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Original Article

Relevance of *KCNE1*, *SCN5A* and *eNOS* gene polymorphisms in Tunisian atrial fibrillation patients

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Abstract: Atrial fibrillation (AF) is the most prevalent arrhythmia in clinical practice. In this complex multifactorial disorder, the association with several genetic factors has been proposed, such as the 112A>G polymorphism in *KCNE1* (S38G variant), 1673A>G in *SCN5A* (H558R variant) and the -786T>C promoter variant in the *eNOS* gene. However, the association of these polymorphisms with AF remains controversial suggesting that the association may depend on the ethnic background. A total of 102 consecutive patients diagnosed with AF and 106 control subjects were recruited for a case-control study. 112A>G genotyping was carried out by direct sequencing, while 1673A>G and -786T>C were performed by PCR-RFLP and confirmed by sequencing as well. Pearson's chi-squared test (χ^2) was used to compare allele frequencies, genotype and polymorphism combination distributions. Association between AF and genetic variations was assessed by logistic regression analysis. The allele and genotype frequencies of each polymorphism did not differ between patients and controls. These results indicate that carrying the polymorphisms 112A>G, 1673A>G or -786T>C individually, did not predispose to AF. However, the combination of the *KCNE1* and *eNOS* polymorphism was more frequent in patients compared to controls, but this trend was not statistically significant. Thus, the results indicate that carrying either the *KCNE1*, *SNC5A* or *eNOS* variant alleles does not increase AF predisposition in the Tunisian population.

Keywords: Atrial fibrillation, polymorphisms, tunisian population, 112A>G, 1673A>G, -786T>C

Introduction

Atrial fibrillation (AF) is the most prevalent arrhythmia in clinical practice, a leading cause of cardiovascular morbidity, and represents a major public health concern [1]. In most cases, AF occurs in association with structural heart disease including hypertension, coronary artery disease (CAD), valvular heart disease and heart failure [2]. However, some patients may develop AF in the absence of any known risk factor suggesting that there are genetic factors predisposing to the arrhythmia [3]. Thus, in case-control studies that compare cohorts of AF to controls, AF risk has been associated with polymorphisms in genes that encode cardiac potassium channel subunits [4], sodium channels [5], and effector molecules such as *GNB3* and endothelial nitric oxide synthase (*eNOS*) [6, 7].

Human cardiac potassium channels play an essential role in the cardiac action potential repolarization. *KCNE1* is a gene located on chromosome 21q22.12 and encodes the β-subunit of the slowly activating delayed rectifier potassium current I_{Ks} [8]. Data from some population studies suggest that the 112A>G polymorphism (NM_000219.5) is associated with AF [4, 9]. This polymorphism leads to a glycine substitution for serine at position 38 in the *KCNE1* subunit; mechanistically, the 112A>G isoform reduces the I_{Ks} current density [10]. This current reduction may delay atrial action potential repolarization leading to slowly developing changes in atrial structure by increasing the Ca^{2+} load, which may promote AF occurrence indirectly [10]. However, the hypothesis that 112A>G forms a risk factor to develop AF is contested by other population studies [11-13].

Whereas potassium channels are essential for cardiac action potential repolarization, the human cardiac voltage-gated sodium channel type 5 (Nav1.5) encoded by the *SCN5A* gene, is responsible for the fast upstroke of the cardiac action potential [5]. Gene mutations that impair channel function disrupt the action potential generation and affect conduction of the impulse in atrial cardiomyocytes [14]. Hence, a large spectrum of cardiovascular disorders, including Brugada syndrome [15], dilated cardiomyopathy [16] and atrial arrhythmias including AF [17], has been associated with *SCN5A* gene mutations and single-nucleotide polymorphisms (SNPs). The 1673A>G (NM_000335.4), a missense polymorphism in the *SCN5A* gene that leads to an arginine substitution for histidine at position 558 of the Nav1.5 channel and has been suggested to increase the vulnerability to AF [17].

