From Genome-Wide Association Studies to Cardiac Electrophysiology: Through the Maze of Biological Complexity

Koen T. Scholman¹, Veronique M. F. Meijborg²,³, Carolina Gálvez-Montón⁴,⁵, Elisabeth M. Lodder² and Bastiaan J. Boukens¹,²*

¹ Department of Medical Biology, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands, ² Department of Experimental Cardiology, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands, ³ Netherlands Heart Institute, Utrecht, Netherlands, ⁴ ICREC Research Program, Germans Trias i Pujol Health Science Research Institute, Badalona, Spain, ⁵ CIBERCV, Instituto de Salud Carlos III, Madrid, Spain

Genome Wide Association Studies (GWAS) have provided an enormous amount of data on genomic loci associated with cardiac electrophysiology and arrhythmias. Clinical relevance, however, remains unclear since GWAS do not provide a mechanistic explanation for this association. Determining the electrophysiological relevance of variants for arrhythmias would aid development of risk stratification models for patients with arrhythmias. In this review, we give an overview of genetic variants related to ECG intervals and arrhythmogenic pathologies and discuss how these variants may influence cardiac electrophysiology and the occurrence of arrhythmias.

Keywords: GWAS, cardiac electrophysiology, arrhythmias, gene expression, genetics

INTRODUCTION

Genome Wide Association Studies (GWAS) can identify genetic variants associated with phenotypic traits, such as electrocardiographic (ECG) intervals (Table 1). Interpretation of GWAS data relies on identification of the target gene affected by the novel discovered variant. Variants can be present in coding DNA, causing amino acid changes affecting protein function – or in non-coding DNA, altering the behavior of regulatory elements, thereby changing expression levels of its target gene(s) (Figure 1A). For many highly significant GWAS loci the gene causing the association with ECG intervals has not been identified yet. This is mostly because genes in or near these loci do not have a proven association with the phenotype. Overlaying such loci with publicly available genome wide data sets of gene expression (GTEx Consortium, 2013; Heinig, 2018), cardiac transcriptional elements (van Duijvenboden et al., 2016), genomic conformation Hi-C data sets (Montefiori et al., 2018), and other GWAS results will likely provide more insight into potential novel candidate genes regulating cardiac electrophysiology (van Ouwerkerk et al., 2019).

Upon discovery, the expression of the target gene is often modified in isolated cardiomyocytes (from an animal model or human induced pluripotent stem cells). These cellular models can be used to assess any electrophysiological phenotypes associated with the potential GWAS candidate. However, ECG intervals reflect the complex electrophysiological interaction of the cardiac cells and tissue structures, complicating extrapolation of single cell measurements to ECG intervals (Opthof et al., 1987). Additionally, discovered variants may affect expression of genes in non-cardiomyocytes, further complicating the interpretation of such experiments in isolated cardiomyocytes (Stroud et al., 2016; Veerman et al., 2016).
To overcome these limitations, *in vivo* models such as transgenic mice are most commonly used to investigate the relation between GWAS variants and electrophysiological phenotypes. However, large electrophysiological differences exist between mice and larger mammals such as human (Boukens et al., 2014), which need to be taken into account when interpreting the resulting data.

In this review, we give an overview of genomic loci related to abnormal cardiac electrophysiology and discuss how these variants may directly or indirectly influence ECG intervals and the occurrence of arrhythmias through their expression pattern and regulation within the heart.

**GWAS LOCI RELATED TO HEART RATE**

The sinus node is located at the border between intercaval area and the right atrium. Action potentials of sinus node cardiomyocytes have low upstroke velocities and occur spontaneously (West, 1955). The mechanism underlying these spontaneous depolarizations is based on the funny current, mediated by the Hyperpolarization-Activated Cyclic Nucleotide-gated channel 4 (*HCN4*), and the calcium clock mediated by e.g., Ryanodine Receptor 2 (*RYR2*) and Phospholamban (*PLN*) (Lakatta and DiFrancesco, 2009). Accordingly, associations between the genetic loci of *HCN4* and *PLN* and heart rate found by GWAS is most likely mediated through variants impacting on the expression and or function of these genes (Eijgelsheim et al., 2010; den Hoed et al., 2013; Nolte et al., 2017). Unfortunately, for the other GWAS loci, the association is not as straightforward. In the proximity of these loci, no genes with a known function in the SAN have been identified. These loci therefore potentially affect SAN function by impacting on other parameters, e.g., electrical coupling with the rest of the atrium and levels of fibrosis (Glukhov et al., 2013).

In smaller mammals – e.g., mice and rabbits – the sinus node is transmural (Bleeker et al., 1980; van Eif et al., 2019), whereas in large mammals – e.g., humans and dogs – it is not (Fedorov et al., 2009, 2010). The geometry of the sinus node affects coupling with atrial myocardium, which influences its electrophysiological behavior (Kirchhof et al., 1987). The delicate interaction between the sinus node and the surrounding atrial myocardium is established by fibrous tissue providing up to five exit pathways in large mammals (Figure 1B; Unudurthi et al., 2014; Csépe et al., 2015; Li et al., 2017). A transitional layer of cells within the exit pathways (Figure 1B) allows for spontaneous depolarization within the sinus node (Joyner and van Capelle, 1986; Kléber and Rudy, 2004). The identity of the transitional cells may depend on the expression of transcription factor Nkx2-5 and GWAS has found variants near NKX2-5 associating with heart rate (Mommersteeg et al., 2007b; den Hoed et al., 2013; Li et al., 2019). Within the exit pathways, high conductance connexins CX43 and CX40 (*GJA1* and *GJA5*) are lower expressed toward the sinus node, whereas the low conductance CX45 is expressed higher toward the sinus node (Chandler et al., 2009, 2011; Allah et al., 2011). This gradual increase of conductance

---

**Table 1** | Level of functional studies for GWAS loci associated with ECG intervals.

| Associated gene | G | C | T |
|-----------------|---|---|---|
| AF              |   |   |   |
| AGBL4           | 1 |   |   |
| AKAP6           | 1 |   |   |
| AHGAP10         | 1 |   |   |
| AHGAP26/NR3C1   | 1 |   |   |
| ASAH1           | 1 |   |   |
| ATXN1           | 1 | 6 |   |
| BEST3           | 1 |   |   |
| C10orf76        | 1 |   |   |
| C2orf68         | 1 |   |   |
| Cioorf7 NDUDT3  | 1 |   |   |
| CSnorf3         | 1 |   |   |
| CAND2           | 1 | 13|   |
| CASC20/BMP2     | 1 | 14|   |
| CASZ1           | 1 |   |   |
| CDK6            | 1 |   |   |
| CEP68           | 1 |   |   |
| CGA/ZNF292      | 1 |   |   |
| COG5            | 1 |   |   |
| CREB5           | 1 |   |   |
| CUL4A           | 1 |   |   |
| CYTH1           | 1 |   |   |
| DGK8            | 1 |   |   |
| DNAH10          | 1 |   |   |
| DPF3            | 1 |   |   |
| EPHA3           | 1 |   |   |
| ERBB4           | 1 | 25| 26|
| FBN2/SLC27A6    | 1 |   |   |
| FBRSL1          | 1 |   |   |
| FBX032          | 1 |   |   |
| GCOM1           | 1 |   |   |
| GJA5            | 1 | 32| 32|
| GOPC            | 1 |   |   |
| GORAB/PRRX1     | 1 | 35|   |
| GTF2I           | 1 |   |   |
| GYPC            | 1 |   |   |
| HAND2           | 1 |   |   |
| HIP1R           | 1 |   |   |
| HSPG2/CELA3B    | 1 |   |   |
| IGF1R           | 1 | 38| 39|
| KND3            | 1 | 40|   |
| KONJ5           | 1 | 45| 46|
| KONN2           | 1 | 47| 48|
| KONN3/PMVK      | 1 | 50| 51|
| KDM1B           | 1 |   |   |
| KIF3C           | 1 |   |   |
| KRR1/PHLDA1     | 1 |   |   |
| LHX3            | 1 |   |   |
| LINC00208/GATA4 | 1 |   |   |
| LINC00326/EYA4  | 1 |   |   |
| LINC00540/BASP1P1 | 1 |   |   |
| LINC00927/ARNT2 | 1 |   |   |

