Risk factors and SCN5A-H558R polymorphism for atrial fibrillation in Tibetans living at different altitudes

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Abstract
Several studies have found associations of genes with atrial fibrillation (AF), including SCN5A-H558R. However, there are limited data of these associations among populations living at different altitudes. We investigated the relationship between the SCN5A-H558R polymorphism and AF in Tibetans living at different altitudes in Qinghai, China. General clinical and genotype data were obtained from 72 patients with AF and 109 non-AF (NAF) individuals at middle altitudes, and from 102 patients with AF and 143 NAF individuals at high altitudes. Multifactor logistic regression was performed to determine associations and AF risk factors. SCN5A-H558R genotypes differed significantly between the AF and NAF groups (P < .0125) and the G allele was an independent AF risk factor (P < .05) at both altitudes, with no significant differences according to altitude (P > .0125). At middle altitudes, age, red blood cell distribution width (RDW-SD), left atrial internal diameter (LAD), and G allele were independent AF risk factors. At high altitudes, age, smoking, hypertension, RDW-SD, free triiodothyronine, LAD, and G allele were independent AF risk factors (P < .05). The G allele of SCN5A-H558R might be an independent risk factor of AF both high and middle altitude, but there are some differences in other clinical risk factors of AF.

Abbreviations: AF = atrial fibrillation, FT3 = free triiodothyronine, LAD = left atrial internal diameter, LDL cholesterol = low-density lipoprotein, LVEF = left ventricular ejection fraction, NAF = non-atrial fibrillation, Plt = platelet count, RBC = red blood cell count, RDW = red blood cell distribution width, TC = total cholesterol, TSH = thyroid-stimulating hormone, UA = uric acid.

Keywords: atrial fibrillation, different elevations, genetic polymorphism, risk factors, SCN5A-H558R

1. Introduction
Atrial fibrillation (AF) is the most common type of intraventricular arrhythmia, characterized by rapid and disordered atrial electrical activity. Epidemiological studies have suggested that the overall AF incidence has increased over time, and its prevalence is expected to increase further. Accordingly, the medical and health burden for treating AF-associated complications such as heart failure and stroke is expected to increase. Although there are diverse treatment strategies for AF, the exact pathogenesis has not yet been fully elucidated. Recently, the relationship between genes and AF has attracted increasing attention, and specific genotypes have been reportedly associated with a higher risk of AF development. SCN5A encodes the α subunit of the human cardiac sodium channel Nav1.5, which regulates gating, cellular localization, intracellular trafficking, and degradation to affect cardiac excitability and conduction. SCN5A mutations are associated with various arrhythmic syndromes, including AF, sick sinus syndrome, long QT syndrome, Brugada syndrome, conduction disease, atrial arrest, mixed arrhythmia phenotype overlap syndrome, and drug arrhythmias. SCN5A-H558R is a loss-of-function mutation associated with reduced sodium current density and a shortened refractory period, decreased atrial conduction velocity, and increased susceptibility to atrial arrhythmias. This mutation also plays a role in the maintenance of AF, thus increasing the risk of AF development. As we all know the SCN5A-H558R polymorphism is significantly associated...
with AF incidence; however, the distribution of the gene frequencies differs significantly among different groups. It may be related to the racial inheritance and living habits of different populations. The Tibetans of Qinghai live in the northeast of the Qinghai-Tibet Plateau, with a strong and persistent living environment pressure caused by cold, dry, low pressure, hypoxia and strong ultraviolet radiation. Some studies at plateau have found some differences in diseases at different altitudes and in different ethnic groups, and genetic polymorphisms associated with AF are different between high altitude and plain populations. To the best of our knowledge, differences in the SCN5A-H558R polymorphism in populations living at different altitudes have not been reported. Therefore, the aim of this study was to analyze the SCN5A-H558R polymorphism in patients with AF living at different altitudes and to better understand the risk factors affecting AF in these patients.

2. Materials and methods

2.1. Study subjects

Between June 2018 and August 2021, we enrolled 72 patients with AF and 109 non-AF (NAF) individuals living at 1800 to 2500 m (middle altitude), and 102 patients with AF and 109 NAF individuals living at 3500 to 4500 m (high altitude).

The inclusion criteria of AF group were as follows: an AF diagnosis by 24-hours dynamic echocardiogram (ECG) or 12-lead ECG, Tibetan ethnicity, aged between 18 and 85 years, with no immediate or extended family history of heterogamy. The exclusion criteria were congenital heart disease, cardio-myopathy, chronic pulmonary heart disease, chronic obstructive pulmonary disease, severe liver and kidney dysfunction, blood disorders, rheumatic immune diseases, hyperthyroidism, hypothyroidism, infectious diseases, or malignant tumors. The non-AF group was hospitalized in the cardiology department without developing AF, and the exclusion criteria were the same as the AF group.