Other studies have examined the association between AF and the *eNOS* polymorphism -786T>C in the gene's promoter region, but likewise the conclusions remains controversial [4, 7, 18]. Nitric oxide (NO) is an important endothelium-derived relaxing factor synthesized by at least three nitric oxide synthase (NOS) isoforms: constitutive neuronal NOS, inducible NOS, and endothelial NOS (eNOS or NOS3) [19]. Attenuation of NO production by *eNOS* - located on the chromosome 7q35-36 - is induced by gene variants leading to a reduction of NO levels in human cardiomyocytes [20, 21]. Several *eNOS* gene polymorphisms have been identified of which the -786T>C (g.6933T>C, NG_011992.1) polymorphism has been associated with ischemic heart disease [22], hypertension and coronary artery disease [23, 24].

It is becoming clear that the individual susceptibility to AF may be associated to several common genetic polymorphisms. Some of the identified associations involve the *KCNE1*, *SCN5A* and *eNOS* genes but it appears that also the ethnic background is an important factor [17, 18, 25]. To the best of our knowledge, the genetic susceptibility of AF has not yet been tested in the Tunisian population. Therefore, the present study was undertaken to investigate the possible role of 112A>G, 1673A>G and -786T>C polymorphisms in the predisposition of AF, and to analyze the potential interaction between *KCNE1*, *SCN5A* and *eNOS* genes in the occurrence of AF in a cohort of a Tunisian population.

Materials and methods

Study subjects

This was a case-control study involving 102 consecutive hemodynamically stable patients (39 males and 63 females). Patients were sampled from a cosmopolitan population in the Sahel of Tunisia admitted to the emergency department of Fattouma Bourguiba University Hospital of Monastir, Tunisia. Patients were included if they had a history of sustained AF confirmed by ECG, or a documented rapid AF (>120 beats per minute) lasting more than 24 hours during their hospital observation. The control group included 106 volunteers (59 males and 47 females) with no personal history of AF. At enrollment, the participants were asked to complete a symptoms questionnaire. All participants were unrelated to each other but came from the same geographic area. The study protocol was approved by the local ethics committee. Both AF and control groups gave written informed consent for participation in the study.

Genetic analysis

The genotypes of 112A>G polymorphism (reference SNP rs1805127) were determined by polymerase chain reaction method. Segments of *KCNE1* were amplified using the sense primer 5' GTGGGATCCTAATGCCCAGGATGATC 3', and antisense primer 5' GTGGTCGACTTCATGGGGAAGGCTTC 3'. The reaction mixture was carried out in 25 µL volumes containing 5 µL of 10× PCR buffer, 2 mM of $MgCl_2$, 0.2 mM of each dNTPs, 0,4 µM of each primer (20 µM/µL), 1 U of Promega Taq DNA polymerase and 2 µL of genomic DNA (50 ng/µL) isolated from peripheral blood using the salting out procedure. Amplification was accomplished by initial incubation at 95°C for 5 minutes followed by 35 sequential cycles of incubation at 94°C, 57°C (annealing) and 73°C (extension) for 45 sec each, followed by a final extension at 72°C for 5 minutes [26]. PCR-products were purified and sequenced by VIB Genetic Service Facility (Antwerp, Belgium) using Applied Biosystems 3730XL DNA Analyzer. The resulting sequences were viewed and analyzed using the Lasergene software (DNAstar, Madison, WI, USA).

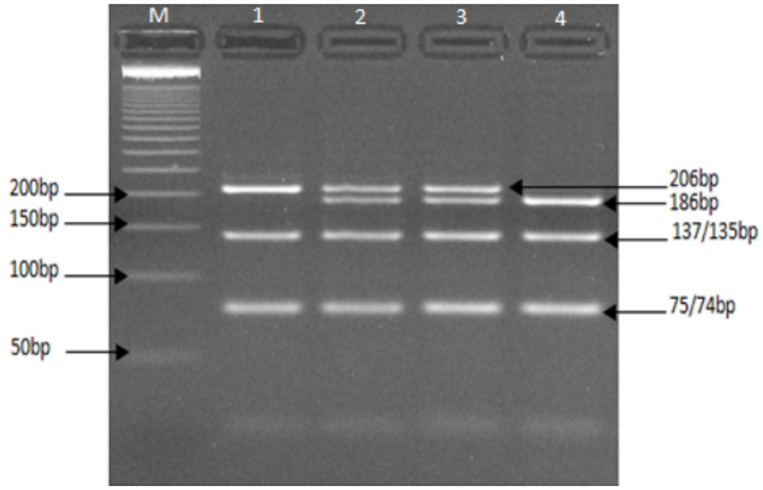

Figure 1. PCR-RFLP of *SCN5A* (1673A>G) gene polymorphism. M: molecular weight marker *DNA Ladder Gene Ruler 50 bp Fermentas marker*, lane 1: AA genotype, lanes 2, 3: AG genotype, lane 4: GG genotype. 4% agarose gel electrophoresis.

