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The emerging genetic landscape underlying cardiac conduction system function

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Abstract

Proper function of an organized Cardiac Conduction System (CCS) is vital to the survival of metazoans ranging from fly to man. The routine use of non-invasive electrocardiogram measures in the diagnosis and monitoring of cardiovascular health has established a trove of reliable CCS functional data in both normal and diseased cardiac states. Recent combination of ECG data with genome wide association studies has identified genomic regions implicated in ECG variability and which impact CCS function. Here we review the substantial recent progress in this area, highlighting the identification of novel loci, confirming the importance of previously implicated loci in CCS function, and exploring potential links between genes with important roles in developmental processes and variation in function of the CCS.

Keywords

Cardiac; heart; conduction; development; transcription factor; T-box; GWAS

Introduction

The form of the conduction system is stereotyped in mammals, and is comprised of the discrete components including: (1) the sinoatrial node (SAN), a pacemaker region at the junction of the vena cava and right atrium; (2) the atrioventricular node (AVN), responsible for atrioventricular conduction delay, (3) the proximal ventricular conduction system (VCS), including the bundle of His and bundle branches, responsible for rapid and coordinated impulse propagation from the AVN to the ventricular apex and (4) the Purkinje fibers, responsible for ramification of the electrical impulse from the proximal VCS into ventricular myocardium (Figure 1).

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ECG (electrocardiogram) measures have been a valuable tool for decades for inferring proper function of the CCS. The routine 12 lead ECG allows analysis of the electrical vectors across the precordium. The P wave and QRS complex reflect atrial and ventricular depolarization, respectively. The broad shaped T wave represents ventricular repolarization. Directly obtainable measures from the routine ECG include the heart rate, PR interval, QRS duration, and QT interval (Figure 1). These measures are functional readouts of CCS and myocardial function. Thus, heart rate and variability are indicative of SA node function, the PR interval encompasses the AVN and proximal VCS function, the QRS duration measures VCS function, and QT interval includes repolarization of the remaining myocardium. Since these intervals are so easily and rapidly determined for large numbers of individuals, the ECG intervals have clear statistical norms; values diverging from these norms imply dysfunction at the level of the associated CCS structure.

Abnormal ECG intervals, directly reflective of CCS dysfunction as noted above, are associated with morbidity and mortality. Increased baseline heart rate has been associated with increased risk of sudden cardiac death in some populations (Jouven et al., 2005). Prolonged PR intervals have been shown to increase risk for pacemaker implantation and cardiac mortality (Cheng et al., 2009). Prolonged QRS intervals are associated with increased mortality. Alterations in the QT interval increase risk of ventricular arrhythmias and sudden cardiac death (Patel and Pavri, 2009; Schwartz et al., 1993).

Our knowledge of the genetic contributions to CCS dysfunction has until recently relied on heritable monogenic diseases. Thus, excellent understanding of the molecular causes of long QT syndrome owes to the presence of families with inherited monogenic disease allowing positional cloning and candidate approaches for identification of disease-causing mutations. The result has been identification of multiple loci, encoding primarily ion channel subunits broadly expressed within the heart (reviewed in Hedley et al., 2009). When mutated, most commonly as dominant disorders, these genes modulate myocardial repolarization and cause lethal long QT-based arrhythmias. These monogenic approaches have yielded direct links between gene and function, but explain a very small minority of functional variability of QT length control in the population at large.

Similar approaches have allowed advances in understanding the molecular regulation of CCS development. For example, monogenic contributions to congenital heart disease (CHD) have implicated the Tbx5 and Nkx2-5 transcription factors in CCS function (Basson et al., 1997; Li et al., 1998; Schott et al., 1998). More recent advances have begun to reveal the molecular machinery required for proper development of the conduction system, including the role of Nkx2-5, Tbx5, and other cardiogenic transcription factors including Tbx3 (reviewed in Hatcher and Basson, 2009). The distinct regions of the CCS appear to develop independently and their specification requires distinct molecular pathways (Moskowitz et al., 2007). In this regard, the molecular pathways governing the function and resulting ECG interval of specific CCS segments may be expected to be distinct as well.

