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ARTICLE

Association of *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A* polymorphisms with QTc interval length in a healthy population

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The QT interval (QT) reflects cardiac ventricular repolarization and varies according to various known factors such as heart rate, gender and age. Nevertheless, a high intrasubject stability of the QT-RR pattern also suggests that a genetic component contributes to individual QT length. To determine whether single nucleotide polymorphisms (SNPs) in genes encoding cardiac ion channels were associated with the heart-rate corrected QT (QTc) length, we analyzed two groups of 200 subjects presenting the shortest and the longest QTc from a cohort of 2008 healthy subjects. A total of 17 polymorphisms were genotyped; they were all in the Hardy–Weinberg equilibrium in both groups. Neither allele nor haplotype frequencies of the 10 *KCNQ1* SNPs showed a significant difference between the two groups. In contrast, *KCNH2* 2690 C (K897T) and *SCN5A* 5457 T (D1819D) minor alleles were significantly more frequent in the group with the shortest QTc interval, whereas *KCNE1* 253 A (D85N), *SCN5A* 1673 G (H558R) and 1141-3 A minor alleles were significantly more frequent in the group with the longest QTc interval. Interestingly, an interaction was also found between the *KCNH2* 2690 A>C SNP and the *KCNQ1* 2031 + 932 A>G SNP suggesting that the effect of the *KCNH2* 2690 C allele on QTc length may occur within a particular genetic background. This suggests that genetic determinants located in *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A* influence QTc length in healthy individuals and may represent risk factors for arrhythmias or cardiac sudden death in patients with cardiovascular diseases.

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Introduction

The QT interval (QT), measured on the electrocardiogram (ECG), reflects cardiac ventricular repolarization. In healthy populations, its length varies widely and several factors such as heart rate, gender and age exert an influence on the QT length.[1,2] Indeed, women present relative QT prolongation compared with men[3,4] and QT shortens in men after puberty.[2] Whereas there exists a substantial interindividual variability of the QT-RR relationship, which represents the relationship between QT duration and heart rate, a high intraindividual stability of the QT-RR pattern has been shown, suggesting that a genetic component might partly determine individual QT length.[5,6] Indeed, Busjahn *et al*[7] have shown a significant linkage in dizygotic twins between heart rate-corrected QT interval (QTc) and

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two loci harboring genes known to cause the long QT syndrome (LQTS).

Ventricular action potential (AP) is under the joint control of the depolarizing Na^+ (I_{Na}) and the repolarizing slow (I_{Ks}) and rapid (I_{Kr}) K^+-delayed rectifier currents. The activity and expression levels of each of these current-underlying channels establish a subtle equilibrium between depolarizing and repolarizing currents determining the AP duration in each individual. Thus, it would not be surprising that polymorphisms in the genes coding for these channels may influence this equilibrium even by weak effects on activity and/or expression level of channels subunits.

The cardiac I_{Na} current is underlined by the α $Na_v1.5$ subunit channel encoded by the *SCN5A* gene. As for the K^+-delayed rectifier current, the I_{Ks} current is elicited by the α KvLQT1 and β minK subunits encoded by the *KCNQ1* and *KCNE1* genes, respectively, and the I_{Kr} current by the α HERG and β Mirp1 subunits encoded, respectively, by the *KCNH2* and *KCNE2* genes.[8–11]

Mutations in these ion channel subunits are responsible for the LQTS, where a decrease in outward K^+ current or an increase in inward Na^+ current results in a prolongation of the AP. This prolongation, leading to a prolonged QT interval on baseline ECG, renders patients at high risk of developing *torsades de pointes* or ventricular fibrillation leading to syncope or sudden death. However, more recently, mutations in *KCNH2* and *KCNQ1*, leading to an increase in outward K^+ current, have also been linked to the short QT syndrome (SQTS). In SQTS, a drastic decrease of the QTc interval is also linked to life-threatening arrhythmias and sudden death.[12,13]