(Continued)
| Associated gene | G | C | T |
|-----------------|---|---|---|
| LOC100506385    | 1 |   |   |
| LOC102467213/EFNA5 | 1 |   |   |
| LRRC74/LRF2BPL  | 1 |   |   |
| MAPT            | 1 |   | 61|
| MBD5            | 1 |   |   |
| METTL1B/LINC01142 | 1 |   |   |
| MEX3C           | 1 |   |   |
| MIR30B          | 1 |   |   |
| MTSS1/LINC00964 | 1 |   |   |
| MY018B          | 1 |   |   |
| NACA            | 1 |   |   |
| NEURL           | 1 |   | 13|
| NUCKS1          | 1 |   |   |
| OPN1SW          | 1 |   |   |
| PAK2            | 1 |   |   |
| PTX2/C4orf32    | 1 |   | 69|
| PKP2            | 1 |   | 70|
| POLR2A/TNFSF12  | 1 |   |   |
| PPFA4           | 1 |   |   |
| PPP2R3A         | 1 |   |   |
| PRDM8/FGF5      | 1 |   |   |
| PSMB7           | 1 |   |   |
| PTEN            | 1 |   |   |
| RBM20           | 1 |   | 81|
| REEP1/KDM3A     | 1 |   |   |
| REEP3           | 1 |   |   |
| RPS2            | 1 |   |   |
| SCM/H1          | 1 |   |   |
| SIRT1           | 1 |   | 2 |
| SLC2A2/MLLT3    | 1 |   |   |
| SLC9B1          | 1 |   |   |
| SLIT3           | 1 |   |   |
| SMAD7           | 1 |   |   |
| SNRNP27         | 1 |   |   |
| SNX6/CFL2       | 1 |   |   |
| SORL1/MIR100HG  | 1 |   |   |
| SSPN            | 1 |   |   |
| SUN1            | 1 |   |   |
| SYNE2           | 1 |   |   |
| SYNP02L         | 1 |   |   |
| TEX41           | 1 |   |   |
| THRB            | 1 |   |   |
| TUBA8           | 1 |   |   |
|UBE4B            | 1 |   |   |
| USP3            | 1 |   |   |
| UST             | 1 |   |   |
| WDR1            | 1 |   | 15|
| WIPF1/CHRNA1    | 1 |   |   |
| WNT7A/NME5      | 1 |   |   |
| XPO1            | 1 |   |   |
| XP07            | 1 |   |   |
| XXYL1T1         | 1 |   |   |
| YWHAECR6       | 1 |   |   |

(Continued)
| Associated gene | G | C | T |
|----------------|---|---|---|
| DKK1           | 7 | 83|   |
| HAND1/SAP3OL   | 7 |   |   |
| HEATR5B/STRN   | 7 |   |   |
| IGFBP3         | 4 |   |   |
| NFIA           | 4 |   |   |
| SETBP1         | 4 |   |   |
| TKT/PRKCD/CACNA1D | 4 | 5 | 5 |
| VT1A           | 4 |   |   |
| QT             |    |   |   |
| ANKR9D9        | 7 |   |   |
| ATP1B1         | 7 | 8 |   |
| ATP2A2         | 7 | 9 | 10|
| AZIN1          | 7 |   |   |
| C30RF75        | 7 |   |   |
| c6orf204       | 7 |   |   |
| CNOT1          | 7,11| 12 |   |
| CREBBP         | 7 |   |   |
| FEN1/FADS2     | 7 |   |   |
| GBF1           | 7 |   |   |
| GFRA3          | 7 |   |   |
| GMRPR          | 7 |   |   |
| KCNE1          | 7,11| 16 | 17|
| KCNQ1          | 7,11| 18 | 19|
| LAPTMB4        | 7 |   |   |
| LG3            | 7,11|   |   |
| LITAF          | 7,11|   |   |
| MKL2           | 7 |   |   |
| NCOA2          | 7 |   |   |
| NOS1AP         | 7,11| 22 | 23|
| RN2F07         | 7,11| 24 | 24|
| SLC4A4         | 7 | 27 |   |
| SLC8A1         | 7 | 28 | 28|
| SMARCAD1       | 7 |   |   |
| SP3            | 7 |   |   |
| TCEA3          | 7 |   |   |
| USP50/TRPM7    | 7 | 33 |   |
| AF+PR          |    |   |   |
| CAMK2D         | 1,29| 36 | 36|
| FRMD4B         | 1,29|   |   |
| MYOCD          | 1,29|   |   |
| NAV2           | 1,29|   |   |
| PHLD22         | 1,29|   |   |
| TLE3/UCAC      | 1,29|   |   |
| AF+HR          |    |   |   |
| GJA1           | 1,41,42| 43 | 44|
| GNB4           | 1,41|   |   |
| MYH7           | 1,42| 49 |   |
| AF+QT          |    |   |   |
| KCN2H           | 1,7,11| 53 | 54|
| KCN2J/CASC17    | 1,7 | 55 | 56|
| SPATS2L         | 1,7 |   |   |
| AF+QRS         |    |   |   |
| C1orf85/RNF11/DKN2C/FAF1 | 1,4 |   |   |

(Continued)
from sinus node toward atrial myocardium enables a proper current-to-load match. Therefore, genes involved in sinus node insulation and exit pathway formation are candidates for being associated with sinus node function (or heart rate) in GWAS. Accordingly, a locus near GJA1 is associated with heart rate in GWAS (den Hoed et al., 2013).

The electrotonic influence of atrial myocardium on sinus node function is important to consider when investigating the relevance of genes provided by GWAS. For instance, common and rare variants in MYH6 – a component of the sarcomere – are associated with heart rate and sick sinus syndrome (Holm et al., 2010, 2011). However, MYH6 is more abundantly expressed in the atrial myocardium than in the sinus node (van Eif et al., 2019). In vitro experiments in atrial-like cells indicate that mutations in MYH6 affect conduction velocity (Ishikawa et al., 2015). It is possible that conduction slowing in the atrium underlies the association between MYH6 and abnormal function of the sinus node. That genes expressed in the atrium but not in the sinus node can affect sinus is further illustrated by Cx40 knock-out mice in which the dominant pacemaker is not always the SAN (Bagwe et al., 2005). Moreover, mutations in SCN5A can result in sick sinus syndrome (Benson et al., 2003) despite the lack of SCN5A expression in the sinus node.

GWAS LOCI RELATED TO PR INTERVAL

The PR interval is mainly determined by conduction through the atrioventricular junction (Meijler and Janse, 1988). The atrioventricular junction is a complex anatomical structure which was first described in 1906 by Sunao Tawara, who found cells forming a compact complex network but also small cells that joined into bundles (Tawara, 1906). These cells were later called compact nodal cells and transitional cells, respectively, and both have distinct electrophysiological properties (de Carvalho and de Almeida, 1960; Greener et al., 2011). A layer of transitional cells can be found in rings around the orifices of the AV valves where expression of Cx40 and SCN5A is low (Aanhaanen et al., 2010; Fedorov et al., 2011). This transitional ring lies on top of an atrioventricular nodal ring providing two conducting pathways with different electrophysiological characteristics both connecting the atria with the His bundle (Denes et al., 1973; Figure 1B). The presence of two conduction pathways illustrates the challenge of relating gene expression of single atrioventricular nodal cells to the electrophysiological phenotype of the atrioventricular node. Moreover, in the atrioventricular junction, not only the compact atrioventricular node contributes to atrioventricular delay but also all other cells present within the AV junction – e.g., transitional cells, fibroblasts and macrophages (Hulsmans et al., 2017). Similar to the sinus node, the function of the atrioventricular node depends on the interaction with these different cell-types.