In contrast to the single gene Mendelian disorders that provide highly useful information in families, genomewide association studies (GWAS) can discern genetic contribution across populations. Although GWAS are very powerful to identify genetic regions that contribute to

the phenotype of interest, by design, these studies identify common variants in the population. To have achieved such prevalence in the population, these DNA variants typically only confer relatively modest effects. The challenge of performing GWAS on relatively rare disorders is illustrated by several studies that have been performed on genetic variation associated with the risk of atrial fibrillation, ventricular fibrillation in the setting of acute myocardial infarction, and protection from sudden cardiac death. Variations near 3 genes that confer a risk to atrial fibrillation have been identified (Gudbjartsson et al., 2007; Benjamin et al., 2009; Ellinor et al., 2010), while single SNPs were found that associate with the risk of ventricular fibrillation in acute MI and protection from sudden cardiac death (Bezzina et al., 2010; Arking et al., 2010). Each of these studies was successful in identifying variants associated with traits that are extremely clinically relevant, but the modest number of genes identified demonstrates the challenge such studies face. In contrast, GWAS are ideal for determining the genetics of complex common traits such as ECG intervals.

The ubiquity and established norms of ECG measures worldwide has provided an excellent platform for investigating the genetic contributions to variations in CCS function. Several groups have exploited this platform and recently reported the results of genome-wide association studies for ECG intervals, including heart rate (Cho et al., 2009; Holm et al., 2010; Newton-Cheh et al., 2007), PR interval (Holm et al., 2010; Newton-Cheh et al., 2007; Pfeufer et al., 2010), QRS interval (Chambers et al., 2010; Holm et al., 2010; Sotoodehnia et al., 2010), and QT duration (Arking et al., 2009; Chambers et al., 2010; Holm et al., 2010; Newton-Cheh et al., 2009; Pfeufer et al., 2009). These studies utilized the latest available technology and were well-powered, allowing variants to be implicated at statistical thresholds accepted by the field as significant in a genome-wide context. Each group was successful in identifying candidate loci contributing to variability of these ECG measurements, and therefore candidates for loci essential in CCS health. We review the salient findings of each and note overlap where present.

Heart Rate

The heart rate (HR) is defined as the number of heart beats during an interval of time, conventionally measured over a minute. Numerous studies have reported an association of elevated heart rate with cardiovascular morbidity and mortality in both the general population as well as patients with cardiovascular diseases (Jouven et al., 2005; Palatini and Julius, 2004), including hypertension and left ventricular dysfunction. Heart rate variability (HRV) reflects the integrity of autonomic function in the heart, and can be measured in a variety of ways. There is evidence of a significant genetic component to HR and HRV measures, with reported heritability indices ranging from 29–77% (Hanson et al., 1989; Havlik et al., 1980; Newton-Cheh et al., 2007; Russell et al., 1998; Singh et al., 1999).

Two recently published GWAS have reported SNPs associated with measures of heart rate, which may implicate genes and molecular pathways important to cardiac autonomic functioning. Cho and colleagues published a GWAS of Asian populations which reported loci significantly associated to eight biomedically important traits, including body mass index, waist hip ratio, height, systolic and diastolic blood pressure, heart rate, and bone

densitometry (Cho et al., 2009). They found two independent SNPs that were associated with elevated HR in their study (Table 1). One of the signals maps to chromosome 1q32.2,, to a region near a cluster of three genes which code receptors of complement activation (RCA). One of these genes, CD34, has been theorized to mediate stem cell attachment to bone marrow extracellular matrix and/or stromal cells, which is important to early hematopoiesis (Dooley et al., 2004). It remains to be elucidated how these pathways contribute to cardiac conduction and automaticity. The second SNP reaching statistical significance is located in an intergenic region ~400 kb upstream of *GJAI*, which encodes the gap junction Cx43. Cx43 is as a major component of cardiac gap junctions and is enriched in non-conduction cardiomyocytes (Coppin et al., 2003). Cardiomyocyte-restricted deletion of Cx43 in the mouse leads to abnormalities in ventricular conduction and sudden arrhythmic death (Gutstein et al., 2001).

