulated by hERG1, moreover microvesicles express hERG1 on their surface and when incubated with leukaemia cells increased their expression of hERG1.

Thus overall hERG1 would seem a target for cancer therapy, though the concomitant risk of proarrhythmia is a hurdle to such an approach (Crociani et al., 2014; Pier et al., 2014; Pillozzi et al., 2011; Wulff et al., 2009). A proposed risk mitigation strategy is hERG1 isoform selective inhibition i.e. targeting hERG1b for treatment of leukaemias where it is the predominant isoform (Gasparoli et al., 2015). The risk/benefit profile of anticancer drugs is of course very different to those drugs used to treat benign conditions e.g. arsenic trioxide, TK inhibitors (Yeh and Bickford, 2009). Though the first in class of these inhibitors imatinib (Gleevec) has little cardiotoxicity, the other standard first-line therapies of newly diagnosed chronic myeloid leukemia dasatinib and nilotinib carry a warning and "black box" warning respectively in their prescribing information of the risk of QT prolongation and sudden death (Yeh and Bickford, 2009; Raschi and De Ponti, 2012). Interestingly the underlying mechanism is through their TK inhibition directly or indirectly down-regulating PI3K signalling which then affects multiple cardiac ion currents with the decrease in I_{Kr} and increase in $I_{Na\text{-}L}$ of particular importance (Lu et al., 2012). Imatinib did not decrease PI3K activity or prolong APD. These effects on canine cardiomyocytes were not immediate, though all three drugs have also been shown to acutely block hERG1 all be it e.g. imatinib at ≥100-fold its FTPC (Dong et al., 2013; Freebern et al., 2007). However imatinib, when incubated at concentrations approximating FTPC with cells of a leukemia cell line, downregulated hERG1 transcription and consequently protein levels (Zheng et al., 2012b). This was accompanied by a decrease in the proliferation and an increase in the apoptosis of the cells as well as a down-regulation of VEGF mRNA and secretion by them. All of these effects, as noted earlier, could be hERG1 mediated so inhibition of hERG1 may contribute to the action of imatinib.

Inhibition of hERG1 may also treat some collateral effects of cancer (Wulff et al., 2009). Skeletal muscle atrophy occurs as a consequence of cancer, other diseases, muscle damage or disuse and aging. In the atrophic skeletal muscle of tumour bearing or limb disuse mice expression of mERG1a protein is upregulated (Wang et al., 2006). mERG1a channel function induced atrophy through an increase in ubiquitin-proteasome proteolysis resulting from the transcription and translation of *Murf1,* which encodes a ubiquitin E3 ligase (Pond et al., 2014; Wang et al., 2006).

3. AIMS OF THE STUDY

The overall aim of this study was to examine different aspects of hERG1 potassium channel functioning, specifically:

I. To investigate the effect of a novel gastrointestinal prokinetic, prucalopride, on hERG1 and hERG1 K897T.

II. To characterise both *in vitro* and *in vivo* the effect of the common hERG1 polymorphism K897T.

III. To elucidate the effect of the endogenous sphingolipid ceramide on hERG1.

IV. To characterise the cardiomyocytes derived from the induced pluripotent stem cells (iPSCs) of a relatively asymptomatic LQT2 patient with a normal QTc interval.

4. METHODS

Cell culture (**I**, **II**, **III**, **IV**)

Human embryonic kidney (HEK 293) and African green monkey kidney (COS-7) cells were transiently transfected (by either lipid reagent or calcium phosphate precipitation methods) with hERG1a WT or K897T cDNA in a pcDNA3 plasmid. Enhanced green fluorescent protein cDNA was also transfected in electrophysiological studies as a marker. Experiments were performed 36-72 hours later. In **III** a stably expressing hERG1a-HEK cell line (generated by G418 antibiotic selection) was used.

In **IV** iPSCs were generated from the primary fibroblasts of a 61-year old male with a R176W mutation of hERG1 by infection with retroviral vectors containing *OCT3/4*, *SOX2*, *KLF4* and *MYC* (as Takahashi et al., 2007). Two patient-specific cell lines were established (UTA.00514.LQT2 and UTA.00525.LQT2). A number of control cell lines were used: the human embryonic stem cell (hESC) line H7 and the iPSC lines FiPS 6-14, UTA.00112.hFF, UTA.01006.WT and UTA.04602.WT. The pluripotency of the iPSC lines was demonstrated at the mRNA (by RT-PCR) and protein (by immunocytochemistry) levels as well as by markers for and tissues from the three germ layers in respectively embryoid body derived cells and teratomas. iPSCs and H7 hESCs were differentiated to cardiomyocytes by co-culturing with the mouse visceral endodermal-like END-2 cells. Subsequently the beating areas were mechanically excised and cells dissociated by collagenase A treatment.

Patch-clamp recordings (**I**, **II**, **III**, **IV**)

Patch-clamp electrophysiology is the gold-standard assay for detailed and quantitative mechanistic insight of ionic currents (Hamill et al., 1981; Neher and Sakmann, 1976), including I_{hERG} (Goineau et al., 2012; Pollard et al., 2010; Priest et al., 2008). In manual patch-clamp a glass pipette is placed on the surface of a single cell and, with suction, a high resistance seal (a giga-ohm seal) is formed enabling the recording of membrane currents through the ion channels in the enclosed patch of membrane (e.g. Fig. 1). From the initial cell-attached configuration other configurations including whole cell, where the membrane patch is ruptured resulting in cytosolic access, may be obtained (Hamill et al., 1981). In whole cell patch-clamp, facilitated by the low resistance electrical access, membrane currents of the entire cell can be recorded. Studies **I**, **II** and **III** in this work have used this configuration in voltage-clamp mode, with an EPC-9 amplifier and Pulse/Pulsefit software, on either fluorescent or stably expressing attached cells at room (22-24 ^{o}C) temperature. Additional studies were also performed at physiological (~36 ^{o}C) temperature. The patch pipettes had resistances of 1.5 to 4 MΩ when filled with a solution containing (in mM): KCl 150, $MgCl_2$ 2, BAPTA 5, HEPES 10 and MgATP 5 (pH 7.2 with KOH). The extracellular solution contained (in mM): NaCl 150, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1 and HEPES 5 (pH 7.4 with NaOH). Capacitance and series resistance were compensated, the latter by ≥70 %. The voltage protocols used are described in the individual studies and detailed in the figures or legends here. The simulated cardiac ventricular-like waveform had an amplitude of 115 mV, from holding potential of −80 mV, and an APD_{90} of ~350 ms. It should be noted that action potential waveform differences, between regions/layers, results in varying hERG current profiles (Lu et al., 2001).

In **IV** to preserve the intracellular milieu of the cardiomyocytes the perforated patch technique was employed. Specifically the antibiotic amphotericin B (0.24 mg/ml) was included in the pipette solution, the resulting pores in the membrane patch are permeable to small monovalent ions, but not to large ions or molecules, and permit electric access to the cell (Rae

et al., 1991). Voltage-clamp (measuring I_{Kr} as the 1 µM E-4031 sensitive current) and current-clamp (for action potentials) recordings from spontaneously beating dissociated cells were conducted with an Axopatch 200B amplifier and pClamp 9.2 software at 36 °C. A coverslip with cells was placed in the recording chamber and perfused with extracellular solution consisting of (in mM) 143 NaCl, 4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 5 glucose, 10 HEPES (pH 7.4 with NaOH; osmolarity adjusted to 301 ± 3 mOsm). The pipette solution consisted of (in mM): 122 K-gluconate, 30 KCl, 1 $MgCl_2$, 5 HEPES (pH 7.2 with KOH; osmolarity adjusted to 290 ± 3 mOsm). Additionally, using cardiomyocytes derived from the FiPS 6-14 and UTA.00514.LQT2 cell line, I_{Kr} was also recorded with the whole cell patch-clamp configuration at room temperature in isotonic caesium conditions for isolation (Zhang, 2006). The extracellular solution contained (in mM): 135 CsCl, 1 $MgCl_2$, 10 glucose, 10 HEPES and 10 µM nifedipine (pH 7.4 with CsOH; osmolarity adjusted to 301 ± 3 mOsm). The intracellular solution contained (in mM): 135 CsCl, 1 $MgCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with CsOH; osmolarity adjusted to 290 ± 3 mOsm).

Field potential recordings (**IV**)

Cardiomyocyte aggregates were plated onto microelectrode arrays and field potentials were subsequently recorded with a Multi Channel Systems platform at 37 ºC. The field potential duration and beating frequency were determined with AxoScope software.

Western Blot (**II**, **III**)

Membrane fractions of cells were isolated (as Zhou et al., 1998a), subjected to SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The separated proteins were incubated with primary (anti-hERG1, against a distal C-terminal epitope) and secondary (anti-rabbit) antibodies.

Immunoprecipitation (**III**)

Cell lysates were incubated overnight with anti-hERG1, the resulting immunocomplexes were precipitated using protein G-Agarose and detected by Western blotting with anti-ubiquitin and anti-HERG antibodies.

Labelling of cell surface proteins (**III**)

Extracellularly exposed lysine residues of cell membrane proteins were tagged with the amine reactive biotinylating reagent Sulfo-NHS-SS-biotin. The cells were lysed, hERG1 protein immunoprecipitated and subjected to SDS-polyacrylamide gel electrophoresis. The hERG1-biotin complex was detected with streptavidin conjugated horseradish peroxidase.

Metabolic labelling (**III**)

Newly synthesised protein was labelled with [^{35}S]methionine and [^{35}S]cysteine and chased for up to 24 hours with unlabelled methionine and cysteine. At various time points cells were lysed and hERG1 protein immunoprecipitated. Following SDS-polyacrylamide gel electrophoresis the ^{35}S- labelled hERG proteins was detected by autoradiography.

Immunocytochemistry **(III, IV)**

In **III** cells on coverslips were fixed, permeabilised and incubated overnight with anti-hERG1 (± anti-Lamp-1) antibody. After blocking of unspecific sites, cells were stained with goat anti-rabbit FITC-conjugated (± anti-mouse Cy3-conjugated) secondary antibody. The

subcellular distribution of hERG was determined by confocal microscopy using NIH-image computer program.

In **IV**, cells were fixed a week after dissociation following differentiation and the expression of cardiac proteins (with anti-cardiac troponin T, anti-α-actinin and anti-connexin-43) assayed. A larger set of cardiac markers, including hERG, was studied at the mRNA level with RT-PCR.

Patients and their evaluation (**II**)

The DNA samples and clinical data of 261 LQTS patients (carriers of KCNQ1 G589D) were available. 53 individuals underwent an exercise stress test with a bicycle ergometer. The QT intervals of these exercise ECGs were measured from the onset of the QRS complex to the end of the T-wave defined by the tangent method and corrected for heart rate with Bazett's formula.

Genetic analysis (**II**, **IV**)

The determination of the hERG K897T SNP and R176W mutation status was by a variant specific test of PCR amplified genomic DNA using a primer-induced restriction assay. In **IV** karyotype analysis of the cell lines was performed.

Statistical Analysis (**I**, **II**, **III, IV**)

The student's t-test (paired and independent data) and analysis of variance for multiple comparisons was used. $p<0.05$ was considered significant. The difference in field potential durations between different aggregate populations was determined by nonlinear regression analysis.

5. RESULTS & DISCUSSION

5.1 PRUCALOPRIDE AND THE hERG1 CHANNEL (I)

PRUCALOPRIDE AND ITS CARDIAC SAFETY

Drug-induced LQTS is an issue of considerable importance for both clinically used drugs and those in development (Pollard et al., 2010; Wallis, 2010). Due to the extremely low incidence of TdP and the lack of a better marker, QT interval prolongation though itself not a safety risk *per se* has been the only surrogate end-point acceptable to pharmaceutical regulatory agencies (Pollard et al., 2010). The evaluation of this index is enshrined in dedicated regulatory guidelines for both clinical (ICH E14), which defines the "thorough QT/QTc (TQT) study", and non-clinical (ICH S7B) studies (Anon, 2005a, b). The association with inhibition of the hERG1 channel is compelling enough that as part of the integrated risk assessment in ICH S7B an *in vitro* hERG assay[6] is recommended to best specifically ascertain the degree and nature of any interaction (Anon, 2005b; Wallis, 2010).

In the wake of cisapride-associated QTc prolongation and arrhythmias (Ahmad and Wolfe, 1995; Napolitano et al., 2000; Olsson and Edwards, 1992; Wysowski et al., 2001; Vitola et al., 1998), the cardiac safety of this therapeutic class- gastrointestinal prokinetics- has been scrutinised and extensively profiled (Beattie et al., 2013; Claassen and Zünkler, 2005; Drolet et al., 2000; Meyers and Hickling, 2007; Potet et al., 2001; Toga et al., 2007). Prucalopride, developed initially at Janssen Pharmaceutica NV of Belgium, is part of the next generation of prokinetics with a serotonin 5-HT_4 receptor agonism mechanism of action (De Maeyer et al., 2008). Unlike its progenitors metoclopramide (Primperan), cisapride (Prepulsid) and tegaserod[7] (Zelnorm) which suffer from a lack of 5-HT_4 selectivity, prucalopride is both potent (~10nM in binding and functional assays) and selective as evident from binding studies (>200-fold difference in affinity from other receptors) and *in vivo* where 5-HT_4 antagonist can block all its induced effects (Beattie et al., 2013; De Maeyer et al., 2008; Pindon et al., 2002). In healthy humans prucalopride, in contrast to cisapride, selectively stimulates colonic transit with little effect on gastric emptying or small bowel transit (Bouras et al., 1999). The facilitation of propulsive activity in the colon is believed to occur predominantly through activation of receptors on both the excitatory cholinergic and inhibitory nitrergic efferent neurones of the myenteric plexus enhancing the neuromuscular transmission (De Maeyer et al., 2008). Prucalopride was developed therefore for treatment of lower, unlike cisapride, GI tract hypomotility disorders.

In **I** the effect of acute application of prucalopride on hERG1 was investigated. Prucalopride exhibited concentration dependent inhibition of the hERG1 current (Fig. 8), both during the depolarising and repolarising steps of the voltage protocol. Thus the peak tail current amplitude

[6] In the drug discovery process the hERG assay provides the earliest experimental indicator of TdP liability and may then be used in the synthesis-screening cycle of the lead optimisation phase to enable a designing out of hERG activity (Pollard et al., 2010).

[7] Post-Vioxx the association of ischaemic cardiovascular events with tegaserod led the FDA to recommend its withdrawal after ~5 years on the market for irritable bowel syndrome and constipation (De Maeyer et al., 2008; Pasricha, 2007). This link has been disputed and the underlying mechanism is unknown (Beattie et al., 2013). Tegaserod was reintroduced later that year (2007) under a restricted access program but is now no longer available.

was reduced e.g. by 3 and 30 µM prucalopride to 0.62±0.05 and 0.23±0.02 (n=6 and 5) of that during control conditions. The hERG tail current IC_{50} value was 4.1 µM with a Hill coefficient of one, suggesting a drug-channel binding stoichiometry of one to one. Previously Potet et al. (2001) reported a hERG IC_{50} value of 5.7 µM for prucalopride using a step pulse voltage protocol with transiently transfected COS-7 cells. One notable difference between **I** and that study was the recording temperature (room temperature in **I** versus 35 ^{o}C; Potet et al., 2001). The comparable potencies would suggest that prucalopride block, like cisapride's (Kirsch et al., 2004; Walker et al., 1999; Windley et al., 2016), is temperature insensitive. The IC_{50} though of certain drugs, e.g. erythromycin, is influenced by temperature (Eap et al., 2007; Kirsch et al., 2004). According to the Committee for Medicinal Products for Human Use assessment report of prucalopride the IC_{50} from various studies using hERG-HEK was from 4.1 to 22 µM (European Medicines Agency, 2009). However no further information is available on this latter value. Also of note is a stated IC_{50} of 10 µM for guinea-pig ventricular myocyte I_{Kr}.

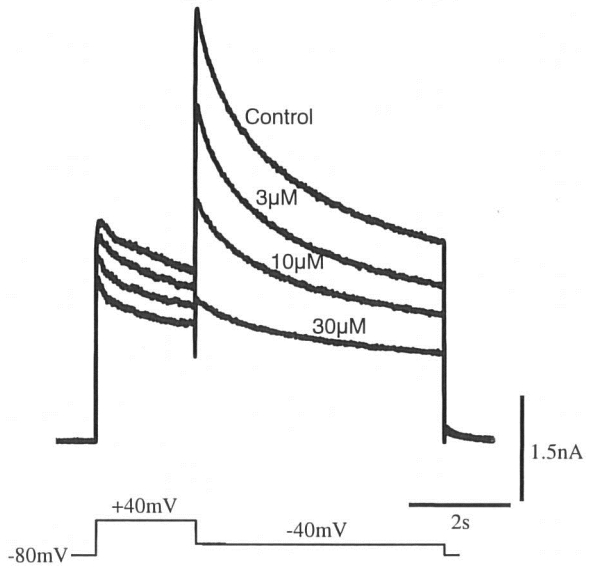

Figure 8. Concentration dependent inhibition by prucalopride of the hERG1a current of a transiently transfected HEK 293 cell. The step pulse voltage protocol used to elicit the hERG current is shown beneath.

Cisapride and domperidone, another, though chemically distinct, prokinetic with action via peripheral dopamine D2 receptor antagonism, are both potent acute hERG1 blockers. The hERG IC_{50} for cisapride is reported to be 10-26 nM (Crumb et al., 2016; Kirsch et al., 2004; Margulis et al., 2010; Walker et al., 1999; Windley et al., 2016), though these are overestimates as the nonphysiological voltage protocols negate its kinetics of block (Milnes et al., 2010; Potet et al., 2001). Domperidone (Motilium) has a hERG IC_{50} value of 57-162 nM (Claassen and Zünkler, 2005; Drolet et al., 2000) and, when intravenously administrated, has been associated with TdP (Redfern et al., 2003). Furthermore domperidone in epidemiological studies was shown to increase by 2.8-fold SCD leading to a reassessment of its risk-benefit ratio and questioning of its continued availability (Hondeghem, 2013).

In **I**, as extensively employed in academia and the pharmaceutical industry, homomeric hERG1a channels were used as a model for the cardiac I_{Kr} channel. Differential drug sensitivities between hERG1a and hERG1a/1b channels occur, though only 1.5 % and 8 % of compounds were reported to be more potent (>2-fold difference) against hERG1a and hERG1a/1b respectively (Abi-Gerges et al., 2011). Cisapride, like most of the diverse set of 50 compounds tested, exhibited similar IC_{50} values with homomeric hERG1a and heteromeric

hERG1a/1b channels. Furthermore the pharmacological sensitivity of heterologously expressed hERG1a seems unaffected by coexpression with WT KCNE1 (Guo et al., 2006; Ulens et al., 1999) or KCNE2 (Scherer et al., 2002; Weerapura et al., 2002). Another assumption often made in these *in vitro* studies (including **I**) is that the nominal concentration of the drug is equivalent to the actual concentration available to interact with hERG. For most compounds this is likely the case (Qu et al., 2011), however some compounds may be poorly soluble in the assay buffer or adhere to glassware and the perfusion system reservoirs/tubing thus reducing exposure and leading to underestimation of the potency towards hERG (Brimecombe et al., 2009; Margulis et al., 2010). Such adsorptive loss may occur with cisapride (Margulis et al., 2010), though as prucalopride is less hydrophobic this source of error is likely not problematic.

A hERG1 IC_{50} value is not sufficient to predict proarrhythmic potential (Mirams et al., 2011). The IC_{50} values of the antipsychotics clozapine and quetiapine (Hill et al., 2014; Kongsamut et al., 2002), the antidepressants imipramine and amitriptyline (Teschemacher et al., 1999) as well as citalopram (Witchel et al., 2002), and the antimalarial chloroquine (Traebert et al., 2004) fall within the same range (2-6 µM) and all are associated with QT interval prolongation/TdP (De Bruin et al., 2005; Raschi et al., 2013; Redfern et al., 2003; Sarganas et al., 2014; van Noord et al., 2009; Yap and Camm, 2003). Furthermore the early prokinetic metoclopramide, which has an IC_{50} of 5.4 µM, is also associated with LQTS/TdP (Claassen and Zünkler, 2005; Sarganas et al., 2014). Of the withdrawn drugs, the antitussive clobutinol and opioid agonist levacetylmethadol also have similar IC_{50} values while the fluoroquinolone antibiotic grepafloxacin has an IC_{50} of 50 µM (Bellocq et al., 2004; Kang et al., 2001b, 2003; Katchman et al., 2002). Of greater significance is the ratio between the hERG IC_{50} and the predicted (when in preclinical development) or measured maximal free therapeutic plasma concentration (van Noord et al., 2011; Wallis, 2010). The hERG IC_{50}/FTPC ratio (or hERG safety margin) for grepafloxacin was 16, for chloroquine is 6 and for cisapride was 2 (Kang et al., 2001b; Margulis et al., 2010; Traebert et al., 2004). From such data Redfern et al. (2003) recommended at least a 30-fold hERG safety margin as drugs with ratios below this have a high torsadogenic potential. Subsequent similar retrospective studies have likewise demonstrated a higher risk, possibly 3-4 times, of serious ventricular arrhythmias and sudden death when a ratio of 30 was taken as a cut-off point for drugs (De Bruin et al., 2005; van Noord et al., 2011). Unsurprisingly the predictivity of TdP risk is substantially improved by an evaluation that also incorporates I_{Na} and I_{CaL} drug block (Kramer et al., 2013; Mirams et al., 2011). The relative potency, which negates the need for FTPC, of a compound on hERG1 versus $Ca_V1.2$ is seen as a major determinant (Kramer et al., 2013).

At 2 mg orally once daily prucalopride increases the frequency of spontaneous complete bowel movements in chronic constipation patients, as well as decreasing the related symptoms and improving patient quality of life (Camilleri et al., 2008; Quigley et al., 2009; Tack et al., 2009). In healthy humans repeated dosing at this therapeutic level resulted in the steady-state plasma concentrations fluctuating between trough and peak values of 2.5 and 7 ng/ml, equivalent to 6.8 and 19.0 nM (Van de Velde et al., 2008). The plasma Cmax value after administration of a single prucalopride dose was 4.34 ng/ml (Frampton, 2009). Drugs can bind to plasma proteins in the circulation, so reducing the available drug concentration and consequently potency towards hERG (Margulis et al., 2010). For drugs which exhibit high plasma protein binding the calculated free fraction may not correlate with hERG1 potency and clinical QTc changes (Kongsamut et al., 2002; Margulis et al., 2010). Prucalopride however has low plasma protein binding (28-33 %; Van de Velde et al., 2008), the free peak plasma concentration after repeated dosing is calculated to be 12.7-13.7 nM resulting in a hERG

IC_{50}/FTPC margin of 299-323. Therefore in regard to direct hERG1 block by prucalopride the relative risk of serious ventricular arrhythmias and sudden death is expected to be negligible (De Bruin et al., 2005).

With the adoption of ICH E14 another clinical outcome a QTc increase of ≥5 ms, which is a level of regulatory concern, has emerged (Gintant, 2011; Wallis, 2010). Analysis of TQT studies has demonstrated that QTc prolonging drugs are 7 times more likely to have a hERG safety margin less than 30, within this margin there was moderate confidence in predicting the QTc risk (Gintant, 2011). Elsewhere it has been suggested that for dofetilide a QT interval prolongation in humans of 20 ms corresponds to 10 % inhibition of hERG, thus the threshold QT change may be more accurately predicted by the hERG IC_5 or IC_{10} value (Wallis, 2010). These values for prucalopride are 0.22 and 0.46 µM which is 16-36 fold above the FTPC, where a cut-off of twofold gives both sensitivity and specificity in predicting the outcome of a clinical QT study. Thus prucalopride should be negative in the TQT study. However the translation of hERG1 safety margins, even in conjunction with data from *in vitro* APD assays and *in vivo* QT studies obtained during the drug discovery process, to human clinical QT studies is imperfect (Gintant, 2011; Trepakova et al., 2009; Wallis, 2010). One confounding factor e.g. is that the most relevant pharmacokinetic parameter for interpretation of hERG data is the intracellular cardiac myocyte concentration not FTPC, unless there is only passive diffusion of the drug and equilibrium is reached (Michaud and Turgeon, 2013). The heart however expresses some CYP isozymes and various drug influx and efflux transporters so differential accumulation of drugs, including cisapride, occurs in the myocardium (McBride et al., 2009; Michaud and Turgeon, 2013; Sugiyama and Hashimoto, 1998; Titier et al., 2004). It should be noted that human atria and ventricles express 5-HT_4 receptors, but only at very low density, and activation could potentially be arrhythmogenic as 5-HT via cAMP increases I_{CaL} that may lead to Ca^{2+} overload (De Maeyer et al., 2008). However in this low efficacy system prucalopride behaves as a partial agonist, whereas in other tissues it is a full agonist, giving a smaller I_{CaL} (EC_{50}=0.2 µM) and APD_{50} increase, and antagonising the effect of the endogenous ligand (Chai et al., 2012; De Maeyer et al., 2008; Pau et al., 2005).

The focus on hERG1 inhibition and QT prolongation, while proving successful in terms of identifying potential torsadogenic risk and abating drug withdrawals from the market, has likely resulted in errors, particularly false positives as hERG alone is not always sufficient and the sensitive QT interval is not specific, leading to unwarranted chemotype/drug attrition and labeling (Gintant, 2011; Kramer et al., 2013; Sager et al., 2014). To address this negative impact a shift in the testing paradigm was proposed that includes the Comprehensive in vitro Proarrhythmia Assay (CiPA) which should result in the revision of S7B (Sager et al., 2014). One component of CiPA, which takes into account multi-ion channel block and various proarrhythmic mechanisms, will require the testing of drug candidates against a recombinant ion channel panel consisting, initially at least, of hERG1 (I_{Kr}), KCNQ1+KCNE1 (I_{Ks}), K_V4.3+KChIP2 (I_{to}), K_{ir}2.1 (I_{K1}), Na_V1.5 (peak and late I_{Na}) and Ca_V1.2 (I_{CaL})(Fermini et al., 2016). The drug effects on the ion channel panel would then be incorporated in an *in silico* human ventricular action potential model and the potential proarrhythmic risk, e.g triangulation and EADs, elucidated. For cisapride it was recently shown that at ~50-fold FTPC little inhibition (<15 %) of any current except for I_{hERG} was exhibited (Table 2; Crumb et al., 2016). Of the 30 drugs assayed 70 % blocked (≥20 %) I_{hERG} at 3x FTPC while each of the other currents was blocked by ≤20 % of the drugs (Crumb et al., 2016). While the other potassium currents were rarely inhibited this is not always the case and thus may contribute to LQTS e.g. norfluoxetine, a metabolite of fluoxetine, which in addition to acute and trafficking hERG block

also blocks I_{Ks} (Rajamani et al., 2006; Veerman et al., 2013). Anyway without a doubt the hERG1 assay will continue to be fundamental in the assessment of proarrhythmic risk for a drug.

Only the effect of acute exposure to prucalopride was investigated in **I**. Direct and trafficking inhibition of hERG1 coexists (Rajamani et al., 2006; Takemasa et al., 2007) and does so for a large proportion of LQTS liable drugs (Wible et al., 2005). It is worth noting that cisapride, at up to 100 μM, has no effect on hERG1 trafficking (Rajamani et al., 2006; Wible et al., 2005). The effects of prolonged hERG1 inhibition may extend beyond LQTS to apoptosis with the consequent loss of cardiomyocytes leading to heart failure. The α_1-adrenoceptor antagonist doxazosin is associated with an increased risk of congestive heart failure and induces apoptosis of human cardiomyocytes *in vitro*. Thomas et al. (2008) showed that doxazosin (which has a hERG1 IC_{50} of 0.3 μM) induced in hERG1 expressing HEK cells, but not in untransfected cells, apoptosis. This hERG1 associated apoptosis can also be induced by amoxapine and desipramine, both of which additionally exhibit direct and forward trafficking block of hERG1 (Obers et al., 2010; Staudacher et al., 2011). The mechanism by which hERG1 inhibition promotes apoptosis and whether it extends to cardiomyocyte apoptosis and heart failure remains unknown.

An adequate cardiac safety profile for prucalopride was borne out in subsequent clinical studies. A TQT study, that included moxifloxacin 400 mg as a positive control, demonstrated no significant differences in the QTc interval in healthy volunteers between prucalopride, at either therapeutic or supratherapeutic (10 mg) doses, and placebo (Mendzelevski et al., 2012). The mean QTc change from baseline was <5 ms at all time points over the ≥24 hr period post final dose. The repeated dosing of 10mg resulted in a mean plasma prucalopride Cmax value of 42.7 ng/ml or 116.1 nM, thus a free concentration of 77.8-83.6 nM and a hERG safety margin of 49-53. Therefore even at this supratherapeutic dose the negative TQT study result (i.e. no effect on ventricular repolarisation) is not surprising. Furthermore two earlier placebo controlled Phase I cardiovascular safety studies in which repeated doses of up to 20 mg daily were given found, as in the TQT study, no QT intervals (Fredericia-corrected) of >500 ms or increases >60 ms (Boyce et al., 2009). In the three 12-week placebo-controlled Phase III studies that have established the efficacy of prucalopride in chronic constipation the incidence of prolonged QTc interval during the treatment period (with 2 and 4 mg/day) was low and similar to placebo (Camilleri et al., 2008; Quigley et al., 2009; Tack et al., 2009). No treatment-related ECG abnormalities or cardiac arrhythmias were found in the TQT or other studies, even when conducted in a potentially high risk patient population (Camilleri et al., 2009). Moreover some evidence of prucalopride's safety is offered by open-label, long-term (up to ~2 years) studies (Frampton, 2009).

Prucalopride (Resolor® from Shire; 1-2 mg/day orally) was approved in Europe, in October 2009, "for the symptomatic treatment of chronic constipation in women in whom laxatives fail to provide adequate relief". In June 2015 the marketing authorisation was extended from women to adults following a phase 3 trial in men where the efficacy and safety was similar to the earlier studies with predominantly female patients (Yiannakou et al., 2015).

PRUCALOPRIDE AND ITS MECHANISM OF BLOCK

The effect of prucalopride (at 10 μM) on hERG1a channel kinetics was investigated. Though the rate of activation was unaffected, deactivation was significantly slowed by exposure to prucalopride. Thus at a membrane potential of −70 mV the fast and slow components of deactivation were increased from means of 168 ± 12 ms and 874 ± 51 ms in control to 303 ± 35 ms and 1328 ± 91 ms ($p<0.05$ and $p<0.005$; n=6; the relative contribution of the fast deactivating current component was not significantly altered). A significant hyperpolarising shift of the half maximal activation potential was seen however as it was only -1.9 mV any physiological relevance is unlikely and a time-dependent effect (e.g. Ferreira et al., 2001; Rodriguez-Menchaca et al., 2014) cannot be excluded as the shift was not reversed on wash-out. Similar to a number of other drugs (see e.g. González et al., 2002; Scherer et al., 2008), prucalopride was shown to modify inactivation. Specifically prucalopride reversibly accelerated the rate of inactivation, particularly at less depolarised membrane potentials, so at 0 mV the time constant decreased from 5.8 ± 0.5 ms to 3.9 ± 0.4 ms ($p<0.005$; n=5; wash-out: 5.7 ± 0.4 ms). As a consequence the extent of inactivation was altered, being significantly increased at -20mV. This would likely augment the inhibitory effect of prucalopride by further reducing outward current during the plateau and repolarisation of the action potential. Moreover prucalopride also slowed the time constant of recovery from inactivation however this was only at unphysiologically negative membrane potentials (−100 to −120 mV).

The mechanism of block by prucalopride had not been detailed previously (or since). Prucalopride was found, as with most hERG1 blockers including cisapride (Milnes et al., 2010; Mohammad et al., 1997; Walker et al., 1999), to preferentially inhibit the activated (open/inactivated) states. This was initially indicated by the voltage dependence of block, where inhibition was significantly weaker at membrane potentials just positive to the threshold of activation i.e. −40 and −30 mV. This is opposite to the voltage dependence of the closed state hERG1 inhibitor BeKm-1's block (Milnes et al., 2003b; Restano-Cassulini et al., 2006). The state dependence of prucalopride was clearly shown in the response to a sustained depolarisation (to 0 mV, where at room temperature 99.9 % of hERG1 channels would be activated; Hill et al., 2014) where the initial outward hERG1 current was unchanged following prucalopride equilibration but subsequently block progressively developed (Fig. 9A). This was also evident in the data from the envelope of tail currents protocol used to determine the time course of activation. Depolarisation to +20 mV resulted in relatively little inhibition with durations up to 40 ms, reinforcing the view that inhibition of the closed state (and the requirement for a binding site distinct from that of the inner cavity, as with BeKm-1; Tseng et al., 2007) plays no significant part (Fig. 9B). After 40 ms the degree of blockade increased and achieved steady-state levels within 600 ms, the development of inhibition had a time constant of ~70 ms (n=3). Typically using a sustained depolarisation pulse voltage protocol, the kinetics of block onset have ranged from time constants of seconds for methanesulfonanilides to tens of milliseconds for bupivacaine, cocaine and ranolazine (González et al., 2002; Kamiya et al., 2006; Rajamani et al., 2008; Snyders and Chaudhary, 1996; Zhang et al., 2001). It should be noted that the kinetics of block exhibit a concentration dependency (Hill et al., 2014; Snyders and Chaudhary, 1996; Windley et al., 2016; Zhang et al., 2001). For prucalopride, at 10 μM, the principal time constant for the onset of block was 192 ms (Fig. 9A), this is comparable to that of chloroquine at 0mV with a concentration resulting in a similar fractional block (Sánchez-Chapula et al., 2002). The kinetics of prucalopride block are much quicker than that of cisapride block (at 22 ^{o}C with 200 nM which inhibited I_{hERG} ~80%: τ = ~4.7 s; Windley et al., 2016).

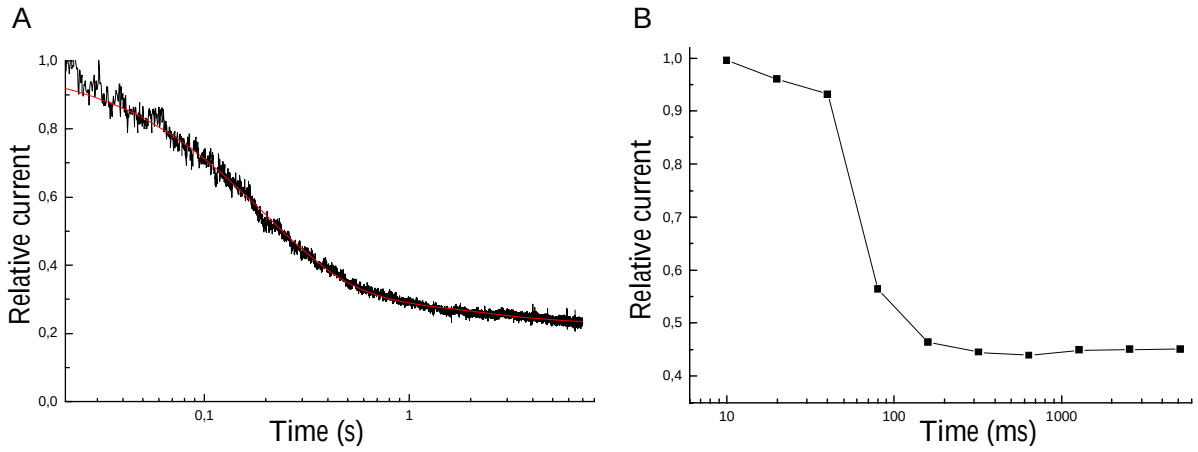

Figure 9. Time dependence of I_{hERG} blockade by prucalopride (10 µM). (A) Development of block over time during a depolarisation step to 0 mV (for 7 s from −80 mV). The data from **I** Fig. 6B replotted: the mean relative current (I_{Pru}/I_{Ctrl}) from 3 cells versus an abscissa of time with a logarithmic scale. The data was fitted with a double exponential (in red). (B) From an envelope of tail currents protocol the relative amplitude of tail currents is plotted as a function of the depolarising pulse duration. The cell was stepped from the holding potential of −80 mV to +20 mV for variable durations with a tail current recorded at −120 mV. The voltage protocols in A and B were first performed in control conditions and then following equilibration with prucalopride.

To clarify whether prucalopride can inhibit hERG1 channels in their inactivated state a voltage protocol with an initial depolarisation step to +80 mV was used. Upon stepping back to 0 mV there was scant evidence of any time dependent development of block as the channels reopen (**I** Fig. 6D). This implies that maximal inhibition occurred already at +80 mV where hERG1 channels are predominantly in the inactivated state. Analogous results with this same protocol are reported with e.g. the benzodiazepine midazolam, the antidepressant mianserin, the hypnotic zolpidem (Jehle et al., 2013; Scherer et al., 2008; Vonderlin et al., 2015). This is in marked contrast to the tricyclic antidepressant desipramine which blocks the open state with little or no affinity for the inactivated state (Staudacher et al., 2011). An interaction of prucalopride with the inactivated state was also evident from the channel kinetic changes, where a stabilisation of that state was indicated. According to Walker et al. (1999) cisapride caused an acceleration of the rate of inactivation and a negative shift in the voltage-dependence of steady-state availability, though in a later study conducted at 37 ^{o}C there was no effect seen (Milnes et al., 2010). Inactivation is reported to increase cisapride affinity for hERG by an estimated 7.8-fold compared to the open state (Perrin et al., 2008), however differential binding to the states is not evident in the kinetics of block and unblock (Windley et al., 2016). While binding of prucalopride may not be monophasic (see Fig. 9A) like for clozapine, which has two distinct kinetic components suggested to represent interaction with the open and inactivated states (Hill et al., 2014), such detailed analysis was not performed. What is clear is that prucalopride binds to hERG1a channels in the open and inactivated states.

As with block, the kinetics of hERG1 channel unblock are drug specific. In the maintained presence of prucalopride I_{hERG} rapidly recovered from block (τ of ~260 ms) upon hyperpolarisation. Prucalopride's dissociation rate from, i.e. lower affinity for, the closed states is similar to that of ranolazine, this is much quicker than cisapride (τ of ~6 s) but slower than cocaine (Milnes et al., 2010; Rajamani et al., 2008; Walker et al., 1999; Zhang et al., 2001).

