Molecular Genetics of Atrial Fibrillation

Chia-Ti Tsai, MD, PhD,* Ling-Ping Lai, MD, PhD,*† Juey-Jen Hwang, MD, PhD,* Jiunn-Lee Lin, MD, PhD,* Fu-Tien Chiang, MD, PhD‡
Taipei, Taiwan

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. There is genetic predisposition for the development of AF. Recently, by linkage analysis, several loci have been mapped for monogenetic AF, including 11p15.5, 21q22, 17q, 7q35–36, 5p13, 6q14–16, and 10q22. Some of these loci encode for subunits of potassium channels (KCNQ1, KCNE2, KCNJ2, and KCNH2 genes), and the remaining are yet unidentified. All of the known mutations are associated with a gain of function of repolarization potassium currents, resulting in a shortening of action potential duration and atrial refractory period, which facilitate multiple re-entrant circuits in AF. In addition to familial AF, common AF often occurs in association with acquired diseases such as hypertension, valvular heart disease, and heart failure. By genetic association study, some genetic variants or polymorphisms related to the mechanism of AF have been found to be associated with common AF, including genes encoding for subunits of potassium or sodium channels, sarcolipin gene, renin-angiotensin system gene, connexin-40 gene, endothelial nitric oxide synthase gene, and interleukin-10 gene. These observations suggest that genes related to ionic channels, calcium handling protein, fibrosis, conduction and inflammation play important roles in the pathogenesis of common AF. The complete elucidation of genetic loci for common AF is still in its infancy. However, the availability of genomewide scans with hundreds or thousands of polymorphisms has made it possible. However, challenges and pitfalls exist in association studies, and consideration of particular features of study design is necessary before making definite conclusions from these studies. (J Am Coll Cardiol 2008;52:241–50) © 2008 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice and therefore represents a major public health problem. The majority of patients with AF have underlying heart disease, such as valvular heart disease, hypertension, or left ventricular dysfunction. However, other patients remain in sinus rhythm despite the presence of significant valvular diseases and/or left ventricular dysfunction. Importantly, some patients develop AF in the absence of any known risk factor (lone AF). Taken together, this suggests there is also a genetic predisposition for the development of AF. A recent Framingham Heart Study of 2,243 participants showed that the relative risk of AF was increased by 85% in individuals with at least 1 parent with a history of AF (1).

According to the patterns of heredity, AF could be categorized into 2 major types. The first type is familial AF with a mendelian hereditary pattern, and the second type is nonfamilial AF. Familial AF is a monogenetic disorder and is often identified as AF being present in many members of the same family. Although uncommon, familial AF sometimes occurs in the setting of other inherited (structural) heart diseases (e.g., in association with dilated or hypertrophic cardiomyopathy) (2–5).

Unlike familial AF, in which genetic factor is the major contributing factor, nonfamilial AF typically occurs in association with underlying cardiovascular disease. Therefore, genetic factors, interacting with nongenetic or environmental factors, contribute to the risk of nonfamilial AF. As such, nonfamilial AF is also called complex-trait, multifactorial, or multigenetic AF. Nonfamilial AF is more commonly encountered in clinical practice than familial AF. In the present review, we present recent work on genetic studies of familial and nonfamilial AF separately, because the molecular mechanisms and methods used to study the genetic basis of these 2 distinct types of AF are different (Table 1). Familial AF in the setting of inherited (structural) heart disease is not the subject of the present review.

General Mechanisms of AF

The underlying mechanisms of AF are complex. Multiple rapidly discharging foci, a focal source with fibrillatory conduction (6,7), or multiple re-entrant circuits (8) have been proposed to explain the maintenance of AF. At present, both the focal source and the multiple re-entrant circuit theories are the generally accepted hypotheses to explain AF.
In the multiple re-entry theory, the stability of AF is determined by the number of wavelets in the atria (8). The wavelength, which is the distance traveled by an impulse during 1 refractory period, is the basal unit of a traveling wavelet, and can be calculated from the product of the refractory period and conduction velocity (7). Therefore, the shorter the wavelength, the more wavelets in the atria, and thus AF will be more stable (8). Consequently, the functional effects of genes that determine changes in refractory period or conduction velocity will affect the wavelength of the traveling wavelet, and therefore the stability of AF. Changes of the electrophysiological properties after repeated episodes of AF, such as the shortening of the atrial refractory period, are referred to as electrical remodeling.

Triggers are required for the initiation of AF and include atrial ectopic foci originating from the junctions between the left atrium and the pulmonary veins or the atria itself. Proposed mechanisms initiating these atrial ectopic foci include increased automaticity, afterdepolarization, and microre-entry. These triggers may initiate multiple re-entrant circuits in the presence of conduction blocks in the atria. Spatial inhomogeneity of atrial refractoriness plays an important role in creating conduction block. Spatial inhomogeneity of atrial refractoriness typically occurs in association with structural remodeling of the atrium. Therefore, the downstream functional effects of genes that contribute to the mechanisms of increased automaticity, afterdepolarization, or structural remodeling will also promote AF.