A recent study in a European population identified a SNP near MYH6 associated with variations in HR (Table 1; Holm et al., 2010). MYH6 codes for the alpha isoform of sarcomeric myosin heavy chain (MyHC) (Kurabayashi et al., 1988). Though prior studies report relatively low expression of alpha-MyHC in the myocardium (Franco et al., 1998; Lompre et al., 1984), more recent evidence suggests that myocardial contractility is highly sensitive to the differential expression of isoforms, and that even a small increase in the alpha isoform expression may improve cardiac function under stress (Herron and McDonald, 2002). Thus, it is plausible that SNPs near MYH6 may affect alpha-MyHC expression and thus impact cardiac function with a compensatory change in heart rate. Furthermore, in 2005, MYH6 mutations were found in cases of hypertrophic and dilated cardiomyopathy (Carniel et al., 2005) and in a family with dominantly inherited atrial septal defect (Ching et al., 2005).

PR Interval

The PR interval represents the time required for impulse propagation from the atria to the ventricles and includes the AV-node and proximal VCS. PR interval prolongation has recently been associated with increased risk of mortality (Cheng et al., 2009). The PR interval is a genetically influenced trait and has a heritability index, h^2 , estimated at around 34% (Havlik et al., 1980; Newton-Cheh et al., 2007).

Rare Mendelian forms of atrioventricular (AV) block and prolonged PR interval have been reported. Nkx2-5, Tbx5, and Gata4, transcription factors required for early events in cardiac development, have each been implicated in atrioventricular conduction. Specifically, deleterious non-synonymous mutations or haploinsufficiency of Nkx2-5 and Tbx5 cause PR prolongation and AV block in humans and mice whereas GATA4 haploinsufficiency results in PR shortening in mice (Jay et al., 2004; Bruneau et al., 2001; Moskowitz et al., 2004; Munshi et al., 2009). The specific CCS role of these transcription factors has been challenging to uncover, as each plays an important role in structural cardiac development as well as CCS function. In the case of Nkx2-5, elegant studies have revealed that a precise level of Nkx2-5 is required for Purkinje cell differentiation in the chick and mouse, and that Nkx2-5 haploinsufficiency in mice specifically impacts conduction in the Purkinje system (Harris et al., 2006; Meysen et al., 2007). Postnatal tamoxifen-inducible deletion of Nkx2-5

has also been shown to have significant effects on conduction system function, particularly in the perinatal period (Briggs et al., 2008; Takeda et al., 2009). Whether these important cardiogenic transcription factors also play a role in PR interval variability in the population at large has been unknown.

Three GWAS simultaneously reported analysis of the PR interval, one from analysis of an Indian Asian cohort (Chambers et al., 2010) and one a direct study of a European cohort (Holm et al., 2010) and another a meta analysis of studies from several European cohorts (Pfeufer et al., 2010). All three identified significant association between PR interval and variants adjacent to or within SCN10A (Table 1). SCN10A encodes the alpha subunit of the tetrodotoxin (TTX)-resistant voltage-gated sodium channel Nav1.8. SCN10A is 70.4% similar to SCN5A (Rabert et al., 1998), the major cardiac voltage-gated sodium channel, which has been linked to several cardiac disorders, including long QT syndrome, Brugada syndrome, sick sinus syndrome, and atrial fibrillation (see below) (Ruan et al., 2009). Chambers reported that three SCN10A common variants in pairwise linkage disequilibrium were associated with PR interval. In particular, non-synonymous SNP rs6795970 (A1073V) was identified in all three studies, providing an attractive candidate for the functional mutation. This SNP was also significantly associated with pathological measures including first-degree AV block, bundle branch block, and bifascicular heart block by Chambers et al., and with pacemaker implantation by Holm et al. These studies also implicated SCN5A in PR variability (Table 1). Importantly, the sentinel SCN5A SNP implicated in PR interval by Pfeufer, rs11708996, is in weak linkage disequilibrium with SCN10A SNP rs6795970, suggesting independent activity of these adjacent genes on PR interval.

