Atrial fibrillation (AF) is the most common sustained and most important arrhythmia in clinical practice. The mechanisms underlying AF initiation and maintenance are known to be complex and heterogeneous. The general understanding of the detailed molecular basis of AF is still incomplete. Recently, these is increasing evidence that small conductance calcium-activated potassium (SK) channels are associated with atrial action potential repolarization and the pathogenesis of AF. Although the functional role of SK channels in the genesis of AF is not entirely clear, new insights into the basic pathophysiological mechanism of AF have been provided. Besides, genome-wide association studies also implicate that genes coding for SK channels are related to the risk of developing AF. This article reviews recent work on the association of SK channels and AF, genetic studies of SK channels, and discuss future investigation and developments regarding this field.

**Key Words:** Atrial fibrillation • Genetics • Small conductance calcium-activated potassium channels

**INTRODUCTION**

Atrial fibrillation (AF) is the most common sustained cardiac dysrhythmia in the general population and causes serious cardiovascular morbidity and mortality. The electrophysiological mechanisms involved in AF pathogenesis are complex and highly heterogeneous, and associated with underlying heart diseases. The general understanding of the molecular basis of AF to date remains incomplete. Over the past ten years, increasing evidence has emerged to link small-conductance calcium-activated potassium (SK) channels to atrial action potential profile and atrial arrhythmogenesis, including the pathogenesis of AF. In addition, during the last few years, a growing volume of epidemiologic evidence has indicated that genetic causes predispose individuals in the general population to the occurrence of AF. Upon closer examination of the relevant application of the recent population-based, case-control genome-wide association studies method, several common genetic variants in 9 distinct loci have been identified to be associated with AF. At one of the AF susceptibility loci, the risk variant is located in the potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3 (KCNN3), which encodes the SK3 channel (KCa 2.3). In this review, we have presented recent work on the association of SK channels and AF, genetic studies of SK channels, and discuss future investigation and developments regarding this field.
Small-conductance calcium-activated potassium channels in the atria

The calcium-activated potassium channels family can be divided into three subfamilies of channel subunits according to their unitary conductance: big conductance (BK, KCa 1.1), intermediate conductance (IK, KCa 3.1), and small conductance (SK, KCa 2) channels. Small-conductance calcium-activated potassium (SK) channels are encoded by 3 different genes, namely KCNN1 (SK1), KCNN2 (SK2), and KCNN3 (SK3). The phylogenetic tree showing the KCa2-KCa3 families is displayed in Figure 1. SK channels are solely activated by an increase of intracellular calcium to give rise to a time- and voltage-independent current. Presently, there is no entirely selective blocker for SK channel subtypes, indicating that their individual physiological roles cannot be clearly delimited.

SK channels have been widely studied in central and peripheral nerve systems and they play an essential role in the firing frequency of neurons. Although it has long been speculated that SK channels exist in cardiac tissue, the functional roles of SK channels have not received much attention as they relate to the heart. Prior to 1999, Wang et al. had reported that SK3 channels were expressed in myocytes from a cell-line derived from embryonic rat ventricle. In 2003, it was first documented by Xu et al. that activation of SK2 channels contributes to the late repolarization phase of the cardiac action potential. They also showed the presence of atrial-selective expression of SK2 channels in human and mouse cardiac myocytes. In 2005, Tuteja et al. provided the first evidence that SK3 channels were detected to have comparable levels of expression in the atria and ventricles, in contrast to atrial-selective expression of SK1 and SK2 channels in mouse heart.

Later, Lu et al. published the first report revealing the molecular mechanisms of the coupling of SK2 channels with voltage-gated calcium channels via cytoskeletal proteins in mouse cardiac myocytes. Abnormal expression of voltage-gated calcium channels would result in dysfunction of SK2 channels and prolongation of repolarization and then atrial arrhythmias. In addition to cardiac myocytes, Zhang et al. also demonstrated that ablation of SK2 channels resulted in prolongation of the action potential of atroventricular nodal cells and a decrease in the firing frequency. In 2010, evidence provided by Tuteja et al. showed the formation of homo- and heteromultimeric complexes by these three SK channel subunits in human atrial myocytes, although their functional significance remains to be discovered.

