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Significant association of rare variant p.Gly8Ser in cardiac sodium channel β4-subunit SCN4B with atrial fibrillation

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Abstract

Atrial fibrillation (AF) affects 33.5 million individuals worldwide. It accounts for 15% of strokes and increases risk of heart failure and sudden death. The voltage-gated cardiac sodium channel

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CONFLICTS OF INTEREST

All authors have declared no conflict of interest.

SUPPORTING INFORMATION

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complex is responsible for the generation and conduction of the cardiac action potential, and composed of the main pore-forming α-subunit $Na_v1.5$ (encoded by the *SCN5A* gene) and one or more auxiliary β-subunits, including $Na_v\beta1$ to $Na_v\beta4$ encoded by *SCN1B* to *SCN4B*, respectively. We and others identified loss-of-function mutations in *SCN1B* and *SCN2B* and dominant-negative mutations in *SCN3B* in patients with AF. Three missense variants in *SCN4B* were identified in sporadic AF patients and small nuclear families; however, the association between *SCN4B* variants and AF remains to be further defined. In this study, we performed mutational analysis in *SCN4B* using a panel of 477 AF patients, and identified one nonsynonymous genomic variant p.Gly8Ser in four patients. To assess the association between the p.Gly8Ser variant and AF, we carried out case-control association studies with two independent populations (944 AF patients vs. 9,81 non-AF controls in the first discovery population and 732 cases and 1,291 controls in the second replication population). Significant association was identified in the two independent populations and in the combined population ($p = 4.16 \times 10^{-4}$, odds ratio [OR] = 3.14) between p.Gly8Ser and common AF as well as lone AF ($p = 0.018$, OR = 2.85). These data suggest that rare variant p.Gly8Ser of *SCN4B* confers a significant risk of AF, and *SCN4B* is a candidate susceptibility gene for AF.

Keywords

atrial fibrillation; case-control association study; genetics; single-nucleotide polymorphism (SNP); sodium channel β4-subunit (SCN4B)

1 | INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia seen at the clinic. The prevalence of AF increases with aging and reaches to 8% of the adult population over the age of 80 years (Peters, Schilling, Kanagaratnam, & Markides, 2002). More than 3 million Americans (Naccarelli, Varker, Lin, & Schulman, 2009), 10 million people in China, and nearly 33.5 million individuals worldwide (corresponding to the estimated global age adjusted prevalence of 0.5%) are affected with AF (Chugh et al., 2014; Hu & Sun, 2008; Zhou & Hu, 2008). AF accounts for approximately 15% of stroke cases (Furie et al., 2011; Wolf, Abbott, & Kannel, 1991). Moreover, the risk of AF-related stroke increases with aging from 1.5% for those aged 50–59 years to 23.5% for those aged 80–89 years (Wolf et al., 1991). AF also increases risk of heart failure and sudden death, and the mortality rate is 1.5- to 1.9-fold higher among patients with AF compared with those with normal sinus rhythm (Benjamin et al., 1998; Wolf et al., 1991). AF is a complex disease caused by both genetic and environmental factors as well as their interactions. The risk factors for AF include the age, valvular heart disease, hypertension, coronary artery disease (CAD), thyroid disease, rheumatic heart disease, heart failure, and cardiomyopathies (Singer & Go, 2001). Lone AF is referred to as AF not associated with other cardiovascular diseases. Lone AF accounts for nearly 30% of all AF cases (Brand, Abbott, Kannel, & Wolf, 1985; Levy et al., 1999). Some AF patients have a positive family history, and several epidemiological studies suggest that genetic factors may play an important role in the development of AF, especially in patients with lone AF (Ali & Antezano, 2006).

Genetic analyses of families with inherited AF have identified disease-causing mutations in multiple ion channel genes *KCNQ1*, *KCNE1*, *KCNJ2*, *KCNA5*, *KCNH2*, and *SCN5A* and the gap junction gene *GJA5* (Parvez & Darbar, 2011). We were the first to show that mutations in non-ion channel gene *NUP155* encoding a key component of the nucleocytoplasmic pore complex also causes AF (Zhang et al., 2008). More-over, genome-wide association studies have identified >20 genomic variants or single-nucleotide polymorphisms (SNPs) associated with susceptibility of AF (Ellinor et al., 2012; Mahida & Ellinor, 2012; Wang et al., 2018). However, these mutations and genomic variants account for a small portion of AF heritability and most genomic variants for AF remain largely unknown.

