Published in final edited form as:
Circ Res. 2014 April 25; 114(9): 1469–1482. doi:10.1161/CIRCRESAHA.114.302225.

Emerging Directions in the Genetics of Atrial Fibrillation

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Abstract

Atrial fibrillation (AF) is the most common arrhythmic disorder, and currently affects nearly 3 million Americans, 8.8 million Europeans, and an estimated 30 million individuals worldwide. The clinical risk factors for AF are numerous, with age, sex, hypertension, obesity, and ischemic heart disease among the most prevalent. Over the last ten years, a preponderance of evidence also suggests a large genetic contribution to AF. The earliest report of familial AF dates to the early 1940s[1]. Since then, it has become apparent that AF in referral populations[2,3] and in the community is heritable[4,5]. Indeed, having a family member with AF is associated with a 40% increased risk for the arrhythmia[6]. Once the heritability was recognized, traditional genetics techniques for the discovery of rare, monogenic causes of AF were used to identify the initial AF genes. These studies in turn, informed candidate gene screening in AF cohorts. To identify additional sources of heritability for AF, large-scale analyses of common variation through genome wide association studies (GWAS) has recently yielded data identifying risk loci in many regions of the genome. In spite of these advances, the combination of these techniques has, as yet, failed to completely identify the heritability of AF in the population. It is the goal of this review to examine the previous studies on rare variants, address the findings of the recent GWAS studies, and describe future avenues towards defining the heritability of AF.

Keywords

Atrial fibrillation; genetics; arrhythmia

Mendelian and Candidate Gene Studies

Classic genetic techniques such as linkage analysis have been used with great success to identify the genetic basis of hypertrophic cardiomyopathy, long QT syndrome and many other heritable conditions. Although there had been sporadic reports of families with AF over the last 60 years, the first application of such methods for AF arose from work by Bob Roberts and colleagues published in the *New England Journal of Medicine* in 1997[7]. In this manuscript, they identified a genetic locus for AF using a series of related families with early-onset AF. Although the specific causative gene at this locus remains unknown, this study helped to firmly establish a genetic basis for some patients with AF.

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Disclosures The authors have no conflicts of interest to report.

In a seminal manuscript published in *Science* in 2003, Yi-Han Chen and colleagues identified the first gene for familial AF[8]. Using a large Chinese kindred with autosomal dominant AF, they found a gain of function mutation in *KCNQ1* or the gene encoding the alpha subunit of the slowly repolarizing potassium channel current, I_{Ks}. The identification of a well-known ion channel mutation for AF quickly led many groups to turn to candidate gene screening of a wide range of cardiac genes. Indeed, several additional gain of function variants have been identified in *KCNQ1*[9–14]. A challenge with the interpretation of these candidate gene studies is that most lack convincing genetic support in the form of variant transmission in extended families. With this limitation in mind, we have provided an overview of the genes related to AF in the following section, and we have included a detailed compendium of known AF variants in Table 1.

Ion channel variation in AF

In the broadest terms, the majority of functionally validated, AF-associated potassium channel variants have a gain of channel function, with an expected shortening of the atrial action potential duration and atrial refraction period. In addition to *KCNQ1*, mutations have been identified in potassium channels genes including *KCNA5*,[15], *KCND3*,[16] and *KCNJ2*[17,18] and accessory subunits *KCNE1*,[19] *KCNE2*,[20] *KCNE3*,[21] and *KCNE5*.[22] Alternatively, it has also been demonstrated that prolongation of atrial action potentials caused by loss of function potassium channel mutations can lead to early after-depolarizations and AF,[23] After an initial description by the Olson laboratory,[24] additional mutations in *KCNA5* of the I_{Kur} current have been reported in subsequent years[15,25,26].

Variation in sodium channel subunits has also been identified as an important factor in the development of familial AF. Voltage-gated sodium channels (NaV) are responsible for initiating the upstroke during phase 0 of cardiac action potential and for the coordinated propagation of the action potential throughout the atria. Cardiac sodium channels are composed of a pore-forming alpha subunit, and beta subunits, which can alter channel trafficking and inactivation kinetics. To date, AF-causing variants have been observed in both the major cardiac sodium channel, encoded by *SCN5A*[27–32], and four of its associated beta subunits[33–37]. Similarly to reports of potassium channel variation, both loss and gain of function variation seem to be capable of creating a pro-arrhythmogenic substrate.

Other Genes Discovered in Individuals and Families with AF

Several variants have also been identified in genes which do not directly alter the atrial action potential, but instead would be expected to instigate the onset of AF through alternative mechanisms. Along these lines, Gollob et al discovered a series of somatic mutations in *GJA5*[38], which encodes the gap junctional protein, Connexin 40. Interestingly, while this mutation was observed in atrial biopsies, it was not found in DNA isolated from blood. The extent to which somatic mutation or mosaicism contribute to the AF is unclear, and further study is often limited by the difficulty in obtaining primary samples. Further lending support to *GJA5* as an AF candidate gene, recently, several reports have identified additional GJA5 loss of function variants that associate with disease. Since gap junctions are responsible for propagation of action potentials between cardiomyocyes, disruption of these

complexes can result in reduced conduction velocity throughout the atrium, conditions that would be predicted to promote reentry.

Another study identified a frameshift mutation which resulted in early truncation of *NPPA* in an extensive family with lone AF[39]. *NPPA* encodes the precursor for atrial naturetic peptide (ANP), an important factor in the regulation of sodium homeostasis and, by association, blood pressure. This mutation was shown to increase the resistance of ANP to degradation, in essence causing an increase in ANP-mediated signaling[40]. In this study, when the mutant, mature ANP was perfused in a rat, whole heart, Langendorff model there was significant shortening of the atrial action-potential duration. While the APD shortening may be the major phenotype observed following acute treatment, prolonged systemic exposure to the mutant ANP could also cause AF-inducing structural remodeling, as seen in canine models[41] and supported by the recent identification of an autosomal recessive mutation in *NPPA* in a family with severe atrial dilated cardiomyopathy[42].