The cells of the atrioventricular junction find their origin in the atrioventricular canal of the embryonic heart. Impulse conduction delay occurs in both the transitional cells and the compact atrioventricular node, therefore, normal development of the atrioventricular canal is crucial for atrioventricular delay in adult hearts (Meijler and Janse, 1988). Accordingly, GWAS for PR interval showed association with 18 of 44 loci which are related to heart development (van Setten et al., 2018), indicating that proper embryonic development is a crucial factor for adult AV conduction. Some of these loci are located near TBX2 and TBX3 – essential transcription factors controlling patterning of the atrioventricular canal during development (Holm et al., 2010; Pfeufer et al., 2010; Aanhaanen et al., 2011; van Setten et al., 2018; Table 1). Other loci are located nearby genes related to electrical function of the adult cardiomyocyte, like SCN5A and CAMK2D or cardiomyocyte contraction, like TTN and MYH6, however, the significance of these associations with PR interval awaits further investigation (Holm et al., 2010; van Setten et al., 2018).

GWAS LOCI RELATED TO QRS DURATION

Total ventricular activation time is visualized on the ECG as the duration of the QRS complex, which comprises conduction in the His-Purkinje system and in the ventricular myocardium. The cardiac sodium channel Na$^+$ – encoded by SCN5A – is the major determinant of conduction in these tissues and, accordingly, QRS duration is associated with loci near SCN5A (Papadatos et al., 2002; Sotoodehnia et al., 2010). Variants in the SCN10A gene – encoding the neuronal sodium channel Na$^+$ – associate with QRS duration as well (Sotoodehnia et al., 2010). These variants are located within an enhancer region that modulates expression of SCN5A which could explain a relation with QRS duration (Sotoodehnia et al., 2010; van den Boogaard et al., 2014). Although variants in these region do not always associate with QRS prolongation (Behr et al., 2015). Other variants related to QRS duration are those near or within genes involved in bundle branch development and working myocardial phenotype, like TBX3, TBX5, TBX20, HAND1, DKK1, and NFIA (Moskowitz et al., 2007; Bakker et al., 2008; Singh et al., 2009; Sotoodehnia et al., 2010). In addition variants in calcium handling genes such as PLN, CACNA1D, STRN, PRKCA, and CASQ2, ATP2A2/ANAPC7 are associated with QRS duration (Sotoodehnia et al., 2010; van Setten et al., 2019). These variants could affect calcium homeostasis resulting in reduced sodium current and slow conduction and thereby prolong QRS duration (King et al., 2013).

The Purkinje network activates the ventricular myocardium via Purkinje muscle junctions composed of transitional cells connecting the Purkinje fibers to ventricular cardiomyocytes (Figure 1B; Martinez-Palomo et al., 1970; Tranum-Jensen et al., 1991). Similar to the AV node and the SAN, the connection of Purkinje cardiomyocytes with ventricular cardiomyocytes requires high resistance – preventing current-to-load mismatch (Rohr et al., 1997). We expect that reduction of electrical coupling in these junctions – by e.g., lower expression of CX40 – will delay ventricular activation, which prolongs QRS duration. Purkinje fibers develop from embryonic trabeculae (Jensen et al., 2012) where e.g., Cx40, Scn5a, Ctnm2 are abundantly present. The primordial Purkinje trabeculae require further specialization
FIGURE 1 | In GWAS, (A) SNPs are associated with ECG intervals, like PR interval, QRS complex, and QT interval. To understand physiological relevance of a SNP three biological levels of organization should be studied (1) Chromatin level: Determine which gene(s) is affected by the SNP. The SNP (red mark) can be located in non-coding or coding DNA. For SNPs in non-coding DNA, it is important to determine which coding genes are affected. (2) Cell level: Determine in which cell types the gene is expressed. The cell type in which the affected gene is expressed is relevant for cellular function of the gene. (3) Tissue level: Determine how gene expression in a cell type affects tissue electrophysiology. Tissue electrophysiology can be related to ECG intervals. (B) Tissue structure is essential for proper conduction. The pulmonary vein has an outer sleeve of cardiomyocytes. These pulmonary vein cardiomyocytes are not as densely packed as the atrial cardiomyocytes – resulting in less coupling. Small reentry circuits or ectopic activity can therefore occur in pulmonary vein myocardium. The sinus node – where it is not insulated by fibrosis – connects with the atrial cardiomyocytes through transitional cells. In the atrioventricular junction, AV nodal cells are electrically connected with the atrial myocardium through a layer of transitional cells. In the Purkinje muscle junction, Purkinje cells connect with ventricular cardiomyocytes through transitional cells. AV, atrioventricular; IVC, inferior vena cava; SNP, Single nucleotide polymorphism; SVC, superior vena cava.
after birth under influence of Nkx2-5 and Irx3 expression and Notch signaling (Zhang et al., 2011; Rentschler et al., 2012). Homozygous Irx3 loss-of-function mice have slowed conduction in Purkinje fibers (Koizumi et al., 2016). We expect that genetic variations affecting expression of these factors – that are involved in development and maturation of the Purkinje fibers – will relate to total ventricular activation time and thereby QRS duration in GWAS.

GWAS LOCI RELATED TO QT INTERVAL

The QT interval is a measure of ventricular repolarization and reflects the time between the first moment of activation to the last moment of repolarization. Accordingly, the QT interval relates to action potential duration (APD) but also to differences in regional conduction velocity. Conduction slowing in regions that repolarize late prolongs QT interval whereas conduction slowing in areas that repolarize early may not affect QT interval. The QT interval has an inverse relation with heart rate. This inverse relation results from shortening of the APD at higher rates due to activation of the slowly delayed rectifier current 1Ks (Boyett and Jewell, 1978; Carmeliet, 2006). QT intervals corrected for heart rate – QTc – or QT interval measured at similar heart rates may increase sensitivity for finding variants related to repolarization (Bazett, 1920). In mice, however, QT interval does not depend on heart rate. Therefore, QT interval – not QTc – should be used as measure for ventricular repolarization in mice (Speerschneider and Thomsen, 2013).

Genome Wide Association Studies for QT interval identified 22 loci (Table 1) of which many are in or near genes encoding for potassium channels or involved in calcium handling (Arking et al., 2014). Increased potassium current shortens QT interval whereas increased calcium current prolongs QT interval (Wemhöner et al., 2015; Landstrom et al., 2016). Nitric Oxide Synthase 1 Adaptor Protein (NOS1AP) – regulating calcium current – is associated with QT interval in GWAS (Arking et al., 2006, 2014). Genetic variation within the NOS1AP gene affects QT interval and is related to arrhythmogenesis in patients with the long QT syndrome (Crotti et al., 2009; Tomás et al., 2010). The effect of NOS1AP on QT interval, however, could also have an extracardiac pathway as its expression is high in brain (Xu et al., 2005) providing the possibility of NOS1AP to affect autonomic modulation of the QT interval (Yagishita et al., 2015).

