Single Nucleotide Polymorphisms and Haplotype of Four Genes Encoding Cardiac Ion Channels in Chinese and their Association with Arrhythmia

Yu Zhang, Ph.D.,* Bingxi Chang, M.D.,† Songnian Hu, Ph.D.,* Duenmei Wang, Ph.D.,* Quan Fang, M.D.,† Xianyong Huang, M.D.,‡§ Qiang Zeng, M.D.,‡ and Ming Qi, Ph.D., F.A.C.M.G.*§¶

*From the *Key Laboratory of Genome Science and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China; †Peking Union Medical College Hospital, Beijing, China; ‡General Hospital of PLA, Beijing, China; §Zhejiang University-Adinovo Center for Genetic & Genomic Medicine, First Affiliated Hospital of Zhejiang University School of Medicine, James Watson Institute of Genome Sciences, Hangzhou, China; and ¶University of Rochester Medical Center, Rochester, New York*

Background: Many studies revealed that variations in cardiac ion channels would cause cardiac arrhythmias or act as genetic risk factors. We hypothesized that specific single nucleotide polymorphisms in cardiac ion channels were associated with cardiac rhythm disturbance in the Chinese population.

Method: We analyzed 160 nonfamilial cardiac arrhythmia patients and 176 healthy individuals from which 81 individuals were selected for association study, and a total of 19 previously reported SNPs in four cardiac ion channel genes (*KCNQ1, KCNH2, SCN5A, KCNE1*) were genotyped.

Results: The frequency of *KCNQ1* 1638G>A, as well as the haplotype harboring *KCNQ1* 1638A, *KCNQ1* 1685 + 23G and 1732 + 43T (haplotype AGT) was significantly higher in healthy controls than in arrhythmia patients. This finding implicated that this haplotype (AGT) might be a protective factor against arrhythmias.

Conclusions: Our study provided important information to elucidate the effect of SNPs of cardiac ion channel genes on channel function and susceptibility to cardiac arrhythmias in Chinese population.

Ann Noninvasive Electrocardiol 2008;13(2):180–190

arrhythmia; cardiac ion channel (*KCNQ1, KCNH2, SCN5A, KCNE1*); single nucleotide polymorphism; haplotype; Chinese population

Cardiac arrhythmias have a high morbidity worldwide and are a common cause of death in developed countries. Sudden cardiac deaths are partly attributed to arrhythmias such as ventricular tachycardia. Long QT syndrome (LQTS) is an inherited cardiac arrhythmia characterized by syncope, prolongation of QT interval, and ventricular tachycardia known as torsades de pointes on ECG and risk of sudden cardiac death. So far 10 genes have been identified for this disorder. They include *KCNQ1*, which is responsible for causing LQT1, *KCNH2* for LQT2, *SCN5A* for LQT3, *KCNE1* for LQT5 and *KCNE2* for LQT6. LQT4 is found to be caused by mutations in *AnkB*. *KCNJ2* is responsible for LQT7.[1] All these genes except *AnkB* are ion channel genes.

Mutations in ion channel genes will lead not only to long QT syndrome but also other arrhythmias such as Brugada syndrome,[2] familial atrial fibrillation,[3] and cardiac conduction defects.[4] However,

Address for reprints: Ming Qi, Ph.D., F.A.C.M.G., Zhejiang University-Adinovo Center for Genetic & Genomic Medicine, First Affiliated Hospital of Zhejiang University School of Medicine and James Watson Institute of Genome Sciences, 79 Qingchun Road, Hangzhou, China 310003. Fax: 86-571-8820 8274; E-mail: qiming_14618@yahoo.com

This study was partially supported by the "985 Project Grant" (Zhejiang University) from the Ministry of Education of China and the "Qiangjiang Research Talent" grant (Dr. Ming Qi) from the Science and Technology Department of Zhejiang Province.