The mechanisms triggering and maintaining AF overlap. For example, a rapidly discharging focus could both initiate and maintain AF. Structural changes of the atria may decrease the conduction velocity, resulting in decreased wavelength (the product of the refractory period and conduction velocity) and thus more wavelets in the atria, which are involved in maintenance of AF. Structural changes of the atria may also create conduction blocks and thus initiate multiple re-entrant circuits and AF.

In the early stage of AF, patients may present with paroxysmal AF due to intermittent episodes of the atrial ectopic foci. After repeated episodes of AF, the atria undergo electrical and structural remodelings, which facilitate the maintenance of AF. Finally, AF undergoes self-perpetuation and becomes persistent or chronic. The whole spectrum of AF can be observed in both familial and nonfamilial AF.

**Familial AF**

Linkage analysis was used in most of the genetic studies for monogenetic AF. The success of this method depends on the establishment of a large pedigree as well as a clear identification of the phenotypes. Although a mutated gene found in one family may not be present in another family or in patients with multifactorial or multigenetic AF, identifying the responsible genes and investigating the functional significance of the mutation will contribute significantly to the understanding of the pathogenesis of AF.

**Unknown gene.** Brugada et al. (9) first reported 3 families with autosomal dominant AF. Linkage analysis revealed 10q22–24 as the genetic locus in these families, although the exact gene responsible remains unclear. A candidate gene approach has been applied to the 10q22–24 region. One of the possible candidate genes was the DLG5 (discs, large [Drosophila] homolog 5) gene, a member of the MAGUK (membrane-associated guanylate kinase) family which mediates intracellular signaling. However, this gene has been excluded as the responsible gene in these families (10). Efforts on positional cloning of other genes in this region are still ongoing.

Darbar et al. (11) also reported 4 multigeneration families with autosomal dominant AF. However, genotyping of these families with deoxyribonucleic acid (DNA) markers spanning the chromosome 10q22–24 region excluded linkage of AF to this locus (11).

Ellinor et al. (12) investigated 34 members in a family with familial AF. They found a logarithm of the odds score of 3.63 at a marker at 6q14–16. The exact gene responsible remains unknown.

**KCNQ1 gene.** Chen et al. (13) studied a 4-generation Chinese family with autosomal dominant AF. The locus was mapped to chromosome 11p15.5, and the KCNQ1 gene, which encodes for the α-subunit of the cardiac slow delayed rectifier potassium channel (IKs) (14), was identified as the responsible gene. This gene is the same as the first genetic locus for congenital long QT syndrome (LQT1) (14). Sequencing studies revealed a missense mutation at nucleotide 418 from adenine to guanine. This missense mutation results in a change of amino acid from serine to glycine at position 140 (S140G). In vitro coexpression of this mutant gene with minK gene (15) (LQT5, the β-subunit of the cardiac IKs channel) in COS7 cells demonstrated that this mutation causes a significant increase in IKs current density. Therefore, a mutation in KCNQ1 with gain-of-function effect is responsible for familial AF in this Chinese family. Interestingly, other mutations in the same gene with loss-of-function effect have been reported to be responsible for congenital LQTS (13). It is speculated that a gain of function on IKs results in a shortening of action potential duration and atrial refractory period, which facilitates multiple re-entrant circuits and wavelets in AF (8,17). This observation implies that IKs
plays an important role in AF and that IKs-blocking agents might be an effective way to treat AF in some patients. **KCN2 gene.** The same group also identified a locus responsible for familial AF on 21q22 encoding for another potassium channel subunit, KCN2 (18). The mutation involved a cytosine to thymine transition at nucleotide 79 of the gene for KCN2. This resulted in an arginine to cysteine substitution at residue 27 (R27C). The KCNE gene family encodes small proteins that function as β-subunits of several voltage-gated cation channels (19). KCN2 encodes for MiRP1, the putative KCNE2 in AF (8,17). Increased expression of the Kir2.1 channel has a gain-of-function consequence on the Kir2.1 current, which might be an effective way to treat AF in some patients. Recently, Ellinor et al. (27) screened for mutations in KCNJ2 and KCNE1 in Chinese AF kindreds for a mutation in KCNJ2, which encodes for the Kir2.1 channel and mediates an inward rectifier potassium current in the heart (IK1). A valine-to-isoleucine mutation at position 93 (V93I) of the Kir2.1 gene was found in all affected members in one kindred. Functional analysis of the V93I mutant also demonstrated a gain-of-function consequence on the Kir2.1 current, which again may result in a shortening of the action potential duration and facilitate multiple wavelets and perpetuation of AF (8,17).