This study was conducted according to the principles stated in the Declaration of Helsinki and was reviewed by the Medical Ethics Committee of Qinghai Provincial People’s Hospital (Xining, China). Before collecting blood samples, written informed consent was obtained from all patients.

2.2. Data collection

Patient information, including sex, age, ethnicity, smoking history, drinking history, as well as the medical history of hypertension, diabetes, and coronary heart disease, was recorded.

Approximately 3 mL of peripheral blood was collected at fasting in Y tubes to extract DNA, determine the genotype, and examine relevant blood indices. Laboratory examinations included white blood cell count, red blood cell (RBC) count, hemoglobin, red blood cell distribution width (RDW-SD), platelet count (PLT), fasting blood glucose, triglyceride, total cholesterol (TC), high-density lipoprotein, low-density lipoprotein (LDL cholesterol), uric acid (UA), thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine.

The study participants also underwent cardiac color Doppler ultrasound, which included measurements of left atrial internal diameter (LAD), ventricular septal thickness, left ventricular posterior wall thickness, and left ventricular ejection fraction (LVEF).

2.3. DNA sequencing

DNA was extracted by professionals of the molecular pathology laboratory of Qinghai Provincial People’s Hospital (Beijing Yaanda Biotechnology Co., Ltd., catalog number: DP348). DNA purity and concentration were determined using a Boao microspectrophotometer (NanoQ Micro, Beijing Boao Jingdian Biotechnology Co., Ltd.). DNA sequencing was performed by Xi’an Zhenpin Biotechnology Co., Ltd. to identify relevant DNA sites. The following primers were used: SCN5A-H558R-Forward primer, 5’-GCCAGTGCCACAAAAAGACAGGCT-3’; SCN5A-H558R-Reverse primer, 5’-GGAACCTGCTGATCAGTTTGGGAGA-3’.

2.4. Statistical analysis

The SPSS statistical analysis software version 21.0 (IBM, Armonk, NY) was used for data analysis. The count data are expressed as a composition ratio; the chi-square test was used for comparisons between 4 groups, and chi-square segmentation was used for pairwise comparisons. Data with a normal distribution are expressed as mean; analysis of variance was used for multiple group comparisons, and the least-significant difference test was used for pairwise comparisons. Data with a skewed distribution are expressed as median and interquartile range (IQR); the Kruskal–Wallis H test was used for intergroup comparisons, and pairwise comparisons between groups were performed after the H test. The Hardy–Weinberg equilibrium for genotype was evaluated using a chi-square test. Binary logistic regression was used to identify independent risk factors for AF. *P < .05 was considered statistically significant.

3. Results

3.1. General participant information

Age significantly differed among all 4 subgroups (P < .05). Moreover, a significant difference was observed between the middle-altitude AF and NAF groups (P < .05), between the high-altitude AF and NAF groups (P < .05), and between the middle- and high-altitude NAF groups (P < .05) (Table 1).

No significant differences were observed between the middle-altitude AF and NAF groups or between the middle- and high-altitude AF groups in any of the measured parameters (P > .0125). Significant differences were noted in smoking status and hypertension occurrence between the high-altitude AF and NAF groups (P < .0125), as well as in terms of smoking status and incidence of diabetes mellitus and coronary heart disease between the middle- and high-altitude NAF groups (P < .0125) (Table 2).

Table 1

| Index | Middle altitude | High altitude |
|-------|-----------------|---------------|
|       | NAF group | AF group | NAF group | AF group | H   | P    |
| Age   | 55.0       | 65.0      | 59.0       | 72.0       | 84.127 | <.001 |
|       | (45.5, 62.5) | (59.3, 75.0) | (50.0, 72.0) | (65.0, 75.0) |       |      |

AF = atrial fibrillation, NAF = non-atrial fibrillation.

Comparison with the middle altitude NAF group, *P < 0.05; ◆Comparison with the high altitude NAF group, P<0.05.
Coronary heart disease 22(20.2)
Diabetes mellitus 37(33.9)
Hypertension 44(40.4) 42(58.3) 45(31.5) 65(63.7)
Smoking 42(38.5)
Sex Male 76(69.7) 44(61.1) 81(56.6) 49(48.0) 10.668 .014

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observed in RBC and UA between the middle- and high-altitude NAF groups (LDL cholesterol, and FT3 between the high-altitude AF and NAF groups was used as the independent variable for binary logistic regression analysis. The results showed that in the high-altitude group, age, smoking, hypertension, RDW-SD, FT3, LAD and G allele significantly affected AF occurrence (P < .05) (Table 7).