GWAS LOCI RELATED TO ARRHYTHMIAS

Atrial Arrhythmias

Atrial fibrillation (AF) is the most common arrhythmia and the prevalence increases with age (Krijthe et al., 2013). AF results from an interplay between electrical (triggers and reentry), structural and hemodynamic remodeling (Schotten et al., 2011). The combination of these pathophysiological changes sets the stage for AF. The trigger for AF is commonly near the connection between the left atrium and pulmonary veins (PVs) (Haïssaguerre et al., 1998). In humans – at this connection – the four PVs are enclosed by an outer sleeve of myocardium (Saito et al., 2000). PV cardiomyocytes are morphologically similar to atrial cardiomyocytes, but have a different developmental history (Verheule et al., 2002; Mommersteeg et al., 2007a). The formation of PV myocardium in mice highly depends on expression of Pitx2 and Nkx2-5 during development (Mommersteeg et al., 2007a). GWAS in more than 65 thousand AF patients identified 97 loci (Roselli et al., 2018) – including PITX2 and NKX2-5, suggesting a relation of AF with PV formation during development. The myocardial sleeves of the PVs are thinner distal to the left atrium and end in single cardiomyocyte protrusions in the PV (Figure 1B; Verheule et al., 2002). These PV myocytes have a high resting membrane potential and low expression of inward rectifier channels (Ehrlich et al., 2003; Melnyk et al., 2005), setting the stage for spontaneous activity. Accordingly, variants near genes encoding inward rectifier channels – e.g., KCNJ2, KCNJ5 – are associated with AF (Christophersen et al., 2017; Roselli et al., 2018).

Electrical remodeling is a cause and effect of reentry circuits that maintain AF. Reentry is facilitated by slow conduction and short APD (Wiener and Rosenblueth, 1946). Accordingly, GWAS for AF identified six genes encoding for potassium channels all involved in atrial APD: KCNJD3, KCNH2, KCNN2, and KCNN3 (Roselli et al., 2018). Genes encoding channels involved in conduction – e.g., GJA1, GJA5, SCN5A, and SCN10A are also associated with AF (Christophersen et al., 2017; Roselli et al., 2018). Moreover, transcription factors involved in spatiotemporal expression of these ion channels – TBX3, TBX5, and PITX2 – associate with AF as well (Tao et al., 2014; Nadadur et al., 2016). Novel findings have indicated that not only genes encoding ion channel are related to AF. Titin (TTN) – a large sarcomere protein – is associated with early onset AF (Choi et al., 2018). TTN dysfunction may predispose to AF by increasing myocardial fibrosis and prolonging PR interval, which are both associated with increased risk for AF (Ahlberg et al., 2018).

Ventricular Arrhythmias

Up to 80% of sudden cardiac arrests (SCA) are caused by acute ischemia resulting from coronary artery disease (Huikuri et al., 2001; Fishman et al., 2010). GWAS in patients with coronary artery disease identified 11 variants related to SCA of which eight were near or within genes related to long QT syndrome (Marsman et al., 2001; Fishman et al., 2010). GWAS in patients with coronary artery disease identified 11 variants related to SCA of which eight were near or within genes related to long QT syndrome (Marsman et al., 2001). It is unclear whether these variants predispose to arrhythmias in general or only in the setting of coronary artery disease. A gene that does specifically relate to arrhythmias in the setting of acute myocardial ischemia is CXADR which encodes the CXADR Ig-like cell adhesion molecule (previously named Coxsockie and adenovirus receptor, CAR) (Bezzina et al., 2010). Reduced expression of CAR lowers sodium channel availability – thereby reducing conduction velocity – and facilitates reentry arrhythmias in the setting of ischemia (Marsman et al., 2014). Non-ischemia induced arrhythmias explain 20% of SCA and comprise a variety of arrhythmogenic syndromes which can be related to structural
abnormal myocardium or genetic mutations (John et al., 2012). GWAS for SCA identified several genes which all associate with risk factors for SCA such as QRS duration and QT interval (Adabag et al., 2010; Arking et al., 2011; Milano et al., 2016; Ashar et al., 2018).

Genome Wide Association Studies for syncope – which is a common symptom of many arrhythmogenic syndromes – identified a genetic variant close in proximity to the gene zinc finger protein 804a (ZNF804A) (Hadj-Turdeghal et al., 2020). Whether a role of ZNF804A in cardiac arrhythmias or e.g., blood pressure regulation explains this association remains to be investigated.

Ventricular fibrillation is associated with IRX3 (Koizumi et al., 2016), which plays a role in conduction in Purkinje fibers. Ablation of Purkinje muscle junctions is a successful treatment for a subset of patients with idiopathic ventricular fibrillation indicating a role of Purkinje cells in these arrhythmias (Haïssaguerre et al., 2002).

Understanding arrhythmogenic mechanisms can guide interpretation of GWAS derived data. This is exemplified by the Brugada syndrome, which is characterized by ST segment elevation in the right precordial leads and ventricular arrhythmias. Initially, the Brugada syndrome was considered an ion channel disease mainly resulting from dysfunction of the cardiac sodium channel. However, only 20% of patients present with mutations in SCN5A (Antzelevitch et al., 2005). A GWAS in Brugada syndrome patients identified several variants in regulatory DNA controlling SCN5A expression (Bezzina et al., 2013). In order to discover causal variants in GWAS with limited number of patients, knowledge on the arrhythmogenic mechanism of the disease is helpful. Mechanistic studies in hearts from Brugada syndrome patients have suggested that arrhythmias occur due to conduction block in the presence of subtle structural abnormalities (Coronel et al., 2005; Hoogendijk et al., 2010). This suggests that variants near genes involved in the formation of fibrosis (TGFB2) or genes affecting safety factor of cardiac conduction (e.g., SCN5A, GJA1, KCHIP1) are important variants to further investigate.

CONCLUSION

Most of the variants provided by GWAS lie near or within expected candidate genes potentially explaining the phenotype they are associated with. However, the electrophysiological characterization of the vast majority of associated genes has not shown direct effects on ECG intervals nor on arrhythmia susceptibility. In this review, we emphasize that mechanistic knowledge of the structure-function relations underlying ECG intervals and arrhythmias should be considered when interpreting experimental characterization of these variants, in order to guide clinical applicability of GWAS data.

AUTHOR CONTRIBUTIONS

KS and BB designed and wrote the manuscript. VM, CG-M, and EL critically revised the manuscript.

FUNDING

This work was supported by the Dutch Heart Foundation (2016T047 to BB), the Dutch Research Council (NWO Talent Scheme VIDI-91718361 to EL), and the CVON (Dutch cardiovascular allowance RESCUED project to EL).