It is now widely recognized that penetrance of these monogenic arrhythmias is highly variable. In other words, among individuals carrying the same causative mutation within a specific affected kindred, some express a clinical phenotype whereas others do not manifest a phenotype.[14] Variability elsewhere in the genome, such as polymorphisms in the same disease-causing gene or in other genes, might contribute to this variable penetrance as well as to the appearance of a clinical phenotype. For example, the *SCN5A* H558R variant which is a common polymorphism in the American population,[15] was shown to mitigate the *in vitro* effects of a nearby mutation on the Na^+ channel function[16] whereas coinheritance of the *SCN5A* D1275N variant with a rare haplotype in the *Cx40* gene was associated with an atrial standstill phenotype in a kindred.[17]

It has also been shown that not only rare mutations[11,15,18] but also polymorphisms in *KCNE1*, *KCNE2* and *SCN5A* could represent risk factors for drug-induced arrhythmias.[18–20]

Some polymorphisms have already been reported to be associated with the QTc length. The K897T amino-acid variant of *KCNH2* was associated with the length of QT interval in Finnish LQT1 patients.[21] Bezzina *et al*[22] reported in healthy women, that homozygote carriers for the T897 allele had shorter QTc than homozygote for the K897 allele. Very recently, single nucleotide polymorphisms (SNPs) in other ion channel subunits were also associated with the QTc length in healthy individuals.[23]

Given the major role of cardiac sodium and potassium channels in ventricular repolarization, we investigated the potential association of 10 *KCNQ1*, two *KCNE1*, four *SCN5A* polymorphisms and the *KCNH2* K897T variant with the QTc length in a healthy French population. We also tested whether these SNPs might interact to influence on the QTc duration.

Material and methods

Study population

Participants of the Data from an Epidemiological Study on the Insulin Resistance syndrome (D.E.S.I.R.) cohort gave informed consent to the clinical and genetic study, which was approved by an ethics committee. The investigations conform to the principles outlined in the Declaration of Helsinki.

The D.E.S.I.R. study

The study subjects were selected from the D.E.S.I.R. cohort, which included French men and women aged 30–64 years. Participants were volunteers who agreed to be followed for 9 years and to have a clinical examination every 3 years. This study involved 10 health examination centers in France.[24,25]

The first examination took place in 1994 (D0) and the second in 1997 (D3). Blood pressure was measured after at least 5 min rest and venous blood samples were collected to determine glucose, triglyceride and HDL-cholesterol concentrations. Disease history (diabetes, hypertension, cardiovascular disease, etc), medical treatment, physical activity, alcohol and tobacco consumption were collected for each subject.

Subjects underwent a 12-lead resting ECG. QT intervals were measured in lead II (ms) by Cardionics® software and corrected for heart rate by use of the Fridericia formula (QTc).[26] Subjects with an ECG recording at D0 and D3 ($n=3478$) form the basis of the study. We excluded the subjects ($n=1399$) with known or detected cardiac pathology, diabetes (defined by use of treatment or a fasting plasma glucose level (≥ 7.0 mmol/l)), treated or untreated high blood pressure as well as the subjects taking medication known to prolong the QT interval such as neuroleptics, antiarrhythmics or antihistaminics, at D0 and D3. Subjects with a difference ≥ 30 ms in QTc between the two examinations were also excluded ($n=71$).

The distribution of the mean QTc values (averaged on measures at D0 and D3) of the remaining healthy subjects ($n=2008$) is shown in the Supplementary Figure 1. Mean QTc values were adjusted for age, separately in men and women, and the residuals were used to select the 100 men

and 100 women with the shortest QTc, and 100 men and 100 women with the longest QTc.

In a preliminary study, the *KCNQ1* exons had been sequenced in the 50 men and 50 women with the longest QTc, and two new *KCNQ1* mutations had been identified (Y148X, ΔS276).[27] The two carriers of these mutations whose QTc interval prolongation was assumed to be due to these mutations were excluded from the study.

