

# Molecular Genetics of Atrial Fibrillation

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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, which include 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 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 reentrant circuits in AF. In addition to familial AF, common AF often occurs in association with acquired diseases such as hypertension, valvular heart disease, or 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, renin-angiotensin-aldosterone system genes, connexin 40 gene, endothelial nitric oxide synthase gene, and interleukin 10 genes. 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 genome-wide scans with hundreds or thousands of polymorphisms will, in the future, make 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.

**Key Words:** Genetics • Atrial fibrillation • Familial • Multifactorial

## INTRODUCTION

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, and left ventricular dysfunction. Therefore, it has traditionally been regarded as a sporadic, non-genetic disorder. However, some 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, these phenomena suggest there is also a genetic predisposition for the development of AF. A recent Framingham Heart study of 2,243 participants also showed that the relative risk of AF was increased by 85% in individuals with at least one parent with a history of AF.<sup>1</sup>

According to the patterns of heredity, AF can be categorized into two major types. The first type is familial AF with a Mendelian hereditary pattern, and the second type non-familial AF. Familial AF is a monogenetic disorder, and is often identified when AF is present in many

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members of the same family. Although uncommon, familial AF sometimes occurs in the setting of other inherited (structural) heart diseases, for example in association with dilated or hypertrophic cardiomyopathy.<sup>2-5</sup>

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

## MECHANISMS OF AF

The underlying mechanisms of AF are complex. Multiple rapidly discharging foci, a focal source with fibrillatory conduction,<sup>6,7</sup> or multiple reentrant circuits<sup>8,9</sup> have been proposed to explain the maintenance of AF. At present, both the focal source and the multiple reentrant circuit theories are the generally accepted hypotheses to explain AF.

In the multiple-reentry theory, the stability of AF is determined by the number of wavelets in the atria.<sup>8</sup> The wavelength, which is the distance traveled by an impulse during one refractory period, is the basal unit of a traveling wavelet, and can be calculated from the product of the refractory period and conduction velocity.<sup>7</sup> Therefore, the shorter the wavelength, the more wavelets in the atria, and thus AF will be more stable.<sup>8</sup> Consequently, the functional effect(s) of gene(s) which determine changes in refractory period or conduction velocity

**Table 1.** Genetics of familial atrial fibrillation

	Functions	Mode	GenBank/dbSNP	Case Number	Race	AF type	Method	UHD	Reference
No locus and gene identified									
FAF 1-4	?	AD	–	4 families	Caucasian	PAF	Screen	Nil	11
Locus identified									
10q 22-24	?	AD	–	3 families	Caucasian	CAF	Link	Nil	9
6q 14-16	?	AD	–	1 family	Caucasian	PAF	Link	Nil	12
Gene identified									
KCNQ1 S140G	$\alpha$ subunit of IKs (KVLQT1) Gain of function	AD	NP_000209.2:p.S140G NM_000218.2:c.418A > G	1 family	Chinese	CAF	Link	Nil	13
KCNQ1 R14C	$\alpha$ subunit of IKs (KVLQT1) Gain of function under stretch	AD	NP_000209.2:p.R14C NM_000218.2:c.40C > G	50 families	Caucasian	PAF	Screen	NTN	28
KCNE2 R27C	$\beta$ subunit of KCQT1-KCNE2 Gain of function	AD	NP_751951.1:p.R27C NM_172201.1:c.79C > T	2 families	Chinese	PAF	Link	Nil	18
KCNJ2 V93I	$\alpha$ subunit of IK1 (Kir2.1) Gain of function	AD	NP_000882.2:p.V93I NM_000891.2:c.277G > A	30 kindreds	Chinese	PAF	Screen	Nil	21
KCNH2 N588K	$\alpha$ subunit of IKr (HERG) Gain of function	AD	NP_000229.2:p.N588K NM_000238.2:c.1764C > A	1 family	Caucasian	PAF	Screen	Nil	26

AD = autosomal dominant; CAF = chronic atrial fibrillation; FAF = familial atrial fibrillation; HTN = hypertension; link = linkage analysis; IKs = slowly activating delayed outward rectifier potassium channel; IKr = rapidly activating delayed outward rectifier potassium channel; IK1 = inward rectifier potassium channel; PAF = paroxysmal atrial fibrillation; UHD = underlying heart disease; VHD = valvular heart disease.

