KCNN3 SNP rs13376333 on Chromosome 1q21 Confers Increased Risk of Atrial Fibrillation

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Summary

To investigate the relationship between KCNN3 SNP (single-nucleotide polymorphism) rs13376333 and risk of atrial fibrillation (AF) and to provide evidence for prevention and treatment for AF.

The PubMed, Embase, OVID, Cochrane library, CNKI, and Wan Fang databases were searched to identify studies on the relationship between KCNN3 SNP rs13376333 polymorphism and atrial fibrillation. Two authors performed independent article reviews and study quality assessment using the Newcastle-Ottawa Scale (NOS) checklist.

Seven studies involving 24,339 individuals were included in the meta-analysis. The overall combined OR of rs13376333 polymorphism was observed for both lone AF (OR: 1.58 [95% CI: 1.37 to 1.82]; P < 0.001; I² = 47.0%) and total AF (OR: 1.33 [95% CI: 1.14 to 1.54]; P < 0.001; I² = 0). Further, when stratified by ethnicity, control sources, sample sizes, and genotyping method, similar results were observed in both subgroups. Sensitivity analysis revealed that the source of control was the source of the heterogeneity for lone AF. Omission of any single study had little effect on the combined risk estimate. No evidence of publication bias was found.

This meta-analysis suggests that KCNN3 SNP rs13376333 polymorphism significantly increases the risk of lone AF and total AF, which suggests the rs13376333 polymorphism of the KCNN3 gene may play an important role in the pathogenesis of AF. (Int Heart J 2015; 56: 511-515)

Key words: Single nucleotide polymorphism, Genetics, Meta analysis

Atrial fibrillation (AF) is the most common cardiac arrhythmia and accounts for approximately one-third of hospitalizations for cardiac rhythm disturbance.1 With its rising prevalence in the general population, AF has been an important risk factor for developing stroke and heart failure worldwide. Known risk factors for typical AF include aging, male gender, obesity, valvular heart disease, hypertension, myocardial infarction, and a family history. However, a subset of cases with AF have no clinically overt structural heart disease, the AF occurs at a younger age, and the patients are diagnosed as lone AF2,3 which accounts for up to 30% of all AF cases.

Over the past few years, it has been reported that P wave analysis in 12-lead electrocardiograms (ECG) can be useful for predicting new onset AF,4 and genetic predisposition is the newest addition to the occurrence of structural or lone AF. Some AF specific genes found may contribute to the development of left atrium remodeling.5,6 and several genetic loci have been identified for monogenetic AF, such as loci on chromosomes 1q21, 3p21, 11p15.5, 12p13, 21q22, 7q35-36, and 6q14-16.7-9 Based on the recent population-based, case-control genome-wide association studies (GWAS), variants in 3 distinct loci on chromosomes 4q25, 16q22, and 1q21 have been identified as being significantly associated with an increased risk of AF.6-9 Although identification of these mutations has offered little explanation with respect to the heritability of AF, it has shed new light on the molecular mechanisms of this complex arrhythmia and provides hope for more targeted rhythm control strategies in the future.

The majority of AF cases result from the interaction between genetic and environmental factors. The recent application of GWAS technology has identified a novel genetic locus in the potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3 (KCNN3) on chromosome 1q21 in European ancestry.10 PITX2, at the chromosome 4q25 locus, encodes a transcription factor, and ZFHX3 at the chromosome 16q22 locus encodes the zinc finger homeobox3. KCNN3, a much more important mutation locus on chromosome 1q21, is thus far the only ionic channel gene identified in the GWAS of AF. Over the last decade, a number of case-control studies have been conducted to investigate the association of SNP (single-nucleotide polymorphism) rs13376333 or KCNN3 with AF.10-13 In addition, two additional European cohorts were able to replicate the results.13 In contrast, another
two case-control studies failed to replicate this association in a Chinese population. A variable association of SNP rs13376333 with AF has been reported. To examine the significance and nature of the association of rs13376333 with AF, we performed this meta-analysis of published case-control studies to ascertain the role of rs13376333 in AF.