As a bridge from the GWAS results to the underlying physiology of cardiac function, the role of SCN10A has been examined in the murine heart. SCN10A expression was examined and reported in human as well as murine heart, and SCN10A was found to be enriched in isolated murine Purkinje cells relative to ventricular myocardium (Chambers et al., 2010; Sotoodehnia et al., 2010). Expression of SCN10A had previously been observed in the left ventricle of individuals with dilated cardiomyopathy (Eckel et al., 2005), but had not been previously described in healthy myocardium. Pharmacologic inhibition of SCN10A resulted in significant prolongation of the QRS complex but did not affect other ECG parameters (Sotoodehnia et al., 2010). In contrast, SCN10A^{-/-} mice were found to have a significantly decreased P-R interval (Chambers et al., 2010); other ECG intervals including QRS complex duration were not affected. Notably, pharmacologic interventions run the risk of off-target effects, while compensatory adaptations may occur following germline loss of SCN10A. These studies lend support to the hypothesis that changes in SCN10A expression may underlie the association of genomic variation near SCN10A with changes in the ECG, although the physiologic role of SCN10A in the conduction system of the heart, as well as the effect of the observed genetic variation on SCN10A expression, is far from clear.

Several variants associated with PR interval were identified adjacent to the same candidate genes in two independent studies (Holm et al., 2010; Pfeufer et al., 2010), including TBX5, CAV1 and CAV2, and ARHGAP24 (Table 1). As noted, the cardiogenic transcription factor Tbx5 and sodium channel SCN5A are known regulators of cardiac conduction (Moskowitz et al., 2004; Ruan et al., 2009). Holm et al reported that Tbx5 variant rs3825214, located in

the last intron of the *Tbx5* gene, associated with PR, QRS, and QT interval lengths. In follow-up case controls, the allele of this variant associated with prolonged PR interval was a risk factor for advanced atrioventricular block. Pfeufer reported association of rs1896312 with PR interval, a variant over 200Kb downstream of the paralogous *TBX5* and *TBX3* genes, making both genes phenotypic candidates.

Both Holm and Pfeufer identified an association between PR interval and a variant (rs3807989) in linkage disequilibrium with *CAV1* and *CAV2* (Table 1). *CAV1* and *CAV2* encode proteins that are members of the caveolin family; caveolae are invaginations of the plasma membrane that play key roles in vesicular trafficking and signal transduction. The sentinel SNP resides in an intron of *CAV1*. *CAV1* is expressed in the heart and *CAV1* deficient mice develop dilated cardiomyopathy (Zhao et al., 2002). *Cav1* deficiency also led to increased systemic nitric oxide (NO) levels; given the strong links between QT interval prolongation and NOS1AP, discussed at length below, this highlights the potential role of genetic variants that affect NO signaling on CCS and myocardial function. In addition to reporting links between variants near *CAV1* and *CAV2*, both studies also identified distinct SNPs adjacent to *ARHGAP24* a Rho GTPase-activating protein (Katoh, 2004) with no known function in the heart.