Association of SK channels and AF

In 2007, Ozgen et al. implied the existence of a link between SK channels and AF pathogenesis for the first time. They provided circumstantial evidence that increased SK2 channel cell-membrane trafficking and thereby elevation of calcium-activated potassium currents by intermittent burst pacing at the pulmonary vein-atrial interface provided an arrhythmogenic substrate for AF in a rabbit model. In 2009, Li et al. gave more direct evidence for the role of SK channels in AF. They illustrated that genetic knockout of the SK2 channel gene caused prolongation of action potential duration (APD) and inducible early afterdepolarizations (EADs) in atrial myocytes isolated from mice. Interestingly, in vivo electrophysiological recordings showed inducible AF by programmed premature extrastimulation. On the contrary, in 2010, Diness et al. showed that in models of AF, in isolated perfused heart from rat, guinea pig, and rabbit, pharmacological inhibition of SK channels resulted in prolongation of atrial effective refractory period (ERP), thereby inhibition of reentry, and then AF prevention and termination. In an in vivo rat model of acutely induced AF, SK channel inhibition could decrease AF susceptibility as well. Moreover, it was worth mentioning that no ventricular tachyarrhythmia was present in the reports by Li et al. and Diness et al., probably due to the nature of atrial-selection distribution of SK channels. Subsequently, two similar studies substantiated that prolongation of atrial ERP caused by SK channel inhibition was associated with antiarrhythmic activity in rat in vivo. Conversely, in 2013, Hsueh et al. indicated that SK channels blockade enhanced the heterogeneity of atrial APD, and then pro-

Figure 1. The phylogenetic tree showing the KCa2-KCa3 families.
moted the development of atrial arrhythmia in isolated canine atrium although SK channels blockade significantly increased atrial APD.21 The distinct elements from these studies regarding SK channels are summarized and compared in Table 1.

To date, the functional role of SK channels in the pathogenesis of AF is not entirely clear. Based on current evidence, both inhibition and activation of SK channel activity can be linked to an increased risk of AF. However, these seemingly contradictory results may not be crucial. We have known that loss or gain of function of potassium channels both are related to AF genesis. Increased potassium current can cause shortening of atrial ERP, which promotes the development of reentry and then AF.22 On the contrary, reduced potassium current leads to AF by 2 mechanisms. First, EADs could occur in this circumstance, which produces ectopic beats and the initiation of AF.23 Second, reduced potassium current may increase heterogeneity of atrial APD and ERP, rendering the atria more susceptible to reentrant arrhythmia, such as AF, in this situation.24

Besides, the species used in these studies were different. The mechanisms underlying AF initiation and maintenance are known to be complex and highly heterogeneous; in different species, the main mechanisms that contribute to AF genesis may vary.18 Furthermore, the distribution and components of SK channel subtypes in the atria could not be the same in different species. In one article, they found that, in human hearts, SK2 and SK3 channels are more abundant than SK1 channels in the atria.25

There are only a few reports on the alternation of SK current in the atrial electrical remodeling of AF. Li et al. reported that in patients with persistent AF, the SK2 current density in atrial myocytes was increased.26 In contrast, data from Yu et al. revealed that SK current was decreased in association with the downregulation of mRNA and protein expression level of SK1 and SK2 in chronic AF patients.27 Consistent with the findings by Ozgen et al., no change of SK3 channel levels was observed in AF patients and controls. However, in a recent report, Ling et al. demonstrated that atrial miRNA-499 was unregulated, resulting in downregulation of SK3 and possible electrical remodeling in chronic AF.28 The reason for this apparent discrepancy was unclear. One possible reason is that there was selection bias in human cardiac tissues including both chronic AF and control groups. Preliminary studies have found that SK channels are considered to be involved in AF electrical remodeling, and these varied findings need to be clarified in a larger patient population.

So far, all published reports with regard to the functional role of SK channels in the pathogenesis of AF focus on the consequences of their inhibition or blockade. These studies underscored the importance of the association of decreased SK channel activities and atrial arrhythmogenesis, but their results are conflicting, partly due to the complexity of AF mechanisms. On the other hand, it is still unknown how activation or enhancement of SK channel function influences the development of atrial arrhythmias. For elucidating the role of one specific ion channel in arrhythmogenesis, the effect of its activation also needs to be assessed by using selective compounds or transgenic models. Further investigation of this topic is mandatory to provide more relevant information and extend our understanding of

Table 1. Comparison of the distinct elements from published studies regarding SK channels

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal</th>
<th>Action</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozgen et al., 200716</td>
<td>Rabbit</td>
<td>Pacing at the pulmonary vein-atrial interface</td>
<td>Regional APD shortening and increased SK2 channel cell-membrane trafficking</td>
</tr>
<tr>
<td>Li et al., 200917</td>
<td>Mouse</td>
<td>Genetic knockout of SK2 channels</td>
<td>Atrial APD prolongation, occurrence of EADs, and AF induction</td>
</tr>
<tr>
<td>Diness et al., 201218</td>
<td>Rat, guinea pig, and rabbit</td>
<td>Pharmacological inhibition of SK channels</td>
<td>Atrial ERP prolongation and AF prevention and termination</td>
</tr>
<tr>
<td>Hsueh et al., 201321</td>
<td>Canine</td>
<td>Pharmacological inhibition of SK channels</td>
<td>Atrial APD prolongation, enhanced heterogeneity of atrial APD, and development of atrial arrhythmias</td>
</tr>
</tbody>
</table>

AF, atrial fibrillation; APD, action potential duration; EADs, early afterdepolarizations; ERP, effective refractory period; SK channels, small-conductance calcium-activated potassium channels.
the functional importance of SK channels in the pathogenesis of AF.

