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## Genome Wide Association Studies in Cardiac Electrophysiology: Recent Discoveries and Implications for Clinical Practice

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### Abstract

Genome wide association studies have been increasingly used to study the genetics of complex human diseases. Within the field of cardiac electrophysiology, this technique has been applied to traits such as AF, the QT interval, and several electrocardiographic parameters. While these studies have identified multiple genomic regions associated with each trait, questions remain including the best way to explore the pathophysiology of each association and the potential for clinical utility. This review will summarize recent genome wide association study results within cardiac electrophysiology, and discuss their broader implications in basic science and clinical medicine.

### Introduction

In the last three years, genome wide association studies (GWAS) have been used to identify the genetic underpinnings of over 100 traits including electrocardiographic parameters and atrial fibrillation (AF). While increasingly popular, a number of fundamental questions exist. Specifically, what are the implications of these genetic studies? Will the results have utility beyond the basic research setting? Should we as electrophysiologists care about the results? Will these studies add clinical information beyond what is currently available in patient care?

In this review, we seek to describe the methods for performing a GWAS, summarize the latest results of GWAS in cardiac electrophysiology, and explore the implications of these studies in research and patient management.

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## What is a genome wide association study (GWAS)?

Traditional approaches to understanding the genetic basis of heritable traits or diseases have focused on single gene defects with clear modes of inheritance. These diseases or traits are often referred to as Mendelian, familial, or monogenic. In electrophysiology, a classic example is autosomal dominant long QT syndrome. Using *linkage analysis*, investigators identify genetic loci and ultimately a single genetic defect that causes the disease. A limitation of this approach is the need for large families with multiple affected individuals, and a definitive diagnosis for affected and unaffected family members. Once a causative gene has been identified in a Mendelian family, investigators will then use this information to guide *candidate gene resequencing* efforts in other families to identify mutations in related genes. As sequencing technology has advanced and costs declined, candidate gene sequencing has been increasingly employed when family structure does not permit linkage analysis.

In contrast to monogenic traits, which are typically rare, many human diseases and traits are genetically complex. Complex conditions are believed to be caused by the joint effects of many common genetic variants, and potentially, environmental factors. Complex traits may be heritable, and yet have no straightforward mode of inheritance. Diseases such as coronary artery disease and hypertension are complex traits that have proven refractory to linkage analysis. The effect sizes of rare, causal genetic variants in monogenic conditions are large, in contrast to the much smaller effect sizes attributable to common modifying variants in complex traits.

The use of GWAS has led to the identification of hundreds of genetic loci for many complex diseases and traits. These loci serve both as markers of novel pathophysiological signaling pathways and as disease susceptibility loci. Advantages of GWAS over linkage analysis include the ability to use available epidemiologic study populations, and the fact that large families are not necessary. Technological advances permit association testing of a million genetic markers simultaneously. These markers are single nucleotide polymorphisms (SNPs) or commonly occurring variations in single bases throughout the genome. SNPs can be directly associated with disease or may serve as proxies for disease-causing variant in close proximity. Genetic variants that lie in close physical proximity to one another are typically inherited together, and when linked in this way are said to be in *linkage disequilibrium*. Thus, in order to demonstrate a genetic association, investigators do not have to identify or even test the causative variant, so long as they test a nearby variant that is closely linked. However, identification of the causative genetic variant is a critical step for moving beyond the statistical association and providing a greater understanding of disease mechanism.

In a typical GWAS, investigators might select individuals with or without myocardial infarction (MI) from a population. Each individual's DNA is characterized at several hundred thousand locations using a *SNP chip*. Each of these SNPs is then tested for association with MI using after adjusting for differences in ancestry between cases and controls. Variants associated with MI beyond a stringent significance threshold that is adjusted for the large number of independent tests performed (e.g.,  $P < 5 \times 10^{-8}$ ) are subsequently tested in an independent replication cohort for association with MI.

Several limitations of GWAS exist. False-positive associations are inevitable with multiple testing, and therefore GWAS must employ very stringent significance thresholds. Because of the stringent significance thresholds and modest effect sizes of each genetic variant, thousands of subjects are generally necessary. An additional disadvantage of GWAS is that racial variations in SNP frequencies and linkage patterns can confound associations unless population ancestry is properly identified. Studies to date have largely been performed in subjects of European descent, so the applicability to other ethnicities may be limited.

Additionally, GWAS are expensive, with genotyping costs at ~\$400 per person. By design, GWAS will not detect rare variants that are associated with a disease. Finally, a missing feature of almost all GWAS is the effect of environmental factors that influence the trait. It is especially difficult to account for gene-environment interactions where a particular genotype modifies the susceptibility to an environmental exposure.

## GWAS in Cardiac Electrophysiology

Within cardiac electrophysiology, GWAS have been applied to several ECG traits as well as specific arrhythmias. Electrocardiographic traits include the QT interval, but also PR and RR intervals. The diseases studied to date include AF and sudden cardiac death (although SCD was not investigated as a GWAS, but in follow-up to the QT studies.) One of the themes that emerges is that the effect sizes of the genetic variants identified are fairly small. Additionally, it is clear that GWAS identify a genetic region, but not the specific gene or SNP that is responsible for the trait.