3.7. Risk prediction model
Moreover, we integrated the above significant factors into nomograms for the prediction of probability of occurrence of AF at middle (Fig. 2) and high altitudes (Fig. 3), respectively. The probability of developing AF was predicted according to the scoring system corresponding to the nomograph (Tables 8–11). The results suggested that the score of the population at middle altitudes was 91 and that of the population at high altitudes was 140, with a probability of AF of 50%. The prediction accuracy (cindex) of the above scoring system was 0.898 (95% CI: 0.850–0.946) and 0.881 (95% CI: 0.837–0.925) for the population at middle and high altitudes, respectively. The accuracy of the above scoring system was verified according to the calibration plot (Figs. 4 and 5).

4. Discussion
AF is a clinically and genetically heterogeneous disease. Both acquired[12] and genetic[13] risk factors have been identified for AF. Multiple studies have confirmed that the susceptibility of AF is related to SCN5A mutations. H558R, a common loss-of-function polymorphism of SCN5A, results in a T-to-C transition, replacing histidine with arginine in the protein sequence. This changes the functions of the cardiac sodium channel and reduces sodium influx, thereby shortening the action potential time range and increasing the AF risk.[14] The H558R polymorphism plays an important role in increasing AF sensitivity, and the TC and CC genotypes are significantly associated with an increased risk of AF.[15] The SCN5A-H558R polymorphism is reportedly more common in patients with early-onset AF lacking traditional risk factors[16] than in controls, and the allele imparts a 1.6-fold increased risk of isolated AF occurrence. Chen et al.[17] reported that the H558R polymorphism plays an important role in increasing AF sensitivity, and the TC and CC genotypes are significantly associated with an increased risk of AF. Yellken et al.[18] reported that in the Han and Uygur populations, AF was associated with the SCN5A-H558R polymorphism, and the G allele was an independent risk factor for AF. Additionally, significant differences were noted in the H558R polymorphism between different ethnic groups, suggesting that the relationship between this polymorphism and susceptibility to AF may differ among different populations.

Here, we found that the SCN5A-H558R polymorphism was more common in patients with AF than in the NAF group, regardless of altitude. The G allele frequency was also significantly higher in the AF group than in the NAF group, suggesting its association with the AF occurrence in the Tibetan population in Qinghai. Furthermore, logistic regression analysis

Table 2
| Index                      | Middle altitude | High altitude | \( \chi^2 \) | \( P \) |
|----------------------------|-----------------|---------------|-------------|-------|
| Sex                        |                 |               |             |       |
| Male                       | 76(69.7)        | 44(61.1)      | 10.668      | .014  |
| Female                     | 33(30.3)        | 28(38.9)      |             |       |
| Smoking                    | 42(38.5)        | 20(27.8)      |             |       |
| Drinking                   | 33(30.3)        | 15(20.8)      |             |       |
| Hypertension               | 44(40.4)        | 42(58.3)      |             |       |
| Diabetes mellitus          | 37(33.9)        | 14(19.4)      |             |       |
| Coronary heart disease     | 22(20.2)        | 17(23.6)      |             |       |

AF = atrial fibrillation, NAF = non-atrial fibrillation.
*Comparison with the middle altitude NAF group, \( P < .0125 \); Comparison with the high altitude NAF group, \( P < .0125 \); \( \chi^2 \)Comparison with the high altitude AF group, \( P < .0125 \).

3.2. General laboratory parameters
Significant differences were observed in RDW-SD, Plt, TC, LDL cholesterol, and UA between the middle- and high-altitude AF and NAF groups (P < .05). Similarly, there were significant differences in RDW-SD, Plt, TC, high-density lipoprotein cholesterol, LDL cholesterol, and FT3 between the high-altitude AF and NAF groups (P < .05). Additionally, significant differences were observed in RBC and UA between the middle- and high-altitude NAF groups (P < .05). However, there were no significant differences between the middle- and high-altitude AF groups (P > .05) (Table 3).

3.3. Cardiac doppler ultrasound indicators
There were significant differences in LAD between the middle-altitude AF and NAF groups (P < .05). Similarly, LAD and LVEF differed significantly between the high-altitude AF and NAF groups (P < .05). However, the indicators between the middle- and high-altitude NAF groups (P > .05) or between the middle- and high-altitude AF groups were comparable (P > .05) (Table 4).