**KCN2 gene.** Because overexpression of the wild-type Kir2.1 in the mouse induced AF (20), Xia et al. (21) studied 30 Chinese AF kindreds for a mutation in KCNJ2, which encodes for the Kir2.1 channel and mediates an inward rectifier potassium current in the heart (IK1). A valine-to-isoleucine mutation at position 93 (V93I) of the Kir2.1 gene was found in all affected members in one kindred. Functional analysis of the V93I mutant also demonstrated a gain-of-function consequence on the Kir2.1 current, which again may result in a shortening of the action potential duration and favor multiple re-entrant circuits and wavelets in AF (8,17). Increased expression of the Kir2.1 channel has also been found in atrial samples from patients with common AF (22–25).

**KCN2 gene.** Recently, Hong et al. (26) identified a family with short QT syndrome in whom 3 members presented with AF. The 17-year-old proband presented with paroxysmal AF. They found that the KCNH2 gene exhibited a missense mutation at nucleotide 1764 with a cytosine to guanine substitution, resulting in a lysine to asparagine mutation at residue 588 (N588K). KCNH2 encodes for the HERG protein, the α-subunit of the cardiac IKr channel, which also contributes to the repolarization of the cardiac action potential. Programmed electrical stimulation was performed in all affected members with N588K mutation, which revealed a remarkably short atrial and ventricular refractory period, and inducibility of atrial and ventricular fibrillation. The mutation therefore confers a gain-of-function of IKr, with a shortening of the effective atrial refractory period.

In summary, all identified genes discussed thus far encode for subunits of potassium channels and the mutations confer a gain of function, shortening the atrial action potential duration and the atrial effective refractory period, therefore promoting an ideal substrate for multiwavelet re-entry. However, most of the AF families with identified potassium channel mutations are from the Chinese population (12,18,20). Recently, Ellinor et al. (27) screened for mutations in KCNJ2 and KCNE1—5 genes in 96 probands of AF families from the Caucasian population. No mutations were found in these genes. Therefore, the potassium channel gene mutation may not be universal for familial AF in all ethnic populations, although other important potassium channel genes, such as the KVLQTI and HERG genes, were not screened. Furthermore, the manifestation of a mutation may also be affected by environmental factors. Recently, R14C missense mutation of the KCNQ1 gene was identified in one AF family with a high prevalence of hypertension. All of the affected members had hypertension. Patch-clamp studies of wild-type or R14C KCNQ1 coexpressed with KCNE1 in Chinese hamster ovary cells showed no significant differences between wild-type and mutant channel kinetics at baseline. After exposure to hypotonic solution to elicit cell swelling/stretch, mutant channels showed a marked increase in current and altered channel kinetics (28). These data suggest that the R14C KCNQ1 mutation alone is insufficient to cause AF. A model of a “second hit,” such as an environmental factor like hypertension, which promotes atrial stretch, along with the inherited defect in ion channel kinetics (the “first hit”), was proposed to explain this phenomenon.

Nevertheless, the above observations demonstrate that potassium channels play a very important role in the pathogenesis of AF in patients without underlying heart disease and provide specific targets for the development of novel drug therapy to treat AF.

**Nonfamilial AF**

This form of AF is distinct from familial AF in terms of its clinical presentation, underlying genetic loci and molecular mechanisms. To find the genetic susceptible loci for common AF, a genetic association study is commonly used instead of linkage analysis. The concept of genetic association is based on the premise that the frequencies of the variants within or close to the susceptibility gene(s) are different between the diseased population and the general population. Furthermore, these genetic variants encode proteins with only mild or minimal functional change and not marked loss or gain of function (such as those encoded by mutant genes in familial AF). This kind of genetic variant is called a polymorphism (instead of mutation). When the variant involves only a nucleotide change, it is called a single nucleotide polymorphism (SNP).