### Atrial fibrillation

The first AF susceptibility region was identified on chromosome 4q25 using a GWAS in 2007.<sup>1</sup> The study included 3,580 subjects with AF and 19,256 controls of European ancestry. The most significantly associated SNP at this locus had a *risk allele* frequency of approximately 20%. The robust association with AF demonstrated in this study has been replicated in subsequent studies,<sup>2–5</sup> including an analysis of 333 cases and 2,836 controls of Asian ancestry ( $P=0.0006$ ). The strongest signal at this locus lies in a large interval that contains no known or putative genes. The closest gene, *PITX2* (paired-like homeodomain transcription factor 2), is located approximately 150,000 bases away and is an interesting candidate gene for AF. *PITX2* is known to play an important role in embryonic development including asymmetric morphogenesis of the gut and heart.<sup>6</sup> One transcript variant of *PITX2* is critical for the suppression of a default sinoatrial node in the left atrium and in specifying pulmonary venous myocardium.<sup>7</sup>

The recognition that large sample sizes are necessary for the identification of common variants associated with disease has prompted investigators from independent cohorts to combine their study populations. Analyses from two independent consortia identified a novel susceptibility locus for AF on chromosome 16q22 in individuals of European ancestry.<sup>4,8</sup> The most significantly associated variants from each study had risk allele frequencies of approximately 20%, and fell within the *ZFHX3* (zinc finger homeobox 3) gene. Little is known about the role of *ZFHX3* in the heart or cardiovascular system, but it is interesting to note that the top variant in one of the two GWAS of AF<sup>8</sup> was also strongly associated with Kawasaki disease in a separate GWAS.<sup>9</sup>

A further collaborative study of lone AF was performed in a meta-analysis of five cohorts using 1,335 cases of lone AF and 12,844 referent subjects. The results of the meta-analysis for lone AF are illustrated in the *Manhattan plot* where each point on the graph indicates the strength of association between a SNP and AF (Figure 2). The strongest association with lone AF was at the chromosome 4q25 or *PITX2* locus. A second locus was identified at chromosome 1q21 near a calcium-activated potassium channel, *KCNN3* (KCa2.3 or SK3). The *KCNN* channels are expressed in the brain, heart and vascular endothelium. *KCNN3* has been shown to participate in regulation of blood pressure via the endothelium derived hyperpolarization factor vasodilator pathway.<sup>10,11</sup> Although *KCNN3* remains a strong candidate gene at this locus, further work will be needed to clarify the relation between *KCNN3* and AF.

## PR interval and relation to AF

Two GWAS of the PR interval conducted in subjects of European ancestry also tested the top PR-related variants for association with AF.<sup>12,13</sup> Variants in six of nine genes associated with the PR interval were also associated with AF, albeit at less stringent thresholds (Table 2).<sup>12</sup> The plausibility of the PR interval as an intermediate phenotype for AF is buttressed by observations that PR interval prolongation is associated with AF in the community,<sup>14,15</sup> though not all SNPs associated with an increased risk of AF were associated with PR prolongation in these GWAS.<sup>12,13</sup>

In both studies that tested for associations between PR-related variants and AF, variants in *CAVI* were identified.<sup>12,13</sup> *CAVI* and *CAV2* encode caveolin proteins, which line small invaginations in cellular membranes involved in signal transduction.<sup>16</sup> *CAVI* is expressed in atrial but not ventricular myocytes. Mice deficient in *CAVI* develop dilated cardiomyopathy,<sup>17</sup> pulmonary hypertension,<sup>17</sup> and cardiac hypertrophy.<sup>18</sup> In the study by Pfeufer et al.,<sup>12</sup> additional variants in *SCN5A*, *SCN10A*, *NKX2-5*, and *SOX5* were associated with both the PR interval and AF. In the study by Holm et al.,<sup>13</sup> variants in *TBX5* were also associated with both phenotypes. *SCN5A* and *SCN10A* are two adjacent voltage gated sodium channels. Genetic variants in *SCN5A* underlie numerous arrhythmias, including the sick sinus syndrome, long QT syndrome, the Brugada syndrome, and AF, as mentioned above.<sup>19</sup> *SCN10A* has not been previously implicated in cardiac arrhythmias. The study by Holm et al.<sup>13</sup> did not replicate the association between variants in *SCN10A* and AF, despite a -high degree of *linkage disequilibrium* between the variant tested in this study and that by Pfeufer et al.<sup>12</sup> Interestingly, three transcription factors necessary for cardiac development were related to PR including *NKX2-5*, *SOX5*, and *TBX5a*.

## QT Interval

The first GWAS reported for an electrocardiographic trait was performed in 200 subjects who were found to lie at the extremes of a population-based QT interval distribution of 3,966 subjects.<sup>20</sup> This GWAS identified a signal that lies upstream of the gene *NOS1AP* (*CAPON*) that was associated with QT interval. *NOS1AP* is a regulator of neuronal nitric oxide synthase, and was a new candidate gene for modulation of cardiac repolarization.

Subsequently, two larger GWAS of the QT interval were reported in early 2009. In the QTGEN study, three genome-wide association studies involving 13,685 individuals of European ancestry were meta-analyzed for SNPs associated with QT interval duration. QTGEN investigators observed associations with variants in or near *NOS1AP*, *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A*.<sup>21–24</sup> All of these genes were previously known to be involved in myocardial repolarization and/or long-QT syndromes.<sup>24,25</sup> Five new loci were also identified on 16q21 near *NDRG4* and *GINS3*, 6q22 near *PLN*, 1p36 near *RNF207*, 16p13 near *LITAF*, and 17q12 near *LIG3* and *RFLL*. In the accompanying paper, genome-wide data from five population-based cohorts with a total of 15,842 individuals of European ancestry confirmed associations between variants in *NOS1AP*, *KCNQ1*, *KCNH2*, *SCN5A* and *KCNJ2* and the QT interval.<sup>26</sup> Two other loci included *ATP1B1* and *PLN*, genes with established electrophysiological function, whereas three mapped to *RNF207*, near *LITAF* and within *NDRG4-GINS3-SETD6-CNOT1*, none of which had previously been implicated in cardiac electrophysiology.

There are several noteworthy findings. First, all three studies of QT interval have consistently identified a region near *NOS1AP*. Second, the two large GWAS of QT interval identified common variants in 5 of the known Long QT syndrome genes. These results can be viewed as a proof of concept since these genes had the highest pretest probability of influencing QT interval in the general population. Finally, even the novel loci in the two

large QT GWAS studies have significant overlap, highlighting genomic regions that do not contain known ion channels or genes with established roles in cardiac electrophysiology.

