Genetics of Atrial Fibrillation
Implications for Future Research Directions and Personalized Medicine

Steven A. Lubitz, MD; Cevher Ozcan, MD; Jared W. Magnani, MD; Stefan Kääb, MD, PhD; Emelia J. Benjamin, MD, ScM; Patrick T. Ellinor, MD, PhD

Atrial fibrillation (AF) was first described in humans approximately 100 years ago, and familial forms of AF were reported more than 70 years ago. Within the last 10 years, 3 critical developments have advanced our understanding of the genetic basis of AF. First, multiple epidemiological studies have demonstrated that AF is heritable. Second, rare mutations predisposing to AF have been identified in potassium and sodium channels, gap junction proteins, and signaling molecules. Finally, population-based, genome-wide association studies (GWAS) have implicated novel biological pathways responsible for AF.

The molecular biology of established mutations underlying AF has been well summarized. In this report, we focus on the approaches used to identify AF susceptibility loci and describe the findings from recent GWAS of AF. We also address future directions in the field of AF genetics that may improve our understanding of AF pathophysiology, risk prediction, prevention, and patient treatment.

Literature Search Methods
We searched entries in PubMed from 1948 through April 1, 2010, with the key words “atrial fibrillation” and “genetic,” “genetics,” or “gene” published in English, yielding 833 references. We scanned the titles and abstracts of these references to select relevant articles for full-text review. We reviewed the reference lists of retrieved articles to identify any additional relevant articles for inclusion. Case reports and abstracts were excluded. Association-based studies reporting genetic associations with AF were included if associations between the same variants and AF were replicated in an independent sample. Thus, the list of references reflects an overview of existing literature relevant to AF genetics.

AF Is Heritable
Whereas individual cases of familial AF were recognized in the mid-20th century, a widespread heritable component underlying AF was reported only recently. In 2004, investigators from the Framingham Heart Study described an increased risk of AF in offspring in whom at least 1 parent had AF, even after accounting for established AF risk factors such as hypertension, diabetes mellitus, and clinically overt heart disease (Figure 1).

Offspring with at least 1 parent affected with AF had an increased risk of AF (odds ratio [OR], 1.85; 95% confidence interval [CI], 1.12 to 3.06) relative to those without a parental history of AF. The OR increased to 3.23 (95% CI, 1.87 to 5.58) when restricting to those in whom parental AF developed before 75 years of age. Similarly, investigators from Iceland observed an increased risk of AF among relatives of an affected individual (OR, 1.77; 95% CI, 1.67 to 1.88) and noted that the risk of AF decreased with each successive degree of relation from the affected individual, consistent with a genetic condition.

Lone atrial fibrillation, or AF in the absence of overt cardiovascular disease, appears to have an even greater heritability than AF observed in association with other risk factors. Investigators at the Mayo Clinic noted a family history of AF in first- or second-degree relatives of those with lone AF in 15% of probands. Familial aggregation of AF in relatives of patients with lone AF was also observed by investigators at the Massachusetts General Hospital. Recently, a report from the Danish Twin Study demonstrated an increased risk of AF among monozygotic as compared to dizygotic twins (concordance rate, 22% versus 12%; P<0.001 for monozygotic versus dizygotic twins, respectively). The Danish investigators estimated the heritability of AF attributable to additive genetic effects to be 62% (95% CI, 55% to 68%). Although estimates of heritability can vary widely depending on study design and study populations, to put this measure into perspective, the heritability of height has been estimated to be ~80%, whereas estimates for fasting glucose and electrocardiographic parameters such as the QT interval are somewhat lower, ranging between ~30% to 35%.

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In sum, over the last 5 years we have come to appreciate that AF is heritable. Although heritability does not discern between environmental or genetic factors that predispose to AF, recognition that AF is heritable has motivated efforts to search for genetic variation underlying AF. Recent genetic studies have begun to yield exciting discoveries that implicate novel genes and pathways in the pathogenesis of AF.