Finally, genes broadly characterized under the umbrella of developmentally related cardiac transcription factors have also been identified as being associated with AF. Specifically, genetic variation in *NKX2.5*[43], *PITX2*[44], *GATA4*[45–47], *GATA5*[48,49], and *GATA6*[50] have been described, although the mechanisms whereby these lead to disease have remained unclear.

Genome-Wide Association Studies of AF

Until the mid-2000s, linkage and candidate gene sequencing methods were the predominant approaches used to identify AF genes. In 2005, a novel technique, termed a genome-wide association study or GWAS, was utilized to identify genetic loci associated with age-related macular degeneration[51]. A GWAS relies on the unbiased comparison of common single nucleotide polymorphisms or SNPs throughout the genome. SNPs that occur with different frequency in individuals with a disease versus controls can localize disease-related genetic loci. While a potentially powerful tool for identifying genetic variation associated with common diseases, careful correction for multiple testing is necessary. Since that initial publication, over 1,700 GWAS have been published listing associations at nearly 12,000 SNPs. Among cardiovascular diseases, this technique has successfully identified risk loci for premature myocardial infarction[52], hypertension[53], lipid levels[54], and electrocardiographic intervals[55–60], among others.

Initial GWAS Studies of AF

The first GWAS performed for AF was published in 2007 and identified a region on chromosome 4q25 (sentinel SNP rs2200733) which was associated with AF in those of European and Asian descent[61]. Subsequently, these findings were broadly replicated in individuals of European[62,63], Asian[64], and African-American[65] descent. Further analysis also identified the same genomic region as being associated with an increased risk of cardioembolic stroke [64,66,67] and a prolonged PR interval [56,68]. In a recent meta-analysis of AF GWAS data, carriers of a single copy of the 4q25 variant had a nearly 65% increased risk of AF (odds ratio (OR) of 1.64 for rs2634073 ($p=1.8\times10^{-74}$) [69]. A follow up fine mapping study of the 4q25 locus identified at least three independent association signals within this region[70]. When these three signals are considered together, there is a subset of

~1% of the population that has all six risk alleles and a nearly six-fold risk of AF (OR of 6.02, $p=1.2\times10^{-36}$).

The 4q25 risk region lies in a relatively gene-sparse intergenic region approximately 150 kb upstream from the *PITX2* gene. Although at present there is no data linking the SNPs in this region to the expression levels of Pitx2, our current understanding of Pitx2 function suggests a plausible link with AF. *PITX2* encodes the paired-like homeodomain 2 protein, a transcription factor which is crucial during embryogenesis and, notably for AF, cardiogenesis[71–75]. Pitx2 expression is near the closing stages of the left/right asymmetry program in vertebrates, with 100 fold higher expression in the left versus the right atrium[76]. Critical roles for Pitx2 have also been identified for formation of the atrial septum, outflow tract, SA node, and the pulmonary vein myocardial sleeves[77,78]. The last of these is of particular note given the prevalence of ectopic electrical foci arising from the pulmonary vein in patients with AF, and the common approach of electrically isolating the pulmonary veins to treat recurrent AF.

Evaluation of Pitx2 knockout mice have also been informative for potential mechanisms whereby misregulation of Pitx2 could contribute to AF. Specifically, whereas homozygous knockout is embryonic lethal, haploinsufficiency of the predominant cardiac isoform, PITX2c, results in a shortened atrial action potential and an increased susceptibility to AF following burst pacing[76]. The same study also identified continued expression of Pitx2c in the left atrial myocardium, but whether altered adult expression in the myocardium contributes to the causation of AF in the absence of developmental differences is unclear. Atrial-specific conditional knockout of Pitx2c also results in perturbation of the action potential and resting membrane potential[79]. Further, deletion of PITX2c expression results in diminished expression of cardiac sodium and potassium channels[80].

Following this initial study, the need for greater statistical power was recognized and led to the formation of the CHARGE-AF or AFGen Consortium. In 2009, two groups independently identified a second locus for AF at 16q22 in Europeans and Han Chinese[81,82]. These results were later replicated in individuals of African-American descent[65]. The AF risk SNP at this locus is intronic to the gene *ZFHX3*, alternatively known as ATBF1, which encodes a zinc finger homeobox transcription factor. ZFHX3 expression has been identified as a factor in the terminal differentiation of both neuronal and striated muscle tissues[83,84], and also reported as a putative tumor suppressor gene[85,86]. Given these roles in other tissues, and its apparent expression within cardiac tissues[87], a developmental role in the atria is possible. However, the lack of availability of model systems with altered ZFHX3 expression has limited the understanding of its potential role in AF. Development of these resources will undoubtedly aid in the discovery of the potential mechanisms whereby this gene, and this susceptibility locus, may be related to AF.

In a separate GWAS from the CHARGE-AF Consortium, patients with early-onset AF were used in hopes of minimizing any sample heterogeneity that may have been seen in previous analyses. In a meta-analysis of five GWAS studies with early-onset AF, a region intronic to the *KCNN3* gene was identified[88]. Similar to the majority of targets identified in candidate gene studies in familial AF, *KCNN3* encodes a potassium channel responsible for membrane

repolarization. The encoded protein, the SK3 channel, is a calcium-activated, small conductance potassium channel which has largely been studied for its role in neuronal electrophysiology. In neurons, SK3 acts in late repolarization to reduce excitability of neurons following repeated stimulation, a phenomenon termed afterhyperpolarization[89]. The role of the KCNN3 in the heart is much less clear, but some evidence exists for a role of SK family members in AF pathogenesis. Among these, studies regarding the deletion of the SK2 channel in mice found a prolongation in cardiac action potentials and increased susceptibility to AF[90]. Further, blockade of the SK family-mediated $I_{K,Ca}$ current also confers an increased risk of atrial arrhythmias in rodents[91] and canines[92]. Finally, recent reports utilizing a mouse model of altered SK3 expression demonstrated alterations in atrial myocyte repolarization[93] and an increased incidence of inducible atrial arrhythmias[94]. Together, these data suggest a mechanism whereby altered expression of SK3 may have important implications on the electrical stability of the atrium.