Currently, genetic testing is able to identify mutations in known long QT genes in approximately 75% of probands. The novel loci identified by these GWAS may contain new long QT genes that might be responsible for some of the remaining cases, though in order to demonstrate this, causal genes will need to be identified. Even if these loci do not identify novel monogenic long QT syndrome genes, the causal genes may modify the severity of the Long QT phenotype, as has been demonstrated for *NOS1AP* in one large South African long QT population.<sup>27</sup> Further work will be required to determine whether this result is applicable outside the single highly inbred family where it was found. These genetic signals may also yield information about susceptibility to drug-induced QT prolongation and torsades de pointes (TdP). It is known that baseline QT prolongation is a risk factor for drug-induced QT prolongation and TdP, but whether any of the genetic associations in baseline QT interval will be major determinants of this risk remains to be seen.

## Sudden Cardiac Death

Longer QT intervals are associated with increased risk of sudden cardiac death.<sup>28–30</sup> Therefore, a logical next step was to determine if variation at the most strongly associated locus, near *NOS1AP*, alters the risk of sudden cardiac death. In a study of two populations of more than 19,000 subjects and 498 episodes of SCD, a significant association was observed with two different SNPs at the *NOS1AP* locus.<sup>31</sup> The first SNP was correlated with longer QT interval and increased risk of sudden death, while the second SNP was not correlated with QT interval, but seemed to be associated with decreased risk of sudden death. The effects were only observed in white subjects and the discordance in the associations between blacks and whites may represent the result of lower statistical power (due to inappropriate tagging SNPs and smaller numbers of events) in blacks, or it may represent a real genetic difference.

## RR interval

A GWAS in 8,842 samples from Korea reported two genomic loci that were associated with heart rate.<sup>32</sup> The strongest signal was found in a region with no likely candidate gene and was responsible for <1 beat per minute change in heart rate per variant allele. The next signal was responsible for approximately 0.5 beats per minute variation. The closest gene has an unknown function, but the connexin gene *GJAI* is 400kb away.<sup>32</sup> A second GWAS examined ~10,000 individuals of European descent and found a single locus for heart rate at the gene encoding alpha-myosin heavy chain (*MYH6*).<sup>13</sup> The two loci identified in the Korean population failed to replicate in this study, perhaps because of population differences. The *MYH6* genetic variant explains about 0.1% of the variability in heart rate. Finally, a third GWAS for heart rate identified a SNP within the *GPR133* gene,<sup>33</sup> a G-protein coupled receptor that interestingly had been previously reported to be associated with height.<sup>34</sup>

## QRS Duration

Researchers at deCODE genetics recently published four genetic loci associated with QRS duration.<sup>13</sup> Two of these loci, near *TBX5* and *SCN10A* were also implicated in PR interval, suggesting potential roles in generalized myocyte impulse propagation. The other two loci did not contain obvious candidate genes. The first linked region contains only a single gene, cyclin dependent kinase inhibitor 1A, and the second region contained several genes, the closest is *DKK1*, an inhibitor of the Wnt signaling pathway. Together these variants explain

only 0.6% of the variance in QRS duration, but they may identify novel signaling, developmental, or regulatory pathways that are important in cardiac electrophysiology.

## Clinical implications of these findings

From a clinical perspective, the value of these findings remains to be determined. While, in general, individual magnitudes of effects are too small to be of clinical predictive value, a combination of genetic risk factors may provide meaningful information. Additional studies will be needed to determine whether these variants might influence response to specific therapies or identify subtypes of disease states.

### Atrial Fibrillation

In the case of AF, the effect sizes for the genetic variants identified to date are sufficiently large to be expected to eventually provide clinically useful risk profiling. Although genotyping at the 4q25 locus is commercially available to identify individuals at risk of AF, we do not believe that routine testing is currently warranted. Genetic risk stratification will ultimately incorporate testing at all of the major AF loci and its utility will be dependent on whether such testing alters clinical practice. Potential applications include prediction of response to pharmacological or procedural therapies, progression of disease, or risk prediction of outcomes such as heart failure, stroke, and death (Figure 4).

### QT Interval and Sudden Cardiac Death

The effect estimates for the common genetic variants associated with the QT interval and SCD are small. Therefore, genotyping NOS1AP to predict SCD risk is unlikely to be useful in clinical testing. However, in the case of QT interval, there are environmental factors, chief among them drug exposure, that pose a significant health hazard to a minority of individuals. It is possible that one or a combination of many SNPs associated with the QT interval may identify individuals at greater risk for drug-induced QT prolongation and TdP, but at present such testing is not warranted.

## Future research directions

Although the clinical utility of GWAS data at present remains limited, each genetic locus that is associated with a phenotype identifies a relevant pathway influencing human physiology. To a large extent, enthusiasm within the scientific community for GWAS is based on the fact that, unlike the study of model organisms in which human relevance is always in question, GWAS is an unbiased approach to understanding physiology and disease in humans. Novel loci uncovered by this method will require further investigation using a variety of animal models, cell culture and genetic approaches to understand the fundamental mechanisms responsible for the original genetic signal. Years of work may lie ahead in unraveling such mechanisms, but their relevance to human physiology is already established (Figure 4).

The modest effect sizes expected with common variants<sup>35</sup> suggest that larger sample sizes may be necessary to identify additional novel signals. These signals will likely have even smaller effect sizes than the ones already discovered and discussed in this review. However, it is hoped that patterns will emerge that allow identification of entire signaling pathways and networks that regulate a given complex trait. Regions of the genome that have not been extensively analyzed, such as the X and Y chromosomes or mitochondria, have the potential to harbor variants that affect phenotypic diversity. Moreover, the search for variants that interact with environmental variables may reveal loci that have effects only among those individuals exposed to specific triggers or with a particular substrate.