REFERENCES

Aanhaanen, W. T. J., Mommersteeg, M. T. M., Norden, J., Wakker, V., de Gier-de Vries, C., Anderson, R. H., et al. (2010). Developmental origin, growth, and three-dimensional architecture of the atrioventricular conduction axis of the mouse heart. Circ. Res. 107, 728–736. doi: 10.1161/CIRCRESAHA.110.222992

Aanhaanen, W. T. J. I., Boukens, B. J. D. D., Sizarov, A., Wakker, V., de Gier-de Vries, C., van Ginneken, A. C., et al. (2011). Defective Tbx2-dependent patterning of the atrioventricular canal myocardium causes accessory pathway formation in mice. J. Clin. Invest. 121, 534–544. doi: 10.1172/JCI44350

Adabag, A. S., Luepker, R. V., Roger, V. L., and Gersh, B. J. (2010). Sudden cardiac death: epidemiology and risk factors. Nat. Rev. Cardiol. 7, 216–225. doi: 10.1038/nrcardio.2010.3

Ahlberg, G., Refsgaard, L., Landeggaard, P. R., Andreassen, L., Ranthe, M. F., Linscheid, N., et al. (2018). Rare truncating variants in the sarcomeric protein titin associate with familial and early-onset atrial fibrillation. Nat. Commun. 9:4316. doi: 10.1038/s41467-018-06618-y

Allah, E. A., Tellez, J. O., Yanni, J., Nelson, T., Monfredi, O., Boyett, M. R., et al. (2011). Changes in the expression of ion channels, connexins and Ca2+-handling proteins in the sino-atrial node during postnatal development. Exp. Physiol. 96, 426–438. doi: 10.1113/expphysiol.2010.055780

Amend, N., Thiermann, H., Worek, F., and Wille, T. (2019). The arrhythmogenic potential of nerve agents and a cardiac safety profile of antidotes – A proof-of-concept study using human induced pluripotent stem cells derived cardiomyocytes (hiPSC-CM). Toxicol. Lett. 308, 1–6. doi: 10.1016/j.toxlet.2019.03.003

Antzelevitch, C., Brugada, P., Borggrefe, M., Brugada, J., Brugada, R., Corrado, D., et al. (2005). Brugada syndrome: report of the second consensus conference: endorsed by the heart rhythm society and the European heart rhythm association. Circulation 111, 659–670. doi: 10.1161/01.CIR.0000152479.54298.51

Arking, D. E., Juntila, M. J., Goyette, P., Huertas-Vazquez, A., Eijgelsheim, M., Blom, M. T., et al. (2011). Identification of a sudden cardiac death susceptibility locus at 2q24.2 through genome-wide association in European ancestry individuals. PLoS Genet. 7:e1002158. doi: 10.1371/journal.pgen.1002158

Arking, D. E., Pfeuffer, A., Post, W., Kao, W. H. L., Newton-Cheh, C., Ikeda, M., et al. (2006). A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. Nat. Genet. 38, 644–651. doi: 10.1038/ng1790

Arking, D. E., Pulit, S. L., Crotti, L., van der Harst, P., Munroe, P. B., Koopmann, T. T., et al. (2014). Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. Nat. Genet. 46, 826–836. doi: 10.1038/ng3014

Ashar, F. N., Mitchell, R. N., Albert, C. M., Newton-Cheh, C., Brody, J. A., Müller-Nurasyid, M., et al. (2018). A comprehensive evaluation of the genetic architecture of sudden cardiac arrest. Eur. Heart J. 39, 3961–3969. doi: 10.1093/eurheartj/ehy474

Bagwe, S., Berenfeld, O., Vaidya, D., Morley, G. E., and Jalife, J. (2005). Altered right atrial excitation and propagation in connexin40 knockout mice. Circulation 112, 2245–2253. doi: 10.1161/CIRCULATIONAHA.104.527325

Bai, Y., Jones, P. P., Guo, J., Zhong, X., Clark, R. B., Zhou, Q., et al. (2013). Phospholamban knockout breaks arrhythmogenic Ca2+ waves and suppresses...
exit pathways that connect the canine sinoatrial node and atria. *Circ. Res.* 104, 915–923. doi: 10.1161/CIRCRESAHA.108.193193
Fishman, G. I., Chugh, S. S., Dimarco, J. P., Albert, C. M., Anderson, M. E., Bonow, R. O., et al. (2010). Sudden cardiac death prediction and prevention: report from a national heart, lung, and blood institute and heart rhythm society workshop. *Circulation* 122, 2335–2348. doi: 10.1161/CIRCULATIONAHA.110.976092
Glukhov, A. V., Hage, L. T., Hansen, B. J., Pedraza-Toscano, A., Vargas-Pinto, P., Hamlin, R. L., et al. (2013). Sinoatrial node reentry in a canine chronic left ventricular infarct model: role of intranodal fibrosis and heterogeneity of refractoriness. *Circ. Arrhythm. Electrophysiol.* 6, 984–994. doi: 10.1161/CIRCEP.113.000404
Goldfarb, R. D., Glock, D., Johnson, K., Creasey, A. A., Carr, C., McCarthy, R. I., et al. (1998). Randomized, blinded, placebo-controlled trial of tissue factor pathway inhibitor in porcine septic shock. *Shock* 10, 258–264. doi: 10.1097/00024382-199810000-00005
Greener, I. D., Monfredi, O., Inada, S., Chandler, N. J., Tellez, J. O., Atkinson, A., et al. (2011). Molecular architecture of the human specialised atrioventricular conduction axis. *J. Mol. Cell. Cardiol.* 50, 642–651. doi: 10.1016/j.yjmcc.2010.12.017
GTEx Consortium (2013). The genotype-tissue expression (GTEx) project. *Nat. Genet.* 45, 580–585. doi: 10.1038/ng.2653
Hadjii-Turdeghal, K., Andreassen, L., Hagen, C. M., Ahlberg, G., Ghouse, J., Bækved-Hansen, M., et al. (2020). Genome-wide association study identifies locus at chromosome 2q32.1 associated with syncope and collapse. *Cardiovasc. Res.* 116, 1138–1148. doi: 10.1093/cvr/cvr106
Haïssaguerre, M., Jais, P., Shah, D. C., Takahashi, A., Hocini, M., Quinou, G., et al. (1998). Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N. Engl. J. Med.* 339, 659–666. doi: 10.1056/NEJM199809033391003
Haïssaguerre, M., Shah, D. C., Jais, P., Shoda, M., Kautzner, J., Arentz, T., et al. (2002). Role of Purkinje conduction system in triggering of idiopathic ventricular fibrillation. *Lancet* 359, 677–678. doi: 10.1016/S0140-6736(02)70807-8
Hattori, T., Makiyama, T., Akao, M., Ebara, E., Ohno, S., Iguchi, M., et al. (2012). A novel gain-of-function KCNQ2 mutation associated with short-QT syndrome impairs inward rectification of Kir2.1 currents. *Cardiovasc. Res.* 93, 666–673. doi: 10.1093/cvr/cvs329
Heimig, M. (2018). Using gene expression to annotate cardiovascular GWAS loci. *Front. Cardiovasc. Med.* 5:59. doi: 10.3389/fcvm.2018.00059
Heron, T. J., Devaney, E., Mundada, L., Arden, E., Day, S., Guererro-Serna, G., et al. (2010). Ca2+-independent positive molecular inotropy for failing rabbit heart. *J. Pharmacol. Exp. Ther.* 334, 316–323. doi: 10.1124/jpet.109.145666
Holm, H., Gudbjartsson, D. F., Arnar, D. O., Thorleifsson, G., Thorgersson, G., Stefansson, H., et al. (2010). Several common variants modulate heart rate, PR interval and QRS duration. *Nat. Genet.* 42, 117–122. doi: 10.1038/ng.511
Holm, H., Gudbjartsson, D. F., Sulem, P., Masson, G., Helgadottir, H. T., Zanon, C., et al. (2011). A rare variant in MYH6 is associated with high risk of sick sinus syndrome. *Nat. Genet.* 43, 316–323. doi: 10.1038/ng.781
Hoogendijk, M. G., Potse, M., Linnenbank, A. C., Verkerk, A. O., den Ruijter, H. W., Gudbjartsson, D. F., Arnar, D. O., Thorleifsson, G., Thorgeirsson, H., et al. (2016). ZFHX3 knockdown increases arrhythmogenesis and dysregulates cardiac homeostasis in HL-1 atrial myocytes. *Int. J. Cardiol.* 210, 85–92. doi: 10.1016/j.ijcard.2016.02.091
Kapoor, A., Sekar, R. B., Hansen, N. F., Fox-Talbot, K., Morley, M., Pihur, V., et al. (2014). An enhancer polymorphism at the cardiacmyocyte intercalated disc protein NOS1AP locus is a major regulator of the QT interval. *Am. J. Hum. Genet.* 94, 854–869. doi: 10.1016/j.ajhg.2014.05.001
Kelly, R. G., Buckingham, M. E., and Moorman, A. F. (2014). Heart fields and cardiac morphogenesis. *Cold Spring Harb. Perspect. Med.* 4:a015750. doi: 10.1101/chspmed.a015750
King, J. H., Wickramarachchi, C., Kua, K., Du, Y., Jeevaramathan, K., Matthews, H. R., et al. (2013). Loss of Nav1.5 expression and function in murine atria containing the RyR2-P23285 gain-of-function mutation. *Cardiovasc. Res.* 99, 751–759. doi: 10.1093/cvr/cvt141
Kirchoff, C. J., Bonke, F. I., Allessie, M. A., and Lammers, W. J. (1987). The influence of the atrial myocardium on impulse formation in the rabbit sinus node. *Pflugers Arch.* 410, 198–203. doi: 10.1007/bf00581916
Kléber, A. G., and Rudy, Y. (2004). Basic mechanisms of cardiac impulse propagation and associated arrhythmias. *Physiol. Rev.* 84, 431–488. doi: 10.1152/physrev.00025.2003
Knollmann, B. C., Casimiro, M. C., Katchman, A. N., Sirenko, S. G., Schober, T., Rong, Q., et al. (2004). Isoprotenerol exacerbates a long QT phenotype in Kcnq1-deficient neonatal mice: possible roles for human-like Kcnq1 isoform 1 and slow delayed rectifier K+ current. *J. Pharmacol. Exp. Ther.* 310, 311–318. doi: 10.1124/jpet.103.063743
Koizumi, A., Sasano, T., Kimura, W., Miyamoto, Y., Aiba, T., Ishikawa, T., et al. (2010). A novel gain-of-function KCNJ2 mutation associated with short-QT syndrome impairs inward rectification of Kir2.1 currents. *Cardiovasc. Res.* 93, 666–673. doi: 10.1093/cvr/cvs329
Koizumi, A., Sasano, T., Kimura, W., Miyamoto, Y., Aiba, T., Ishikawa, T., et al. (2016). Genetic defects in a His-Purkinje system transcription factor, IRX3, cause lethal cardiac arrhythmias. *Eur. Heart J.* 37, 1469–1475. doi: 10.1093/eurheartj/ehv449
Krijthe, B. P., Kunst, A., Benjamin, E. J., Lip, G. Y. H., Franco, O. H., Hofman, A., et al. (2013). Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. *Eur. Heart J.* 34, 2746–2751. doi: 10.1093/eurheartj/eht280
Lakatta, E. G., and DiFrancesco, D. (2009). What keeps us ticking: a funny current, a calcium clock, or both? *J. Mol. Cell. Cardiol.* 47, 157–170. doi: 10.1016/j.yjmcc.2009.03.022
Landstrom, A. P., Boczek, N. J., Ye, D., Miyake, C. Y., De la Uz, C. M., Allen, H. D., et al. (2016). Novel long QT syndrome-associated missense mutation, L762F, in the cardiac ryanodine receptor 2a (RyR2) altered calcium homeostasis and induced arrhythmias. *Eur. Heart J.* 37, 1989–2000. doi: 10.1093/eurheartj/ehv047
Li, H., Li, D., Wang, Y., Huang, Z., Xu, J., Yang, T., et al. (2019). Nkx2-5 defines a subpopulation of pacemaker cells and is essential for the physiological function of the sinoatrial node in mice. *Development* 146, dev178145. doi: 10.1242/dev.178145
Liu, N., Hansen, B. J., Copee, T. A., Zhao, J., Ignozzi, A. J., Sul, I. V., et al. (2017). Redundant and diverse intranodal pacemakers and conduction pathways protect the human sinoatrial node from failure. *Sci. Transl. Med.* 9, eaam5607. doi: 10.1126/scitranslmed.aan5607