Table 1. Characteristics of the Arrhythmia Patients and Healthy Controls

	Males		Females		Total	
	Number	Average Age	Number	Average Age	Number	Average Age
Patients	91	61.70 ± 14.92	69	59.20 ± 16.05	160	60.75 ± 15.34
Controls	52	62.47 ± 7.06	29	62.56 ± 9.84	81	62.48 ± 7.35

single nucleotide polymorphisms (SNPs) may mediate arrhythmia susceptibility. For example, some variants may influence the susceptibility to develop of acquired LQTS, which is caused by drug administration,[5,6] electrolyte disturbances, and other heart diseases. A sodium channel variant, *SCN5A* S1103Y, found in individuals of African origin was associated with a high risk of arrhythmia.[7] Another example, the presence of the H558R polymorphisms in the *SCN5A* gene was reported to restore normal trafficking and normalized Na^+ current that was changed by mutation M1766L.[8]

Studies on variants in cardiac ion channel genes in different ethnic groups have been performed in recent years.[9–12] It is of importance to identify the specific risk factor for arrhythmias in a specific population. However, the frequencies of polymorphisms in cardiac ion channel genes in Chinese population remain unknown. In this study, we aim to estimate the frequencies of some reported SNPs of ion channel related genes in a Chinese population and compared the frequencies of the SNPs and the haplotypes between the arrhythmia patients and healthy controls. We hope these data may provide some information for future functional and pharmacogenetic research on the mechanism of ion channel variation and susceptibility to cardiac arrhythmia.

METHODS

Subjects

One hundred sixty unrelated Chinese arrhythmic patients were recruited from the Peking Union Medical College Hospital. Informed consent was obtained from all patients. The patients were diagnosed using electrocardiographic criteria. The mean age of patients is shown in Table 1. According to the cellular mechanisms of cardiac arrhythmia and the ECG findings, patients were divided into several subgroups (Table 2). As for the original causes of the arrhythmias, hypertension was found in 83 patients, coronary atherosclerotic heart disease (including angina, myocardial infarction) in 55 patients, heart failure in 9 patients, cardiomyopathy in 25 patients, and valvular heart disease in 22 patients. Twenty-seven patients have more than one original causes.

The group of 176 healthy individuals of 98 males (mean age 52.2 ± 16.3 years) and 78 females (mean age 37.1 ± 15.3 years) was also recruited from the Peking Union Medical College Hospital. No family history or history of syncope or any atrial or ventricular arrhythmias and normal ECG were exclusion criteria. Informed consents were also obtained from these participants. We selected 81 individuals from the group of 176 individuals whose age was compatible with that of patients for association study. The features of the 81 individuals were also shown in Table 1. The ethics committee of Beijing Genomics Institute, Chinese Academy of Science approved this study.

SNPs Selection

Iwasa reported 20 SNPs of four genes and their allele frequencies in Japanese population.[13] Maekawa sequenced the entire SCN5A coding

Table 2. Clinical Subgroups of Arrhythmia Patients

Cellular Mechanism	ECG Findings	Number	Percentage (%)
Repolarization abnormalities	Atrial flutter and atrial fibrillation	100	59.2
	Premature ventricular contraction	26	15.3
	Supraventricular premature contraction	11	6.5
	Ventricular tachycardiac	11	6.5
Conduction abnormalities	Atrioventricular block	5	3.0
	Intraventricular block	16	9.5

Table 3. Selected SNPs for Genotyping and their Locations in Genes

Gene	SNP	Amino Acid Change	SNP Database	Region
KCNQ1	435C>T	I145I	rs17221868	S1-S2
	1110G>A	A370A	rs1805118	C-terminal
	1394–12C>T	Intronic variant		Intron
	1638G>A	S546S	rs1057128	C-terminal
	1685 + 23G>A	Intronic variant		Intron
	1732 + 43 T>C	Intronic variant	rs81204	Intron
	1927G>A	G643S	rs1800172	C-terminal
KCNH2	1539T>C	F513F	rs1805120	S3
	1692G>A	L564L	rs1805121	S5
	1956C>T	Y652Y	rs17424631	S6
SCN5A	703 + 130G>A	Intronic variant		Intron
	1673A>G	H558R	rs1805124	DI-DII
	3269C>T	P1090L	rs1805125	DII-DIII
	4299 + 53T>C	Intronic variant		Intron
	5457C>T	D1819D	rs1805126	C-terminal
	5851G>T	V1951L		C-terminal
	5963T>G	L1988R		C-terminal
KCNE1	112G>A	G38S	rs1805127	Extracellular
	253G>A	D85N	rs1805128	Cytoplasmatic

exons and their flanking introns in Japanese arrhythmia patients and healthy controls.[14] They found two SNPs, 703 + 130G>A and 5963T>G have significant different frequencies between patients and controls. Japanese and Chinese are both Asians and are ethnically similar, thus we chose 19 SNPs from the studies of Japanese to test in our study. These SNPs included synonymous SNPs, nonsynonymous, and intronic ones. We tried to speculate on the relationship between common variants of ion channel genes and susceptibility of cardiac arrhythmias from different mechanisms. Among 19 SNPs, 14 SNPs had been published in dbSNP public database (Table 3).