The *SCN5A* gene encodes the voltage-gated cardiac sodium channel ($Na_v1.5$) responsible for the initiation and propagation of the cardiac action potentials (Catterall, 2000; Grant, 2009). $Na_v1.5$ is the main pore-forming α-subunit of the sodium channel complex, which is also composed of one or more auxiliary β-subunits, including $Na_v\beta1$ to $Na_v\beta4$ encoded by *SCN1B* to *SCN4B*, respectively (Catterall, 2000). *SCN5A* mutations were reported to cause AF (Savio-Galimberti & Darbar, 2014). Loss-of-function mutations in *SCN1B* and *SCN2B* were identified in AF patients (Watanabe et al., 2009). We previously showed that mutation in *SCN3B* causes AF (Wang et al., 2010). A similar finding was reported later by another independent group (Olesen et al., 2011). Three missense variants were reported in *SCN4B* in a sporadic AF patient and two small nuclear families (Husser et al., 2017; Li et al., 2013); however, they were not characterized by functional studies. Therefore, much more studies are needed to establish the association between *SCN4B* variants and AF.

We hypothesized that rare variants in *SCN4B* are associated with AF. We screened a panel of 477 AF patients in *SCN4B*, and identified a rare, nonsynonymous variant, p.Gly8Ser. Case-control association studies using two independent populations demonstrated significant association between variant p.Gly8Ser and common AF as well as lone AF, establishing *SCN4B* as a susceptibility gene for AF.

2 | MATERIALS AND METHODS

2.1 | Study subjects and isolation of human genomic DNA

The AF and non-AF control study subjects involved in this study were selected from the GeneID database, one of the largest databases for identification of genes for cardiovascular diseases in China (Chen et al., 2016; Li et al., 2011; Wang et al., 2016; Xu et al., 2014; Yin et al., 2017). All study subjects, including 477 AF patients for mutation identification using Sanger sequencing, 944 AF cases and 981 controls for the discovery population used for analyzing the association between *SCN4B* variant p.Gly8Ser and AF, and the second population of 732 AF patients and 1,291 controls without AF for further validation of the significant association between p.Gly8Ser and AF, are of the ethnic origin of Han by self-description (Chen et al., 2016; Li et al., 2011; Wang et al., 2016; Xu et al., 2014; Yin et al., 2017). This study was approved by the Ethics Committee of Huazhong University of Science and Technology and other appropriate local ethics committees on human subject research and conformed to the guidelines set forth by the Declaration of Helsinki. All study subjects signed written informed consent.

AF was diagnosed using the data from electrocardiograms (ECGs) and/or Holter ECG recordings following the ACC/AHA/ESC guidelines for AF by expert cardiologists (January et al., 2014). Individuals with ECG features of irregular RR intervals, the absence of P waves, and/or faster f-waves were diagnosed as the affected (January et al., 2014). Individuals without AF were considered as normal controls. Patients with other types of cardiac arrhythmias, valvulopathies, and cardiomyopathies detected by ECG, Holter monitoring, echocardiography, magnetic resonance imaging, and/or X-ray computed tomography were excluded from this study. AF patients without CAD, congenital heart disease, hypertensive heart disease, hyperthyroidism, and chronic obstructive pulmonary disease were classified as lone AF.

Human genomic DNA was isolated from peripheral blood samples using the Wizard Genomic DNA Purification Kit as described by the manufacturer (Promega Corporation, Madison, WI).

2.2 | Mutational analysis of *SCN4B* and genotyping of variant p.Gly8Ser

Highly sensitive high-resolution melt (HRM) analysis was used for mutational analysis using DNA samples from a panel of 477 AF patients as described (Li et al., 2018; Wang et al., 2016). All exons and exon–intron boundaries were amplified using polymerase chain reactions using the primers listed in Supplementary Table S1.

HRM analysis was also used for genotyping of *SCN4B* variant p.Gly8Ser in two populations of AF cases and controls as described (Chen et al., 2015, 2016; Naji et al., 2018; Wang et al., 2016; Xiong et al., 2013, 2018; Xu et al., 2014).

HRM analysis was carried out using a Rotor-Gene 6200 System (Corbett Life Science, Sydney, Australia) as described by us previously (Chen et al., 2015, 2016; Naji et al., 2018; Wang et al., 2016; Xiong et al., 2013, 2018; Xu et al., 2014). The samples showing abnormal HRM peaks were subjected to direct DNA sequence analysis to identify the exact sequence changes (Chen et al., 2015, 2016; Naji et al., 2018; Wang et al., 2016; Xiong et al., 2013, 2018; Xu et al., 2014). The primers used for sequence analyses are listed in Supplementary Table S2.

DNA sequencing analysis was performed using a BigDye Terminator Cycle Sequencing Kit by Shanghai Sangni Biotechnology (Applied Biosystems, Foster City, CA) as described (Li et al., 2018; Wang et al., 2016). Sequencing data were directly viewed using Sequencing Analysis (version 5.1.1, Applied Biosystems) to identify potential variants.