A preponderance of transcriptional regulators and genes implicated in cardiac development were identified as candidate effectors of PR interval (Table 1). *Nkx2-5*, a well-studied regulator of atrioventricular node development (Jay et al., 2004), is an excellent candidate as part of a linkage disequilibrium block implicated by a SNP associated with PR interval by Pfeuffer et al., Interestingly, three independent loci suggest that Wnt signaling may be important to the function of the CCS: SNPs associated with variation in P-R interval were found in the first intron of *WNT11* (Pfeufer et al., 2010), near *DKK1* (Holm et al., 2010), and in the eighth intron of *MEIS1*. *DKK1* is member of a small gene family of soluble Wnt/ beta-catenin inhibitors with important roles in vertebrate embryonic pattern formation, and *MEIS1* (Pfeufer et al., 2010) encodes a member of a homeobox-containing transcription factor family required to transduce Wnt signals in some developmental contexts (Elkouby et al., 2010). *WNT11* is a WNT-family ligand that induces non-canonical Wnt-signaling responses and is an inducer of cardiogenesis in *Xenopus* (Pandur et al., 2002). Intriguingly, several studies suggest that non-canonical Wnt signaling may be involved in the development of the conduction system. *Wnt11*, as well as another non-canonical Wnt ligand, *Wnt7a*, are upregulated in association with the differentiation of the His bundle and Purkinje fibers during embryogenesis, suggesting they may play role in patterning the embryonic conduction system (Bond et al., 2003). Furthermore, recent studies in zebrafish have revealed an essential role for *Wnt11* in establishing a zone of fast conduction at the outer curvature of the developing ventricle through effects on transmembrane Ca^{2+} conductance (Panakova et al., 2010). The aforementioned GWAS, as well as the studies discussed on non-canonical Wnt signaling, suggest a potential link between the development and function of the CCS and non-canonical Wnt signaling.

The SNPs found to have significant associations with P-R interval (Table) have recently been tested in a study that linked DNA repositories to electronic medical systems (Denny et al., 2010). SNPs near *SCN10A* had a significant association with P-R duration in this study,

validating previous results, while SNPs near *TBX5* and *ARHGAP24* had p values for association of 0.02. An additional 6 SNPs, all in intergenic regions, were identified in their unadjusted data with p values for association of $<10^{-5}$. Given the relatively small sample size included in this study, these results establish that GWAS conducted in conjunction with electronic medical records are an attractive platform for performing genotype-phenotype analyses.

QRS Interval

The QRS interval represents the time required for ventricular depolarization through the Purkinje system and ventricular myocardium. Aberrations in the ventricular conduction system can manifest as life-threatening brady- or tachy-arrhythmias, ranging from heart block to ventricular fibrillation. Furthermore, prolongation of the QRS interval independent from dysrhythmia has been associated with mortality (Desai et al., 2006), sudden death (Elhendy et al., 2005; Oikarinen et al., 2004), and heart failure (Dhingra et al., 2006). While early studies did not find a heritable component to QRS duration (Havlik et al., 1980; Russell et al., 1998), more recent studies have suggested heritability indices, h^2 , of 33–43% (Hanson et al., 1989; Li et al., 2009; Mutikainen et al., 2009).

Three GWAS have recently been published, all of which reported significant associations between common genetic variants and QRS interval (Chambers et al., 2010; Holm et al., 2010; Pfeufer et al., 2009). All three studies reported significant associations between SNPs in the vicinity of *SCN10A* and QRS duration, and two of the three studies reported that SNPs near *Tbx5*, *Dkk1*, and *CDKN1A* were associated with variation in QRS duration (Table 1). SNPs in the vicinity of *SCN10A* were also associated with increased risk of ventricular arrhythmias, bundle branch block and bifascicular heart block, and interestingly, protection against ventricular fibrillation in the setting of acute myocardial infarction (Chambers et al., 2010). As noted previously, *Tbx5* encodes a transcription factor key to cardiac development, and is widely expressed in the ventricular conduction system in mice (Moskowitz et al., 2004). Mutations in this gene have been linked to clinical phenotypes, specifically cardiac malformations and conduction disorders seen in Holt-Oram syndrome, which can include sinus dysfunction, varying severity of AV block, as well as bundle branch block (Basson et al., 1997; Basson et al., 1994; Li et al., 1997). As discussed above, *Dkk1* is involved in the Wnt signaling pathway, and the association of genetic variation near *DKK1* with QRS duration suggests that Wnt signaling may influence conduction through the AV bundle, bundle branches, and ventricular myocardium. The reported variant at chromosome 6p21 shares an LD block with the *CDKN1A* gene, which encodes a protein mediating cellular response to DNA damage and tumor growth suppression via the p53-dependent cell cycle arrest mechanism. It is yet to be elucidated how this last locus may be influencing biologically important pathways in cardiac conduction.