Genetic studies of SK channels
In 2010, in European ancestry populations consisting of 1335 individuals with lone AF and 12844 unrelated individuals without AF, Ellinor et al. (in their genome-wide association studies) discovered the single nucleotide polymorphism (SNP) rs13376333 most significantly associated with lone AF in a novel genetic locus in KCNN3 on chromosome 1q21.9 SNP rs13376333 showed a significant association with lone AF at a raised odds ratio (OR) of 1.56 (p = 6.3 × 10^{-12}). The genetic variant rs13376333 is an intronic SNP located between the first and second exon of the gene KCNN3. The association had also been independently replicated in two additional European cohorts.9 Besides, the relation between SNP rs13376333 and structural AF was found to be much weaker with an OR of 1.13 (p = 0.006). In another article by Olesen et al., they found that the synonymous SNP rs11318220 in KCNN3 was associated with lone AF with an OR of 2.85 (p = 0.026).9

Our previous study showed that there are significant associations between SNP rs13376333 and the risk of both lone and structural AF in the Taiwanese population.30 The minor allele T of SNP rs13376333 conferred significant risks for both lone (p < 0.001; OR, 3.02) and structural AF (p = 0.004; OR, 2.18). In concordance with the results from Ellinor et al., SNP rs13376333 had a greater effect on lone AF risk than on structural AF risk. However, the magnitude of the associations between this genetic variant and both lone and structural AF seemed to be stronger in the Taiwanese population. The reasons for this difference are unclear, but interesting. There has been some evidence that intrinsic racial differences in myocardial membrane stability, myocardial conduction pathways, or genetic polymorphisms cause different susceptibility to the development of AF.31 Another possibility is that the difference could be a result of complicated gene-gene and gene-environment interactions, which can dilute or accentuate genetic effects in complex traits such as AF.32 The difference in risk contribution of SNP rs13376333 to AF may indicate a relatively greater impact of this pathogenetic pathway on individual AF risk in the Taiwanese population.

In the report of Ellinor et al. among the Caucasian AF populations, the allele frequency of minor allele T of SNP rs13376333 was approximately 30%, much higher than the allele frequency of 7-8% in the Taiwanese AF population. Although the cause and significance of the apparent lower frequency of the minor allele T of SNP rs13376333 in the Taiwanese and Chinese Han populations may not be important, these findings suggest that this SNP and its related gene, KCNN3, provide a much lower overall population attributable risk in some general populations of Asia.

On the contrary, a recent case-control study conducted in a mainland Chinese Han population could not replicate the significant association between SNP rs13376333 and AF risk.33 In the Taiwanese population the frequency of the minor allele T of SNP rs13376333 was around 7-9% in patients with AF compared with 3% in unaffected controls, in contrast to 4-5% in AF patients compared with 3% in controls in that mainland Chinese Han population. Although the Taiwanese and Chinese Han populations are closely related in ethnicity, differences of genetic susceptibility appear to exist in terms of AF risk. Further investigations including additional different Asian ethnic groups and even larger sample sizes are required to validate the impact of SNP rs13376333 on the risk of structural or lone AF in Asian populations.

CONCLUSIONS
Over the past few years, growing evidence of the role of SK channels in atrial repolarization and atrial arrhythmias has provided us new insight into the pathophysiology of AF. However, the exact roles that SK channels play in AF pathogenesis remain to be resolved. In addition, the mechanisms through which SNP rs13367333 increases AF risk are still unclear. Prior studies regarding the association of SK channels and AF have presented some paradoxical and inconsistent results. Therefore, future research is necessary to further elucidate how SK channels contribute to the genesis of AF and involve the electrical remodeling of AF, and whether they modulate clinical manifestations of AF.

Blockade of SK channel function has been suggested as a novel target for AF therapy. Due to the differential expression of SK channels in the atrium versus the ventricle, modification of their activities are expected to
provide an exceptional opportunity to treat atrial arrhythmias. Only by advancing our understanding of the role of SK channels in AF pathogenesis will there be an opportunity for future development of new antiarrhythmic drugs. Furthermore, based on preliminary evidence from genetic studies, the factor of ethnic difference may need to be considered for future individually tethered pharmacological therapy for AF.

REFERENCES


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