The Role of Mendelian AF, Candidate Gene Resequencing, and Rare Variants

On recognition that a condition is heritable, there are a number of techniques that can be used to search for causal genetic variants including linkage analysis, candidate gene resequencing, and association studies. Linkage analysis is a traditional method that has been used with great success to identify the causative genes for familial forms of hypertrophic cardiomyopathy, long-QT syndrome, and in some cases, AF. Although linkage analysis is a powerful technique, it is limited by the fact that large families with multiple affected individuals with AF are uncommon. In contrast to rare monogenic forms of AF, many cases of AF are believed to result from the aggregate and perhaps subtle effects of numerous common genetic variants and environmental factors. Despite these limitations, Mendelian families have informed our understanding of AF pathophysiology by facilitating the discovery of mutations underlying monogenic forms of AF (Table).14–31 After the identification of ion channel mutations in monogenic forms of AF, investigators began broadly resequencing other ion channel candidate genes for mutations associated with AF.15–17,21–28,31

Potassium channel mutations underlying AF are predominantly characterized by gain-of-function changes and are usually predicted to promote repolarization, shorten the atrial action potential, and facilitate reentry,15,16,22–24,50 a major factor in the maintenance of AF.51 However, atrial remodeling in the setting of heart failure, a predisposing condition for AF,52 is accompanied by prolongation of the atrial action potential.53 Consequently, triggered activity may be involved in the pathogenesis of some forms of AF.54 Indeed, a loss-of-function mutation in \( \text{KCNA5} \) associated with AF results in decreased ultrarapid delayed rectifier potassium current \( (I_{Kur}) \), prolongation of the atrial action potential, and facilitates early afterdepolarizations.21 Recent discoveries have also revealed mechanisms by which mutations in sodium channels may influence AF susceptibility. Two studies re-

### Table. Summary of Genes and Genetic Loci Associated With Atrial Fibrillation

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<th>Mendelian AF, Candidate Gene Resequencing, and Rare Variants</th>
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<th><strong>Genome-Wide Association Studies</strong></th>
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<td><strong>Chr 16q22</strong></td>
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<td><strong>Chr 1p21</strong></td>
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Chr indicates chromosome.

*Only replicated associations between genetic variants and AF are displayed.
†Associated at a genome-wide significance threshold of \( P<5 \times 10^{-8} \).
Candidate Gene Association Studies

In contrast to linkage analysis or candidate gene resequencing, investigators have sought to identify common genetic variants underlying AF by using candidate gene association studies (Table). Plausible candidate genes are selected for study based on biologically driven hypotheses, and genetic variants are tested for association with the condition of interest. Candidate gene association studies generally have a low pretest probability, since with nearly 30,000 genes and millions of common polymorphisms in the genome, it is unlikely that any single chosen variant is involved in the pathogenesis of AF. As such, many reported associations from candidate gene association studies may represent false-positive results stemming from positive publication bias, small sample sizes, multiple statistical testing, lack of control for population stratification, and absence of replication. Few variants associated with AF using a candidate gene approach have been replicated in independent samples (Table), an important criterion in the current era for establishing the validity of reported associations.

In one notable exception, investigators identified and replicated an association between a common nonsynonymous variant in KCNH2 (p.K897T, ~20% minor allele frequency) and AF in a 2-stage candidate gene study that included 1207 AF case subjects and 2475 control subjects (minor allele odds ratio: OR = 1.68; 95% CI, 1.37 to 2.05; I² = 81%). This study indicates that genes with rare variants underlying Mendelian disease may harbor common variants that contribute to AF. The observation that rare and common variants associated with conditions may occur at the same loci is not unique to AF. Some of the observed associations may represent false-positives, but the substantial number of studies that have replicated these results suggests that some of the associations may be biologically meaningful.

Genome-Wide Association Studies

GWAS have been central to advancing our understanding of AF genetics by testing across the genome. In addition to testing for association in candidate genes, GWAS also test for association in intergenic regions. Typically, single nucleotide polymorphisms (SNPs), commonly occurring (~1% population frequency) variations in single bases throughout the genome, are tested for association between affected and unaffected subjects. SNPs serve as proxies for presumed disease-causing variants in close proximity and are well suited for association analyses because they are abundant in
the genome (at least 10 million common SNPs), relatively easy to genotype, and catalogued in a reference database for different ancestral populations provided by the international HapMap project.\textsuperscript{61} GWAS permit the identification of common genetic variants in entire populations rather than in individuals or families. In general, the SNPs tested for association in GWAS are expected to confer modest effects on disease risk.\textsuperscript{62}

In 2007, investigators identified an AF susceptibility locus on chromosome 4q25 in a GWAS.\textsuperscript{63} SNP rs2200733 was the most significantly associated polymorphism with AF in 3 European populations (minor allele: OR, 1.72; 95% CI, 1.59 to 1.86; \(P=3.3\times10^{-11}\); Figure 2). In further study, this SNP was associated with AF in a sample of Asian individuals from Hong Kong (minor allele: OR, 1.42; 95% CI, 1.16 to 1.73; \(P=0.00064\)). The AF risk allele of SNP rs2200733 occurred with a frequency of about 20% in individuals of European ancestry and 60% in individuals of Asian ancestry.