Genotyping

Peripheral blood samples were collected from study subjects and genomic DNA was extracted from blood. Genotyping was performed on a Sequenom MassArray Genotyping System. Primers were designed according to the genomic sequence of *KCNQ1* (NT_009237), *KCNH2* (NT_007914), *SCN5A* (NT_022517), and *KCNE1* (NT_011512) using SpectroDESIGNER (Sequenom, San Diego, CA). Product size was approximately 100 bp with 50 bp flanking the identified SNP. Extension primers were designed again using SpectroDESIGNER (Sequenom). The primer sets used in genotyping are listed in supplementary Table 1. The PCR amplification (10 μL total volume) contained 1× HotStar Taq PCR buffer, 1.5 mM $MgCl_2$, 250 μM each dNTP (Amersham, Piscataway, NJ), 0.125 U Enzyme HotStar Taq polymerase (Takara, Otsu, Shiga, Japan), 100 nM each forward and reverse extension primer, and 3 ng of genomic DNA. The PCR amplification was performed in 384-well plates using the following conditions: 95°C for 15 minutes and

Table 4. Allele Frequencies of SNPs of *KCNQ1* Gene in Chinese Arrhythmia Patients and Healthy Controls*

Gene	SNP	Amino Acid Change	MAF of Cases	MAF of Controls	P Value	OR	95%CI
KCNQ1	435C>T	I145I	0.038	0.071	0.119	1.934	0.834–4.488
	1110G>A	A370A	0.003	0.000	1.000	0.997	0.991–1.003
	1392–12C>T		0.000	0.000	–	–	–
	1638G>A	S546S	0.242	0.367	**0.005**	1.818	1.199–2.757
	1685 + 23G>A		0.018	0.013	1.000	0.701	0.134–3.657
	1732 + 43T>C		0.297	0.244	0.217	0.761	0.493–1.175
	1927G>A	G643S	0.032	0.031	0.944	0.963	0.323–2.863

MAF = minor allele frequency; OR = odds ration; CI = confidence interval. The bold representation is statistical significance.

Table 5. Allele Frequency of SNP 1638G>A of *KCNQ1* Gene in Different Arrhythmia Subgroups and Healthy Controls*

Subgroup	MAF of Cases	MAF of Controls	P Value	OR	95%CI
Atrial fibrillation and atrial flutter	0.229	0.367	**0.005**	1.956	1.223–3.128
Premature contraction (ventricular and supraventricular)	0.288	0.367	0.255	1.435	0.769–2.676
Ventricular tachycardiac	0.300	0.367	0.556	1.353	0.493–3.714
Atrioventricular and bunch blocks	0.184	0.367	**0.032**	2.569	1.064–6.203

MAF = minor allele frequency; OR = odds ration; CI = confidence interval. The bold representation is statistical significance.

45 cycles of 95°C for 20 seconds, 56°C for 30 seconds, 72°C for 1 minute, followed by a final extension step of 72°C for 3 minutes. PCR products were treated with shrimp alkaline phosphatase (USB, Cleveland, OH) at 37°C for 30 minutes to remove excess dNTPs. Extension reaction condition was 94°C for 2 minutes, followed by 94°C for 5 seconds, 52°C for 5 seconds, and 72°C for 5 seconds for 40 cycles. Base extension products were treated with the SpectroCLEAN (Sequenom) resin to remove salts in the reaction buffer. 10 nL of reaction solution was dispensed onto a 384-format SpectroCHIP (Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid by using a SpectroPoint (Sequenom) nanodispenser. Biflex matrix-assisted laser desorption ionization/time-of-flight MS (Bruker, Billerica, MA) was used for data acquisitions from the SpectroCHIP. The expected molecular weights of all relevant peaks were calculated before the analysis and identified from the mass spectrum. Discordance between blind duplicate samples included in the genotyping was <1% and the call rate (the percentage of SNPs called on the array) for each assay was set at >90%. Average call rate was 97.6% (range from 92.0% to 99.7%).