Li, N., Timofeyev, V., Tuteja, D., Xu, D., Lu, L., Zhang, Q., et al. (2009). Ablation of a Ca2+-activated K+ channel (SK2 channel) results in action potential prolongation in atrial myocytes and atrial fibrillation. *J. Physiol.* 587, 1087–1100. doi: 10.1113/jphysiol.2008.167718

Liu, G.-Z., Hou, T.-T., Yuan, Y., Hang, P.-Z., Zhao, J.-J., Sun, L., et al. (2016). Fenofibrate inhibits atrial metabolic remodelling in atrial fibrillation through PPAR-α/sirtuin 1/PGC-1α pathway. *Br. J. Pharmacol.* 173, 1095–1109. doi: 10.1111/bph.13438

Liu, Z., Hutt, J. A., Rajeshkumar, B., Azuma, Y., Duan, K. L., and Donahue, Li, N., Hansen, B. J., Csepe, T. A., Zhao, J., Ignozzi, A. J., Sul, L. V., et al. (2017). Novel mutant Na+/HCO3⁻ co-transporter NBC2 in a case of compound-heterozygous inheritance of proximal renal tubular acidosis. *J. Physiol.* 594, 6267–6286. doi: 10.1113/JP272252

Nadadur, R. D., Broman, M. T., Boukens, B., Mazurek, S. R., Yang, X., Van Den Boogaard, M., et al. (2016). Ptx2 mutants display a Thx5-dependent gene regulatory network to maintain atrial rhythm. *Sci. Transl. Med.* 8:354ra15. doi: 10.1126/scitranslmed.aaf4891

Newton-Cheh, C., Eijgelsheim, M., Rice, K. M., De Bakker, P. I. W., Yin, X., Estrada, K., et al. (2009). Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nat. Genet.* 41, 399–406. doi: 10.1038/ng.364

Nolte, I. M., Munoz, M. L., Tragante, V., Amante, A. T., Jansen, R., Vaze, A., et al. (2017). Genetic loci associated with heart rate variability and their effects on cardiac disease risk. *Nat. Commun.* 8:15805. doi: 10.1038/ncomms15805

Noulredin, M., Chen, H., and Bai, D. (2018). Functional characterization of novel atrial fibrillation-linked GJAX (Cx60) mutants. *Int. J. Mol. Sci.* 19:977. doi: 10.3390/ijms1904977

Ophoth, T., VanGinneken, A. C., Bouman, L. N., and Jongsm, H. J. (1987). The intrinsic cycle length in small pieces isolated from the rabbit sinoatrial node. *J. Mol. Cell. Cardiol.* 19, 923–934. doi: 10.1016/0022-2828(87)90121-1

Orvos, P., Kohajda, Z., Szőlővész, J., Gazdag, P., Árpádföldi-Lóvas, T., Tóth, D., et al. (2019). Evaluation of possible proarrhythmic potency: comparison of the effect of dofetilide, cisapride, sotalol, terfenadine, and verapamil on βEGR and native IKr currents and on cardiac action potential. *Toxicol. Sci.* 168, 365–380. doi: 10.1093/toxsci/kfy299

Papadatos, A. G., Wallerstein, P. M. R., Head, C. E. G., Ratcliff, P., Brady, P. A., Benndorf, K., et al. (2002). Slowed conduction and ventricular tachycardia after targeted disruption of the cardiac sodium channel gene Scn5a. *Proc. Natl. Acad. Sci. USA.* 99, 6210–6215. doi: 10.1073/pnas.082121299

Park, D. S., Woodman, S. E., Schubert, W., Cohen, A. W., Frank, P. G., Chandra, M., et al. (2002). Caveolin-1/3 double-knockout mice are viable, but lack both muscle and non-muscle caveolae, and develop a severe cardiomyopathic phenotype. *Am. J. Pathol.* 160, 2207–2217. doi: 10.1016/S0002-9440(10)6168-6