3.4. SCN5A-h558r polymorphism
The genotype frequencies of SCN5A-H558R of the 4 groups were consistent with the Hardy–Weinberg equilibrium law with identity (P > .05). The genotype and allele distribution frequency of the SCN5A-H558R polymorphism differed significantly between the middle-altitude AF and NAF groups (P < .0125) and between the high-altitude AF and NAF groups (P < .0125). The genotype and allele distribution frequency of the SCN5A-H558R polymorphism were not significantly different between the middle- NAF and high-altitude NAF groups (P > .0125) or between the middle- and high-altitude AF groups (P > .0125) (Fig. 1) (Table 5).

3.5. Risk factors in the middle-altitude AF group
The index of significant differences between the middle-altitude AF and NAF groups was used as an independent variable and AF was used as the dependent variable for binary logistic regression analysis. The results showed that in the high-altitude group, age, smoking, hypertension, RDW-SD, FT3, LAD and G allele significantly affected AF occurrence (P < .05) (Table 6).

3.6. Risk factors in the high-altitude AF group
The index of significant differences between the high-altitude AF and NAF groups was used as the independent variable and AF was used as the dependent variable for binary logistic regression analysis. The results showed that in the high-altitude group, age, smoking, hypertension, RDW-SD, FT3, LAD and G allele significantly affected AF occurrence (P < .05) (Table 7).
showed that the G allele was an independent risk factor for AF occurrence in the Tibetan population regardless of altitude. Early identification of AF susceptibility in a population carrying the G allele may help to regulate other controllable AF risk factors to delay disease progression, thereby reducing AF occurrence.

As the R558 allele may decrease the baseline cardiac sodium current density, it may increase the sensitivity to sodium channel block arrhythmias when patients with AF are treated with class I antiarrhythmic drugs. Therefore, analyzing the relationship between the H558R polymorphism and AF occurrence is important for guiding the individualized use of antiarrhythmic drugs.

Different geographical environments and lifestyle habits at various altitudes may affect the genotype and phenotype and their association. Qinghai Tibetans have lived under low oxygen pressure (hypoxia) conditions, which may alter the occurrence and development of certain diseases when compared with those of the lowland population. However, in our study, the genotype and allele frequencies of -H558R did not differ significantly between the AF and NAF groups at different altitudes.

### Table 3
Comparison of the general laboratory indicators M(P25, P75).

| Index | NAF group | AF group | NAF group | AF group | $H$ | $P$ |
|-------|-----------|----------|-----------|----------|-----|-----|
| WBC ($\times 10^9$/L) | 5.69 | 5.69 | 6.11 | 5.90 | 4.82 | .185 |
| (4.75,7.01) | (4.34,7.34) | (5.05,7.50) | (5.08,7.33) | |
| RBC ($\times 10^5$/L) | 5.01 | 4.72 | 5.35 | 5.09 | 13.62 | .003 |
| (4.49,5.49) * | (4.36,5.67) | (4.85,5.84) | (4.54,5.83) | |
| Hb (g/L) | 154.00 (138.00,168.00) | 153.00 (137.50,165.00) | 157.00 | 154.50 (132.75,171.00) | 3.32 | .345 |
| RDW-SD (FL) | 44.60 | 49.40 | 43.00 | 47.65 | 53.30 | <.001 |
| (41.75,48.55) | (44.43,56.20) * | (40.50,47.20) | (44.00,54.55) * | |
| Pt ($\times 10^9$/L) | 186.00 | 152.00 | 207.00 | 155.50 (110.75,209.25) * | 36.76 | <.001 |
| (145.50,238.00) | (121.25,203.25) * | (163.00,254.00) | |
| FBG (mmol/L) | 4.98 | 5.09 | 4.93 | 4.92 | 1.94 | .584 |
| (4.46,7.39) | (4.60,7.11) | (4.51,6.27) | (4.41,5.82) | |
| TG (mmol/L) | 1.21 | 1.08 | 1.23 | 1.07 | 11.44 | .010 |
| (0.90,1.69) | (0.82,1.33) | (0.92,1.63) | (0.77,1.52) | |
| TC (mmol/L) | 4.12 | 3.02 | 4.24 | 3.36 | 65.97 | <.001 |
| (3.46,4.95) | (2.61,4.07) * | (3.55,4.97) | (2.66,4.11) * | |
| HDL (mmol/L) | 1.01 | 0.92 | 0.99 | 0.87 | 21.44 | <.001 |
| (0.79,1.22) | (0.73,1.10) | (0.83,1.20) | (0.70,1.02) * | |
| LDL (mmol/L) | 2.52 | 1.95 | 2.67 | 2.07 | 45.97 | <.001 |
| (1.82,3.05) | (1.37,2.43) * | (2.10,3.27) | (1.64,2.64) * | |
| UA (μmol/L) | 312.00 | 384.5 (311.75,454.25) * | 383.00 (285.0,444.00) | 375.50 | 21.49 | <.001 |
| (256.50,399.00) * | (314.00,508.25) | |
| TSH (μIU/L) | 11.35 | 12.19 (10.95,13.90) | 12.02 (10.19,14.68) | 12.47 | 12.44 | .006 |
| (10.03,12.81) | (9.00,13.79) | (10.90,13.55) | (10.59,13.10) | |
| FT3 (pmol/L) | 4.49 | 4.85 | 4.36 | 4.58 | 20.39 | <.001 |
| (4.12,5.01) | (4.35,5.29) | (3.74,4.90) | (4.21,5.12) * | |