Meta-analysis Identification of Novel AF Loci

In 2010, the AFGen Consortium published a meta-analysis of GWAS data from 16 different studies meta-analysis in which six novel AF loci were identified in individuals of European and Japanese descent[69]. The following section will detail the identified loci and possible mechanisms how they might contribute to AF.

Genetic variants at the 1q24 locus, approximately 46kb upstream of the *PRRX1* gene, were associated with a modest, 14% increased risk of AF ($p= 8.4\times10^{-14}$). *PRRX1* encodes a member of the paired related homeobox gene family, transcription factors which broadly contribute to differentiation and developmental patterning. In humans, mutations in *PRRX1* lead to agnathia-otocephaly[95,96], a generally fatal condition characterized by severely altered craniofacial development. In rodent models, homozygous deletion of *PRRX1* results in early postnatal death, and abnormal development of craniofacial, limb and vertebral structures[97]. In addition, *PRRX1* is highly expressed in the developing great vessels and is essential to the proper formation of the pulmonary vein[98,99]. As discussed for *PITX2*, ectopic depolarizations within the pulmonary venous regions are often responsible for the initiation of AF. It remains unclear whether *PRRX1* regulatory variation is related to congenital alterations in the pulmonary vein structure or function during development, or is instead associated with altered activity later in life.

Another association signal was localized intronic to *HCN4* on 15q24. Hcn4 is highly expressed in both sinoatrial and atrioventricular nodes and is responsible for the funny current (I_f) that controls cardiac pacemaking. Interestingly, mutations in *HCN4* have been found in individuals and families with sick sinus syndrome[100–102], tachy-brady syndrome and AF[103]. Whether the risk locus for AF alters overall HCN4 expression levels to a sufficient extent to confer a risk for AF, or if this region results in critical expression differences in a tissue-specific manner remains to be determined.

A novel locus was also located on 7q31 intronic to the *CAV1* gene that encodes Caveolin-1, a protein essential for the formation and maintenance of caveolae. The caveolae are regions of the membrane with unique phospholipid composition that act as mediators of clathrin-

independent endocytosis and as scaffolds for cellular, particularly integrin-mediated, signaling. In addition to these roles, caveolae also harbor many ion channels[104], including those responsible for all phases of the cardiac action potential. Studies of cardiovascular function in CAV1-null mice reported aberrant calcium signaling, and an alteration in myogenic tone[105]. Dilated cardiomyopathy, right ventricular hypertrophy and pulmonary hypertension have also been observed[106]. Further evaluation of the risk locus may aid in determination of the tissue-localized effect of CAV1 which leads to an increased risk of AF.

SNPs significantly associated with AF were identified on chromosome 14q23 intronic to *SYNE2*. The *SYNE2* gene encodes Nesprin2, a KASH protein family member that localizes to the nuclear outer membrane. Through its binding with the cytoskeleton, Nesprins are thought to provide a stable nuclear localization in the cell[107,108] and also are crucial for microtubule-mediated migration of the nucleus during differentiation[109]. Missense mutations in SYNE2 have been reported to cause familial Emery-Dreifuss muscular dystrophy[110], a disease which is also characterized by a spectrum of arrhythmic disorders, including AF.

On chromosome 9q22, an association signal with AF was identified within the gene *C9ORF3* (rs10821415, OR=1.11, p=4.2×10-11). However, this region is relatively gene rich, with 3 additional genes and 3 identified MIRs within 300kb of the sentinel SNP. Since none of the genes in the region have an obvious relationship with cardiovascular function or development, further investigation of this locus will be necessary.

Genetic variants associated with AF were also localized to an intergenic region between two genes known to play crucial roles in striated muscle physiology, *SYNPO2L* and *MYOZ1* (10q22, rs10824026, OR=0.87, $p=4.0\times10^{-9}$). This *SYNPO2L/MYOZ1* locus illustrates the utility of expression quantitative trait loci (eQTL) data to identify a disease-associated gene. Many intronic and intergenic SNPs identified by GWAS are thought to mediate their effects by regulating the transcription of a gene in the region. Sometimes there can be many genes at a locus so it can be difficult to know which is related to disease. Therefore, in eQTL mapping, one examines the relation between a SNP genotype and transcript levels of all genes at the locus, ideally from a relevant tissue. If a disease-related SNP is associated with transcriptional differences in a gene, this cis-eQTL association provides compelling support for the role of this gene in disease.

Initially, a SNP in LD with the sentinel SNP at *SYNPO2L/MYOZ1* locus was found to correlate with alterations in the expression of both genes. However, this data was derived from lymphoblastoid cell lines, a tissue type unlikely to truly reflect the transcriptional alterations associated with AF. Recently, an eQTL analysis from left atrial tissue found that the AF SNP was associated with transcriptional differences in *MYOZ1* expression alone[111]. Therefore, it is likely that *MYOZ1* is the AF related gene at this locus. The encoded protein, myozenin 1, is a cardiac-enriched, z-disk localized protein which aids in the binding of α-actinin and γ-filamin to confer stable sarcomeric organization[112]. Although no known disease causing variants of *MYOZ1* have been identified, mutations in *MYOZ2* result in familial hypertrophic cardiomyopathy[113], and replication of these mutations or ablation of

expression in a murine model[114] resulted in hypertrophic program activation and disruption of z-disk structure in ventricular myocytes.