A candidate gene approach is commonly used to test the association of specific genetic variants or SNPs with the disease. This approach is performed by choosing candidate genes which encode for proteins that have been determined to be mechanistically linked to the pathogenesis of the disease. This approach is different from the so-called genome-wide approach, in which all genes or makers from the whole genome are tested without an a priori assumption regarding which genes are possibly responsible.
<table>
<thead>
<tr>
<th>Functions</th>
<th>Mode</th>
<th>GenBank/dbSNP</th>
<th>No. of Cases</th>
<th>Race</th>
<th>AF Type</th>
<th>Method</th>
<th>UHD</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial AF</td>
<td>No locus and gene identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAF1–4</td>
<td>?</td>
<td>AD</td>
<td>—</td>
<td>4 families</td>
<td>Caucasian</td>
<td>PAF</td>
<td>Screen</td>
<td>Nil</td>
</tr>
<tr>
<td>10q22–24</td>
<td>?</td>
<td>AD</td>
<td>—</td>
<td>3 families</td>
<td>Caucasian</td>
<td>CAF</td>
<td>Link</td>
<td>Nil</td>
</tr>
<tr>
<td>6q14–16</td>
<td>?</td>
<td>AD</td>
<td>—</td>
<td>1 family</td>
<td>Caucasian</td>
<td>PAF</td>
<td>Screen</td>
<td>HTN</td>
</tr>
<tr>
<td>Gene identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNQ1 S140G</td>
<td>(\alpha)-subunit of IKs (KVLQT1)</td>
<td>Gain of function</td>
<td>AD</td>
<td>NP_000209.2:p.S140G NM_000218.2:c.418A&gt;G</td>
<td>1 family</td>
<td>Chinese</td>
<td>CAF</td>
<td>Link</td>
</tr>
<tr>
<td>KCNQ1 R14C</td>
<td>(\alpha)-subunit of IKs (KVLQT1)</td>
<td>Gain of function under stretch</td>
<td>AD</td>
<td>NP_000209.2:p.R14C NM_000218.2:c.40C&gt;T</td>
<td>50 families</td>
<td>Caucasian</td>
<td>PAF</td>
<td>Screen</td>
</tr>
<tr>
<td>KCNE2 R27C</td>
<td>(\beta)-subunit of KCNQ1-KCNE2</td>
<td>Gain of function</td>
<td>AD</td>
<td>NP_751951.1:p.R27C NM_172201.1:c.79C&gt;T</td>
<td>2 families</td>
<td>Chinese</td>
<td>PAF</td>
<td>Link</td>
</tr>
<tr>
<td>KCNJ2 V93I</td>
<td>(\alpha)-subunit of IK1 (Kir2.1)</td>
<td>Gain of function</td>
<td>AD</td>
<td>NP_000882.1:p.V93I NM_000891.2:c.277G&gt;A</td>
<td>30 kindreds</td>
<td>Chinese</td>
<td>PAF</td>
<td>Screen</td>
</tr>
</tbody>
</table>

| Nonfamilial AF |  |  |  |  |  |  |  |  |
| Candidate gene approach |  |  |  |  |  |  |  |  |
| Ionic channels/calcium handling proteins |  |  |  |  |  |  |  |  |
| KCNE1 (A112G or S38G) | \(\beta\)-subunit of IKs (KVLQT1) | Decreased function | Poly | rs1805127 or rs17846179 | 108 case-control pairs | Taiwanese | PAF = CAF | Asso | VHD, CHF, HTN | 29,31 |
| G protein beta(3)-subunit (C825T) | Regulatory protein of IK1 | Decreased function | Poly | rs5443 | 291 cases/292 controls | Caucasian | PAF = CAF | Asso | HTN | 34 |
| SCNSA (A1867G or H558R) | \(\alpha\)-subunit of INa | Decreased function | Poly | rs1805124 | 157 cases/314 controls | Caucasian | PAF = CAF | Asso | Nil | 36 |
| Sarcolipin (G-65C) | Inhibitor of SERCA | Functional significance unknown | Poly | rs583362 | 147 cases/92 controls | Caucasian | PAF = CAF | Screen | Asso | Nil | 37 |

| Nonionic channels/calcium handling proteins |  |  |  |  |  |  |  |  |
| Renin-angiotensin system | AngII biosynthesis pathway |  | Poly | rs1799752 | 250 case-control pairs | Taiwanese | PAF = CAF | Asso | VHD, CHF, HTN | 38,42 |
| (ACE I/D) |  |  | Poly | rs1799752 |  |  |  |  |  |
| (AGT G-6A, A-20C, G-152A, and G-217A) | AngII augments iCaL |  | Poly | rs699 (M239T) and rs4762 (T174M) |  |  |  |  |  |
| (AT1R A1166C) |  |  | Poly | rs1799752 |  |  |  |  |  |