AF = atrial fibrillation, RBC = red blood cell count, TC = total cholesterol, TC = total cholesterol, TG = triglyceride, TC = triiodothyronine, TSH = thyroid-stimulating hormone, UA = uric acid, WBC = white blood cell count.

*Comparison with the middle altitude NAF group, $P < .05$; †Comparison with the high altitude AF group, $P < .05$; ‡Comparison with the middle altitude AF group, $P < .05$.

### Table 4
Comparison of cardiac doppler ultrasound indicators M(P25, P75).

| Index | NAF group | AF group | NAF group | AF group | $H$ | $P$ |
|-------|-----------|----------|-----------|----------|-----|-----|
| LAD (mm) | 35.00 (32.00,38.00) | 45.00 | 35.00 | 42.00 | 118.11 | <.001 |
| (10.63,4.62) | (41.00,48.75) * | (31.00,40.00) | (38.00,48.00) | |
| IVS (mm) | 10.00 | 10.00 | 10.00 | 11.00 | 10.54 | .014 |
| (9.00,11.00) | (9.00,11.75) | (9.00,12.00) | (10.00,13.00) | |
| LVPWT (mm) | 10.00 | 10.00 | 10.00 | 10.00 | 4.94 | .176 |
| (9.00,11.00) | (9.00,11.00) | (9.00,11.00) | (9.00,12.00) | |
| LVEF (%) | 64.00 | 62.00 | 63.00 | 60.00 | 22.74 | <.001 |
| (60.00,67.00) | (56.00,67.00) | (60.00,67.00) | (55.75,63.78) | |

AF = atrial fibrillation, IVS = ventricular septal thickness, LVEF = left ventricular ejection fraction, LVPWT = left ventricular posterior wall thickness, NAF = non-atrial fibrillation.

*Comparison with the middle altitude NAF group, $P < .05$; †Comparison with the high altitude AF group, $P < .05$; ‡Comparison with the high altitude AF group, $P < .05$. 
Table 5

Comparison of genotype and allele of SCN5A-H558R (%).

| Group          | Genotype | H-W | Allele |
|----------------|----------|-----|--------|
|                | AA | AG | GG | $\chi^2$ | $P$ | A | G |
| **Middle altitude** |     |     |     |         |     |    |    |
| NAF-group      | 72(66.1) | 34(31.2) | 3(2.8) | 0.183 | .913 | 178(81.7) | 40(18.3) |
| AF-group       | 34(47.2) | 28(38.9) | 10(13.9) | 1.125 | .570 | 96(66.7) | 48(33.3) |
| **High altitude** |     |     |     |         |     |    |    |
| NAF-group      | 99(69.2) | 39(27.3) | 5(3.5) | 1.805 | .406 | 237(82.9) | 49(17.1) |
| AF-group       | 53(52.0) | 35(34.3) | 14(13.7) | 3.927 | .140 | 141(63.1) | 63(30.9) |
| $\chi^2$       | 23.440 |     |     |         | .001 |     |     |
| $P$            |     |     |     |         | <.001 |     |     |

AF = atrial fibrillation, H-W = Hardy-Weinberg balance test, NAF = non-atrial fibrillation.

*Comparison with the middle altitude NAF group, $P < .0125$; ♦Comparison with the high altitude NAF group, $P < .0125$.  