Statistical Analysis

Statistical analyses were conducted using SPSS (version 11.5) and a P value <0.05 was considered to be significant. Allele frequencies were calculated for each SNP site by the allele counting method. SNP frequencies in both of the groups were tested for deviation from Hardy-Weinberg equilibrium using chi-square test. Differences in genotype frequency and allele frequency between patients and controls were tested by Fisher's exact test. Pairwise linkage disequilibrium and haplotypes analyses were performed by using Haploview software (version 3.2).[15]

RESULTS

KCNQ1 Polymorphisms

Genotype Distributions of All Loci Were in Hardy-Weinberg Equilibrium

One SNP, 1392–12C>T, was nonpolymorphic in the population we studied. Another SNP, 1110G>A, was nonpolymorphic in healthy controls and was rare (0.003) in patients. 1638G>A showed significantly different frequencies between the patients and controls. The healthy controls were more likely to have the 1638A allele compared to the patients with P value of 0.005. The odds ratio was 1.818 with 95% confidence interval (95%CI) of 1.199–2.757 (Table 4). However, the association became nonsignificant after Bonferroni correction (the P value is 0.05/19, i.e., 0.002).

Table 6. Haplotype Analysis of Block in *KCNQ1* Gene

Haplotype	Select SNPs			Haplotype Frequency (%)			
	1638G>A	1685 + 23G>A	1732 + 43T>C	All Subjects	Patients	Controls	P Value
GGT	G	G	T	0.421	0.443	0.377	0.165
AGT	A	G	T	0.284	0.242	0.367	**0.004**
GGC	G	G	C	0.279	0.297	0.244	0.219
GAT	G	A	T	0.016	0.017	0.012	0.672

The bold representation is statistical significance.

Table 7. Summary of SNPs in *KCNH2* Gene Genotyped in Different Arrhythmia Subgroups and Healthy Controls*

Gene	SNP	Amino Acid Change	MAF of Cases	MAF of Controls	P Value	OR	95%CI
KCNH2	1539T>C	F513F	0.338	0.295	0.352	0.820	0.539–1.246
	1692G>A	L564L	0.104	0.100	0.898	1.042	0.555–1.956
	1956C>T	Y652Y	0.066	0.101	0.201	0.625	0.314–1.243

MAF = minor allele frequency; OR = odds ration; CI = confidence interval.

Different arrhythmia types may have different molecular etiologies. The patients were thus divided into four subgroups according to their diagnoses, atrial flutter and atrial fibrillation, premature contraction (ventricular and supraventricular included), ventricular tachycardia, and block (atrioventricular and bunch included). Allele frequencies of each subgroup were compared to those in healthy controls. A significantly different frequency of 1638G>A between patients and healthy was obtained (0.005) in atrial flutter and atrial fibrillation subgroup (Table 5).

We selected five SNPs for linkage disequilibrium analysis and haplotype construction of *KCNQ1*. The other two SNPs were not selected due to nonpolymorphisms (1392–12C>T) or low minor allele frequencies (1110G>A). Three SNPs, 1638G>A, 1685 + 23G>A, and 1732 + 43T>C were in a haplotype block, which refers to sites of closely located SNPs that are inherited together. Regions corresponding to blocks have a few common haplotypes, which account for a large proportion of chromosomes. In our research the block had three major haplotypes with frequencies >5% (Table 6). The frequency of AGT carrying minor allele of SNP 1638G>A in healthy control group was significantly higher than that in patient group ($P = 0.0043$).

KCNH2 Polymorphisms

Genotype distributions of all loci were in Hardy-Weinberg equilibrium. No significant difference between patients and healthy controls among these SNPs was found (Table 7). Allele frequencies comparisons in each subgroup were done and no significant difference was found.

All of the three SNPs of *KCNH2* gene were selected for linkage disequilibrium analysis and haplotype construction. There were no significant differences of haplotype frequencies between patients and healthy controls.