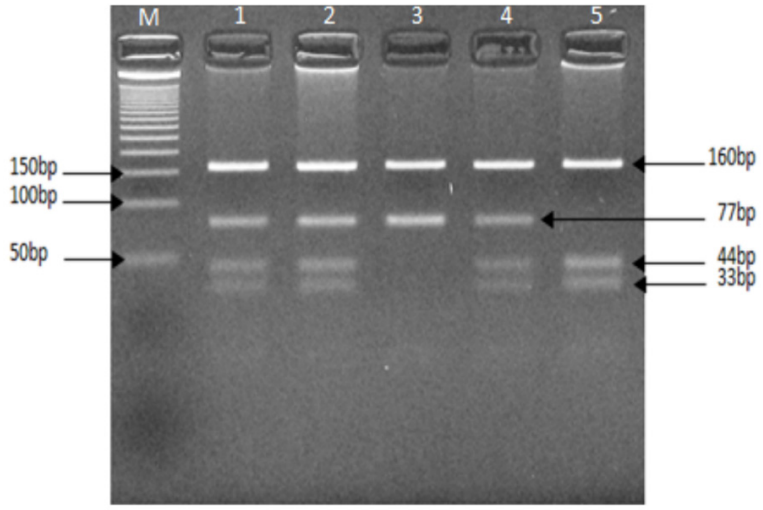

Figure 2. PCR-RFLP of *eNOS* (-786T>C) gene polymorphism. M: molecular weight marker *DNA Ladder Gene Ruler 50 bp Fermentas marker*, lanes 1, 2 and 4: TC genotype, lane 3: TT genotype, lane 5: CC genotype. 3% agarose gel electrophoresis.

1673A>G and -786T>C genotype analysis was performed by PCR-RFLP analysis, using *Hae III* and *AciI* digestion, respectively. Genotypes for 1673A>G polymorphism (rs1805124) in the exon 12 of *SCN5A* gene were determined by PCR amplification using a final volume of 25 µL reaction mixture containing 5 µL of Taq buffer (10×), 0.4 µM of Forward 5' GCCAGTGGCACAAAAGACAGGCT 3' and Reverse primer 5' CCTGGGCACTGGTCCGGCGCA 3', 200 µM of dNTPs, 3 mM of $MgCl_2$, 1 U of Taq DNA polymerase and 100 ng of DNA. Cycling conditions were: initial denaturation at 95°C for 5 min, then, 35 cycles of cyclic denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 45 sec and a final extension at 72°C for 10 min. The amplification reaction was followed by 16 hours of digestion at 37°C with 10 U of *AciI* (Fermentas) restriction enzyme, producing fragments of 206, 137, 135, 75, 74, 22 and 2 bp for the wild-type allele (allele A), or 186, 137, 75, 74, 22 and 2 bp in the case of a polymorphic variant (allele G). Fragments were separated by electrophoresis on 4% agarose gels with ethidium bromide staining. The RFLP analysis was carried out in the research unit (UR12EF10) at the Faculty of Medicine Monastir (Tunisia) (**Figure 1**).