Figure 1. Gene sequence plots.
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common in the AF group. The statistical results suggest a clear correlation between the \textit{SCN5A-H558R} gene polymorphism and the occurrence of AF in this region. After binary logistic regression analysis of the AF risk factors with significant differences, the results showed that G allele was a strong independent risk factor of AF. Early identification of susceptible individuals carrying the G allele could help to prevent other controllable AF risk factors to delay and reduce the occurrence of AF. Moreover, with the continued development of genetic molecular technology, these findings may also provide therapeutic targets for the upstream treatment of AF. In addition, carrying the susceptible allele may affect the baseline heart sodium current density. Since the susceptibility gene affects channel function, antiarrhythmic drugs such as class I antiarrhythmic drugs that inhibit sodium ion channels may increase the sensitivity of sodium channel block arrhythmia, leading to the occurrence of other arrhythmic events. Therefore, analyzing the relationship between the \textit{SCN5A-H558R} gene polymorphism and AF in this

| Table 6 | Logistic regression analysis of the risk factors for middle altitude presence of AF. |
|---------|-------------------------------------------------------------|
| Variable | \textit{b} | \textit{sb} | Wald | \textit{P} | OR(95%CI) |
| Age | 0.078 | 0.019 | 16.131 | <0.001 | 1.081 (1.041, 1.123) |
| RDW-SD | 0.061 | 0.030 | 4.120 | .042 | 1.063 (1.002, 1.127) |
| LAD | 0.167 | 0.038 | 19.764 | <.001 | 1.182 (1.098, 1.273) |
| G allele | 0.852 | 0.428 | 3.966 | .046 | 2.344 (1.014, 5.421) |
| Constant | −16.469 | 2.709 | 36.951 | <.001 | 1.000 (0.000, 973) |

\textit{AF} = atrial fibrillation, \textit{LAD} = left atrial internal diameter, \textit{RDW} = red blood cell distribution width.

| Table 7 | Logistic regression analysis of the risk factors for high altitude presence of AF. |
|---------|-------------------------------------------------------------|
| Variable | \textit{b} | \textit{sb} | Wald | \textit{P} | OR(95%CI) |
| Age | 0.042 | 0.014 | 8.761 | .003 | 1.043 (1.014, 1.073) |
| Smoking | 1.372 | 0.399 | 11.850 | .001 | 3.943 (1.806, 8.612) |
| Hypertension | 1.122 | 0.353 | 10.094 | .001 | 3.070 (1.537, 6.133) |
| RDW-SD | 0.053 | 0.026 | 4.251 | .039 | 1.054 (1.003, 1.108) |
| FT3 | 0.000 | 0.025 | 9.648 | .002 | 2.224 (1.343, 3.685) |
| LAD | 0.108 | 0.027 | 16.184 | <.001 | 1.114 (1.057, 1.174) |
| G allele | 1.072 | 0.369 | 8.438 | .004 | 2.921 (1.417, 6.021) |
| Constant | −13.886 | 2.413 | 33.123 | <.001 | 1.000 (0.001, 157) |

\textit{AF} = atrial fibrillation, \textit{FT3} = free triiodothyronine, \textit{LAD} = left atrial internal diameter, \textit{RDW} = red blood cell distribution width.

Figure 2. Nomogram of middle altitude (0 means A, 1 means G).
region also plays an important role in guiding the individualized use of antiarrhythmic drugs in patients with AF.

Moreover, age is a well-known risk factor for AF.\textsuperscript{[20,21]} The prevalence of AF increases with increasing age; in China, the prevalence of AF in adults aged < 55 years was reported to be 0.1%, compared to 9% in those aged 80 years.\textsuperscript{[22]} The lifetime risk of AF increases significantly in individuals aged ≥ 75 years.\textsuperscript{[20,21]} Consistently, age was identified as an independent risk factor for AF at both middle and high altitudes in our study. We hypothesize that the effect of age on AF may be related to age-specific myocardial remodeling, an increase in reactive oxygen species production, and decreased antioxidation capacity. These age-related physiological changes can affect the expression of certain genes and activate specific factors to promote atrial fibrosis, ultimately increasing the AF risk.\textsuperscript{[24]}

Figure 3. Nomogram of high altitude (Smoking: 0 means no, 1 means yes; hypertension: 0 means no, 1 means yes; allele: 0 means A, and 1 means G).