2.3 | Statistical analysis

The allelic association and genotypic association under three different inheritance models (dominant, recessive, or additive) were analyzed between the *SCN4B* variant p.Gly8Ser and AF using 2 × 2 or 2 × 3 contingence table chi-square tests implemented in SPSS (version 17.0) as described (Chen et al., 2015, 2016; Naji et al., 2018; Wang et al., 2016; Xiong et al., 2013, 2018; Xu et al., 2014). Multivariate logistic regression analysis was used to adjust for covariates of age and sex using SPSS (version 17.0). Hardy–Weinberg equilibrium tests in the control groups were performed using PLINK (version 1.06). Power and sample size

calculations for case-control studies used PS software (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize).

3 | RESULTS

3.1 | Mutational analysis of *SCN4B* in a panel of 477 AF patients

To identify genomic variants of *SCN4B* that are associated with AF, we used HRM analysis followed by direct Sanger DNA sequence analysis to screen for genomic variants in a panel of 477 AF patients. All exons and exon–intron boundaries of *SCN4B* were analyzed for genomic variants and the results are shown in Table 1. Mutational analysis of *SCN4B* identified six genomic variants, including one missense variant in exon 1, p.Gly8Ser (Figure 1a), two intronic variants 62 (−124) C > T and 62 (−93) A > G, two synonymous variants (p.C58C in exon 2 and p.G217G in exon 5), and one variant in the 3′-UTR in exon 5 (694 A > G) (Table 1). *SCN4B* variant p.Gly8Ser was identified in four of 477 AF patients (0.84%), suggesting that p.Gly8Ser is a rare variant. Variant p.Gly8Ser is located in the signal peptide sequence of $Na_v\beta4$. It occurred at a highly conserved residue across species during evolution (Figure 1b). The majority of bioinformatic programs predicted that the p.Gly8Ser variant was possibly damaging (Table 2). Therefore, we pursued the *SCN4B* variant p.Gly8Ser in further genetic studies.

3.2 | Significant allelic association between *SCN4B* variant p.Gly8Ser and AF

Population-based association studies are a highly effective strategy to determine whether a genomic variant confers a risk of the disease under study. The frequency of variant p.Gly8Ser in the AF population (4/477 = 0.84%) appeared to be much higher than its frequency of 0.30% in the East Asian populations (Figure 2). Therefore, we used a case-control association study design to determine whether *SCN4B* variant p.Gly8Ser is a significant risk variant for AF. We employed a two-stage case-control association analysis to determine whether *SCN4B* variant p.Gly8Ser is a susceptibility factor for AF. The study includes the AF discovery population with 944 AF cases and 981 non-AF controls, and the second validation population with 732 AF patients and 1,291 controls without AF (Table 3). The detailed demographic and clinical characteristics of the two populations are shown in Table 3. In the first discovery population, 36.1% (341) of AF patients qualified to be lone AF cases. In the second validation population, 36.5% (267) of cases qualified to be lone AF cases.

The genotyping data for *SCN4B* variant p.Gly8Ser were in Hardy–Weinberg equilibrium in controls from both the discovery population and the validation populations ($p = 1.00$) (Table 4). A 2 × 2 contingence table chi-square test detected a significant allelic association between p.Gly8Ser and AF with an observed P_{obs} of 0.017 and a high OR of 3.66 (Table 4). After adjusting for age and gender, the association remained significant ($P_{adj} = 0.022$, OR = 3.79) (Table 4).

The initial significant association between p.Gly8Ser and AF in the discovery population was replicated in the second validation population with 732 AF patients and 1,291 non-AF controls. A 2 × 2 contingence table chi-square test identified that significant allelic

association was detected between p.Gly8Ser and AF with an observed P_{obs} of 3.84×10^{-3} and an OR of 3.15 (Table 4). After adjusting for age and gender, the association remained significant ($P_{adj} = 6.60 \times 10^{-3}$, OR = 3.44) (Table 4).

In the combined discovery and validation populations together (1,676 AF cases and 2,272 non-AF controls), a 2 × 2 contingence table chi-square test showed that the association between p.Gly8Ser and AF became more significant ($P_{obs} = 2.87 \times 10^{-4}$, OR = 3.14; $P_{adj} = 4.16 \times 10^{-4}$, OR = 3.60) (Table 4).

3.3 | Significant genotypic association between *SCN4B* variant p.Gly8Ser and AF

To further investigate how *SCN4B* variant p.Gly8Ser confers genetic risks to AF, we conducted genotypic association analysis under three common genetic models of autosomal-dominant, recessive, and additive inheritance using 2 × 3 contingence table chi-square tests. Significant genotypic association was detected between p.Gly8Ser and AF in the discovery population with 944 AF cases and 981 non-AF controls under an additive ($P_{obs} = 0.017$; $P_{adj} = 0.022$) or dominant model ($P_{obs} = 0.017$; $P_{adj} = 0.022$) (Table 5). Significant genotypic association was confirmed in the second validation population with 732 AF patients and 1,291 non-AF controls with $P_{obs} = 3.73 \times 10^{-3}$ and $P_{adj} = 6.41 \times 10^{-3}$ under the additive model and $P_{obs} = 3.73 \times 10^{-3}$ and $P_{adj} = 6.41 \times 10^{-3}$ under the dominant model (Table 5). The genotypic association between p.Gly8Ser and AF became more significant in the combined population (1,676 AF cases and 2,272 non-AF controls) ($P_{obs} = 2.76 \times 10^{-4}$; $P_{adj} = 3.99 \times 10^{-4}$) under the additive and dominant models (Table 5).