**Table 2.** Genetics of non-familial atrial fibrillation

	Functions	Mode	GenBank/dbSNP	Case Number	Race	AF type	Method	UHD	Reference
Candidate gene approach									
Ionic channels/calcium handing proteins									
KCE1 (A112G or S38G)	$\beta$ subunit of IK1 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
SCN5A (A1867G or H558R)	$\alpha$ subunit of Na Decreased function	Poly	rs1805124	157 cases /314 controls	Caucasian	PAF+CAF	Asso	Nil	36, 37
Sarcolipin (G-65C)	Inhibitor of SERCA Functional significance unknown	Poly	rs583362	147 cases /92 controls	Caucasian	PAF+CAF	Screen Asso	Nil	38
Non-ionic channels/calcium handing protrins									
Renin-angiotensin-aldosterone system (ACE I/D)	AngII biosynthesis pathway AngII augments ICaL	Poly	rs1799752	250 case-control pairs	Taiwanese	PAF+CAF	Asso	VHD, CHF, HTN	39, 43, 48
(AGT G-6A, A-20C, G-152A and G-217A)		Poly	rs699 (M235T) and rs4762 (T174M)						
(AT1R A1166C) (CYP 11B2 T-344C)	Synthesis of aldosterone	Poly	rs1799752	196 patients with CHF (63 AF/133 no~AF)	Caucasian	PAF+CAF	Asso	CHF	53
Cx40	Cx40 gene proomoter haplotype Decreased promoter functon	Poly	–	173 cases /232 controls	Taiwanese	PAF+CAF	Asso	VHD, CHF, HTN	54
(–44A/+71G and –44G/+71A)									
(P88S, M163V, G38D and A96S)	Mutant ZCx40 protein Impaired intracellular transport	S.Mut	–	15 cases	Caucasian	PAF	Screen	Nil	56

eNOS	Anti-inflammatory pathway									
(T-786C, interaction with KCNE1 S38G)	Functional significance unknown	Poly	–	331 cases /441 controls	Caucasian	PAF+CAF	Asso	HTN, CHF	31	
(G894T or E298D)		Poly	rs1799983	340 patients with CHF (51 AF/289 no~AF)	Caucasian	PAF+CAF	Asso	CHF	57	
MMP2 (C1306T)	Inflammatory and fibrotic pathways	Poly	–	196 cases /873 controls	Japanese	CAF		Nil	58	
and IL 10(A-592C)	Functional significance unknown	Poly	rs1800872							
Genomewide scan. Illumina Hap300 BreadChip SNPs	Underlying gene uncertain			2801 cases /17714 controls (Iceland)	Caucasian	AF+AFL	Asso	Stroke, HTN	61	
rs2200733	Nearby genes PITX2 and ENPEP	Poly	rs2200733	143 cases /738 controls (Sweden)						
rs10033464	Morphogenesis of the heart (PITX2)	Poly	rs10033464	636 cases /804 controls (US)	Chinese			Stroke, DM		
	Breakdown of AngII(ENPEP)			333 cases /2836 controls						

ACE =angiotensin converting enzyme; 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; CYP11B2 = aldosterone synthase; eNOS = endothelial nitric oxide synthase; HTN = hypertension; eNOS = endothelial nitric oxide synthase; Ical = L-type calcium current; I/D = insertion/deletion; IKs = slowly activating delayed outward rectifier potassium channel; IL-10 = interleukin 10; MMP2 matrix metalloproteinase 2; PAF = paroxysmal atrial fibrillation; poly = polygenetic SERCA = sarcoplasmic reticulum calcium ATPase; SLN = sarcolipin; S. Mut = somatic mutation; UHD = underlying heart disease; US = United States; VHD = valvular heart disease.