Table 8
The scoring system corresponding to the above nomogram is as follows.

| Factor | Score | Factor | Score |
|--------|-------|--------|-------|
| Age    |       | LAD    |       |
| 10     | 0     | 20     | 0     |
| 20     | 8     | 25     | 8     |
| 30     | 15    | 30     | 17    |
| 40     | 23    | 35     | 25    |
| 50     | 31    | 40     | 33    |
| 60     | 39    | 45     | 42    |
| 70     | 46    | 50     | 50    |
| 80     | 54    | 55     | 58    |
| 90     | 62    | 60     | 67    |
| RDW-SD |       |        |       |
| 30     | 0     | 65     | 75    |
| 35     | 3     | 70     | 83    |
| 40     | 5     | 75     | 92    |
| 45     | 8     | 80     | 100   |
| 50     | 10    | Allele |       |
| 55     | 13    | A      | 0     |
| 60     | 15    | G      | 9     |
| 65     | 18    |        |       |
| 70     | 21    |        |       |
| 75     | 23    |        |       |
| 80     | 26    |        |       |

LAD = left atrial internal diameter, RDW = red blood cell distribution width.
Smoking can also increase the AF risk.\cite{12,25,26} In our study, smoking was an independent risk factor for AF in the high-altitude group only. In a large cohort study, the AF incidence was 9.5% in smokers and was 7.8% in nonsmokers, suggesting that smoking may be associated with an increased risk of AF.\cite{27} However, this association was less significant after adjusting for cardiovascular risk factors. The authors suggested that this may be due to smoking-related exacerbation of cardiovascular risk factors associated with AF, such as increased oxidative stress, inflammation, and atrial fibrosis. At high altitudes, the hemodynamic load and elevated sympathetic stress can cause oxidative stress, and hemodynamic overload can also trigger an inflammatory response, leading to atrial electrical and structural remodeling, providing a theoretical basis for the occurrence and development of AF. Thus, we considered that the association of smoking with AF at high altitudes might be attributed to the above-mentioned causes. Moreover, nicotine reportedly exerts certain cardiovascular effects.\cite{24} However, a direct link between smoking and AF pathogenesis has not been reported.

The pathological changes associated with hypertension affect the electrical activity of the myocardium, eventually leading to AF development.\cite{29} The Framingham Heart Study\cite{30} showed that both sustained increases in systolic pressure and prolonged antihypertensive treatment were associated with an increased risk of AF. A study in China has shown that highlanders (especially the working class) have a higher prevalence of hypertension.\cite{31} Moreover, the lack of hypertension treatment and control rates due to poor health awareness may explain why hypertension significantly affects AF at high altitudes. Therefore, measures to control the risk factors for hypertension and regulate blood pressure would effectively reduce AF occurrence.

Elevated levels of RDW are often associated with impaired erythropoiesis and degradation. Oxidative stress and inflammation can inhibit the maturation of erythrocytes, and the accumulation of immature erythrocytes will lead to a significant increase in RDW levels in the blood. Studies suggest that higher levels of RDW may reflect an increased risk of AF, and that RDW may serve as an independent predictor of AF after cardiac surgery or others surgery.\cite{32,33} In another study comparing individuals living at different altitudes,\cite{34} RDW was also suggested as an independent risk factor for the occurrence of AF. RDW-SD was suggested as a risk factor for AF at both middle and high altitudes. However, another study showed no obvious difference in RDW of Tibetans living at different altitudes in this region, which is consistent with our results.

Hyperthyroidism is associated with a significantly increased AF risk. A large cohort study reported that AF risk depends on TSH and free thyroxine levels; a decrease in TSH levels increases the AF risk. However, AF risk is lower in patients with overt and subclinical hypothyroidism, suggesting that hypothyroidism may protect against AF. A survey study reported that the normal T3, T4, FT3, and TSH levels of healthy Tibetan adults in

### Table 9
The probability of AF corresponding to the above scoring system is as follows.

| Normo score | Probability of AF |
|-------------|------------------|
| 60          | 5.00%            |
| 68          | 10.00%           |
| 72          | 15.00%           |
| 76          | 20.00%           |
| 82          | 30.00%           |
| 86          | 40.00%           |
| 91          | 50.00%           |
| 95          | 60.00%           |
| 99          | 70.00%           |
| 105         | 80.00%           |
| 113         | 90.00%           |
| 121         | 95.00%           |
| 138         | 99.00%           |

\(AF = \text{atrial fibrillation.}\)