SCN5A Polymorphisms

Genotype Distributions of All Loci Were in Hardy-Weinberg Equilibrium

Two SNPs, 5851G>T and 5963T>G were nonpolymorphic in the population we studied. All of the SNPs we tested did not show significantly different frequencies between the patient and control groups (Table 8). Allele frequencies comparison in subgroups was performed as well and no significant difference was found.

Five SNPs of SCN5A gene were selected for haplotype construction and the other two were omitted from analysis because of nonpolymorphisms (5851G>T and 5963T>G). There were no

Table 8. Summary of SNPs in *SCN5A* Gene Genotyped in Different Arrhythmia Subgroups and Healthy Controls*

Gene	SNP	Amino Acid Change	MAF of Cases	MAF of Controls	P Value	OR	95%CI
SCN5A	703 + 130G>A		0.096	0.133	0.271	1.451	0.801–2.628
	1673A>G	H558R	0.144	0.141	0.947	0.982	0.571–1.689
	3269C>T	P1090L	0.013	0.019	0.691	1.510	0.334–6.829
	4299 + 53T>C		0.327	0.315	0.786	0.945	0.630–1.419
	5457C>T	D1819D	0.449	0.407	0.385	0.844	0.574–1.239
	5851G>T	V1951L	0.000	0.000	–	–	–
	5963T>G	L1988R	0.000	0.000	–	–	–

MAF = minor allele frequency; OR = odds ration; CI = confidence interval.

Table 9. Summary of SNPs in *KCNE1* Gene Genotyped in Different Arrhythmia Subgroups and Healthy Controls*

Gene	SNP	Amino Acid Change	MAF of Cases	MAF of Controls	P Value	OR	95%CI
KCNE1	112G>A	G38S	0.293	0.263	0.503	1.160	0.751–1.793
	253G>A	D85N	0.000	0.013	0.113	1.013	0.995–1.030

MAF = minor allele frequency; OR = odds ration; CI = confidence interval.

significant differences of haplotype frequencies between patient and control groups.

KCNE1 Polymorphisms

Genotype Frequencies Did Not Deviate from the Hardy-Weinberg Disequilibrium in either Group

Allele frequency of the 112G>A was 0.293 in patients and 0.263 in healthy controls (Table 9). They did not differ significantly between the two groups. 253G>A did not show polymorphism in patients but has an allele frequency of 0.013 in healthy controls. Allele frequencies comparisons within different subgroups were also done and no significant difference was found.

Haplotype of *KCNE1* was not constructed due to nonpolymorphism of 253G>A in patients.

Comparison among Different Ethnicities

We compared the allele frequencies (176 healthy individuals) in our study with those that were previously reported in other ethnicities including Asian, Caucasian, and African (Table 10). We also compared our results to the data from Hapmap project (www.hapmap.org), which studied four populations.

Of six SNPs in *KCNQ1*, all in Chinese population had similar frequencies with those of Japanese except 1927G>A, which was lower in Chinese than in Japanese. This variant was reported to be associated with life-threatening arrhythmias and was much more frequent in Japanese than in other populations.[16] Another SNP, 1638G>A had a relatively higher frequency in Asian populations including Chinese and Japanese than in Caucasian. Of three SNPs in *KCNH2*, two (1692G>A and 1956C>T) were more frequent in Caucasian than in Chinese of our study, while another one 1539T>C had an opposite result. All these three SNPs in our study were not totally consistent with other Asian population in different studies. The 1673A>G was one of the most frequently reported sites in *SCN5A* in different publications and had a similar frequency in Chinese and other Asian populations compared to higher ones in Caucasian and African origin. The intronic SNP, 4299 + 53T>C was higher in Asians than in Caucasians. The two SNPs of *KCNE1* varied significantly in different populations by different studies.

DISCUSSION

It is important to identify the genetic factors influencing the susceptibility to cardiac arrhythmia because of its high morbidity. Focus on ion channels of the heart for identification of those factors is important as these proteins are concerned with the cardiac action potential. In this study we screened SNPs in Chinese population.