Pfeiffer, M. J., Quananta, R., Piccioni, I., Fell, J., Rao, J., Röpke, A., et al. (2018). Cardiogenic programming of human pluripotent stem cells by dose-controlled activation of EOMES. *Nat. Commun.* 9:440. doi: 10.1038/s41467-017-02812-6

Pfeiffer, A., van Noord, C., Marcianò, K. D., Arking, D. E., Larson, M. G., Smith, A. V., et al. (2010). Genome-wide association study of PR interval. *Nat. Genet.* 42, 153–159. doi: 10.1038/ng.957

Posukhova, E., Ng, D., Opel, A., Masuho, I., Tinker, A., Biesecker, L. G., et al. (2013). Essential role of the m2R-RGS6-1AKaCh pathway in controlling intrinsic heart rate variability. *PloS One* 8:e67973. doi: 10.1371/journal.pone.0076973

Posukhova, E., Wydeven, N., Allen, K. L., Wickman, K., and Martemyanov, K. A. (2010). RGS6/Gq5 complex accelerates IαCh gating kinetics in atrial myocytes and modulates parasympathetic regulation of heart rate. *Circ. Res.* 107, 1350–1354. doi: 10.1161/CIRCRESAHA.110.224212

Pott, C., Ren, X., Tran, D. X., Yang, M.-J., Henderson, S., Jordan, M. C., et al. (2007). Mechanism of shortened action potential duration in Na+-Ca2+ exchanger knockout mice. *Am. J. Physiol. Cell Physiol.* 292, C696–C697. doi: 10.1152/ajpcell.00177.2006

Purohit, A., Rokita, A. G., Guan, X., Chen, B., Koval, O. M., Voigt, N., et al. (2013). Oxidized Ca2+/-calmodulin-dependent protein kinase II triggers atrial fibrillation. *Circulation* 128, 1748–1757. doi: 10.1161/CIRCULATIONAHA.113.033313

Rentschler, S., Yen, A. H., Lu, J., Petrenko, N. B., Lu, M. M., Manderfield, L. J., et al. (2012). Myocardial Notch signaling reprograms cardiomyocytes and modulates parasympathetic regulation of heart rate. *Circ. Res.* 110, 385–393. doi: 10.1161/CIRCRESAHA.112.103333

Rochen, A. B., Crimmins, J. L., Amatruda, J. M., Kinson, M. S., Todd-Brown, M. A., Lewis, J., et al. (2010). Structural and functional changes in tau mutant mice neurons are not linked to the presence of NFTs. *Exp. Neurol.* 223, 385–393. doi: 10.1016/j.expneurol.2009.07.029
Scholman et al.

GWAS to Function

Roder, K., Werdich, A. A., Li, W., Liu, M., Kim, T. Y., Organ-Darling, L. E., et al. (2014). RING finger protein RNF207, a novel regulator of cardiac excitation. J. Biol. Chem. 289, 33730–33740. doi: 10.1074/jbc.M114.592295

Rohr, S., Kucera, J. P., Fast, V. G., and Kleber, A. G. (1997). Paradoxical improvement of impulse conduction in cardiac tissue by partial cellular uncoupling. Science 275, 841–844. doi: 10.1126/science.275.5301.841

Roselli, C., Chaffin, M. D., Weng, L.-C., Aeschbacher, S., Ahlberg, G., Albert, C. M., et al. (2018). Multi-ethnic genome-wide association study for atrial fibrillation. Nat. Genet. 50, 1225–1233, doi: 10.1038/s41588-018-0133-9

Sah, R., Mesirca, P., Van den Boogert, M., Rosen, J., Mably, J., Mangoni, M. E., et al. (2013). Ion channel-kinease TRPM7 is required for maintaining cardiac automaticity. Proc. Natl. Acad. Sci. U.S.A. 110, E3037–E3046. doi: 10.1073/pnas.1311865110

Saito, T., Waki, K., and Becker, A. E. (2000). Left atrial myocardial extension onto pulmonary veins in humans: anatomic observations relevant for atrial arrhythmias. J. Cardiovasc. Electrophysiol. 11, 888–894. doi: 10.1111/j.1540-8167.2000.tb00668.x

Sapra, G., Tham, Y. K., Cemerlang, N., Matsumoto, A., Kiriazis, H., Bernardo, B. C., et al. (2011). Contrasting Nav1.8 activity in Scn10a-/- ventricular myocytes and the intact heart. J. Clin. Invest. 117, 1814–1823. doi: 10.1172/JCI32715

Shin, J.-H., Bostick, B., Yue, Y., Hajjar, R., and Duan, D. (2011). SERCA2a gene transfer improves electrophysiologic performance in aged mdx mice. J. Transl. Med. 9:132. doi: 10.1186/1479-5876-9-132

Singh, R., Horsttuis, T., Farin, H. F., Grieskamp, T., Norden, J., Petry, M., et al. (2009). Loss of plakophilin-2 expression leads to decreased sodium current and slower conduction velocity in cultured cardiac myocytes. Circ. Res. 105, 523–526. doi: 10.1161/CIRCRESAHA.109.204148

van den Hoogenhof, M. M. G., Beqqali, A., Amin, A. S., van der Made, I., Aufiero, D., et al. (2014). Diminished PRKX1 expression is associated with increased risk of atrial fibrillation and shortening of the cardiac action potential. Circ. Res. 115, e01902. doi: 10.1161/CIRCGENETICS.117.001902

Tawara, S. (1906). Das Reizleitungsstystem des Säugertierherzens. Jena: Fisher.

Tomás, M., Napolitano, C., De Gugli, L., Böese, R., Subiran, L., Malovini, A., et al. (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. doi: 10.1016/j.jacc.2009.12.065

Tranum-Jensen, J., Wilde, A. M. M., Vermeulen, J. T., and Janse, M. J. (1991). Morphology of electrophysiologically identified junctions between Purkinje fibers and ventricular muscle in rabbit and pig hearts. Circ. Res. 69, 429–437. doi: 10.1161/01.RES.69.2.429

Tucker, N. R., Dolmatova, E. V., Lin, H., Cooper, R. R., Ye, J., Tucker, W. J., et al. (2017). Reduced PRRX1 expression is associated with increased risk of atrial fibrillation and tachycardia in the congenital G650S mutant (Oculudentodigital dysplasia) mouse. J. Physiol. Heart Circ. Physiol. 300, H1402–H1411. doi: 10.1115/aphy.2014.00946

van den Boogaard, M., Smemo, S., Burnicka-Turek, O., Arnoldos, D. E., van der Werken, H. J. G., Klous, P., et al. (2014). A common genetic variant within SCN10A modulates cardiac SCN5A expression. J. Clin. Invest. 124, 1844–1852. doi: 10.1172/JCI73140

van den Hoogenhof, M. G. M., Beqqali, A., Amin, A. S., van der Made, I., Aufiero, S., Khan, M. A. F., et al. (2018). RBM20 mutations induce an arrhythmogenic dilated cardiomyopathy related to disturbed calcium handling. Circulation 138, 1330–1342. doi: 10.1161/CIRCULATIONAHA.117.031947

van Duijenboden, K., de Boer, B. A., Capon, N., Ruiter, J. M., and Christoffers, V. M. (2016). EMERGE: a flexible modelling framework to predict genomic regulatory elements from genomic signatures. Nucleic Acids Res. 44:442. doi: 10.1093/nar/gkv1144

van Duijenboden, K., Bakker, M., Bakker, V., Gier-de Vries, C., et al. (2019). Transcriptome analysis of mouse and human sinoatrial node cells reveals a conserved genetic program. Development 146:dev173161. doi: 10.1242/dev.173161

van Ouwerkerk, A. F., Bosada, F. M., van Duijenboden, K., Hill, M. C., Montefiori, L. E., Scholman, K. T., et al. (2019). Identification of atrial fibrillation associated genes and functional non-coding variants. Nat. Commun. 10:4755. doi: 10.1038/s41467-019-10476-9

van Setten, J., Brody, J. A., Jamshidi, Y., Swenson, B. R., Butler, A. M., Campbell, H., et al. (2018). PR interval genome-wide association meta-analysis identifies 50 loci associated with atrial and atrioventricular electrical activity. Nat. Commun. 9:2904. doi: 10.1038/s41467-018-04766-9

van Setten, J., Verweij, N., Mbaraek, H., Niemeijer, M. N., Trompet, S., Arking, D. E., et al. (2019). Genome-wide association meta-analysis of 30,000 samples identifies seven novel loci for quantitative ECG traits. Eur. J. Hum. Genet. 27, 952–962. doi: 10.1038/s41431-018-0295-z