Studying the genetic basis of disease in populations of non-European ancestry has great potential to enhance our understanding of the biological mechanisms underlying disease. Most of the understanding of AF epidemiology, genetic and otherwise, is derived from the study of individuals of European descent.<sup>36</sup> As a result of the migration patterns of human populations, linkage disequilibrium patterns differ between different racial and ethnic groups. As such, high density genotyping, or fine mapping, in different ancestral populations may help narrow the boundaries of a common disease susceptibility locus. AF prevalence has significant racial and ethnic differences,<sup>37</sup> which may potentially be explained by different frequencies of common susceptibility variants or distinct susceptibility loci.

Genetic variation only represents one aspect of a complementary network of signals that influence phenotypic variation.<sup>38</sup> Epigenetic phenomena such as DNA methylation and gene silencing, as well as complex interactions between distinct genetic elements, differentially expressed transcripts, and metabolic and proteomic signals, may also explain a substantial proportion of phenotypic diversity. Bioinformatic techniques, improved phenotyping informed by molecular, electrophysiological, or refined clinical features may enhance the discovery of loci and networks that govern disease pathogenesis.

## Conclusions

GWAS have identified novel biological signals involved in human electrophysiology and disease. Findings from GWAS present tremendous opportunities to understand the mechanisms by which these novel pathways relate to human health and disease. Future research will focus on whether consideration of variants identified in GWAS can facilitate the identification of individuals at risk for disease, guide clinical management decisions, and relate to prognosis.

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## Glossary

Candidate gene resequencing	A candidate gene is hypothesized to be biologically related to a particular condition, based on preexisting knowledge of physiology, proximity to an association signal, or some other prior information. Candidate gene resequencing is the screening of genes for mutations in a cohort of individuals with a given condition.
Genome wide significance	When performing multiple tests of association, the chances for a false positive finding are increased. Adjusting the significance threshold for the number of tests performed will mitigate false positives, but results in smaller P value requirements in order to declare true associations. GWAS usually employ a conservative Bonferroni adjustment, dividing the traditional significance threshold of 0.05 by the number of independent tests performed. A commonly accepted threshold is $P < 5 \times 10^{-8}$ .
Haplotype	Alleles on the same chromosome that are inherited together form a haplotype. Haplotypes are bounded by areas where recombination is likely to occur during meiosis.

Human Genome Project	The large scale effort to sequence every one of the 3 billion nucleotides in the human genome. The completion of this project has provided investigators a roadmap for the genome that is now readily searchable at <a href="http://www.ucsc.genome.org">www.ucsc.genome.org</a> or <a href="http://www.ncbi.org">www.ncbi.org</a> .
Linkage analysis	A technique to identify a locus or genetic region associated with a disease. In a Mendelian family, the disease gene and the known marker will be linked or shared in common with all affected individuals and not by the unaffected individuals.
Linkage disequilibrium	The non-random association of two alleles. Alleles in linkage disequilibrium are found together more often than expected by chance alone.
Manhattan plot	A plot of the $-\log_{10}$ of the p value versus chromosomal location for each SNP. Higher points on the Y axis indicate stronger associations (resembling the Manhattan skyline).
Single nucleotide polymorphism (SNP)	Common genetic variation at a single base pair of DNA in the genome. Typically SNPs are biallelic or due to the presence of one of two variations at a given base pair. For example: GATTC versus GACTC. SNPs account for an estimated 90% of the variation between individuals.
Risk allele	The form of a single nucleotide polymorphism that is associated with disease.