Continued on next page
<table>
<thead>
<tr>
<th>Functions</th>
<th>Mode</th>
<th>GenBank/dbSNP</th>
<th>No. of Cases</th>
<th>Race</th>
<th>AF Type</th>
<th>Method</th>
<th>UHD</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx40 (+44A/-71G and -44G/-71A)</td>
<td>Decreased promoter function</td>
<td>Poly</td>
<td>—</td>
<td>173 cases/232 controls</td>
<td>Taiwanese</td>
<td>PAF + CAF</td>
<td>Asso</td>
<td>VHD, CHF, HTN</td>
</tr>
<tr>
<td>(P88S, M163V, G38D, and A96S)</td>
<td>Mutant Cx40 protein</td>
<td>S. Mut</td>
<td>—</td>
<td>15 cases</td>
<td>Caucasian</td>
<td>PAF</td>
<td>Screen</td>
<td>Nil</td>
</tr>
<tr>
<td>eNOS (T-786C, Interaction with KCNE1 S38G)</td>
<td>Antiinflammatory pathway</td>
<td>Poly</td>
<td>—</td>
<td>331 cases/441 controls</td>
<td>Caucasian</td>
<td>PAF + CAF</td>
<td>Asso</td>
<td>HTN, CHF</td>
</tr>
<tr>
<td>MMP2 (C-1306T) and IL10 (A-592C)</td>
<td>Inflammatory and fibrotic pathways</td>
<td>Poly</td>
<td>—</td>
<td>196 cases/873 controls</td>
<td>Japanese</td>
<td>CAF</td>
<td>Asso</td>
<td>Nil</td>
</tr>
<tr>
<td>Genome-wide scan</td>
<td>Underlying gene uncertain</td>
<td>Poly</td>
<td>rs1800872</td>
<td>2,801 cases/17,714 controls</td>
<td>Caucasian</td>
<td>AF + AFL</td>
<td>Asso</td>
<td>Stroke, HTN</td>
</tr>
</tbody>
</table>

ACE = angiotensin-converting enzyme; AD = autosomal dominant; AF = atrial fibrillation; AFL = atrial flutter; AGT = angiotensinogen; AngII = angiotensin II; Asso = association study; AT1R = angiotensin type 1 receptor; CAF = chronic atrial fibrillation; CHF = congestive heart failure; Cx40 = connexin 40; eNOS = endothelial nitric oxide synthase; FAF = familial atrial fibrillation; HTN = hypertension; ICaL = L-type calcium current; I/D = insertion/deletion; IKr = rapidly activating delayed outward rectifier potassium channel; IKs = slowly activating delayed outward rectifier potassium channel; ILK1 = inward rectifier potassium channel; IL10 = interleukin 10; link = linkage analysis; MMP2 = matrix metalloproteinase 2; PAF = paroxysmal atrial fibrillation; poly = polygenic; SERCA = sarcoplasmic reticulum calcium ATPase; SLN = sarcolipin; S. Mut = somatic mutation; UHD = underlying heart disease; VHD = valvular heart disease.
Genes of potassium channel subunits and regulatory proteins. Lai et al. (29) first investigated the association between minK gene (KCN1E1) polymorphism and nonfamilial AF in the Taiwanese population. The polymorphism involves an adenine to guanine transition at position 112 in the KCNE1 gene, resulting in a glycine to serine amino acid substitution at position 38 of the minK peptide (S38G). This study also used a candidate gene approach, and minK gene was selected because it is the β-subunit of the cardiac IKs (15,30). In this study, the authors used an individually matched design to decrease the effects of confounding factors. The case and control groups were individually matched regarding age, gender, left ventricular dysfunction, and significant valvular heart disease. These parameters are known nongenetic risk factors for the development of AF. There were a total of 108 patients with AF and 108 matched control subjects. The results demonstrated that the minK 38G allele was associated with an increased risk for AF. The minK 38G allele frequency was significantly higher in the AF group than in the control group (76.4% vs. 63.0%; p < 0.01), and patients with at least 1 38G allele had a higher risk to develop AF (odds ratio [OR]: 1.8; 95% confidence interval [CI]: 1.2 to 2.7; p < 0.0046).

Recently Fatini et al. (31) studied 331 patients with nonvalvular AF and 441 control subjects from the Caucasian population and similarly found that minK gene 38G allele was associated with AF (dominant model: OR: 1.73; 95% CI: 1.19 to 2.53; p = 0.004; recessive model: OR: 1.59; 95% CI: 1.15 to 2.27; p = 0.006). These results are interesting, and indicate that a polymorphism (29) and a mutation (12) on genes (KCN1E1 and KCNQ1, respectively) encoding different subunits of the same ion channel (IKs) may be responsible for the development of nonfamilial and familial AF, respectively. From these results it is also implied that familial and nonfamilial AF may share some common mechanism.

A functional study of this minK (KCN1E1) gene polymorphism was performed by another group (32). Ehrlich et al. (32) reported a smaller IKs current density when 38G minK was coexpressed with KVLQT1 in Chinese hamster ovary cells, prolonging simulated atrial action potential duration and favoring occurrence of early afterdepolarizations under some conditions. This result is contrary to that reported by Chen et al. (12), where a gain of function of IKs from a mutation in the KCNQ1 gene was found to cause familial AF. However, it has also been reported that mice with deletion of the KCNE1 protein (KCN1E1−/−; complete loss of function) have spontaneous episodes of AF despite normal atrial size and structure (33). KCN1E1−/− mice displayed unexpectedly shortened atrial action potentials and had spontaneous episodes of AF. Chromanol 293B (a KCNQ1 blocker) sensitive potassium currents were also significantly increased in atrial cells from KCNE1−/− mice. Furthermore, cells expressing KCNQ1 alone displayed marked current accumulation at a fast rate (10 Hz), which was not found in cells expressing KCNQ1 and KCNE1. These results indicate that multiple factors related to genetic variations of IKs subunit genes may underlie the molecular mechanism of AF. Further functional studies are warranted to find alternative explanations for these contradictory results.