3.4 | Significant allelic and genotypic association between *SCN4B* variant p.Gly8Ser and lone AF

We analyzed the association between *SCN4B* variant p.Gly8Ser and lone AF without structural heart disease. There are 608 patients with lone AF and 2,272 non-AF controls in the combined discovery and validation population (Table 4). A 2 × 2 contingence table chi-square test showed that significant allelic association was detected between variant p.Gly8Ser and lone AF ($P_{obs} = 2.96 \times 10^{-3}$, OR = 3.18; $P_{adj} = 0.018$, OR = 2.85). The 2 × 3 contingence table chi-square tests identified that significant genotypic association was also detected between p.Gly8Ser and lone AF under an additive ($P_{obs} = 2.90 \times 10^{-3}$; $P_{adj} = 0.018$) or dominant model ($P_{obs} = 2.90 \times 10^{-3}$; $P_{adj} = 0.018$) (Table 5). All together, these studies provide strong genetic evidence that *SCN4B* is a susceptibility gene for common AF.

4 | DISCUSSION

In this study, we performed mutational screening for all exons and exon–intron boundaries of *SCN4B* and identified one rare nonsynonymous variant, p.Gly8Ser, in four of 477 AF patients. We then used a case-control association study design to determine whether *SCN4B* variant p.Gly8Ser is a significant risk variant for AF. In two independent AF populations (the discovery population with 944 AF cases and 981 non-AF controls and the second validation population of 732 AF patients and 1,291 non-AF controls), we identified significant allelic and genotypic associations between rare variant p.Gly8Ser in *SCN4B* and AF in the two independent AF populations before and after adjusting for important

covariates for AF (Tables 4 and 5). When the two subpopulations were combined (1,676 AF cases and 2,272 non-AF controls), the *P* values for the associations became more significant ($P_{adj} = 4.16 \times 10^{-4}$, OR = 3.60 for allelic association; $P_{adj} = 3.99 \times 10^{-4}$, OR = 3.63, and $P_{adj} = 6.41 \times 10^{-3}$, OR = 3.63 under a dominant model and additive model, respectively) (Tables 4 and 5). Moreover, we also identified significant allelic and genotypic associations between rare variant p.Gly8Ser in *SCN4B* and lone AF (Tables 4 and 5). Together, these data provide strong genetic evidence to suggest that *SCN4B* is a susceptibility gene for AF. The data also strongly support the hypothesis that rare variants with large effects are involved in the genetic determination of AF.

Five different variants in *SCN4B* have been previously reported and listed in Tables 1 and 2. The first reported *SCN4B* variant was p.Leu179Phe identified in a 21-month-old infant with long QT syndrome (LQTS) (Medeiros-Domingo et al., 2007). Variant p.Leu179Phe is located in the transmembrane segment of $Na_v\beta4$ and was found to generate persistent late I_{Na} and a 3.42-mV positive shift of inactivation. The second variant is p.Ser206Leu identified in a 5-month-old African-American male infant and located in the C-terminal intra-cellular domain of $Na_v\beta4$, and was found to generate late persistent I_{Na} and a positive shift of inactivation (Tan et al., 2010). Two heterozygous *SCN4B* variants p.Val162Gly and p.Ile166Leu were identified in patients with familial AF (Li et al., 2013), but the functional effect of these variants were not studied. The p.Val162Gly variant was identified in a small family with only three AF patients and one of the patients were affected with both long QT syndrome and AF (Li et al., 2013). The p.Ile166Leu variant was also identified in a small family with three AF patients (Tables 1 and 2) (Li et al., 2013). However, a substitution of an isoleucine residue by a leucine residue is considered as a minor change. Recently, another variant of p.Thr211Arg was identified in one patient with AF (Husser et al., 2017) (Tables 1 and 2), but again its functional effect is unknown. Considering the genetic evidence was relatively weak because of the very small sizes of families involved and lack of functional data, the association between *SCN4B* variants p.Val162Gly and p.Ile166Leu may need to be further studied. We also performed mutation screening for all exons and exon–intron boundaries of *SCN4B* in 199 patients with ventricular tachycardia (VT) and identified the same p.Gly8Ser variant in two patients (manuscript submitted). Follow-up case-control studies showed that the MAF of p.Gly8Ser was 1.68%–1.84% in the VT populations, which was even higher than that in the AF population, and identified significant association between *SCN4B* variant p.Gly8Ser and VT ($P_{obs} = 1.20 \times 10^{-7}$, OR = 6.42; $P_{adj} = 3.09 \times 10^{-5}$, OR = 6.17) (manuscript submitted).