For the -786T>C polymorphism (rs2070744) in the 5-flanking region of *eNOS* gene, DNA was amplified using the following primers 5' ATGCTCCCACCAGGGCATCA 3' (Forward) and 5' GTCCTTGAGTCTGACATTAGGG 3' (reverse). The reaction mixture (25 µL) included 5 µL of Taq buffer (10×), 1.5 µL of $MgCl_2$ (20 mmol/L), 0.5 µM of each primer, 0.5 µL of dNTP (0.2 mmol/L) mix, 1 U of Taq polymerase (Promega, Madison, USA), H_2O, and 50 ng of sample DNA [27]. The amplification ran under the following conditions: an initial denaturation carried out at 94°C for 7 minutes then 30 cycles each comprising denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension 72°C for 30 seconds, followed by final extension time of 7 minutes at 72°C. PCR products of 237 bp were digested with the restriction enzyme *Hae III* (Fermentas) at 37°C for 16 hours and separated on 3% agarose gel and identified by ethidium bromide staining, the T alleles shows two

Table 1. Clinical and demographic parameters of the study population

Parameters	Cases (n=102)	Controls (n=106)	*P*-value
Male/Female (n)	39/63	59/47	0.013
Age (years)	64 ± 15	64 ± 13	Ns
BMI (kg/m^2)	28.3 ± 5.3	27.1 ± 3.4	0.067
CAD (%)	9.8	0	0.001
Hypertension (%)	44	7.5	<0.001
Diabetes (%)	23.5	1.9	<0.001
Heart failure (%)	11.8	0.9	0.001
Valvular Disease (%)	8.8	0	0.001
Smoke (%)	13.7	2.8	0.004
LVEF (cm)	55 ± 13	57 ± 16	Ns

Data are expressed as mean ± SD or numbers and percentages. NS: not statistically significant (*P*>0.05). CAD: coronary artery disease, LVEF: left ventricular ejection fraction.

fragments of 160 and 77 bp while the C alleles yielded three fragments of 160, 44 and 33 bp (**Figure 2**).

PCR-RFLP results of both SNPs in *SCN5A* and *eNOS* were confirmed by direct sequencing using the amplification products and the PCR primers. Amplicons were purified and sequenced by VIB Genetic Service Facility at the University of Antwerp and sequencing data were analyzed with the Lasergene software (DNAstar, Madison, WI, USA).

Statistical analysis

Continuous variables were expressed as mean ± SD and compared with the unpaired (student) t-test. Categorical variables data were presented as percentages and Pearson's chi-squared test (χ^2) was used to assess intergroup significance. χ^2 was also used to evaluate allele and genotype frequencies, and to test the deviation of genotype distribution from Hardy-Weinberg equilibrium. The association between *KCNE1*, *SCN5A*, *eNOS* gene polymorphisms and AF was estimated first under the wild-type, heterozygote and homozygote genotype categories and then grouped into two categories of dominant and recessive genetic models. The dominant model represents the wild type genotype (baseline group) versus the combination of the polymorphic homozygous and the heterozygous genotype, while the recessive model compares the homozygous polymorphic genotype with the combined wild type and heterozygous genotype that form the baseline group. Logistic univariate and multivariate regression analyses were used to calculate Odds Ratio (OR) with 95% confidence interval (CI). Crude and adjusted models for gender, BMI, CAD, hypertension, diabetes, heart failure, valvular disease and smoking were respectively determined (OR, OR^a). The distribution of combinations between each pair of *KCNE1*, *SCN5A* and *eNOS* polymorphisms was revealed using the χ^2-test under the dominant and recessive genetic model in order to assess the potential of gene-gene interactions as suggested by Fatini and colleagues [4]. Differences were considered to be statistically significant when $P \leq 0.05$. Statistical analysis was performed using SPSS software (version 20.0, Chicago, USA).

Results

Clinical characteristics

The baseline characteristics of the study population are summarized in **Table 1**. No significant differences were seen between patients and control subjects with regard to age, body mass index (BMI) and left ventricular ejection fraction (LVEF). However, the prevalence of coronary artery disease (CAD), hypertension, diabetes, heart failure, valvular disease and smoking were significantly higher in patients than in the control group (*P*<0.01).

Genotype and risk of AF

The genotype distributions and allele frequencies in AF patients and controls are shown in **Table 2**. The genotype distributions of 112A>G and -786T>C polymorphisms were in agreement with those predicted by the Hardy Weinberg equilibrium (HWE) in AF patients and controls (*P*>0.05). For 1673A>G polymorphism, the AF group deviated from the HWE (*P*<0.05) but the control group did not (*P*>0.05). In the primary analysis, there was no statistical significant difference between patients and controls in the distribution of genotype and allele frequencies of each of the three SNPs (*P*>0.05). No statistically significant difference was found in the distribution of genotypes between AF patients and controls, neither in the dominant, nor in the recessive genetic model. The risk of developing AF was estimated for each of these models and there were no associations found between AF risk and the variant allele for these polymorphisms (**Table 2**).