Genetic analysis

Genomic DNA amplification Genomic DNA was prepared from peripheral blood lymphocytes by standard procedures. Previously designed primers were used to amplify the *KCNQ1* exons by PCR.[28] Primers used to amplify *KCNQ1* 3′UTR region, *KCNE1*, *SCN5A* and *KCNH2* DNA sequences are given on the European Journal of Human Genetics website.

Sequence analysis DNA sequences containing the *KCNQ1* 2031+479 G>A, 2031+875 A>G and 2031+932 A>G, the *KCNE1* 112 G>A and 253 G>A, and the *SCN5A* −92 C>A polymorphisms were amplified by PCR using a GeneAmp ThermoCycler 9700 (Applied Biosystems). The PCR products were cleaned up using Bio-Gel® P-100 Gel (Bio-Rad) spin columns in a MultiScreen™ 96-well filter plate (Milipore). The sequencing reactions were performed by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides according to the dGTP Big Dye® Terminator v3.0 protocol, then cleaned up using Sephadex™ G50 Superfine (Amersham Biosciences) spin columns in a MultiScreen™ 96-well filter plate. The reactions were run on an ABI 377 (Applied Biosystems).

Fluorescent-SSCP (F-SSCP) DNA sequences containing the *KCNQ1* 477+80 insGG, 1514+46 A>G, 1590+14 T>C, 1638 G>A, 1732+43 T>C and 1794+32 G>T were amplified by PCR using ThermoCycler™ 9700 (Applied Biosystem). PCR was carried out using fluorescent-dye-labeled sense and antisense primers specific for the DNA region of interest (6FAM™, HEX™). In all, 0.5–2 μl of PCR amplicons were mixed to a denaturing buffer (50% formamide, 25 μg dextran blue (50 mg/ml), 2.5 mM EDTA (25 mM)) and to a DNA molecular weight standard (GENESCAN-500 Rox, Applied Biosystem). Samples were then denatured 5 min at 94°C and quenched on ice. In all, 1 μl of samples was applied to a nondenaturing gel (MDE 0.5X, MGDA) and electrophoresed on the ABI 377 system. The mobility of F-SSCP fragments was measured relative to ROX size standard by use of Genescan 3.1.2 software (Applied Biosystems).

Fluorescence resonance energy transfer and probe melting curves The *KCNQ1* 1986 C>T and 2031+875 A>G ($n=400$ and 200, respectively), the *KCNH2* 2690 A>C and the *SCN5A* 1673 A>G, 5457 C>T and 1141–3 C>A polymorphisms were analyzed by Fluorescence Resonance Energy Transfer within a LightCycler (Roche Diagnostics). In all, 40 ng of sample DNA was used to perform a 25 μl PCR reaction containing the detection probe and the anchor probe for the *KCNQ1* 1986 C>T, *KCNH2* 2690 C>A, *SCN5A* 1673 A>G, 5457 C>T and 1141-3 C>A polymorphisms in a GeneAmp ThermoCycler 9700 system (Perkin Elmer). PCR amplicons were then loaded into glass capillary cuvettes (Roche Diagnostics). After an initial denaturation step at 95°C for 30 s, a melting curve was recorded by cooling the reaction mixture to 40°C at 20°C/s, holding it at 40°C for 120 s, and then slowly heating it to 85°C at 0.1°C/s. Fluorescence was measured continuously during the slow temperature ramp to monitor the dissociation of the fluorescein-labeled detection probe. For the *KCNQ1* 2031+875 A>G, 40 ng of sample DNA was used to do PCR reactions in the LightCycler in a reaction volume of 20 μl with 0.4 μmol/l of each primer, 0.06 μmol/l anchor and detection probes, 3 mmol/l $MgCl_2$ and 2 μl of the Master Mix Hybridyzation Probes (Roche Diagnostics). After an initial denaturation step at 95°C for 30 s, amplification was performed using 40 cycles of denaturation (95°C for 3 s), annealing (55°C for 10 s) and extension (72°C for 15 s). The temperature transition rates were programmed at 20°C/s from denaturation to annealing, 5°C/s from annealing to extension, and 20°C/s from extension to denaturation. After amplification was complete, a final melting curve was recorded as described above.