KCNQ1 Polymorphisms

Of seven selected SNPs of *KCNQ1,* one is nonsynonymous SNP G643S. Kubota reported that G643S was found in 11% of Japanese population and might reduce the outward K^+ current density and accelerate the deactivation process resulting in a dominant-negative effect on the heteromultimeric channel complexes.[16] They demonstrated that G643S might predispose gene carriers to life-threatening arrhythmias in the presence of appropriate precipitating factors such as hypokalemia. In our study, the allele frequency of G643S in arrhythmic patients (0.032) was similar to that in healthy controls (0.031). It is likely that this SNP was not directly involved in arrhythmogenesis in our study. More studies are required to validate whether it is related to arrhythmia susceptibility in Chinese population.

The synonymous SNP S546S had an allele frequency of 0.242 in patients and 0.367 in controls, which are significant ($P = 0.005$). Haplotype analysis using the 3 SNPs, 1638G>A (S546S), 1685 + 23G>A, and 1732 + 43T>C showed that the frequency of haplotype AGT was significantly lower

Table 10. SNPs and their Frequencies in Four Ion Channel Genes in Chinese Population and Comparison with Other Populations

Gene	SNPs	Amino Acid Change	Location	Minor Allele Frequency	Chromosomes Tested	Previously Reported Allele Frequencies (Ethnicity; No. of subjects) [Reference]*		
						Asian	Caucasian	African
KCNQ1	435C>T	I145I	Exon2	0.061	352	0.06(Japanese;50)[13] 0.055(Chinese;265)[12] 0.025(Malay;118)[12] 0.014(Indian;139)[12]	NA	NA
	1110G>A	A370A	Exon8	0.003	352	0.04(Japanese;50)[13]	NA	NA
	1394–12C>T	Intronic variant	Intron11	0.000	352	0.04(Japanese;50)[13]	NA	NA
	1638G>A	S546S	Exon13	0.304	352	0.28(Japanese;50)[13] 0.33(Japanese;44)[H] 0.289(Chinese;45)[H] 0.174(Malay;118)[12] 0.194(Indian;139)[12]	0.117(Caucasian;60)[H] 0.19(Caucasian;32)[11] 0.21(Caucasian;400)[23] 0.243(Chinese;265)[12] 0.20(Caucasian;282)[25]	0(Nigeria;60)[H]
	1685 + 23G>A	Intronic variant	Intron13	0.024	352	0.04(Japanese;50)[13]	NA	NA
	1732 + 43 T>C	Intronic variant	Intron14	0.269	352	0.23(Japanese;50)[13]	NA	NA
	1927G>A	G643S	Exon16	0.032	352	0.09(Japanese;50)[13] 0.11(Japanese;110)[16] 0.008(Chinese;265)[12] 0.008(Malay;118)[12] 6.0%(0.035)†(Asian;134)[9]	0%(0.000)†(Caucasian; 187)[9]	5.9%(0.031)†(African-American;305)[9]
KCNH2	1539T>C	F513F	Exon6	0.337	352	0.28(Japanese;50)[13] 0.351(Chinese;175)[12] 0.441(Malay;118)[12] 0.327(Indian;139)[12]	0.19(Caucasian;32)[11] 0.19(Caucasian;282)[25] 0.43†(0.313)(Dutch/Belgian;32)[26] 0.82(Danish;46)[27]	NA
	1692G>A	L564L	Exon7	0.113	352	0.94(Japanese;50)[13] 0.143(Chinese;175)[12] 0.233(Malay;118)[12] 0.511(Indian;139)[12]	0.36(Caucasian; 32)[11] 0.50†(0.50)(Dutch/Belgian;32)[26] 0.82(Danish;46)[27]	NA
	1956C>T	Y652Y	Exon8	0.098	352	0.88(Japanese;49)[13] 0.085(Chinese;175)[12] 0.144(Malay;118)[12] 0.331(Indian;139)[12]	0.41(Caucasian;32)[11] 0.57(Caucasian;282)[25] 0.65(Dutch/Belgian;32)[26] 0.58(Danish;46)[27]	NA
SCN5A	703 + 130G>A	Intronic variant	Intron6	0.124	352	0.086(Japanese;232)[14]	NA	NA
	1673A>G	H558R	Exon12	0.132	352	0.04(Japanese;50)[13] 0.103(Japanese;232)[14]	0.20(Caucasian;282)[25] 0.204(Caucasian;295)[10]	0.29(African-American;319)[10] 0.246(Nigeria;[H]

Continued.