will affect the wavelength of the traveling wavelet, and therefore 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 cardiac veins, such as pulmonary veins and superior vena cava, or the atria itself. Proposed mechanisms initiating these atrial ectopic foci include increased automaticity, afterdepolarization and microentry. These triggers may initi-

ate multiple reentrant 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 changes of the atrium, which are referred to as structural remodeling. Therefore, the downstream functional effect(s) of gene(s) which contribute to the mechanisms of increased automaticity, afterdepolarization, microentry and structural remodeling will also promote AF.

The mechanisms triggering and maintaining AF

overlap. For example, a rapidly discharging focus can 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 is involved in maintenance of AF. Structural changes in the junction between the left atrium and the pulmonary vein may also create conduction block and thus initiate microreentry as a trigger of 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 remodeling, 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 non-familial AF.

## FAMILIAL AF

Linkage analysis was used in most of the genetic studies of monogenetic AF. The success of this method depends on the establishment of a large pedigree as well as a clear identification of the phenotypes.<sup>10</sup> Although a mutated gene found in one family may not be present in another family or in patients with multi-factorial or multi-genetic 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.<sup>11</sup> first reported 3 families with autosomal dominant AF. Linkage analysis revealed 10q22-q24 as the genetic locus in these families, although the exact gene responsible remains unclear. A candidate gene approach has been applied to the 10q22-q24 region. One of the possible candidate genes was the DLG5 gene (Discs, Large (Drosophila) Homolog 5), a member of the MAGUKs (membrane-associated guanylate kinase) family which mediates intracellular signaling. However, this gene has been excluded as the responsible gene in these families.<sup>12</sup> Efforts at positional cloning of other genes in this region are still ongoing.

Darbar et al. also reported four multi-generation

families with autosomal dominant AF.<sup>13</sup> However, genotyping of these families with deoxyribonucleic acid markers spanning the chromosome 10q22-q24 region excluded linkage of AF to this locus.<sup>13</sup>

Ellinor et al.<sup>14</sup> investigated 34 members in a family with familial AF. They found a LOD score of 3.63 at a marker at 6q14-16. The exact gene responsible remains unknown.

### KCNQ1 gene

Chen et al.<sup>15</sup> studied a four-generation Chinese family with autosomal dominant AF. The locus was mapped to chromosome 11p15.5 and the gene, KCNQ1, which encodes for the  $\alpha$  subunit of the cardiac slow delayed-rectifier potassium channel (IKs)<sup>16,17</sup> and was identified as the responsible gene. This gene is the same as the first genetic locus for congenital long QT syndrome (LQT1).<sup>16,17</sup> 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<sup>18</sup> (LQT5, the  $\beta$ -subunit of the cardiac IKs channel) or minK-related protein 1<sup>19</sup> (MiRP1, the  $\beta$ -subunit of several cardiac potassium channels [its identity as the  $\beta$ -subunit of various potassium channels in humans in-vivo is speculative]) in COS-7 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 long QT syndrome.<sup>15</sup> 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 reentrant circuits and wavelets in AF.<sup>8,20</sup> 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.

### KCNE2 gene

The same group also identified a locus responsible for familial AF on 21q22 encoding for another potassium channel subunit-KCNE2.<sup>21</sup> The mutation involved a cytosine to thymine transition at nucleotide 79 of the gene for KCNE2. This resulted in an arginine to cysteine

substitution at residue 27 (R27C). The KCNE gene family encodes small proteins that function as  $\beta$ -subunits of several voltage-gated cation channels.<sup>22</sup> KCNE2 encodes for MiRP1, the  $\beta$ -subunit of the rapid component of the delayed rectifier current (IKr)<sup>19</sup> and the KCNQ1-KCNE2 channel, which produces background potassium current. Functional analyses also revealed a gain-of-function effect, which may result in a shortening of the action potential duration and facilitate multiple wavelets and perpetuation of AF.<sup>8,20</sup>