The affinity of many hERG1 blockers for the activated states is evident as use dependence whether frequency dependent, e.g. as with cisapride and verapamil, or independent, e.g. as with E-4031 and terfenadine (Stork et al., 2007; Walker et al., 1999; Zhang et al., 1999). Underlying the use and frequency dependence of block is overlap of the stimulation protocol and rates with the rates of drug-channel interaction. Thus consistent with its rapid kinetics prucalopride does not exhibit at 0.1 and 1 Hz frequency or use dependence i.e. steady-state block is reached by end of each 300 ms test pulse to +40 mV with rapid dissociation from the channel during the interpulse period at −80 mV. A lack of use and frequency dependence of hERG block is similarly observed with ranolazine, cocaine and other compounds (Katchman et al., 2002; Rajamani et al., 2008; Scherer et al., 2008; Vonderlin et al., 2015; Zhang et al., 2001). The use of higher rates (up to 3 Hz) and a more physiological voltage waveform would also be of interest.

Overall this suggests that prucalopride is not trapped within the hERG1 inner cavity at rest by the activation gate like methanesulfonanilides and terfenadine (Kamiya et al., 2006; Mitcheson et al., 2000b; Stork et al., 2007), where block is not associated with a change in the deactivation rate and is practically irreversible. The time-dependent unbinding of prucalopride on repolarisation resulting in loss of inhibition and an increase in I_{hERG} may explain the apparent slowing of deactivation. A number of drugs e.g. bupivacaine, chloroquine, cocaine and ketoconazole also slow deactivation (Ferreira et al., 2001; González et al., 2002; Ridley et al., 2006; Sánchez-Chapula et al., 2002; Zhang et al., 2001). The slowing of hERG1 deactivation by untrapped, but not trapped, drugs with rapid binding kinetics has also been shown *in silico* (Di Veroli et al., 2014). Though cisapride is untrapped, its relatively slow dissociation from the channel means that it has no effect on the rate of deactivation (Milnes et al., 2010; Stork et al., 2007; Walker et al., 1999).

While the importance of potency at hERG1 and its relation to QT prolongation and TdP was expounded earlier, the mode of action (i.e. whether trapped), binding kinetics and state dependence of a drug may also play a part in proarrhythmia (Di Veroli et al., 2014; Fermini et al., 2016; Lee et al., 2016). Whereas slow binding/unbinding kinetics and the transient occupancy of the activated state during the cardiac cycle results in inhibition equilibrium never being reached, this is not the case when kinetics are rapid as block is achieved during depolarisation. Thus at IC_{50}, according to a simulation study, an untrapped drug with fast binding kinetics would prolong the APD at 1 Hz compared to one with slow kinetics by >4-fold (Di Veroli et al., 2014). This enhanced prolongation is similar to that found with the trapped mode, which is independent of binding kinetics. Another simulation study likewise demonstrated that rapid drug-channel interaction reduced I_{Kr} and delayed its peak resulting in greater APD prolongation than expected (Lee et al., 2016). For prucalopride, the kinetics of binding to and unbinding from hERG1 are unlikely then to provide any additional benefit to the substantial potency derived safety margin. It should be remembered that **I** was performed at room temperature. At physiological temperature the kinetics and drug-channel interaction may be different as recently shown for cisapride (Windley et al., 2016). A potential consequence of state dependency is differential sensitivity to heteromeric hERG1/I_{Kr} channels. The kinetic changes arising from the fewer eag domains in hERG1a/1b channels results in a reduction in inactivation and increased occupancy of the open state which decreases the affinity of E-4031 and dofetilide but increases the affinity of fluoxetine and ebastine (Abi-Gerges et al., 2011; Sale et al., 2008).

Though the molecular determinants (e.g. Mitcheson et al., 2000a) or even sideness (Crumb, 2014; Melgari et al., 2015; Zhang et al., 1999, 2001) of prucalopride binding to hERG1 were not investigated in **I** the results are consistent with the site being within the inner cavity as access, determined clearly by the activation gate, is critical for the association and dissociation of prucalopride from the channel. Prucalopride is an analogue of cisapride (Fig. 10) which, from mutagenesis studies, interacts with Thr623 and Ser624 at the base of the pore helix, via hydrogen bonding, and Tyr652 and Phe656 (Imai et al., 2009; Kamiya et al., 2008; Mitcheson et al., 2000a). For the aromatic residues their position in S6 and their nature, the hydrophobic volume of the 656 side chain and the aromaticity of the 652 side chain, as well as the interaction between them determines sensitivity to cisapride (Chen et al., 2002; Fernandez et al., 2004; Myokai et al., 2008). Furthermore as cisapride is asymmetric it interacts unequally with residues of the four subunits so cisapride interacts with Y652 residues, but not F656, from adjacent subunits (Dempsey et al., 2014; Imai et al., 2009; Myokai et al., 2008). Very recently an additional novel binding mode was proposed based on interactions of hERG blockers, including cisapride, with F557 of S5 where the drug is positioned immediately below the pore helix and protruding into the lateral fenestration between adjacent subunits (Saxena et al., 2016). The primary structural feature distinguishing prucalopride from cisapride is the loss of the fluorophenyl ring (‡ Fig. 10). This moiety, from docking in homology models, interacts with F557 or conventionally with F656 of the third subunit via π-π interaction (Imai et al., 2009; Saxena et al., 2016). This latter interaction is important in cisapride binding as the hERG IC_{50} is shifted from 10 nM in WT to 1.7 μM for the homotetrameric F656A mutant (Imai et al., 2009). The absence of this interaction in prucalopride binding is further indicated by unblocking kinetics which for cisapride are markedly faster with the F656A mutant (Myokai et al., 2008). Moreover the integrity of interaction with Y652 (the aromatic ring of prucalopride, denoted by bold asterisk for cisapride in Fig. 10) is suggested by the presence of voltage-dependence of block which is proposed to occur by depolarisation induced changes in the orientation of the residue (Myokai et al., 2008; Sánchez-Chapula et al., 2002, 2003).

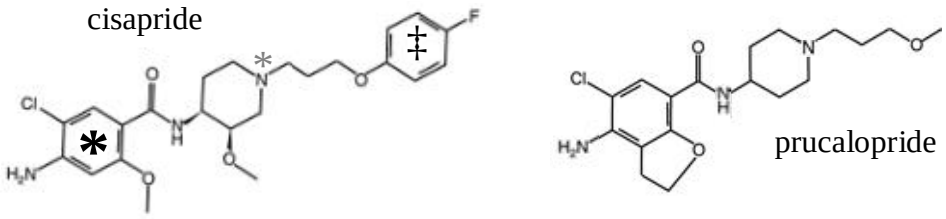

Figure 10. The structure of cisapride and prucalopride. From the structure-activity relationships of known hERG blockers structural features of drugs such as hydrophobic or aromatic groups radiating from a central nitrogen, the tertiary amine rendering compounds protonated at physiological pH, are predictive of hERG1 block (Aronov, 2008). The important features of this classic pharmacophore model as exhibited by cisapride, a benzamide derivative, are denoted. One strategy, as employed by the cisapride analog mosapride, to decrease hERG potency, but retain 5-HT_4 receptor activity, is to shorten the linker (between * and ‡**)** which affects the interactions of the drug's aromatic moieties (Durdagi et al., 2014; Potet et al., 2001). Prucalopride though differs and is the first representative of the dihydrobenzofurancarboxamide chemical class (De Maeyer et al., 2008). It is also less lipophilic (clogP 1.44 vs 3.81 for cisapride), a physicochemical property that correlates with hERG1 blockade potency (Aronov, 2008).

Other *in silico* studies have suggested more extensive cisapride-hERG interactions and alternate orientation of the bound cisapride so that the fluorophenyl ring interacts with Y652 (Dempsey et al., 2014; Durdagi et al., 2014; Stary et al., 2010). While also other structural differences between cisapride and prucalopride (conversion of methoxy to furan and/or loss of the other methoxy) may contribute to the shift in potency. It should be noted that significant effects on potency at and interactions with hERG1 are achievable by just single substitutions within a compound and by enantiomers e.g. with quinidine/quinine, and methadone (Eap et al., 2007; Karczewski et al., 2009; Sánchez-Chapula et al., 2003).

5.2 THE hERG1 K897T POLYMORPHISM (I, II)

Single-nucleotide polymorphisms (SNPs) are nucleotide changes that occur in >1 % of the population that may (nonsynonymous) or may not (synonymous) change the amino acid sequence of a protein. Even synonymous SNPs may have an effect e.g. large scale synonymous changes in hERG1 mRNA were shown to differentially affect translation rate and protein maturation/trafficking (Sroubek et al., 2013). SNPs generally do not result in overt disease but may contribute to the variable penetrance or expressivity of a disorder. For example the minor alleles of 3´ UTR SNPs in KCNQ1 act as modifiers of LQT1 phenotype depending whether they occur in *cis* or in *trans* with the mutation by decreasing the expression of either respectively the mutant or WT allele (Amin et al., 2012).

The C-terminus of hERG1a beyond the cNBD contains 16 SNPs as well as 5 SIDS-linked missense mutations and over 30 LQT2-linked missense mutations (C. Anderson et al., 2014). One of these SNPs results in the changing of the conserved positively charged lysine at residue 897 to an uncharged polar threonine (K897T; rs1805123) as adenine is substituted by cytosine at nucleotide 2690 in exon 11 (Iwasa et al., 2000; Laitinen et al., 2000). The K897T SNP is highly prevalent in European and other populations e.g. the minor allele frequency is 27 % in the French, 24 % in the German and 16 % in the Finnish and Indian populations (Bezzina et al., 2003; Gouas et al., 2005; Koo et al., 2006; Laitinen et al., 2000). The minor allele frequency though does exhibit ethnic dependent distribution being 4-5 times greater in Caucasians than African Americans (Ackerman et al., 2003; D. Wang et al., 2014**)**. A lower frequency is also found in Japanese (2 %) and Chinese (5 %) populations (Iwasa et al., 2000; Koo et al., 2006).

In **II** a hERG1a K897T cDNA construct was transiently expressed in HEK 293 cells and functionally studied using patch-clamp at room temperature. While the hERG1 current evoked by depolarising steps and during repolarisation was qualitatively similar (**II** Fig. 1A and B), immediately apparent was a difference in the current amplitude between hERG K897T and WT. The hERG1 tail current density was significantly decreased by the minor variant (34 ± 4 pA/pF compared to 64 ± 10 pA/pF; $p<0.005$). No difference was found in the voltage-dependence of activation or activation rate at +20 mV. For hERG K897T and WT the mean activation time constants were, respectively, 191.7 ± 39.4 ms and 170.5 ± 24.6 ms ($n = 4$ and 5; $p = 0.6$). There was however a difference in the rate of deactivation, though only at less negative membrane potentials. At −40 mV both the fast and slow time constants of deactivation were significantly slower for hERG K897T.

The C-terminus is known to affect inactivation gating and voltage dependence (Aydar and Palmer, 2001; Bhuiyan et al., 2008; Verkerk et al., 2005). The K897T variant induced a change

in the voltage dependence of recovery from inactivation. This is reflected in the free energy difference in channel gating transition:

$$\Delta G = RT\ (V_{½}/k)$$

This takes into account a shift in both $V_{½}$ and k (though limitations are evident), values taken from the Boltzmann fit (**II** Fig. 3 legend) and RT = 0.592 kcal/mol. For hERG K897T the perturbation ($\Delta\Delta G = \Delta G_{var} - \Delta G_{wt}$) in the free energy of inactivation is -0.6 kcal/mol, a significant perturbation in inactivation gating (Piper et al., 2005) and equivalent to the presence of the N-terminus (Wang et al., 1998). The negative value of $\Delta\Delta G$ indicates stabilisation of the hERG1a channel inactivated state(s) relative to the open state(s). This is, however, not paralleled by the time constants of inactivation gating. There was no change in the rate of recovery from inactivation and the inactivation rate of hERG K897T was significantly slower, infering stabilisation of the open state. An explanation for this paradox may be that hERG K897T destabilises a transition state. Moreover the complex nature of the inactivation process means that the measured rates may not be the forward and reverse rates of the same step in the process. Therefore if hERG K897T destabilises the transition state for the rate limiting step in inactivation, but this same step is not rate limiting in recovery from inactivation then the differential rate effects can also be explained.

Residue 897 is expected to lie close to the cytoplasmic surface of the plasma membrane as the immediate proximal region (amino acids 883-894) is a binding site for PIP_2 and proposed to interact with the negatively charged phosphate groups of the membrane phospholipid's head (Bian et al., 2004). Also in the immediate vicinity are two PKA phosphorylation sites (S890 and T895), the K897T polymorphism results in a novel consensus PKA phosphorylation site (Cui et al., 2000; Thomas et al., 1999). This raises the possibility that the SNP may have an additive effect on the PKA modulation of hERG1. However, in **II** when the adenylyl cyclase activator forskolin was present no differential shift of the half maximal activation voltage between hERG K897T and WT was evident. This is in agreement with Scherer et al. (2002), who showed with *Xenopus* oocytes that both forskolin and the phorbol ester PMA produced similar depolarising shifts of the activation curve for WT and hERG K897T. Consequently the sensitivity of hERG1a channels to PKA is unaltered by the K897T polymorphism. The SNP was found to disrupt a Rho-stimulated protein kinase (PKN) consensus site but create a phosphorylation site for Akt (protein kinase B) resulting in an alteration of the hormonal regulation of hERG1 (Gentile et al., 2008). Thus whereas thyroid hormone, via PI3K, increases WT current by dephosphorylation of T895 through protein phosphatase 5, it decreases the 897T variant current through Akt.

Additional experiments were conducted at physiological temperature (Fig. 11). Consistent with hERG K897T exhibiting more complete inactivation the current amplitude at potentials in the negative slope conductance region of the steady-state current-voltage relationship were decreased compared to WT (Fig. 11C). However such biophysical changes do not give rise to any apparent gross changes in the hERG1a current waveform evoked by a voltage command modelled on a cardiac ventricular action potential (Fig. 11D). This is consistent with Anson et al. (2004) who showed that the variants had qualitatively similar current waveforms, including the timing of the peak, though differences in the current at a few time points were found. It should be noted that as hERG1 (and K897T) recovery from inactivation is so fast the voltage-dependence of channel availability determines the number of channels that recover from inactivation. Therefore, the expectation is that the resurgent current of hERG K897T during an

action potential would be reduced as in the repolarisation voltage range of −10 to −85 mV more hERG channels would reside in the inactivated state. This was shown for the G584S LQT2 mutation which most noticeably results in a large negative shift in the voltage dependence of inactivation (Zhao et al., 2009). *In silico* modeling predicted only modest action potential prolongation by this mutation and a similar response to premature stimuli as WT. This mild *in vitro* phenotype of hERG G584S appears to translate clinically, which differentiates it from the associated severity of other pore domain mutations.

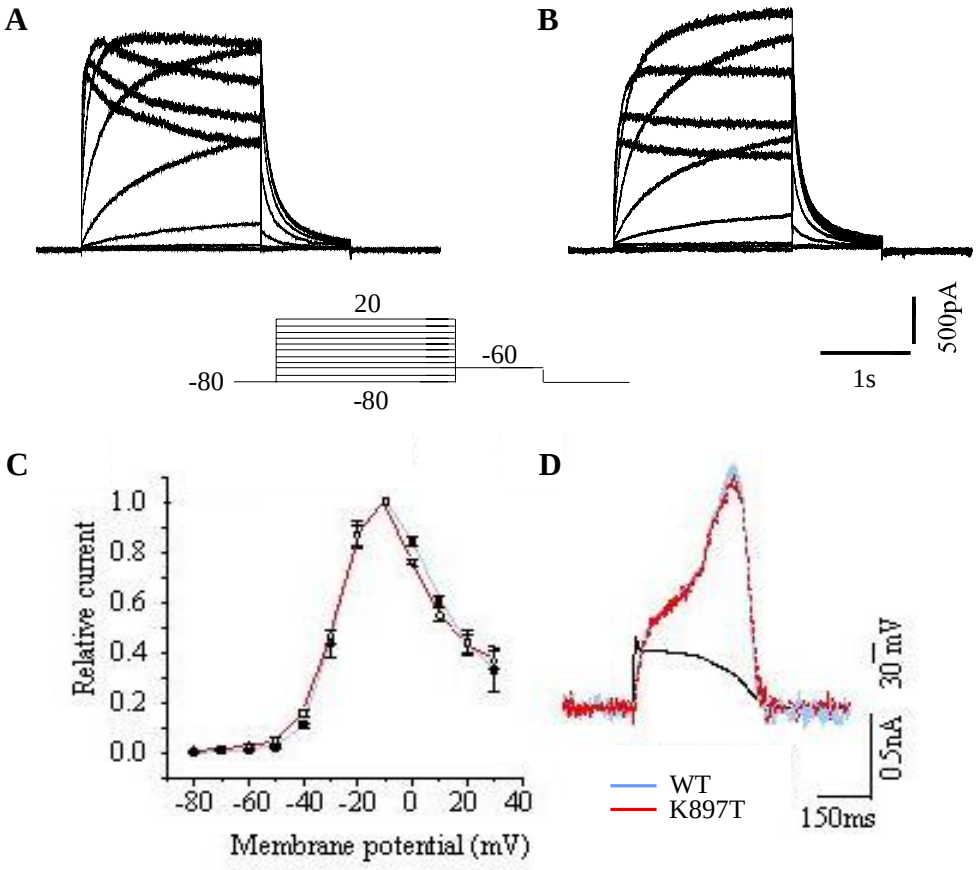

Figure 11. The hERG1a K897T SNP at physiological temperature. I_{hERG} recorded from a WT (A) and a K897T (B) transfected HEK 293 cell. The voltage protocol is shown below. (C) The mean current-voltage relationship of the two variants (n = 3) with current measured at the end of the 2 s depolarizing step. The current was normalised to the value at −10 mV. (D) Currents (leak subtracted) evoked by a simulated cardiac ventricular-like action potential waveform from COS-7 transfected cells.

As referenced above several contemporary and subsequent studies also investigated the *in vitro* effects of hERG1 K897T (Table 3). Discrepancies between the studies are evident and may in part be a consequence of the expression system used as well as differences in the experimental set-ups, protocols and analysis e.g. though the experiments of Bezzina et al. (2003) used transiently expressing HEK 293 cells as in **II** they were conducted at 36 ^{o}C with the reported faster activation time course of hERG K897T occurring at membrane potentials negative to −10 mV. In the full I-V relationship of Cordeiro et al. (2010) greater rectification of hERG K897T in the negative slope region is apparent though a positive shift in the voltage-dependence of channel availability for that variant was found. On the whole any changes in the biophysical properties of the hERG K897T channel appear to be relatively minor which is in

line with its polymorphism status. Furthermore all the other hERG1 polymorphisms functionally characterised are more WT-like (Anson et al., 2004; Männikkö et al., 2010).

Paper	$I_{Density}$	Activation	Deactivation	Inactivation/ Recovery	Inhibition
Scherer et al., 2002	No Δ	No $\Delta V_{½}$	No Δ (at -80)	No Δ/-	No Δ[a]
Bezzina et al., 2003	No Δ	$\Delta V_{½}$ −7mV* Faster rate*	Faster at −40* No Δ ≤−60	No Δ in rates or $V_{½}$	
II & III	53%*	No Δ $V_{½}$ or rate (at +20)	Slower at −40* No Δ ≤−60	Slower/No Δ $\Delta V_{½}$ -12mV	No Δ[b]
Anson et al., 2004	61%	$\Delta V_{½}$ −6mV*	No Δ	Both Faster*	No Δ[c]
Crotti et al., 2005	75%*	No Δ $V_{½}$	No Δ ≤−60	No Δ in rates or $V_{½}$	
Cordeiro et al., 2010	59%*			$\Delta V_{½}$ +8mV	
Männikkö et al., 2010		No $\Delta V_{½}$ or rate (at +10)	No Δ (at −100)	No Δ rates (at +10/-100) or $V_{½}$	No Δ[d]

Table 3. Comparison of the reported electrophysiological properties of the HERG K897T polymorphism (i.e. relative to WT). * significant difference; Drugs used: [a] terfenadine, [b] prucalopride, [c] cisapride, [d] 48 compounds including terfenadine, though slight difference with dofetilide.

The most consistent differential effect of hERG K897T, identified first in **II**, is the decrease in current density (Table 3). The reported reduction in tail current density ranging from 25-47 % (**II**; Anson et al., 2004; Cordeiro et al., 2010; Crotti et al., 2005). The lack of a difference in hERG K897T current amplitude reported by Scherer et al. (2002) may emanate from their usage of the *Xenopus* oocytes expression system which can yield misleading results with trafficking defective hERG1 variants (e.g. hERG-USO) and LQT2 mutations (e.g. R534C) that demonstrate functional expression due to the lower temperature (~18 ^{o}C) at which the oocytes are cultured (Anderson et al., 2006; Aydar and Palmer, 2006; Nakajima et al., 1999). It is noteworthy in regard to the origin of this decrease in current density that the C-terminus plays an important role in the assembly, trafficking and stability of hERG1 channels (Akhavan et al., 2003; Aydar and Palmer, 2001; Biliczki et al., 2008; Christé et al., 2008; Gong et al., 2004b; Kupershmidt et al., 2002; Mihic et al., 2011). To address this hERG1 protein expression was investigated in HEK 293 and COS-7 cells. Though hERG K897T was present as both immature core-glycosylated and mature fully glycosylated forms in Western blots (as respectively 135 and 155 kDa bands) a marked reduction in protein expression and the ratio of mature membrane-associated to immature forms was evident with the two cell lines. This implies there is inefficient biosynthesis or rapid degradation and defective trafficking of hERG K897T which consequently decreases the number of functional channels available. A similar, but more complete, effect occurs with the deletion of residues 860-899 which results in the absence of

surface expression (no mature protein form or I_{hERG}) and decreased immature protein (Akhavan et al., 2003). However no such changes in Western blots from transfected HEK cells were reported in other studies, with hERG K897T undergoing similar biochemical processing as WT (Anson et al., 2004; Scherer et al., 2002).

This *in vitro* part of **II** was performed on homomeric hERG1a K897T channels only. While applicable given that e.g. 3.3 % of the Finnish population is homozygous for T897 (Marjamaa et al., 2009a), of wider consequence (28.8 % of that population) would be the study of the hERG K897T-WT heteromer. The subtle effects noted here may well be attenuated, indeed according to Nof et al. (2010b) there was no significant difference in current density compared to WT alone when hERG K897T and WT were coexpressed.

The decrease in the I_{hERG} should give rise to a loss-of-function phenotype with hERG K897T. Indeed clinically a 50 % reduction of hERG1 (as expected for heterozygous nonsense LQT2 patients) results in a 16.5 % prolongation of the mean QTc interval (480 ms vs 412 ms for controls; Mann et al., 2016). Given the prevalence of K897T such an extreme effect is unlikely. In a dynamic clamp study with guinea pig ventricular myocytes a 30 % decrease of the modelled I_{Kr} conductance did not significantly affect APD but increased the temporal variability in repolarisation (Altomare et al., 2015). Thus while reducing the repolarisation reserve, overall a subclinical effect of K897T may be expected. Consequently in **II** the clinical effects of the K897T SNP were studied in a LQT1 patient cohort (n = 261), all carriers of the KCNQ1 G589D mutation. Within this population it was previously suggested that homozygous 897T females, but not males, may have a shorter QTc interval at rest (Laitinen et al., 2000). In **II** it was shown that genotype had no significant impact on the risk of cardiac events with 23 % of patients carrying the 897T allele and 31 % of homozygous WT being symptomatic. As I_{Ks} is critical to cardiac repolarisation under β–adrenergic stimulation and this modulation is disrupted by G589D any deficit in hERG1 may become evident by these patients undergoing an exercise test (Banyasz et al., 2014; Jost et al., 2005; Marx et al., 2002). Therefore the QT-RR interval relationship of 53 patients was investigated. Only in a subgroup, those with a prolonged QTc (>440 ms at rest, n = 39), were genotype differences seen. The 897T genotypes had a significant longer QT interval during exercise, but not during recovery, than homozygous WT. The premise of conducting this clinical study in the Finnish KCNQ1 G589D founder population appears correct as a larger study of 1,975 heterogeneous Finnish patients found no effect of genotype during exercise (Koskela et al., 2008). The only significant difference was seen at rest in women where K897T was associated with a longer QTc interval, consistent with an earlier report (Pietilä et al., 2002).

Subsequent studies also suggest that K897T forms a latent genetic background. As a consequence family members who are negative for the family-associated LQTS mutation have an 11-fold increase in the risk of recurrent syncope, apparently without significant QTc prolongation (Barsheshet et al., 2011). Clinical case reports identify hERG K897T as a genetic modifier unmasking latent LQT1, which resulted in an overlapping ECG phenotype of both LQT1 and LQT2 morphologies, and LQT2 (Cordeiro et al., 2010; Crotti et al., 2005; Nof et al., 2010b). An outcome of the coexistence of hERG P926AfsX14 and K897T was sudden infant death, while the carriers (parents and siblings) of either were asymptomatic (Nof et al., 2010b). It should be noted that there was no difference in the allele frequency of 897T in SIDS cases (or drug-induced LQTS) compared to reference populations i.e. no enrichment (Arnestad et al., 2007; Paulussen et al., 2004; Yang et al., 2002). The K897T SNP may also increase the severity of symptoms in Andersen-Tawil syndrome, whereas it may mask the abbreviated QT intervals

of SQT mutation carriers (Antzelevitch et al., 2007; Hu et al., 2011; Jagodzińska et al., 2016). Finally this SNP may exacerbate arrhythmic risk in the days following myocardial infarction. K897T in this setting (i.e. a remodelled substrate) was associated with an 8-fold increase in risk, as 69 % of all patients who developed TdP had an 897T allele compared to 35 % without this complication (Crotti et al., 2012).

The effect of hERG K897T beyond the heart however has seldom been investigated (Atalar et al., 2010; Erdem et al., 2015; Sarzani et al., 2006). One may speculate that the loss-of-function phenotype of hERG1 K897T would result in an oncogenic potential that is less favourable. Consistent with such a supposition is the reduction in the hERG1b overexpression of 897T paediatric acute myeloid leukaemia patients compared to 897K homozygote patients (Erdem et al., 2015). Moreover possibly in line with only subtle kinetic alterations, this SNP is not associated with schizophrenia (Atalar et al., 2010).

However, in contrast to the above Bezzina et al. (2003) reported that healthy female 897T homozygotes had an abbreviated QTc (by ~14 ms) compared to heterozygotes and WT homozygotes. Their observation of altered hERG activation was proposed, aided by *in silico* human ventricular cell simulations, as underlying this clinical phenotype. Though using the same electrophysiological data Glinka and Polak (2012) obtained contradictory simulation results. Other population studies have also shown the 897T allele to be associated with shorter QTc intervals (Gouas et al., 2005; Hajj et al., 2014; Marjamaa et al., 2009a; Newton-Cheh et al., 2007; Pfeufer et al., 2005). Interestingly Gouas et al. (2005) suggested the effect of 897T was dependent on the major allele of a 3´ UTR SNP in *KCNQ1* i.e. not suppression of KCNQ1 expression (Amin et al., 2012).

It appears similarly complex in regard to AF. While no difference in the prevalence of K897T in AF probands compared to healthy controls was found (Mann et al., 2012), in other studies this SNP was associated with risk of AF. The effect of the minor allele may differ in populations having been suggested to be protective in AF but to increase the risk of early onset lone AF (Andreasen et al., 2013; Sinner et al., 2008). These findings may be explained by either loss-of-function (decreased I_{hERG}) or gain-of-function (via biophysical properties) with K897T. Of note hERG1 T895M which is associated with lone AF and SIDS was recently electrophysiologically determined to be a gain-of-function mutation (Hayashi et al., 2015; Otagiri et al., 2008). Analogous to hERG K897T (**II**), this included a significantly slowed deactivation at −40 mV but not at more hyperpolarised potentials (Hayashi et al., 2015).

The hERG K897T polymorphism did not affect the potency of prucalopride (IC_{50} = 2.7 µM), with no significant differences in the extent of inhibition at any of the tested concentrations between the variants. Furthermore the prucalopride induced changes in hERG1 channel kinetics described earlier, i.e. slowing of deactivation and acceleration of inactivation, were also evident with hERG K897T (Fig. 12). Other studies have also shown that hERG1 K897T exhibits a similar pharmacology as the WT hERG1a channel (Table 3), including one by Männikkö et al. (2010) that tested 48 compounds using an automated patch-clamp device. This is reassuring as this is an assumption made in the preclinical cardiovascular safety assessment of NCEs. Only for dofetilide did the IC_{50} not overlap with WT, though the difference was <1.5-fold. The similarity may not be surprising given that the site of the polymorphism is remote from the canonical binding site, the inner cavity. It is also consistent with the view that only subtle alterations in channel kinetics and occupancy of the activated states occur between WT and K897T given the dependence/mechanism of block of the

compounds. Differential kinetics can result in pharmacological differences e.g. steady-state inactivation mutation hERG1 N588K increased the IC_{50} of both cisapride and terfenadine by 2.7-fold compared to WT (Perrin et al., 2008).

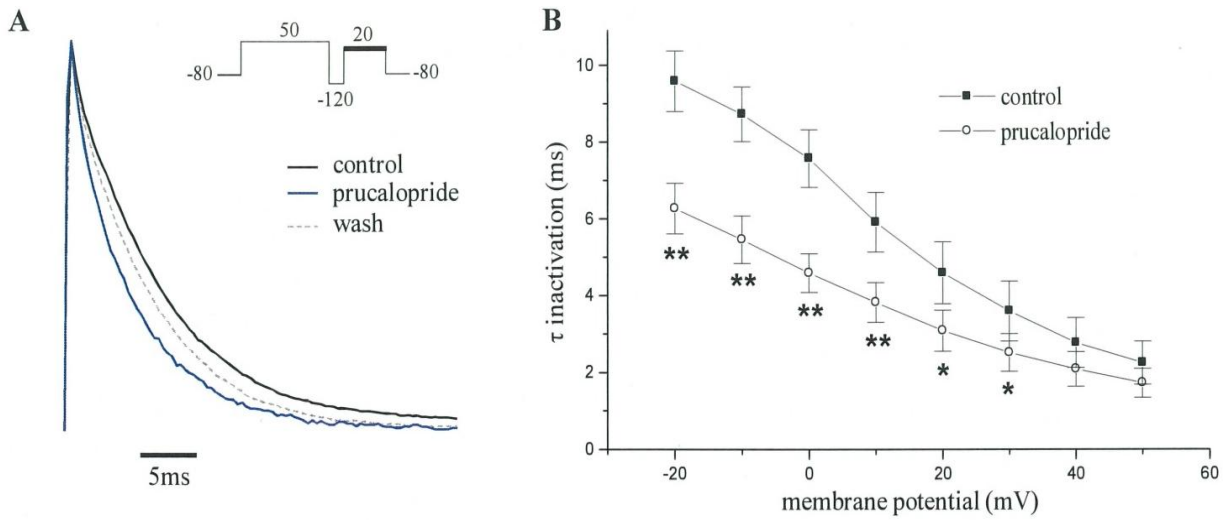

Figure 12. The effect of prucalopride (10 µM) on hERG K897T inactivation. (A) Inactivating currents at +20 mV in the absence, presence of prucalopride and with wash-out. The peak currents have been aligned to illustrate the timecourse. The three step voltage protocol is shown inset and consisted of a 2 s depolarisation to +50 mV, a 7 ms repolarisation to −120 mV and depolarisation to potentials from −20 to +50 mV (though only +20 mV is highlighted). (B) Plot of the hERG K897T time constant of inactivation at various membrane potentials in the absence and presence of prucalopride (n = 5; *p<0.05, **p<0.005). A single exponential was fitted to the inactivating current of the last voltage step.

5.3 CERAMIDE AND THE hERG1 CHANNEL (III)

Sphingolipids, of which there are over 4000 species, are biologically active components of cell membranes (Borodzicz et al., 2015; Chaurasia and Summers, 2015). The biosynthesis of these sphingolipids (little is absorbed in the diet) initially occurs in the ER and generates ceramide (Fig. 13) which is trafficked to the Golgi for conversion to sphingomyelin and other complex sphingolipids (Chaurasia and Summers, 2015). Ceramide can be re-formed from membrane sphingomyelin by hydrolysis via sphingomyelinase in response to e.g. proinflammatory cytokines such as TNF-α and interleukin-1β. In addition to its role as a precursor, ceramide is an important second messenger mediating through various signalling cascades processes such as proliferation, differentiation and apoptosis (Borodzicz et al., 2015). Ceramide has a significant involvement in pathophysiology, for instance in lipotoxicity, i.e. where excess fat in obesity and dyslipidemia is supplied to the pancreatic islets, heart etc resulting in dysfunction, and therefore diabetes, insulin resistance, cardiomyopathy, and atherosclerosis (Chaurasia and Summers, 2015). Ceramide accumulates in ischaemia/reperfusion injury and in advanced human heart failure the intracellular ceramide level was found to be increased by 50 % (Borodzicz et al., 2015; Chokshi et al., 2012). It is therefore of great interest to study the effect of ceramide on cardiac ion channels and the resulting contribution to the electrophysiological remodelling seen in these disease states. Prior to **III** acute exposure to ceramide was shown to have no effect on I_{hERG} (at 50 µM) but to substantially reduce (by ~70 % at 10 µM) the I_{ERG} of a rat lactotroph cell line (Wang et al., 2001; Wu et al., 2001).

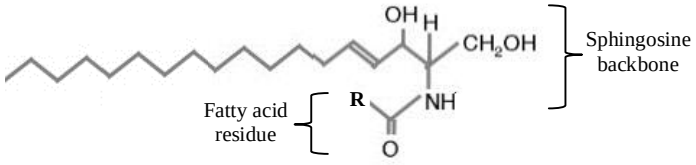

Figure 13. Ceramide. For the cell permeable C_6-ceramide used in **III** R = C_5H_{11}.

In **III** at a concentration of 10 µM, which mimicks the intracellular level reached during physiological and pathophysiological stimulation (Hannun, 1996), C_6-ceramide was shown to induce a time dependent decrease of the hERG1a current in stably expressing HEK 293 cells. Thus the peak tail current density in ceramide was reduced significantly more than current rundown: after 15mins by 11.4 ± 3.2 % (vs 2.7 ± 1.0 % vehicle; n=6 for both; $p<0.05$) and after 60 mins by 30.2 ± 4.4 % (n=4; vs 13.1 ± 3.7 % vehicle; n= 5; $p<0.05$). Furthermore the reduction in I_{hERG} was not prevented by the PKC inhibitor calphostin C (0.1 µM). A detailed electrophysiological characterisation was undertaken of cells incubated for 1 hour in 10 µM ceramide or vehicle. The hERG1 activating current evoked by a depolarising step, the tail current and the instantaneous current following relief of inactivation were all significantly decreased by ceramide e.g. the current density was decreased by 22 % with depolarisation to 0 mV and the tail current by 30 % after depolarisation to +40 mV. The specificity of this response was demonstrated with the ineffectiveness of the inactive analog dihydro-ceramide on hERG1

current. Moreover a concentration dependent reduction in hERG1 current was evident with ceramide, though significance was only achieved at 10 µM. Ceramide did not alter hERG1 steady-state activation or inactivation, but significantly accelerated the deactivation time constants. The latter exhibiting a concentration dependent effect, thus the fast and slow time constants were respectively 16 and 20 % faster at 0.3 µM and 33 and 42 % faster at 10 µM. Of note similar electrophysiological results were subsequently reported with exposure to a DAG analogue, however the decrease in I_{hERG} was PKC dependent (Ramström et al., 2010).

The ceramide-induced decrease in I_{hERG} paralleled a reduction in hERG cell membrane protein expression as determined by western blot (a 30 % loss of intensity of the 155 kDa band), biotinylation (a 42 % decrease in labelled hERG) and immunostainings. These cell surface expression effects of ceramide on hERG1 may reflect protein synthesis and/or trafficking disruption or increased degradation of membrane protein. The unchanged levels of the core-glycosylated, immature 135 kDa form of hERG protein from pre-Golgi compartments argues against an effect on channel synthesis. Analogous results are seen with hERG trafficking inhibitors such as pentamidine, ketoconazole, probucol and cardiac glycosides (J. Guo et al., 2007; Kuryshev et al., 2005; Takemasa et al., 2007; Wang et al., 2007). However a fundamental difference between these trafficking inhibitors and ceramide is the temporal aspect of the hERG decrement. A similar reduction in mature hERG protein occurs for ceramide at 1 hr as for ketoconazole (30 µM, its approximate maximal effective concentration) at 8-24 hr (**III** Fig. 3A cf Takemasa et al., 2007 Fig. 5A). Unsurprisingly when synthesis and processing of hERG was explored more directly with pulse-chase experiments it was shown to be unaltered by ceramide. Thus whether control or ceramide exposed similar quantities of the core glycosylated hERG form were initially synthesised (at t_0) and subsequently processed (e.g. t_{8hr} ~27 % remained) to the mature 155 kDa form giving at 8hrs an increase of ≈50 %. This therefore does not explain the results of the above steady-state experiments and differs from hERG trafficking inhibitors (Ficker et al., 2004). Instead ceramide was shown to increase hERG1 ubiquitination (according to immunoprecipitation experiments) with subsequent targeting of the protein to lysosomes. The lysosomal inhibitor bafilomycin A1, but not the proteosomal blocker MG132, augmented hERG1 ubiquitination and prevented the reduction in hERG protein staining induced by ceramide. Finally, exposure to ceramide resulted in hERG1 protein co-localising, according to immunostainings, with the lysosomal-associated membrane protein Lamp 1. Thus it is suggested that in hERG1a-HEK cells ceramide causes the ubiquitination of hERG1 leading to channel internalisation and targeting to lysosomes.

Subsequent to **III** the regulation of hERG1 channel expression at the cell membrane has been increasing explored (see section 2.2.4). Key to this regulation is Nedd4-2, which mediates hERG1 ubiquitination (Albesa et al., 2011; Guo et al., 2012; Kang et al., 2015). Modulation of Nedd4-2 activity either positively, e.g. by AMP-dependent protein kinase, or negatively, e.g. by the PKC pathway and SGKs, results respectively in a decrease or increase in I_{hERG} (Almilaji et al., 2013; Lamothe and Zhang, 2013; T. Wang et al., 2014). The effectors of the ceramide-induced ubiquitination of hERG1 were not identified in **III**, thus with the current knowledge an investigation of the role of Nedd4-2 and the enhancement of its activity may be a starting point. Interestingly, but contrary to the expectation with hERG1, ceramide (C_4) was shown to increase the trafficking of ΔF508 mutant cystic fibrosis transmembrane conductance (CFTR) to the cell surface via activation of SGK1 and resultant Nedd4-2 inactivation (Caohuy et al., 2014). Of note the trafficking of CFTR shares many similarities with hERG1 (Delisle et al., 2009), recently Nedd4-2 recruitment and activation at the Golgi apparatus by Nedd4 family interacting proteins was suggested to likewise reduce trafficking to the cell membrane (Fig. 7; Kang et al.,

2015). However MG132 has been shown to prevent the down-regulation of the mature hERG1 protein by Nedd4-2 suggesting it targets hERG1 to the proteosome for degradation (Albesa et al., 2011). Whether under different conditions different targeting by Nedd4-2 occurs or if a different ligase (e.g. CHIP which appears to be important in peripheral quality control; Apaja et al., 2013) is involved in this instance is unknown.