Integrating GWAS Data to Stratify AF Risk

In summary, genome wide studies have identified 9 genetic loci associated with AF. Although the odds ratios for any given region are modest, the potential risk in a given individual may be much higher when the AF SNPs are considered together. Ultimately, utilizing these combined data would be important in a clinical setting, where risk could be stratified based upon a combination of genetic and clinical risk factors. Along these lines, Dr. Albert and colleagues derived a clinical risk score for AF in women without previous cardiovascular disease. The addition of a genetic risk score, consisting of the top 9 GWAS variants, improved AF risk prediction, but it did alter the reclassification into ten year risk categories[115].

Interestingly, a recent large-scale conditional analysis in 17 cohorts from the AFGen Consortium, found that there are at least four different risk alleles at the 4q25/PITX2 locus for AF[116]. Consideration of these *PITX2* SNPs plus the other 8 GWAS SNPs resulted in a nearly five-fold gradient in the risk of AF among individuals of European descent (Figure 1, European). The application of these same SNPs to a large Japanese population provided similar results (Figure 1, Japanese). As discussed below, with such a marked variation in AF risk in the population, it will be possible to identify individuals with both a marked increased and decreased risk for AF. Such genetic stratification of AF risk may ultimately enable an improved assessment of different treatment approaches or outcomes based on one's risk.

Future Directions for the Genetics of AF

Great strides have been made in determining the genetic risk for the development of AF; however, many challenges remain. In the following section, we outline a series of selected potential future directions for genetics studies of AF (Figure 1).

Identification of Additional AF Genetic Loci

A qualitative viewing of the Manhattan plot from the latest publication by the AFGen Consortium reveals several association signals that rise well above the milieu of background noise, but do not exceed a genome-wide significance threshold (Figure 3). A logical extension of this work would then be to determine if these "subthreshold" loci are additional potential AF genetic risk loci. Genotyping AF associated SNPs from these subthreshold loci in a larger number of patient samples would likely lead a strengthening of an association signal for some loci. While genotyping these SNPs in additional cases is straightforward, the subthreshold loci that are found are likely to contribute to an ever decreasing fraction of AF risk. Thus, while newly identified loci are unlikely to have a large impact on clinical risk prediction, they could still be helpful to identifying more members of the molecular pathways that underlie AF.

Future work should also focus on the identification of AF risk variants in different races and ethnicities. To date, the majority of discovery has been performed in populations of European descent, with limited work being done in individuals of Asian and African-American descent. Since AF prevalence varies greatly among races, it remains unclear whether the results from the studies of Europeans translate to other races, or if a different combination of risk loci are instead present. Studies in other races and ethnicities will be particularly important for the future application of genetic data to clinical care.

The Challenge of Causal Variant Identification at GWAS Loci

There are currently 9 identified genetic loci that are significantly associated with AF. However, despite the publication of the 4q25/*PITX2* locus over 6 years ago, the causative variants at all of the AF loci remain unknown. One challenge is the sheer size of these genomic regions, as the *PITX2* locus alone comprises a region of nearly 150 kilobases. Another challenge is that the top SNP identified by GWAS is rarely the causative variant, rather it is usually serving as a surrogate for a nearby causal variant. A final challenge is that the genetic mechanism for the association with AF is also unknown. We typically assume that AF risk is mediated by a SNP, but it is also quite possible that the association with AF could be due to a non-coding insertion or deletion, a genetic rearrangement, a variation in copy number, or an epigenetic modification.

To address these challenges a combination of techniques will be required (Figure 3). One approach could be to refine the genetic signal by fine mapping or increasing the density of SNPs within a target locus. This could be done directly genotyping more SNPs at a locus in a large population of cases and controls. Such an approach was used to identify multiple susceptibility signals at the 4q25 [70,116]. However, with the coverage of current genotyping platforms used for GWAS that consist of 1 to 5 million SNPs and the increasing resolution provided by the 1000 Genomes project, additional genotyping may have a limited incremental benefit.

As the turnaround time from submission to results-in-hand is now measured in weeks and the cost continues to drop, a viable complementary approach would be to sequence an entire disease locus. Importantly, sequencing would provide nucleotide-level resolution of the genetic architecture within AF risk loci. Thus, it would be expected that sequencing of AF risk loci in a large number of cases and controls will aid the identification of the causative haplotypes and variants associated with AF. Since the genetic variants identified by GWAS are markers of an association rather than a causative variant, one would anticipate that a causative SNP identified by sequencing would have a greater effect size and significance than the original GWAS signal. Sequencing a locus could also identify insertions/deletions or copy number variants that associate with disease and may be poorly described in current public databases. Finally, it is possible that sequencing could reveal multiple causative variants within a given locus, something that may not be identifiable by fine mapping. While such large-scale sequencing is currently feasible, the overall benefits remain unclear particularly given the significant cost of such projects.

In addition to refinement of the loci with fine-mapping and sequencing, it will be essential to integrate the vast amount of emerging regulatory data. Although coding variation is a possibility at some loci, a large majority of GWAS loci reside in intergenic or non-coding areas of the genome. This observation led to the assumption that these associations may be due to alterations of regulatory elements such as enhancers or promoters which, in turn, alter the activity of distant genes. Indeed some GWAS loci have been shown to alter transcription factor binding sites that in turn lead to differential expression of an adjacent gene [117]. For this purpose, *in silico* analyses of data provided by the ENCODE project can be incredibly useful for determining the causal mechanism of variation at a genetic locus. Genomic regions with high mammalian conservation, increased DNase hypersensivity, increased H3K27-acetylation, and identification of transcription factor binding sites through chromatin immunoprecipitation sequencing can prove useful for identifying altered functional elements within a risk locus.