Table 2. Genotype distribution and relative allele frequencies of KCNE1, SCN5A and eNOS gene polymorphisms among AF patients and controls and crude and adjusted estimations for AF

SNP	Allele/Genotype	Patients	Controls	OR (95% CI)	*P*	OR[a] (95% CI)	*P*[a]
112A>G	A n (%)	71 (34.8)	77 (36.3)	1			
	G n (%)	133 (65.2)	135 (63.7)	1.06 (0.71-1.59)	0.748		
	Codominant model						
	AA n (%)	10 (9.8)	16 (15)	1		1	
	AG n (%)	51 (50)	45 (42.5)	0.55 (0.22-1.33)	0.188	1.03 (0.35-2.99)	0.957
	GG n (%)	41 (40.2)	45 (42.5)	0.68 (0.28-1.68)	0.410	1.39 (0.46-4.16)	0.554
	Dominant model						
	AA n (%)	10 (9.8)	16 (15.1)	1		1	
	AG+GG n (%)	92 (90.2)	90 (84.9)	0.61 (0.26-1.41)	0.252	1.17 (0.42-3.25)	0.752
	Recessive model						
	AA+AG n (%)	61 (59.8)	61 (57.5)	1		1	
	GG n (%)	41 (40.2)	45 (42.5)	1.09 (0.63-1.90)	0.741	1.35 (0.66-2.74)	0.406
	Allele/Genotype	Patients	Controls	OR (95% CI)	*P*	OR[a] (95% CI)	*P*[a]
1673A>G	A n (%)	161 (78.9)	170 (80.2)				
	G n (%)	43 (21.1)	42 (19.8)	1,08 (0.66-1.74)	0,750		
	Codominant model						
	AA n (%)	67 (65.7)	69 (65.1)	1		1	
	AG n (%)	27 (26.5)	32 (30.2)	1.15 (0.62-2.12)	0.653	0.96 (0.45-2.03)	0.925
	GG n (%)	8 (7.8)	5 (4.7)	0.60 (0.18-1.94)	0.420	0.56 (0.14-2.13)	0.398
	Dominant model						
	AA n (%)	67 (65.7)	69 (65.1)	1		1	
	AG+GG n (%)	35 (34.2)	37 (34.9)	1.02 (0.58-1.81)	0.929	0.85 (0.42-1.72)	0.668
	Recessive model						
	AA+AG n (%)	94 (92.2)	101 (95.3)	1		1	
	GG n (%)	8 (7.8)	5 (4.7)	0.58 (0.18-1.84)	0.357	0.50 (0.13-1.86)	0.303
	Allele/Genotype	Patients	Controls	OR (95% CI)	*P*	OR[a] (95% CI)	*P*[a]
-786T>C	T n (%)	115 (56.4)	120 (56.6)	1			
	C n (%)	89 (43.6)	92 (43.4)	1 (0.68-1.48)	0.962		
	Codominant model						
	TT n (%)	31 (30.4)	36 (34)	1		1	
	TC n (%)	53 (52)	48 (45.3)	0.78 (0.42-1.44)	0.431	0.77 (0.36-1.65)	0.510
	CC n (%)	18 (17.6)	22 (20.8)	1.05 (0.47-2.31)	0.899	0.81 (0.31-2.11)	0.676
	Dominant model						
	TT n (%)	31 (30.4)	36 (34)	1		1	
	TC+CC n (%)	71 (69.6)	70 (66)	0.84 (0.47-1.52)	0.582	0.79 (0.38-1.61)	0.518
	Recessive model						
	TT+TC n (%)	85 (82.5)	84 (79.2)	1		1	
	CC n (%)	18 (17.5)	22 (20.8)	1.22 (0.61-2.44)	0.570	1.01 (0.43-2.35)	0.982

[a]Adjusted for gender, BMI, CAD, hypertension, diabetes, heart failure, valvular disease and smoking habit.