As mentioned above the specifics of the ubiquitination and internalisation mechanism in regards to ceramide exposure remain to be determined, as does whether the internalised hERG channels can be recycled back to the cell membrane. The most extensively studied situation has been hypokalaemia. Chronic exposure to K^+_o below 5 mM decreases I_{hERG} (with an IC_{50} of 2 mM) through enhanced ubiquitin-mediated internalisation via endocytosis and lysosomal degradation of hERG1 channels (Apaja et al., 2013; Guo et al., 2009). The trigger for internalisation is the entry of the hERG1 channels into a novel nonconducting state, which likely results in the exposure of ubiquitin modification site(s) (Massaeli et al., 2010b). Internalisation of hERG was shown to be a dynamin-dependent Cav mechanism (Massaeli et al., 2010a). Another analogous case to **III** is drug-induced internalisation of hERG1 which can occur acutely (≥30 min) with e.g. the antidepressants amoxapine and desipramine (Obers et al., 2010; Staudacher et al., 2011). Furthermore desipramine in hERG-HEK cells induced rapid ubiquitination of the recycling hERG channels and transit to lysosomes for degradation (Dennis et al., 2011).

Two other reports have examined the effect of ceramide on hERG1a-HEK cells. As in their earlier paper, Bai et al. (2007) found that ceramide (C_2) exposure for up to 20 mins had no significant effect on I_{hERG} but with prolonged incubation caused a concentration dependent reduction (IC_{50} = 19.5 µM). We though observed a small but significant decrease already after 15 mins, it is noteworthy however that the extent of inhibition by 10 µM ceramide after 1 hr (**III**) or 10 hrs (Bai et al., 2007) incubation was similar (~30 %). One clear discrepancy between the electrophysiological data of the two studies is in regard to the half maximum activation voltage. This likely reflects the ceramide incubation time difference i.e. a depolarising shift becomes evident with >1 hr exposure, but could arise from the different ceramide isoforms used. The prolonged exposure to ceramide resulted in increased ROS generation which was shown to partly, at least, underlie the C_2-induced I_{hERG} decrease. Certainly inconsistent with Bai et al. (2007) and greater than in **III**, Ganapathi et al. (2010) reported a more rapid effect of ceramide (C_6) with a maximal 36 % reduction of I_{hERG} within 10 mins. In this initial time period, there was also a hyperpolarising shift of the voltage dependence of activation and, as in **III**, an acceleration of the deactivation kinetics. Underlying these gating changes was a recruitment of hERG1 to lipid rafts where the exogenous ceramide accumulates assuming a biophysical role (Ganapathi et al., 2010). The ceramide-induced I_{hERG} decrease had a distinct unknown mechanism, as puzzlingly the authors were unable to replicate with up to 1 hr ceramide exposure the loss of cell surface hERG expression proposed in **III**. The only longer time point (24 hrs) did exhibit a 44 % reduction in hERG protein using a biotinylation assay.

5.4 hERG1 R176W AND iPS-CARDIOMYOCYTES (IV)

The use of a heterologous expression system, as in studies **I** – **III**, affords a number of advantages beginning with defining the cDNA introduced, thus the human form (whether WT or mutant) can be expressed and investigated. The controlled nature with large amplitude currents and minimal contaminating currents lends itself to detailed structure based (e.g. mutation) analyses of ion channel function and interaction (e.g. drug). Though conventionally used the results from such artificial models may poorly correlate or appear discrepant, at least initially, with the clinical phenotype as the native situation is not fully recapitulated (Biliczki et al., 2008; Watanabe et al., 2011; Zarraga et al., 2011). One explanation may be the cellular context e.g. cell type dependence in the components involved in the TRH regulation of ERG channels was found suggesting cell specific protein composition and availability modulates the elements in signal transduction pathways (Carretero et al., 2012). This demonstrates the necessity of studying modulation in native systems and it remains to be explored whether the effects of ceramide obtained in heterologous expression systems translates to cardiomyocytes. Another important point is that functional characterisation of *hERG1* mutations and polymorphisms have almost exclusive been described in the context of hERG1a isoform though most also occur within the coding regions for hERG1b and the other isoforms. A few examples employing other hERG1 isoforms exist (McPate et al., 2009; Stump et al., 2012a), thus while the effect of the N588K mutation was similar to that found with hERG1a alone there was greatly enhanced attenuation of inactivation by this SQT1 mutation in heteromeric hERG1a/1b channels (McPate et al., 2009).

Ideally heterologous expression studies would be complemented by more integrated approaches encompassing the *in vivo* complexity of the channel and its physiological effects. Hitherto insights into the pathogenesis of LQT2 though have come almost entirely from heterologous expression systems with transgenic animal models scarcely used (Babij et al., 1998; Brunner et al., 2008; Teng et al., 2008). LQT2 mutants have also been exogenously expressed in mouse and rat cardiomyocytes (Balijepalli et al., 2012; Lin et al., 2010). The use of cardiomyocytes differentiated from human pluripotent stem cells, whether embryonic or induced (i.e. generated by reprogramming of the mature somatic cells of an individual; Takahashi et al., 2007; Yu et al., 2007) pluripotent stem (ES or iPS) cells, has great potential as a human *in vitro* integrated model system (e.g. Liang et al., 2013; Ma et al., 2011; Peng et al., 2010). Indeed use of human iPSC derived ventricular myocytes will be incorporated in the forthcoming CiPA where they will provide confirmation of the ion channel and *in silico* results, and reveal any unforeseen effects (Fermini et al., 2016; Sager et al., 2014). Also the exogenous expression of the LQT2 mutants hERG1 R56Q and hERG1b-R25W in human iPSC derived cardiomyocytes has taken place (Jones et al., 2016; Liu and Trudeau, 2015). Furthermore iPS has offered a unique opportunity to study ion channel (or other) mutations in the disease relevant cellular context by differentiation of patient-specific human iPSC lines (Hoekstra et al., 2012; Takahashi et al., 2007). Moretti et al. (2010) first demonstrated that the LQTS phenotype of individuals could be reproduced *in vitro* i.e. the atrial- and ventricular-like action potentials of cardiomyocytes derived from iPSCs with a KCNQ1 R190Q mutation were prolonged.

In **IV** iPSC lines were established from a 61-year old man carrying an arginine to tryptophan substitution in the N-terminal proximal domain at position 176 (R176W; a result of the exon 4 change 526C>T) of hERG1. He, apart from occasional palpitations, was asymptomatic with a QTc of 437 ms. His sister had overt LQTS (with QTc intervals up to 582

ms) and died suddenly at 32 years when awoken by a telephone. His father also died suddenly at the age of 40. In Finland hERG1 R176W together with three mutations (G589D and IVS7-2A>G of KCNQ1, and hERG1 L552S) accounts for over 70 % of the genotyped LQTS patients (Fodstad et al., 2004). hERG1 R176W was present in ~8 % of these genotyped LQTS patients, with 40 % of male and 22 % of female carriers having a prolonged QTc and overall 16 % symptomatic (Marjamaa et al., 2009b). In line with its founder mutation status hERG1 R176W, in the heterozygous form, was associated with a low incidence of cardiac events (0 % syncope, 0 % life threatening; this compares to 29 % and 0 % for non-Finnish founder hERG1 mutations) up to 18 years old (Koponen et al., 2015). Furthermore though prevalent (1 in 568) in the general adult Finnish population and associated with a 22 ms longer QT, R176W is not a risk factor for sudden cardiac death (Lahtinen et al., 2013; Marjamaa et al., 2009b). Similarly elsewhere hERG1 R176W (rs36210422) was found as a common variant (frequency of ~0.5 %) in apparently healthy Caucasian populations (Ackerman et al., 2003; Mank-Seymour et al., 2006). While enriched in the Finnish LQTS population, hERG1 R176W was also found in one case of SUD and one of SUD in epilepsy as well as in dofetilide and terodiline induced TdP cases (Mank-Seymour et al., 2006; Männikkö et al., 2010; Tester et al., 2012; Tu et al., 2011). In addition hERG1 R176W was found, but no further mutations in *hERG1* or the other genes screened, in a case of a 12-year old girl who presented with a life-threatening cardiac event following emotional stress (Donner et al., 2012). The above suggests hERG1 R176W exhibits variable penetrance and expressivity. Moreover a more severe phenotype is reported in compound heterozygotes i.e. when in combination with KCNQ1 mutations (Fodstad et al., 2006).

The differentiation of the patient and control iPSCs as well as H7 hESCs towards the cardiac lineage was by co-culture with END-2 cells. Within 2 weeks spontaneously beating areas were evident, these were excised and dissociated. The electrophysiological properties of these spontaneously beating dissociated cells was characterised with patch-clamp. In current-clamp two action potential configurations were seen: ventricular-like, where a plateau phase was evident; and atrial-like, with a triangular shape. While the upstroke velocity, action potential amplitude and maximum diastolic potential of the LQT2-iPSC derived cardiomyocytes exhibited no difference from that of the control cardiomyocytes, the APD did. The APD at 50 % and 90 % repolarisation of the atrial-like LQT2-iPSC derived cardiomyocytes was also prolonged (APD_{50} and APD_{90} by 34 %) but did not reach statistical significance. At a spontaneous action potential firing frequency of ~1 Hz, the APD of the ventricular-like LQT2-iPSC derived cardiomyocytes was significantly prolonged (Fig. 14A; APD_{50}: 436.4 ± 24.9 ms vs hESC 291.9 ± 18.2 ms and control 264.2 ± 17.7 ms, $p<0.001$, hESC vs control $p=0.71$; APD_{90} 516.5 ± 26.1 ms vs hESC 338.6 ± 19.9 ms, control 310.5 ± 19.6 ms, $p<0.001$, hESC vs control $p=0.73$). As shown in figure 14A the APD did not differ between cardiomyocytes derived from the two LQT2-iPSC lines or the various control cell lines. Furthermore in one LQT2-iPSC derived cardiomyocyte, but in no control iPSC derived cardiomyocytes (n=20 for both), EADs were evident.

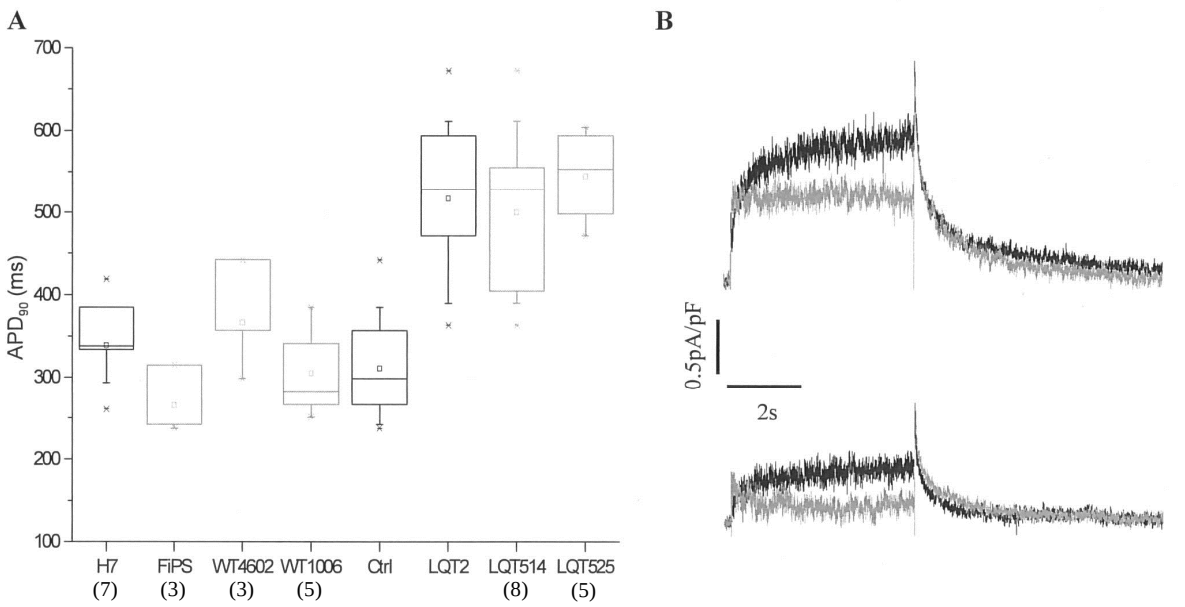

Figure 14. (A) Box graph of the APD_{90} of cardiomyocytes derived from hESC, control iPSC and LQT2 iPSC lines that had a frequency of ~1 Hz. The summary data and that from the individual cell lines (the number of cells is shown beneath) are in black and grey respectively. (B) The I_{Kr} evoked from a control (UTA.04602.WT; above) and LQT2 (UTA.00525.LQT2; below) iPSC derived cardiomyocyte by depolarisation to 0 and + 20mV (grey trace) followed by a return to the holding potential of −40 mV.

To confirm a loss-of-function was associated with hERG1 R176W resulting in APD prolongation, the I_{Kr} of ventricular-like cardiomyocytes was recorded using two isolation methods and different cell lines. As seen in figure 14B, the cardiomyocyte I_{Kr} (as E-4031-sensitive current) exhibited inward rectification and large tail currents. The I_{Kr} magnitude of LQT2-iPSC derived cardiomyocyte was significantly decreased compared to control. Depolarisation to +20mV and the subsequent repolarisation generated I_{Kr} of 0.75 ± 0.05 pA/pF and 1.80 ± 0.14 pA/pF from control iPSC derived cardiomyocytes compared with 0.35 ± 0.09 pA/pF and 1.08 ± 0.11 pA/pF from LQT2-iPSC derived cardiomyocytes ($p<0.05$ and <0.005, n=3-5). Importantly when a step-ramp voltage protocol was employed a reduction in the resurgent I_{Kr} was evident. A reduction in I_{Kr} was also observed when isotonic caesium solutions were used to isolate I_{Kr}. Thus the tail current density after depolarisation to +20 mV was 4.1 ± 0.6 pA/pF and 2.5 ± 0.5 pA/pF for control and LQT2-iPSC derived cardiomyocytes respectively ($p>0.05$, n=3 and 4).

The delayed repolarisation of the LQT2-iPSC derived cardiomyocytes was confirmed at the multicellular level in field potential recordings, being most evident at low spontaneous beating rates. No arrhythmogenicity was seen at baseline with either control or LQT2-iPSC derived cardiomyocytes on the microelectrode arrays. However EADs were more readily induced in LQT2-iPSC derived cardiomyocyte aggregates, as in other studies (e.g. Matsa et al., 2011; Mehta et al., 2014), compared to control on exposure to E-4031 or sotalol.

This study provides further support that a heritable condition, LQT2, can be modelled *in vitro* with human iPSC derived cardiomyocytes and is consistent with the growing number of recent reports (Table 4). In one study iPSC lines were created from a proband and mother,

though the latter also carried the A561T mutation and had a prolonged (though not to the same extent) QTc she was asymptomatic (Matsa et al., 2011). The derived cardiomyocytes recapitulated the phenotypes *in vitro* with the APD of the maternal origin cells being shorter, but longer than that of control cardiomyocytes, and EADs being non-inducible. The intermediate phenotype is comparable to the one obtained in **IV**. The relatively mild electrophysiological profile of the R176W iPSC derived cardiomyocytes bears most resemblance to that of cardiomyocytes derived from the latent LQTS N996I carrier (Table 4). Patient symptomaticity appears in general to correlate *in vitro* with greater APD prolongation and prevalence of EADs, even for the same hERG1 mutation (Chai et al., 2015; Matsa et al., 2011). Thus in contrast to R176W and N996I, the substantial reduction in cardiomyocyte I_{Kr} observed with A561V and A614V results in a greatly prolonged APD and frequent EADs mirroring the overt LQTS of the patients from which they were derived (Table 4).

Mutation	**Patient**	**ΔAPD_{90}**	**$\downarrow I_{Kr}$ tail**	**EADs**
R176W (**IV**)	QTc=437 ms Asymptomatic/Palpitations	166 %	40 %	5 %
A422T (Spencer et al., 2014)	QTc=493 ms Syncope	a	45 %	70 %
A561P (Jouni et al., 2015)	QTc=628 ms diTdP	~156 %	~50 %	29 %
A561T (Matsa et al., 2011)	QTc=571 ms Recurrent syncope, VT QTc=514 ms Asymptomatic	a		b
A561V (Mehta et al., 2014)	QTc=584 ms TdP	191 %	~90 %	b
A614V (Itzhaki et al., 2011)	QTc=520 ms TdP	~200 %	64 %	66 %
N996I (Bellin et al., 2013)	QTc=617 ms Asymptomatic	142 %	33 %	0 %

Table 4. Comparison of the electrophysiologically characterised LQT2 iPSC derived cardiomyocyte data. APD_{90} of LQT2 iPSC derived cardiomyocyte relative to control at ~1 Hz otherwise a, APD prolongation but frequency not disclosed. The % of LQT2 iPSC derived cardiomyocytes in which EADs were observed otherwise b, spontaneously occurring EADs noted but no figure given. diTdP refers to drug (clobutinol) induced TdP.

The electrophysiological phenotype described in **IV** appears more severe than the patient phenotype, likely a consequence of various factors. Firstly dissociated single cardiomyocytes may exhibit an exaggerated phenotype (Matsa et al., 2011; Spencer et al., 2014). Of note in this regard was that the prolongation was much less (only 10–20 %) in multielectrode array measurements where cellular coupling was preserved. Also though the QTc of the patient is reported within the normal range, variability of the QTc interval with hERG1 R176W on repeated measurement occurs (Donner et al., 2012). Furthermore the control iPSC lines are suboptimal as their genetic backgrounds differ from the patient so differences may be over-

estimated. The use of isogenic cell lines, as in recent studies (e.g. Bellin et al., 2013), would negate this and could provide insight into the contribution of the epistatic effects of background to the heterogeneity of R176W's clinical phenotype.

Previously hERG1 R176W was studied by heterologous expression in COS-7 and CHO cells (Fodstad et al., 2006; Männikkö et al., 2010). hERG1 R176W was found to decrease the tail current density by ~75 % compared to hERG1 WT, the decrement though was lessened (to ~23 %) and became insignificant upon co-expression (Fodstad et al., 2006). In **IV,** native expression in cardiomyocytes derived from a heterozygous R176W individual, a 40 % reduction in I_{Kr} density was found. The slight difference may result from the differing composition of the channels (hERG1a vs native hERG1a/1b + accessory subunits/associated proteins). How hERG1a R176W interacts with hERG1b is unknown and as the R176W variant does not occur in hERG1b the relative abundance of this subunit may then give rise to variability. The extent of the reduction (<50 %) is indicative of haploinsufficiency rather than a dominant negative effect of the R176W subunits on the WT subunits. The steady-state voltage-dependence or kinetics of I_{Kr} were not studied in **IV** due to the small amplitude of the current, particularly that of R176W. In the earlier studies no significant (or slight) differences were seen at room temperature between R176W and WT (Fodstad et al., 2006; Männikkö et al., 2010). The lack of any major biophysical alterations to account for the loss of I_{hERG} may suggest hERG1 R176W is trafficking defective, however this has not been tested. A similarity with the hERG1 R273Q mutation, identified in a SIDS case (Arnestad et al., 2007), is evident. When this mutant was co-expressed it also generated WT-like current but with a tail current density reduced by ~35 % (Rhodes et al., 2008). So far though when specifically studied no N-terminal proximal domain variants affect protein synthesis or channel trafficking (Anderson et al., 2006; Mechakra et al., 2014; Osterbur et al., 2015; Perry et al., 2016).

While iPSC derived cardiomyocytes exhibit an I_{Kr} density that is comparable to native human cardiomyocytes due, at least in part, to the expression and functionality of hERG1b, their electrophysiological phenotype is immature as exemplified by their spontaneous beating (Cordeiro et al., 2013b; Doss et al., 2012; Hoekstra et al., 2012; Jones et al., 2014). Thus in **IV** and elsewhere (e.g. Itzhaki et al., 2011; Jouni et al., 2015; Mehta et al., 2014) the ventricular-like cardiomyocyte maximum diastolic potential was depolarised (-63 mV) and the maximal upstroke velocity reduced (being up to 27 V/s) compared to the values (~-85 mV and >220 V/s) of human ventricular cells (Drouin et al., 1995; Peeters et al., 1995). Strategies to improve the maximum diastolic potential (and consequently the upstroke velocity) include using clusters of cells and enhancing the small I_{K1} (Jones et al., 2014; Spencer et al., 2014; Vaidyanathan et al., 2016) but the primary on-going goal is to obtain mature iPSC derived cardiomyocytes. Meanwhile of note in regards to CiPA is the introduction of a human *ex-vivo* ventricular trabeculae action potential model (Page et al., 2016), which also enables investigation of drug-induced effects in the context of comorbidities such as type 2 diabetes.

6. CONCLUSIONS AND FUTURE DIRECTIONS

The I_{Kr}/hERG1 channel plays an essential role in the repolarisation of the cardiac action potential and in the excitability of neurones, smooth muscle and endocrine cells. Loss or gain-of-function of hERG1 is associated with arrhythmogenesis and sudden cardiac death, while its overexpression and ectopic expression is associated with cancer. This thesis provides insights into the effect of genetic variation, drug and regulation on hERG1 function and cardiac electrophysiology.

The gastrointestinal prokinetic drug prucalopride exhibited concentration dependent inhibition of hERG1a with rapid block and unblock of the activated states. At therapeutic concentrations this should pose little proarrhythmic risk as the hERG safety margin is ≥300. This has translated to the clinic with a negative TQT study and a benign cardiac safety profile in the various phase III studies.

The sphingolipid ceramide was shown to induce a time dependent decrease in hERG1a current as channels are ubiquitinated and internalised. Whether this also occurs in cardiomyocytes or other native cell types remains to be determined. This mechanism could contribute to the QT prolongation (and apoptosis) in diabetic cardiomyopathy, myocardial ischaemia and HF.

Two common genetic variants of hERG1 were investigated. The hERG1 SNP K897T was found have a decreased hERG1 current density compared to wild-type when heterologously expressed. While this loss-of-function *in vitro* phenotype may translate clinically to certain subgroups (e.g. during exercise to KCNQ1 G589D carriers with prolonged resting QTc or individuals following myocardial infarction), in the general population the minor allele is associated with shorter QTc intervals. In a proof-of-concept study, using human iPSC derived cardiomyocytes from a relatively asymptomatic individual, the variant hERG1 R176W caused a reduction in I_{Kr} and action potential prolongation. In line with expectation the extent of the reduction and prolongation (as well as EAD occurrence) was less than reported for iPSC derived cardiomyocytes from highly symptomatic LQT2 patients. Of note hERG1 K897T has now also been studied with iPSC derived cardiomyocytes from a proband (with a *de novo* mutation in *SCN5A* and heterozygous for K897T), his WT father and homozygous K897T mother (Terrenoire et al., 2013). No significant differences were seen in the I_{Kr} I-V curves (density or rectification) or in the voltage-dependence of activation between cardiomyocytes from the family members.

A more integrated *in vitro* approach to evaluate the proarrhythmic risk of drugs (e.g. with CiPA) and genetic variants has begun and will develop as the maturity of iPSC derived cardiomyocytes improves along with the advances in assaying technologies (e.g. optogenetics). Thus novel drug-induced cardiac repolarisation prolongation mechanisms, e.g. as with histone deacetylase inhibitors (Kopliar et al., 2016; Spence et al., 2016), may be detected earlier. Together with increasingly complex *in silico* modelling (from cell models to heart simulator in model torso; e.g. Okada et al., 2015), the prediction of the action of a drug or variant would be more informed and testable under physiological and pathophysiological conditions.

Finally it should be remembered that the application of iPSCs to hERG1 study is not limited to cardiomyocytes as hERG1/I_{Kr} is widely expressed and when inhibited yields multisystem phenotypes at least *in vitro*/*ex vivo* systems. Thus e.g. human iPSC derived neurones could provide a system to investigate endogenous hERG1-3.1 and the link between LQT2 and epilepsy. It is surprising even with a homozygous nonsense mutation (Q1070X) which, due to NMD, would result in near complete loss of hERG1 channels that the phenotype was apparently cardiac restricted (Bhuiyan et al., 2008).

7. ACKNOWLEDGEMENTS

This work was carried out in the Institute of Biotechnology, University of Helsinki, the Minerva Foundation Institute for Medical Research, Biomedicum Helsinki and the Department of In Vitro Biology, Orion Pharma, Turku.

I am most grateful to my supervisor Dr Michael Pasternack- firstly for his friendship and support through the years, then for introducing me to hERG1 which would subsequently form the basis for this work. Of course there is also the matter of bring me to Finland.

I thank Prof Eero Mervaala and Prof Fredrik Elinder for carefully reviewing my thesis manuscript and for their suggestions and comments. I would also like to thank Prof Juha Voipio for his help particularly during the dissertation process.

I wish to express my gratitude to my co-authors, especially Cia Ramström, Kid Törnquist, Kristian Paavonen, Kimmo Kontula and Ari Koivisto.

In getting me to initially stay in Finland Michael was ably assisted by members of his former group in Viikki, all of whom made the time both in and outside the lab so pleasurable and to them I am deeply indebted. There are many others from both the Institute of Biotechnology and Minerva who also made my time in Helsinki a joy. Furthermore I warmly acknowledge and thank my colleagues at Orion Pharma for their help and interest.

Finally I thank my family for their continuous love, support and patience. To my parents and grandfather I will always be beholden. To Anita thank you, the end is near! To Elsa thank you for being in my life, I love you.

REFERENCES

Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SAN (1999) MiRP1 forms I_{Kr} potassium channels with HERG and is associated with cardiac arrhythmia. Cell 97: 175 - 187.

Abi-Gerges N, Holkham H, Jones EMC, Pollard CE, Valentin J-P, Robertson GA. (2011) hERG subunit composition determines differential drug sensitivity. Br J Pharmacol 164: 419 – 432.

Ackerman MJ, Tester DJ, Jones GS, Will ML, Burrow CR, Curran ME (2003) Ethnic differences in cardiac potassium channel variants: implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. Mayo Clin Proc 78: 1479 – 1487.

Adsit GS, Vaidyanathan R, Galler CM, Kyle JW, Makielski JC (2013) Channelopathies from mutations in the cardiac sodium channel protein complex. J Mol Cell Cardiol 61: 34 – 43.

Afrasiabi E, Hietamäki M, Viitanen T, Sukumaran P, Bergelin N, Törnquist K (2010) Expression and significance of HERG (KCNH2) potassium channels in the regulation of MDA-MB-435S melanoma cell proliferation and migration. Cell Signal 22: 57 - 64.

Ahmad SR, Wolfe SM (1995) Cisapride and torsades de pointes (Letter). Lancet 345: 508.

Akbarali HI, Thatte H, He XD, Giles WR, Goyal RK. (1999) Role of HERG-like K^+ currents in opossum esophageal circular smooth muscle. Am J Physiol Cell Physiol 277: C1284 – C1290.

Akhavan A, Atanasiu R, Shrier A (2003) Identification of a COOH-terminal segment involved in maturation and stability of human ether-a-go-go-related gene potassium channels. J Biol Chem 278: 40105 – 40112.

Albesa M, Grilo LS, Gavillet B, Abriel H (2011) Nedd4-2-dependent ubiquitylation and regulation of the cardiac potassium channel hERG1. J Mol Cell Cardiol 51: 90 – 98.

Almilaji A, Munoz C, Elvira B, Fajol A, Pakladok T, Honisch S, Shumilina E, Lang F, Föller M (2013) AMP-activated protein kinase regulates hERG potassium channel. Pflügers Arch 465: 1573 – 1582.

Altmann HM, Tester DJ, Will ML, Middha S, Evans JM, Eckloff BW, Ackerman MJ (2015) Homozygous/compound heterozygous triadin mutations associated with autosomal-recessive long-QT syndrome and pediatric sudden cardiac arrest: elucidation of the triadin knockout syndrome. Circulation 131: 2051 – 2060.

Altomare C, Bartolucci C, Sala L, Bernardi J, Mostacciuolo G, Rocchetti M, Severi S, Zaza A (2015) I_{Kr} impact on repolarization and its variability assessed by dynamic clamp. Circ Arrhythm Electrophysiol 8: 1265 – 1275.

Amin AS, Giudicessi JR, Tijsen AJ, Spanjaart AM, Reckman YJ, Klemens CA, Tanck MW, Kapplinger JD, Hofman N, Sinner MF, Müller M, Wijnen WJ, Tan HL, Bezzina CR, Creemers EE, Wilde AAM, Ackerman MJ, Pinto YM (2012) Variants in the 3´ untranslated region of the *KCNQ1*-encoded $K_V7.1$ potassium channel modify disease severity in patients with type 1 long QT syndrome in an allele-specific manner. Eur Heart J 33: 714 – 723.

Anderson CL, Delisle BP, Anson BD, Kilby JA, Will ML, Tester DJ, Gong Q, Zhou Z, Ackerman MJ, January CT (2006) Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. Circulation 113: 365 – 373.

Anderson CL, Kuzmicki CE, Childs RR, Hintz CJ, Delisle BP, January CT (2014) Large-scale mutational analysis of Kv11.1 reveals molecular insights into type 2 long QT syndrome. Nat Commun 5: 5535.

Anderson JH, Bos JM, Meyer FB, Cascino GD, Ackerman MJ (2012) Concealed long QT syndrome and intractable partial epilepsy: a case report. Mayo Clin Proc 87: 1128 – 1131.

Anderson JH, Bos JM, Cascino GD, Ackerman MJ (2014) Prevalence and spectrum of electroencephalogram-identified epileptiform activity amongst patients with long QT syndrome. Heart Rhythm 11: 53 – 57.

Andrade J, Khairy P, Dobrev D, Nattel S (2014) The clinical profile and pathophysiology of atrial fibrillation: relationships among clinical features, epidemiology, and mechanisms. Circ Res 114: 1453 – 1468.

Andreasen L, Nielsen JB, Christophersen IE, Holst AG, Sajadieh A, Tveit A, Haunsø S, Svendsen JH, Schmitt N, Olesen MS (2013) Genetic modifier of the QTc interval associated with early-onset atrial fibrillation. Can J Cardiol 29: 1234 – 1240.

Ando F, Kuruma A, Kawano S (2011) Synergic effects of β-estradiol and erythromycin on hERG currents. J Membr Biol 241: 31 – 38.

Anon (2005a) ICH E14: The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. CPMP/ICH/2/04.

Anon (2005b) ICH S7B: The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. CPMP/ICH/423/02.

Anson BD, Ackerman MJ, Tester DJ, Will ML, Delisle BP, Anderson CI, January CT (2004) Molecular and functional characterization of common polymorphisms in HERG (KCNH2) potassium channels. Am J Physiol Heart Circ Physiol 286: H2434 – H2441.

Antzelevitch C (2007) Ionic, molecular, and cellular basis of QT-interval prolongation and torsade de pointes. Europace 9: Suppl 4 iv4 – 15.

Antzelevitch C, Sicouri S (2012) Mechanisms underlying arrhythmogenesis in long QT syndrome. Card Electrophysiol Clin 4: 17 – 27.

Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeiffer R, Oliva A, Wollnik B, Gelber P, Bonaros EP, Burashnikov E, Wu Y, Sargent JD, Schickel S, Oberheiden R, Bhatia A, Hsu L-F, Haïssaguerre M, Schimpf R, Borggrefe M, Wolpert C (2007) Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation 115: 442 – 449.

Antzelevitch C, Nesterenko V, Shryock JC, Rajamani S, Song Y, Belardinelli L (2014) The role of late I_{Na} in development of cardiac arrhythmias. Handb Exp Pharmacol 221: 137 – 168.

Apaja PM, Foo B, Okiyoneda T, Valinsky WC, Barriere H, Atanasiu R, Ficker E, Lukacs GL, Shrier A (2013) Ubiquitination-dependent quality control of hERG K^+ channel with acquired and inherited conformational defect at the plasma membrane. Mol Biol Cell 24: 3787 – 3804.

Apud JA, Zhang F, Decot H, Bigos KL, Weinberger DR (2012) Genetic variation in KCNH2 associated with expression in the brain of a unique hERG isoform modulates treatment response in patients with schizophrenia. Am J Psychiatry 169: 1 – 10.

Arcangeli A (2011) Ion channels and transporters in cancer. 3. Ion channels in the tumor cell-microenvironment cross talk. Am J Physiol Cell Physiol 301: C762 – C771.

Arcangeli A, Bianchi L, Becchetti A, Faravelli L, Coronnello M, Mini E, Olivotto M, Wanke E (1995) A novel inward-rectifying K^+ current with a cell-cycle dependence governs the resting potential of mammalian neuroblastoma cells. J Physiol 489: 455 – 471.

Arcangeli A, Romoli MR, Boni L, Gerlini G, Tofani L, Urso C, Borgognoni L (2013) High hERG1 expression in advanced melanoma. Melanoma Res 23: 185 – 190.

Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Wang DW, Rhodes TE, George AL, Schwartz PJ (2007) Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. Circulation 115: 361 – 367.

Aronov AM (2008) Tuning out of hERG. Curr Opin Drug Discov Devel 11: 128 – 140.

Asghar MY, Viitanen T, Kemppainen K, Törnquist K (2012) Sphingosine 1-phosphate and human ether-à-go-go-related gene potassium channels modulate migration in human anaplastic thyroid cancer cells. Endocr Relat Cancer 19: 667 - 680.

Ashcroft FM (2006) From molecule to malady. Nature 440: 440 - 447.

Asher V, Warren A, Shaw R, Sowter H, Bali A, Khan R (2011) The role of Eag and HERG channels in cell proliferation and apoptotic cell death in SK-OV-3 ovarian cancer cell line. Cancer Cell Int 11: 6.

Atalar F, Acuner TT, Cine N, Oncu F, Yesilbursa D, Ozbek U, Turkcan S (2010) Two four-marker haplotypes on 7q36.1 region indicate that the potassium channel gene *HERG1* (*KCNH2*, Kv11.1) is related to schizophrenia: a case control study. Behav Brain Funct 6: 27.

Auerbach DS, McNitt S, Gross RA, Zareba W, Dirksen RT, Moss AJ (2016) Genetic biomarkers for the risk of seizure in long QT syndrome. Neurology 87: 1660 - 1668.

Aydar E, Palmer C. (2001) Functional characterization of the C-terminus of the human *ether-à-go-go*-related gene K^+ channel (HERG). J Physiol 534: 1 – 14.

Aydar E, Palmer C. (2006) Expression and functional characterization of the human *ether-à-go-go*-related gene (*HERG*) K^+ channel cardiac splice variant in *Xenopus laevis* oocytes. J Membrane Biol 211: 115 – 126.

Babij P, Askew GR, Nieuwenhuijsen B, Su C-M, Bridal TR, Jow B, Argentieri TM, Kulik J, DeGennaro LJ, Spinelli W, Colatsky TJ (1998) Inhibition of cardiac delayed rectifier K^+ current by overexpression of the long-QT syndrome HERG G628S mutation in transgenic mice. Circ Res 83: 668 – 678.

Bai Y, Wang J, Shan H, Lu Y, Zhang Y, Luo X, Yang B, Wang Z (2007) Sphingolipid metabolite ceramide causes metabolic perturbation contributing to hERG K^+ channel dysfunction. Cell Physiol Biochem 20: 429 – 440.

Balijepalli RC, Delisle BP, Balijepalli SY, Foell JD, Slind JK, Kamp TJ, January CT (2007) Kv11.1 (ERG1) K^+ channels localize in cholesterol and sphingolipid enriched membranes and are modulated by membrane cholesterol. Channels 1: 263 – 272.

Balijepalli SY, Lim E, Concannon SP, Chew CL, Holzem KE, Tester DJ, Ackerman MJ, Delisle BP, Balijepalli RC, January CT (2012) Mechanism of loss of Kv11.1 K^+ current in mutant T421M-Kv11.1-expressing rat ventricular myocytes: interaction of trafficking and gating. Circulation 126: 2809 – 2818.

Banyasz T, Jian Z, Horvath B, Khabbaz S, Izu LT, Chen-Izu Y (2014) Beta-adrenergic stimulation reverses the I_{Kr}-I_{Ks} dominant pattern during cardiac action potential. Pflugers Arch 466: 2067 – 2076.

Barros F, Gómez-Varela D, Viloria CG, Palomero T, Giráldez T, de la Peña P (1998) Modulation of human erg K^+ channel gating by activation of a G protein-coupled receptor and protein kinase C. J Physiol 511: 333 – 346.

Barsheshet A, Moss AJ, McNitt S, Polonsky S, Lopes CM, Zareba W, Robinson JL, Ackerman MJ, Benhorin J, Kaufman ES, Towbin JA, Vincent GM, Qi M, Goldenberg I (2011) Risk of syncope in family members who are genotype-negative for a family-associated long-QT syndrome mutation. Circ Cardiovasc Genet 4: 491 – 499.

Bauer CK, Schäfer R, Schiemann D, Reid G, Hanganu I, Schwarz JR. (1999) A functional role of the erg-like inward-rectifying K^+ current in prolactin secretion from rat lactotrophs. Mol Cell Endocrinol 148: 37 – 45.

Bauer CK, Wulfsen I, Schäfer R, Glassmeier G, Wimmers S, Flitsch J, Lüdecke D, Schwarz J (2003) HERG K^+ currents in human prolactin-secreting adenoma cells. Pflügers Arch 445: 589 – 600.

Beattie DT, Higgins DL, Ero MP, Amagasu SM, Vickery RG, Kersey K, Hopkins A, Smith JAM (2013) An in vitro investigation of the cardiovascular effects of the 5-HT_4 receptor selective agonists, velusetrag and TD-8954. Vascul Pharmacol 58: 150 – 156.

Becchetti A, De Fusco M, Crociani O, Cherubini A, Restano-Cassulini R, Lecchi M, Masi A, Arcangeli A, Casari G, Wanke E (2002) The functional properties of the human *ether-à-go-go*-like (HELK2) K^+ channel. Eur J Neurosci 16: 415 - 428.

Bellin M, Casini S, Davis RP, D'Aniello C, Haas J, Ward-van Oostwaard D, Tertoolen LGJ, Jung CB, Elliott DA, Welling A, Laugwitz K-L, Moretti A, Mummery CL (2013) Isogenic human pluripotent stem cell pairs reveal the role of a KCNH2 mutation in long-QT syndrome. EMBO J 32: 3161 – 3175.