We used patch-clamping to characterize the potential effect of *SCN4B* variant p.Gly8Ser on cardiac sodium channel $Na_v1.5$, but did not find any effect on the densities of cardiac sodium current or persistent late I_{Na} (manuscript submitted). Therefore, the molecular mechanism by which *SCN4B* variant p.Gly8Ser affects AF remains unknown. Because $Na_v\beta1$ was found to modulate the functions of several potassium channels, including Kv1, Kv7, Kv4.3, and Kv11.1 (Deschenes & Tomaselli, 2002; Nguyen et al., 2012), future studies may focus on characterization of *SCN4B* variant p.Gly8Ser identified in this study and three other previously reported variants for their effects on other ionic channels including potassium channels. These studies may identify novel molecular mechanisms underlying the pathogenesis of AF.

In conclusion, we have identified a rare nonsynonymous variant p.Gly8Ser in *SCN4B* associated with risk of AF. Population-based case-control association studies in two independent AF populations demonstrated that the rare variant p.Gly8Ser conferred a significant risk of AF, including lone AF, with a large effect (high ORs). These data provide strong genetic evidence that *SCN4B* is a susceptibility gene for AF. Our results suggest that rare variants with large effects can contribute importantly to development of AF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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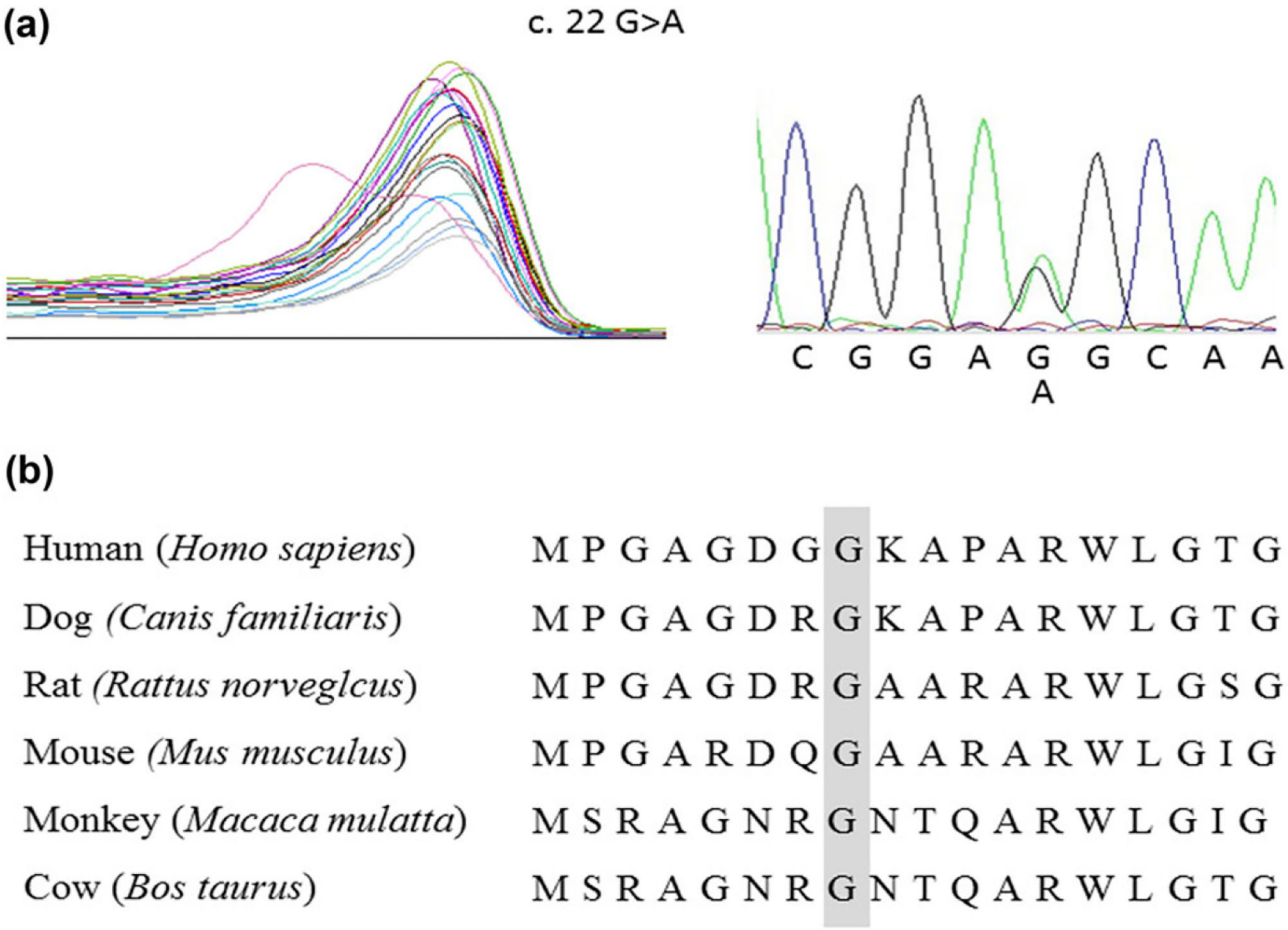

FIGURE 1.