Veerman, C. C., Mengarelli, I., Guan, K., Stauske, M., Dohman, J. H., et al. (2016). hiPSC-derived cardiomyocytes from Brugada syndrome patients without identified mutations do not exhibit clear cellular electrophysiological abnormalities. Sci. Rep. 6:30967. doi: 10.1038/srep30967

Verheule, S., Wilson, E. E., Arora, R., Engle, S. K., Scott, L. R., and Olgin, J. E. (2010). Pathophysiological mechanisms of atrial fibrillation: a translational approach. Physiol. Rev. 91, 727–789. doi: 10.1152/physrev.00130.2009

Verheule, S., Wilson, E. E., Arora, R., Engle, S. K., Scott, L. R., and Olgin, J. E. (2010). Pathophysiological mechanisms of atrial fibrillation: a translational approach. Physiol. Rev. 91, 727–789. doi: 10.1152/physrev.00130.2009

Vermeulen, J., Janse, M. J., and Verheule, S., Wilson, E. E., Arora, R., Engle, S. K., Scott, L. R., and Olgin, J. E. (2010). Pathophysiological mechanisms of atrial fibrillation: a translational approach. Physiol. Rev. 91, 727–789. doi: 10.1152/physrev.00130.2009

Wang, H., Qin, J., Gong, S., Fang, B., Zhang, Y., and Tao, J. (2014). Insulin-like growth factor-1 receptor-mediated inhibition of A-type K(+) current induces sensory neuronal hyperexcitability through the phosphatidylinositol 3-kinase and extracellular signal-regulated kinase 1/2 pathways, independently of Akt. Endocrinology 155, 168–179. doi: 10.1210/en.2013-1559
Wemhöner, K., Friedrich, C., Stallmeyer, B., Coffey, A. J., Grace, A., Zumhagen, S., et al. (2015). Gain-of-function mutations in the calcium channel CACNA1C (Cav1.2) cause non-syndromic long-QT but not Timothy syndrome. J. Mol. Cell. Cardiol. 80, 186–195. doi: 10.1016/j.yjmcc.2015.01.002

West, T. C. (1955). Ultramicroelectrode recording from the cardiac pacemaker. J. Pharmacol. Exp. Ther. 115, 283–290.

Wiener, N., and Rosenblueth, A. (1946). The mathematical formulation of the problem of conduction of impulses in a network of connected excitable elements, specifically in cardiac muscle. Arch. Inst. Cardiol. Mex 16, 205–265.

Xu, B., Wratten, N., Charych, E. I., Buyske, S., Firestein, B. L., and Brzustowicz, L. M. (2005). Increased expression in dorsolateral prefrontal cortex of CAPON in schizophrenia and bipolar disorder. PLoS Med. 2:e263. doi: 10.1371/journal.pmed.0020263

Yagishita, D., Chui, R. W., Yamakawa, K., Rajendran, P. S., Ajijola, O. A., Nakamura, K., et al. (2015). Sympathetic nerve stimulation, not circulating norepinephrine, modulates T-peak to T-end interval by increasing global dispersion of repolarization. Circ. Arrhythm. Electrophysiol. 8, 174–185. doi: 10.1161/CIRCEP.114.002195

Yamaguchi, T., Suzuki, T., Sato, T., Takahashi, A., Watanabe, H., Kadowaki, A., et al. (2018). The CCR4-NOT deadenylase complex controls Atg7-dependent cell death and heart function. Sci. Signal. 11:eaan3638. doi: 10.1126/scisignal.aan3638

Yang, J.-M., Shen, C.-J., Chen, X.-J., Kong, Y., Liu, Y.-S., Li, X.-W., et al. (2019). erbB4 deficits in chandelier cells of the medial prefrontal cortex confer cognitive dysfunctions: implications for schizophrenia. Cereb. Cortex 29, 4334–4346. doi: 10.1093/cercor/bhy316

Yang, K.-C., Breibart, A., De Lange, W. J., Hofsteene, P., Futakuchi-Tsuchida, A., Xu, J., et al. (2018). Novel adult-onset systolic cardiomyopathy due to MYH7 E848G mutation in patient-derived induced pluripotent stem cells. JACC Basic Transl. Sci. 3, 728–740. doi: 10.1016/j.jacbts.2018.08.008

Yi, F., Ling, T.-Y., Lu, T., Wang, X.-L., Li, J., Claycomb, W. C., et al. (2015). Down-regulation of the small conductance calcium-activated potassium channels in diabetic mouse atria. J. Biol. Chem. 290, 7016–7026. doi: 10.1074/jbc.M114. 607952

Zaritsky, J. I., Redell, J. B., Tempel, R. L., and Schwarz, T. L. (2001). The consequences of disrupting cardiac inwardly rectifying K(+) current (I(K1)) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. J. Physiol. 533, 697–710. doi: 10.1111/j.1469-7793.2001.00697.x

Zhang, S., Kim, K., Rosen, A., Smyth, J. W., Sakuma, R., Delgado-Olguín, P., et al. (2011). Iroquois homebox gene 3 establishes fast conduction in the cardiac His-Purkinje network. Proc. Natl. Acad. Sci. U.S.A. 108, 13576–13581. doi: 10.1073/pnas.1106911108

Zheng, Z., He, Y., Tuteja, D., Xu, D., Timofeyev, V., Zhang, Q., et al. (2005). Functional roles of Cav1.3(alpha1D) calcium channels in atria: insights gained from gene-targeted null mutant mice. Circulation 112, 1936–1944. doi: 10.1161/CIRCULATIONAHA.105.540070

Zhang, Z., He, Y., Tuteja, D., Xu, D., Timofeyev, V., Zhang, Q., et al. (2005). Functional roles of Cav1.3(alpha1D) calcium channels in atria: insights gained from gene-targeted null mutant mice. Circulation 112, 1936–1944. doi: 10.1161/CIRCULATIONAHA.105.540070

Zheng, Z., Wang, Z.-M., and Delbono, O. (2002). Charge movement and transcription regulation of L-type calcium channel alpha1S in skeletal muscle cells. J. Physiol. 540, 397–409. doi: 10.1113/jphysiol.2001.013464

Zhou, X., Wang, Z., Huang, B., Yuan, S., Sheng, X., Yu, L., et al. (2018). Regulation of the NRG1/ErbB4 pathway in the intrinsic cardiac nervous system is a potential treatment for atrial fibrillation. Front. Physiol. 9:1082. doi: 10.3389/fphys.2018.01082

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Scholman, Meijborg, Gálvez-Montón, Lodder and Boukens. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.