A consortium replicated the association between the chromosome 4q25 locus and AF in individuals of European ancestry,\textsuperscript{65} and subsequent GWAS have confirmed this region as an AF susceptibility locus.\textsuperscript{47,48} The 4q25 locus also has been associated with prevalent AF and atrial flutter in a small study from Italy\textsuperscript{66} and with incident AF after coronary artery bypass grafting.\textsuperscript{67} Replication of the association between rs2200733 and AF in a Chinese population also was recently demonstrated.\textsuperscript{68} Variants on chromosome 4q25 appear to have a stronger effect in those with early AF onset.\textsuperscript{63,65}

Figure 2 summarizes reported associations between SNP rs2200733 at the chromosome 4q25 locus and AF. Each copy of the minor allele of SNP rs2200733 conveys a 1.68-fold increased risk of AF (95% CI, 1.50 to 1.87) compared with those without the variant (Figure 2). Variants on chromosome 4q25 appear to have a stronger effect in those with early AF onset.\textsuperscript{63,65} Subjects with 2 copies of the risk allele have an estimated 2.8-fold relative risk of AF (quantified as 1.68\(^2\)). The effect sizes seen with this single genetic variant are comparable to other established risk factors for AF, including each increasing decade of age (OR\(\approx2\)), male sex (OR\(\approx1.5\)), hypertension (OR\(\approx1.5\)) and diabetes mellitus (OR\(\approx1.5\)).\textsuperscript{69}

How the 4q25 locus confers AF risk has not been established. The locus is devoid of any known genes, but the closest gene, paired-like homeodomain 2 (\textit{PITX2}), is a plausible candidate for AF. \textit{PITX2} encodes a transcription factor expressed in the heart and lungs and is involved in...
A particularly intriguing observation considering that AF is commonly triggered by ectopic foci originating in the pulmonary venous myocardium. Mutations in PITX2 cause Axonfeld-Reiger syndrome, the Peter anomaly and other Mendelian disorders, all characterized by ocular abnormalities; arrhythmias or cardiac abnormalities are not primary features of these disorders. The 4q25 locus is marked by regions of substantial phylogenetic conservation, which may indicate that the locus harbors regulatory elements with effects on unknown target genetic sequences. Indeed, there is emerging evidence that highly conserved noncoding regions contain regulatory elements that can underlie phenotypic diversity.

Two separate GWAS have established an AF susceptibility locus on chromosome 16q22. The top signals were located within or around the zinc finger homeobox 3 (ZFHX3) gene. In both studies, the most significantly associated variants at this locus were located in intron 1, were in high linkage disequilibrium with one another (r²=0.78, HapMap3 CEU panel) and occurred with a frequency of approximately 20% in subjects of European ancestry. These studies included 8222 individuals with AF and 58 439 referent subjects without AF. ZFHX3 encodes a transcription factor that was originally identified as a regulator of α-fetoprotein expression. Common genetic variants in ZFHX3 have been associated with Kawasaki disease, as well as with malignancies such as prostate cancer. The expression patterns of ZFHX3 in human cardiac and pulmonary tissue are not clear, nor are the mechanisms by which variants in this gene may predispose to cardiovascular pathology.

Recently, we reported the identification of a novel genetic locus for lone AF on chromosome 1q21 in a GWAS comprising 13 35 subjects with lone AF and 12 844 without AF of European ancestry. The SNP most significantly associated with AF was rs13376333; each minor allele of the SNP conferred an approximately 50% increase in the odds of lone AF (minor allele: OR, 1.52; 95% CI, 1.40 to 1.64; P=1.83×10⁻²¹). rs13376333 is located between the first and second exon of KCNN3, a calcium-activated, small conductance potassium channel. These channels are expressed in the heart, and pharmacological inhibition of the KCNN family of channels results in altered action potential properties in a rabbit burst pacing model of pulmonary venous ectopy. Further work will be necessary to determine the role of this channel in atrial repolarization and the potential utility of developing drugs that target KCNN3 for the pharmacological treatment of AF.