Schreieck et al. (34) reported an association between C825T polymorphism in the coding region of the G-protein β3-subunit (GNB3) gene and nonfamilial AF. This polymorphism has been reported to affect atrial inward rectifier potassium currents, because it has been shown that patients with TT genotype have a higher IK1 current density in the right atrium (35). There were 291 AF patients and 292 control patients in the study and both groups had a similar profile of cardiovascular risk factors (hypertension, hypercholesterolemia, and high body mass index). Patients with coronary heart disease, valvular heart disease, or cardiomyopathy were excluded from the study. They found that the TT genotype was associated with a 54% decrease in the adjusted risk (OR: 0.46; 95% CI: 0.24 to 0.87; p = 0.02) for the occurrence of AF. Again, this result is contrary to the result reported by Xia et al. (21), where a gain of function of IK1 from a mutation in the KCN12 gene was found to cause familial AF. Nevertheless, these results demonstrate that alternation of the inward rectifier potassium channel current contributes not only to familial AF (21), but also to nonfamilial AF (34). It is also possible that other than the electrophysiological mechanisms, many of the genetic variations or mutations (including ion channel mutations) induce secondary morphologic, signaling, and/or mechanical changes that predispose to AF.

Genes of sodium channel subunits. The cardiac sodium channel (SCN5A) is a target for the treatment of arrhythmias (class I antiarrhythmic drugs). Recently, Chen et al. (36) studied 157 patients with early-onset AF who lacked traditional risk factors and 314 matched control subjects. They found an association between a common loss-of-function H558R amino acid polymorphism of the SCN5A gene and nonfamilial lone AF. The R558 allele was more common in AF patients than in control subjects (30% vs. 21%; p = 0.002), conferring an OR for AF of 1.6 (95% CI: 1.2 to 2.2). It is speculated that decreased sodium channel current may result in a slower rate of the upstroke of phase 0 depolarization, a decreased conduction velocity, and thus a shorter wavelength of a conduction impulse (7). As mentioned, the shorter the wavelength, the more wavelets in the atria, and AF will be more stable (8,17).

Gene of the sarcoplasmic reticulum calcium ATPase (SERCA2) regulatory protein. Sarcolipin (SLN) is a 31-amino acid protein, a homologous peptide of phospholamban. Like phospholamban, SLN is an effective inhibitor of SERCA2a. However, it is more highly expressed in atrial myocytes compared with phospholamban (37). The association study between the SLN gene variation and AF was first shown by Nyberg et al. (38). The coding region of SLN was screened for mutations using single-strand conforma-
tion polymorphism/heteroduplex analysis on the genomic DNA from 147 patients with AF and 92 control subjects. Aberrant conformers were sequenced. No mutations or polymorphisms were found in the coding sequence. A polymorphism, a guanine-to-cytosine transversion at position −65 in the promoter region was found, with a G allele frequency of 0.48. A significant difference in genotype distribution of this polymorphism (G-65C) was found between the AF group and control subjects (CC genotype: 15% in control subjects and 32% in AF patients; p = 0.011), although no significant difference of allele frequency was found. No promoter functional assay was performed for this polymorphism. Studies involving larger series of patients from different ethnic populations are warranted to confirm the role of SLN gene variations in AF.

Renin–angiotensin system (RAS) genes. Our group (39) also used a risk-factor matched design and reported an association between genetic polymorphisms within genes of the RAS and nonfamilial AF. The choice of RAS genes was based on recent findings that AF is associated with the activation of RAS in the atria of humans (40) and animal models of AF (41,42). Angiotensin II induces atrial fibrosis (41–43), which may result in an increase in conduction heterogeneity, conduction block, and facilitation of re-entry. Compared with a previous study with a similar risk-factor matched design (29), the case number and number of genetic polymorphisms in this study were substantially increased (250 case-control pairs and 8 polymorphisms).

Special statistical methods were adopted to accommodate the higher number of polymorphisms in the genetic association study, which included haplotype analysis (44,45), multifactor dimensionality reduction (46), and multilocus genotype disequilibrium tests (47,48).