### KCNJ2 gene

Because overexpression of the wild-type Kir2.1 in mice induced AF,<sup>23</sup> Xia et al. studied thirty Chinese AF kindreds for a mutation in KCNJ2,<sup>24</sup> which encodes for the Kir 2.1 channel and mediates an inward rectifier potassium current in the heart (IK1). A valine-to-isoleucine mutation at position 93 (V93I) of 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 reentrant circuits and wavelets in AF.<sup>8,20</sup> Increased expression of the Kir2.1 channel has also been found in atrial samples from patients with common AF.<sup>25-28</sup>

### KCNH2 gene

Recently, Hong et al.<sup>29</sup> identified a family with short QT syndrome (SQTS) in which three 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). The KCNH2 encodes for the HERG protein, the  $\alpha$ -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, revealing 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 the 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, and therefore promoting an ideal substrate for multi-wavelet reentry. However, most of the AF families with identified potassium channel mutations are from the Chinese population.<sup>14,21,23</sup> Recently, Ellinor et al. screened 96 probands of AF families from the Caucasian population, and found no mutation in either KCNJ2 or KCNE1-5 genes.<sup>30</sup> Therefore, the potassium channel gene mutation may not be universal for familial AF in all ethnic populations. 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 have hypertension. Patch-clamp studies of wild-type or R14C KCNQ1 co-expressed with KCNE1 in CHO 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.<sup>31</sup> 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.

## NON-FAMILIAL 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, instead of linkage analysis, a genetic association study is commonly used. 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, this variant 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 the genes or makers from the whole genome are tested without an a priori assumption regarding which genes are possibly responsible.

### Genes of potassium channel subunits and regulatory proteins

Lai et al.<sup>32</sup> first investigated the association between minK gene (KCNE1) polymorphism and non-familial 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  $\beta$ -subunit of the cardiac IKs.<sup>18,33</sup> 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 with regards to age, sex, left ventricular dysfunction and significant valvular heart disease. These parameters are known non-genetic risk factors for the development of AF. There were a total of 108 patients with AF and 108 matched controls. The results demonstrated that the minK 38G allele was associated with an increased risk for AF. MinK 38G allele frequency was significantly higher in the AF than in the control groups (76.4% vs 63.0%;  $P < 0.01$ ), and patients with at least one 38G allele had a higher risk to develop AF (odds ratio [OR] 1.8, 95% confidence interval [CI] 1.2-2.7,  $P < 0.0046$ ).

Recently, Fatini et al. studied 331 patients with non-valvular AF and 441 controls from the Caucasian population, and similarly found that minK gene 38G allele was associated with AF (dominant model: OR =

1.73, 95% CI 1.19-2.53,  $P = 0.004$  and recessive model: OR = 1.59, 95% CI 1.15-2.27,  $P = 0.006$ ).<sup>34</sup> These results are interesting, and indicate that a polymorphism<sup>29</sup> and a mutation<sup>14</sup> on genes (KCNE1 and KCNQ1, respectively) encoding different subunits of the same ionic channel (IKs) may be responsible for the development of non-familial and familial AF, respectively. From these results, it is also implied that familial and non-familial AF may share some common mechanism(s).

A functional study of this minK (KCNE1) gene polymorphism was performed by Ehrlich et al.<sup>35</sup> They reported a smaller IKs current density when 38G minK was co-expressed with KVLQT1 in Chinese hamster ovary cells, prolonging simulated atrial action potential duration and favoring occurrence of early afterdepolarizations under some conditions.<sup>35</sup> This result is contrary to that reported by Chen et al., where a gain of function of IKs from a mutation in the KCNQ1 gene was found to cause familial AF.<sup>14</sup> However, it has also been reported that mice with deletion of the KCNE1 protein (KCNE1<sup>-/-</sup>; complete loss of function) have spontaneous episodes of AF despite normal atrial size and structure.<sup>36</sup> KCNE1<sup>-/-</sup> 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<sup>-/-</sup> 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 underlie the molecular mechanism of AF. Further functional studies are warranted to find alternative explanations for these contradictory results.

