Journal of Interventional Cardiac Electrophysiology (2019) 54:283–288
https://doi.org/10.1007/s10840-018-0484-2

Genotype-phenotype associations in atrial fibrillation: meta-analysis

Zhen Hu[1] · Deling Zou[1]

Received: 27 May 2018 / Accepted: 30 October 2018 / Published online: 17 November 2018

Abstract

Purpose Genome-wide association studies have identified several single-nucleotide polymorphisms (SNPs) associated with atrial fibrillation (AF). The relationship between SNPs and the incidence of stroke, heart failure, and the recurrence rate of AF after cardioversion has been reported. This meta-analysis focuses on the genotype-phenotype associations in AF.

Methods We searched PubMed/Medline and Embase for literature providing the phenotypic parameters and genotypes of *RS10033464*, *RS13376333*, *RS2106261*, *RS2200733*, and *RS7193343*. We selected literature published in English and reviewed the full text of included studies to perform a meta-analysis.

Results Fifteen papers, and 7034 patients with AF, were included. The mean risk gene frequency of the investigated variants was between 12 and 43%. The mean age of patients was between 50 and 70 and 70–80% of them were male. The stroke and heart failure frequencies in AF patients with *RS2200733* were 10 and 7%, respectively. There was no significant difference in left ventricular ejection fraction and left ventricular end-diastolic diameter for all risk genotypes. For the AF recurrence after cardioversion treatment with direct current electric conversion, catheter ablation therapy, and anti-arrhythmic drugs. The early AF recurrence rate was 46% in *RS10033464* and *RS13376333* patients, and the late AF recurrence rate was 53% in *RS2200733* patients.

Conclusions Pooled analysis showed a significantly high prevalence of stroke (10%) in *RS2200733* AF patients. AF patients with the studied SNPs had preserved left ventricular systolic function (i.e., ejection fraction greater than 50%). AF patients with *RS10033464* presented larger left atrium diameter (44 mm (95% CI 42.02–45.98)) than those with other SNPs. The late AF recurrence rate was highest in *RS2200733* patients (53% (95% CI 0.43–0.64)). This study aids our understanding of the existing genetic findings and the function-altering "strongest" SNPs.

Keywords Atrial fibrillation · Genotype-phenotype association · SNPs · Meta-analysis

1 Introduction

Atrial fibrillation (AF) is the most common arrhythmia associated with stroke and heart failure (HF). AF leads to a significant increase in mortality and morbidity complicated by stroke and HF. Relapse often happens after cardioversion treatment. With the help of a genetic risk score, the latent AF diagnosis is improved and the cardiogenic cerebral embolism evaluation is more specific [1, 2]. Over the past several years, genome-wide association studies (GWAS) have identified several single-nucleotide polymorphisms (SNPs) associated with AF. Although these loci are in introns or intergenic regions, they likely act to regulate the expression of nearby disease-causing genes [3–5]. PITX2, ZFHX3, and *KCNN3* are high-profile genes in this field. *PITX2* is located on chromosome 4q25 and encodes a protein involved in the development of left-right heart asymmetry, left atrium differentiation, and SA node formation [6]. *RS10033464* and *RS2200733* are physically close to *PITX2* and are strongly associated with AF in different populations [3]. *ZFHX3* is another AF susceptibility-conferring gene located on chromosome 16q22. The nearby SNPs, *RS2106261* and *RS7193343*, are associated with the occurrence and recurrence of AF [4, 7]. Located on chromosome 1q21, *RS13376333* is another promising SNP associated with AF. It is located close to *KCNN3*, which encodes K^+ channels involved in atrial repolarization [5]. It is hypothesized that different genotypes result in different phenotypes in AF, as observed in dilated cardiomyopathy [8]. We select the five most highly studied SNPs in AF to perform a meta-analysis investigation of the genotype-phenotype associations.