Interaction between KCNE1, SCN5A and eNOS genotypes

To examine whether a combination of 112A>G, 1673A>G and -786T>C gene polymorphisms predisposes to AF risk, we evaluated the gene-gene interaction analysis between each pair of genes. The distribution of the 112A>G and -786T>C combination showed that AGGG/TCCC was more frequent in AF patients compared to

Table 3. Distribution of the genetic combination between 112A>G/1673A>G, 112A>G/-786T>C and 1673A>G/-786T>C polymorphisms according to dominant and recessive genetic models among AF patients and controls

Genetics model	Genetic Combination		Patients n (%)	Controls n (%)	*P*
	112AG	1673A>G			
Dominant model	AA	AA	5 (4.9)	10 (9.4)	0.621
	AA	AGGG	5 (4.9)	6 (5.7)	
	AGGG	AA	62 (60.8)	59 (55.7)	
	AGGG	AGGG	30 (29.4)	31 (29.2)	
Recessive model	AAAG	AAAG	55 (53.9)	58 (54.7)	0.753
	AAAG	GG	6 (5.9)	3 (2.8)	
	GG	AAAG	39 (38.2)	43 (40.6)	
	GG	GG	2 (2)	2 (1.9)	
	Genetic Combination		Patients n (%)	Controls n (%)	*P*
	112A>G	T-786C			
Dominant model	AA	TT	5 (4.9)	3 (2.8)	0.199
	AA	TCCC	5 (4.9)	12 (11.3)	
	AGGG	TT	26 (25.5)	33 (31.1)	
	AGGG	TCCC	66 (64.7)	58 (54.7)	
Recessive model	AAAG	TTTC	48 (47.1)	49 (46.2)	0.647
	AAAG	CC	13 (12.7)	12 (11.3)	
	GG	TTTC	36 (35.3)	35 (33)	
	GG	CC	5 (4.9)	10 (9.4)	
	Genetic Combination		Patients n (%)	Controls n (%)	*P*
	1673A>G	T-786C			
Dominant model	AA	TT	24 (23.5)	24 (22.6)	0.723
	AA	TCCC	44 (43.1)	45 (42.5)	
	AGGG	TT	7 (6.9)	12 (11.3)	
	AGGG	TCCC	27 (26.5)	25 (23.6)	
Recessive model	AAAG	TTTC	78 (76.5)	80 (75.5)	0.715
	AAAG	CC	16 (15.7)	21 (19.8)	
	GG	TTTC	6 (5.9)	4 (3.8)	
	GG	CC	2 (2)	1 (0.9)	

P: Pearson chi-square test.

the control group under the dominant model (64.7 versus 54.7 %); however, this finding did not reach statistical significance (**Table 3**). On the other hand, there was no significant association between the genetic distribution of 1673A>G combined with either 112A>G or -786T>C and the presence of AF. The predisposition of the combination of all three genes could not be evaluated because the numbers of subjects within the combination subgroups were insufficient precluding meaningful analysis (data not shown).

Discussion

Evidence of a genetic susceptibility to AF was first demonstrated in 1943 by Wolff in a familial form of AF [28]; subsequently, several responsible genes have been identified [3]. In this context, several SNPs that been evaluated for their susceptibility to AF with the *KCNE1* 112A>G polymorphism being one of the most investigated variants [29]. Our data show that the G allele was the most common one in our Tunisian population, which is in agreement with previous findings from Asian, Italian and Polish cohorts [4, 25, 26, 30, 31]. However, in our study there was no marked difference in allele and genotype distribution of 112A>G between AF and controls (**Table 2**). Moreover, the adjusted logistic regression analysis revealed that the 112GG isoform had no influence on AF occurrence. This finding is in agreement with several other reports but at the same time contests others. Similarly, no significant difference in the G allele distribution was observed between AF patients and controls in some different Chinese populations [11, 12, 13] concluding the GG genotype not to be an independent risk factor for AF predisposition. In contrast, other Chinese reports showed that the G allele was more abundant in AF patients compared to control subjects and that the 112GG isoform was associated with a higher risk for developing AF [9, 32, 33]. Moreover, Polish Caucasian patients carrying the GG genotype have also been proposed to have a greater risk for developing AF than the GA or AA gen-

otypes [26]. Thus, based on the functional impact of 112A>G polymorphism, the G allele is most likely a predictor of AF risk factor [10]. However, the results apparently vary among ethnic backgrounds and geographical regions.