Identification of a novel, nonsynonymous genomic variant p.Gly8Ser in *SCN4B*. (a) High-resolution melt (HRM) traces are shown on the *left*. HRM analysis identified an abnormal HRM pattern in an AF patient (*left*). Sanger sequencing data are shown on the *right*. DNA sequence analysis revealed the presence of variant p.Gly8Ser in exon 1 of *SCN4B*. (b) Variants p.Gly8Ser of *SCN4B* occur at a residue that is highly conserved across different species during evolution

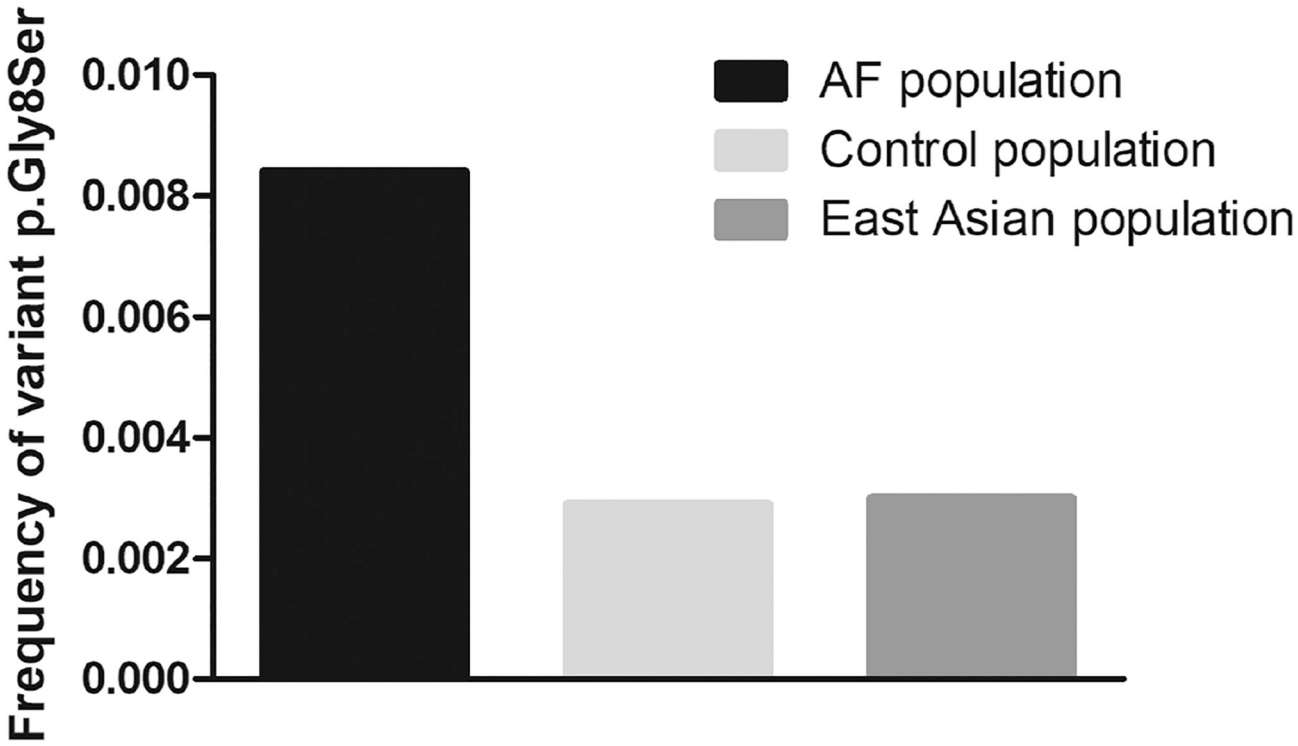

FIGURE 2.