✉ Deling Zou
zoudl@sj-hospital.org

[1] Department of Cardiology, ShengJing Hospital of China Medical University, Shenyang, China

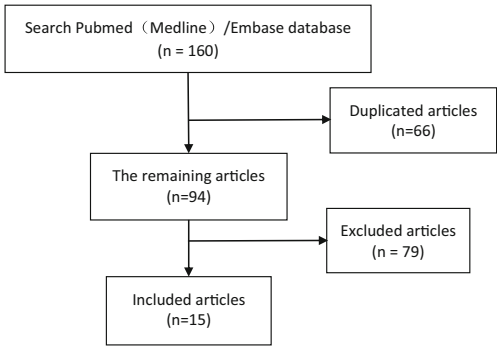

Fig. 1 Flow diagram of study selection

2 Methods

2.1 Study design

We searched Pubmed/Medline and Embase for human studies published in English prior to March 2018. We searched for the terms "*RS10033464*", "*RS13376333*", "*RS2106261*", "*RS2200733*", "*RS7193343*", and "atrial fibrillation." We carefully reviewed the meta-analyses for the missing articles. Duplicated research (66) were excluded. Two researchers independently reviewed the full texts of 94 published papers, 79 of which were excluded as reviews, meta-analyses, and unrelated articles. Articles that only provided gene frequencies were also excluded. Fifteen articles compared the phenotypic features in *RS10033464*, *RS13376333*, *RS2106261*, *RS2200733*, and *RS7193343*. Five articles were included for *RS10033464* [9–13], six for *RS13376333* [9, 11, 12, 14–16], four for *RS2106261* [14, 15, 17, 18], 13 for *RS2200733* [9–15, 17, 19–23], and five for *RS7193343* [9, 11, 12, 14, 20] (Fig. 1). The 15 included articles included a total of 7034 patients. Phenotypic data were extracted for *RS10033464* (GT + TT), *RS13376333* (CT + TT), *RS2106261* (AG + AA), *RS2200733* (CT + TT), and *RS7193343* (CT + TT) including risk gene frequency, age, sex, cardiogenic stroke or HF, left ventricular ejection fraction (LVEF), left atrial diameter (LAD), left ventricular end-diastolic diameter (LVEDD), early recurrence of atrial fibrillation (ERAF), and late recurrence of atrial fibrillation (LRAF).

2.2 Statistical analysis

The meta-analysis was reported according to the MOOSE statement and conducted using the meta package in R software (version 3.4.3, The R Foundation for Statistical Computing 2017) for the combination of means and proportions. Heterogeneity was quantified by Q test and I^2 statistics. The fixed effect model was selected only if $p > 0.1$ and $I^2 \leq 50\%$, otherwise the random effect model was used. Because of the limited number of articles included in this study, we did not use the funnel plot.

3 Results

3.1 Risk gene frequency, mean age, and sex distribution

Twenty-eight studies, including 14,170 AF patients and corresponding controls, investigated the risk gene frequency of *RS10033464* (T), *RS13376333* (T), *RS2106261* (A), *RS2200733* (T), and *RS7193343* (T), resulting in pooled frequencies of 13% (95% CI 10–16%), 12% (95% CI 3–24%), 43% (95% CI 36–95%), 39% (95% CI 28–51%), and 22% (95% CI 19–24%), respectively. Ten studies provided data on the mean age at AF diagnosis, which was 56 years (95% CI 54.54–58.89), 57 years (95% CI 55.94–58.26 and 95% CI 54.12–61.36), and 62 years (95% CI 50.20–74.70) in the *RS10033464*, *RS2106261* and *RS2200733*, and the *RS13376333* groups, respectively. There was no significant difference in sex distribution between the investigated SNPs. No data for sex were collected for the *RS7193343* group, but the male proportion of AF patients in the other SNP groups was 80% (95% CI 69–89%) for *RS10033464*, 71% (95% CI 54–85%) for *RS13376333*, 70% (95% CI 68–77%) for *RS2106261*, and 72% (95% CI 67–76%) for *RS2200733*.

It is important to note that a single article may contain several studies and that one person may carry different SNPs.