Although both sequencing and *in silico* analyses can provide a higher resolution map of a genetic locus, there may still be many candidate regulatory regions across the locus. Studies that can identify the functional role of a regulatory region will be a critical next step. For example, one could postulate that, at the 4q25/*PITX2* locus, sequencing would allow the identification of the critical haplotypes that are associated with AF. An *in silico* analysis would then identify a number of highly conserved regions with enhancer activity. One could then examine these potential enhancers for activity in a model system such as mice, zebrafish or in an atrial or cardiomyocyte cell line. The causative genetic variant would then likely be one that is both significantly associated with disease and results in an alteration in enhancer activity.

While methods currently exist for each of the steps outlined above, sequencing, *in silico* analyses and functional follow up is expensive, slow, and challenging. The limited number of causative variants that have been identified at GWAS loci is not a problem specific to AF. Indeed, thousands of GWAS loci have been described, but causative variants have only been identified at a handful. Ultimately, a larger scale effort to systematically identify causative variants at GWAS loci will be necessary to overcome the obstacles faced by any single laboratory.

Atrial and Pulmonary Vein Specific eQTL Maps

As detailed above for the *MYOZ1* locus for AF, eQTL maps, which examine the changes in tissue specific expression of nearby genes when a given SNP genotype is present, can provide a useful link between GWAS loci and potential gene targets. Such analyses of gene expression have been useful in studies of atrial identity [111] and other cardiovascular traits [118,119]. While these eQTL associations at genetic loci can be helpful if they are present, the tissue-specificity of an eQTL signal is critical. Current publically available datasets such as the eQTL browser or the GTEx repository [120] have a limited tissue composition that reflects the challenge in obtaining relevant human tissue samples, but they are quickly expanding. For AF, it would be ideal to have eQTL data from much more specific tissue sources that are more plausibly involved in the pathogenesis of the arrhythmia. One would expect that the generation of publically available left atrial,

pulmonary venous, or AV nodal eQTL datasets would greatly aid in the discovery of the mechanism of causal variation in AF.

The Exome Chip will Enable Large-scale Assessment of Rare Coding Variation in AF

The evaluation of GWAS loci discussed above was focused on non-coding regions, but it is important to realize that many loci are in linkage disequilibrium, and thus effectively overlap, with coding region of one or more genes. In these cases, it is possible that the GWAS SNP is a marker or proxy for a coding SNP that actually underlies the association signal. SNPs within a gene could have many potential effects including non-synonymous variation that directly alters protein function, synonymous variation that alters splicing, affects transcript stability or influences codon efficiency, or untranslated region variation that affect translational efficiency or interactions with non-coding regulatory RNAs.

Once a locus is identified that overlaps with a gene, one could genotype every SNP within the gene in a large number of cases and controls to see if it has a stronger association with AF than that identified by GWAS. While straightforward in concept, in practice, GWAS loci are large, they may contain many genes each of which can have many rare and common variants and the cost of genotyping remains relatively expensive. One solution to address this issue has been the development of an exome genotyping array or exome chip.

In a GWAS genotyping array, SNPs are captured throughout the entire genome, while in an exome array, the focus is largely on coding SNPs. Current exome arrays include over a quarter of a million SNPs that essentially capture almost all of the common and rare coding variants for every gene in the genome. Within the past year, hundreds of thousands of individuals have been genotyped with these arrays. Much like a GWAS analysis, by comparing a large number of cases and controls, one can quickly identify any coding changes associated with AF. The exome chip analysis can be considered with the GWAS results to simultaneously identify the coding variants within all of the known AF loci.

Exome genotyping arrays will be incredibly powerful at systematically identifying any known coding variation for AF and we can expect to see the initial results of these studies within the next year. However, since these arrays are only genotyping known SNPs, they would not be useful for studying sporadic or novel genetic variation in an individual or family. Detection of such variation would require direct sequencing of individual genes, exomes, or genomes.

Candidate Gene Screening will be Replaced by Exome and Genome Sequencing

As described earlier, many mutations described for AF have been identified using a candidate gene approach. In brief, the coding region of a gene is sequenced in AF cases, a unique variant is identified, and that variant is then shown to alter the function of a protein. While such studies are straightforward, they are limited by 1) the time and cost restraints of sequencing that restrict the analysis to a small number of genes, 2) the inability to detect

polygenic causative variation, 3) the focus on coding variation, and, perhaps most importantly, 4) the limited likelihood that a particular candidate gene or variant within a gene is pathologically related to AF.

In the upcoming years, the continually decreasing cost and improving quality of next generation sequencing will enable the widespread adoption of sequencing the exome or protein coding region of the genome. We can expect that exome sequencing of cohorts of individuals with early-onset AF will provide a more comprehensive initial approach for relating rare genetic variation to AF; however, several challenges remain. For every individual sequenced, one can expect to find hundreds of unique non-synonymous SNPs or insertion deletions that have never been described in publicly available resources such as the Exome Variant Server. Thus, determining which variants are truly related to AF and which are genetic noise can be difficult. The identification of multiple hits in a given gene or genetic pathway across individuals can provide compelling evidence for the role of the gene or pathway in AF, yet large, well-powered studies will be required to make definitive conclusions. Improvements in the yield of such efforts may come from sequencing extremes of a phenotype such as cases of early-onset AF.