Bellocq C, Wilders R, Schott J-J, Louérat-Oriou B, Boisseau P, Le Marec H, Escande D, Baró I (2004) A common antitussive drug, clobutinol, precipitates the long QT syndrome 2. Mol Pharmacol 66: 1093 – 1102.

Bentzen BH, Bahrke S, Wu K, Larsen AP, Odening KE, Franke G, van's Gravesande KS, Biermann J, Peng X, Koren G, Zehender M, Bode C, Grunnet M, Brunner M (2011) Pharmacological activation of Kv11.1 in transgenic long QT-1 rabbits. J Cardiovasc Pharmacol 57: 223 – 230.

Berecki G, Zegers JG, Verkerk AO, Bhuiyan ZA, de Jonge B, Veldkamp MW, Wilders R, van Ginneken ACG (2005) HERG channel (dys)function revealed by dynamic action potential clamp technique. Biophys J 88: 566 - 578.

Bernèche S, Roux B (2001) Energetics of ion conduction through the K^+ channel. Nature 414: 73 - 77.

Bethge N, Honne H, Hilden V, Trøen G, Eknæs M, Liestøl K, Holte H, Delabie J, Smeland EB, Lind GE (2013) Identification of highly methylated genes across various types of B-cell non-Hodgkin lymphoma. PLoS ONE 8: e79602.

Bett GCL, Zhou Q, Rasmusson RL (2011) Models of HERG gating. Biophys J 101: 631 – 642.

Bezzina CR, Verkerk AO, Busjahn A, Jeron A, Erdmann J, Koopmann TT, Bhuiyan ZA, Wilders R, Mannens MMAM, Tan HL, Luft FC, Schunkert H, Wilde AAM (2003) A common polymorphism in *KCNH2* (HERG) hastens cardiac repolarization. Cardiovasc Res 59: 27 – 36.

Bhuiyan ZA, Momenah TS, Gong Q, Amin AS, Al Ghamdi S, Carvalho JS, Homfray T, Mannens MMAM, Zhou Z, Wilde AAM (2008) Recurrent intrauterine fetal loss due to near absence of HERG: clinical and functional characterization of a homozygous nonsense HERG Q1070X mutation. Heart Rhythm 5: 553 – 561.

Bian J-S, Cui J, McDonald TV (2001) HERG K^+ channel activity is regulated by changes in phosphatidyl inositol 4,5-bisphosphate. Circ Res 89: 1168 – 1176.

Bian J-S, Kagan A, McDonald TV (2004) Molecular analysis of PIP_2 regulation of HERG and I_{Kr}. Am J Physiol Heart Circ Physiol 287: H2154 - H2163.

Bianchi L, Wible B, Arcangeli A, Taglialatela M, Morra F, Castaldo P, Crociani O, Rosati B, Faravelli L, Olivotto M, Wanke E (1998) *herg* encodes a K^+ current highly conserved in tumors of different histogenesis: a selective advantage for cancer cells? Cancer Res 58: 815 – 822.

Biliczki P, Girmatsion Z, Harenkamp S, Anneken L, Brandes RP, Varro A, Marschall C, Herrera D, Hohnloser SH, Nattel S, Ehrlich JR (2008) Cellular properties of C-terminal KCNH2 long QT syndrome mutations: description and divergence from clinical phenotypes. Heart Rhythm 5: 1159 – 1167.

Biliczki P, Girmatsion Z, Brandes RP, Harenkamp S, Pitard B, Charpentier F, Hébert TE, Hohnloser SH, Baró I, Nattel S, Ehrlich JR (2009) Trafficking-deficient long QT syndrome mutation KCNQ1-T587M confers severe clinical phenotype by impairment of KCNH2

membrane localization: evidence for clinically significant I_{Kr}-I_{Ks} α-subunit interaction. Heart Rhythm 6: 1792 – 1801.

Boczek NJ, Best JM, Tester DJ, Giudicessi JR, Middha S, Evans JM, Kamp TJ, Ackerman MJ (2013) Exome sequencing and systems biology converge to identify novel mutations in the L-type calcium channel, CACNA1C, linked to autosomal dominant long QT syndrome. Circ Cardiovasc Genet 6: 279 – 289.

Borodzicz S, Czarzasta K, Kuch M, Cudnoch-Jedrzejewska A (2015) Sphingolipids in cardiovascular diseases and metabolic disorders. Lipids Health Dis 14:55.

Boyce MJ, Kerstens R, Beyens G, Ausma J, Vandeplassche L (2009) Cardiovascular safety of prucalopride in healthy subjects: results from two randomized, double-blind, placebo-controlled, cross-over trials. Gastroenterol 136 Suppl. 1: A-535.

Brahmajothi MV, Morales MJ, Reimer KA, Strauss HC (1997) Regional localization of ERG, the channel protein responsible for the rapid component of the delayed rectifier K^+ current, in the ferret heart. Circ Res 81: 128 – 135.

Brelidze TI, Carlson AE, Zagotta WN (2009) Absence of direct cyclic nucleotide modulation of mEAG1 and hERG1 channels revealed with fluorescence and electrophysiological methods. J Biol Chem 284: 27989 – 27997.

Brelidze TI, Gianulis EC, DiMaio F, Trudeau MC, Zagotta WN (2013) Structure of the C-terminal region of an ERG channel and functional implications. Proc Natl Acad Sci USA 110: 11648 – 11653.

Brimecombe JC, Kirsch GE, Brown AM (2009) Test article concentrations in the hERG assay: Losses through the perfusion, solubility and stability. J Pharmacol Toxicol Methods 59: 29 – 34.

Brink PA, Schwartz PJ (2009) Of founder populations, long QT syndrome, and destiny. Heart Rhythm 6: S25 – S33.

Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, Menendez TM, Brugada J, Pollevick GD, Wolpert C, Burashnikov E, Matsuo K, Wu YS, Guerchicoff A, Bianchi F, Giustetto C, Schimpf R, Brugada P, Antzelevitch C (2004) Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation 109: 30 – 35.

Brunner M, Peng X, Liu GX, Ren X-Q, Ziv O, Choi BR, Mathur R, Hajiri M, Odening KE, Steinberg E, Folco EJ, Pringa E, Centracchio J, Macharzina RR, Donahay T, Schofield L, Rana N, Kirk M, Mitchell GF, Poppas A, Zehender M, Koren G (2008) Mechanisms of cardiac arrhythmias and sudden death in transgenic rabbits with long QT syndrome. J Clin Invest 118: 2246 – 2259.

Cai Y, Wang Y, Xu J, Zuo X, Xu Y (2014) Down-regulation of *ether-a-go-go-related* gene potassium channel protein through sustained stimulation of AT_1 receptor by angiotensin II. Biochem Biophys Res Commun 452: 852 – 857.

Calcaterra NE, Hoeppner DJ, Wei H, Jaffe AE, Maher BJ, Barrow JC (2016) Schizophrenai-associated hERG channel Kv11.1-3.1 exhibits a unique trafficking deficit that is rescued through proteasome inhibition for high throughput screening. Sci Rep 6: 19976.

Camilleri M, Kerstens R, Rykx A, Vandeplassche L (2008) A placebo-controlled trial of prucalopride for severe chronic constipation. N Engl J Med 358: 2344 – 2354.

Camilleri M, Beyens G, Kerstens R, Robinson P, Vandeplassche L (2009) Safety assessment of prucalopride in elderly patients with constipation: a double-blind, placebo-controlled study. Neurogastroenterol Motil 21: 1256 – e117.

Caohuy H, Yang Q, Eudy Y, Ha TA, Xu AE, Glover M, Frizzell RA, Jozwik C, Pollard HB (2014) Activation of 3-phosphoinositide-dependent kinase 1 (PDK1) and serum- and glucocorticoid-induced protein kinase 1 (SGK1) by short-chain sphingolipid C4-ceramide

rescues the trafficking defect of ΔF508-cystic fibrosis transmembrane conductance regulator (ΔF508-CFTR). J Biol Chem 289: 35953 – 35968.

Carretero L, Barros F, Miranda P, Fernández-Trillo J, Machín A, de la Peña P, Domínguez P (2012) Cell type influences the molecular mechanisms involved in hormonal regulation of ERG K^+ channels. Pflügers Arch 463: 685 – 702.

Ceccarini L, Masetti M, Cavalli A, Recanatini M (2012) Ion conduction through the hERG potassium channel. PLoS One 7: e49017.

Chai S, Wan X, Ficker E, Ramirez-Navarro A, Kaufman ES, George AL, Deschenes I (2015) Identification of novel genetic modifiers of LQT2 by combining patient derived iPS derived cardiomyocytes and exome sequencing. Circulation 132: A12669.

Chai W, Chan KY, de Vries R, van den Bogeardt AJ, de Maeyer JH, Schuurkes JAJ, Villalón CM, Saxena PR, AHJ Danser, MaassenVanDenBrink A (2012) Inotropic effects of prokinetic agents with 5-HT_4 receptor agonist actions on human isolated myocardial trabeculae. Life Sci 90: 538 – 544.

Champeroux P, Ouillé A, Martel E, Fowler JSL, Maurin A, Richard S, Le Guennec JY (2011) A step towards characterisation of electrophysiological profile of torsadogenic drugs. J Pharmacol Toxicol Methods 63: 269 – 278.

Champeroux P, Thireau J, Judé S, Laigot-Barbé C, Maurin A, Sola ML, Fowler JSL, Richard S, Le Guennec JY (2015) Short-term variability in QT interval and ventricular arrhythmias induced by dofetilide are dependent on high-frequency autonomic oscillations. Br J Pharmacol 172: 2878 – 2891.

Chandler NJ, Greener ID, Tellez JO, Inada S, Musa H, Molenaar P, Difrancesco D, Baruscotti M, Longhi R, Anderson RH, Billeter R, Sharma V, Sigg DC, Boyett MR, Dobrzynski H (2009) Molecular architecture of the human sinus node: insights into the function of the cardiac pacemaker. Circulation 119: 1562 – 1575.

Chaurasia B, Summers SA (2015) Ceramides- lipotoxic inducers of metabolic disorders. Trends Endocrinol Metab 26: 538 – 550.

Chen J, Seebohm G, Sanguinetti MC (2002) Position of aromatic residues in the S6 domain, not inactivation, dictates cisapride sensitivity of HERG and eag potassium channels. Proc Natl Acad Sci USA 99: 12461 - 12466.

Chen J, Sroubek J, Krishnan Y, Li Y, Bian J, McDonald TV (2009) PKA phosphorylation of HERG protein regulates the rate of channel synthesis. Am J Physiol Heart Circ Physiol 296: H1244 – H1254.

Chen J, Chen K, Sroubek J, Wu Z-Y, Thomas D, Bian JS, McDonald TV (2010) Post-transcriptional control of human ether-a-go-go-related gene potassium channel protein by α-adrenergic receptor stimulation. Mol Pharmacol 78: 186 – 197.

Chen J, Guo J, Yang T, Li W, Lamothe SM, Kang Y, Szendrey JA, Zhang S (2015) Rab11-dependent recycling of the human ether-a-go-go-related gene (hERG) channel. J Biol Chem 290: 21101 – 21113.

Chen X, Wang Q, Ni F, Ma J (2010) Structure of the full-length Shaker potassium channel Kv1.2 by normal-mode-based X-ray crystallographic refinement. Proc Natl Acad Sci USA 107: 11352 – 11357.

Cheng YM, Hull CM, Niven CM, Qi J, Allard CR, Claydon TW (2013) Functional interactions of the voltage sensor charges with an S2 hydrophobic plug in hERG channels. J Gen Physiol 142: 289 – 303.

Cherubini A, Taddei GL, Crociani O, Paglierani M, Buccoliero AM, Fontana L, Noci I, Borri P, Borrani E, Giachi M, Becchetti A, Rosati B, Wanke E, Olivotto M, Arcangeli A (2000) HERG potassium channels are more frequently expressed in human endometrial cancer as compared to non-cancerous endometrium. Br J Cancer 83: 1722 – 1729.

Chiesa N, Rosati B, Arcangeli A, Olivotto M, Wanke E (1997) A novel role for HERG K^+ channels: spike-frequency adaptation. J Physiol 501: 313 – 318.

Choe C-U, Schulze-Bahr E, Neu A, Xu J, Zhu ZI, Sauter K, Bähring R, Priori S, Guicheney P, Mönnig G, Napolitano C, Heidemann J, Clancy CE, Pongs O, Isbrandt D (2006) C-terminal *HERG* (LQT2) mutations disrupt I_{Kr} channel regulation through 14-3-3ε. Hum Mol Genet 15: 2888 - 2902.

Chokshi A, Drosatos K, Cheema FH, Ji R, Khawaja T, Yu S, Kato T, Khan R, Takayama H, Knöll R, Milting H, Chung CS, Jorde U, Naka Y, Mancini DM, Goldberg IJ, Schulze PC (2012) Ventricular assist device implantation corrects myocardial lipotoxicity, reverses insulin resistance, and normalizes cardiac metabolism in patients with advanced heart failure. Circulation 125: 2844 – 2853.

Christé G, Thériault O, Chahine M, Millat G, Rodriguez-Lafrasse C, Rousson R, Deschênes I, Ficker E, Chevalier P (2008) A new C-terminal hERG mutation A915fs+47X associated with symptomatic LQT2 and auditory-trigger syncope. Heart Rhythm 5: 1577 – 1586.

Christiansen M, Hedley PL, Theilade J, Stoevring B, Leren TP, Eschen O, Sørensen KM, Tybjærg-Hansen A, Ousager LB, Pedersen LN, Frikke-Schmidt R, Aidt FH, Hansen MG, Hansen J, Thomsen PE, Toft E, Henriksen FL, Bundgaard H, Jensen HK, Kanters JK (2014) Mutations in Danish patients with long QT syndrome and the identification of a large founder family with pF29L in *KCNH2*. BMC Med Genet 15: 31.

Claassen S, Zünkler BJ (2005) Comparison of the effects of metoclopramide and domperidone on HERG channels. Pharmacol 74: 31 - 36.

Clarke CE, Hill AP, Zhao J, Kondo M, Subbiah RN, Campbell TJ, Vandenberg JI (2006) Effect of S5P α-helix charge mutants on inactivation of hERG K^+ channels. J Physiol 573: 291 – 304.

Cockerill SL, Tobin AB, Torrecilla I, Willars GB, Standen NB, Mitcheson JS (2007) Modulation of hERG potassium currents in HEK-293 cells by protein kinase C: evidence for direct phosphorylation of pore forming subunits. J Physiol 581: 479 - 493.

Cookson J, Hodgson R, Wildgust HJ (2012) Prolactin, hyperprolactinaemia and antipsychotic treatment: a review and lessons for treatment of early psychosis. J Psychopharmacol 26 (Suppl): 42 – 51.

Cordeiro JM, Brugada R, Wu YS, Hong K, Dumaine R (2005) Modulation of I_{Kr} inactivation by mutation N588K in KCNH2: a link to arrhythmogenesis in short QT syndrome. Cardiovasc Res 67: 498 – 509.

Cordeiro JM, Perez GJ, Schmitt N, Pfeiffer R, Nesterenko VV, Burashnikov E, Veltmann C, Borggrefe M, Wolpert C, Schimpf R, Antzelevitch C (2010) Overlapping LQT1 and LQT2 phenotype in a patient with long QT syndrome associated with loss-of-function variations in KCNQ1 and KCNH2. Can J Physiol Pharmacol 88: 1181 – 1190.

Cordeiro JM, Panama BK, Goodrow RJ, Zygmunt AC, White C, Treat JA, Zeina T, Nesterenko VV, Di Diego JM, Burashnikov A, Antzelevitch C (2013a) Developmental changes in expression and biophysics of ion channels in the canine ventricle. J Mol Cell Cardiol 64: 79 – 89.

Cordeiro JM, Nesterenko VV, Sicouri S, Goodrow RJ, Treat JA, Desai M, Wu Y, Doss MX, Antzelevitch C, Di Diego JM (2013b) Identification and characterization of a transient outward K^+ current in human induced pluripotent stem cell-derived cardiomyocytes. J Mol Cell Cardiol 60: 36 – 46.

Cordeiro S, Guseva D, Wulfsen I, Bauer CK (2011) Expression pattern of Kv11 (ether à-go-go-related gene; erg) K^+ channels in the mouse retina. PloS One 6: e29490.

Cordero-Morales JF, Cuello LG, Zhao Y, Jogini V, Cortes DM, Roux B, Perozo E (2006) Molecular determinants of gating at the potassium-channel selectivity filter. Nat Struct Mol Biol 13: 311 - 318.

Cordero-Morales JF, Jogini V, Lewis A, Vásquez V, Cortes DM, Roux B, Perozo E (2007) Molecular driving forces determining potassium channel slow inactivation. Nat Struct Mol Biol 14: 1062 - 1069.

Cordes JS, Sun Z, Lloyd DB, Bradley JA, Opsahl AC, Tengowski MW, Chen X, Zhou J (2005) Pentamidine reduces hERG expression to prolong the QT interval. Br J Pharmacol 145: 15 - 23.

Couette B, Marger L, Nargeot J, Mangoni ME (2006) Physiological and pharmacological insights into the role of ionic channels in cardiac pacemaker activity. Cardiovasc Hematol Disord Drug Targets 6: 169 - 190.

Crociani O, Guasti L, Balzi M, Becchetti A, Wanke E, Olivotto M, Wymore RS, Arcangeli A (2003) Cell cycle-dependent expression of HERG1 and HERG1B isoforms in tumor cells. J Biol Chem 278: 2947 – 2955.

Crociani O, Zanieri F, Pillozzi S, Lastraioli E, Stefanini M, Fiore A, Fortunato A, D'Amico M, Masselli M, De Lorenzo E, Gasparoli L, Chiu M, Bussolati O, Becchetti A, Arcangeli A (2013) hERG1 channels modulate integrin signaling to trigger angiogenesis and tumor progression in colorectal cancer. Sci Rep 3: 3308.

Crociani O, Lastraioli E, Boni L, Pillozzi S, Romoli MR, D'Amico M, Stefanini M, Crescioli S, Masi A, Taddei A, Bencini L, Bernini M, Farsi M, Beghello S, Scarpa A, Messerini L, Tomezzoli A, Vindigni C, Morgagni P, Saragoni L, Giommoni E, Gasperoni S, Di Costanzo F, Roviello F, de Manzoni G, Bechi P, Arcangeli A (2014) hERG1 channels regulate VEGF-A secretion in human gastric cancer: clinicopathological correlations and therapeutical implications. Clin Cancer Res 20: 1 - 11.

Crottès D, Martial S, Rapetti-Mauss R, Pisani DF, Loriol C, Pellissier B, Martin P, Chevet E, Borgese F, Soriani O (2011) Sig1R protein regulates hERG channel expression through a post-translational mechanism in leukemic cells. J Biol Chem 286: 27947 – 27958.

Crotti L, Lundquist AL, Insolia R, Pedrazzini M, Ferrandi C, De Ferrari GM, Vicentini A, Yang P, Roden DM, George AL, Schwartz PJ (2005) *KCNH2*-K897T is a genetic modifier of latent congenital long-QT syndrome. Circulation 112: 1251 – 1258.

Crotti L, Lewandowska MA, Schwartz PJ, Insolia R, Pedrazzini M, Bussani E, Dagradi F, George AL, Pagani F (2009a) A *KCNH2* branch point mutation causing aberrant splicing contributes to an explanation of genotype-negative long QT syndrome. Heart Rhythm 6: 212 - 218.

Crotti L, Monti MC, Insolia R, Peljto A, Goosen A, Brink PA, Greenberg DA, Schwartz PJ, George AL (2009b) *NOS1AP* is a genetic modifier of the long-QT syndrome. Circulation 120: 1657 – 1663.

Crotti L, Hu D, Barajas-Martinez H, De Ferrari GM, Oliva A, Insolia R, Pollevick GD, Dagradi F, Guerchicoff A, Greco F, Schwartz PJ, Viskin S, Antzelevitch C (2012) Torsades de pointes following acute myocardial infarction: evidence for a deadly link with a common genetic variant. Heart Rhythm 9: 1104 – 1112.

Crotti L, Tester DJ, White WM, Bartos DC, Insolia R, Besana A, Kunic JD, Will ML, Velasco EJ, Bair JJ, Ghidoni A, Cetin I, Van Dyke DL, Wick MJ, Brost B, Delisle BP, Facchinetti F, George AL, Schwartz PJ, Ackerman MJ (2013) Long QT syndrome-associated mutations in intrauterine death. JAMA 309: 1473 – 1482.

Crumb WJ (2014) Allosteric effects of erythromycin pretreatment on thioridazine block of hERG potassium channels. Br J Pharmacol 171: 1668 – 1675.

Crumb WJ, Vicente J, Johannesen L, Strauss DG (2016) An evaluation of 30 clinical drugs against the comprehensive *in vitro* proarrhythmia assay (CiPA) proposed ion channel panel. J Pharmacol Toxicol Methods 81: 251 - 262.

Cuello LG, Jogini V, Cortes DM, Perozo E (2010) Structural mechanism of C-type inactivation in K^+ channels. Nature 466: 203 – 208.

Cui J, Melman Y, Palma E, Fishman GI, McDonald TV (2000) Cyclic AMP regulates the HERG K^+ channel by dual pathways. Curr Biol 10: 671 - 674.

Cui J, Kagan A, Qin D, Mathew J, Melman YF, McDonald TV (2001) Analysis of the cyclic nucleotide binding domain of the HERG potassium channel and interactions with KCNE2. J Biol Chem 276: 17244 – 17251.

Curran J, Mohler PJ (2011) Coordinating electrical activity of the heart: ankyrin polypeptides in human cardiac disease. Expert Opin Ther Targets 15: 789 – 801.

Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT (1995) A molecular basis for cardiac arrhythmia: hERG mutations cause long QT syndrome. Cell 80: 795 – 803.

Danielsson BR, Lansdell K, Patmore L, Tomson T (2005) Effects of the antiepileptic drugs lamotrigine, topiramate and gabapentin on hERG potaasium currents. Epilepsy Res 63: 17 – 25.

Danielsson BR, Danielsson C, Nilsson MF. (2007) Embryonic cardiac arrhythmia and generation of reactive oxygen species: common teratogenic mechanism for I_{Kr} blocking drugs. Reprod Toxicol 24: 42 – 56.

Danielsson C, Brask J, Sköld A-C, Genead R, Andersson A, Andersson U, Stockling K, Pehrson R, Grinnemo KH, Salari S, Hellmold H, Danielsson B, Sylvén C, Elinder F (2013) Exploration of human, rat and rabbit embryonic cardiomyocytes suggests K-channel block as a common teratogenic mechanism. Cardiovasc Res 97: 23 – 32.

Dausse E, Berthet M, Denjoy I, André-Fouet X, Cruaud C, Bennaceur M, Fauré S, Coumel P, Schwartz K, Guicheney P (1996) A mutation in HERG associated with notched T waves in long QT syndrome. J Mol Cell Cardiol 28: 1609 – 1615.

De Bruin ML, Pettersson M, Meyboom RHB, Hoes AW, Leufkens HGM (2005) Anti-HERG activity and the risk of drug-induced arrhythmias and sudden death. Eur Heart J 26: 590 - 597.

de la Peña P, Machín A, Fernández-Trillo J, Domínguez P, Barros F (2015) Interactions between the N-terminal tail and the gating machinery of hERG K^+ channels both in closed and open/inactivated states. Pflügers Arch 467: 1747 – 1756.

Delisle BP, Anderson CL, Balijepalli RC, Anson BD, Kamp TJ, January CT (2003) Thapsigargin selectively rescues the trafficking defective LQT2 channels G601S and F805C. J Biol Chem 278: 35749 – 35754.

Delisle BP, Underkofler HAS, Moungey BM, Slind JK, Kilby JA, Best JM, Foell JD, Balijepalli RC, Kamp TJ, January CT (2009) Small GTPase determinants for the Golgi processing and plasmalemmal expression of human ether-a-go-go related (hERG) K^+ channels. J Biol Chem 284: 2844 – 2853.

De Maeyer JH, Lefebvre RA, Schuurkes JAJ (2008) 5-HT4 receptor agonists: similar but not the same. Neurogastroenterol Motil 20: 99 - 112.

Dempsey CE, Wright D, Colenso CK, Sessions RB, Hancox JC (2014) Assessing hERG pore models as templates for drug docking using published experimental constraints: The inactivated state in the context of drug block. J Chem Inf Model 54: 601 – 612.

Dennis AT, Nassal D, Deschenes I, Thomas D, Ficker E (2011) Antidepressant-induced ubiquitination and degradation of the cardiac potassium channel hERG. J Biol Chem 286: 34413 – 34425.

Dennis AT, Wang L, Wan H, Nassal D, Deschenes I, Ficker E (2012) Molecular determinants of pentamidine-induced hERG trafficking inhibition. Mol Pharmacol 81: 198 – 209.

Deo R, Albert CM (2014) Epidemology and genetics of sudden cardiac death. Circulation 125: 620 – 637.

Devaraneni PK, Komarov AG, Costantino CA, Devereaux JJ, Matulef K, Valiyaveetil FI (2013) Semisynthetic K^+ channels show that the constricted conformation of the selectivity filter is not the C-type inactivated state. Proc Natl Acad Sci USA 110: 15698 – 15703.

Ding X-W, Yang W-B, Gao S, Wang W, Li Z, Hu W-M (2010) Prognostic significance of hERG1 expression in gastric cancer. Dig Dis Sci 55: 1004 – 1010.

Di Veroli GY, Davies MR, Zhang H, Abi-Gerges N, Boyett MR (2014) hERG inhibitors with similar potency but different binding kinetics do not pose the same proarrhythmic risk: implications for drug safety assessment. J Cardiovasc Electrophysiol 25: 197 – 207.

Dolderer JH, Schuldes H, Bockhorn H, Altmannsberger M, Lambers C, von Zabern D, Jonas D, Schwegler H, Linke R, Schröder UH (2010) HERG1 gene expression as a specific tumor marker in colorectal tissues. Eur J Surg Oncol 36: 72 – 79.

Dong Q, Fu X-X, Du L-L, Zhao N, Xia C-K, Yu K-W, Cheng L-X, Du Y-M (2013) Blocking of the human ether-à-go-go-related gene channel by imatinib mesylate. Biol Pharm Bull 36: 268 – 275.

Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M, Chivoret G, Schwartz K, Coumel P, Guicheney P (1997) *KvLQT1* C-terminal missense mutation causes a forme fruste long-QT syndrome. Circulation 96: 2778 – 2781.

Donner BC, Marshall C, Schmidt KG (2012) A presumably benign human ether-a-go-go-related gene mutation (R176W) with a malignant primary manifestation of long QT syndrome. Cardiol Young 22: 360 – 363.

Doss MX, Di Diego JM, Goodrow RJ, Wu Y, Cordeiro JM, Nesterenko VV, Barajas-Martinez H, Hu D, Urrutia J, Desai M, Treat JA, Sachinidis A, Antzelevitch C (2012) Maximum diastolic potential of human induced pluripotent stem cell-derived cardiomyocytes depends critically on I_{Kr}. PLoS ONE 7: e40288.

Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: Molecular basis K^+ conduction and selectivity. Science 280: 69 - 77.

Drolet B, Rousseau G, Daleau P, Cardinal R, Turgeon J (2000) Domperidone should not be considered a no-risk alternative to cisapride in the treatment of gastrointestinal motility disorders. Circulation 102: 1883 - 1885.

Drouin E, Charpentier F, Gauthier C, Laurent K, Le Marec H (1995) Electrophysiologic characteristics of cells spanning the left ventricular wall of human heart: evidence for presence of M cells.J Am Coll Cardiol 26: 185 – 192.

Du CY, El Harchi A, Zhang H, Hancox JC (2013) Modification by KCNE1 variants of the hERG potassium channel response to premature stimulation and to pharmacological inhibition. Physiol Rep 1: e00175.

Duncan RS, Ridley JM, Dempsey CE, Leishman DJ, Leaney JL, Hancox JC, Witchel HJ (2006) Erythromycin block of the HERG K^+ channel: accessibility to F656 and Y652. Biochem Biophys Res Commun 341: 500 – 506.

Durdagi S, Randall T, Duff HJ, Chamberlin A, Noskov SY (2014) Rehabilitating drug-induced long QT promoters: in-silico design of hERG-neutral cisapride analogues with retained pharmacological activity. BMC Pharmacol Toxicol 15: 14.

Eap CB, Crettol S, Rougier J-S, Schläpfer J, Sintra Grilo L, Déglon J-J, Besson J, Croquette-Krokar M, Carrupt P-A, Abriel H (2007) Stereoselective block of hERG channel by

(S)-methadone and QT interval prolongation in CYP2B6 slow metabolizers. Clin Pharmacol Ther 81: 719 - 728.

Earle N, Han DY, Pilbrow A, Crawford J, Smith W, Shelling AN, Cameron V, Love DR, Skinner JR (2014) Single nucleotide polymorphisms in arrhythmia genes modify the risk of cardiac events and sudden death in long QT syndrome. Heart Rhythm 11: 76 -82.

Eddy C-A, MacCormick JM, Chung S-K, Crawford JR, Love DR, Rees MI, Skinner JR, Shelling AN (2008) Identification of large gene deletions and duplications in *KCNQ1* and *KCNH2* in patients with long QT syndrome. Heart Rhythm 5: 1275 – 1281.

Ehret GB, Voide C, Gex-Fabry M, Chabert J, Shah D, Broers B, Piguet V, Musset T, Gaspoz J-M, Perrier A, Dayer P, Desmeules JA (2006) Drug-induced long QT syndrome in injection drug users receiving methadone. Arch Intern Med 166: 1280 - 1287.

Ehrlich JR, Pourrier M, Weerapura M, Ethier N, Marmabachi AM, Hebert TE, Nattel S (2004) KvLQT1 modulates the distribution and biophysical properties of HERG: a novel alpha-subunit interaction between delayed rectifier currents. J Biol Chem 279: 1233 – 1241.

Einarsen K, Calloe K, Grunnet M, Olesen S-P, Schmitt M (2009) Functional properties of human neuronal Kv11 channels. Pflügers Arch 458: 689 – 700.

El Harchi A, Melgari D, Zhang YH, Zhang H, Hancox JC (2012) Action potential clamp and pharmacology of the variant 1 short QT syndrome T618I hERG K^+ channel. PloS ONE 7: e52451.

Elliott DJS, Dondas NY, Munsey TS, Sivaprasadarao A (2009) Movement of the S4 segment in the hERG potassium channel during membrane depolarization. Mol Membr Biol 26: 435 – 447.

Elmedyb P, Olesen S-P, Grunnet M (2007) Activation of ERG2 potassium channels by the diphenylurea NS1643. Neuropharmacol 53: 283 – 294.

Emmi A, Wenzel HJ, Schwartzkroin PA, Taglialatela M, Castaldo P, Bianchi L, Nerbonne J, Robertson GA, Janigro D (2000) Do glia have heart? Expression and functional role for *ether-à-go-go* currents in hippocampal astrocytes. J Neurosci 20: 3915 – 3925.

European Medicines Agency (2009) CHMP assessment report for Resolor. Document reference: EMEA/664892/2009 (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001012/WC500053997.pdf)

Erdem M, Tekiner TA, Fejzullahu A, Akan G, Anak S, Saribeyoglu ET, Ozbek U, Atalar F (2015) *herg1b* expression as a potential specific marker in pediatric acute myeloid leukemia patients with hERG 897K/K genotype. Pediatr Hematol Oncol 32: 182 – 192.

Es-Salah-Lamoureux Z, Fougere R, Xiong PY, Robertson GA, Fedida D (2010) Fluorescence-tracking of activation gating in human ERG channels reveals rapid S4 movement and slow pore opening. PloS One 5: e10876.

Es-Salah-Lamoureux Z, Xiong PY, Goodchild SJ, Ahern CA, Fedida D (2011) Blockade of permeation by potassium but normal gating of the G628S nonconducting hERG channel mutant. Biophys J 101: 662 – 670.

Etheridge SP, Compton SJ, Tristani-Firouzi M, Mason JW (2003) A new oral therapy for long QT syndrome: long-term oral potassium improves repolarization in patients with *HERG* mutations. J Am Coll Cardiol 42: 1777 – 1782.

Fano S, Çalişkan G, Heinemann U (2012) Differential effects of blockade of ERG channels on gamma oscillations and excitability in rat hippocampal slices. Eur J Neurosci 36: 3628 – 3635.

Farrelly AM, Ro S, Callaghan BP, Khoyi MA, Fleming N, Horowitz B, Sanders KM, Keef KD (2003) Expression and function of KCNH2 (HERG) in the human jejunum. Am J Physiol Gastrointest Liver Physiol 284: G883 – G895.

Feigenspan A, Trümpler J, Dirks P, Weiler R (2009) *Ether-à-go-go-related* gene (erg1) potassium channels shape the dark response of horizontal cells in the mammalian retina. Pflügers Arch 458: 359 – 377.

Feng J, Yu J, Pan X, Li Z, Chen Z, Zhang W, Wang B, Yang L, Xu H, Zhang G, Xu Z (2014) HERG1 functions as an oncogene in pancreatic cancer and is downregulated by miR-96. Oncotarget 5: 5832 – 5844.

Fermini B, Hancox JC, Abi-Gerges N, Bridgland-Taylor M, Chaudhary KW, Colatsky T, Correll K, Crumb W, Damiano B, Erdemli G, Gintant G, Imredy J, Koerner J, Kramer J, Levesque P, Li Z, Lindqvist A, Obejero-Paz C, Rampe D, Sawada K, Strauss DG, Vandenberg JI (2016) A new perspective in the field of cardiac safety testing through the comprehensive in vitro proarrhythmia assay paradigm. J Biomol Screen 21: 1 – 11.

Fernandez D, Ghanta A, Kauffman GW, Sanguinetti MC (2004) Physicochemical features of the hERG channel drug binding site. J Biol Chem 279: 10120 - 10127.

Fernandez D, Ghanta A, Kinard KI, Sanguinetti MC (2005) Molecular mapping of a site for Cd^{2+}- induced modification of human *ether-à-go-go*-related gene (hERG) channel activation. J Physiol 567: 737 - 755.

Ferreira S, Crumb WJ, Carlton CG, Clarkson CW (2001) Effects of cocaine and its major metabolites on the HERG-encoded potassium channel. J Pharmacol Exp Ther 299: 220 – 226.

Ficker E, Jarolimek W, Kiehn J, Baumann A, Brown AM (1998) Molecular determinants of dofetilide block of HERG K^+ channels. Circ Res 82: 386 – 395.

Ficker E, Jarolimek W, Brown AM (2001) Molecular determinants of inactivation and dofetilide block in ether a-go-go (EAG) channels and EAG-related K^+ channels. Mol Pharmacol 60: 1343 – 1348.

Ficker E, Obejero-Paz CA, Zhao S, Brown AM (2002) The binding site for channel blockers that rescue misprocessed human long QT syndrome type 2 *ether-a-gogo*-related gene (HERG) mutations. J Biol Chem 277: 4989 – 4998.

Ficker E, Dennis AT, Wang L, Brown AM (2003) Role of the cytosolic chaperones Hsp70 and Hsp 90 in maturation of the cardiac potassium channel hERG. Circ Res 92: e87 – e100.

Ficker E, Kuryshev YA, Dennis AT, Obejero-Paz C, Wang L, Hawryluk P, Wible BA, Brown AM (2004) Mechanisms of arsenic-induced prolongation of cardiac repolarization. Mol Pharmacol 66: 33 - 44.

Finley MR, Li Y, Hua F, Lillich J, Mitchell KE, Ganta S, Gilmour RF, Freeman LC (2002) Expression and coassociation of ERG1, KCNQ1, and KCNE1 potassium channel proteins in horse heart. Am J Physiol Heart Circ Physiol 283: H126 – H138.

Fodstad H, Swan H, Laitinen P, Piippo K, Paavonen K, Viitasalo M, Toivonen L, Kontula K (2004) Four potassium channel mutations account for 73% of the genetic spectrum underlying long-QT syndrome (LQTS) and provide evidence for a strong founder effect in Finland. Ann Med 36 (suppl 1): 53 - 63.

Fodstad H, Bendahhou S, Rougier J-S, Laitinen-Forsblom PJ, Barhanin J, Abriel H, Schild L, Kontula K, Swan H (2006) Molecular characterization of two founder mutations causing long QT syndrome and identification of compound heterozygous patients. Ann Med 38: 294 – 304.

Fontana L, D'Amico M, Crociani O, Biagiotti T, Solazzo M, Rosati B, Arcangeli A, Wanke E, Olivotto M (2001) Long-term modulation of HERG channel gating in hypoxia. Biochem Biophys Res Commun 286: 857 – 862.

Frampton JE (2009) Prucalopride. Drugs 69: 2463 - 2476.

Freebern WJ, Fang HS, Slade MD, Wells S, Canale J, Megill J, Grubor B, Shi H, Fletcher A, Lombardo L, Levesque P, Lee FY, Sasseville VG (2007) In vitro cardiotoxicity potential

comparative assessments of chronic myelogenous leukemia tyrosine kinase inhibitor therapies: dasatinib, imatinib and nilotinib. Blood 110: 4582.

Friederich P, Solth A, Schillemeit S, Isbrandt D (2004) Local anaesthetic sensitivities of cloned hERG channels from human heart: comparison with hERG/MiRP1 and hERG/MiRP1$_{T8A}$. Br J Anaesth 92: 93 - 101.

Furlan F, Guasti L, Avossa D, Becchetti A, Cilia E, Ballerini L, Arcangeli A (2005) Interneurons transiently express the ERG K^+ channels during development of mouse spinal networks in vitro. Neurosci 135: 1179 – 1192.

Furlan F, Taccola G, Grandolfo M, Guasti L, Arcangeli A, Nistri A, Ballerini L (2007) ERG conductance expression modulates the excitability of ventral horn GABAergic interneurons that control rhythmic oscillations in the developing mouse spinal cord. J Neurosci 27: 919 – 928.

Furtado MB, Wilmanns JC, Chandran A, Tonta M, Biben C, Eichenlaub M, Coleman HA, Berger S, Bouveret R, Singh R, Harvey RP, Ramialison M, Pearson JT, Parkington HC, Rosenthal NA, Costa MW (2016) A novel conditional mouse model for *Nkx2-5* reveals transcriptional regulation of cardiac ion channels. Differentiation 91: 29 – 41.