Table 10. Continued

Gene	SNPs	Amino Acid Change	Location	Minor Allele Frequency	Chromo somes Tested	Previously Reported Allele Frequencies (Ethnicity; No. of subjects) [Reference]*		
						Asian	Caucasian	African
						0.092(Asian;112)[10] 0.150(Japanese;40)[H] 0.141(Chinese;38)[H] 0.104(Chinese;120)[28]	0.44(Caucasian;32)[11] 0.205(Caucasian;400)[23] 0.179(Caucasian;53)[H]	
	3269C>T	P1090L	Exon18	0.041	352	0.04(Japanese;50)[13] 0.024(Japanese;232)[14] 0.022(Asian;112)[10]	0(Caucasian;295)[10]	0(African-American; 319)[10]
	4299 + 53T>C	Intronic variant	Intron24	0.320	352	0.27(Japanese;50)[13] 0.276(Japanese;232)[14]	0.05(Caucasian;282)[25] 0.05(Caucasian;32)[11]	NA
	5457C>T	D1819D	Exon28	0.415	352	0.46(Japanese;50)[13] 0.496(Japanese;232)[14] 0.466(Japanese;44)[H] 0.389(Chinese;45)[H] 0.413(Chinese;115)[28]	0.38(Caucasian;32)[11] 0.358(Caucasian;400)[23] 0.683(Caucasian;60)[H]	0.242(Nigeria;60)[H]
	5851G>T	V1951L	Exon28	0.003	352	0.005(Japanese;50)[13] 0.004(Japanese;232)[14] 0(Asian;112)[10]	0(Caucasian;295)[10]	0(African-American; 319)[10]
	5963T>G	L1988R	Exon28	0.000	352	0.024(Japanese;232)[14]	NA	NA
KCNE1	112G>A	G38S	Exon1	0.290	352	0.81(Japanese;49)[13] 0.306(Chinese;265)[12] 0.246(Malay;118)[12] 0.255(Indian;139)[12] 16.4%(0.090)†(Asian;134)[9]	0.62(Caucasian;282)[25] 0.33(Caucasian;32)[11] 0.363(Caucasian;400)[23] 44.9%(0.340)† (Caucasian;187)[9]	36.1%(0.236)†(African-American;[9]
	253G>A	D85N	Exon1	0.014	352	0.02(Japanese;50)[13] 0.004(Chinese;265)[12] 0(Malay;118)[12] 0(Indian;139)[12] 0.7%(0.004)†(Asian;134)[9] 0.011(Japanese;44)[H] 0 (Chinese;45)[H]	0.01(Caucasian;282)[25] 0(Caucasian;32)[11] 0.008(Caucasian;400)[23] 1.1%(0.006)†(Caucasian; 187)[9] 0.025(Caucasian;60)[H]	0.7%(0.004)†(African-American;305)[9] 0(Nigeria; 60)[H]

*[H], Hapmap project.

†Heterozygous frequency, the minor allele frequency were calculated through heterozygous frequency and showed in the following bracket.

in the patients than that in the controls ($P = 0.004$). Further work is required to study whether the haplotype AGT had been positively selected because of its protective effect against arrhythmias. Although 1638G>A (S546S) is a synonymous variant and not involved in an amino acid substitution, there are evidence for the hypothesis that synonymous mutations might affect the thermodynamic stability of mRNA secondary structures or affect splicing such as exon skipping and may not be neutral in evolution.[17] We speculate that 1638G>A perhaps affect the encoding of a functional protein through the mechanisms described above. Besides Schmitt identified a small domain between residues 589 and 620 in the KCNQ1 C terminus that may function as an assembly domain for KCNQ1 subunits.[18] Without this domain KCNQ1 C termini do not assemble and KCNQ1 subunits do not express functional potassium channels. S546S is located in the KCNQ1 C terminus and is close to that domain, which is contained in the haplotype block.