As costs continue to further decrease, genome sequencing will also become more realistic in cohort studies, yet with an even greater number of variants identified, assigning causality to noncoding variants will prove even more difficult. Given the continued challenges with large-scale sequencing approaches, an important step forward would be the creation of a centralized repository of exome and genome sequencing-derived variants identified in patients with AF. Comparison of variation in larger datasets on the scale of thousands rather than tens or hundreds of patients will aid in determining variants and genes that are truly causative for the arrhythmia.

Families can Provide a Unique Window into the Mechanisms of AF

Although much of our discussion has focused on using genetics to identify risk markers for AF in populations, familial forms of AF remain an important investigational tool. While families with autosomal dominant AF are rare, even a single family can shed light on the underlying molecular mechanisms for AF. To date, convincing evidence from families has identified the role of *KCNQ1* and ANP in AF. Challenges with using families to identify AF genes include the rarity of the families, the limited number of individuals with AF, and the difficulty in ensuring that all family members have a common genetic basis for the disease. The last point is particularly pertinent given that the background prevalence of AF can be as high as 10%. Traditional linkage analysis has become increasingly easy to perform by using SNP chips to genotype family members at a high density. Furthermore, exome and genome sequencing can be quickly performed in affected family members. Although with exome sequencing one will still identify hundreds of variants in each person, the familial transmission of disease enables a focus on those variants shared among all affected family members. A combination of using linkage analysis to identify a genetic locus and exome or genome sequencing, can further narrow the search for an underlying mutation. Ultimately, once identified, functional evaluation of a mutation on protein function will be necessary to provide convincing evidence of the role of gene in the pathogenesis of AF.

Given that only a handful of causative mutations have been identified in families with AF, the current HRS/EHRA consensus guideline states that there is no clinical utility for screening known AF-associated genes in patients with AF[121]. This includes utilization of any currently available commercial testing panels for AF genes and risk loci. As these gene panels are systematically tested in larger cohorts of individuals with familial AF, future evidence may emerge regarding the utility of commercial testing.

Other Forms of Genetic Variation

In addition to analyses of common and rare genetic variants described above, there are multiple other potential genetic analyses that could be considered to identify more of the heritability of AF. Variations in copy number have not been systematically examined in AF patients. High-resolution detection of deletions, insertions and duplications either in coding or non-coding regions has become increasingly straightforward using array-based or next generation sequencing methods. The major barriers to analyses of copy number variation at present are largely centered on cost and sample size necessary to ensure adequate statistical power.

The detection of epigenetic DNA methylation patterns in a tissue is also a straightforward technique; however, as DNA methylation is a highly tissue-specific process, multiple challenges exist with respect to AF. Ideally one would want to analyze left atrial or pulmonary venous tissue from both patients with and without lone AF, yet it is not practical to obtain these samples. Rather, most samples are obtained at the time of cardiac surgery for coronary disease, valvular heart disease, or transplant, and as such the analyses are limited by the inherent co-morbidities present with each type of patient population.

Given that AF increases in prevalence with age, it is possible that somatic or acquired mutations underlie some portion of the heritability of AF. In an intriguing paper, Dr. Gollob and colleagues found somatic mutations in GJA5 among patients with lone atrial fibrillation [38]. Presently, one could identify total somatic variation by whole genome or whole exome sequencing rather than on a gene-by-gene basis; however, the same challenges mentioned above regarding the need for left atrial tissue from healthy individuals will limit these analyses.

Finally, it will be interesting to determine whether *de novo* genetic variation could be responsible for AF. With each successive generation, there is a background rate of spontaneous genetic variation that occurs. By performing exome or genome sequencing in an affected child and unaffected parents, it is possible to identify the handful of novel coding variants present in the child, but not in the parents that may be associated with a disease. Recently, such an approach has identified a novel pathway for autism spectrum disorders[122].

Integration of Genetic Data to Predict AF and Outcomes

One ultimate goal of research into the genetic basis of AF is the potential return of this data to clinical arena. It is hoped that with the current trajectory of novel findings and the integration of the additional studies outlined above, that SNP data could be clinically useful in the near future. However, it is important to note that, in addition to not recommending the

testing of known AF genes, the current HRS/EHRA guidelines recommend against the testing of individual GWAS-associated SNPs in AF patients. This decision was likely based on the small number of AF SNPs that had been identified at that time, and the limited data on the clinical utility of these variants.

Since the publication of these guidelines, there have been a number of studies examining the relation between AF SNPs and treatment outcomes. Specifically, the risk of AF recurrence after cardioversion [123], pulmonary vein isolation [124,125], or the intiation of antiarrhythmic medication [126] has been studied; however, the observed sample and effect sizes have limited the applicability of these results to the broader population. More compelling results have been seen in stroke patients. Interestingly, the top two genetic variants identified in a large GWAS for cardioembolic stroke are also the top two regions (*PITX2* and *ZFHX3*) associated with AF [64,66,67,82].

Over the last five years, it has become clear that clinical risk factors[127], biomarkers[128] and now genetic variants can all help to identify individuals at risk for AF. Rather than using any single one of these approaches alone, we should seek to combine each of these risk factors to enhance the detection of AF. One could imagine that in high-risk populations, such as cryptogenic stroke, that we will be able to stratify patients into varying degrees of AF risk, and in turn consider alternative strategies to AF monitoring or anticoagulation.

Conclusions

Recent studies have identified a number of rare and common genetic variants associated with AF. However, the present data only account for a limited percentage of the heritability of AF. Integration of next generation sequencing technologies, improved gene expression data repositories, the identification of additional AF risk loci and a more complete understanding of causative mechanisms behind AF risk loci will be required. Ultimately, with a more complete picture of the genetic risk for AF, we can seek to develop genetically-driven clinical interventions and treatment strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Julie Herndon for the creation of original artwork utilized in accompanying figures.