In addition to identifying SNPs near *SCN10A*, *CDKN1A*, *TBX5*, and *DKK1*, a meta-analysis of 14 studies performed in individuals of European descent identified an additional 18 loci associated with variation in QRS duration (Table 1). These genes include ion channels, transcription factors, calcium-handling proteins, kinase inhibitors, and genes related to tumorigenesis. It is notable that 6 (*Tbx3*, *Tbx5*, *Tbx20*, *HAND1*, *CRIM1*, and

DKK1) of these 22 loci are near genes with known roles in heart development or in pathways that regulate heart development. As previously discussed, *Tbx5* plays a central role in heart development and is implicated in Holt-Oram syndrome in humans, and *DKK1* is linked to Wnt signaling. *Tbx3* encodes a transcriptional repressor that is expressed throughout the developing conduction system and plays an essential role in the specification of the ventricular conduction (Bakker et al., 2008). In humans, mutations in *Tbx3* are linked to Ulnar-Mammary syndrome (Bamshad et al., 1997), which classically includes ulnar defects, inverted nipples, and short stature, but which also may include conduction system defects (Linden et al., 2009). *Tbx20* is necessary for heart tube looping and growth (Cai et al., 2005), and mutations in *Tbx20* are linked to a range of congenital heart malformations in human including defects in septation, chamber growth, and valvulogenesis (Kirk et al., 2007). *HAND1* is expressed throughout the outer curvature of the developing heart, and loss of myocardial *HAND1* results in an array of congenital heart malformations in mice, including septal, valvular, and outflow tract defects (McFadden et al., 2005). In humans, mutations in *HAND1* are associated with congenital heart defects including hypoplastic heart and septation defects (Reamon-Buettner et al., 2008; Reamon-Buettner et al., 2009). Finally, *CRIMI* which is a transmembrane regulator of BMP signaling (Wilkinson et al., 2003), is expressed in the developing heart (Pennisi et al., 2007). BMP signaling plays an important role in early cardiogenesis and may play a role in AV nodal function (Klaus and Birchmeier, 2009; Stroud et al., 2007; van Wijk et al., 2007). The precise role, if any, of *CRIMI* in heart development, however, is unclear.

QT Interval

The Q-T interval includes the QRS interval as well as the time required for ventricular repolarization, and both long and short QT intervals are associated with increased risk for cardiac mortality in multiple populations (Dekker et al., 2004; Dekker et al., 1994; Elming et al., 1998; Karjalainen et al., 1997; Okin et al., 2000; Schouten et al., 1991; Sharp et al., 1998). The QT interval is a genetically influenced trait and has a heritability index, h^2 , that has been estimated at around 30% (Holm et al., 2010; Newton-Cheh et al., 2005).

Familial forms of long and short QT syndromes encompass a range of findings in addition to alterations in the QT interval and are associated with increased risk of ventricular arrhythmias and sudden cardiac death (Patel and Pavri, 2009; Schwartz et al., 1993). The incidence of long QT syndrome has been estimated as high as 1 per 2000 individuals (Schwartz et al., 2009) while short QT syndrome is considerably more rare. Currently over 700 mutations (generally “private,” or specific to an individual family) have been described in 13 genes in association with long and short QT syndrome (Hedley et al., 2009). All of these mutations are in ion channels, ion channels adaptors, or scaffolding proteins, as would be expected for a disorder involving ventricular repolarization. While these studies have shed important light on the molecular mechanisms involved in ventricular repolarization, the genetic underpinnings of QT variation in the general population have only recently begun to be explored (Arking et al., 2006; Newton-Cheh et al., 2009; Pfeufer et al., 2009).