We genotyped angiotensin-converting enzyme gene I/D polymorphism and T174M, M235T, G-6A, A-20C, G-152A, and G-217A polymorphisms of the angiotensinogen gene as well as A1166C polymorphism of the angiotensin II type I receptor gene. We demonstrated that the frequencies of M235, G-6, and G-217 alleles in the angiotensinogen gene were significantly higher in AF patients than in matched control subjects. The ORs for AF were 2.5 (95% CI: 1.7 to 3.3) with M235/M235 plus M235/T235 genotype, 3.3 (95% CI: 1.3 to 10.0) with G-6/G-6 genotype, and 2.0 (95% CI: 1.3 to 2.5) with G-217/G-217 genotype. Furthermore, significant gene–gene interactions were detected by the multifactor–dimensionality reduction method and multilocus genotype disequilibrium tests. In another substudy, instead of using an individually matched design, a regression approach was used to evaluate the independent effects of genetic factors while adjusting for the confounding effects of nongenetic factors (45).

Regarding their functional significance, polymorphisms in the RAS genes may affect the serum angiotensin II level. In addition to the profibrotic effect on the atrium (39), our group (49) also found that angiotensin II augments L-type calcium current and calcium transient through increasing the expression of the pore-forming α1C-subunit of L-type calcium channel. Using a computer simulation model, it has been demonstrated that increased L-type calcium current plays a critical role in induction of dynamic spatial dispersions of repolarization in the atrium, which causes conduction block, re-entry, and initiation of AF (50). However, it has yet to be demonstrated that polymorphisms of the RAS genes promote AF via alterations of atrial angiotensin II levels. Therefore, the possibility that angiotensin II promotes AF through increasing L-type calcium current and calcium overload remains speculative. More studies are warranted to elucidate whether there is a mechanistic link between the observed association of RAS gene polymorphisms and AF.

Connexin-40 gene. Juang et al. (51) also reported an association between polymorphisms in the proximal promoter region of connexin-40 gene with nonfamilial AF. Connexin-40 plays an important role in electrical coupling between atrial myocytes. In this study, there were 173 AF and 232 control subjects, each with similar baseline characteristics, including the percentage of patients with valvular heart disease and similar mean left ventricular ejection fraction. The researchers found that the frequency of connexin-40 gene proximal promoter haplotype (−44A, +71G) was significantly higher in the AF group than in the control group (OR: 1.51; 95% CI: 1.13 to 2.04; p < 0.006). Juang et al. (51) also performed functional studies, demonstrating that the (−44A,+71G) promoter haplotype was associated with a lower promoter activity by luciferase assay. Furthermore, Firouzi et al. (52), using electrophoretic mobility shift and luciferase reporter assays, showed that Sp1 and GATA4 are important regulators of human connexin-40 gene transcription and that the −44G-to-A polymorphism negatively affects the promoter regulation by the transcription factors Sp1 and GATA4. This result indicates that genetic variants in the connexin-40 gene may cause a decrease of connexin-40 expression. It is speculated that decreased connexin expression may impair electrical coupling between atrial myocytes and create conduction heterogeneity, which may provide the substrate for AF.

Recently, somatic mutations in the connexin-40 gene were found in atrial tissue specimens, but not in lymphocytes, from patients with lone AF. Gollob et al. (53) sequenced human connexin-40 gene from genomic DNA isolated from surgically resected atrial tissue and peripheral lymphocytes from 15 patients with lone AF. Identified mutations were transfected into a gap-junction-deficient cell line to assess their functional effects on protein transport and intercellular electrical coupling. Four novel heterozygous missense mutations, P88S (in 2 subjects), M163V (in 1 subject), G38D (in 1 subject), and A96S (in 1 subject), were identified. Three variants, P88S, M163V, and G38D, were found only in the atrial tissue specimens, but not in the
lymphocytes, indicating a somatic source of the genetic defects. One variant, A96S, was detected in both atrial tissue and lymphocytes, suggesting a germ-line origin. Analysis of the expression of mutant proteins revealed impaired intracellular transport or reduced intercellular electrical coupling in P88S, G38D, and A96S variants. These results suggest that in addition to traditionally considered germ-line mutations or variants, somatic mutations also play an important role in predisposition to common diseases such as AF.

**Genes related to inflammation.** Fatini et al. (31), in addition to the minK 38G polymorphism, also studied SNPs in the endothelial nitric oxide synthase gene (eNOS), which plays an important role in antioxidation and inflammation. They found that the eNOS $-786C$ allele weakly influenced the risk of nonvalvular AF. However, the contemporaneous presence of minK 38G and eNOS $-786C$ alleles synergistically increased the predisposition to nonvalvular AF (OR: 2.11; 95% CI: 1.48 to 3.02; p < 0.0001; OR: 2.58; 95% CI: 1.37 to 4.88; p = 0.003; OR: 3.08; 95% CI: 1.49 to 6.33; p = 0.002; according to dominant, recessive, and additive models, respectively). Bedi et al. (54) also reported an association of the eNOS G894T polymorphism (OR: 3.2; 95% CI: 1.7 to 6.2; p < 0.001; for GG genotype) with AF in 340 unselected unrelated patients with congestive heart failure. However, they found no association of eNOS gene T-786C polymorphism with AF in their heart failure population. No functional data were available in these studies.