SOURCES OF FUNDING Dr. Tucker is supported by a T32 training grant from the National Heart, Lung, and Blood Institute (T32HL007208). Dr. Ellinor is supported by grants from the National Institutes of Health (R01HL092577, R01HL104156, 1K24HL105780) and the American Heart Association to (13EIA14220013).

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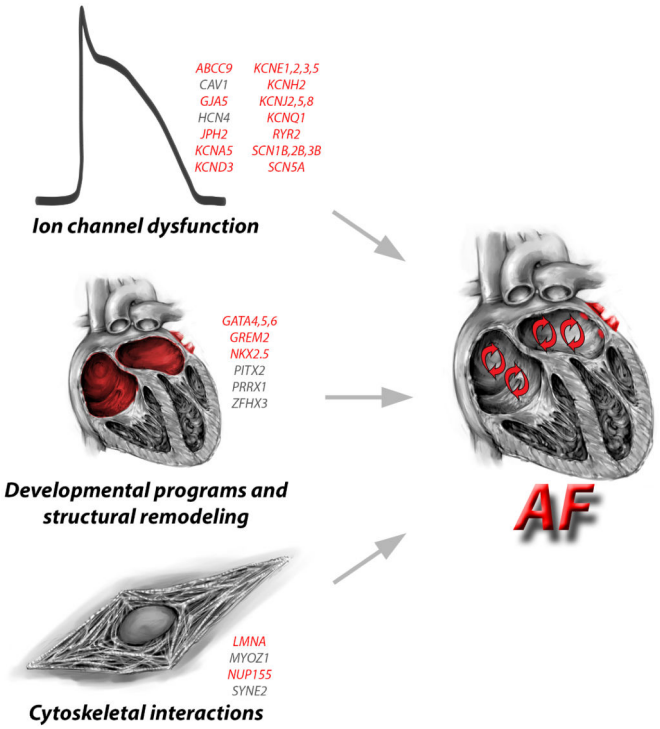

Figure 1. Known genetic pathways for AF pathogenesis

Schematic of known AF-related genes derived from previous studies. Genes listed include those where coding variation was identified in familial AF and candidate gene screens, as well as the genes suggested to be implicated in AF based upon GWAS. Names listed in red indicate those identified by familial studies and candidate gene screens. Those listed in gray are gene targets implicated by GWAS.

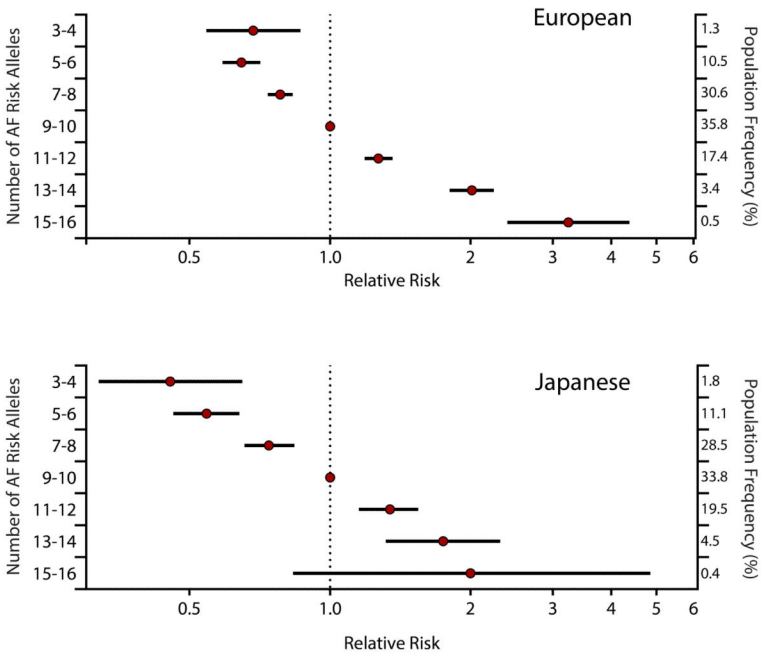

Figure 2. Graded relative risk of atrial fibrillation in European and Japanese populations

The risk of atrial fibrillation is plotted according to the estimated atrial fibrillation risk alleles, relative to that among individuals with the most common number of estimated risk alleles for all genome-wide significant atrial fibrillation susceptibility loci. Data is plotted from individuals of European ancestry from AFGen and Japanese ancestry from BioBank Japan. Right axis denotes the population frequency of each category. Error bars represent the 95% confidence intervals. Adapted from Lubitz et. al, *JACC* 2014[116].