Methods

We followed the proposed MOOSE (Meta-Analysis of Observational Studies in Epidemiology) 10 guidelines to report the present meta-analysis. 10

Data section: We searched PubMed, Embase, OVID, and Web of Science through December 2014, and systematically identified case-control studies. The following search terms were used: 1) KCNN3 or potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3, or SNP rs13376333; 2) polymorphism or variant; 3) atrial fibrillation or AF. The search was limited to English articles. Additional publications were sought using the reference lists of identified papers and published reviews on the topic.

Study selection: We first performed an initial screening of titles or abstracts. A second screening was based on full-text review. Studies were considered eligible if they met the following criteria: 1) Evaluate the association between rs13376333, a single nucleotide polymorphism on chromosome 1q21, with AF. 2) The study should have a case-control or population-based design. 3) Report an estimate of risk for AF (odds ratio [OR]) with its 95% confidence interval (CI). 4) If the same population was studied in more than one study, we included the study with the larger sample size and more comprehensive outcome evaluation. Any discordance between reviewers was resolved by consensus.

Data extraction and quality assessment: Outcomes of interest in this study included total AF and lone AF. Data extraction was then performed using a standardized data-collection form: first author’s name, year of publication, ethnicity, total number of patients, baseline clinical characteristics of the patients, the presence of polymorphism, and the adjusted relative risk measure (OR) of AF with its 95% CI. Two investigators independently conducted the data extraction and any disagreements were resolved by discussion. Study quality was evaluated according to the 9-point Newcastle-Ottawa Scale (NOS). 30 The quality of each study was assessed based on 3 broad perspectives including selection, comparability, and exposure. A total score of 7 or greater indicated that a study was of high quality.

Statistical analyses: OR was used as a common measure of the association between rs13376333 and risk of AF (or lone AF). The hazard ratios were directly considered as ORs. SNP was modeled using an additive genetic effect. We calculated ORs for one study in which only ORs under a dominant model were reported. For another study that reported ORs for 3 different cohorts, we included each OR for our analysis. ORs and corresponding SEs, which were derived from 95% CI or P values, were logarithmically transformed to stabilize variance and normalize the distribution.

The Cochran Q statistic was used to evaluate the heterogeneity between studies. When the P value was < 0.10, the heterogeneity was considered as significant and then the results were pooled using a random effect model; otherwise, the fixed model was used. The I² statistic, which is a quantitative measure of inconsistency across studies, was also calculated. Therefore, I² takes values between 0% and 100%, values of 25%, 50%, and 75% being considered to be low, moderate, and high, respectively. Because the characteristics of the populations and adjustments for confounding factors were not consistent between studies, we further conducted a sensitivity analysis to explore possible explanations for heterogeneity and to examine the influence of various exclusion criteria on the overall risk estimate. We also investigated the influence of a single study on the overall risk estimate by omitting 1 study in each turn. The sensitivity analysis was only performed for lone AF because of the rather small numbers of studies for total AF.

Potential publication bias was assessed by visual inspection of the Begg funnel plots in which the log ORs were plotted against their SEs. We also performed the Begg rank correlation test and Egger linear regression test at the P < 0.10 level of significance. All analyses were performed using STATA version 12.0 (StataCorp LP, College Station, TX, USA). A P value < 0.05 was considered statistically significant, except where otherwise specified.

Results

Study characteristics: Initially, the searched keywords identified 18 articles. After reviewing the titles and abstracts, 4 articles were excluded. After reviewing the full texts and data, 9 more articles were excluded (4 were not case-control studies, 3 not case-control studies about SNP polymorphism, and 2 were not not-case-control studies on KCNN3 rs13376333 polymorphism and AF risk). Finally, 5 articles including 7 case-control studies met our inclusion criteria and were included in this meta-analysis. These studies were published between 2010 and 2014. Four studies were conducted in Europe, two in mainland China, and one in Taiwan. The sizes of the cases ranged from 187 to 1335 (total 4662). Among the 7 studies included here, 6 reported lone AF events and 3 reported total AF events. All studies had a case-control design. Genotype frequencies of the control subjects were all in HWE (all P > 0.05). The NOS scores of all studies were all more than 7 (high quality). The characteristics of the 7 case-control studies are presented in Table I.