Schreieck et al. reported an association between C825T polymorphism in the coding region of the G-protein beta(3) subunit (GNB3) gene and non-familial AF.<sup>37</sup> 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.<sup>38</sup> 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 body mass index) hypothesized that nudction eriod period. ock, which resulted in. Patients with coronary heart disease,

valvular heart disease, or cardiomyopathy were excluded from the study. The investigators found that the TT genotype was associated with a 54% decrease in the adjusted risk (OR 0.46, 95% CI 0.24-0.87,  $P = 0.02$ ) for the occurrence of AF. Again, this result is contrary to the result reported by Xia et al., where a gain of function of IK1 from a mutation in the KCNJ2 gene was found to cause familial AF.<sup>24</sup> Nevertheless, these results demonstrate that alternation of the inward rectifier potassium channel current contributes not only to familial AF,<sup>24</sup> but also to non-familial AF.<sup>37</sup> It is also possible that other than the electrophysiological mechanisms, many of the genetic variations or mutations (including ion channel mutations) induce secondary morphological, 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 anti-arrhythmic drugs). Recently, Chen et al. studied 157 patients with early-onset AF who lacked traditional risk factors and 314 matched controls. They found an association between a common loss-of-function H558R amino acid polymorphism of the SCN5A gene and non-familial lone AF.<sup>39</sup> The R558 allele was more common in AF patients than in controls (30% vs. 21%,  $P = 0.002$ ), conferring an OR for AF of 1.6 (95% CI 1.2-2.2). 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.<sup>7</sup> As mentioned above, the shorter the wavelength, the more wavelets in the atria, and AF will be more stable.<sup>8,20</sup>

In another study,<sup>40</sup> Darbar et al. sequenced the entire SCN5A coding region in 375 subjects with either lone AF ( $n = 118$ ) or AF associated with heart disease ( $n = 257$ ). Controls ( $n = 360$ ) from the same population were then genotyped for the presence of mutations or rare variants identified in the AF cases. They identified 8 novel SCN5A variants in the AF cohort. Because these 8 variants affect highly conserved residues, they are predicted to perturb cardiac sodium channel function.

### Gene of the sarcoplasmic reticulum calcium ATPase (SERCA2) regulatory protein

Sarcoplipin (SLN) is a 31-amino acid protein, a ho-

mologous peptide of phospholamban. SLN is an effective inhibitor of SERCA2a when it forms a ternary complex with phospholamban and SERCA2a. Altered SERCA2a function results in impaired calcium homeostasis and cycling, which contributes to arrhythmogenesis and contractile dysfunction during AF.

Nyberg et al. examined the genetic variation of SLN in patients with long QT syndrome (LQTS), sudden arrhythmia death syndrome, and AF.<sup>41</sup> The coding region of SLN was screened for mutations using single strand conformation polymorphism/heteroduplex analysis on the genomic DNA from 95 unrelated LQTS patients, 59 patients with sudden arrhythmia death syndrome, 147 patients with AF and 92 controls. Aberrant conformers were sequenced. No mutations or polymorphisms were found in the coding sequence. A guanine-to-cytosine transversion in the highly conserved position +1 of the 3' untranslated region was found in two patients with sudden arrhythmia death syndrome. A polymorphism, a guanine-to-cytosine transversion at position -65 in the promoter region was also 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 controls (CC genotype 15% in controls and 32% in AF patients,  $P = 0.011$ ). Studies involving larger series of patients from different ethnic populations are warranted to confirm the role of SLN gene variations in AF.