Additional loci that may be associated with AF were recently described in 2 GWAS of the PR interval, though associations were not demonstrated at genome-wide significance thresholds. These GWAS demonstrate that examination of intermediate phenotypes for AF, such as PR interval duration, may facilitate the discovery of novel variants associated with AF. Future meta-analyses and replication will be necessary to establish these regions as bona fide AF loci.

In summary, common genetic variants have been associated with AF risk, and many confer a risk of AF comparable to those of other widely accepted risk factors for the arrhythmia. Although the exact mechanisms by which these variants lead to AF are unknown, recent GWAS have focused our attention on at least 3 previously unrecognized genomic regions involved in the pathogenesis of AF.

**Future Directions**

The identification of rare variants underlying familial AF has facilitated the understanding of electrophysiological mechanisms that influence AF, whereas findings from GWAS have led to the identification of novel and poorly described biological mechanisms potentially underlying AF. Large gaps in knowledge remain in the application of genetic discoveries to the evidence-based practice of genotype-guided patient management (Figure 4).
Understanding the genetic basis of AF has the potential to impact patient care by facilitating improved risk stratification, elucidation of pathophysiological mechanisms, and prediction of therapeutic responses. As AF is associated with substantial morbidity, mortality, and health care costs, prevention of AF is of public health importance.\(^9\) Whereas genetic risk scores using a few top variants identified in GWAS have not typically enhanced risk prediction for disease,\(^9\) emerging analytic techniques that incorporate the genotypes of numerous variants appear to explain substantially more variability in phenotypes than was previously appreciated.\(^91,92\) Investigators from the Framingham Heart Study recently developed a risk score for AF based on clinical factors that performed well as a prediction algorithm on the basis of discrimination and calibration metrics.\(^93\) Evaluating whether the predictive performance of this tool is improved with the addition of biomarkers associated with AF, including genetic variants, is warranted. Aggressive risk factor control or surveillance for AF in high-risk populations may be of clinical value. Similarly, identification of genetic susceptibility loci will aid basic and translational efforts to understand the biological mechanisms that underlie AF, which may ultimately result in new targets for disease prevention.

Determining the relations between AF-related variants and AF-related morbidity may permit the prevention of morbidity. For example, associations between AF-related variants on chromosomes 4 and 16, and ischemic stroke, have been described in some\(^48,94\) though not all studies.\(^95–97\) Efforts to test whether genotypes or biomarker information enhance existing thromboembolism prophylaxis algorithms are warranted.\(^98\)

Genetic information may influence clinical care by guiding therapy selection based on potential efficacy and toxicity. For instance, the consideration of both clinical factors as well as genotypes at loci involved in the metabolism of warfarin explain a striking proportion of the variability in warfarin dose requirements.\(^99\) Evaluation of the clinical impact of gene-based warfarin dosing is ongoing, with the hope that it may reduce morbidity from subtherapeutic or supratherapeutic dosing during warfarin initiation. The principles and potential applications of drug-related cardiovascular pharmacogenomics have been recently reviewed.\(^100\) Similar principles can be applied to the use of antiarrhythmic drugs, catheter ablation techniques, and other therapies used in the care of patients with AF.

Conclusions
The abundance of genetic data informing the pathophysiology of AF is growing rapidly. The study of AF through candidate gene association studies has revealed a number of genetic variants that predispose to AF, most of which have been observed or are presumed to affect the atrial action potential duration. Epidemiological observations and GWAS have contributed to the understanding of common genetic variation as it relates to AF in individuals of European descent. Discovery of these loci has implicated biological pathways involved in the pathogenesis of AF that have yet to be characterized. Future avenues of research in AF genetics will involve the further identification of AF susceptibility loci, determination of underlying biological pathways linking genetic variation to disease, and application of findings to AF prevention and the clinical treatment of patients. Genotype risk scores that include multiple genetic variants hold promise for the prospects of genotype-guided risk prediction of disease incidence, recurrence, and efficacy and toxicity of therapies.

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None.

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