The first GWAS study to analyze the QT interval was performed in a European cohort, and identified a variant near *NOS1AP*, encoding the nitric oxide synthase 1 adaptor protein,

associated with variation in the QT interval (Arking et al., 2006). SNPs near *NOS1AP* have consistently been identified in subsequent studies, which have been conducted in European, US, Icelandic, and Indian populations (Chambers et al., 2010; Holm et al., 2010; Newton-Cheh et al., 2009; Pfeufer et al., 2009). In addition to confirming variation near the *NOS1AP* gene, common variants near an additional 12 loci (Table 1) have been identified that influence QT variation. Of these, five (*SCN5A*, *KCNQ1*, *KCNH2*, *KCNE1*, and *KCNJ1*) are located near genes encoding ion channels in which mutations are associated with hereditary Long QT Syndrome, highlighting the potential importance of these genes for ventricular repolarization in a broader population. Of the remaining loci, only *Tbx5*, *PLN*, and *ATP1B1* have previously been linked to cardiac function. *PLN* encodes phospholamban, a critical regulator of the cardiac sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA2a). Phospholamban plays a critical role in regulating cardiac contractility and Ca^{2+} handling (MacLennan and Kranias 2003) and mutations in phospholamban are associated with dilated cardiomyopathy and heart failure (Schmitt et al., 2003). *ATP1B1* encodes the beta1 subunit of the Na^+/K^+ ATPase. Genetic manipulation of *ATP1B1*, as well as other components of the Na^+/K^+ ATPase in the mouse have revealed a role for the Na^+/K^+ ATPase in regulating cardiac contractility through effects on Ca^{2+} currents mediated by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Barwe et al., 2009; James et al., 1999). The lack of known links between *LITAF*, *CNOT1*, *RNF207*, *LIG3*, and *NDRG4* and cardiac function suggests the power of GWAS to identify variations near novel genes without previously identified roles in cardiac physiology, as well as the challenge of linking these common variants to the underlying biology.

Discussion

A revolution in our understanding of the molecular basis of cardiac conduction system function is underway. Enabled by ease of quantitative ECG data collection, the recent highly powered GWAS reviewed here have provided a major early step. These studies have implicated large numbers of loci with potentially fundamental roles in explaining variability in ECG parameters and therefore CCS function. Previously, analysis of rare and highly penetrant mutations has shaped our classical understanding of the influence of genetic mutation on disease states. While this has led to a growing understanding of the molecular mechanisms that operate in health and that are altered in pathological states, the majority of genetic variation lies not in rare mutations with large phenotypic effects, but rather in common variations with more subtle phenotypic effects. With the current era of GWAS, we have now begun to grasp the role of common variants on the function of the conduction system.

Several interesting patterns emerged in reviewing the implicated loci across the evaluated studies. (i) The number of genes implicated in development of the heart and developmental processes more generally is remarkable. *Tbx3*, *Tbx5*, *Tbx20*, *Nkx2-5*, and *Hand1* all have well established roles in cardiac development and for *Tbx3*, *Tbx5*, and *Nkx2-5* roles in the development of the CCS specifically. (ii) Several genes adjacent to identified variants share signaling or transcriptional pathways providing more credence to their candidacy for phenotypic relevance. *Tbx5* directly regulates the transcription of *MYH6*, and both are implicated in PR interval. The identification of variants near *CAV1* and *NOS1AP* points to a potential role for nitric oxide signaling in the regulation of PR interval. Several implicated

loci (WNT11, DKK1, MEIS1) point to a possible role for Wnt signaling in conduction regulation. Future investigations will determine the direct biological relevance of these pathways in CCS function. (iii) Many loci were implicated in more than one region of the CCS. In particular, Holm et al reported SNPs at three loci (SCN10A, Tbx5, and CAV1) associated with both PR interval and QRS duration despite the weak correlation between the loci contributing to these measures, suggesting independent regional activity of these genes in the CCS.

The recent GWAS have revealed a number of variants that impact conduction system function and pointed to a potential role for genes important in developmental processes to the function of the CCS. Now the challenge of fully understanding the significance of these variants may begin. The majority of SNPs identified, for instance, are in intergenic regions or in introns, so their functional significance for gene expression needs to be determined. They are likely to be acting in the transcriptional regulation of adjacent genes, and potentially at long distances. This understanding opens the door to genes outside of the linkage disequilibrium blocks defined by the associated variants in the search for biologically relevant candidates. The nearby genes discussed herein are probably over represented for those with known roles, specifically in cardiac development. Furthermore, the described genetic variants account for only a small fraction of the functional variation in ECG parameters. Nevertheless, several variants associated not only with ECG measurements but also with cardiac conduction disease, highlighting their potential clinical importance. These studies have significantly broadened our understanding of genetic influences on conduction system function, and provided numerous avenues for further research.