Comparison of the frequency of *SCN4B* variant p.Gly8Ser in the atrial fibrillation (AF) population (477 GeneID Chinese Han subjects) and that in an East Asian non-AF population (2,272 East Asian subjects from the GnomAD-Genomes database from dbSNP [the Single-Nucleotide Polymorphism database])

TABLE 1

List of *SCN4B* variants identified in 477 Chinese AF patients and previously reported variants

	Exon	Variant (NT)	Variant (AA)	Variant type	Number of carriers	Frequency in dbSNP (Global GnomAD-Exomes)	Associated disease
Variant identified in this study	1	22 G > A	p.Gly8Ser	Nonsynonymous	4	rs149868494 (0.00043)	AF
	2	62 (-124) T > C	–	Intronic	3	rs1793137 (0.33892)	None
	2	62 (-93) G > A	–	Intronic	1	rs955917 (0.15679)	None
	2	174 G > A	p.Cys58Cys	Synonymous	6	rs45539032 (0.03543)	None
	5	650 C > G	p.Gly217Gly	Synonymous	1	NA	None
	5	694 G > A	–	3′-UTR	1	rs79071006 (0.00008)	None
Previously reported variant	5	632 C > G	p.Thr211Arg	Nonsynonymous	1 (137)	rs201454653 (0.00013)	AF
	5	617 C > T	p.Ser206Leu	Nonsynonymous	1 (292)	rs140348243 (0.00007)	SID
	4	535 C > T	p.Leu179Phe	Nonsynonymous	7 in 22 peoples of a 4-generation family	rs121434386 (NA)	LQTS
	4	496 A > C	p.Ile166Leu	Nonsynonymous	3 (170)	rs587777560 (NA)	AF
	4	485 T > G	p.Val162Gly	Nonsynonymous	3 (170)	rs587777559 (NA)	AF

Note. NT, variant denoted at the nucleotide level; AA, variant denoted at the amino acid level; AF, atrial fibrillation; SID, sudden infant death; LQTS, long QT syndrome; NA, data not available; dbSNP, the Single-Nucleotide Polymorphism database (dbSNP).

TABLE 2

Bioinformatic analyses of *SCN4B* variants identified in this study and previously reported

Variant	**p.Gly8Ser**	**NA**	**NA**	**NA**	**NA**	**p.Thr211Arg**	**p.Val162Gly**	**p.Ile166Leu**	**p.ser206Leu**	**p.Leu179Phe**
SNPID	rs149868494	rs1793137	rs955917	rs45539032	rs79071006	rs201454653	rs587777559	rs587777560	rs140348243	rs121434386
Associated disease	AF	AF	AF	AF	AF	AF	AF	AF	SID	LQTS
Frequency in gnomAD	0.00043	0.33892	0.15679	0.03543	0.00008	0.00013	NA	NA	7.30E-05	NA
Frequency in ExAC	0.00043	NA/NA	NA/NA	NA/NA	NA/NA	0.00012	NA	NA	6.60E-05	NA
SIFT_pred	T/T	NA/NA	NA/NA	NA/NA	NA/NA	D/D	T/D	T/D	D/D	T/T
Polyphen2_HDIV_pred	D/NA	NA/NA	NA/NA	NA/NA	NA/NA	D/NA	P/NA	D/NA	D/NA	B/NA
PolyPhen	NA/P	NA/NA	NA/NA	NA/NA	NA/NA	NA/D	NA/P	NA/B	NA/D	NA/B
MutationAssessor_pred	L/NA	NA/NA	NA/NA	NA/NA	NA/NA	L/NA	N/NA	L/NA	L/NA	N/NA
FATHMM_pred	D/D	NA/NA	NA/NA	NA/NA	NA/NA	D/D	D/D	D/D	D/D	D/D
PROVEAN_pred	N/N	NA/NA	NA/NA	NA/NA	NA/NA	D/D	N/D	N/N	D/D	N/N
fathmm-MKL_coding_pred	D/D	NA/NA	NA/NA	NA/NA	NA/NA	D/D	D/D	D/D	D/D	N/N
MetaSVM_pred	D/D	NA/NA	NA/NA	NA/NA	NA/NA	D/D	D/D	D/D	D/D	D/D
MetaLR_pred	D/D	NA/NA	NA/NA	NA/NA	NA/NA	D/D	D/D	D/D	D/D	D/D

Note. Data analysis was performed using and Annovar (http://www.openbioinformatics.org/annovar) and VEP (variant effector predictor; https://useast.ensembl.org/info/docs/tools/vep/index.html); SNP, single-nucleotide polymorphism; gnomAD, the Genome Aggregation Database; ExAC, The Exome Aggregation Consortium (ExAC) database; SIFT: D, deleterious; T, tolerated; Polyphen2_HDIV/HVAR: D, probably damaging; P, possibly damaging; B, benign; MutationAssessor: H, high; M, medium; L, low; N, neutral; FATHMM: D, deleterious; T, tolerated; Lower values are more deleterious; PROVEAN: D, deleterious; N, neutral; Higher values are more deleterious; fathmm-MKL: D, deleterious; T, tolerated; MetaSVM: D, deleterious; T, tolerated: Higher scores are more deleterious; MetaLR: D, deleterious; T, tolerated; Higher scores are more deleterious; NA, data not available; AF, atrial fibrillation; SID, sudden infant death; LQTS, long QT syndrome.