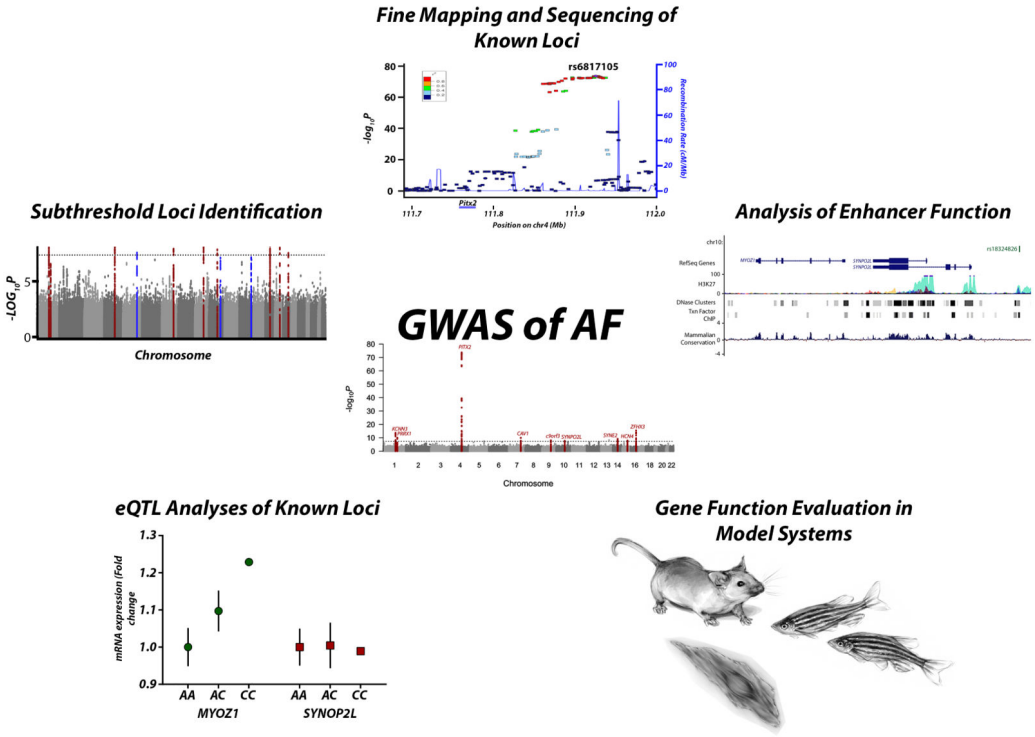

Figure 3. Future directions for the study of GWAS risk loci

Initial analyses of common variation have yielded 9 susceptibility loci for atrial fibrillation. Future pathways for confirming the causative variation include: Identification of subthreshold loci by increasing sample size or reduced sample heterogeneity in GWAS, fine mapping or direct sequencing of known risk loci for increased resolution of the causal region, *in silico* analyses of locus function to determine potential regulatory regions/causal variation, evaluation of AF candidate genes in model systems, and expression quantitative trait loci mapping to link common variation to altered gene expression in relevant tissues.

Table 1

Compendium of AF genetic variants identified in families and individuals.

Gene	Gene Name	Function	Citation(s)
ABCC9	ATP-binding cassette, subfamily C, member 9	I_{KATP} current	129
GATA4	Transcription factor GATA-4	Cardiac development	45–47,130
GATA5	Transcription factor GATA-5	Cardiac development	48,49,131
GATA6	Transcription factor GATA-6	Cardiac development	50,132,133
GJA5	Connexin 40	Formation of atrial gap junctions	38,134–138
GREM2	Gremlin-2	BMP antagonist	139
HCN4	Hyperpolarization activated cyclic nucleotide-gated potassium channel 4	I_f current	103
JPH2	Junctophilin-2	Ca^{2+} homeostasis	140
KCNA5	Potassium voltage-gated channel, shaker-related subfamily, member 5	I_{Kur} current	15,24–26
KCND3	Potassium voltage-gated channel, Shal-related subfamily, member 3	I_{to1} current	16
KCNE1	Potassium voltage-gated channel, Isk-related family, member 1	K_v channel activity modulation	19
KCNE2	Potassium voltage-gated channel, Isk-related family, member 2	K_v channel activity modulation	20
KCNE3	Potassium voltage-gated channel, Isk-related family, member 3	K_v channel activity modulation	21
KCNE5	KCNE1-like	K_v channel activity modulation	22
KCNH2	Potassium voltage-gated channel, subfamily H (eag-related), member 2	I_{Kr} current	141,142
KCNJ2	Potassium inwardly-rectifying channel, subfamily J, member 2	I_{K1} current	17,18
KCNJ5	Potassium inwardly-rectifying channel, subfamily J, member 5	I_{KACh} current	143
KCNJ8	Potassium inwardly-rectifying channel, subfamily J, member 8	I_{KATP} current	144
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1	I_{Ks} current	8 – 14
LMNA	Lamin A/B	Nuclear envelope structure	145,146
NKX2.5	Homeobox protein Nkx2.5	Cardiac development	43
NPPA	Natriuretic Peptide Precursor A	Systemic sodium homeostasis	39,147
NUP155	Nucleoporin 155	Nuclear pore formation	148
PITX2c	Paired-like homeodomain 2c	Great vein development, left-right asymmetry	44
RYR2	Ryanodine Receptor 2	Ca^{2+} release from sarcoplasmic reticulum	149
SCN1B	Sodium channel, voltage-gated, type I, beta subunit	I_{Na} current modulation	34,37
SCN2B	Sodium channel, voltage-gated, type II, beta subunit	I_{Na} current modulation	37
SCN3B	Sodium channel, voltage-gated, type III, beta subunit	I_{Na} current modulation	35,36
SCN4B	Sodium channel, voltage-gated, type IV,	I_{Na} current modulation	33

Gene	Gene Name	Function	Citation(s)
	beta subunit		
SCN5A	Sodium channel, voltage-gated, type V, alpha subunit	I_{Na} current	[27 – 32]

Table 2

GWAS-derived risk loci for AF

Locus	Sentinel SNP	RR	P-value	Nearest gene symbol	Relative location	Citations
4q25	rs6817105	1.64	1.8×10-74	*PITX2*	150kb upstream	61–65,69,70,81,82,88
16q22	rs2106261	1.24	3.2×10-16	*ZFHX3*	Intronic	65,69,82
1q21	rs6666258	1.18	2.0×10-14	*KCNN3*	Intronic	65,69,88
1q24	rs3903239	1.14	8.4×10-14	*PRRX1*	46kb upstream	69
7q31	rs3807989	0.90	3.6×10-12	*CAV1*	Intronic	69
14q23	rs1152591	1.13	5.8×10-13	*SYNE2*	Intronic	69
9q22	rs10821415	1.11	4.2×10-11	*C9orf3*	Intronic	69
15q24	rs7164883	1.19	2.8×10-17	*HCN4*	Intronic	69
10q22	rs10824026	0.87	4.0×10-9	*MYOZ1*	20kb upstream	69