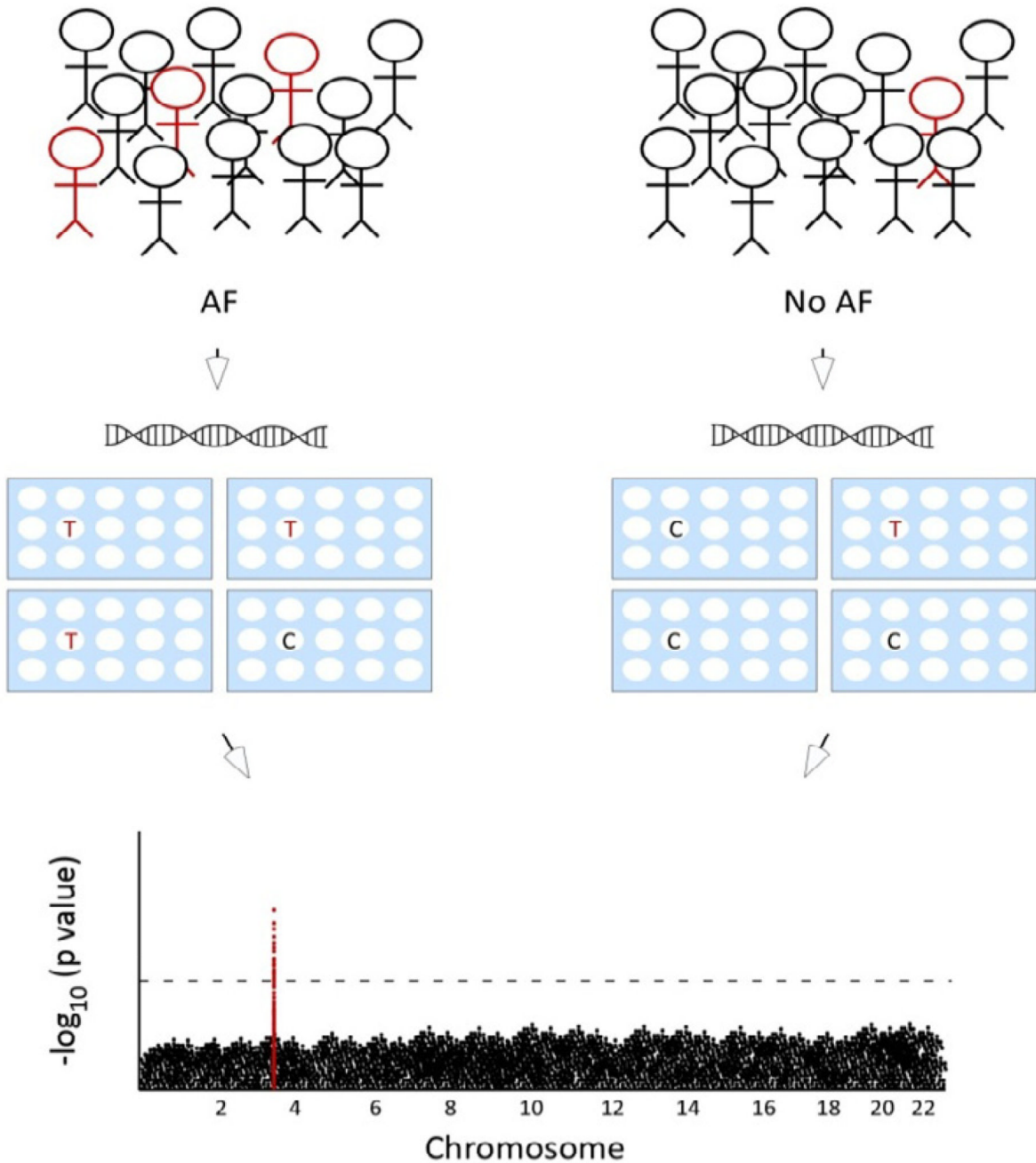
## References

- Gudbjartsson DF, Arnar DO, Helgadóttir A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature* 2007;448:353–357. [PubMed: 17603472]
- Kaab S, Darbar D, van Noord C, et al. Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. *Eur Heart J* 2009;30:813–819. [PubMed: 19141561]
- Body SC, Collard CD, Shernan SK, et al. Variation in the 4q25 chromosomal locus predicts atrial fibrillation after coronary artery bypass graft surgery. *Circ Cardiovasc Genet* 2009;2:499–506. [PubMed: 20031626]
- Benjamin EJ, Rice KM, Arking DE, et al. Variants in ZFHX3 are associated with atrial fibrillation in individuals of European ancestry. *Nat Genet* 2009;41:879–881. [PubMed: 19597492]
- Ellinor PT, Lunetta KL, N.L G, et al. Common Variants in KCNN3 are Associated with Lone Atrial Fibrillation. *Nat Genet* 2010;42:240–244. [PubMed: 20173747]
- Logan M, Pagan-Westphal SM, Smith DM, Paganessi L, Tabin CJ. The transcription factor Pitx2 mediates situs-specific morphogenesis in response to left-right asymmetric signals. *Cell* 1998;94:307–317. [PubMed: 9708733]
- Mommersteeg MT, Brown NA, Prall OW, et al. Pitx2c and Nkx2-5 are required for the formation and identity of the pulmonary myocardium. *Circ Res* 2007;101:902–909. [PubMed: 17823370]
- Gudbjartsson DF, Holm H, Gretarsdóttir S, et al. A sequence variant in ZFHX3 on 16q22 associates with atrial fibrillation and ischemic stroke. *Nat Genet* 2009;41:876–878. [PubMed: 19597491]
- Burgner D, Davila S, Breunis WB, et al. A genome-wide association study identifies novel and functionally related susceptibility Loci for Kawasaki disease. *PLoS Genet* 2009;5:e1000319. [PubMed: 19132087]
- Brahler S, Kaistha A, Schmidt VJ, et al. Genetic deficit of SK3 and IK1 channels disrupts the endothelium-derived hyperpolarizing factor vasodilator pathway and causes hypertension. *Circulation* 2009;119:2323–2332. [PubMed: 19380617]