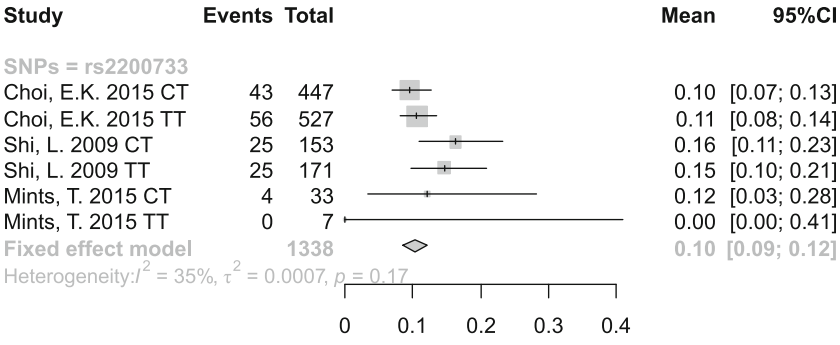

Fig. 2 Forest plot of incidence of stroke of AF patients in *RS2200733*. Ten percent of AF patients with *RS2200733* showed ischemic stroke. *CI*, confidence interval

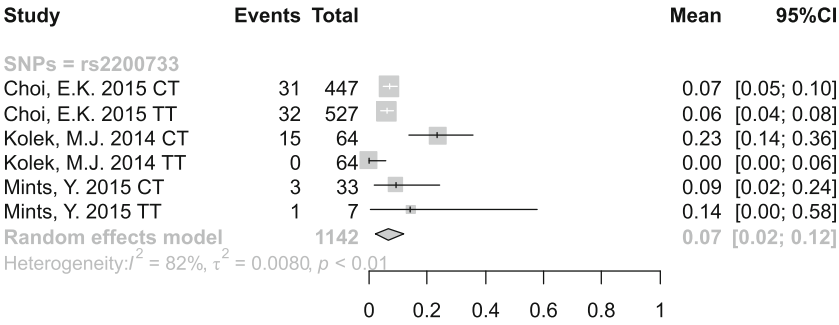

Fig. 3 Forest plot of incidence of HF of AF patients in *RS2200733*. Seven percent of AF patients with *RS2200733* progressed to a diagnosis of HF. *CI*, confidence interval

3.2 Clinical phenotypes and prognosis

Three studies reported ischemic stroke rate and HF rate in AF patients with *RS2200733* (Figs. 2 and 3). As many as 10% (95% CI 9–12%) of AF patients with *RS2200733* showed ischemic stroke and 7% (95% CI 2–12%) progressed to a diagnosis of HF.

In studies including more than 2000 patients with AF, the LVEF of *RS10033464*, *RS13376333*, *RS2106261*, *RS2200733*, and *RS7193343* were 58.21 (95% CI 55.37–61.04), 61.73 (95% CI 60.99–62.46), 63.28 (95% CI 62.69–63.86), 59.91 (95% CI 57.38–62.44), and 71.52 (95% CI 63.79–79.25), respectively. AF patients in the *RS10033464* group showed a LAD of 44 mm (95% CI 42.02–45.98) which was greater than that observed in patients in the *RS13376333*, *RS2106261*, *RS2200733*, and *RS7193343* groups (Table 1).

The LVEDD of *RS13376333*, *RS2106261*, *RS2200733*, and *RS7193343* patients was 48.68 mm (95% CI 47.49–49.14), 49.9 3 mm (95% CI 49.62–50.24), 49.87 mm (95% CI 49.44–50.29), and 49.53 mm (95% CI 48.37–50.7), respectively.

We focused on the AF recurrence rate in patients after antiarrhythmic drugs, direct current electric conversion, and catheter ablation cardioversion therapy. ERAF was defined as AF recurrence within 3 months of cardioversion, and LRAF was AF recurrence after 3 months. Given our small sample sizes and the limited studies available for analysis, the ERAF analysis is listed for reference (Table 2). In pooled datasets of 2418 patients, the LRAF of *RS2200733* was highest at 53% (95% CI 0.43–0.64), followed by *RS2106261* (49% (95% CI 0.27–0.71)), and *RS10033464* (46% (95% CI 0.33–0.59)) (Fig. 4).