However, the association between 1638G>A and disease became nonsignificant after Bonferroni correction. The Bonferroni correction is a multiple-comparison correction used when several dependent or independent statistical tests are being performed simultaneously. In order to avoid a lot of spurious positives, the alpha value needs to be lowered to account for the number of comparisons being performed. However, Bonferroni correction is conservative, and has a risk of discarding interesting results as nonsignificant.

Thus, whether the SNP is linked with an unidentified variant that is associated with susceptibility to arrhythmias in that domain needs further study.

KCNE1 Polymorphisms

KCNE1 encodes beta subunits that coassemble with KCNQ1 alpha subunit to form cardiac potassium channel I_{Ks}. Two nonsynonymous SNPs, 112G>A and 253G>A, lead to the amino acid changes of G38S and D85N. G38 allele was shown to be associated with atrial fibrillation in a Chinese population of Taiwan,[19] and some experiments supported that KCNE1 G38 isoform was associated with reduced I_{Ks}, likely due to decreased KCNQ1 membrane expression.[20] Friedlander found that G38S polymorphism was associated with QTc interval length in an Israeli population study.[21] In our study, the frequency of G38S was slightly higher in atrial fibrillation patients than that in healthy controls, although a significant difference was not obtained. Whether it is associated with atrial fibrillation in Chinese population, sample sizes need to be larger.

D85N polymorphism was thought to modify the I_{Ks} channel function leading to a prolongation of the QTc interval length. Functional studies revealed that the channel in which D85N-minK coexpressed with wild-type KCNQ1 in CHO cells activated slower and deactivated faster than wild-type channels.[22] In Gouas et al.'s study, D85N was found to be associated with a longer QTc.[23] Furthermore, D85N was considered to be a risk factor of drug-induced LQTS. More than one study has found that D85N was more prevalent in acquired LQTS patients than in controls.[11] Its frequency was low in most of the populations throughout the world. As for our study, D85N was not found in patients and the allele frequency was low in controls. We speculate that this variant did not contribute importantly to arrhythmogenesis in the Chinese population.

KCNH2 Polymorphisms

Three SNPs of *KCNH2* that were analyzed are synonymous SNPs. Since all these three SNPs did not show significant differences between patients and healthy controls in our study, we speculated that they were not involved in the pathogenesis of arrhythmia in the population we studied.

SCN5A Polymorphisms

Of seven selected SNPs of *SCN5A*, four are nonsynonymous SNPs (H558R, P1090L, V1951L, and L1988R), one is synonymous SNP (D1819D), and the other two are intronic variants (703 + 130G>A and 4299 + 53T>C). H558R does not change the voltage properties of the channel, but it can modulate alterations of sodium channel caused by other variations. Ye et al. reported that H558R restored the trafficking defect caused by the LQT-3 variant M1766L.[8] Viswanathan showed that H558R attenuated the abnormal gating effect caused by the proximal variant Thr512Ile.[24] As for our study, the frequency of H558R did not show a significant difference between patients and healthy controls. In Maekawa et al.'s study of Japanese, the haplotype in which L1988R was combined with H558R was speculated to be associated with arrhythmia.[14] However, L1988R did not exist in the population of our study. 703 + 130G>A observed to have signifi-

cantly different allele frequency between Japanese arrhythmia patients and controls, whereas it did not show significant difference between these two groups in our study. And there was no difference between these two groups in both genders and different age layers (data not shown). The inconsistency may reflect genetic heterogeneity between the studied populations, differences in their age or gender compositions.

In conclusion, we screened some most relevant SNPs within four ion channel genes, *KCNQ1*, *KCNH2*, *SCN5A,* and *KCNE1*, in Chinese arrhythmia patients and healthy controls. It is the first comprehensive study of the frequency, haplotype analysis of cardiac potassium, and sodium channel SNPs in Chinese population. The frequency of 1638G>A was significantly higher in the healthy controls than in patients. The analysis of haplotype structures of *KCNQ1* revealed that the haplotype harboring 1638A was associated with a factor against arrhythmias. These variants might also modulate the effects of ion channel mutations leading to arrhythmias. Our results will facilitate continued epidemiologic and functional studies of cardiac ion channel variants.

Acknowledgment: *We thank all the patients and healthy control participants. We are grateful to Dr. Arno Motulsky for his helpful commons on this article.*

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Supplementary Table 1. Primers Used in Genotyping System.