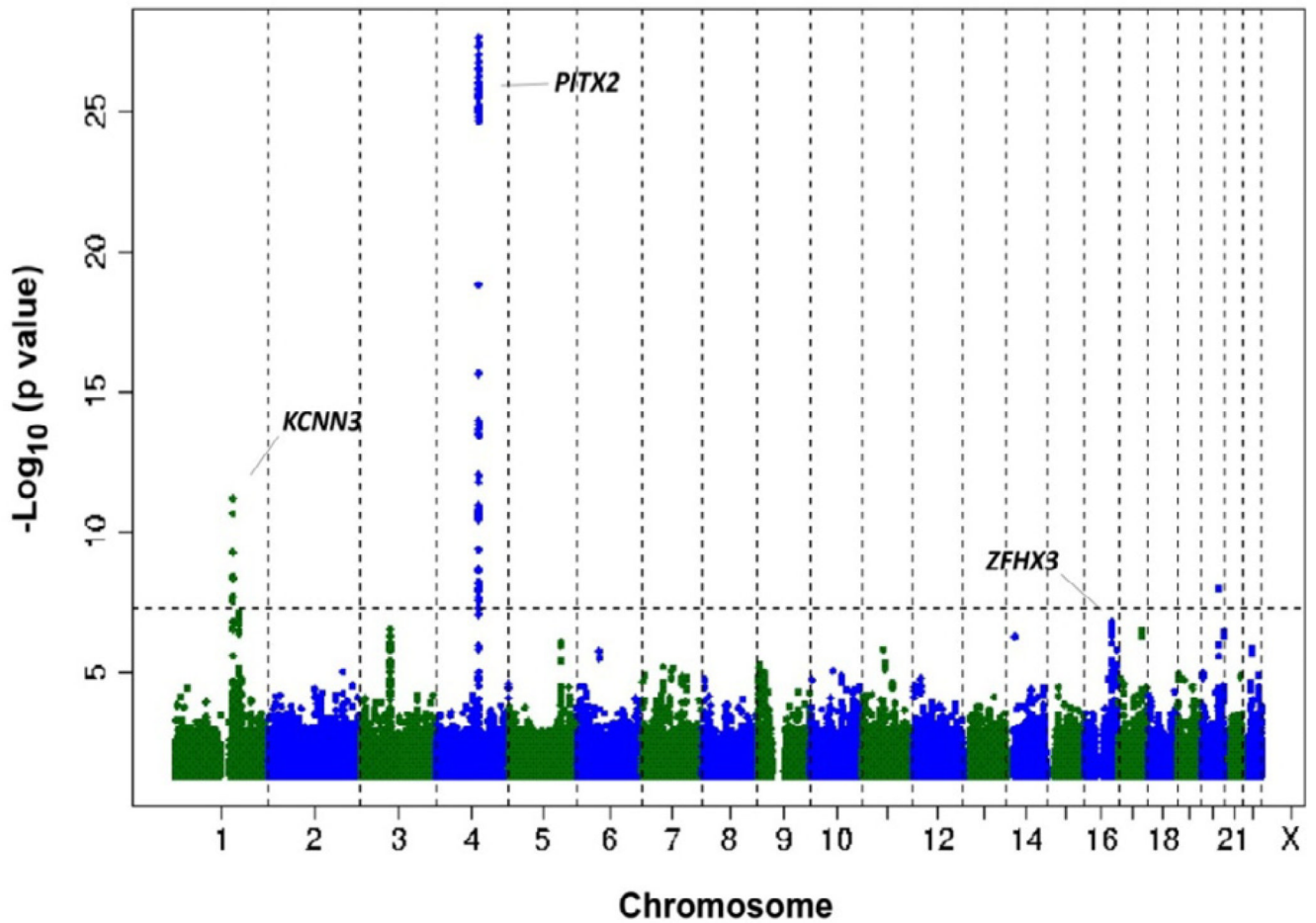
11. Taylor MS, Bonev AD, Gross TP, et al. Altered expression of small-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> (SK3) channels modulates arterial tone and blood pressure. *Circ Res* 2003;93:124–131. [PubMed: 12805243]
12. Pfeufer A, van Noord C, Marcianti KD, et al. Genome-wide association study of PR interval. *Nat Genet* 2010;42:153–159. [PubMed: 20062060]
13. Holm H, Gudbjartsson DF, Arnar DO, et al. Several common variants modulate heart rate, PR interval and QRS duration. *Nat Genet* 2010;42:117–122. [PubMed: 20062063]
14. Schnabel RB, Sullivan LM, Levy D, et al. Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. *Lancet* 2009;373:739–745. [PubMed: 19249635]
15. Cheng S, Keyes MJ, Larson MG, et al. Long-term outcomes in individuals with prolonged PR interval or first-degree atrioventricular block. *JAMA* 2009;301:2571–2577. [PubMed: 19549974]
16. Gratton JP, Bernatchez P, Sessa WC. Caveolae and caveolins in the cardiovascular system. *Circ Res* 2004;94:1408–1417. [PubMed: 15192036]
17. Zhao YY, Liu Y, Stan RV, et al. Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice. *Proc Natl Acad Sci U S A* 2002;99:11375–11380. [PubMed: 12177436]
18. Cohen AW, Park DS, Woodman SE, et al. Caveolin-1 null mice develop cardiac hypertrophy with hyperactivation of p42/44 MAP kinase in cardiac fibroblasts. *Am J Physiol Cell Physiol* 2003;284:C457–C474. [PubMed: 12388077]
19. Ruan Y, Liu N, Priori SG. Sodium channel mutations and arrhythmias. *Nat Rev Cardiol* 2009;6:337–348. [PubMed: 19377496]
20. Arking DE, Pfeufer A, Post W, et al. A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. *Nat Genet* 2006;38:644–651. [PubMed: 16648850]
21. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. *Cell* 1995;81:299–307. [PubMed: 7736582]
22. Wang Q, Curran ME, Splawski I, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet* 1996;12:17–23. [PubMed: 8528244]
23. Splawski I, Timothy KW, Tateyama M, et al. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. *Science* 2002;297:1333–1336. [PubMed: 12193783]
24. Splawski I, Tristani-Firouzi M, Lehmann MH, Sanguinetti MC, Keating MT. Mutations in the hminK gene cause long QT syndrome and suppress IKs function. *Nat Genet* 1997;17:338–340. [PubMed: 9354802]
25. Splawski I, Timothy KW, Vincent GM, Atkinson DL, Keating MT. Molecular basis of the long-QT syndrome associated with deafness. *N Engl J Med* 1997;336:1562–1567. [PubMed: 9164812]
26. Pfeufer A, Sanna S, Arking DE, et al. Common variants at ten loci modulate the QT interval duration in the QTSCD Study. *Nat Genet* 2009;41:407–414. [PubMed: 19305409]
27. Crotti L, Monti MC, Insolia R, et al. NOS1AP is a genetic modifier of the long-QT syndrome. *Circulation* 2009;120:1657–1663. [PubMed: 19822806]
28. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J. QTc prolongation measured by standard 12-lead electrocardiography is an independent risk factor for sudden death due to cardiac arrest. *Circulation* 1991;83:1888–1894. [PubMed: 2040041]
29. Schouten EG, Dekker JM, Meppelink P, et al. QT interval prolongation predicts cardiovascular mortality in an apparently healthy population. *Circulation* 1991;84:1516–1523. [PubMed: 1914093]
30. Straus SM, Kors JA, De Bruin ML, et al. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. *J Am Coll Cardiol* 2006;47:362–367. [PubMed: 16412861]
31. Kao WH, Arking DE, Post W, et al. Genetic variations in nitric oxide synthase 1 adaptor protein are associated with sudden cardiac death in US white community-based populations. *Circulation* 2009;119:940–951. [PubMed: 19204306]
32. Cho YS, Go MJ, Kim YJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet* 2009;41:527–534. [PubMed: 19396169]