Table 1 The LAD of AF patients in different SNPs

SNPs	Total (*n*)	Studies (*n*)	Mean	SD	ERAF 95%CI
RS10033464	63	1	44.00	8.00	[42.02;45.98]
RS13376333	134	2	41.22	6.58	[40.13;42.32]
RS2106261	914	4	41.43	6.06	[41.04;41.82]
RS2200733	1367	11	41.55	6.46	[40.19;42.92]
RS7193343	50	2	41.22	5.83	[40.05;43.18]

The LAD of AF patients in the *RS10033464* group (44 mm) was larger than that observed in patients in other groups (41 mm). *LAD*, left atrium diameter

4 Discussion

AF is a common clinical arrhythmia. With aging societies, AF is an epidemic disorder that results in enormous medical burdens worldwide. Linkage analysis and candidate gene screening studies identified several rare variants in patients with sporadic and familial AF. Following this, GWASs identified numerous common variants in the general population. The risk gene frequencies of the five SNPs we focused on in this meta-analysis are all higher than 1%, which is consistent with the theory: "common diseases, common variation." GWAS was used to analyze the genome for SNPs associated with AF. Previously, GWAS was used to quantify the 25 known AF susceptibility loci and explained 5.3% (95% CI 4.2–6.5%) of the total variance in AF susceptibility [24]. Further assessment of each locus was required to identify function-altering, or "strongest", SNPs. We chose the five most highly studied SNPs for our meta-analysis. Previously, it was estimated that the *RS10033464* TT genotype was significantly correlated with early age of ischemic stroke onset (60.76 years for GG, 61.74 years for GT, 55.47 years for TT, TT vs. GT: $P = 0.043$) [25]. We performed pooled analyses of the mean age of patients with AF to determine if certain SNPs were associated with early age of onset. AF patients with *RS10033464*, *RS2106261*, and *RS2200733* were in their 50s, while AF patients with *RS13376333* were in their 60s. This result might indicate an association between *RS10033464*, *RS2106261*, and *RS2200733* and the early age at onset of AF. Our analysis also showed a trend toward male predominance in all four SNPs analyzed for sex distribution. There were four times as many male patients with AF and the *RS10033464* SNP. Although the four SNPs were not on the sex chromosomes, there might be an SNP mutation tendency in males that leads to AF occurrence. Further studies are required to validate our results.

Research indicated *RS10033464*, but not *RS2200733*, was associated with left atrial volume [10]. Our findings were consistent with the results of previous research. The LAD of

Table 2 The ERAF of AF patients with different SNPs

SNPs	Study	Year	AF type	Genotype	Event	Total	ERAF 95%CI
rs10033464	Husser, D.	2010	ERAF	GT	29	63	0.46 [0.33;0.59]
rs13376333	Choi, E. K.	2015	ERAF	CT	22	48	0.46 [0.31;0.61]
rs2106261	Choi, E. K.	2015	ERAF	AG	146	520	0.28 [0.25;0.31]
				AA	70	251	
rs2200733	Choi, E. K.	2015	ERAF	CT	126	447	0.32 [0.26;0.39]
				TT	155	527	
	Husser, D.	2010	ERAF	CT + TT	39	87	

Summary of the ERAF after anti-arrhythmic drugs (AADs), direct current electric conversion (DCCV), and catheter ablation cardioversion therapy

ERAF, early recurrence rate of AF (within 3 months of cardioversion); *CI*, confidence interval

patients in the *RS10033464* group was 44 mm, which was higher than the LAD of patients with other SNPs. This indicates that *RS10033464* might promote AF patients to left atrial dilatation and AF recurrence after ablation.

Previous meta-analyses suggested that chromosome 4q25 variants were associated with ischemic stroke risk, especially cardioembolic stroke [26]. The mechanism underlying these associations is unclear. For the first time, we quantified the frequency of stroke in *RS2200733* AF patients. The average annual stroke rate of anticoagulated AF patients with the *RS2200733* SNP (10%) was significantly higher than that observed in previous studies (1.5%) [27]. With the high rate of stroke, genetic tests to identify SNPs that might influence outcomes could be recommended for the enhancement of antithrombotic treatment. Investigation of cardiac function revealed that the incidence of HF in AF patients with *RS2200733* was 7%, which is lower than that identified in previous studies (20–30%) [28]. Furthermore, the mean LVEFs of the five SNPs examined here were all no less than 50%. The evidence presented here indicates that these SNPs might not have any specific influence on left ventricular function in AF patients.