Results of meta-analysis: Figure 1 shows the results from the random-effect model combining the OR for lone AF. Among the 6 studies, 5 showed a significant positive relation between SNP rs13376333 and risk of lone AF. However, the P values for the association varied from < 0.001 to 0.869. Compared with the reference group, people with SNP rs13376333 experienced a significantly increased risk for developing lone AF (OR: 1.58 [95%CI: 1.37 to 1.82]; P < 0.001). Moderate heterogeneity was observed (I² = 47.0%). Figure 2 shows the result from the fixed-effect model combining the OR for total AF. In contrast to lone AF, among the 3 studies included, only 1 reported a significant positive relation between SNP rs13376333 and risk of total AF. The overall combined OR in relation to rs13376333 was 1.33 (95%CI: 1.14 to 1.54; P < 0.001) for total AF. No evidence of heterogeneity was observed. Further analyses using the random-effect model yielded an identical result.

The results of subgroup analyses according to ethnicity,
source of control, sample size, and genotyping method are presented in Table II. When stratified by ethnicity, the same associations between the rs1337633 polymorphism and risk of lone AF were found in both Asian (OR: 1.87 [95% CI: 1.05 to 3.31]; \( P = 0.032 \)) and Caucasian populations (OR: 1.51 [95% CI: 1.39 to 1.65]; \( P < 0.001 \)) (Table II).

When the sources of the control groups were considered, there was a significant association between the rs1337633 polymorphism and increased risk of lone AF both in the HB and PB studies (Table II). Stratified analyses by sample size and genotyping method also suggested that the rs1337633 polymorphism of KCNN3 increased the risk of lone AF both in large and small studies (Table II). Overall, the association between rs1337633 and AF was not significantly modified by these variables.

Sensitivity analyses: Sensitivity analyses were conducted to explore potential sources of heterogeneity in the association between SNP rs1337633 and lone AF in order to examine the influence of various exclusion criteria on the overall risk estimate. Exclusion of 2 studies \(^{2,3}\) with relatively small sample

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Number</th>
<th>odds ratio (OR)</th>
<th>95%CI</th>
<th>( P )</th>
<th>( I^2 ) (%)</th>
</tr>
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<tbody>
<tr>
<td>Total</td>
<td>6</td>
<td>1.58</td>
<td>1.37</td>
<td>1.82</td>
<td>&lt;0.001</td>
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<td>Ethnicity</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Asian</td>
<td>3</td>
<td>1.87</td>
<td>1.05</td>
<td>3.31</td>
<td>0.032</td>
</tr>
<tr>
<td>Caucasian</td>
<td>3</td>
<td>1.51</td>
<td>1.39</td>
<td>1.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Source of control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB</td>
<td>2</td>
<td>2.36</td>
<td>1.68</td>
<td>3.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PB</td>
<td>4</td>
<td>1.50</td>
<td>1.38</td>
<td>1.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sample size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1000</td>
<td>4</td>
<td>1.53</td>
<td>1.32</td>
<td>1.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt; 1000</td>
<td>2</td>
<td>1.99</td>
<td>1.06</td>
<td>3.75</td>
<td>0.033</td>
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<td>Genotyping method</td>
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<tr>
<td>Taqman</td>
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<td>1.53</td>
<td>1.36</td>
<td>1.72</td>
<td>&lt;0.001</td>
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<tr>
<td>non-Taqman</td>
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</table>

HB indicates hospital-based; and PB, population-based.
sizes showed a similar risk (OR: 1.53 [95% CI: 1.32 to 1.78]; \( P < 0.001 \)) with substantial evidence of heterogeneity (\( P = 0.119, I^2 = 48.7\% \)). Exclusion of two studies with hospital-based controls had little effect on the overall risk estimate (OR: 1.50 [95% CI: 1.38 to 1.63]; \( P < 0.001 \)), but no evidence of heterogeneity was observed among the remaining studies (\( I^2 = 0\% \)). Further exclusion of the other studies one by one did not materially alter the overall combined OR, with a range from 1.51 (95% CI: 1.33 to 1.91) to 1.65 (95% CI: 1.34 to 2.05) (Figure 3).