Furutani M, Trudeau MC, Hagiwara N, Seki A, Gong Q, Zhou Z, Imamura S, Nagashima H, Kasanuki H, Takao A, Momma K, January CT, Robertson GA, Matsuoka R (1999) Novel mechanism associated with an inherited cardiac arrhythmia: Defective protein trafficking by the mutant hERG (G601S) potassium channel. Circulation 99: 2290 – 2294.

Garborit N, Le Bouter S, Szuts V, Varro A, Escande D, Nattel S, Demolombe S (2007) Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. J Physiol 582: 675 – 693.

Gaborit N, Varro A, le Bouter S, Szuts V, Escande D, Nattel S, Demolombe S (2010) Gender-related differences in ion-channel and transporter subunit expression in non-diseased human hearts. J Mol Cell Cardiol 49: 639 – 646.

Ganapathi SB, Fox TE, Kester M, Elmslie KS (2010) Ceramide modulates HERG potassium channel gating by translocation into lipid rafts. Am J Physiol Cell Physiol 299: C74 – C86.

Ganetzky B, Robertson GA, Wilson GF, Trudeau MC, Titus SA (1999) The eag family of K^+ channels in *Drosophila* and mammals. Ann NY Acad Sci 868: 356 – 369.

Gang H, Zhang S (2006) Na^+ permeation and block of hERG potassium channels. J Gen Physiol 128: 55 – 71.

Gao Y, Zhang P, Li X-B, Wu C-C, Guo J-H (2013) A novel deletion-frameshift mutation in the S1 region of *HERG* gene in a chinese family with long QT syndrome. Chin Med J 126: 3093 – 3096.

Garberoglio L, Giustetto C, Wolpert C, Gaita F (2007) Is acquired short QT due to digitalis intoxication responsible for malignant ventricular arrhythmias? J Electrocardiol 40: 43 – 46.

Garg V, Stary-Weinzinger A, Sachse F, Sanguinetti MC (2011) Molecular determinants for activation of human *ether-à-go-go-related* gene 1 potassium channels by 3-nitro-*N*-(4-phenoxyphenyl) benzamide. Mol Pharmacol 80: 630 – 637.

Gasparoli L, D'Amico M, Masselli M, Pillozzi S, Caves R, Khuwaileh R, Tiedke W, Mugridge K, Pratesi A, Mitcheson JS, Basso G, Becchetti A, Arcangeli A (2015) New pyrimido-indole compound CD-160130 preferentially inhibits the Kv11.1B isoform and produces antileukemic effects without cardiotoxicity. Mol Pharmacol 87: 183 – 196.

Gentile S, Martin N, Scappini E, Williams J, Erxleben C, Armstrong DL (2008) The human ERG1 channel polymorphism, K897T, creates a phosphorylation site that inhibits channel activity. Proc Natl Acad Sci USA 105: 14704 – 14708.

Gerlach AC, Stoehr SJ, Castle NA (2010) Pharmacological removal of human ether-à-go-go-related gene potassium channel inactivation by 3-nitro-*N*-(4-phenoxyphenyl) benzamide (ICA-105574). Mol Pharmacol 77: 58 – 68.

Gessner G, Heinemann SH (2003) Inhibition of hEAG1 and hERG1 potassium channels by clofilium and its tertiary analogue LY97241. Br J Pharmacol 138: 161 – 171.

Gianulis EC, Trudeau MC (2011) Rescue of aberrant gating by a genetically encoded PAS (Per-Arnt-Sim) domain in several long QT syndrome mutant human *ether-á-go-go*-related gene potassium channels. J Biol Chem 286: 22160 – 22169.

Gianulis EC, Liu Q, Trudeau MC (2013) Direct interaction of eag domains and cyclic nucleotide-binding homology domains regulate deactivation gating in hERG channels. J Gen Physiol 142: 351 – 366.

Gintant G (2000) Characterization and functional consequences of delayed rectifier current transient in ventricular repolarization. Am J Physiol Heart Circ Physiol 278: H806 – 817.

Gintant G (2011) An evaluation of hERG current assay performance: translating preclinical safety studies to clinical QT prolongation. Pharmacol Ther 129: 109 – 119.

Giustetto C, Schimpf R, Mazzanti A, Scrocco C, Maury P, Anttonen O, Probst V, Blanc JJ, Sbragia P, Dalmasso P, Borggrefe M, Gaita F (2011) Long-term follow-up of patients with short QT syndrome. J Am Coll Cardiol 58: 587 – 595.

Gladding PA, Evans C-A, Crawford J, Chung SK, Vaughan A, Webster D, Neas K, Love DR, Rees MI, Shelling AN, Skinner JR (2010) Posthumous diagnosis of long QT syndrome from neonatal screening cards. Heart Rhythm 7: 481 - 486.

Glassmeier G, Hempel K, Wulfsen I, Bauer CK, Schumacher U, Schwarz JR (2012) Inhibition of HERG1 K^+ channel protein expression decreases cell proliferation of human small cell lung cancer cells. Pflügers Arch 463: 365 – 376.

Glinka A, Polak S (2012) Wild type and K897T polymorphisms of the hERG gene: modeling the APD in Caucasians. Bioinformation 8: 1062 – 1065.

Glukhov AV, Fedorov VV, Lou Q, Ravikumar VK, Kalish PW, Schuessler RB, Moazami N, Efimov IR (2010) Transmural dispersion of repolarization in falling and nonfalling human ventricle. Circ Res 106: 981 – 991.

Goineau S, Legrand C, Froget G (2012) Whole-cell configuration of the patch-clamp technique in the hERG channel assay to predict the ability of a compound to prolong QT interval. Curr Protoc Pharmacol 10.15.1 – 10.15.14.

Goldenberg I, Moss AJ, Peterson, DR, McNitt S, Zareba, W, Andrews ML, Robinson JL, Locati EH, Ackerman MJ, Benhorin J, Kaufman ES, Napolitano C, Priori SG, Qi M, Schwartz PJ, Towbin JA, Vincent GM, Zhang L (2008) Risk factors for aborted cardiac arrest and sudden cardiac death in children with congenital long-QT syndrome. Circulation 117: 2184 – 2191.

Goldenberg I, Horr S, Moss AJ, Lopes CM, Barsheshet A, McNitt S, Zareba, W, Andrews ML, Robinson JL, Locati EH, Ackerman MJ, Benhorin J, Kaufman ES, Napolitano C, Platonov PG, Priori SG, Qi M, Schwartz PJ, Shimizu W, Towbin JA, Vincent GM, Wilde AA, Zhang L (2011) Risk for life-threatening cardiac events in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. J Am Coll Cardiol 57: 51 – 59.

Gómez-Varela D, Barros F, Viloria CG, Giráldez T, Manso DG, Dupuy SG, Miranda P, de la Peña P (2003) Relevance of the proximal domain in the amino-terminus of HERG channels for regulation by phospholipase C-coupled hormone receptor. FEBS Lett 535: 125 – 130.

Gómez-Varela D, Contreras-Jurado C, Furini S, Garcia-Ferreiro R, Stühmer W, Pardo LA (2006) Different relevance of inactivation and F468 residue in the mechanisms of hEag1 channel blockage by astemizole, imipramine and dofetilide. FEBS Lett 580: 5059 – 5066.

Gong Q, Anderson CL, January CT, Zhou Z (2002) Role of glycosylation in cell surface expression and stability of HERG potassium channels. Am J Physiol Heart Circ Physiol 283: H77 – H84.

Gong Q, Anderson CL, January CT, Zhou Z (2004a) Pharmacological rescue of trafficking defective HERG channels formed by coassembly of wild-type and long QT mutant N470D subunits. Am J Physiol Heart Circ Physiol 287: H652 – H658.

Gong Q, Keeney DR, Robinson JC, Zhou Z (2004b) Defective assembly and trafficking of mutant HERG channels with C-terminal truncations in long QT syndrome. J Mol Cell Cardiol 37: 1225 – 1233.

Gong Q, Keeney DR, Molinari M, Zhou Z (2005) Degradation of trafficking-defective long QT syndrome type II mutant channels by the ubiquitin-proteasome pathway. J Biol Chem 280: 19419 – 19425.

Gong Q, Jones MA, Zhou Z (2006) Mechanisms of pharmacological rescue of trafficking-defective hERG mutant channels in human long QT syndrome. J Biol Chem 281: 4069 – 4074.

Gong Q, Zhang L, Vincent GM, Horne BD, Zhou Z (2007) Nonsense mutations in hERG cause a decrease in mutant mRNA transcripts by nonsense-mediated mRNA decay in human long QT syndrome. Circulation 116: 17 – 24.

Gong Q, Zhang L, Moss AJ, Vincent GM, Ackerman MJ, Robinson JC, Jones MA, Tester DJ, Zhou Z (2008) A splice site mutation in hERG leads to cryptic splicing in human long QT syndrome. J Mol Cell Cardiol 44: 502 – 509.

Gong Q, Stump MR, Dunn AR, Deng V, Zhou Z (2010) Alternative splicing and polyadenylation contribute to the generation of hERG1 C-terminal isoforms. J Biol Chem 285: 32233 – 32241.

Gong Q, Stump MR, Zhou Z (2011) Inhibition of nonsense-mediated mRNA decay by antisense morpholino oligonucleotides restores functional expression of hERG nonsense and frameshift mutations in long-QT syndrome. J Mol Cell Cardiol 50: 223 - 229.

Gong Q, Stump MR, Deng V, Zhang L, Zhou Z (2014a) Identification of Kv11.1 isoform switch as a novel pathogenic mechanism of long-QT syndrome. Circ Cardiovasc Genet 7: 482 - 490.

Gong Q, Stump MR, Zhou Z (2014b) Position of premature termination codons determines susceptibility of hERG mutations to nonsense-mediated mRNA decay in long QT syndrome. Gene 539: 190 - 197.

González T, Arias C, Caballero R, Moreno I, Delpón E, Tamargo J, Valenzuela C (2002) Effects of levobupivacaine, ropivacaine and bupivacaine on *HERG* channels: stereoselective bupivacaine block. Br J Pharmacol 137: 1269 – 1279.

Goodchild SJ, Macdonald LC, Fedida D (2015) Sequence of gating charge movement and pore gating in hERG activation and deactivation pathways. Biophys J 108: 1435 – 1447.

Gordon E, Panaghie G, Deng L, Bee KJ, Roepke TK, Krogh-Madsen T, Christini DJ, Ostrer H, Basson CT, Chung W, Abbott GW (2008) A KCNE2 mutation in a patient with cardiac arrhythmia induced by auditory stimuli and serum electrolyte imbalance. Cardiovasc Res 77: 98 – 106.

Gouas L, Nicaud V, Berthet M, Forhan A, Tiret L, Balkau B, Guicheney P, the D.E.S.I.R. study group (2005) Association of *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A* polymorphisms with QTc interval length in a healthy population. Eur J Hum Genet 13: 1213 – 1222.

Grant AO (2009) Cardiac ion channels. Circ Arrhythm Electrophysiol 2: 185 – 194.

Greenwood IA, Yeung SY, Tribe RM, Ohya S. (2009) Loss of function K^+ channels encoded by *ether-à-go-go-related* genes in mouse myometrium prior to labour onset. J Physiol 587: 2313 – 2326.

Grunnet M, Diness TG, Hansen RS, Olesen S-P (2008) Biophysical characterization of the short QT mutation hERG-N588K reveals a mixed gain- and loss-of-function. Cell Physiol Biochem 22: 611 – 624.

Guasti L, Cilia E, Crociani O, Hofmann G, Polvani S, Becchetti A, Wanke E, Tempia F, Arcangeli A (2005) Expression pattern of the ether-a-go-go-related (ERG) family proteins in the adult mouse central nervous system: evidence for coassembly of different subunits. J Comp Neurol 491: 157 – 174.

Guasti L, Crociani O, Redaelli E, Pillozzi S, Polvani S, Masselli M, Mello T, Galli A, Amedei A, Wymore RS, Wanke E, Arcangeli A (2008) Identification of a posttranslational mechanism for the regulation of hERG1 K^+ channel expression and hERG1 current density in tumor cells. Mol Cell Biol 28: 5043 – 5060.

Gullo F, Ales E, Rosati B, Lecchi M, Masi A, Guasti L, Cano-Abad MF, Arcangeli A, Lopez MG, Wanke E (2003) ERG K^+ channel blockade enhances firing and epinephrine secretion in rat chromaffin cells: the missing link to LQT2-related sudden death? FASEB J 17: 330 – 332.

Guo D, Zhao X, Wu Y, Liu T, Kowey PR, Yan GX (2007) L-type calcium current reactivation contributes to arrhythmogenesis associated with action potential triangulation. J cardiovasc Electrophysiol 18: 196 – 203.

Guo J, Gang H, Zhang S (2006) Molecular determinants of cocaine block of human ether-á-go-go-related gene potassium channels. J Pharmacol Exp Ther 317: 865 – 874.

Guo J, Massaeli H, Li W, Xu J, Luo T, Shaw J, Kirshenbaum LA, Zhang S (2007) Identification of I_{Kr} and its trafficking disruption induced by probucol in cultured neonatal rat cardiomyocytes. J Pharmacol Exp Ther 321: 911 - 920.

Guo J, Massaeli H, Xu J, Jia Z, Wigle JT, Mesaeli N, Zhang S (2009) Extracellular K^+ concentration controls cell surface density of I_{Kr} in rabbit hearts and of the HERG channel in human cell lines. J Clin Invest 119: 2745 – 2757.

Guo J, Li X, Shallow H, Xu J, Yang T, Massaeli H, Li W, Sun T, Pierce GN, Zhang S (2011a) Involvement of caveolin in probucol-induced reduction in hERG plasma-membrane expression. Mol Pharmacol 79: 806 – 813.

Guo J, Wang T, Yang T, Xu J, Li W, Fridman MD, Fisher JT, Zhang S (2011b) Interaction between the cardiac rapidly (I_{Kr}) and slowly (I_{Ks}) activating delayed rectifier potassium channels revealed by low K^+-induced hERG endocytic degradation. J Biol Chem 286: 34664 – 34674.

Guo J, Wang T, Li X, Shallow H, Yang T, Li W, Xu J, Fridman MD, Yang X, Zhang S (2012) Cell surface expression of human ether-a-go-go-related gene (hERG) channels is regulated by caveolin-3 protein via the ubiquitin ligase Nedd4-2. J Biol Chem 287: 33132 – 33141.

Gussak I, Brugada P, Brugada J, Wright RS, Kopecky SL, Chaitman BR, Bjerregaard P (2000) Idiopathic short QT interval: a new clinical syndrome? Cardiology 94: 99 – 102.

Gustina AS, Trudeau MC (2011) hERG potassium channel gating is mediated by N- and C-terminal region interactions. J Gen Physiol 137: 315 – 325.

Gutman GA, Chandy KG, Grissmer S, Lazdunski M, McKinnon D, Pardo LA, Robertson GA, Rudy B, Sanguinetti MC, Stuhmer W, Wang X (2005) International union of pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. Pharmacol Rev 57: 473 - 508.

Hagendorf S, Fluegge D, Engelhardt C, Spehr M (2009) Homeostatic control of sensory output in basal vomeronasal neurons: activity-dependent expression of *ether-à-go-go*-related gene potassium channels. J Neurosci 29: 206 – 221.

Hajj A, Ksouda K, Peoc'h K, Curis E, Messali A, Deveaux LL, Bloch V, Prince N, Mouly S, Scherrmann J-M, Lépine J-P, Laplanche J-L, Drici M-D, Vorspan F (2014) KCNH2 polymorphism and methadone dosage interact to enhance QT duration. Drug Alcohol Depend 141: 34 – 38.

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391: 85 - 100.

Hancox JC, Levi AJ, Witchel HJ (1998) Time course and voltage dependence of expressed HERG current compared with native "rapid" delayed rectifier K current during the cardiac ventricular action potential. Pflügers Arch 436: 843 – 853.

Hannun YA (1996) Functions of ceramide in coordinating cellular reponses to stress. Science 274: 1855 – 1859.

Hardman RM, Forsythe ID (2009) *Ether-à-go-go*-related gene K^+ channels contribute to threshold excitability of mouse auditory brainstem neurons. J Physiol 587: 2487 - 2497.

Hardman RM, Stansfeld PJ, Dalibalta S, Sutcliffe MJ, Mitcheson JS (2007) Activation gating of hERG potassium channels. J Biol Chem 282: 31972 - 31981.

Hardy AB, Fox JEM, Giglou PR, Wijesekara N, Bhattacharjee A, Sultan S, Gyulkhandanyan AV, Gaisano HY, MacDonald PE, Wheeler MB (2009) Characterization of ERG K^+ channels in α- and β-cells of mouse and human islets. J Biol Chem 284: 30441 – 30452.

Harley CA, Jesus CSH, Carvalho R, Brito RMM, Morais-Cabral JH (2012) Changes in channel trafficking and protein stability caused by LQT2 mutations in the PAS domain of the hERG channel. PLoS ONE 7: e32654.

Harmati G, Bányász T, Bárándi L, Szentandrássy N, Horváth B, Szabó G, Szentmiklósi JA, Szénási G, Nánási PP, Magyar J (2011) Effects of β-adrenoceptor stimulation on delayed rectifier K^+ currents in canine ventricular cardiomyocytes. Br J Pharmacol 162: 890 – 896.

Harrell DT, Ashihara T, Ishikawa T, Tominaga I, Mazzanti A, Takahashi K, Oginosawa Y, Abe H, Maemura K, Sumitomo N, Uno K, Takano M, Priori SG, Makita N (2015) Genotype-dependent differences in age of manifestation and arrhythmia complications in short QT syndrome. Int J Cardiol 190: 393 – 402.

Hashimoto R, Ohi K, Yasuda Y, Fukumoto M, Yamamori H, Kamino K, Morihara T, Iwase M,Kazui H, Takeda M (2011) The KCNH2 gene is associated with neurocognition and the risk of schizophrenia. World J Biol Psychiatry 14: 114 – 120.

Haugaa KH, Amlie JP, Berge KE, Leren TP, Smiseth OA, Edvardsen T (2010) Transmural differences in myocardial contraction in long QT syndrome: mechanical consequences of ion channel dysfunction. Circulation 122: 1355 – 1363.

Haugaa KH, Vestervik TT, Andersson S, Amlie JP, Jørum E, Gjerstad L, Taubøll E (2013) Abnormal electroencephalograms in patients with long QT syndrome. Heart Rhythm 10: 1877 – 1883.

Hayashi K, Shuai W, Sakamoto Y, Higashida H, Yamagishi M, Kupershmidt S (2010) Trafficking-competent KCNQ1 variably influences the function of HERG long QT alleles. Heart Rhythm 7: 973 - 980.

Hayashi K, Konno T, Tada H, Tani S, Liu L, Fujino N, Nohara A, Hodatsu A, Tsuda T, Tanaka Y, Kawashiri MA, Ino H, Makita N, Yamagishi M (2015) Functional characterization of rare variants implicated in susceptibility to lone atrial fibrillation. Circ Arrhythm Electrophysiol 8: 1095 - 1104.

Heath BM, Terrar DA (2000) Protein kinase C enhances the rapidly activating delayed rectifier potassium current, I_{Kr}, through a reduction in C-type inactivation in guinea-pig ventricular myocytes. J Physiol 522: 391 – 402.

Hedley PL, Jørgensen P, Schlamowitz S, Wangari R, Moolman-Smook J, Brink PA, Kanters JK, Corfield VA, Christiansen M (2009) The genetic basis of long QT and short QT syndromes: a mutation update. Hum Mutat 30: 1486 - 1511.

Heide J, Mann SA, Vandenberg JI (2012) The schizophrenia-associated Kv11.1-3.1 isoform results in reduced current accumulation during repetitive brief depolarizations. PLoS ONE 7: e45624.

Heide J, Zhang F, Bigos KL, Mann SA, Carr VJ, Shannon Weickert C, Green MJ, Weinberger DR, Vandenberg JI (2016) Differential response to risperidone in schizophrenia patients by *KCNH2* genotype and drug metabolizer status. Am J Psychiatry 173: 53 – 59.

Heo DH, Kang T-C (2012) The changes of ERG channel expression after administration of antiepileptic drugs in the hippocampus of epilepsy gerbil model. Neurosci Lett 507: 27 – 32.

Hesdorffer DC, Tomson T (2013) Sudden unexpected death in epilepsy: potential role of antiepileptic drugs. CNS Drugs 27: 113 – 119.

Hill AP, Perrin MJ, Heide J, Campbell TJ, Mann SA, Vandenberg JI (2014) Kinetics of drug interaction with the Kv11.1 potassium channel. Mol Pharmacol 85: 769 – 776.

Himmel HM, Hoffmann M (2010) QTc shortening with a new investigational cancer drug: A brief case study. J Pharmacol Toxicol Methods 62: 72 – 81.

Hirdes W, Schweizer M, Schuricht KS, Guddat SS, Wulfsen I, Bauer CK, Schwarz JR (2005) Fast erg K^+ currents in rat embryonic serotonergic neurones. J Physiol 564: 33 - 49.

Hirdes W, Napp N, Wulfsen I, Schweizer M, Schwarz JR, Bauer CK (2009) Erg K^+ currents modulate excitability in mouse mitral/tufted neurons. Pflügers Arch 459: 55 - 70.

Hirdes W, Dinu C, Bauer CK, Boehm U, Schwarz JR (2010) Gonadotropin-releasing hormone inhibits ether-à-go-go-related gene K^+ currents in mouse gonadotropes. Endocrinol 151: 1079 - 1088.

Hoekstra M, Mummery CL, Wilde AAM, Bezzina CR, Verkerk AO (2012) Induced pluripotent stem cell derived cardiomyocytes as models for cardiac arrhythmias. Front Physiol 3: 346.

Holbrook M, Malik M, Shah RR, Valentin J-P (2009) Drug induced shortening of the QT/QTc interval: An emerging safety issue warranting further modelling and evaluation in drug research and development? J Pharmacol Toxicol Methods 59: 21 – 28.

Holzem KM, Gomez JF, Glukhov AV, Madden EJ, Koppel AC, Ewald GA, Trenor B, Efimov IR (2016) Reduced response to I_{Kr} blockade and altered hERG1a/1b stoichiometry in human heart failure. J Mol Cell Cardiol 96: 82 – 92.

Hondeghem LM. (2013) Domperidone: limited benefits with significant risk for sudden cardiac death. J Cardiovasc Pharmacol 61: 218 – 225.

Hong K, Bjerregaard P, Gussak I, Brugada R (2005) Short QT syndrome and atrial fibrillation caused by mutation in KCNH2. J Cardiovasc Electrophysiol 16: 394 – 396.

Hu D, Barajas-Martinez H, Pfeiffer R, Gollob MH, Healey J, Leopold HB, Giustetto C, Gaita F, Antzelevitch C (2011) A mutation hotspot in KCNH2 associated with short QT syndrome, SCD and SIDS. Heart Rhythm 8: S322 – S323.

Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V, Lipska BK, Hyde TM, Song J, Rujescu D, Giegling I, Mayilyan K, Proust MJ, Soghoyan A, Caforio G, Callicott JH, Bertolino A, Meyer-Lindenberg A, Chang J, Ji Y, Egan MF, Goldberg TE, Kleinman JE, Lu B, Weinberger DR (2009) A primate-specific, brain isoform of *KCNH2* affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. Nat Med 15: 509 – 518.

Imai YN, Ryu S, Oiki S (2009) Docking model of drug binding to the human ether-à-go-go potassium channel guided by tandem dimer mutant patch-clamp data: a synergic approach. J Med Chem 52: 1630 - 1638.

Islas LD (2016) Functional diversity of potassium channel voltage-sensing domains. Channels 10: 202 – 213.

Itoh H, Sakaguchi T, Ding W-G, Watanabe E, Watanabe I, Nishio Y, Makiyama T, Ohno S, Akao M, Higashi Y, Zenda N, Kubota T, Mori C, Okajima K, Haruna T, Miyamoto A, Kawamura M, Ishida K, Nagaoka I, Oka Y, Nakazawa Y, Yao T, Jo H, Sugimoto Y, Ashihara T, Hayashi H, Ito M, Imoto K, Matsuura H, Horie M (2009a) Latent genetic backgrounds and molecular pathogenesis in drug-induced long QT syndrome. Circ Arrhythmia Electrophysiol 2: 511 – 523.

Itoh H, Sakaguchi T, Ashihara T, Ding W-G, Nagaoka I, Oka Y, Nakazawa Y, Yao T, Jo H, Ito M, Nakamura K, Ohe T, Matsuura H, Horie M (2009b) A novel *KCNH2* mutation as a modifier for short QT interval. Int J Cardiol 137: 83 – 85.

Itoh H, Shimizu W, Hayashi K, Yamagata K, Sakaguchi T, Ohno S, Makiyama T, Akao M, Ai T, Noda T, Miyazaki A, Miyamoto Y, Yamagishi M, Kamakura S, Horie M (2010) Long QT syndrome with compound mutations is associated with a more severe phenotype: a Japanese multicenter study. Heart Rhythm 7: 1411 – 1418.

Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, Feldman O, Gepstein A, Arbel G, Hammerman H, Boulos M, Gepstein L (2011) Modelling the long QT syndrome with induced pluripotent stem cells. Nature 471: 225 - 229.

Iwai C, Li P, Hoshikawa Y, Morikawa K, Maharani N, Higaki K, Sasano T, Notsu T, Ishido Y, Miake J, Yamamoto Y, Shirayoshi Y, Ninomiya H, Nakai A, Murata S, Yoshida A, Yamamoto K, Hiraoka M, Hisatome I (2013) Hsp90 prevents interaction between CHIP and HERG proteins to facilitate maturation of wild-type and mutant HERG proteins. Cardiovasc Res 100: 520 – 528.

Iwasa H, Itoh T, Nagai R, Nakamura Y, Tanaka T (2000) Twenty single nucleotide polymorphisms (SNPs) and their allelic frequencies in four genes that are responsible for familial long QT syndrome in the Japanese population. J Hum Genet 45: 182 – 183.

Jagodzińska M, Szperl M, Ponińska J, Kosiec A, Gajda R, Kukla P, Biernacka EK (2016) Coexistence of Andersen-Tawil syndrome with polymorphisms in *hERG1* gene (K897T) and *SCN5A* gene (H558R) in one family. Ann Noninvasive Electrocardiol 21: 189 – 195.

Jamshidi Y, Nolte IM, Dalageorgou C, Zheng D, Johnson T, Bastiaenen R, Ruddy S, Talbott D, Norris KJ, Snieder H, George AL, Marshall V, Shakir S, Kannankeril PJ, Munroe PB, Camm AJ, Jeffery S, Roden DM, Behr ER (2012) Common variation in the *NOS1AP* gene is associated with drug-induced QT prolongation and ventricular arrhythmia. J Am Coll Cardiol 60: 841 – 850.

January CT, Riddle JM (1989) Early afterdepolarizations: mechanism of induction and block. Circ Res 64: 977 – 990.

Jehle J, Ficker E, Wan X, Deschenes I, Kisselbach J, Wiedmann F, Staudacher I, Schmidt C, Schweizer PA, Becker R, Katus HA, Thomas D (2013) Mechanisms of zolpidem-induced long QT syndrome: acute inhibition of recombinant hERG K^+ channels and action potential prolongation in human cardiomyocytes derived from induced pluripotent stem cells. Br J Pharmacol 168: 1215 – 1229.

Jensen MØ, Borhani DW, Lindorff-Larsen K, Maragakis P, Jogini V, Eastwood MP, Dror RO, Shaw DE (2010) Principles of conduction and hydrophobic gating in K^+ channels. Proc Natl Acad Sci USA 107: 5883 – 5838.

Jensen MØ, Jogini V, Borhani DW, Leffler AE, Dror RO, Shaw DE (2012) Mechanism of voltage gating in potassium channels. Science 336: 229 – 233.

Jervell A, Lange-Nielsen F (1957) Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. Am Heart J 54: 59 - 68.

Ji H, Tucker KR, Putzier I, Huertas MA, Horn JP, Canavier CC, Levitan ES, Shepard PD (2012) Functional characterization of ether-à-go-go-related gene potassium channels in midbrain dopamine neurons- implications for a role in depolarization block. Eur J Neurosci 36: 2906 – 2916.

Jiang C, Atkinson D, Towbin JA, Splawski I, Lehmann MH, Li H, Timothy K, Taggart RT, Schwartz PJ, Vincent GM, Moss AJ, Keating MT (1994) Two long QT syndrome loci map to chromosome 3 and 7 with evidence for further heterogeneity. Nat Genet 8: 141 – 147.

Jiang M, Zhang M, Tang DG, Clemo HF, Liu J, Holwitt D, Kasirajan V, Pond AL, Wettwer E, Tseng G-N (2004) KCNE2 protein is expressed in ventricles of different species, and changes in its expression contribute to electrical remodelling in diseased hearts. Circulation 109: 1783 - 1788.

Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R (2002) The open pore conformation of potassium channels. Nature 417: 523 - 526.

Johnson JN, Tester DJ, Perry J, Salisbury BA, Reed CR, Ackerman MJ (2008) Prevalence of early-onset atrial fibrillation in congenital long QT syndrome. Heart Rhythm 5: 704 – 709.

Johnson JN, Hofman N, Haglund CM, Cascino GD, Wilde AAM, Ackerman MJ (2009) Identification of a possible pathogenic link between congenital long QT syndrome and epilepsy. Neurol 72: 224 – 231.

Jones EM, Roti Roti EC, Wang J, Delfosse SA, Robertson GA (2004) Cardiac IKr channels minimally comprise hERG1a and 1b subunits. J Biol Chem 279: 44690 – 44694.

Jones DK, Liu F, Vaidyanathan R, Eckhardt LL, Trudeau MC, Robertson GA (2014) hERG1b is critcal for human cardiac repolarization. Proc Natl Acad Sci USA 111: 18073 – 18077.

Jones DK, Liu F, Dombrowski N, Joshi S, Robertson GA (2016) Dominant negative consequences of a hERG1b-specific mutation associated with intrauterine fetal death. Prog Biophys Mol Biol 120: 67 – 76.

Jost N, Virág L, Bitay M, Takács J, Lengyel C, Biliczki P, Nagy Z, Bogáts G, Lathrop DA, Papp JG, Varró A (2005) Restricting excessive cardiac action potential and QT prolongation: a vital role for I_{Ks} in human ventricular muscle. Circulation 112: 1392 – 1399.

Jouni M, Si-Tayeb K, Es-Salah-Lamoureux Z, Latypova X, Champon B, Caillaud A, Rungoat A, Charpentier F, Loussouarn G, Baró I, Zibara K, Lemarchand P, Gaborit N (2015) Toward personalized medicine: using cardiomyocytes differentiated from urine-derived pluripotent stem cells to recapitulate electrophysiological characteristics of type 2 long QT syndrome. J Am Heart Assoc 4: e002159.

Kagan A, Melman YF, Krumerman A, McDonald TV (2002) 14-3-3 amplifies and prolongs adrenergic stimulation of HERG K^+ channel activity. EMBO J 21: 1889 - 1898.

Kamiya K, Niwa R, Mitcheson JS, Sanguinetti MC (2006) Molecular determinants of hERG channel block. Mol Pharmacol 69: 1709 - 1716.

Kamiya K, Niwa R, Morishima M, Honjo H, Sanguinetti MC (2008) Molecular determinants of hERG channel block by terfenadine and cisapride. J Pharmacol Sci 108: 301 – 307.

Kang J, Chen X-L, Rampe D (2001a) The antipsychotic drugs sertindole and pimozide block *erg3*, a human brain K^+ channel. Biochem Biophys Res Commun 286: 499 – 504.

Kang J, Wang L, Chen X-L, Triggle DJ, Rampe D (2001b) Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K^+ channel HERG. Mol Pharmacol 59: 122 – 126.

Kang J, Chen X-L, Wang H, Rampe D (2003) Interactions of the narcotic *l*-*α*-acetylmethadol with human cardiac K^+ channels. Eur J Pharmacol 458: 25 – 29.

Kang J, Chen X-L, Wang H, Ji J, Cheng H, Incardona J, Reynolds W, Viviani F, Tabart M, Rampe D (2005) Discovery of a small molecule activator of the human *ether-a-go-go*-related gene (HERG) cardiac K^+ channel. Mol Pharmacol 67: 827 – 836.

Kang Y, Guo J, Yang T, Li W, Zhang S (2015) Regulation of the human ether-a-go-go-related gene (hERG) potassium channel by Nedd4 family interacting proteins (Ndfips). Biochem J 472: 71 – 82.

Kannankeril PJ, Roden DM, Norris KJ, Whalen SP, George AL, Murray KT (2005) Genetic susceptibility to acquired long QT syndrome: pharmacologic challenge in first-degree relatives. Heart Rhythm 2: 134 – 140.

Kapa S, Tester DJ, Salisbury BA, Harris-Kerr C, Pungliya MS, Alders M, Wilde AAM, Ackerman MJ (2009) Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. Circulation 120: 1752 - 1760.

Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, Wilde AAM, Ackerman MJ (2009) Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION® long QT syndrome genetic test. Heart Rhythm 6: 1297 - 1303.

Karczewski J, Wang J, Kane SA, Kiss L, Koblan KS, Culberson JC, Spencer RH (2009) Analogs of MK-499 are differentially affected by a mutation in the S6 domain of the hERG K^+ channel. Biochem Pharmacol 77: 1602 - 1611.

Karle CA, Zitron E, Zhang W, Kathöfer S, Schoels W, Kiehn J (2002) Rapid component I_{Kr} of the guinea-pig cardiac delayed rectifier K^+ current is inhibited by β_1-adrenoceptor activation, via cAMP/protein kinase A-dependent pathways. Cardiovasc Res 53: 355 – 362.

Karnik R, Ludlow MJ, Abuarab N, Smith AJ, Hardy MEL, Elliott DJS, Sivaprasadarao A (2013) Endocytosis of hERG is clathrin-independent and involves Arf6. PloS One 8: e85630.

Katchman AN, McGroary KA, Kilborn MJ, Kornick CA, Manfredi PL, Woosley RL, Ebert SN (2002) Influence of opioid agonists on cardiac human *ether-a-go-go*-related gene K^+ currents. J Pharmacol Exp Ther 303: 688 – 694.

Katritsis D, Morgan J, Brachmann J, Bygrave A, O'Farrell D, Rowland E, Camm AJ (1997) Electrophysiological effects of E4031, a drug with selective class III properties, in man. Pacing Clin Electrophysiol 20: 930 – 937.

Ke Y, Ng CA, Hunter MJ, Mann SA, Heide J, Hill AP, Vandenberg JI (2013) Trafficking defects in PAS domain mutant $K_v11.1$ channels: roles of reduced domain stability and altered domain-domain interactions. Biochem J 454: 69 – 77.

Keller SH, Platoshyn O, Yuan JX-J (2005) Long QT syndrome-associated I593R mutation in HERG potassium channel activates ER stress pathways. Cell Biochem Biophys 43: 365 – 377.

Kiehn J, Lacerda AE, Wible B, Brown AM (1996) Molecular physiology and pharmacology of HERG: single-channel currents and block by dofetilide. Circulation 94: 2572 – 2579.

Kiehn J, Lacerda AE, Brown AM (1999) Pathways of HERG inactivation. Am J Physiol Heart Circ Physiol 277: H199 – H210.

Kim JA, Lopes CM, Moss AJ, McNitt S, Barsheshet A, Robinson JL, Zareba W, Ackerman MJ, Kaufman ES, Towbin JA, Vincent M, Goldenberg I (2010) Trigger-specific risk factors and response to therapy in long QT syndrome type 2. Heart Rhythm 7: 1797 – 1805.

Kim JJ, Němec J, Li Q, Salama G (2015) Synchronous systolic subcellular Ca^{2+}-elevations underlie ventricular arrhythmia in drug-induced long QT type 2. Circ Arrhythm Electrophysiol 8: 703 – 712.

Kirchhof P, Eckardt L, Mönnig G, Johna R, Loh P, Schulze-Bahr E, Breithardt G, Borggrefe M, Haverkamp W (2000) A patient with "atrial torsades de pointes". J Cardiovasc Electrophysiol 11: 806 – 811.

Kirchhof P, Eckardt L, Franz MR, Mönnig G, Loh P, Wedekind H, Schulze-Bahr E, Breithardt G, Haverkamp W (2003) Prolonged atrial action potential durations and polymorphic atrial tachyarrhythmias in patients with long QT syndrome. J Cardiovasc Electrophysiol 14: 1027 – 1033.

Kirsch GE, Trepakova ES, Brimecombe JC, Sidach SS, Erickson HD, Kochan MC, Shyjka LM, Lacerda AE, Brown AM (2004) Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. J Pharmacol Toxicol Methods 50: 93 – 101.

Kolder ICRM, Tanck MWT, Postema PG, Barc J, Sinner MF, Zumhagen S, Husemann A, Stallmeyer B, Koopmann TT, Hofman N, Pfeufer A, Lichtner P, Meitinger T, Beckmann BM, Myerburg RJ, Bishopric NH, Roden DM, Kääb S, Wilde AAM, Schott J-J, Schulze-Bahr E, Bezzina CR (2015) Analysis for genetic modifiers of disease severity in patients with long-QT syndrome type 2. Circ Cardiovasc Genet 8: 447 – 456.

Kongsamut S, Kang J, Chen X-L, Roehr J, Rampe D (2002) A comparison of the receptor binding and HERG channel affinities for a series of antipsychotic drugs. Eur J Pharmacol 450: 37 – 41.

Koo SH, Ho WF, Lee EJD (2006) Genetic polymorphisms in KCNQ1, HERG, KCNE1 and KCNE2 genes in the Chinese, Malay and Indian populations of Singapore. Br J Clin Pharmacol 61: 301 – 308.

Koopmann TT, Alders M, Jongbloed RJ, Guerrero S, Mannens MM, Wilde AAM, Bezzina CR (2006) Long QT syndrome caused by a large duplication in the *KCNH2* (HERG) gene undetectable by current polymerase chain reaction-based exon-scanning methodologies. Heart Rhythm 3: 52 – 55.

Kopliar I, Gallacher DJ, De Bondt A, Cougnaud L, Vlaminckx E, Van den Wyngaert I, Lu HR (2016) Functional and transcriptional characterization of histone deacetylase inhibitor-mediated cardiac adverse effects in human induced pluripotent stem cell-derived cardiomyocytes. Stem Cells Transl Med 5: 602 – 612.

Koponen M, Marjamaa A, Hiippala A, Happonen J-M, Havulinna AS, Salomaa V, Lahtinen AM, Hintsa T, Viitasalo M, Toivonen L, Kontula K, Swan H (2015) Follow-up of 316 molecularly defined pediatric long-QT syndrome patients: clinical course, treatments, and side effects. Circ Arrhythm Electrophysiol 8: 815 – 823.