Mutations in *SCN5A* gene are known to underlie various cardiac diseases including AF [34]. Functional biophysical studies of 1673A>G polymorphism located in the intracellular linker connecting the first and second domain of the sodium channel, revealed its ability to modulate the effects of concomitant *SCN5A* mutations associated with heart disease [35-37]. One study suggested that the 1673A>G increases the vulnerability to AF by shortening the atrial reentrant wavelength, which would be the consequence of a reduced conduction velocity [17]. It was reported that 1673A>G have been detected in several ethnic groups including Asians, African Americans, Caucasians of European ancestry and Hispanics with minor G allele frequencies of 9.2%, 29%, 20.4% and 23.1%, respectively [35]. Genotype AA, AG, and GG frequencies were 53%, 40% and 7% among African Americans and 65%, 30% and 5% in Caucasians [38]. We observed a similar frequency of the G allele (20.1%) with a similar distribution between control group and patients in the Tunisian population. In contrast, it was reported that the GG genotype resulted in a 1.6-fold increased susceptibility to lone AF. In addition, the 1673GG isoform was associated with a younger age in patients with paroxysmal AF [17]. More recently, a study in a Chinese population established a correlation between 1673GG isoform and AF [11]. To our knowledge, we report the first case-control study that investigated the association of 1673A>G polymorphism with the development of AF in a Tunisian population. When comparisons were made between patients and controls, we did not identify a significant difference either in the allelic or genotypic distributions of 1673A>G (**Table 2**). Also, the results of multivariate regression analysis under dominant and recessive genetic models showed that 1673A>G does not increase vulnerability to AF in Tunisian patients.

The third polymorphism that was tested for its predisposition of AF was the-786T>C variant located in the promoter region of the *eNOS* gene which reduces the promoter activity by approximately 50% resulting most likely in lower eNOS expression and NO levels in human cardiomyocytes [20, 21]. The NO deficiency may modulate the electrophysiological phenotype contributing to the vulnerability to arrhythmia [4, 39], e.g. by affecting the L-type calcium channels that are essential for normal sinus node function and myocyte contractility [40]. In this context, a study conducted in Italy reported that -786T>C polymorphism was weakly associated to the occurrence of AF [4]. However, we failed to find any differences in the allele and genotype distributions of -786T>C variant between patients and controls. The absence of a correlation is in agreement with the findings of a similar study conducted in a Caucasian population [7, 41]. Furthermore, -786T>C did not associate with hypertension in our cohort population, as has been suggested previously [27]. Thus, the *eNOS* -786T>C variant alone does not increase the vulnerability to either AF or hypertension in the Tunisian population.

However, since the vulnerability to AF arises most likely from multiple genes, it remains important to identify the role of common variations within the genome and evaluate the effect of genetic combinations. Our findings revealed that there were no statistically significant association between AF and genotype combination of 112A>G with either 1673A>G or -786T>C and the combination of 1673A>G with -786T>C. The AGGG/TCCC combination of *KCNE1* and *eNOS* polymorphisms was more prevalent in the AF group under the dominant model, but this trend was not statistically significant and extra studies are required to confirm this. Fatini and coworkers reported that the simultaneous presence of the *KCNE1* 112GG isoform and the *eNOS* -786T>C variant influenced the susceptibility to non-valvular AF suggesting a combined effect of *eNOS* and *KCNE1* genes in influencing heart rate variability [4]. In contrast, a lack of association between AF and CETP TaqIB, *KCNE1* 112A>G and *eNOS* -786T>C polymorphisms combination was reported for a Chinese population [12].

Conclusion

In conclusion, our findings show that the 112A>G, 1673A>G and -786T>C gene polymorphisms are unlikely to increase AF risk in the Tunisian population by themselves. Nevertheless, the results hint that carrying both

the 112A>G and the -786T>C polymorphisms influences AF susceptibility in the Tunisian population.

Disclosure of conflict of interest

None.

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