RS10033464, *RS2106261*, and *RS2200733*, but not *RS13376333 and RS7193343*, are associated with AF

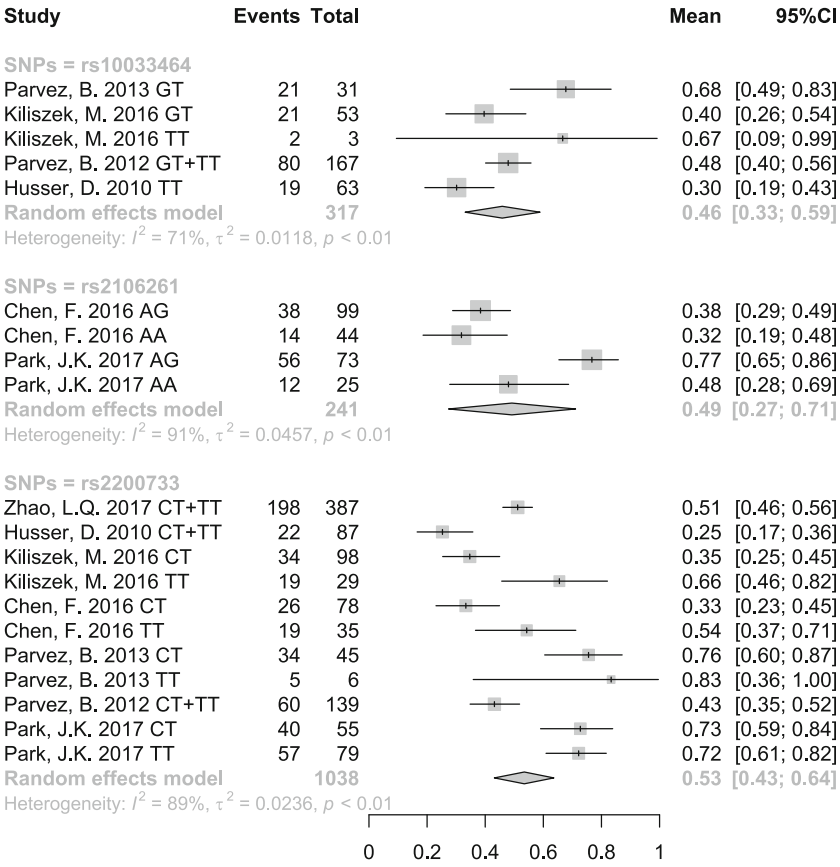

Fig. 4 The LRAF of AF patients with different SNPs. Summary of the LRAF after anti-arrhythmic drugs (AADs), direct current electric conversion (DCCV), and catheter ablation cardioversion therapy. LRAF: The late recurrence rate of AF (3 months or more after cardioversion). *CI*, confidence interval

recurrence after catheter ablation. We found that of the three SNPs associated with AF recurrence, the LRAF of *RS2200733* was the highest. The exact mechanism through which these SNPs modulate the susceptibility of patients with AF for relapse after cardioversion is not known. Indeed, patients with the *RS2200733* risk allele had larger superior pulmonary vein diameters [17], and the nearby *PITX2* gene may play a role in the development of pulmonary myocardial sleeves [29], which may contribute to AF occurrence and relapse. It is tempting to speculate that patients with AF and the *RS2200733* allele showed high AF recurrence rates due their pulmonary vein phenotype (i.e., diameter and extension of pulmonary vein myocardium), which differs from that of patients with AF and the *RS10033464* or *RS2106261* SNPs. This result needs to be confirmed by further studies.