Koskela J, Laiho J, Kähonen M, Rontu R, Lehtinen R, Viik J, Niemi M, Niemelä K, Kööbi T, Turjanmaa V, Pörsti I, Lehtimäki T, Nieminen T (2008) Potassium channel KCNH2 K897T polymorphism and cardiac repolarization during exercise test: the Finnish cardiovascular study. Scand J Clin Lab Invest 68: 31 – 38.

Kramer J, Obejero-Paz CA, Myatt G, Kuryshev YA, Bruening-Wright A, Verducci JS, Brown AM (2013) MICE models: superior to the HERG model in predicting torsade de pointes. Sci Report 3: 2100.

Krishnamurthy G, Patberg KW, Obreztchikova MN, Rybin AV, Rosen MR (2004) Developmental evolution of the delayed rectifier current I_{Ks} in canine heart appears dependent on the β subunit minK. Heart Rhythm 1: 704 -711.

Krishnan Y, Li Y, Zheng R, Kanda V, McDonald TV (2012) Mechanisms underlying the protein-kinase mediated regulation of the HERG potassium channel. Biochim Biophys Acta 1823: 1273 – 1284.

Kruse M, Hille B (2013) The phosphoinositide-sensitivity of the Kv channel family. Channels 7: 530 – 536.

Kupershmidt S, Snyders DJ, Raes A, Roden DM (1998) A K^+ channel splice variant common in human heart lacks a C-terminal domain required for expression of rapidly activating delayed rectifier current. J Biol Chem 273: 27231 – 27235.

Kupershmidt S, Yang T, Chanthaphaychith S, Wang Z, Towbin JA, Roden DM (2002) Defective human ether-à-go-go-related gene trafficking linked to an endoplasmic reticulum retention signal in the C terminus. J Biol Chem 277: 27442 – 27448.

Kupershmidt S, Yang IC-H, Hayashi K, Wei J, Chanthaphaychith S, Petersen CI, Johns DC, George AL, Roden DM, Balser JR (2003) The I_{Kr} drug response is modulated by *KCR1* in transfected cardiac and noncardiac cell lines. FASEB J 17: 2263 – 2265.

Kurokawa J, Tamagawa M, Harada N, Honda S-I, Bai C-X, Nakaya H, Furukawa T (2008) Acute effects of oestrogen on the guinea pig and human I_{Kr} channels and drug-induced prolongation of cardiac repolarization. J Physiol 586: 2961 – 2973.

Kuryshev YA, Ficker E, Wang L, Hawryluk P, Dennis AT, Wible BA, Brown AM, Kang J, Chen X-L, Sawamura K, Reynolds W, Rampe D (2005) Pentamidine-induced long QT syndrome and block of hERG trafficking. J Pharmacol Exp Ther 312: 316 - 323.

Kuryshev YA, Wang L, Wible BA, Wan X, Ficker E (2006) Antimony-based antileishmanial compounds prolong the cardiac action potential by an increase in cardiac calcium currents. Mol Pharmacol 69: 1216 - 1225.

Kääb S, Crawford DC, Sinner MF, Behr ER, Kannankeril PJ, Wilde AAM, Bezzina CR, Schulze-Bahr E, Guicheney P, Bishopric NH, Myerburg RJ, Schott J-J, Pfeufer A, Beckmann B-M, Martens E, Zhang T, Stallmeyer B, Zumhagen S, Denjoy I, Bardai A, Van Gelder IC, Jamshidi Y, Dalageorgou C, Marshall V, Jeffery S, Shakir S, Camm AJ, Steinbeck G, Perz S, Lichtner P, Meitinger T, Peters A, Wichmann H-E, Ingram C, Bradford Y, Carter S, Norris K, Ritchie MD, George AL, Roden DM (2012) A large candidate gene survey identifies the *KCNE1* D85N polymorphism as a possible modulator of drug-induced torsades de pointes. Circ Cardiovasc Genet 5: 91 – 99.

Köpfer DA, Hahn U, Ohmert I, Vriend G, Pongs O, de Groot BL, Zachariae U (2012) A molecular switch driving inactivation in the cardiac K^+ channel hERG. PLoS One 7: e41023.

Köpfer DA, Song C, Gruene T, Sheldrick GM, Zachariae U, de Groot BL (2014) Ion permeation in K^+ channels occurs by direct Coulomb knock-on. Science 346: 352 - 355.

Lacerda AE, Kramer J, Shen K-Z, Thomas D, Brown AM (2001) Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs. Eur Heart J 3 (suppl K): K23 - K30.

Lacerda AE, Kuryshev YA, Chen Y, Renganathan M, Eng H, Danthi SJ, Kramer JW, Yang T, Brown AM (2008) Alfuzosin delays cardiac repolarization by a novel mechanism. J Pharmacol Exp Ther 324: 427 - 433.

Lahtinen AM, Havulinna AS, Noseworthy PA, Jula A, Karhunen PJ, Perola M, Newton-Cheh C, Salomaa V, Kontula K (2013) Prevalence of arrhythmia-associated gene mutations and risk of sudden cardiac death in the Finnish population. Ann Med 45: 328 – 335.

Laitinen P, Fodstad H, Piippo K, Swan H, Toivonen L, Viitasalo M, Kaprio J, Kontula K (2000) Survey of the coding region of the HERG gene in long QT syndrome reveals six novel mutations and an amino acid polymorphism with possible phenotypic effects. Hum Mutat 15: 580 – 581.

Lamothe SM, Zhang S (2013) The serum- and glucocorticoid-inducible kinases SGK1 and SGK3 regulate hERG channel expression via ubiquitin ligase Nedd4-2 and GTPase Rab11. J Biol Chem 288: 15075 – 15084.

Larsen AP, Olesen S-P (2010) Differential expression of hERG1 channel isoforms reproduces properties of native I_{Kr} and modulates cardiac action potential characteristics. PLoS ONE 5: e9021.

Larsen AP, Olesen S-P, Grunnet M, Jespersen T (2008) Characterization of hERG1a and hERG1b potassium channels- a possible role for hERG1b in the I_{Kr} current. Pflügers Arch 456: 1137 – 1148.

Larsen AP, Olesen S-P, Grunnet M, Poelzing S (2010a) Pharmacological activation of I_{Kr} impairs conduction in guinea pig hearts. J Cardiovasc Electrophysiol 21: 923 - 929.

Larsen AP, Bentzen BH, Grunnet M (2010b) Differential effects of K_v11.1 activators on K_v11.1a, K_v11.1b and K_v11.1a/K_v11.1b channels. Br J Pharmacol 161: 614 - 628.

Lastraioli E, Guasti L, Crociani O, Polvani S, Hofmann G, Witchel H, Bencini L, Calistri M, Messerini L, Scatizzi M, Moretti R, Wanke E, Olivotto M, Mugnai G, Arcangeli A (2004) *herg1* gene and HERG 1 protein are overexpressed in colorectal cancers and regulate cell invasion of tumor cells. Cancer Res 64: 606 – 611.

Lastraioli E, Taddei A, Messerini L, Comin CE, Festini M, Giannelli M, Tomezzoli A, Paglierani M, Mugnai G, De Manzoni G, Bechi P, Arcangeli A (2006) hERG1 channels in human esophagus: evidence for their aberrant expression in the malignant progression of Barrett's esophagus. J Cell Physiol 209: 398 – 404.

Lastraioli E, Bencini L, Bianchini E, Romoli MF, Crociani O, Giommoni E, Messerini L, Gasperoni S, Moretti R, Di Costanzo F, Boni L, Arcangeli A (2012) hERG1 channels and Glut-1 as independent prognostic indicators of worst outcome in stage I and II colorectal cancer: a plot study. Transl Oncol 5: 105 – 112.

Lastraioli E, Perrone G, Sette A, Fiore A, Crociani O, Manoli S, D'Amico M, Masselli M, Iorio J, Callea M, Borzomati D, Nappo G, Bartolozzi F, Santini D, Bencini L, Farsi M, Boni L, Di Costanzo F, Schwab A, Onetti Muda A, Coppola R, Arcangeli A (2015) hERG1 channels drive tumour malignancy and may serve as prognostic factor in pancreatic ductal adenocarcinoma. Br J Cancer 112: 1076 – 1087.

Lazzerini PE, Yue Y, Srivastava U, Fabris F, Capecchi PL, Bertolozzi I, Bacarelli MR, Morozzi G, Acampa M, Natale M, El-Sherif N, Galeazzi M, Laghi-Pasini F, Boutjdir M (2016) Arrhythmogenicity of anti-Ro/SSA antibodies in patients with torsades de pointes. Circ Arrhythm Electrophysiol 9: e003419.

Lee W, Mann SA, Windley MJ, Imtiaz MS, Vandenberg JI, Hill AP (2016) In silico assessment of kinetics and state dependent binding properties of drugs causing acquired LQTS. Prog Biophys Mol Biol 120: 89 – 99.

Lees-Miller JP, Kondo C, Wang L, Duff HJ (1997) Electrophysiological characterization of an alternatively processed ERG K^+ channel in mouse and human hearts. Circ Res 81: 719 - 726.

Lees-Miller JP, Duan Y, Teng GQ, Duff HJ (2000) Molecular determinant of high-affinity dofetilide binding to HERG1 expressed in *Xenopus* oocytes: involvement of S6 sites. Mol Pharmacol 57: 367 - 374.

Leren IS, Hasselberg NE, Saberniak J, Håland TF, Kongsgård E, Smiseth OA, Edvardsen T, Haugaa KH (2015) Cardiac mechanical alterations and genotype specific differences in subjects with long QT syndrome. JACC Cardiovasc Imaging 8: 501 – 510.

Li G-R, Feng J, Yue L, Carrier M, Nattel S (1996) Evidence for two components of delayed rectifier K^+ current in human ventricular myocytes. Circ Res 78: 689 – 696.

Li G-R, Lau C-P, Ducharme A, Tardif J-C, Nattel S (2002) Transmural action potential and ionic current remodeling in ventricles of failing canine hearts. Am J Physiol Heart Circ Physiol 283: H1031 – H1041.

Li G-R, Lau C-P, Leung T-K, Nattel S (2004) Ionic current abnormalities associated with prolonged action potentials in cardiomyocytes from diseased human right ventricles. Heart Rhythm 4: 460 – 468.

Li H, Liu L, Guo T, Zhang J, Li X, Du W, Liu W, Chen X, Huang S (2007) Expression and functional role of HERG1, K^+ channels in leukemic cells and leukemic stem cells. J Huazhong Univ Sci Technolog 27: 257 – 260.

Li H, Du Y-M, Guo L, Jie S, Zhang S, Du W, Chen X, Liu W, Fan L, Zhu J, Zou A, Huang S (2009) The role of hERG1 K^+ channels and a functional link between hERG1 K^+ channels and SDF-1 in acute leukemic cell migration. Exp Cell Res 315: 2256 – 2264.

Li P, Ninomiya H, Kurata Y, Kato M, Miake J, Yamamoto Y, Igawa O, Nakai A, Higaki K, Toyoda F, Wu J, Horie M, Matsuura H, Yoshida A, Shirayoshi Y, Hiraoka M, Hisatome I (2011) Reciprocal control of hERG stability by Hsp70 and Hsc70 with implication for restoration of LQT2 mutant stability. Circ Res 108: 458 – 468.

Li Q, Gayen S, Chen AS, Huang Q, Raida M, Kang C (2010) NMR solution structure of the N-terminal domain of hERG and its interaction with the S4-S5 linker. Biochem Biophys Res Commun 403: 126 – 132.

Li Q, Ng HQ, Yoon HS, Kang C (2014) Insight into the molecular interaction between the cyclic nucleotide-binding homology domain and the eag domain of the hERG channel. FEBS Lett 588: 2782 – 2788.

Li Y, Ng HQ, Li Q, Kang C (2016) Structure of the cyclic nucleotide-binding homology domain of the hERG channel and its insight into type 2 long QT syndrome. Sci Rep 6: 23712.

Li Y, Sroubek J, Krishnan Y, McDonald TV (2008) A-kinase anchoring protein targeting of protein kinase A and regulation of HERG channels. J Membr Biol 223: 107 – 116.

Liang B, Nissen JD, Laursen M, Wang X, Skibsbye L, Hearing MC, Andersen MN, Rasmussen HB, Wickman K, Grunnet M, Olesen S-P, Jespersen T (2014) G-protein-coupled inward rectifier potassium current contributes to ventricular repolarization. Cardiovasc Res 101: 175 – 184.

Liang P, Lan F, Lee AS, Gong T, Sanchez-Freire V, Wang Y, Diecke S, Sallam K, Knowles JW, Wang PJ, Nguyen PK, Bers DM, Robbins RC, Wu JC (2013) Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. Circulation 127: 1677 – 1691.

Lieve KV, Williams L, Daly A, Richard G, Bale S, Macaya D, Chung WK (2013) Results of genetic testing in 855 consecutive unrelated patients referred for long QT syndrome in a clinical laboratory. Genet Test Mol Biomarkers 17: 553 – 561.

Limpitikul WB, Dick IE, Joshi-Mukherjee R, Overgaard MT, George AL, Yue DT (2014) Calmodulin mutations associated with long QT syndrome prevent inactivation of cardiac L-type Ca^{2+} currents and promote proarrhythmic behavior in ventricular myocytes. J Mol Cell Cardiol 74: 115 -124.

Lin EC, Holzem KM, Anson BD, Moungey BM, Balijepalli SY, Tester DJ, Ackerman MJ, Delisle BP, Balijepalli RC, January CT (2010) Properties of WT and mutant hERG K^+ channels expressed in neonatal mouse cardiomyocytes. Am J Physiol Heart Circ Physiol 298: H1842 – H1849.

Lin J, Guo J, Gang H, Wojciechowski P, Wigle JT, Zhang S (2005) Intracellular K^+ is required for the inactivation-induced high-affinity binding of cisapride to HERG channels. Mol Pharmacol 68: 855 - 865.

Lin J, Lin S, Choy PC, Shen X, Deng C, Kuang S, Wu J, Xu W (2008) The regulation of the cardiac potassium channel (HERG) by caveolin-1. Biochem Cell Biol 86: 405 - 415.

Lin T-F, Lin I-W, Chen S-C, Wu H-H, Yang C-S, Fang H-Y, Chiu M-M, Jeng C-J (2014) The subfamily-specific assembly of eag and erg K^+ channels is determined by both the amino and the carboxyl recognition domains. J Biol Chem 289: 22815 – 22834.

Liu F, Jones DK, de Lange WJ, Robertson GA (2016) Cotranslational association of mRNA encoding subunits of heteromeric ion channels. Proc Natl Acad Sci USA 113: 4859 – 4864.

Liu J, Zhang M, Jiang M, Tseng G-N (2002) Structural and functional role of the extracellular S5-P linker in the HERG potassium channel. J Gen Physiol 120: 723 – 737.

Liu J, Zhang M, Jiang M, Tseng G-N (2003) Negative charges in the transmembrane domains of the HERG K channel are involved in the activation- and deactivation-gating processes. J Gen Physiol 121: 599 – 614.

Liu QN, Trudeau MC (2015) Eag domains regulate LQT mutant hERG channels in human induced pluripotent stem cell-derived cardiomyocytes. PLoS One 10: e0123951.

Lockless SW (2015) Determinants of cation transport selectivity: equilibrium binding and transport kinetics. J Gen Physiol 146: 3 – 13.

London B, Trudeau MC, Newton KP, Beyer AK, Copeland NG, Gilbert DJ, Jenkins NA, Satler CA, Robertson GA (1997) Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K^+ current. Circ Res 81: 870 - 878.

Long SB, Campbell EB, MacKinnon R (2005a) Crystal structure of a mammalian voltage-dependent *Shaker* family K^+ channel. Science 309: 897 - 903.

Long SB, Campbell EB, MacKinnon R (2005b) Voltage sensor of Kv1.2: structural basis of electromechanical coupling. Science 309: 903-908.

Long SB, Tao X, Campbell EB, MacKinnon R (2007) Atomic structure of a voltage-dependent K^+ channel in a lipid membrane-like environment. Nature 450: 376 - 382.

Lu HR, Vlaminckx E, Hermans AN, Rohrbacher J, Van Ammel K, Towart R, Pugsley M, Gallacher DJ (2008) Predicting drug-induced changes in QT interval and arrhythmias: QT-shortening drugs point to gaps in the ICHS7B guidelines. Br J Pharmacol 154: 1427 - 1438.

Lu Y, Mahaut-Smith MP, Varghese A, Huang CL-H, Kemp PR, Vandenberg JI (2001) Effects of premature stimulation on HERG K^+ channels. J Physiol 537: 843 – 851.

Lu Y, Mahaut-Smith MP, Huang CL-H, Vandenberg JI (2003) Mutant MiRP1 subunits modulate HERG K^+ channel gating: a mechanism for pro-arrhythmia in long QT syndrome type 6. J Physiol 551: 253 - 262.

Lu Z, Wu C-Y, Jiang Y-P, Ballou LM, Clausen C, Cohen IS, Lin RZ (2012) Suppression of phosphoinositide 3-kinase signaling and alteration of multiple ion currents in drug-induced long QT syndrome. Sci Transl Med 4: 131ra50.

Luo X, Xiao J, Lin H, Lu Y, Yang B, Wang Z. (2008) Genomic structure, transcriptional control, and tissue distribution of *HERG1* and *KCNQ1* genes. Am J Physiol Heart Circ Physiol 294: H1371 – H1380.

Lymperopoulos A, Rengo G, Koch WJ (2013) Adrenergic nervous system in heart failure: pathophysiology and therapy. Circ Res 113: 739 – 753.

Lörinczi É, Gómez-Posada JC, de la Peña P, Tomczak AP, Fernández-Trillo J, Leipscher U, Stühmer W, Barros F, Pardo LA (2015) Voltage-dependent gating of KCNH potassium channels lacking a covalent link between voltage-sensing and pore domains. Nat Commun 6: 6672.

Ma J, Guo L, Fiene SJ, Anson BD, Thomson JA, Kamp TJ, Kolaja KL, Swanson BJ, January CT (2011) High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. Am J Physiol Heart Circ Physiol 301: H2006 – H2017.

MacCormick JM, McAlister H, Crawford J, French JK, Crozier I, Shelling AN, Eddy CA, Rees MI, Skinner JR (2009) Misdiagnosis of long QT syndrome as epilepsy at first presentation. Ann Emerg Med 54: 26 – 32.

Magyar J, Iost N, Körtvély Á, Bányász T, Virág L, Szigligeti P, Varró A, Opincariu M, Szécsi J, Papp JG, Nánási PP (2000) Effects of endothelin-1 on calcium and potassium currents in undiseased human ventricular myocytes. Pflügers Arch 441: 144 – 149.

Mank-Seymour AR, Richmond JL, Wood LS, Reynolds JM, Fan Y-T, Warnes GR, Milos PM, Thompson JF (2006) Association of torsades de pointes with novel and known single nucleotide polymorphisms in long QT syndrome genes. Am Heart J 152: 1116 – 1122.

Mann SA, Otway R, Guo G, Soka M, Karlsdotter L, Trivedi G, Ohanian M, Zodgekar P, Smith RA, Wouters MA, Subbiah R, Walker B, Kuchar D, Sanders P, Griffiths L, Vandenberg JI, Fatkin D (2012) Epistatic effects of potassium channel variation on cardiac repolarization and atrial fibrillation risk. J Am Coll Cardiol 59: 1017 – 1025.

Mann SA, Imtiaz M, Winbo A, Rydberg A, Perry MD, Couderc J-P, Polonsky B, McNitt S, Zareba W, Hill AP, Vandenberg JI (2016) Convergence of models of human ventricular myocyte electrophysiology after global optimization to recapitulate clinical long QT phenotypes. J Mol Cell Cardiol 100: 25 – 34.

Marbán E (2002) Cardiac channelopathies. Nature 415: 213 – 218.

Margulis M, Sorota S, Chu I, Soares A, Priestley T, Nomeir AA (2010) Protein binding-dependent decreases in hERG channel blocker potency assessed by whole-cell voltage clamp in serum. J Cardiovasc Pharmacol 55: 368 – 376.

Marjamaa A, Newton-Cheh C, Porthan K, Reunanen A, Lahermo P, Väänänen H, Jula A, Karanko H, Swan H, Toivonen L, Nieminen MS, Viitasalo M, Peltonen L, Oikarinen L, Palotie A, Kontula K, Salomaa V (2009a) Common candidate gene variants are associated with QT interval duration in the general population. J Intern Med 265: 448 – 458.

Marjamaa A, Salomaa V, Newton-Cheh C, Porthan K, Reunanen A, Karanko H, Jula A, Lahermo P, Väänänen H, Toivonen L, Swan H, Viitasalo M, Nieminen MS, Peltonen L, Oikarinen L, Palotie A, Kontula K, (2009b) High prevalence of four long QT syndrome founder mutations in the Finnish population. Ann Med 41: 234 – 240.

Martinez HB, Hu D, Gollob M, Antzelevitch C (2011) Novel gain-of-function N-terminal *KCNH2* mutation associated with the short QT syndrome. Circulation 124: A12845.

Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS (2002) Requirement of a macromolecular signaling complex for β-adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. Science 295: 496 – 499.

Masi A, Becchetti A, Restano-Cassulini R, Polvani S, Hofmann G, Buccoliero AM, Paglierani M, Polio B, Taddei GL, Gallina P, Di Lorenzo N, Franceschetti S, Wanke E, Arcangeli A (2005) hERG1 channels are overexpressed in glioblastoma multiforme and modulate VEGF secretion in glioblastoma cell lines. Br J Cancer 93: 781 – 792.

Massaeli H, Sun T, Li X, Shallow H, Wu J, Xu J, Li W, Hanson C, Guo J, Zhang S (2010a) Involvement of caveolin in low K^+-induced endocytic degradation cell-surface human ether-a-go-go-related gene (hERG) channels. J Biol Chem 285: 27259 – 27264.

Massaeli H, Guo J, Xu J, Zhang S (2010b) Extracellular K^+ is a prerequisite for the function and plasma membrane stability of HERG channels. Circ Res 106: 1072 – 1082.

Matsa E, Rajamohan D, Dick E, Young L, Mellor I, Staniforth A, Denning C (2011) Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. Eur Heart J 32: 952 - 962.

Mazhari R, Greenstein JL, Winslow RL, Marbán E, Nuss HB (2001) Molecular interactions between two long-QT syndrome gene products, *HERG* and *KCNE2*, rationalized by in vitro and in silico analysis. Circ Res 89: 33 - 38.

Mazzanti A, Kanthan A, Monteforte N, Memmi M, Bloise R, Novelli V, Miceli C, O'Rourke S, Bono G, Zienciuk-Krajka A, Curcio A, Surducan AE, Colombo M, Napolitano C, Priori SG (2014) Novel insight into the natural history of short QT syndrome. J Am Coll Cardiol 63: 1300 – 1308.

McBride BF, Yang T, Liu K, Urban TJ, Giacomini KM, Kim RB, Roden DM (2009) The organic cation transporter, OCTN1, expressed in the human heart, potentiates antagonism of the HERG potassium channel. J Cardiovasc Pharmacol 54: 63 – 71.

McDonald TV, Yu Z, Ming Z, Palma E, Meyers MB, Wang K-W, Goldstein SAN, Fishman GI (1997) A minK-HERG complex regulates the cardiac potassium current I_{Kr}. Nature 388: 289 - 292.

McPate MJ, Duncan RS, Milnes JT, Witchel HJ, Hancox JC (2005) The N588K-HERG K^+ channel mutation in the 'short QT syndrome': mechanism of gain-in-function determined at 37^{o}C. Biochem Biophys Res Commun 334: 441 – 449.

McPate MJ, Duncan RS, Witchel HJ, Hancox JC (2006) Disopyramide is an effective inhibitor of mutant HERG K^+ channels involved in variant 1 short QT syndrome. J Mol Cell Cardiol 41: 563 – 566.

McPate MJ, Duncan RS, Hancox JC, Witchel HJ (2008) Pharmacology of the short QT syndrome N588K-hERG K^+ channel mutation: differential impact on selected class I and class III antiarrhythmic drugs. Br J Pharmacol 155: 957 – 966.

McPate MJ, Zhang H, Cordeiro JM, Dempsey CE, Witchel HJ, Hancox JC (2009) hERG1a/1b heteromeric currents exhibit amplified attenuation of inactivation in variant 1 short QT syndrome. Biochem Biophys Res Commun 386: 111 – 117.

Mechakra A, Vincent Y, Chevalier P, Millat G, Ficker E, Jastrzebski M, Poulin H, Pouliot V, Chahine M, Christé G (2014) The variant *hERG*/R148W associated with LQTS is a mutation that reduces current density on co-expression with the WT. Gene 536: 348 – 356.

Medlock MM, Tester DJ, Will ML, Bos JM, Ackerman MJ (2012) Repeat long QT syndrome genetic testing of phenotype-positive cases: prevalence and etiology of detection misses. Heart Rhythm 9: 1977 – 1982.

Mehta A, Sequiera GL, Ramachandra CJA, Sudibyo Y, Chung Y, Sheng J, Wong KY, Tan TH, Wong P, Liew R, Shim W (2014) Re-trafficking of hERG reverses long QT syndrome 2 phenotype in human iPS-derived cardiomyocytes. Cardiovasc Res 102: 497 – 506.

Melgari D, Zhang Y, El Harchi A, Dempsey CE, Hancox JC (2015) Molecular basis of hERG potassium channel blockade by the class 1c antiarrhythmic flecainide. J Mol Cell Cardiol 86: 42 – 53.

Mendzelevski B, Ausma J, Chanter DO, Robinson P, Kerstens R, Vandeplassche L, Camm J (2012) Assessment of the cardiac safety of prucalopride in healthy volunteers: a randomized, double-blind, placebo- and positive-controlled thorough QT study. Br J Clin Pharmacol 73: 203 – 209.

Mewe M, Wulfsen I, Schuster AME, Middendorff R, Glassmeier G, Schwarz JR, Bauer CK (2008) ERG K^+ channels modulate contractile activity in the bovine epididymal duct. Am J Physiol Regul Integr Comp Physiol 294: R895 – R904.

Meyers NL, Hickling RI (2007) The cardiovascular safety profile of renzapride, a novel treatment for irritable bowel syndrome. J Int Med Res 35: 848 - 866.

Michael G, Xiao L, Qi X-Y, Dobrev D, Nattel S (2009) Remodelling of cardiac repolarization: how homeostatic responses can lead to arrhythmogenesis. Cardiovasc Res 81: 491 – 499.

Michaud V, Turgeon J. (2013) Domperidone and sudden cardiac death: how much longer should we wait? J Cardiovasc Pharmacol 61: 215 – 217.

Migdalovich D, Moss AJ, Lopes CM, Costa J, Ouellet G, Barsheshet A, McNitt S, Polonsky S, Robinson JL, Zareba, W, Ackerman MJ, Benhorin J, Kaufman ES, Napolitano C, Platonov PG, Shimizu W, Towbin JA, Vincent GM, Wilde AA, Goldenberg I (2011) Mutation and gender-specific risk in type 2 long QT syndrome: implications for risk stratification for life-threatening cardiac events in patients with long QT syndrome. Heart Rhythm 8: 1537 – 1543.

Mihic A, Chauhan VS, Gao X, Oudit GY, Tsushima RG (2011) Trafficking defect and proteasomal degradation contribute to the phenotype of a novel KCNH2 long QT syndrome mutation. PLoS ONE 6: e18273.

Milnes JT, Crociani O, Arcangeli A, Hancox JC, Witchel HJ (2003a) Blockade of HERG potassium currents by fluvoxamine: incomplete attenuation by S6 mutations at F656 or Y652. Br J Pharmacol 139: 887 – 898.

Milnes JT, Dempsey CE, Ridley JM, Crociani O, Arcangeli A, Hancox JC, Witchel HJ (2003b) Preferential closed channel blockade of HERG potassium currents by chemically synthesised BeKm-1 scorpion toxin. FEBS Lett 547: 20 – 26.

Milnes JT, Witchel HJ, Leaney JL, Leishman DJ, Hancox JC (2010) Investigating dynamic protocol-dependence of hERG potassium channel inhibition at 37^{o}C: cisapride versus dofetilide. J Pharmacol Toxicol Methods 61: 178 – 191.

Mirams GR, Cui Y, Sher A, Fink M, Cooper J, Heath BM, McMahon NC, Gavaghan DJ, Noble D (2011) Simulation of multiple ion channel block provides improved early prediction of compounds' clinical torsadogenic risk. Cardiovasc Res 91: 53 – 61.

Mitcheson JS (2008) hERG potassium channels and the structural basis of drug-induced arrhythmias. Chem Res Toxicol 21: 1005 – 1010.

Mitcheson JS, Chen J, Lin M, Culberson C, Sanguinetti MC (2000a) A structural basis for drug-induced long QT syndrome. Proc Natl Acad Sci USA 97: 12329 - 12333.

Mitcheson JS, Chen J, Sanguinetti MC (2000b) Trapping of a methanesulfonanilide by closure of the HERG potassium channel activation gate. J Gen Physiol 115: 229 - 240.

Mizusawa Y, Horie M, Wilde AAM (2014) Genetic and clinical advances in congenital long QT syndrome. Circ J 78: 2827 – 2833.

Mohammad S, Zhou Z, Gong Q, January CT (1997) Blockage of the HERG human cardiac K^+ channel by the gastrointestinal prokinetic agent cisapride. Am J Physiol Heart Circ Physiol 42: H2534 – H2538.

Moungey BM, Lin EC, Balijepalli RC, January CT, Delisle BP (2008) hERG and MiRP1 do not associate prior to export out of the endoplasmic reticulum. Circulation 118: A1524.

Morais-Cabral JH, Lee A, Cohen SL, Chait BT, Li M, MacKinnon R (1998) Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. Cell 95: 649 - 655.

Morais-Cabral JH, Zhou Y, MacKinnon R (2001) Energetic optimization of ion conduction rate by the K^+ selectivity filter. Nature 414: 37 - 42.

Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flügel L, Dorn T, Goedel A, Höhnke C, Hofmann F, Seyfarth M, Sinnecker D, Schömig A, Laugwitz K-L (2010) Patient-specific induced pluripotent stem-cell models for long-QT syndrome. New Engl J Med 363: 1397 – 1409.

Moric-Janiszewska E, Glogowska-Ligus J, Paul-Samojedny M, Węglarz L, Markiewicz-Loskot G, Szydlowski L (2011) Age- and sex-dependent mRNA expression of KCNQ1 and HERG in patients with long QT syndrome type 1 and 2. Arch Med Sci 7: 941 – 947.

Mullally J, Goldenberg I, Moss AJ, Lopes CM, Ackerman MJ, Zareba W, McNitt S, Robinson JL, Benhorin J, Kaufman ES, Towbin JA, Barsheshet A (2013) Risk of life-threatening cardiac events among patients with long QT syndrome and multiple mutations. Heart Rhythm 10: 378 – 382.

Muskett FW, Thouta S, Thomson SJ, Bowen A, Stansfeld PJ, Mitcheson JS (2011) Mechanistic insight into human ether-à-go-go-related gene (hERG) K^+ channel deactivation gating from the solution structure of the EAG domain. J Biol Chem 286: 6184 – 6191.

Myokai T, Ryu S, Shimizu H, Oiki S (2008) Topological mapping of the asymmetric drug binding to the human ether-à-go-go-related gene product (HERG) potassium channel by use of tandem dimers. Mol Pharmacol 73: 1643 - 1651.

Männikkö R, Overend G, Perrey C, Gavaghan CL, Valentin J-P, Morten J, Armstrong M, Pollard CE (2010) Pharmacological and electrophysiological characterization of nine, single nucleotide polymorphisms of the hERG-encoded potassium channel. Br J Pharmacol 159: 102 – 114.

Nakajima T, Furukawa T, Hirano Y, Tanaka T, Sakurada H, Takahashi T, Nagai R, Itoh T, Katayama Y, Nakamura Y, Hiraoka M (1999) Voltage-shift of the current activation in *HERG* S4 mutation (R534C) in LQT2. Cardiovasc Res 44: 283 – 293.

Napolitano C, Schwartz PJ, Brown AM, Ronchetti E, Bianchi L, Pinnavaia A, Acquaro G, Priori SG (2000) Evidence for a cardiaci on channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. J Cardiovasc Electrophysiol 11: 691 – 696.

Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, Bottelli G, Cerrone M, Leonardi S (2005) Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. JAMA 294: 2975 – 2980.

Nawathe PA, Kryukova Y, Oren RV, Milanesi R, Clancy CE, Lu JT, Moss AJ, DiFrancesco D, Robinson RB (2013) An LQTS6 MiRP1 mutation suppresses pacemaker current and is associated with sinus bradycardia. J Cardiovasc Electrophysiol 24: 1021 – 1027.

Nedergaard S (2004) A Ca^{2+}-independent slow afterhyperpolarization in substantia nigra compacta neurons. Neurosci 125: 841 – 852.

Neher E, Sakmann B (1976) Single-channel currents recorded from membrane of denervated frog muscle fibres. Nature 260: 799 - 801.

Nerbonne JM, Kass RS (2005) Molecular physiology of cardiac repolarization. Physiol Rev 85: 1205 – 1253.

Newton-Cheh C, Guo C-Y, Larson MG, Musone SL, Surti A, Camargo AL, Drake JA, Benjamin EJ, Levy D, D'Ágostino RB, Hirschhorn JN, O'Donnell CJ (2007) Common genetic variation in *KCNH2* is associated with QT interval duration: the Framingham heart study. Circulation 116: 1128 – 1136.

Ng CA, Hunter MJ, Perry MD, Mobli M, Ke Y, Kuchel PW, King GF, Stock D, Vandenberg JI (2011) The N-terminal tail of hERG contains an amphipathic α-helix that regulates channel deactivation. PLoS One 6: e16191.

Ng CA, Phan K, Hill AP, Vandenberg JI, Perry MD (2014) Multiple interactions between cytoplasmic domains regulate slow deactivation of Kv11.1 channels. J Biol Chem 289: 25822 - 25832.

Niculescu D, Hirdes W, Hornig S, Pongs O, Schwarz JR (2013) Erg potassium currents of neonatal mouse Purkinje cells exhibit fast gating kinetics and are inhibited by mGluR1 activation. J Neuroscience 33: 16729 – 16740.

Nie L, Gratton MA, Mu KJ, Dinglasan JN, Feng W, Yamoah EN (2005) Expression and functional phenotype of mouse *ERG* K^+ channels in the inner ear: potential role in K^+ regulation in the inner ear. J Neurosci 25: 8671 – 8679.

Nishio Y, Makiyama T, Itoh H, Sakaguchi T, Ohno S, Gong YZ, Yamamoto S, Ozawa T, Ding WG, Toyoda F, Karamura M, Akao M, Matsuura H, Kimura T, Kita T, Horie M (2009) D85N, a KCNE1 polymorphism, is a disease-causing gene variant in long QT syndrome. J Am Coll Cardiol 54: 812 – 819.

Nof E, Burashnikov A, Antzelevitch C (2010a) Cellular basis for atrial fibrillation in an experimental model of short QT1: implications for a pharmacological approach to therapy. Heart Rhythm 7: 251 – 257.

Nof E, Cordeiro JM, Pérez GJ, Scornik FS, Calloe K, Love B, Burashnikov E, Caceres G, Gunsburg M, Antzelevitch C (2010b) A common single nucleotide polymorphism can exacerbate long-QT type 2 syndrome leading to sudden infant death. Circ Cardiovasc Genet 3: 199 – 206.

Nof E, Barajas-Martinez H, Eldar M, Urrutia J, Caceres G, Rosenfeld G, Bar-Lev D, Feinberg M, Burashnikov E, Casis O, Hu D, Glikson M, Antzelevitch C (2011) LQT5 masquerading as LQT2: a dominant negative effect of *KCNE1*-D85N rare polymorphism on *KCNH2* current. Europace 13: 1478 – 1483.

Noskov SY, Roux B (2006) Ion selectivity in potassium channels. Biophys Chem 124: 279 – 291.

Obejero-Paz CA, Bruening-Wright A, Kramer J, Hawryluk P, Tatalovic M, Dittrich HC, Brown AM (2015) Quantitative profiling of the effects of vanoxerine on human cardiac ion channels and its application to cardiac risk. Sci Rep 5: 17623.

Obers S, Staudacher I, Ficker E, Dennis A, Koschny R, Erdal H, Bloehs R, Kisselbach J, Karle CA, Schweizer PA, Katus HA, Thomas D (2010) Multiple mechanisms of hERG liability: K^+ current inhibition, disruption of protein trafficking, and apoptosis induced by amoxapine. Naunyn-Schmied Arch Pharmacol 381: 385 – 400.

Obreztchikova MN, Sosunov EA, Plotnikov A, Anyukhovsky EP, Gainullin RZ, Danilo P, Yeom Z-H, Robinson RB, Rosen MR (2003) Developmental changes in I_{Kr} and I_{Ks} contribute to age-related expression of dofetilide effects on repolarization and proarrhythmia. Cardiovasc Res 59: 339 – 350.

Ohno S, Zankov DP, Yoshida H, Tsuji K, Makiyama T, Itoh H, Akao M, Hancox JC, Kita T, Horie M (2007) N- and C-terminal *KCNE1* mutations cause distinct phenotypes of long QT syndrome. Heart Rhythm 4: 332 – 340.

Ohya S, Horowitz B, Greenwood IA. (2002a) Functional and molecular identification of ERG channels in murine portal vein myocytes. Am J Physiol Cell Physiol 283: C866 – C877.

Ohya S, Asakura K, Muraki K, Watanabe M, Imaizumi Y. (2002b) Molecular and functional characterization of ERG, KCNQ, and KCNE subtypes in rat stomach smooth muscle. Am J Physiol Gastrointest Liver Physiol 282: C277 – C287.

Ohyama H, Kajita H, Omori K, Takumi T, Hiramoto N, Iwasaka T, Matsuda H (2001) Inhibition of cardiac delayed rectifier K^+ currents by an antisense oligodeoxynucleotide against IsK (minK) and over-expression of IsK mutant D77N in neonatal mouse hearts. Pflügers Arch 442: 329 – 335.

Okada J-I, Yoshinaga T, Kurokawa J, Washio T, Furukawa T, Sawada K, Sugiura S, Hisada T (2015) Screening system for drug-induced arrhythmogenic risk combining a patch clamp and heart simulator. Sci Adv 1: e1400142.

Olesen MS, Nielsen MW, Haunsø S, Svendsen JH (2014a) Atrial fibrillation: the role of common and rare genetic variants. Eur J Hum Genet 22: 297 – 306.