### Table 10
The scoring system corresponding to the above nomogram is as follows.

| Factor       | Score | FT3  | Score |
|--------------|-------|------|-------|
| Age          | 20    | 0    | 2.5   | 0     |
|              | 30    | 7    | 3.0   | 6     |
|              | 40    | 15   | 3.5   | 12    |
|              | 50    | 22   | 4.0   | 19    |
|              | 60    | 30   | 4.5   | 25    |
|              | 70    | 37   | 5.0   | 31    |
|              | 80    | 44   | 5.5   | 37    |
|              | 90    | 52   | 6.0   | 44    |
|              | 100   | 59   | 6.5   | 50    |
| Smoking      | 0     | 0    | 7.0   | 56    |
|              | 1     | 20   | 15    | 0     |
| Hypertension | 0     | 0    | 20    | 8     |
|              | 1     | 17   | 25    | 17    |
| RDW-SD       | 30    | 0    | 30    | 25    |
|              | 35    | 5    | 35    | 33    |
|              | 40    | 10   | 40    | 42    |
|              | 45    | 14   | 45    | 50    |
|              | 50    | 19   | 50    | 58    |
|              | 55    | 24   | 55    | 67    |
|              | 60    | 29   | 60    | 75    |
|              | 65    | 33   | 65    | 83    |
|              | 70    | 38   | 70    | 92    |
|              | 75    | 43   | 75    | 100   |
|              | 80    | 48   | Allele | A | 0 |
|              | 85    | 52   |       | G | 16 |
| 90          | 57    |      |       |     |
| 95          | 62    |      |       |     |

\(FT3 = \text{free triiodothyronine, LAD = left atrial internal diameter, RDW = red blood cell distribution width.}\)
Lhasa are slightly lower than those of individuals living in the plain areas.[37] It is worth noting that the Tibetan individuals living in the hypoxic environment of the plateau may have evolved a reduced adaptive thyroid function, which can help them to maintain a low metabolic rate and avoid excessive metabolic consumption. In this study, the results showed that FT3 was a risk factor affecting AF occurrence in the high-altitude group, suggesting that the high FT3 levels may have more pronounced effects on the risk of AF occurrence at high altitudes.

The Framingham Heart Study[38] reported that increased LAD and left ventricular posterior wall thickness and decreased LVEF predict the risk of non-valvular AF. Additionally, these echocardiographic indicators can provide important information on AF risk factors; for instance, the LAD of patients with heart failure combined with AF is significantly higher than that of patients with heart failure combined with sinus rhythm.[39] Our results suggest that LAD affects AF independently in Tibetans at middle and high altitudes, and increased LAD is associated with an increased AF risk. An increase in LAD is also reportedly associated with alcohol consumption,[40] age, male sex, higher body mass index, higher systolic blood pressure, and lower diastolic blood pressure.[41] Unique lifestyle habits and the generally high blood pressure in our study population may have led to increased LAD levels, thus increasing the AF risk. Increased LAD is also associated with an increased risk of mortality in other cardiovascular diseases.[42] Therefore, early prevention and delayed atrial remodeling by actively regulating LAD-associated factors may have important implications in AF and other heart diseases.

This study had certain limitations. The number of Tibetans living in middle-altitude areas is far lower than that in high-altitude areas; thus, the sample size was limited during the study period. In addition, the design of our study only included altitude observation groups at middle and high altitudes, lacking a low-altitude group.

This study indicates that the SCN5A-H558R gene polymorphism and other traditional risk factors significantly influence the occurrence of AF. Logistic regression analysis combined with risk factors showed that several risk factors, including the G allele, independently influenced the occurrence of AF. Using the nomogram diagram to obtain a relevant scoring system, based on the corresponding score of each risk factor value, the total number was summed to derive the probability of final AF, which can help to identify the probability of developing AF in people with susceptibility factors. Prediction of disease occurrence by specific theoretical values will have important implications for the early identification and active treatment of AF-susceptible populations in this region.

5. Conclusion
We found that AF occurrence in Tibetans at middle and high altitudes in Qinghai is associated with the SCN5A-H558R polymorphism. The G allele significantly increases the risk of AF in these individuals. However, we did not find differences in the

| Normo score | Probability of AF |
|-------------|-------------------|
| 94          | 5.00%             |
| 106         | 10.00%            |
| 113         | 15.00%            |
| 118         | 20.00%            |
| 127         | 30.00%            |
| 133         | 40.00%            |
| 140         | 50.00%            |
| 146         | 60.00%            |
| 153         | 70.00%            |
| 161         | 80.00%            |
| 173         | 90.00%            |
| 185         | 95.00%            |
| 210         | 99.00%            |

AF = atrial fibrillation.

Figure 4. Calibration plot.
SCN5A-H558R polymorphism between individuals living at different elevations. However, we also identified differences in some traditional AF risk factors associated with different altitudes in Tibetans in Qinghai.

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