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Figure 1. Schematic Representations of the Structure of the Cardiac Conduction System its Correlation to the Surface Electrocardiogram (ECG)

(A) Impulse propagation through the mammalian heart can be traced through the surface ECG.

(B) The cardiac conduction system is comprised of the sino-atrial node (SAN), AV node (AVN), AV bundle, bundle branches, and Purkinje fibers (A).

Table 1
Genes Near Loci Associated with Variation in ECG Intervals in Genome Wide Association Studies

HR	P-R	QRS		Q-T
<i>MYH6</i> ¹	<i>SCN10A</i> ^{1,2,3,10}	<i>SCN10A</i> ^{1,3,4}	<i>SETBP1</i> ⁴	<i>SCN5A</i> ^{1,3,5,6}
<i>GJA1</i> ⁹	<i>SCN5A</i> ²	<i>SCN5A</i> ⁴	<i>TKT-PRKCD</i>	<i>C6orf204-SCL35F1</i> -
<i>CD46-CD34</i> ⁹	<u><i>Tbx5</i></u> ^{1,2}	<u><i>Tbx5</i></u> ^{1,4}	<i>CACNA1D</i> ⁴	<i>PLN-BRD7P3</i> ^{1,3,5,6,7}
	<u><i>Tbx3</i></u> ²	<u><i>Tbx3</i></u> ⁴	<u><i>CRIM1</i></u> ⁴	<u><i>Tbx5</i></u> ¹
	<i>CAVI</i> ^{1,2}	<i>C6orf204-SCL35F1</i> -	<i>C1orf185-RNF11</i>	<i>NOS1AP</i> ^{1,3,5,6,7,8}
	<i>ARGHAP24</i> ^{1,2}	<i>PLN-BRD7P3</i> ⁴	<i>CDKN2C-FAF1</i> ⁴	<i>ATP1B1</i> ^{1,3,6}
	<u><i>MEIS1</i></u> ²	<i>CDKN1A</i> ^{1,4}	<i>PRKCA</i> ⁴	<i>KCNH2</i> ^{1,3,5,6}
	<u><i>WNT11</i></u> ²	<i>NFIA</i> ⁴	<i>IGFBP3</i> ⁴	<i>LITAF</i> ^{1,3,5,6}
	<i>SOX5</i> ²	<u><i>HAND1-SAP30L</i></u> ⁴	<i>CASQ2</i> ⁴	<i>KCNJ2</i> ^{1,3,6}
	<u><i>NKX2-5</i></u> ²	<i>SIPA1L1</i> ⁴	<i>KLF12</i> ⁴	<i>KCNQ1</i> ^{1,5,6}
		<u><i>Tbx20</i></u> ⁴	<i>LRG1-SLC25A26</i> ⁴	<i>CNOT1</i> ⁵
		<i>VTG1A</i> ⁴	<u><i>DKK1</i></u> ⁴	<i>RNF207</i> ^{1,5,6}
		<i>GOSR2</i> ⁴	<i>HEATR5B-STRN</i> ⁴	<i>KCNE1</i> ^{1,5}
				<i>LIG3</i> ^{5,6}
				<i>NDRG4-GINS3</i> ^{5,6}

Loci listed in bold have been associated with more than one ECG interval. Underlined loci are linked to developmental processes (see text for details). Superscript numbers reference studies reporting significant genome wide association with the indicated locus as follows:

- (1) Holm et al., 2010
- (2) Pfeufer et al., 2010
- (3) Chambers et al., 2010
- (4) Sotodohenia et al., 2010
- (5) Newton-Cheh et al., 2009
- (6) Pfeufer et al., 2009
- (7) Nolte et al., 2009
- (8) Arking et al., 2006
- (9) Cho et al., 2009
- (10) Denny et al., 2010