Statistical analysis

The difference between the mean QTc value between the two groups for men and women was assessed by a Mann–Whitney Rank Sum test (SygmaStat). Deviation from the Hardy–Weinberg equilibrium was tested by χ^2 analysis with 1 degree of freedom in the shortest and longest QTc groups separately. Pairwise linkage disequilibrium (LD) coefficients between polymorphisms were estimated by log-linear model analysis[29] and the extent of disequilibrium was expressed in terms of $D' = D/D_{max}$ or $-D/D_{min}$. Allele frequencies were estimated from genotype frequencies by gene counting. The study was analyzed as a case/control study comparing the longest and the shortest QTc groups. Genotype and allele frequencies were compared between the two groups by χ^2 test. Homogeneity of the association according to gender was systematically tested and since there was no heterogeneity for any of the polymorphisms, men and women were pooled for analysis. The odds ratio (OR) [95% CI] for longest QTc associated with genotype was estimated from logistic regression analysis adjusted for age and sex. A P-value <0.05 was considered statistically significant. All computations were carried out with the SAS software (SAS Institute, Cary, NC, USA).

To analyze simultaneously all polymorphisms of a gene, haplotype analyses were performed by use of a maximum likelihood model[30] implemented in the THESIAS program (http://www.genecanvas.org). In these analyses, the haplotype combining the most frequent alleles at each locus was used as the reference.

We applied the *d*etection of *i*nformative *c*ombined *e*ffect (DICE) method[31] to investigate high-order interactions between polymorphisms of the different genes on the phenotype. Briefly, the DICE algorithm explores in an automated way all combinations of polymorphisms acting either in an additive or in an interactive way (three-order interaction at maximum). Models of increasing complexity are successively fitted to data and the difference Δ_s of the Akaike's information criterion between models indicates whether the fit is substantially improved, that is, when Δ_s exceeds a predetermined threshold. The algorithm stops when no model leads to a Δ_s higher than the fixed threshold. Given the large number of polymorphisms investigated, a rather stringent threshold was chosen ($\Delta_s > 6$). In this analysis, a dominant coding scheme for polymorphisms was adopted (frequent homozygotes opposed to carriers of the minor allele).

Results

Baseline characteristics of the two groups are presented in Table 1.

KCNQ1 polymorphisms

In a preliminary study, the *KCNQ1* exons had been sequenced in the 100 subjects with the longest QTc intervals and 18 SNPs were found. In Table 2 we report the SNP position, the SNP Identity (ID) from public databases, when available, and their allele frequencies. According to arrhythmia literature, the National Center for

Table 1 Mean ($\pm$SD) ages, heart rates and QTc intervals in men and women of the shortest and longest QTc groups

	Shortest QTc group		*Longest QTc group*	
	Men	*Women*	*Men*	*Women*
	N = 100	*N* = 100	*N* = 99	*N* = 99
Age	45 $\pm$ 10	46 $\pm$ 10	44 $\pm$ 9	46 $\pm$ 10
Heart rate (beats/min)	66 $\pm$ 10	69 $\pm$ 11	61 $\pm$ 10	67 $\pm$ 10
QTc interval (ms)	353 $\pm$ 6	357 $\pm$ 6	406 $\pm$ 9	410 $\pm$ 7
QTc range	333–363	336–365	394–433	400–432

Mean QTc interval values are statistically different between the groups with the shortest and the longest QTc intervals for men and women ($P < 0.001$).