TABLE 3

Demographical and clinical characteristics of the two case-control study populations for AF

	AF population 1: Discovery		AF population 2: Replication	
	Cases	**Controls**	**Cases**	**Controls**
Characteristics	***n* = 944**	***n* = 981**	***n* = 732**	***n* = 1291**
Age[a], mean (SD)	62 (10.3)	51 (8.4)	65 (14.1)	56 (21.5)
Sex[a], male *n*, %	576 (61.0)	604 (61.6)	473 (64.4)	897 (69.5)
Lone AF *n*, %	341 (36.1)	NA	267 (36.5)	NA
Hypertension[b] *n*, %	504 (53.4)	63 (6.4)	288 (39.2)	312 (24.2)
CAD *n*, %	348 (36.9)	NA	218 (29.7)	167 (13.0)
Diabetes[c] *n*, %	109 (11.5)	NA	65 (8.9)	123 (9.5)

[a]Data are shown as mean ± standard deviation (SD) for quantitative variables and percent (%) for qualitative variables.

[b]Hypertension was defined as a systolic blood pressure of ≧140 mmHg or a diastolic blood pressure of ≧90 mmHg.

[c]Diabetes was defined as ongoing therapy of diabetes or a fasting plasma glucose level of ≥7.0 mmol/L.

Note. NA, data not available; AF, atrial fibrillation; CAD, coronary artery disease.

TABLE 4

Analysis of allelic association of *SCN4B* variant p.Gly8Ser with AF

				Before adjustment[a]		After adjustment[b]	
Population	**Sample size (case/control)**	**RA frequency (case/control)**	P_{HWE}	P_{obs}	**OR (95% CI)**	P_{adj}	**OR (95% CI)**
AF population 1 (discovery)	944/981	0.74%/0.20%	1	0.017	3.66 (1.20–11.11)	0.022	3.79 (1.21–11.91)
AF population 2 (validation)	732/1291	1.10%/0.35%	1	3.84E-03	3.15 (1.39–7.14)	6.60E-03	3.44 (1.41–8.41)
Combined population	1676/2272	0.89%/0.29%	1	2.87E-04	3.14 (1.64–6.03)	4.16E-04	3.60 (1.77–7.33)
Lone AF	608/2272	0.90%/0.29%	1	2.96E-0	3.18 (1.42–7.11)	0.018	2.85 (1.19–6.78)

[a]Uncorrected *p*-value and odds ratio (OR) obtained using chi-square tests with Pearson's 2 × 2 tables.

[b]*p*-Value and OR after adjustment of age and sex by multivariate logistic regression analysis.

Note. RA, risk allele; P_{HWE}, *P* value from Hardy–Weinberg disequilibrium test; P_{obs}, *p*-value observed; P_{adj}, *p*-value after adjustment of covariates; OR, odds ratio; CI, confidence interval; AF, atrial fibrillation.

TABLE 5

Analysis of genotypic association of *SCN4B* variant p.Gly8Ser with AF under three different inheritance models (an autosomal dominant, recessive, or additive model)

Population (*n*, cases vs. controls)	Model	P_{obs} [a]	OR (95% CI)	P_{adj} [b]	OR (95% CI)
AF population 1 (discovery)	Dominant	0.017[a]	3.68 (1.20–11.21)	0.022	3.81 (1.21–12.00)
944 cases versus 981 controls	Recessive	NA	NA	NA	NA
	Additive	0.017	3.68 (1.20–11.21)	0.022	3.81 (1.21–12.00)
AF population 2 (validation)	Dominant	3.73E-03	3.17 (1.39–7.21)	6.41E-03	3.48 (1.42–8.52)
732 cases versus 1,291 controls	Recessive	NA	NA	NA	NA
	Additive	3.73E-03	3.17 (1.39–7.21)	6.41E-03	3.48 (1.42–8.52)
AF combined population	Dominant	2.76E-04	3.16 (1.64–6.08)	3.99E-04	3.63 (1.78–7.40)
1,676 cases versus 2,272 controls	Recessive	NA	NA	NA	NA
	Additive	2.76E-04	3.16 (1.64–6.08)	3.99E-04	3.63 (1.78–7.40)
Lone AF population	Dominant	2.90E-03	3.20 (1.43–7.18)	0.018	2.86 (1.20–6.84)
608 cases versus 2,272 controls	Recessive	NA	NA	NA	NA
	Additive	2.90E-03	3.20 (1.43–7.18)	0.018	2.86 (1.20–6.84)

[a]Uncorrected *p*-value and odds ratio (OR) obtained using a Pearson 2 × 3 contingence table chi-square test.

[b]*p*-Value and OR after adjustment of age and sex by multivariate logistic regression analysis.

Note. NA, data not available; P_{obs}, *p*-value observed; P_{adj}, *p*-value after adjustment of covariates; OR, odds ratio; CI, confidence interval; AF, atrial fibrillation.