**Publication bias:** Visual inspection of the Begg funnel plot did not identify substantial asymmetry. The Begg rank correlation test and Egger linear regression test also indicated no evidence of publication bias among studies of SNP rs13376333 and lone AF (Begg, \( P = 0.707 \); Egger, \( P = 0.429 \)) (Figure 4).

**Discussion**

The KCNN3 gene encodes a member of voltage-independent calcium-activated potassium channels, which are expressed in brain and heart. The genetic variant rs13376333 is an intron SNP located between the first and second exon of the KCNN3 gene (also known as SK3). Small conductance calcium-activated potassium channels have 3 different subtypes, SK1, SK2 and SK3 channels. SK3 channels were first detected in atria and ventricles. Subsequently, another study showed that AF risk could be reduced by SK channel inhibition, and animal studies revealed that inhibition of SK could prolong the atrial effective refractory period.

A more recent study demonstrated that SK3 channels were overexpressed in atrial myocytes from SK3 mutant mice. Using patch-clamp techniques, they further showed that the mutant mice had a shortened action potential during repolarization compared to the wild type mice. All of these results indicate that KCNN3 rs13376333 is important in the regulation of heart rhythm.

After Ellinior, et al first reported the association of rs13376333 in the GWAS analysis of lone AF, a number of groups have conducted investigations into the risk of AF related to KCNN3 rs13376333, with several investigators showing increased risk of AF development. However, a lack of associations was also demonstrated in multiple studies. There are several possible explanations for these discrepancies. First, it is known that individual susceptibility to AF differs substantially among different populations. Second, complicated gene-gene and gene-environment interactions could result in the difference, which may dilute or accentuate the genetic effects in AF. To clarify these discrepancies, we performed this study to determine whether SNP rs13376333 influences susceptibility to AF.

In this set of meta-analyses including 24,339 individuals, robust association was observed between SNP rs13376333 and both lone AF and total AF. The T allele carriers of SNP rs13376333 may be a strong independent predictive factor for lone AF. In addition, we conducted an assessment of the quality of the studies using the Newcastle-Ottawa Scale and found that all studies had good quality.

In subgroup analyses of ethnicity, a significant association was observed both in Asian and Caucasian populations. In addition, when stratified by the sources of the control groups, our results showed that the rs13376333 polymorphism was dramatically associated with lone AF both in PB and HB controls. However, other heart diseases may also be an incentive of AF. Thus, studies of HB control could not match all high risk factors of AF between the case group and control group. Consequently, using HB persons as a control group is more representative of the result and involves less bias. However, we obtain consistent results between PB and HB controls, which means our conclusion is reliable.

However, the underlying mechanisms involved in the association between KCNN3 rs13376333 and risk of lone AF are uncertain. Lone AF may be caused by irregular ionic currents. On the other hand, common AF is caused by atrial structural remodeling.

For heterogeneity, in the overall analysis, substantial heterogeneity was observed across studies. Our sensitivity analyses suggested that 2 studies conducted with hospital-based controls probably contributed to the heterogeneity. Because the 2 studies with hospital-based controls were all conducted in...
Asia and using a non-Taqman genotyping method, the heterogeneity was effectively decreased or removed after subgroup analyses by ethnicity and genotyping method.

Above all, our findings suggest that KCNN3 rs13376333 polymorphism may contribute significantly to the susceptibility of AF, and as an ion channel, it could be a potential drug target in the clinical management of AF and thus open up a new strategy for AF therapy.

Although this is the first meta-analysis focusing on the association between KCNN3 SNP rs13376333 and AF, our study still has several limitations. First, all included studies were published in English and studies published in other languages or unpublished studies might have been missed. Second, AF is considered to be a multi-factorial disease, and whether there was a relationship between AF and the coexistence of other SNPs at the same time with KCNN3 is unknown. Third, in Asia, only Chinese research data were included so research data from other Asian countries should also be included in future studies.

In conclusion, our meta-analysis shows that KCNN3 SNP rs13376333 is related to increased risk of lone AF and total AF, and this finding suggests that KCNN3 rs13376333 polymorphism may play an important role in the pathogenesis of AF. Considering the limitations mentioned above, more studies are needed to confirm this relationship in the future.

ACKNOWLEDGMENT

The authors have declared that no competing interests exist.

REFERENCES