### Renin-angiotensin-aldosterone system genes

Tsai et al. also used a risk-factor matched design, and reported an association between genetic polymorphisms within genes of the renin-angiotensin system (RAS) and non-familial AF.<sup>42,43</sup> The choice of RAS genes was based on recent findings that AF was associated with the activation of RAS in the atria of humans<sup>44</sup> and in a dog model of AF.<sup>45</sup> Angiotensin II induces atrial fibrosis,<sup>45</sup> which may result in an increase in conduction heterogeneity, conduction block, and facilitation of reentry. The case number in this study was higher than that of Lai's study (250 AF patients and 250 matched controls), and the number of genetic polymorphisms used was up to 8.

Special statistical methods were adopted to accommodate the higher number of polymorphisms in the genetic association study, which included haplotype analy-

sis,<sup>46,47</sup> multifactor dimensionality reduction<sup>48,49</sup> and multilocus genotype disequilibrium tests.<sup>50,51</sup>

They genotyped angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism, T174M, M235T, G-6A, A-20C, G-152A, and G-217A polymorphisms of the angiotensinogen gene, and A1166C polymorphism of the angiotensin II type I receptor gene. They 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 controls. The ORs for AF were 2.5 (95% CI 1.7–3.3) with M235/M235 plus M235/T235 genotype, 3.3 (95% CI 1.3–10.0) with G-6/G-6 genotype, and 2.0 (95% CI 1.3–2.5) 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 sub-study, 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 non-genetic factors.<sup>47</sup>

In addition to the Taiwanese population, the association between RAS gene polymorphisms and AF was replicated in a large Danish population.<sup>52</sup> In the Copenhagen City Heart Study, the A-20C, G-6A, T174M, and M235T polymorphisms in the angiotensinogen gene and the I/D polymorphism in the ACE gene were genotyped in 9235 individuals from the Danish general population. Participants had sinus rhythm at inclusion. During 26 years of follow-up, 968 individuals developed AF. Multifactorially adjusted hazard ratios for AF for A-20C AC and CC versus AA genotype were 1.1 (95% CI 1.0–1.3;  $P = 0.05$ ) and 1.5 (1.1–2.1;  $P = 0.01$ ).

Regarding the functional significance, polymorphisms in the RAS genes may affect the serum angiotensin II level. In addition to the pro-fibrotic effect on the atrium,<sup>42</sup> Tsai et al. also found that angiotensin II augments L-type calcium current and calcium transient through increasing the expression of the pore-forming  $\alpha_1C$  subunit of the L-type calcium channel.<sup>53</sup> Augmented calcium transient has been reported to initiate late phase 3 early afterdepolarization,<sup>54</sup> which is an important mechanism of rapid firing in the pulmonary veins.<sup>55</sup> Furthermore, 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, reentry, and initiation of AF.<sup>56</sup> 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.

Aldosterone is another component of the renin-angiotensin-aldosterone system. Amir et al. analyzed the possible association between aldosterone synthase (CYP11B2) T-344C polymorphism, which is associated with increased aldosterone activity, and the prevalence of AF in 196 consecutive patients who had symptomatic systolic heart failure (left ventricular ejection fraction < 40%).<sup>57</sup> AF was present in 63 patients (33%) with heart failure. They found the -344 CC genotype to be a strong independent marker for AF. Multivariate regression analysis showed that the CYP11B2 CC genotype was an independent predictor of AF (adjusted OR 2.35, 95% CI 1.57–3.51,  $P = 0.03$ ). Therefore, CYP11B2 T-344C promoter polymorphism may predispose to AF in patients with HF.