Olesen MS, Andreasen L, Jabbari J, Refsgaard L, Haunsø S, Olesen SP, Nielsen JB, Schmitt N, Svendsen JH (2014b) Very early-onset lone atrial fibrillation patients have a high prevalence of rare variants in genes previously associated with atrial fibrillation. Heart Rhythm 11: 246 – 251.

Olsson S, Edwards IR (1992) Tachycardia during cisapride treatment. Br Med J 305: 748 – 749.

Osterbur ML, Zheng R, Marion R, Walsh C, McDonald TV (2015) An interdomain *KCNH2* mutation produces an intermediate long QT syndrome. Hum Mutat 36: 764 – 773.

Organ-Darling LE, Vernon AN, Giovanniello JR, Lu Y, Moshal K, Roder K, Li W, Koren G (2013) Interactions between hERG and KCNQ1 α-subunits are mediated by their COOH termini and modulated by cAMP. Am J Physiol Heart Circ Physiol 304: H589 – H599.

Otagiri T, Kijima K, Osawa M, Ishii K, Makita N, Matoba R, Umetsu K, Hayasaka K (2008) Cardiac ion channel gene mutations in sudden infant death syndrome. Pediatr Res 64: 482 – 487.

Page G, Ratchada P, Miron Y, Steiner G, Ghetti A, Miller PE, Reynolds JA, Wang K, Greiter-Wilke A, Polonchuk L, Traebert M, Gintant GA, Abi-Gerges N (2016) Human ex-vivo action potential model for pro-arrhythmia risk assessment. J Pharmacol Toxicol Methods 81: 183 – 195.

Pakladok T, Almilaji A, Munoz C, Alesutan I, Lang F (2013) PIKfyve sensitivity of hERG channels. Cell Physiol Biochem 31: 785 – 794.

Pan Z, Zhang M, Ma T, Xue Z-Y, Li G-F, Hao L-Y, Zhu L-J, Li Y-Q, Ding H-L, Cao J-L (2016) Hydroxymethylation of microRNA-365-3p regulates nociceptive behaviors via Kcnh2. J Neurosci 36: 2769 – 2781.

Pareja K, Chu E, Dodyk K, Richter K, Miller A (2013) Role of the activation gate in determining the extracellular potassium dependency of block of HERG by trapped drugs. Channels 7: 23 – 33.

Parkington HC, Stevenson J, Tonta MA, Paul J, Butler T, Maiti K, Chan EC, Sheehan PM, Brennecke SP, Coleman HA, Smith R (2014) Diminished hERG K^+ channel activity facilitates strong human labour contractions but is dysregulated in obese women. Nat Commun 5: 4108.

Parr E, Pozo MJ, Horowitz B, Nelson MT, Mawe GM (2003) ERG K^+ channels modulate the electrical and contractile activities of gallbladder smooth muscle. Am J Physiol Gastrointest Liver Physiol 284: G392 – G398.

Partemi S, Cestèle S, Pezzella M, Campuzano O, Paravidino R, Pascali VL, Zara F, Tassinari CA, Striano S, Oliva A, Brugada R, Mantegazza M, Striano P (2013) Loss-of-function *KCNH2* mutation in a family with long QT syndrome, epilepsy, and sudden death. Epilepsia 54: e112 – e116.

Pasricha PJ (2007) Desperately seeking serotonin…a commentary on the withdrawal of tegaserod and the state of drug development for functional and motility disorders. Gastroenterol 132: 2287 - 2290.

Patel C, Antzelevitch C (2008) Cellular basis for arrhythmogenesis in an experimental model of the SQT1 form of the short QT syndrome. Heart Rhythm 5: 585 – 590.

Pathak MM, Yarov-Yarovoy V, Agarwal G, Roux B, Barth P, Kohout S, Tombola F, Isacoff EY (2007) Closing in on the resting state of the Shaker K^+ channel. Neuron 56: 124 - 140.

Pau D, Workman AJ, Kane KA, Rankin AC (2005) Electrophysiological effects of prucalopride, a novel enterokinetic agent, on isolated atrial myocytes from patients treated with β-adrenoceptor antagonists. J Pharmacol Exp Ther 313: 146 - 153.

Paulussen ADC, Gilissen RAHJ, Armstrong M, Doevendans PA, Verhasselt P, Smeets HJM, Schulze-Bahr E, Haverkamp W, Breithardt G, Cohen N, Aerssens J (2004) Genetic variations of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* in drug-induced long QT syndrome patients. J Mol Med 82: 182 – 188.

Peeters GA, Sanguinetti MC, Eki Y, Konarzewska H, Renlund DG, Karwande SV, Barry WH (1995) Method for isolation of human ventricular myocytes from single endocardial and epicardial biopsies. Am J Physiol Heart Circ Physiol 268: H1757 – H1764.

Peng S, Lacerda AE, Kirsch GE, Brown AM, Bruening-Wright A (2010) The action potential and comparative pharmacology of stem cell-derived human cardiomyocytes. J Pharmacol Toxicol Methods 61: 277 - 286.

Perrin MJ, Kuchel PW, Campbell TJ, Vandenberg JI (2008) Drug binding to the inactivated state is necessary but not sufficient for high-affinity binding to human *ether-à-go-go*-related gene channels. Mol Pharmacol 74: 1443 – 1452.

Perry M, de Groot MJ, Helliwell R, Leishman D, Tristani-Firouzi M, Sanguinetti MC, Mitcheson J (2004) Structural determinants of HERG channel block by clofilium and ibutilide. Mol Pharmacol 66: 240 - 249.

Perry MD, Wong S, Ng CA, Vandenberg JI (2013a) Hydrophobic interactions between the voltage sensor and pore mediate inactivation in Kv11.1 channels. J Gen Physiol 142: 275 – 288.

Perry MD, Ng CA, Vandenberg JI (2013b) Pore helices play a dynamic role as integrators of domain motion during Kv11.1 channel inactivation gating. J Biol Chem 288: 11482 – 11491.

Perry MD, Ng CA, Phan K, David E, Steer K, Hunter MJ, Mann SA, Imtiaz M, Hill AP, Ke Y, Vandenberg JI (2016) Rescue of protein expression defects may not be enough to abolish the pro-arrhythmic phenotype of long QT type 2 mutations. J Physiol 594: 4031 – 4049.

Pessia M, Servettini I, Panichi R, Guasti L, Grassi S, Arcangeli A, Wanke E, Pettorossi VE (2008) ERG voltage-gated K^+ channels regulate excitability and discharge dynamics of the medial vestibular nucleus neurones. J Physiol 586: 4877 – 4890.

Peterson LB, Eskew JD, Vielhauer GA, Blagg BSJ (2012) The hERG channel is dependent upon the Hsp90α isoform for maturation and trafficking. Mol Pharmaceutics 9: 1841 – 1846.

Petrecca K, Atanasiu R, Akhavan A, Shrier A (1999) N-linked glycosylation sites determine HERG channel surface membrane expression. J Physiol 515: 41 - 48.

Pfeufer A, Jalilzadeh S, Perz S, Mueller JC, Hinterseer M, Illig T, Akyol M, Huth C, Schöpfer-Wendels A, Kuch B, Steinbeck G, Holle R, Näbauer M, Wichmann H-E, Meitinger T, Kääb S (2005) Common variants in myocardial ion channel genes modify the QT interval in the general population: results from the KORA study. Circ Res 96: 693 – 701.

Phartiyal P, Jones EMC, Robertson GA (2007) Heteromeric assembly of human ether-á-go-go-related gene (hERG) 1a/1b channels occurs cotranslationally via N-terminal interactions. J Biol Chem 282: 9874 - 9882.

Phartiyal P, Sale H, Jones EMC, Robertson GA (2008) Endoplasmic reticulum retention and rescue by heteromeric assembly regulate human ERG 1a/1b surface channel composition. J Biol Chem 283: 3702 - 3707.

Pier DM, Shehatou GSG, Giblett S, Pullar CE, Trezise DJ, Pritchard CA, Challiss RAJ, Mitcheson JS (2014) Long-term channel block is required to inhibit cellular transformation by human ether-à-go-go-related gene (hERG1) potassium channels. Mol Pharmacol 86:211 – 221.

Pietilä E, Fodstad H, Niskasaari E, Laitinen PJ, Swan H, Savolainen M, Kesäniemi YA, Kontula K, Huikuri HV (2002) Association between HERG K897T polymorphism and QT interval in middle-aged Finnish women. J Am Coll Cardiol 40: 511 – 514.

Pillozzi S, Arcangeli A (2010) Physical and functional interaction between integrins and hERG1 channels in cancer cells. Adv Exp Med Biol 674: 55 – 67.

Pillozzi S, Brizzi MF, Balzi M, Crociani O, Cherubini A, Guasti L, Bartolozzi B, Becchetti A, Wanke E, Bernabei PA, Olivotto M, Pegoraro L, Arcangeli A (2002) HERG potassium channels are constitutively expressed in primary human acute myeloid leukemias and regulate cell proliferation of normal and leukemic hemopoietic progenitors. Leukemia 16: 1791 – 1798.

Pillozzi S, Brizzi MF, Bernabei PA, Bartolozzi B, Caporale R, Basile V, Boddi V, Pegoraro L, Becchetti A, Arcangeli A (2007) VEGFR-1 (FLT-1), β_1 integrin, and hERG K^+ channel for a macromolecular signaling complex in acute myeloid leukemia: role in cell migration and clinical outcome. Blood 110: 1238 – 1250.

Pillozzi S, Masselli M, De Lorenzo E, Accordi B, Cilia E, Crociani O, Amedei A, Veltroni M, D'Amico M, Basso G, Becchetti A, Campana D, Arcangeli A (2011) Chemotherapy resistance in acute lymphoblastic leukemia requires hERG1 channels and is overcome by hERG1 blockers. Blood 117: 902 – 914.

Pindon A, Van Hecke G, Van Gompel P, Lesage AS, Leysen JE, Jurzak M (2002) Differences in signal transduction of two 5-HT_4 receptor splice variants: compound specificity and dual coupling with Gαs- and Gαi/o-proteins. Mol Pharmacol 61: 85 - 96.

Piper DR, Varghese A, Sanguinetti MC, Tristani-Firouzi M (2003) Gating currents associated with intramembrane charge displacement in HERG potassium channels. Proc Natl Acad Sci USA 100: 10534 - 10539.

Piper DR, Hinz WA, Tallurri CK, Sanguinetti MC, Tristani-Firouzi M (2005) Regional specificity of human *ether-á-go-go*-related gene channel activation and inactivation gating. J Biol Chem 280: 7206 - 7217.

Pollard CE, Abi Gerges N, Bridgland-Taylor MH, Easter A, Hammond TG, Valentin J-P (2010) An introduction to QT interval prolongation and non-clinical approaches to assessing and reducing risk. Br J Pharmacol 159: 12 – 21.

Poluzzi E, Raschi E, Moretti U, De Ponti F (2009) Drug-induced torsades de pointes: data mining of the public version of the FDA adverse event reporting system (AERS). Pharmacoepidemiol Drug Saf 18: 512 – 518.

Polvani S, Masi A, Pillozzi S, Gragnani L, Crociani O, Olivotto M, Becchetti A, Wanke E, Arcangeli A (2003) Developmentally regulated expression of the mouse homologues of the potassium channel encoding genes *m-erg1*, *m-erg2* and *m-erg3*. Gene Expr Patterns 3: 767 - 776.

Pond AL, Scheve BK, Benedict AT, Petrecca K, Van Wagoner DR, Shrier A, Nerbonne JM (2000) Expression of distinct ERG proteins in rat, mouse, and human heart. J Biol Chem 275: 5997 – 6006.

Pond AL, Nedele C, Wang W-H, Wang X, Walther C, Jaeger C, Bradley KS, Du H, Fujita N, Hockerman GH, Hannon KM (2014) The mERG1a channel modulates skeletal muscle *MuRF1*, but not *MAFbx*, expression. Muscle Nerve 49: 378 – 388.

Potet F, Bouyssou T, Escande D, Baró I (2001) Gastrointestinal prokinetic drugs have different affinity for the cardiac human ether-á-gogo K^+ channel. J Pharmacol Exp Ther 299: 1007 - 1012.

Pourrier M, Zicha S, Ehrlich J, Han W, Nattel S (2003) Canine ventricular KCNE2 expression resides predominantly in Purkinje fibers. Circ Res 93: 189 - 191.

Pratt CM, Al-Khalidi HR, Brum JM, Holroyde MJ, Schwartz PJ, Marcello SR, Borggrefe M, Dorian P, Camm AJ (2006) Cumulative experience of azimilide-associated torsades de pointes ventricular tachycardia in the 19 clinical studies comprising the azimilide database. J Am Coll Cardiol 48: 471 – 477.

Priest BT, Bell IM, Garcia ML (2008) Role of hERG potassium channel assays in drug development. Channels 2: 87 – 93.

Priest JR, Gawad C, Kahlig KM, Yu JK, O'Hara T, Boyle PM, Rajamani S, Clark MJ, Garcia STK, Ceresnak S, Harris J, Boyle S, Dewey FE, Malloy-Walton L, Dunn K, Grove M, Perez MV, Neff NF, Chen R, Maeda K, Dubin A, Belardinelli L, West J, Antolik C, Macaya D, Quertermous T, Trayanova NA, Quake SR, Ashley EA (2016) Early somatic mosaicism is a rare cause of long-QT syndrome. Proc Natl Acad Sci USA 113: 11555 – 11560.

Priori SG, Napolitano C, Schwartz PJ (1999) Low penetrance in the long-QT syndrome: clinical impact. Circulation 99: 529 – 533.

Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D (2003) Risk stratification in the long-QT syndrome. N Engl J Med 348: 1866 – 1874.

Priori SG, Napolitano C, Schwartz PJ, Grillo M, Bloise R, Ronchetti E, Moncalvo C, Tulipani C, Veia A, Bottelli G, Nastoli J (2004) Association of long-QT syndrome loci and cardiac events among patients treated with β-blockers. JAMA 292: 1341 – 1344.

Qu Y, Schnier P, Zanon R, Vargas HM (2011) hERG potency estimates based upon dose solution analysis: what have we learned? J Pharmacol Toxicol Method 64: 251 – 257.

Quigley EMM, Vandeplassche L, Kerstens R, Ausma J (2009) Clinical trial: the efficacy, impact on quality of life, and safety and tolerability of prucalopride in severe chronic constipation- a 12-week, randomized, double-lind, placebo-controlled study. Aliment Pharmacol Ther 29: 315 – 328.

Rae J, Cooper K, Gates P, Watsky M (1991) Low access resistance perforated patch recordings using amphotericin B. J Neurosci Methods 37: 15 – 26.

Rajamani S, Anderson CL, Anson BD, January CT (2002) Pharmacological rescue of human K^+ channel long-QT2 mutations: human ether-a-go-go-related gene rescue without block. Circulation 105: 2830 – 2835.

Rajamani S, Eckhardt LL, Valdivia CR, Klemens CA, Gillman BM, Anderson CL, Holzem KM, Delisle BP, Anson BD, Makielski JC, January CT (2006) Drug-induced long QT syndrome: hERG K^+ channel block and disruption of protein trafficking by fluoxetine and norfluoxetine. Br J Pharmacol 149: 481 – 489.

Rajamani S, Shryock JC, Belardinelli L (2008) Rapid kinetic interactions of ranolazine with HERG K^+ current. J Cardiovasc Pharmacol 51: 581 – 589.

Ramirez AH, Shaffer CM, Delaney JT, Sexton DP, Levy SE, Rieder MJ, Nickerson DA, George AL, Roden DM (2013) Novel rare variants in congenital cardiac arrhythmia genes are frequent in drug-induced torsades de pointes. Pharmacogenomics J 13: 325 – 329.

Ramström C, Chapman H, Viitanen T, Afrasiabi E, Fox H, Kivelä J, Soini S, Korhonen L, Lindholm D, Pasternack M, Törnquist K (2010) Regulation of HERG (KCNH2) potassium channel surface expression by diacylglycerol. Cell Mol Life Sci 67: 157 – 169.

Raschi E, Poluzzi E, Koci A, Boriani G, De Ponti F (2011) QT interval shortening in spontaneous reports submitted to the FDA: the need for consensus. Br J Clin Pharmacol 72: 839 – 841.

Raschi E, De Ponti F (2012) Cardiovascular toxicity of anticancer-targeted therapy: emerging issues in the era of cardio-oncology. Intern Emerg Med 7: 113 – 131.

Raschi E, Poluzzi E, Godman B, Koci A, Moretti U, Kalaba M, Bennie M, Barbui C; Wettermark B, Sturkenboom M, De Ponti F (2013) Torsadogenic risk of antipsychotics: combining adverse event reports with drug utilization data across Europe. PloS One 8: e81208.

Rasmussen HB, Møller M, Knaus HG, Jensen BS, Olesen SP, Jørgensen NK (2004) Subcellular localization of the delayed rectifier K^+ channels KCNQ1 and ERG1 in the rat heart. Am J Physiol Heart Circ Physiol 286: H1300 – H1309.

Redaelli E, Restano-Cassulini R, Silva DF, Clement H, Schiavon E, Zamudio FZ, Odell G, Arcangeli A, Clare JJ, Alagón A, de la Vega RC, Possani LD, Wanke E (2010) Target promiscuity and heterogeneous effects of tarantula venom peptides affecting Na^+ and K^+ ion channels. J Biol Chem 285: 4130 – 4142.

Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, Siegl PKS, Strang I, Sullivan AT, Wallis R, Camm AJ, Hammond TG (2003) Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc Res 58: 32 - 45.

Reed GJ, Boczek NJ, Etheridge SP, Ackerman MJ (2015) *CALM3* mutation associated with long QT syndrome. Heart Rhythm 12: 419 – 422.

Refsgaard L, Holst AG, Sadjadieh G, Haunsø S, Nielsen JB, Olesen MS (2012) High prevalence of genetic variants previously associated with LQT syndrome in new exome data. Eur J Hum Genet 20: 905 – 908.

Ren X-Q, Liu GX, Organ-Darling LE, Zheng R, Roder K, Jindal HK, Centracchio J, McDonald TV, Koren G (2010) Pore mutants of hERG and KvLQT1 downregulate the reciprocal currents in stable cell lines. Am J Physiol Heart Circ Physiol 299: H1525 – H1534.

Restano-Cassulini R, Korolkova YV, Diochot S, Gurrola G, Guasti L, Possani LD, Lazdunski M, Grishin EV, Arcangeli A, Wanke E (2006) Species diversity and peptide toxins blocking selectivity of ether-à-go-go-related gene subfamily K^+ channels in the central nervous system. Mol Pharmacol 69: 1673 - 1683.

Rhodes TE, Abraham RA, Welch RC, Vanoye CG, Crotti L, Arnestad M, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Rognum TO, Roden DM, Schwartz PJ, George AL (2008) Cardiac potassium channel dysfunction in sudden infant death syndrome. J Mol Cell Cardiol 44: 571 – 581.

Ridley JM, Milnes JT, Witchel HJ, Hancox JC (2004) High affinity HERG K^+ channel blockade by the antiarrhythmic agent dronedarone: resistance to mutations of the S6 residues Y652 and F656. Biochem Biophys Res Commun 325: 883 – 891.

Ridley JM, Milnes JT, Duncan RS, McPate MJ, James AF, Witchel HJ, Hancox JC (2006) Inhibition of the HERG K^+ channel by the antifungal drug ketoconazole depends on channel gating and involves the S6 residue F656. FEBS Lett 580: 1999 – 2005.

Riera AR, Uchida AH, Ferreira C, Ferreira Filho C, Schapachnik E, Dubner S, Zhang L, Moffa PJ (2008) Relationship among amiodarone, new class III antiarrhythmics, miscellaneous agents and acquired long QT syndrome. Cardiol J 15: 209 – 219.

Roden DM (1998) Taking the “idio” out of “idiosyncratic”: predicting torsades de pointes. Pacing Clin Electrophysiol 21: 1029 – 1034.

Roden DM (2004) Drug-induced prolongation of the QT interval. New Engl J Med 350: 1013 - 1022.

Roden DM (2006) Long QT syndrome: reduced repolarization reserve and the genetic link. J Intern Med 259: 59 – 69.

Roden DM, Lazzara R, Rosen M, Schwartz PJ, Towbin J, Vincent GM (1996) Multiple mechanisms in the long-QT syndrome. Current knowledge, gaps, and future directions. Circulation 94: 1996 - 2012.

Rodriguez N, Amarouch MY, Montnach J, Piron J, Labro AJ, Charpentier F, Mérot J, Baró I, Loussouarn G (2010) Phosphatidylinositol-4,5-bisphosphate (PIP_2) stabilizes the open pore conformation of the Kv11.1 (hERG) channel. Biophys J 99: 1110 – 1118.

Rodriguez-Menchaca AA, Ferrer T, Navarro-Polanco RA, Sanchez-Chapula JA, Moreno-Galindo EG (2014) Impact of the whole-cell patch-clamp configuration on the pharmacological assessment of the hERG channel: trazodone as a case example. J Pharmacol Toxicol Methods 69: 237 – 244.

Roepke TK, Kontogeorgis A, Ovanez C, Xu X, Young JB, Purtell K, Goldstein PA, Christini DJ, Peters NS, Akar FG, Gutstein DE, Lerner DJ, Abbott GW (2008) Targeted deletion of *kcne2* impairs ventricular repolarization via disruption of $I_{K,slow1}$ and $I_{to,f}$. FASEB J 22: 3648 – 3660.

Romano C, Gemme G, Pongiglione R (1963) Artimie cardiach rare dell’eta pediatrica. II. Accessi sincopali per fibrillazione ventricolare parossitica. Clin Pediatr 45: 656 - 683.

Rosati B, Marchetti P, Crociani O, Lecchi M, Lupi R, Arcangeli A, Olivotto M, Wanke E (2000) Glucose- and arginine-induced insulin secretion by human pancreatic beta-cells: the role of HERG K^+ channels in firing and release. FASEB J 14: 2601 – 2610.

Roti Roti EC, Myers CD, Ayers RA, Boatman DE, Delfosse SA, Chan EKL, Ackerman MJ, January CT, Robertson GA (2002) Interaction with GM130 during HERG ion channel trafficking. J Biol Chem 277: 47779 -47785.

Roussel J, Labarthe F, Thireau J, Ferro F, Farah C, Roy J, Horiuchi M, Tardieu M, Lefort B, Benoist JF, Lacampagne A, Richard S, Fauconnier J, Babuty D, Le Guennec JY (2016) Carnitine deficiency induces a short QT syndrome. Heart Rhythm 13: 165 – 174.

Roy J, Vantol B, Cowley E, Blay J, Linsdell P (2008) Pharmacological separation of hEAG and hERG K^+ channel function in the human mammary carcinoma cell line MCF-7. Oncol Rep 19: 1511 – 1516.

Sacco T, Bruno A, Wanke E, Tempia F. (2003) Functional roles of an ERG current isolated in cerebellar Purkinje neurons. J Neurophysiol 90: 1817 – 1828.

Sager PT, Gintant G, Turner JR, Pettit S, Stockbridge N (2014) Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the cardiac safety research consortium. Am Heart J 167: 292 – 300.

Sakata S, Kurata Y, Li P, Notsu T, Morikawa K, Miake J, Higaki K, Yamamoto Y, Yoshida A, Shirayoshi Y, Yamamoto K, Horie M, Ninomiya H, Kanzaki S, Hisatome I (2014) Instability of KCNE1-D85N that causes long QT syndrome: stabilization by verapamil. Pacing Clin Electrophysiol 37: 853 – 863.

Sale H, Wang J, O'Hara TJ, Tester DJ, Phartiyal P, He JQ, Rudy Y, Ackerman MJ, Robertson GA (2008) Physiological properties of hERG 1a/1b heteromeric currents and a hERG1b-specific mutation associated with Long-QT syndrome. Circ Res 103: e81 – e95.

Sánchez-Chapula JA, Navarro-Polanco RA, Culberson C, Chen J, Sanguinetti MC (2002) Molecular determinants of voltage-dependent human *ether-a-go-go*-related gene (HERG) K^+ channel block. J Biol Chem 277: 23587 - 23595.

Sánchez-Chapula JA, Ferrer T, Navarro-Polanco RA, Sanguinetti MC (2003) Voltage-dependent profile of human *ether-a-go-go*-related gene channel block is influenced by a single residue in the S6 transmembrane domain. Mol Pharmacol 63: 1051 - 1058.

Sanguinetti MC (2014) HERG1 channel agonists and cardiac arrhythmia. Curr Opin Pharmacol 15: 22 – 27.

Sanguinetti MC, Jurkiewicz NK (1990) Two components of cardiac delayed rectifier K^+ current: Differential sensitivity to block by class III antiarrhythmic agents. J Gen Physiol 96: 195 – 215.

Sanguinetti MC, Tristani-Firouzi M (2006) hERG potassium channels and cardiac arrhythmia. Nature 440: 463 – 469.

Sanguinetti MC, Jiang C, Curran ME, Keating MT (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: hERG encodes the I_{Kr} potassium channel. Cell 81: 299 – 307.

Sarganas G, Garbe E, Klimpel A, Hering RC, Bronder E, Haverkamp W (2014) Epidemiology of symptomatic drug-induced long QT syndrome and torsade de pointes in Germany. Europace 16: 101 – 108.

Sartiani L, Bettiol E, Stillitano F, Mugelli A, Cerbai E, Jaconi ME (2007) Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: a molecular and electrophysiological approach. Stem Cells 25: 1136 – 1144.

Sarzani R, Pietrucci F, Corinaldesi C, Francioni M, Letizia C, D'Erasmo E, Dessi-Fulgheri P, Rappelli A (2006) The functional HERG variant 897T is associated with Conn's adenoma. J Hypertens 24: 479 – 487.

Saxena P, Zangerl-Plessl E-M, Linder T, Windisch A, Hohaus A, Timin E, Hering S, Stary-Weinzinger A (2016) New potential binding determinant for hERG channel inhibitors. Sci Rep 6: 24182.

Scherer CR, Lerche C, Decher N, Dennis AT, Maier P, Ficker E, Busch AE, Wollnik B, Steinmeyer K (2002) The antihistamine fexofenadine does not affect I_{Kr} currents in a case report of drug-induced cardiac arrhythmia. Br J Pharmacol 137: 892 – 900.

Scherer D, von Löwenstern K, Zitron E, Scholz EP, Bloehs R, Kathöfer S, Thomas D, Bauer A, Katus HA, Karle CA, Kiesecker C (2008) Inhibition of cardiac hERG potassium channels by tetracyclic antidepressant mianserin. Naunyn-Schmied Arch Pharmacol 378: 73 – 83.

Schimpf R, Veltmann C, Giustetto C, Gaita F, Borggrefe M, Wolpert C (2007) In vivo effects of mutant hERG K^+ channel inhibition by disopyramide in patients with a short QT-1 syndrome: a pilot study. J Cardiovasc Electrophysiol 18: 1157 – 1160.

Schimpf R, Veltmann C, Papavassiliu T, Rudic B, Göksu T, Kuschyk J, Wolpert C, Antzelevitch C, Ebner A, Borggrefe M, Brandt C (2012) Drug-induced QT-interval shortening following antiepileptic treatment with oral rufinamide. Heart Rhythm 9: 776 – 781.

Schledermann W, Wulfsen I, Schwarz JR, Bauer CK. (2001) Modulation of rat erg1, erg2, erg3 and HERG K^+ currents by thyrotropin-releasing hormone in anterior pituitary cells via the native signal cascade. J Physiol 532: 143 – 163.

Schmitt N, Grunnet M, Olesen S-P (2014) Cardiac potassium channel subtypes: new roles in repolarization and arrhythmia. Physiol Rev 94: 609 – 653.

Schram G, Pourrier M, Melnyk P, Nattel S (2002) Differential distribution of cardiac ion channel expression as a basis for region specialization in electrical function. Circ Res 90: 939 – 950.

Schroder EA, Burgess DE, Zhang X, Lefta M, Smith JL, Patwardhan A, Bartos DC, Elayi CS, Esser KA, Delisle BP (2015) The cardiomyocyte molecular clock regulates the circadian expression of *kcnh2* and contributes to ventricular repolarization. Heart Rhythm 12: 1306 – 1314.

Schuster AM, Glassmeier G, Bauer CK. (2011) Strong activation of *ether-à-go-go*-related gene 1 K^+ channel isoforms by NS1643 in human embryonic kidney 293 and chinese hamster ovary cells. Mol Pharmacol 80: 930 – 942.

Schwartz PJ, Crotti L (2014) Long and short QT syndromes. In Cardiac Electrophysiology: From cell to bedside (6^{th} edition) p935 - 946.

Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AAM, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R (2001) Genotype-phenotype correlation in the long-QT syndrome: Gene-specific triggers for life-threatening arrhythmias. Circulation 103: 89 – 95.

Schwartz PJ, Spazzolini C, Crotti L, Bathen J, Amlie JP, Timothy K, Shkolnikova M, Berul CI, Bitner-Glindzicz M, Toivonen L, Horie M, Schulze-Bahr E, Denjoy I (2006) The Jervell Lange-Nielsen syndrome: Natural history, molecular basis and clinical outcome. Circulation 113: 783 – 790.

Schwartz PJ, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Bosi G, Gabbarini F, Goulene K, Insolia R, Mannarino S, Mosca F, Nespoli L, Rimini A, Rosati E, Salice P, Spazzolini C (2009) Prevalence of the congenital long-QT syndrome. Circulation 120: 1761 - 1767.

Schwartz PJ, Ackerman MJ, George AL, Wilde AAM (2013) Impact of genetics on the clinical management of channelopathies. J Am Coll Cardiol 62: 169 – 180.

Schäfer R, Wulfsen I, Behrens S, Weinsberg F, Bauer CK, Schwarz JR (1999) The erg-like potassium current in rat lactotrophs. J Physiol 518: 401 - 416.

Schönherr R, Heinemann SH (1996) Molecular determinants for activation and inactivation of HERG, a human inward rectifier potassium channel. J Physiol 493: 635 – 642.

Schönherr R, Rosati B, Hehl S, Rao VG, Arcangeli A, Olivotto M, Heinemann SH, Wanke E (1999) Functional role of the slow activation property of ERG K^+ channels. Eur J Neurosci 11: 753 – 760.

Schönherr R, Gessner G, Löber K, Heinemann SH (2002) Functional distinction of human EAG1 and EAG2 potassium channels. FEBS Lett 514: 204 - 208.

Selyanko AA, Hadley JK, Wood IC, Abogadie FC, Delmas P, Buckley NJ, London B, Brown DA (1999) Two types of K^+ channel subunit, Erg1 and KCNQ2/3, contribute to the M-like current in a mammalian neuronal cell. J Neurosci 19: 7742 – 7756.

Sesti F, Abbott GW, Wei J, Murray KT, Saksena S, Schwartz PJ, Priori SG, Roden DM, George AL, Goldstein SAN (2000) A common polymorphism associated with antibiotic-induced cardiac arrhythmia. Proc Natl Acad Sci USA 97: 10613 – 10618.

Seth R, Moss AJ, McNitt S, Zareba W, Andrews ML, Qi M, Robinson JL, Goldenberg I, Ackerman MJ, Benhorin J, Kaufman ES, Locati EH, Napolitano C, Priori SG, Schwartz PJ, Towbin JA, Vincent GM, Zhang L (2007) Long QT syndrome and pregnancy. J Am Coll Cardiol 49: 1092 – 1098.

Shah RR (2005) Drug-induced QT interval prolongation- regulatory guidance and perspectives on hERG channel studies. In The hERG cardiac potassium channel: structure, function and long QT syndrome (Novartis Foundation Symposium 266) p251 - 285.

Shah RR (2010) Drug-induced QT interval shortening: potential harbinger of proarrhythmia and regulatory perspectives. Br J Pharmacol 159: 58 – 69.

Shah RR, Hondeghem LM (2005) Refining detection of drug-inducd proarrhythmia: QT interval and TRIaD. Heart Rhythm 2: 758 – 772.

Shan H, Zhang Y, Cai B, Chen X, Fan Y, Yang L, Chen X, Liang H, Zhang Y, Song X, Xu C, LuY, Yang B, Du Z (2013) Upregulation of microRNA-1 and microRNA-133 contributes to arsenic-induced cardiac electrical remodeling. Int J Cardiol 167: 2798 – 2805.

Shao X-D, Wu K-C, Hao Z-M, Hong L, Zhang J, Fan D-M (2005) The potent inhibitory effects of cisapride, a specific blocker for human ether-a-go-go-related gene (HERG) channel, on gastric cancer. Cancer Biol Ther 4: 295 – 301.

Shao X-D, Wu K-C, Guo X-Z, Xie M-J, Zhang J, Fan D-M (2008) Expression and significance of HERG protein in gastric cancer. Cancer Biol Ther 7: 45 – 50.

Shao M, Wei R, Tisdale JE, Liu W, Overholser BR (2013) miR-362-3p decreases hERG expression and is associated with an unidentified form of congenital long-QT syndrome. Circulation 128: A18234.

Shepard PD, Canavier CC, Levitan ES (2007) *Ether-a-go-go*-related gene potassium channels: what's all the buzz about? Schizophr Bull 33: 1263 – 1269.

Shi W, Wymore RS, Wang H-S, Pan Z, Cohen IS, McKinnon D, Dixon JE (1997) Identification of two nervous system-specific members of the *erg* potassium channel gene family. J Neurosci 17: 9423 - 9432.

Shi YP, Cheng YM, Van Slyke AC, Claydon TW (2014) External protons destabilize the activated voltage sensor in hERG channels. Eur Biophys J 43: 59 - 69.

Shimizu W, Moss AJ, Wilde AAM, Towbin JA, Ackerman MJ, January CT, Tester DJ, Zareba W, Robinson JL, Qi M, Vincent GM, Kaufman ES, Hofman N, Noda T, Kamakura S, Miyamoto Y, Shah S, Amin V, Goldenberg I, Andrews ML, McNitt S (2009) Genotype-phenotype aspects of type 2 long QT syndrome. J Am Coll Cardiol 54: 2052 - 2062.

Sicouri S, Glass A, Ferreiro M, Antzelevitch C (2010) Transseptal dispersion of repolarization and its role in the developmental of torsade de pointes arrhythmias. J Cardiovasc Electrophysiol 21: 441 – 447.

Sinner MF, Pfeufer A, Akyol M, Beckmann B-M, Hinterseer M, Wacker A, Perz S, Sauter W, Illig T, Näbauer M, Schmitt C, Wichmann H-E, Schömig A, Steinbeck G, Meitinger T,

Kääb S (2008) The non-synonymous coding I_{Kr}-channel variant *KCNH2*-K897T is associated with atrial fibrillation: results from a systematic candidate gene-based analysis of *KCNH2* (*HERG*). Eur Heart J 29: 907 – 914.

Smith GAM, Tsui H-W, Newell EW, Jiang X, Zhu XP, Tsui FW, Schlichter LC (2002) Functional up-regulation of HERG K^+ channels in neoplastic hematopoietic cells. J Biol Chem 277: 18528 - 18534.

Smith JL, McBride CM, Nataraj PS, Bartos DC, January CT, Delisle BP (2011) Trafficking-deficient hERG K^+ channels linked to long QT syndrome are regulated by a microtubule-dependent quality control compartment in the ER. Am J Physiol Cell Physiol 301: C75 – C85.

Smith JL, Reloj AR, Nataraj PS, Bartos DC, Schroder EA, Moss AJ, Ohno S, Horie M, Anderson CL, January CT, Delisle BP (2013) Pharmacological correction of long QT-linked mutations in KCNH2 (hERG) increases the trafficking of Kv11.1 channels stored in the transitional endoplasmic reticulum. Am J Physiol Cell Physiol 305: C919 – C930.

Smith PL, Yellen G (2002) Fast and slow voltage sensor movements in HERG potassium channels. J Gen Physiol 119: 275 - 293.

Smith PL, Baukrowitz T, Yellen G (1996) The inward rectification mechanism of the HERG cardiac potassium channel. Nature 379: 833 – 836.

Snyders DJ, Chaudhary A (1996) High affinity open channel block by dofetilide of *HERG* expressed in a human cell line. Mol Pharmacol 49: 949 – 955.

Spector PS, Curran ME, Keating MT, Sanguinetti MC (1996a) Class III antiarrhythmic drugs block HERG, a human cardiac delayed rectifier K^+ channel: open-channel block by methanesulfonanilides. Circ Res 78: 499 -503.

Spector PS, Curran ME, Zou A, Keating MT, Sanguinetti MC (1996b) Fast inactivation causes rectification of the I_{Kr} channel. J Gen Physiol 107: 611 – 619.

Spence S, Deurinck M, Ju H, Traebert M, McLean L, Marlowe J, Emotte C, Tritto E, Tseng M, Shultz M, Friedrichs GS (2016) Histone deacetylase inhibitors prolong cardiac repolarization through transcriptional mechanisms. Toxicol Sci 153: 39 – 54.

Spencer CI, Baba S, Nakamura K, Hua EA, Sears MAF, Fu C-C, Zhang J, Balijepalli S, Tomoda K, Hayashi Y, Lizarraga P, Wojciak J, Scheinman MM, Aalto-Setälä K, Makielski JC, January CT, Healy KE, Kamp TJ, Yamanaka S, Conklin BR (2014) Calcium transients closely reflect prolonged action potentials in iPSC models of inherited cardiac arrhythmia. Stem Cell Rep 3: 269 – 281.

Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT (1998) Genomic structure of three long QT syndrome genes: *KVLQT1*, *HERG*, and *KCNE1*. Genomics 51: 86 – 97.

Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, Keating MT (2000) Spectrum of mutations in long-QT syndrome genes: *KVLQT1*, *HERG*, *SCN5A*, *KCNE1*, and *KCNE2*. Circulation 102: 1178 – 1185.

Sroubek J, Krishnan Y, McDonald TV (2013) Sequence and structure-specific elements of HERG mRNA determine channel synthesis and trafficking efficiency. FASEB J 27: 3039 – 3053.

Stansfeld PJ, Gedeck P, Gosling M, Cox B, Mitcheson JS, Sutcliffe MJ (2007) Drug block of the hERG potassium channel: insight from modeling. Proteins 68: 568 – 580.

Stansfeld PJ, Grottesi A, Sands ZA, Sansom MSP, Gedeck P, Gosling M, Cox B, Stanfield PR, Mitcheson JS, Sutcliffe MJ (2008) Insight into the mechanism of inactivation and pH sensitivity in potassium channels from molecular dynamics simulations. Biochemistry 47: 7414 – 7422.

Stary A, Wacker SJ, Boukharta L, Zachariae U, Karimi-Nejad Y, Åqvist J, Vriend G, de Groot BL (2010) Toward a consensus model of the hERG potassium channel. ChemMedChem 5: 455 - 467.