Recently, Kato et al. (55) studied 196 subjects with chronic lone AF and 873 control subjects, and genotyped 40 polymorphisms of 32 candidate genes by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. After multivariable logistic regression analysis with adjustment for age, gender, body mass index, prevalence of smoking, hypertension, diabetes mellitus, and hypercholesterolemia, the C-1306T polymorphism of the matrix metalloproteinase-2 gene (MMP2) and the A-592C polymorphism of the interleukin-10 gene (IL10) were significantly associated with the prevalence of AF. The T-allele of the MMP2 polymorphism and the C-allele of the IL10 polymorphism were, respectively, a risk factor for and a protective factor against AF. There were also no functional data available in this study.

In summary, these observations suggest that some form of genetic control exists in the pathogenesis of the more common type of AF. It is important to recognize that most of these studies had small sample sizes and used a case-control design, making the results sensitive to the methods used to adjust for confounding effects and differences in the population histories. However, these data are promising and may help to clarify why some people develop AF and others do not in the general population.

**Future Aspects**

The results of genetic studies of AF may provide insights into the mechanism of AF. For familial AF, most of the identified genes encode for ionic channel subunits. In these families, AF is a presentation of a channelopathy. The mutations of these genes may result in a shorter atrial refractory period, which facilitates the maintenance of multiple re-entries. For those families with the absence of ion channel mutation or those in which the responsible genes have not been identified, the mechanism of AF is unknown. Regarding the mechanism of nonfamilial or common AF, in addition to the ionic current changes, conduction delay and block related to atrial fibrosis, inflammation, and altered expression of connexin facilitate the initiation and maintenance of multiple re-entries.

With the current availability of abundant genome-wide SNP markers, dissection of the underlying genetic loci for AF is possible. Recently, Gudbjartsson et al. (56) performed a genome-wide association scan, with the use of the Illumina Hap300 BeadChip (San Diego, California) (316,515 SNPs) in a sample of 350 patients with AF and/or atrial flutter and 4,476 control subjects from Iceland. This was followed by replication studies in additional samples from the Icelandic (2,251 case and 13,238 control subjects), Swedish (143 case and 738 control subjects), U.S. (636 case and 804 control subjects) and Chinese (333 case and 2,836 control subjects) populations. They found a strong association between 2 sequence variants on chromosome 4q25 (rs2200733 and rs10033464) and AF in the European-descent populations. The risk of AF increased by 1.72 and 1.39 per copy, respectively. The association with the stronger variant was replicated in the Chinese population, where the risk of AF was increased by 1.42 per copy. There is no known gene present in the haplotype block containing the 2 sequence variants. The closest genes located in the adjacent upstream haplotype block are the PITX2 and ENPEP genes. The protein encoded by the PITX2 (paired-like homeodomain transcription factor–2) gene is important in cardiac development by directing the asymmetric morphogenesis of the heart. The protein encoded by the ENPEP gene is an aminopeptidase responsible for the breakdown of angiotensin II in the vascular endothelium. Whether PITX2 or ENPEP is truly the responsible gene and the yet to be described functional mechanism underlying these mutations warrants further study.

Based upon a pharmacogenetic point of view (57), genetic studies may also help determine which patients may benefit most from the nonchannel blocking agents. For example, RAS gene polymorphisms in patients with AF may determine their response to angiotensin-converting enzyme inhibitor therapy (58). It is also possible that variations in genes encoding for ionic channels may also identify high-risk patients who are susceptible to the potential side effects of channel–blocking antiarrhythmic drugs (59).
However, many challenges are present, especially in the studies with a case-control design. Care must be taken to avoid false-positive or false-negative results, including: 1) a very clear definition of the phenotypes, such as the attack type (paroxysmal, persistent, or chronic AF), family history (familial or nonfamilial), and underlying cardiovascular diseases or nongenetic factors (AF with underlying heart disease or lone AF); 2) use of a homogenous population with the same genetic background or ethnicity; 3) a large sample size with adequate power; 4) correction for p values in the context of multiple testing for association; 5) methods to adjust the confounding effects from environmental factors; 6) evaluation of gene–gene or gene–environment interactions; 7) choice of SNPs or genes with functional significance or a mechanistic link to the disease mechanism; and 8) association that is replicated or confirmed in other studies, especially in different ethnic populations. These elements are essential when performing a genetic association study and necessary to allow definite conclusions.

Reprint requests and correspondence: Dr. Fu-Tien Chiang, Department of Laboratory Medicine, National Taiwan University Hospital, No. 1, Section 1, Jen-Ai Road, Taipei 100, Taiwan. E-mail: futenc@ntuh.gov.tw.

REFERENCES


Key Words: genetics • atrial fibrillation • familial • multifactorial.