Here, we have consolidated reported data to produce the largest dataset available concerning genotype-phenotype associations in AF. Our data show a significantly high prevalence of stroke and the highest LRAF rate in AF patients with the *RS2200733* SNP. Moreover, the *RS10033464* SNP might be related to left atrial dilatation in AF patients. Genome sequencing is informative, not only in cases of AF but also to provide more general information about the human genome. Our results represent the beginning chapter in understanding the functional effects of genetic variants and determining their prognostic significance.

5 Limitations

The studies included studies in this meta-analysis differed in the race of patients included, the selection of SNPs, study design, and genotyping methods. The English language restriction and the linkage effect of multiple SNPs might lead to bias.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Muse ED, Wineinger NE, Spencer EG, Peters M, Henderson R, Zhang Y, et al. Validation of a genetic risk score for atrial fibrillation: a prospective multicenter cohort study [J]. PLoS Med. 2018;15(3):e1002525.
2. Lubitz SA, Parsons OE, Anderson CD, Benjamin EJ, Malik R, Weng LC, et al. WTCCC2, International Stroke Genetics Consortium, and AFGen Consortia. Atrial fibrillation genetic risk and ischemic stroke mechanisms [J]. Stroke. 2017;48(6):1451–6.
3. Gudbjartsson DF, Arnar DO, Helgadottir A, Gretarsdottir S, Holm H, Sigurdsson A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25 [J]. Nature. 2007;448(7151):353–7.
4. Benjamin EJ, Rice KM, Arking DE, Pfeufer A, van Noord C, Smith AV, et al. Variants in ZFHX3 are associated with atrial fibrillation in individuals of European ancestry[J]. Nat Genet. 2009;41(8):879–81.
5. Ellinor PT, Lunetta KL, Glazer NL, Pfeufer A, Alonso A, Chung MK, et al. Common variants in KCNN3 are associated with lone atrial fibrillation [J]. Nat Genet. 2010;42(3):240–4.
6. Paludan-Muller C, Svendsen JH, Olesen MS. The role of common genetic variants in atrial fibrillation [J]. J Electrocardiol. 2016;49(6):864–70.
7. Gudbjartsson DF, Holm H, Gretarsdottir S, Thorleifsson G, Walters GB, Thorgeirsson G, et al. A sequence variant in ZFHX3 on 16q22 associates with atrial fibrillation and ischemic stroke [J]. Nat Genet. 2009;41(8):876–8.
8. Kayvanpour E, Sedaghat-Hamedani F, Amr A, Lai A, Haas J, Holzer DB, et al. Genotype-phenotype associations in dilated cardiomyopathy: meta-analysis on more than 8000 individuals [J]. Clin Res Cardiol. 2017;106(2):127–39.
9. Kiliszek M, Kozluk E, Franaszczyk M, Lodzinski P, Piatkowska A, Ploski R, et al. The 4q25, 1q21, and 16q22 polymorphisms and recurrence of atrial fibrillation after pulmonary vein isolation [J]. Arch Med Sci. 2016;12(1):38–44.
10. Mints Y, Yarmohammadi H, Khurram IM, Hoyt H, Hansford R, Zimmerman SL, et al. Association of common variations on chromosome 4q25 and left atrial volume in patients with atrial fibrillation [J]. Clin Med Insights: Cardiol. 2015;9:39–45.
11. Parvez B, Shoemaker MB, Muhammad R, Richardson R, Jiang L, Blair MA, et al. Common genetic polymorphism at 4q25 locus predicts atrial fibrillation recurrence after successful cardioversion [J]. Heart Rhythm. 2013;10(6):849–55.
12. Parvez B, Vaglio J, Rowan S, Muhammad R, Kucera G, Stubblefield T, et al. Symptomatic response to antiarrhythmic drug therapy is modulated by a common single nucleotide polymorphism in atrial fibrillation [J]. J Am Coll Cardiol. 2012;60(6):539–45.
13. Husser D, Adams V, Piorkowski C, Hindricks G, Bollmann A. Chromosome 4q25 variants and atrial fibrillation recurrence after catheter ablation [J]. J Am Coll Cardiol. 2010;55(8):747–53.
14. Park JK, Lee JY, Yang PS, Kim TH, Shin E, Park J, et al. Good responders to catheter ablation for long-standing persistent atrial fibrillation: clinical and genetic characteristics [J]. J Cardiol. 2017;69(3):584–90.
15. Choi EK, Park JH, Lee JY, et al. Korean atrial fibrillation (AF) network: genetic variants for AF do not predict ablation success [J]. J Am Heart Assoc. 2015;4(8):e2046.
16. Luo Z, Yan C, Zhang W, Shen X, Zheng W, Chen F, et al. Association between SNP rs13376333 and rs1131820 in the KCNN3 gene and atrial fibrillation in the Chinese Han population [J]. Clin Chem Lab Med. 2014;52(12):1867–73.
17. Chen F, Yang Y, Zhang R, Zhang S, Dong Y, Yin X, et al. Polymorphism rs2200733 at chromosome 4q25 is associated with atrial fibrillation recurrence after radiofrequency catheter ablation in the Chinese Han population [J]. Am J Transl Res. 2016;8(2):688–97.
18. Liu Y, Ni B, Lin Y, Chen XG, Fang Z, Zhao L, et al. Genetic polymorphisms in ZFHX3 are associated with atrial fibrillation in a Chinese Han population [J]. PLoS ONE. 2014;9(7). https://doi.org/10.1371/journal.pone.0101318.
19. Zhao LQ, Zhang GB, Wen ZJ, Huang CK, Wu HQ, Xu J, et al. Common variants predict recurrence after nonfamilial atrial fibrillation ablation in Chinese Han population [J]. Int J Cardiol. 2017;227:360–6.
20. Ferran A, Alegret JM, Subirana I, Aragones G, Lluis-Ganella C, Romero-Menor C, et al. Association between rs2200733 and rs7193343 genetic variants and atrial fibrillation in a Spanish population, and meta-analysis of previous studies [J]. Rev Esp Cardiol (Engl Ed). 2014;67(10):822–9.