Stattin E-L, Boström IM, Winbo A, Cederquist K, Jonasson J, Jonsson B-A, Diamant U-B, Jensen SM, Rydberg A, Norberg A (2012) Founder mutations characterize the mutation panorama in 200 Swedish index cases referred for long QT syndrome genetic testing. BMC Cardiovasc Disord 12: 95.

Staudacher I, Wang L, Wan X, Obers S, Wenzel W, Tristram F, Koschny R, Staudacher K, Kisselbach J, Koelsch P, Schweizer PA, Katus HA, Ficker E, Thomas D (2011) hERG K^+ channel-associated cardiac effects of the antidepressant drug desipramine. Naunyn-Schmied Arch Pharmacol 383: 119 – 139.

Stecker EC, Reinier K, Marijon E, Narayanan K, Teodorescu C, Uy-Evanado A, Gunson K, Jui J, Chugh SS (2014) Public health burden of sudden cardiac death in the United States. Circ Arrhythm Electrophysiol 7: 212 – 217.

Stockbridge N, Morganroth J, Shah RR, Garnett C (2013) Dealing with global safety issues: was the response to QT-liability of non-cardiac drugs well coordinated? Drug Saf 36: 167 – 182.

Stork D, Timin EN, Berjukow S, Huber C, Hohaus A, Auer M, Hering S (2007) State dependent dissociation of HERG channel inhibitors. Br J Pharmacol 151: 1368 - 1376.

Stump MR, Gong Q, Zhou Z (2011) Multiple splicing defects caused by hERG splice site mutation 2592 + 1G>A associated with long QT syndrome. Am J Physiol Heart Circ Physiol 300: H312 – H318..

Stump MR, Gong Q, Zhou Z (2012a) Isoform-specific dominant-negative effects associated with hERG1 G628S mutation in long QT syndrome. PLoS ONE 7: e42552.

Stump MR, Gong Q, Packer JD, Zhou Z. (2012b) Early LQT2 nonsense mutation generates N-terminally truncated hERG channels with altered gating properties by the reinitiation of translation. J Mol Cell Cardiol 53: 725 - 733.

Stump MR, Gong Q, Zhou Z (2013) LQT2 nonsense mutations generate trafficking defective NH_2-terminally truncated channels by the reinitiation of translation. Am J Physiol Heart Circ Physiol 305: H1397 – H1404.

Sugiyama A, Hashimoto K. (1998) Effects of gastrointestinal prokinetic agents, TKS159 and cisapride on the *in situ* canine heart assessed by cardiohemodynamic and electrophysiological monitoring. Toxicol Appl Pharmacol 152: 261 – 269.

Sugiyama H, Nakamura K, Morita H, Akagi S, Tani Y, Katayama Y, Nishii N, Miyoshi T, Nagase S, Kohno K, Kusano KF, Ohe T, Kurokawa J, Furukawa T, Ito H (2011) Circulating KCNH2 current-activating factor in patients with heart failure and ventricular tachyarrhythmia. PloS One 6: e19897.

Sun Y, Zhang P, Li X, Zhang H, Li J, Liu G, Guo J (2009) A novel nonsense mutation Y652X in the S6/pore region of human ether-go-go gene found in a long QT syndrome family. Scand Cardiovasc J 43: 181 – 186.

Sun Y, Quan X-Q, Fromme S, Cox RH, Zhang P, Zhang L, Guo D, Guo J, Patel C, Kowey PR, Yan GX (2011) A novel mutation in the *KCNH2* gene associated with short QT syndrome. J Mol Cell Cardiol 50: 433 – 441.

Suzuki H, Hoshina S, Ozawa J, Sato A, Minamino T, Aizawa Y, Saitoh A (2014) Short QT syndrome in a boy diagnosed on screening for heart disease. Pediatr Int 56: 774 – 776.

Szabó G, Szentandrássy N, Biró T, Tóth BI, Czifra G, Magyar J, Bányász T, Varró A, Kovács L, Nánási PP (2005) Asymmetrical distribution of ion channels in canine and human left-ventricular wall: epicardium versus midmyocardium. Pflügers Arch 450: 307 – 316.

Szentandrássy N, Bányász T, Biró T, Szabó G, Tóth BI, Magyar J, Lazar J, Varró A, Kovács L, Nánási PP (2005) Apico-basal inhomogeneity in distribution of ion channels in canine and human ventricular myocardium. Cardiovasc Res 65: 651 – 660.

Tack J, van Outryve M, Beyens G, Kerstens R, Vandeplassche L (2009) Prucalopride (Resolor) in the treatment of severe chronic constipation in patients dissatisfied with laxatives. Gut 58: 357 – 365.

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861 – 872.

Takemasa H, Nagatomo T, Abe H, Kawakami K, Igarashi T, Tsurugi T, Kabashima N, Tamura M, Okazaki M, Delisle BP, January CT, Otsuji Y (2007) Coexistence of hERG current block and disruption of protein trafficking in ketoconazole-induced long QT syndrome. Br J Pharmacol 153: 439 – 447.

Tao X, Lee A, Limapichat W, Dougherty DA, MacKinnon R (2010) A gating charge transfer center in voltage sensors. Science 328: 67 – 73.

Teng GQ, Zhao X, Lees-Miller JP, Quinn FR, Li P, Rancourt DE, London B, Cross JC, Duff HJ (2008) Homozygous missense N629D hERG (KCNH2) potassium channel mutation causes developmental defects in the right ventricle and its outflow tract and embryonic lethality. Circ Res 103: 1483 – 1491.

Terentyev D, Rees CM, Li W, Cooper LL, Jindal HK, Peng X, Lu Y, Terentyeva R, Odening KE, Daley J, Bist K, Choi B-K, Karma A, Koern G (2014) Hyperphosphorylation of RyRs underlies triggered activity in transgenic rabbit model of LQT2 syndrome. Circ Res 115: 919 – 928.

Terrenoire C, Clancy CE, Cormier JW, Sampson KJ, Kass RS (2005) Autonomic control of cardiac action potentials: Role of potassium channel kinetics in response to sympathetic stimulation. Circ Res 96: e25 - e34.

Terrenoire C, Wang K, Tung KWC, Chung WK, Pass RH, Lu JT, Jean JC, Omari A, Sampson KJ, Kotton DN, Keller G, Kass RS (2013) Induced pluripotent stem cells used to reveal drug actions in a long QT syndrome family with complex genetics. J Gen Physiol 141: 61 – 72.

Teschemacher AG, Seward EP, Hancox JC, Witchel HJ (1999) Inhibition of the current of heterologously expressed HERG potassium channels by imipramine and amitriptyline. Br J Pharmacol 128: 479 – 485.

Tester DJ, Ackerman MJ (2007) Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. J Am Coll Cardiol 49: 240 - 246.

Tester DJ, Ackerman MJ (2014) Genetics of long QT syndrome. Methodist Debakey Cardiovasc J 10: 29 - 33.

Tester DJ, Will ML, Haglund CM, Ackerman MJ (2005) Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. Heart Rhythm 2: 507 - 517.

Tester DJ, Will ML, Haglund CM, Ackerman MJ (2006) Effect of clinical phenotype on yield of long QT syndrome genetic testing. J Am Coll Cardiol 47: 764 - 768.

Tester DJ, Medeiros-Domingo A, Will ML, Haglund CM, Ackerman MJ (2012) Cardiac channel molecular autopsy: insights from 173 consecutive cases of autopsy-negative sudden unexplained death referred for postmortem genetic testing. Mayo Clin Proc 87: 524 - 539.

Thomas D, Zhang W, Karle CA, Kathöfer S, Schöls W, Kübler W, Kiehn J (1999) Deletion of protein kinase A phosphorylation sites in the HERG potassium channel inhibits activation shift by protein kinase A. J Biol Chem 274: 27457 – 27462.

Thomas D, Zhang W, Wu K, Wimmer A-B, Gut B, Wendt-Nordahl G, Kathöfer S, Kreye VA, Katus HA, Schoels W, Kiehn J, Karle CA (2003) Regulation of HERG potassium channel activation by protein kinase C independent of direct phosphorylation of the channel protein. Cardiovasc Res 59: 14 – 26.

Thomas D, Wu K, Wimmer A-B, Zitron E, Hammerling BC, Kathöfer S, Lueck S, Bloehs R, Kreye VA, Kiehn J, Katus HA, Schoels W, Karle CA (2004) Activation of cardiac human ether-a-go-go related gene potassium currents is regulated by α_{1A}-adrenceptors. J Mol Med 82: 826 – 837.

Thomas D, Bloehs R, Koschny R, Ficker E, Sykora J, Kiehn J, Schlömer K, Gierten J, Kathöfer S, Zitron E, Scholz EP, Kiesecker C, Katus HA, Karle CA (2008) Doxazosin induces apoptosis of cells expressing hERG K^+ channels. Eur J Pharmacol 579: 98 – 103.

Thomson SJ, Hansen A, Sanguinetti MC (2014) Concerted all-or-none subunit interactions mediate slow deactivation of human ether-à-go-go-related gene K^+ channels. J Biol Chem 289: 23428 – 23436.

Thouta S, Sokolov S, Abe Y, Clark SJ, Cheng YM, Claydon TW (2014) Proline scan of the hERG channel S6 helix reveals the location of the intracellular pore gate. Biophys J 106: 1057 – 1069.

Titier K, Canal M, Déridet E, Abouelfath A, Gromb S, Molimard M, Moore N (2004) Determination of myocardium to plasma concentration ratios of five antipsychotic drugs: comparison with their ability to induce arrhythmia and sudden death in clinical practice. Toxicol Appl Pharmacol 199: 52 - 60.

Toga T, Kohmura Y, Kawatsu R (2007) The 5-HT_4 agonists cisapride, mosapride, and CJ-033466, a novel potent compound, exhibit different human ether-a-go-go-related gene (hERG)-blocking activities. J Pharmacol Sci 105: 207 - 210.

Tomás M, Napolitano C, De Giuli L, Bloise R, Subirana I, Malovini A, Bellazzi R, Arking DE, Marban E, Chakravarti A, Spooner PM, Priori SG (2010) Polymorphisms in the *NOS1AP* gene modulate QT interval duration and risk of arrhythmias in the long QT syndrome. J Am Coll Cardiol 55: 2745 – 2752.

Tomuschat C, O'Donnell AM, Coyle D, Puri P (2016) Reduced expression of voltage-gated Kv11.1 (hERG) K^+ channels in aganglionic colon in Hirschsprung's disease. Pediatr Surg Int 32: 9 – 16.

Tong W-C, Tribe RM, Smith R, Taggart MJ (2014) Computational modeling reveals key contributions of KCNQ and hERG currents to the malleability of uterine action potentials underpinning labor. PLoS One 9: e114034.

Torres AM, Bansal PS, Sunde M, Clarke CE, Bursill JA, Smith DJ, Bauskin A, Breit SN, Campbell TJ, Alewood PF, Kuchel PW, Vandenberg JI (2003) Structure of the HERG K^+ channel S5P extracellular linker: role of an amphipathic alpha-helix in C-type inactivation. J Biol Chem 278: 42136 – 42148.

Traebert M, Dumotier B, Meister L, Hoffmann P, Dominguez-Estevez M, Suter W (2004) Inhibition of hERG K^+ currents by antimalarial drugs in stably transfected HEK293 cells. Eur J Pharmacol 484: 41 – 48.

Trepakova ES, Koerner J, Pettit SD, Valentin J-P (2009) A HESI consortium approach to assess the human predictive value of non-clinical repolarization assays. J Pharmacol Toxicol Methods 60: 45 - 50.

Trudeau MC, Warmke JW, Ganetzky B, Roberson GA (1995) HERG, a human inward rectifier in the voltage-gated potassium channel family. Science 269: 92 – 95.

Trudeau MC, Leung LM, Roti Roti E, Robertson GA (2011) hERG1a N-terminal eag domain-containing polypeptides regulate homomeric hERG1b and heteromeric

hERG1a/hERG1b channels: a possible mechanism for long QT syndrome. J Gen Physiol 138: 581 – 592.

Tseng G-N (2001) I_{Kr}: the hERG channel. J Mol Cell Cardiol 33: 835 – 849.

Tseng G-N, Sonawane KD, Korolkova YV, Zhang M, Liu J, Grishin EV, Guy HR (2007) Probing the outer mouth structure of the hERG channel with peptide toxin footprinting and molecular modeling. Biophys J 92: 3524 – 3540.

Tsuji Y, Opthof T, Kamiya K, Yasui K, Liu W, Lu Z, Kodama I (2000) Pacing-induced heart failure causes a reduction of delayed rectifier potassium currents along with decreases in calcium and transient outward currents in rabbit ventricle. Cardiovasc Res 48: 300 – 309.

Tu E, Bagnall RD, Duflou J, Semsarian C (2011) Post-mortem review and genetic analysis of sudden unexplained death in epilepsy (SUDEP) cases. Brain Pathol 21: 201 – 208.

Tutor AS, Delpón E, Caballero R, Gómez R, Núñez L, Vaquero M, Tamargo J, Mayor F, Penela P (2006) Association of 14-3-3 proteins to β_1-adrenergic receptors modulates Kv11.1 K^+ channel activity in recombinant systems. Mol Biol Cell 17: 4666 - 4674.

Ulens C, Daenens P, Tytgat J (1999) Norpropoxyphene-induced cardiotoxicity is associated with changes in ion-selectivity and gating of HERG currents. Cardiovasc Res 44: 568 – 578.

Ulens C, Tytgat J (2000) Redox state dependency of HERG S631C channel pharmacology: relation to C-type inactivation. FEBS Lett 474: 111 – 115.

Um SY, McDonald TV (2007) Differential association between HERG and KCNE1 or KCNE2. PLoS One 2: e933.

Vaidyanathan R, Markandeya YS, Kamp TJ, Makielski JC, January CT, Eckhardt LL (2016) I_{K1}-enhanced human-induced pluripotent stem cell-derived cardiomyocytes: an improved cardiomyocyte model to investigate inherited arrhythmia syndromes. Am J Physiol Heart Circ Physiol 310: H1611 – H1621.

Vandenberg JI, Perry MD, Perrin MJ, Mann SA, Ke Y, Hill AP (2012) hERG K^+ channels: structure, function, and clinical significance. Physiol Rev 92: 1393 – 1478.

Van de Velde V, Ausma J, Vandeplassche L (2008) Pharmacokinetics of prucalopride (Resolor®) in man. Gut 57 suppl. II: A282.

van Haarst AD, van't Klooster GAE, van Gerven JMA, Schoemaker RC, van Oene JC, Burggraaf J, Coene M-C, Cohen AF (1998) The influence of cisapride and clarithromycin on QT intervals in healthy volunteers. Clin Pharmacol Ther 64: 542 – 546.

van Noord C, Straus SMJM, Sturkenboom MCJM, Hofman A, Aarnoudse AJ, Bagnardi V, Kors JA, Newton-Cheh C, Witteman JCM, Stricker BH (2009) Psychotropic drugs associated with corrected QT interval prolongation. J Clin Psychopharmacol 29: 9 – 15.

van Noord C, Sturkenboom MCJM, Straus SMJM, Witteman JCM, Stricker BH (2011) Non-cardiovascular drugs that inhibit hERG-encoded potassium channels and risk of sudden cardiac death. Heart 97: 215 – 220.

Veldkamp MW, van Ginneken AC, Opthof T, Bouman LN (1995) Delayed rectifier channels in human ventricular myocytes. Circulation 92: 3497 – 3504.

Veerman CC, Verkerk AO, Blom MT, Klemens CA, Langendijk PNJ, van Ginneken ACG, Wilders R, Tan HL (2013) Slow delayed rectifier potassium current blockade contributes importantly to drug-induced long QT syndrome. Circ Arrhythm Electrophysiol 6: 1002 – 1009.

Verkerk AO, Wilders R, Schulze-Bahr E, Beekman L, Bhuiyan ZA, Bertrand J, Eckardt L, Lin D, Borggrefe M, Breithardt G, Mannens MMAM, Tan HL, Wilde AAM, Bezzina CR (2005) Role of sequence variations in the *human ether-a-go-go*-related gene (HERG, KCNH2) in the Brugada syndrome. Cardiovasc Res 68: 441 – 453.

Vicente J, Johannesen L, Mason JW, Crumb WJ, Pueyo E, Stockbridge N, Strauss DG (2015) Comprehensive T wave morphology assessment in a randomized clinical study of dofetilide, quinidine, ranolazine, and verapamil. J Am Heart Assoc 4: e001615.

Vijayakumar R, Silva JNA, Desouza KA, Abraham RL, Strom M, Sacher F, Van Hare GF, Haïssaguerre M, Roden DM, Rudy Y (2014) Electrophysiologic substrate in congenital long QT syndrome: non-invasive mapping with electrocardiographic imaging (ECGI). Circulation 130: 1936 – 1943.

Vitola J, Vukanovic J, Roden DM (1998) Cisapride-induced torsades de pointes. J Cardiovasc Pharmacol 9: 1109 – 1113.

Vonderlin N, Fischer F, Zitron E, Seyler C, Scherer D, Thomas D, Katus HA, Scholz EP (2015) Anesthetic drug midazolam inhibits cardiac human ether-à-go-go-related gene channels: mode of action. Drug Des Devel Ther 9: 867 – 877.

Walker BD, Singleton CB, Bursill JA, Wyse KR, Valenzuela SM, Qiu MR, Breit SN, Campbell TJ (1999) Inhibition of the human ether-a-go-go-related gene (HERG) potassium channel by cisapride: affinity for open and inactivated states. Br J Pharmacol 128: 444 – 450.

Walker VE, Atanasiu R, Lam H, Shrier A (2007) Co-chaperone FKBP38 promotes hERG trafficking. J Biol Chem 282: 23509 – 23516.

Walker VE, Wong MJH, Atanasiu R, Hantouche C, Young JC, Shrier A (2010) Hsp40 chaperones promote degradation of the hERG potassium channel. J Biol Chem 285: 3319 – 3329.

Wallis RM (2010) Integrated risk assessment and predictive value to humans of non-clinical repolarization assays. Br J Pharmacol 159: 115 - 121.

Wang D, Shah KR, Um SY, Eng LS, Zhou B, Lin Y, Mitchell AA, Nicaj L, Prinz M, McDonald TV, Sampson BA, Tang Y (2014) Cardiac channelopathy testing in 274 ethnically diverse sudden unexplained deaths. Forensic Sci Int 237: 90 – 99.

Wang DT, Hill AP, Mann SA, Tan PS, Vandenberg JI (2011) Mapping the sequence of conformational changes underlying selectivity filter gating in the Kv11.1 potassium channel. Nat Struct Mol Biol 18: 35 – 41.

Wang H, Zhang Y, Cao L, Han H, Wang J, Yang B, Nattel S, Wang Z (2002) HERG K^+ channel, a regulator of tumor cell apoptosis and proliferation. Cancer Res 62: 4843 – 4848.

Wang H, Chen Y, Zhu H, Wang S, Zhang X, Xu D, Cao K, Zou J (2012) Increased response to β_2-adrenoreceptor stimulation augments inhibition of I_{Kr} in heart failure ventricular myocytes. PLoS One 7: e46186.

Wang J, Trudeau MC, Zappia AM, Robertson GA (1998) Regulation of deactivation by an amino terminal domain in human *ether-á-go-go* related gene potassium channels. J Gen Physiol 112: 637 - 647.

Wang J, Myers CD, Robertson GA (2000) Dynamic control of deactivation gating by a soluble amino-terminal domain in *HERG* K^+ channels. J Gen Physiol 115: 749 - 758.

Wang J, Wang H, Han H, Zhang Y, Yang B, Nattel S, Wang Z (2001) Phospholipid metabolite 1-palmitoyl-lysophosphatidylcholine enhances human ether-a-go-go-related gene (HERG) K^+ channel function. Circulation 104: 2645 – 2648.

Wang J, Li Y, Jiang C (2015) MiR-133b contributes to arsenic-induced apoptosis in U251 glioma cells by targeting the hERG channel. J Mol Neurosci 55: 985 – 994.

Wang L, Feng Z-P, Kondo CS, Sheldon RS, Duff HJ (1996) Developmental changes in the delayed rectifier K^+ channels in mouse heart. Circ Res 79: 79 – 85.

Wang L, Wible BA, Wan X, Ficker E (2007) Cardiac glycosides as novel inhibitors of human *ether-a-go-go*-related gene channel trafficking. J Pharmacol Exp Ther 320: 525 - 534.

Wang L, Dennis AT, Trieu P, Charron F, Ethier N, Hebert TE, Wan X, Ficker E (2009) Intracellular potassium stabilizes human *ether-à-go-go*-related gene channels for export from endoplasmic reticulum. Mol Pharmacol 75: 927 – 937.

Wang Q, Ohno S, Ding W-G, Fukuyama M, Miyamoto A, Itoh H, Makiyama T, Wu J, Bai J, Hasegawa K, Shinohara T, Takahashi N, Shimizu A, Matsuura H, Horie M (2014) Gain-of-

function *KCNH2* mutations in patients with Brugada syndrome. J Cardiovasc Electrophysiol 25: 522 – 530.

Wang S, Liu S, Morales MJ, Strauss HC, Rasmusson RL (1997a) A quantitative analysis of the activation and inactivation kinetics of *HERG* expressed in *Xenopus* oocytes. J Physiol 502: 45 – 60.

Wang S, Morales MJ, Liu S, Strauss HC, Rasmusson RL (1997b) Modulation of HERG affinity for E-4031 by $[K^+]_o$ and C-type inactivation. FEBS Lett 417: 43 – 47.

Wang S, Xu D-J, Cai J-B, Huang Y-Z, Zou J-G, Cao K-J (2009) Rapid component I_{Kr} of cardiac delayed rectifier potassium currents in guinea-pig is inhibited by α_1-adrenoreceptor activation via protein kinase A and protein kinase C-dependent pathways. Eur J Pharmacol 608: 1 – 6.

Wang T, Hogan-Cann A, Kang Y, Cui Z, Guo J, Yang T, Lamothe SM, Li W, Ma A, Fisher JT, Zhang S (2014) Muscarinic receptor activation increases hERG channel expression through phosphorylation of ubiquitin ligase Nedd4-2. Mol Pharmacol 85: 877 – 886.

Wang X, Hockerman GH, Green HW, Babbs CF, Mohammad SI, Gerrard D, Latour MA, London B, Hannon KM, Pond AL (2006) Merg1a K^+ channel induces skeletal muscle atrophy by activating the ubiquitin proteasome pathway. FASEB J 20: 1531 – 1533.

Wang X, Xu R, Abernathey G, Taylor J, Alzghoul MB, Hannon K, Hockerman GH, Pond AL (2008) Kv11.1 channel subunit composition includes minK and varies developmentally in mouse cardiac muscle. Dev Dyn 237: 2430 – 2437.

Wang Y, Huang X, Zhou J, Yang X, Li D, Mao H, Sun HH, Liu N, Lian J (2012) Trafficking-deficient G572R-hERG and E637K-hERG activate stress and clearance pathways in endoplasmic reticulum. PLoS One 7: e29885.

Wang YH, Shi CX, Dong F, Sheng JW, Xu YF (2008) Inhibition of the rapid component of the delayed rectifier potassium current in ventricular myocytes by angiotensin II via the AT_1 receptor. Br J Pharmacol 154: 429 – 439.

Wang Z, Fermini B, Nattel S (1994) Rapid and slow components of delayed rectifier current in human atrial myocytes. Cardiovasc Res 28: 1540 – 1546.

Wang Z, Dou Y, Goodchild SJ, Es-Salah-Lamoureux Z, Fedida D (2013) Components of gating charge movement and S4 voltage-sensor exposure during activation of hERG channels. J Gen Physiol 141: 431 – 443.

Ward OC (1964) A new familial cardiac syndrome in children. J Ir Med Assoc 54: 103 – 106.

Warmke JE, Ganetzky B (1994) A family of potassium channel genes related to eag in *Drosophila* and mammals. Proc Natl Acad Sci USA 91: 3438 – 3442.

Watanabe H, Yang T, Stroud DM, Lowe JS, Harris L, Atack TC, Wang DW, Hipkens SB, Leake B, Hall L, Kupershmidt S, Chopra N, Magnuson MA, Tanabe N, Knollmann BC, George AL, Roden DM (2011) Striking in vivo phenotype of a disease-associated human *SCN5A* mutation producing minimal changes in vitro. Circulation 124: 1001 – 1011.

Weeke P, Mosley JD, Hanna D, Delaney JT, Shaffer C, Wells QS, Van Driest S, Karnes JH, Ingram C, Guo Y, Shyr Y, Norris K, Kannankeril PJ, Ramirez AH, Smith JD, Mardis ER, Nickerson D, Georger AL, Roden DM (2014) Exome sequencing implicates an increased burden of rare potassium channel variants in the risk of drug-induced long QT interval syndrome. J Am Coll Cardiol 63: 1430 – 1437.

Weeke P, Denny JC, Basterache L, Shaffer C, Bowton E, Ingram C, Darbar D, Roden DM (2015) Examining rare and low-frequency genetic variants previously associated with lone or familial forms of atrial fibrillation in an electronic medical record system: a cautionary note. Circ Cardiovasc Genet 8: 58 – 63.

Weerapura M, Nattel S, Chartier D, Caballero R, Hébert TE (2002) A comparison of currents carried by HERG, with and without coexpression of MiRP1, and the native rapid delayed rectifier current. J Physiol 540: 15 - 27.

Whicher JR, MacKinnon R (2016) Structure of the voltage-gated K^+ channel Eag1 reveals an alternative voltage sensing mechanism. Science 353: 664 – 669.

White EJ, Park SJ, Foster JA, Huizinga JD (2008) Ether-a-go-go-related gene 3 is the main candidate for the E-4031-sensitive potassium current in the pacemaker interstitial cells of Cajal. Am J Physiol Gastrointest Liver Physiol 295: G691 – G699.

Wible BA, Hawryluk P, Ficker E, Kuryshev YA, Kirsch G, Brown AM (2005) HERG-Lite: a novel comprehensive high-throughput screen for drug-induced hERG risk. J Pharmacol Toxicol Methods 52: 136 – 145.

Wilde AAM, Jongbloed RJE, Doevendans PA, Düren DR, Hauer RNW, van Langen IM, van Tintelen JP, Smeets HJM, Meyer H, Geelen JLM (1999) Auditory stimuli as a trigger for arrhythmic events differentiate HERG-related (LQTS2) patients from KvLQT1-related patients (LQTS1). J Am Coll Cardiol 33: 327 – 332.

Wilders R, Verkerk AO (2010) Role of the R1135H *KCNH2* mutation in Brugada syndrome. Int J Cardiol 144: 149 – 151.

Wimmers S, Wulfsen I, Bauer CK, Schwarz JR (2001) Erg1, erg2 and erg3 K channel subunits are able to form heteromultimers. Pflügers Arch 441: 450 - 455.

Wimmers S, Bauer CK, Schwarz JR (2002) Biophysical properties of heteromultimeric erg K^+ channels. Pflügers Arch 445: 423 - 430.

Windley MJ, Mann SA, Vandenberg JI, Hill AP (2016) Temperature effects on kinetics of $K_V11.1$ drug block have important consequences for *in silico* proarrhythmic risk prediction. Mol Pharmacol 90: 1 – 11.

Witchel HJ, Pabbathi VK, Hofmann G, Paul AA, Hancox JC (2002) Inhibitory actions of the selective serotonin re-uptake inhibitor citalopram on HERG and ventricular L-type calcium currents. FEBS Lett 512: 59 - 66.

Wolpert C, Schimpf R, Giustetto C, Antzelevitch C, Cordeiro J, Dumaine R, Brugada R, Hong K, Bauersfeld U, Gaita F, Borggrefe M (2005) Further insights into the effect of quinidine in short QT syndrome caused by a mutation in HERG. J Cardiovasc Electrophysiol 16: 54 – 58.

Wu S-N, Lo Y-K, Kuo BI-T, Chiang H-T (2001) Ceramide inhibits the inwardly rectifying potassium current in GH_3 lactotrophs. Endocrinology 142: 4785 – 4794.

Wu W, Gardner A, Sanguinetti MC (2014) Cooperative subunit interactions mediate fast C-type inactivation of hERG1 K^+ channels. J Physiol 592: 4465 – 4480.

Wu W, Gardner A, Sanguinetti MC (2015) Concatenated hERG1 tetramers reveal stoichiometry of altered channel gating by RPR-260243. Mol Pharmacol 87: 401 – 409.

Wulff H, Castle NA, Pardo LA (2009) Voltage-gated potassium channels as therapeutic targets. Nat Rev Drug Discov 8: 982 – 1001.

Wymore RS, Gintant GA, Wymore RT, Dixon JE, McKinnon D, Cohen IS (1997) Tissue and species distribution of mRNA for the I_{Kr}-like K^+ channel, *erg*. Circ Res 80: 261 – 268.

Wynia-Smith SL, Gillian-Daniel AL, Satyshur KA, Robertson GA (2008) hERG gating microdomains defined by S6 mutagenesis and molecular modeling. J Gen Physiol 132: 507 - 520.

Wysowski DK, Corken A, Gallo-Torres H, Talarico L, Rodriguez EM (2001) Postmarketing reports of QT prolongation and ventricular arrhythmia in association with cisapride and Food and Drug Administration regulatory actions. Am J Gastroenterol 96: 1698 - 1703.

Xiao L, Xiao J, Luo X, Lin H, Wang Z, Nattel S (2008) Feedback remodeling of cardiac potassium current expression: a novel mechanism for control of repolarization reserve. Circulation 118: 983 – 992.

Xie Y, Sato D, Garfinkel A, Qu Z, Weiss JN (2010) So little source, so much sink: requirements for afterdepolarizations to propagate in tissue. Biophys J 99: 1408 – 1415.

Yamashita M, Oki Y, Iino K, Hayashi C, Matsushita F, Faje A, Nakamura H (2009) The role of ether-a-go-go-related gene K^+ channels in glucocorticoid inhibition of adrenocorticotropin release by rat pituitary cells. Regul Pept 152: 73 – 78.

Yang B-F, Xu D-H, Xu C-Q, Li Z, Du Z-M, Wang H-Z, Dong D-L (2004) Inactivation gating determines drug potency: a common mechanism for drug blockade of HERG channels. Acta Pharmacol Sin 25: 554 – 560.

Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, Hohnloser SH, Shimizu W, Schwartz PJ, Stanton M, Murray KT, Norris K, George AL, Roden DM (2002) Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. Circulation 105: 1943 – 1948.

Yang T, Chun YW, Stroud DM, Mosley JD, Knollmann BC, Hong C, Roden DM (2014) Screening for acute I_{Kr} block is insufficient to detect torsades de pointes liability: role of late sodium current. Circulation 130: 224 – 234.

Yang Y, Xia M, Jin Q, Bendahhou S, Shi J, Chen Y, Liang B, Lin J, Liu B, Zhou Q, Zhang D, Wang R, Ma N, Su X, Niu K, Pei Y, Xu W, Chen Z, Wan H, Cui J, Barhanin J, Chen Y (2004) Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. Am J Hum Genet 75: 899 – 905.

Yap YG, Camm AJ (2003) Drug induced QT prolongation and torsades de pointes. Heart 89: 1363 – 1372.

Yeh ETH, Bickford CL (2009) Cardiovascular complications of cancer therapy. J Am Coll Cardiol 53: 2231 – 2247.

Yellen G (2002) The voltage-gated potassium channels and their relatives. Nature 419: 35 - 42.

Yeung SYM, Greenwood IA (2007) Pharmacological and biophysical isolation of K^+ currents encoded by *ether-à-go-go*-related genes in murine hepatic portal vein smooth muscle cells. Am J Physiol Cell Physiol 292: C468 – C476.

Yiannakou Y, Piessevaux H, Bouchoucha M, Schiefke I, Filip R, Gabalec L, Dina I, Stephenson D, Kerstens R, Etherson K, Levine A (2015) A randomized, double-blind, placebo-controlled, phase 3 trial to evaluate the efficacy, safety, and tolerability of prucalopride in men with chronic constipation. Am J Gastroenterol 110: 741 – 748.

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318: 1917 – 1920.

Yu Z, Liu J, van Veldhoven JPD, Ijzerman A, Schalij MJ, Pijnappels DA, Heitman LH, de Vries AAF (2016) Allosteric modulation of Kv11.1 (hERG) channels protects against drug-induced ventricular arrhythmias. Circ Arrhythm Electrophysiol 9: e003439.

Yue Y, Castrichini M, Srivastava U, Fabris F, Shah K, Li Z, Qu Y, El-Sherif N, Zhou Z, January C, Hussain MM, Jiang X-C, Sobie EA, Wahren-Herlenius M, Chahine M, Capecchi P-L, Laghi-Pasini F, Lazzerini P-E, Boutjdir M (2015) Pathogenesis of the novel autoimmune-associated long-QT syndrome. Circulation 132: 230 – 240.

Zamorano-León JJ, Yañez R, Jaime G, Rodriguez-Sierra P, Calatrava-Ledrado L, Alvarez-Granada RR, Mateos-Cáceres PJ, Macaya C, López-Farré AJ (2012) KCNH2 gene mutation: a potential link between epilepsy and long QT-2 syndrome. J Neurogenet 26: 382 - 386.

Zankov DP, Yoshida H, Tsuji K, Toyoda F, Ding W-G, Matsuura H, Horie M (2009) Adrenergic regulation of the rapid component of delayed rectifier K^+ current: implications for arrhythmogenesis in LQT2 patients. Heart Rhythm 6: 1038 – 1046.

Zarraga IG, Zhang L, Stump MR, Gong Q, Vincent GM, Zhou Z (2011) Nonsense-mediated mRNA decay caused by a frameshift mutation in a large kindred of type 2 long QT syndrome. Heart Rhythm 8: 1200 - 1206.

Zeltser D, Justo D, Halkin A, Prokhorov V, Heller K, Viskin S (2003) Torsade de pointes due to noncardiac drugs: most patients have easily identifiable risk factors. Medicine 82: 282 – 290.

Zhang H, Zou B, Yu H, Moretti A, Wang X, Yan W, Babcock JJ, Bellin M, McManus OB, Tomaselli G, Nan F, Laugwitz KL, Li M (2012) Modulation of hERG potassium channel gating normalizes action potential duration prolonged by dysfunctional KCNQ1 potassium channel. Proc Natl Acad Sci USA 109: 11866 – 11871.

Zhang L, Vincent GM, Baralle M, Baralle FE, Anson BD, Benson DW, Whiting B, Timothy KW, Carlquist J, January CT, Keating MT, Splawski I (2004) An intronic mutation causes long QT syndrome. J Am Coll Cardiol 44: 1283 – 1291.

Zhang M, Liu J, Tseng G-N (2004) Gating charges in the activation and inactivation processes of the hERG channel. J Gen Physiol 124: 703 - 718.

Zhang M, Liu J, Jiang M, Wu D-M, Sonawane K, Guy HR, Tseng G-N (2005) Interactions between charged residues in the transmembrane segments of the voltage-sensing domain in the hERG channel. J Membr Biol 207: 169 - 181.

Zhang R, Tian P, Chi Q, Wang J, Wang Y, Sun L, Liu Y, Tian S, Zhang Q (2012) Human ether- à-go-go-related gene expression is essential for cisplatin to induce apoptosis in human gastric cancer. Oncol Rep 27: 433 – 440.

Zhang S (2006) Isolation and characterization of I_{Kr} in cardiac myocytes by Cs^+ permeation. Am J Physiol Heart Circ Physiol 290: H1038 – H1049.

Zhang S, Zhou Z, Gong Q, Makielski JC, January CT (1999) Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. Circ Res 84: 989 – 998.

Zhang S, Rajamani S, Chen Y, Gong Q, Rong Y, Zhou Z, Ruoho A, January CT (2001) Cocaine blocks HERG, but not K_VLQT1+minK, potassium channels. Mol Pharmacol 59: 1069 – 1076.

Zhao JT, Hill AP, Varghese A, Cooper AA, Swan H, Laitinen-Forsblom PJ, Rees MI, Skinner JR, Campbell TJ, Vandenberg JI (2009) Not all hERG pore domain mutations have a severe phenotype. J Cardiovasc Electrophysiol 20: 923 – 930.

Zheng F, Li H, Du W, Huang S (2011) Role of hERG1 K^+ channels in leukemia cells as a positive regulator in SDF-1a-induced proliferation. Hematology 16: 177 – 184.

Zheng F, Li J, Du W, Wang N, Li H, Huang S (2012a) Human ether-a-go-go-related gene K^+ channels regulate shedding of leukemia cell-derived microvesicles. Leuk Lymphoma 53: 1592 – 1598.

Zheng F, Li H, Liang K, Du Y, Guo D, Huang S (2012b) Imatinib has the potential to exert its antileukemia effects by down-regulating hERG1 K^+ channels chronic myelogenous leukemia. Med Oncol 29: 2127 – 2135.

Zhou W, Cayabyab FS, Pennefather PS, Schlichter LC, DeCoursey TE. (1998) HERG-like K^+ channels in microglia. J Gen Physiol 111: 781 – 794.

Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R (2001) Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0Å resolution. Nature 414: 43 - 48.

Zhou Z, Gong Q, Ye B, Fan Z, Makielski JC, Robertson GA, January CT (1998a) Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. Biophys J 74: 230 - 241.

Zhou Z, Gong Q, Epstein ML, January CT (1998b) HERG channel dysfunction in human long QT syndrome: Intracellular transport and functional defects. J Biol Chem 273: 21061-21066.

Zhou Z, Gong Q, January CT (1999) Correction of defective protein trafficking of a mutant HERG potassium channel in human long QT syndrome: Pharmacological and temperature effects. J Biol Chem 274: 31123-31126.

Zicha S, Moss I, Allen B, Varró A, Papp J, Dumaine R, Antzelevitch C, Nattel S (2003) Molecular basis of species-specific expression of repolarizing K^+ currents in heart. Am J Physiol Heart Circ Physiol 285: H1641 – H1649.

Zou A, Curran ME, Keating MT, Sanguinetti MC (1997) Single HERG delayed rectifier K^+ channels expressed in Xenopus oocytes. Am J Physiol Heart Circ Physiol 272: H1309 – H1314.

Zou A, Lin Z, Humble M, Creech CD, Wagoner PK, Krafte D, Jegla TJ, Wickenden AD (2003) Distribution and functional properties of human KCNH8 (Elk1) potassium channels. Am J Physiol Cell Physiol 285: C1356 - C1366.

Ördög B, Brutyó E, Puskás LG, Papp JG, Varró A, Szabad J, Boldogkoi Z (2006) Gene expression profiling of human cardiac potassium and sodium channels. Int J Cardiol 111: 386 – 393.