33. Marroni F, Pfeufer A, Aulchenko YS, et al. A genome-wide association scan of RR and QT interval duration in 3 European genetically isolated populations: the EUROSPAN project. *Circ Cardiovasc Genet* 2009;2:322–328. [PubMed: 20031603]
34. Tonjes A, Koriath M, Schleinitz D, et al. Genetic variation in GPR133 is associated with height: genome wide association study in the self-contained population of Sorbs. *Hum Mol Genet* 2009;18:4662–4668. [PubMed: 19729412]
35. Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. *Science* 2008;322:881–888. [PubMed: 18988837]
36. Benjamin EJ, Chen PS, Bild DE, et al. Prevention of atrial fibrillation: report from a National Heart, Lung, and Blood Institute workshop. *Circulation* 2009;119:606–618. [PubMed: 19188521]
37. Soliman EZ, Alonso A, Goff DC Jr. Atrial fibrillation and ethnicity: the known, the unknown and the paradox. *Future Cardiol* 2009;5:547–556. [PubMed: 19886781]
38. Oti M, Huynen MA, Brunner HG. Phenome connections. *Trends Genet* 2008;24:103–106. [PubMed: 18243400]
39. Newton-Cheh C, Eijgelsheim M, Rice KM, et al. Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nat Genet* 2009;41:399–406. [PubMed: 19305408]
40. Nolte IM, Wallace C, Newhouse SJ, et al. Common genetic variation near the phospholamban gene is associated with cardiac repolarisation: meta-analysis of three genome-wide association studies. *PLoS One* 2009;4:e6138. [PubMed: 19587794]
41. Kaab S, Darbar D, van Noord C, et al. Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. *Eur Heart J*. 2009



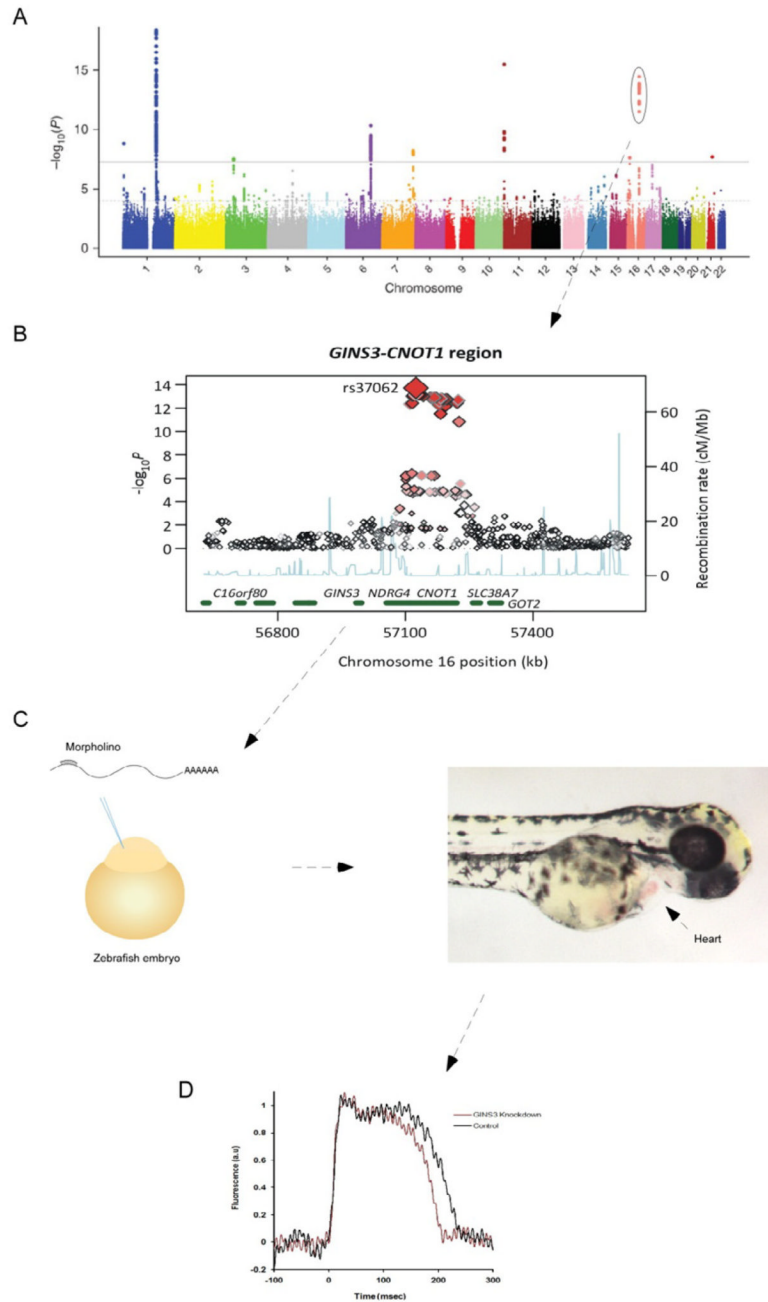
**Figure 1. Overview of a GWAS**

To perform a GWAS, cases and control subjects are genotyped at 500,000 to 1 million common SNPs throughout the genome to identify regions associated with disease. On top is a population with carriers of a risk allele indicated in red. In the middle, a sample of DNA is obtained from each subject and genome wide genotyping is performed. On bottom, a comparison is made of the frequency of each SNP throughout the genome in cases versus controls. The genetic interval associated with disease is indicated in red.