21. Kolek MJ, Parvez B, Muhammad R, Shoemaker MB, Blair MA, Stubblefield T, et al. A common variant on chromosome 4q25 is associated with prolonged PR interval in subjects with and without atrial fibrillation [J]. Am J Cardiol. 2014;113(2):309–13.
22. Goodloe AH, Herron KJ, Olson TM. Uncovering an intermediate phenotype associated with rs2200733 at 4q25 in lone atrial fibrillation [J]. Am J Cardiol. 2011;107(12):1802–5.
23. Shi L, Li C, Wang C, Xia Y, Wu G, Wang F, et al. Assessment of association of rs2200733 on chromosome 4q25 with atrial fibrillation and ischemic stroke in a Chinese Han population [J]. Hum Genet. 2009;126(6):843–9.
24. Weng LC, Choi SH, Klarin D, Smith JG, Loh PR, Chaffin M, et al. Heritability of atrial fibrillation [J]. Circ Cardiovasc Genet. 2017;10(6). https://doi.org/10.1161/CIRCGENETICS.117.001838.
25. Sun L, Tian L, Xu J, Zhang Z, Liu X. Chromosome 4q25 variants and age at onset of ischemic stroke [J]. Mol Neurobiol. 2017;54(5):3388–94.
26. Sun L, Zhang Z, Xu J, Xu G, Liu X. Chromosome 4q25 variants rs2200733, rs10033464, and rs1906591 contribute to ischemic stroke risk [J]. Mol Neurobiol. 2016;53(6):3882–90.
27. Kirchhof P, Breithardt G, Camm AJ, Crijns HJ, Kuck KH, Vardas P, et al. Improving outcomes in patients with atrial fibrillation: rationale and design of the early treatment of atrial fibrillation for stroke prevention trial [J]. Am Heart J. 2013;166(3):442–8.
28. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC guidelines for the management of atrial fibrillation developed in collaboration with EACTS [J]. Eur Heart J. 2016;37(38):2893–962.
29. Mommersteeg MT, Brown NA, Prall OW, et al. Pitx2c and Nkx2-5 are required for the formation and identity of the pulmonary myocardium [J]. Circ Res. 2007;101(9):902–9.