### Connexin 40 gene

Juang et al. also reported an association between polymorphisms in the proximal promoter region of connexin 40 gene with non-familial AF.<sup>58</sup> Connexin 40 plays an important role in electrical coupling between atrial myocytes. In this study, there were 173 AF and 232 control, 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 1.13–2.04,  $P < 0.006$ ). Juang et al. 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., using electrophoretic mobility shift and luciferase reporter assays, showed that Sp1 and GATA4 are important regulators of human Cx40 gene transcription and that the -44 G-to-A poly-

morphism negatively affects the promoter regulation by the transcription factors Sp1 and GATA4.<sup>59</sup> 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.<sup>60</sup> Gollob et al. 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 the 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

In Fatini's study,<sup>34</sup> in addition to the minK S38G polymorphism, they also studied SNPs in the endothelial nitric oxide synthase gene (eNOS), which plays an important role in anti-oxidation and inflammation. They found that the eNOS -786C allele weakly influenced the risk of non-valvular AF. However, the contemporary presence of minK 38G and eNOS -786C alleles synergistically increased the predisposition to non-valvular AF (OR = 2.11, 95% CI 1.48-3.02,  $P < 0.0001$ ; OR = 2.58, 95% CI 1.37-4.88,  $P = 0.003$ ; OR = 3.08, 95% CI 1.49-6.33,  $P = 0.002$ , according to dominant, recessive, and additive models, respectively). Bedi et al. also re-

ported an association of the eNOS G894T polymorphism (OR = 3.2, 95% CI 1.7-6.2,  $P < 0.001$  for GG genotype) with AF in 340 unselected, unrelated patients with congestive heart failure.<sup>61</sup> 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. studied 196 subjects with chronic lone AF and 873 controls, 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.<sup>62</sup> After multivariable logistic regression analysis with adjustment for age, sex, 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 a risk factor for and protective factor against AF, respectively. There were also no functional data available in this study.

In summary, the above 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 while 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 reentries. 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 non-familial 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 reentries.

The results of the genetic studies may have a significant impact on the treatment of AF.<sup>63</sup> After the initial discovery of the association between RAS gene polymorphisms and AF, there have been more and more clinical trials showing the efficacy of angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor blockers to treat or prevent AF.<sup>64,65</sup> Recently, statin, a potent antioxidant and anti-inflammatory agent, has also been shown to be effective in the treatment or prevention of AF.<sup>66,67</sup>

With the current availability of abundant genome-wide SNP markers, dissection of all the underlying genetic loci for AF is possible. Recently, Gudbjartsson et al. performed a genome-wide association scan, with the use of the Illumina Hap300 BeadChip (316515 SNPs), in a sample of 550 patients with AF and/or atrial flutter and 4476 controls from Iceland.<sup>68</sup> This was followed by replication studies in additional samples from the Icelandic (2251 cases and 13,238 controls), Swedish (143 cases and 738 controls), United States (636 cases and 804 controls) and Chinese (333 cases and 2836 controls) populations (56). They found a strong association between two sequence variants on chromosome 4q25 (rs2200733 and rs10033464) and AF in the European descents. 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 two sequence variants. The closest genes located in the adjacent upstream haplotype block are the PITX2 and ENPEP genes. The protein encoded by the PITX2 gene, the paired-like homeodomain transcription factor 2, 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 may be interesting and war-

rants further study.

Furthermore, based upon a pharmacogenetic point of view,<sup>69</sup> genetic studies may also help determine which patients may benefit most from the non-channel blocking agents. For example, RAS gene polymorphisms in patients with AF may determine their response to ACE inhibitor therapy.<sup>70</sup> 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.<sup>71</sup>

However, many challenges are present, especially in the studies with a case control design.<sup>72</sup> Care must be taken to avoid false positive or false negative results, which include: (1) a very clear definition of the phenotypes, such as the attack type (paroxysmal, persistent or chronic AF), family history (familial or non-familial), and underlying cardiovascular diseases or non-genetic 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 links to the disease mechanism; and (8) the association should be 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.

Finally, AF is a complex-trait disease, where dilution of genetic effects by etiologic heterogeneity and environmental factors make systematic approach challenging. Phenotype clarification is further complicated by the paroxysmal and often asymptomatic nature of AF. Stable endophenotypes, such as signal-averaged P-wave duration,<sup>73</sup> which may be directly related to the underlying myocardial disorder, may be required for a comprehensive understanding of the genetic nature of AF.

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