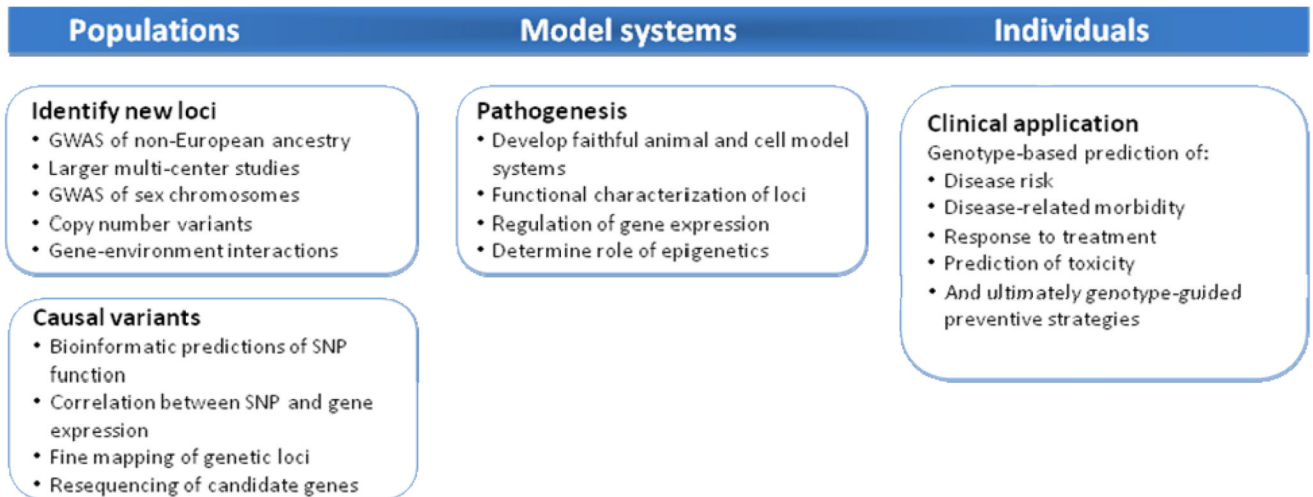


**Figure 2. Genetic loci for AF identified by GWAS**

To date, three genetic loci associated with AF have been identified near *KCNN3*, *PITX2*, and *ZFHX3*. The  $-\log_{10}(p \text{ value})$  is plotted against the physical positions of each SNP on each chromosome. The threshold for genome-wide significance,  $P < 5 \times 10^{-8}$ , is indicated by the dashed line. Reprinted with permission from *Nature Genetics* 42, 240–244 (2010).



**Figure 3. Functional characterization of a genetic locus for the QT interval**  
 Manhattan plot of SNPs associated with the QT interval with the *GINS3* locus encircled (A). A regional plot of the *GINS3* locus illustrating the SNPs associated with the QT interval (B). Genes in this region were knocked down in the zebrafish embryo by injecting a morpholino directed against *GINS3* (C). Hearts from 48 hour old zebrafish were optically mapped and the action potential duration determined in wild-type and *GINS3* morpholino knockout fish (D). Knockdown of *GINS3* results in a shortening of the action potential duration. Panels A and B reprinted with permission from *Nature Genetics* 41, 399–406 (2009).



**Figure 4. Future directions in AF genomics**

An outline of potential research questions and clinical applications to arise from studies of AF genetics.

**Table 1**

## GWAS Results for Electrocardiographic Intervals.

ECG traits / Locus	Gene name	References
<b>PR interval</b>		
<i>MEIS1</i>	Meis homeobox 1	12
<i>SCN5A</i>	Sodium channel, voltage-gated, type V	12
<i>SCN10A</i>	Sodium channel, voltage-gated, type X	12
<i>ARHGAP24</i>	Rho GTPase activating protein 24	12
<i>NKX2-5</i>	NK2 transcription factor related, locus 5	12
<i>CAV1/CAV2</i>	Caveolin 1, 2	12
<i>WNT11</i>	Wingless-type MMTV integration site family, member 11	12
<i>SOX5</i>	Sex determining region Y-box 5	12
<i>TBX5/TBX3</i>	T-box 5, 3	12
<b>QT interval</b>		
<i>RNF207</i>	Ring finger protein 207	26,39
<i>NOS1AP</i>	Nitric oxide synthase 1 adaptor protein	26,39
<i>ATP1B1</i>	ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, beta 1 polypeptide	26
<i>SCN5A-SCN10A</i>	Sodium channel, voltage gated, types V and X	26,39
<i>PLN</i>	Phospholamban	26,39,40
<i>KCNH2</i>	HERG / Potassium voltage-gated channel, subfamily H (eag-related), member 2	26,39
<i>KCNQ1</i>	Potassium voltage-gated channel, KQT-like subfamily, member 1	26,39
<i>LITAF</i>	Lipopolysaccharide-induced TNF factor	26,39
<i>NDRG4</i>	NDRG family member 4	26,39
<i>RFFL</i>	Ring finger and FYVE-like domain containing 1	39
<i>KCNJ2</i>	Potassium inwardly-rectifying channel, subfamily J, member 2	26
<i>KCNE1</i>	Potassium voltage-gated channel, Isk-related family, member 1	39
<b>RR interval</b>		
<i>MYH6</i>	Alpha myosin heavy chain	13
<i>GJA1</i>	Gap junction protein, alpha 1, 43kDa	32
<i>CD34</i>	CD34 molecule	32
<i>GPR133</i>	G-protein Coupled Receptor	32
<b>QRS duration</b>		
<i>TBX5</i>	T-box 5	13
<i>SCN10A</i>	Sodium channel, voltage-gated, type X	13
<i>CDKN1A</i>	Cyclin Dependent Kinase Inhibitor 1A	13
<i>DKK1</i>	Dickkopf-related protein 1	13

**Table 2**

## GWAS Results for Atrial Fibrillation

<b>Locus</b>	<b>Chromosome</b>	<b>OR (per allele)</b>	<b>Gene name</b>	<b>References</b>
<i>PITX2</i>	4	1.68	Paired-like homeodomain 2	1,41
<i>ZFHX3</i>	16	1.25	Zinc finger homeobox 3	4,8
<i>KCNN3</i>	1	1.52	Small conductance calcium-activated potassium channel protein 3	5