Table 2 *KCNQ1* variants identified in the D.E.S.I.R. population

Nucleotide change	*Region*	*Coding polymorphism*	*Frequency of minor allele*	*Number of chromosomes examined*	*Previous reports*	*SNP ID*
459 G>T	Exon 1	T153T	0.00375	800		
477+20 A>G	Intron 1		0.00125	800		
477+80 insGG[a]	Intron 1		0.31	800		
780+77 A>G	Intron 4		0.27	200		rs12786951
811 C>T	Exon 5	L271L	0.005	200	Jongbloed *et al* (2002)	
918+13 C>T	Intron 5		0.005	200		
984 C>T	Exon 6	I328I	0.005	200		
1514+7 G>T	Intron 10		0.005	200		
1514+14 G>T	Intron 10		0.005	200		
1514+46 A>G[a]	Intron 10		0.42	800	Aydin *et al* (2004)	rs760419
1590+14 T>C[a]	Intron 11		0.08	800	Jongbloed *et al* (2002)	rs11024034
1638 G>A[a]	Exon 12	S546S	0.21	800	Lee *et al* (1997); Iwasa *et al* (2000); Jongbloed *et al* (2002)	rs1057128
1732+43 T>C[a]	Intron 13		0.26	800	Iwasa *et al* (2000); Jongbloed *et al* (2002)	rs81204
1794+32 G>T[a]	Intron 14		0.05	800	Jongbloed *et al* (2002)	
1986 C>T[a]	Exon 15	Y662Y	0.25	800	Jongbloed *et al* (2002)	rs11601907
2031+479 G>A[a]	3'UTR		0.06	800		
2031+875 A>G[a]	3'UTR		0.30	800		rs2519184
2031+932 A>G[a]	3'UTR		0.32	800		rs10798

Nucleotide numbering starts from the ATG start codon (Genbank accession AF000571).
[a]Polymorphisms analyzed in this study.

Biotechnology Information (http://www.ncbi.nlm.nih.gov) and the inherited arrhythmias databases (http://pc4.fsm.it:81/cardmoc/), eight of the 18 SNPs were unknown. Six SNPs were rare (<0.01) and consisted in a synonymous G-to-T substitution at position 459 in exon 1 (T153T), a A-to-G substitution at position 477+21 in intron 1, a C-to-T substitution at position 918+13 in intron 5, a synonymous C-to-T substitution at position 984 in exon 6 (I328I), a G-to-T substitution at position 1514+7 and 1514+14 in intron 10. Two polymorphisms were more frequent (>0.01) and consisted in a GG insertion/deletion at position 477+80 of intron 1 and a G-to-A substitution at position 2031+479 in the 3′UTR part.

After exclusion of rare polymorphisms, two synonymous polymorphisms in the coding region of *KCNQ1,* S546S and Y662Y (1638 G>A and 1986 C>T, respectively), five SNPs in intronic sequences (477+80 insGG in intron 1, 1514+46 A>G in intron 10, 1590+14 T>C in intron 11, 1732+43 T>C in intron 13, 1794+32 G>T in intron 14) and three SNPs in the 3′UTR part (2031+479 G>A, 2031+875 A>G and 2031+932 A>G) of the *KCNQ1* gene were studied.

There was no deviation from the Hardy–Weinberg equilibrium for any of the polymorphisms considered. The frequency of the minor allele ranged from 0.05 to 0.42 (Table 2). The three polymorphisms in the 3′ end of the gene and the polymorphism 1986 C>T in exon 15 were in strong LD with each other (absolute pairwise LD coefficients ranging from 0.68 to 0.90). Moreover, the 2031+875 A>G and 2031+932 A>G SNPs were in nearly complete association (only 9.8% of the subjects were recombinants). Conversely, the polymorphisms located upstream from exon 15 exhibited very weak LD. The detailed LD coefficients can be found in the web supplement (Table A, web supplement).

None of the *KCNQ1* polymorphisms exhibited significant difference of genotype or allele frequencies between the groups with the shortest and the longest QTc intervals (data not shown). Given the strong LD between polymorphisms located in exon 15 and 3′UTR, a haplotype analysis of these four polymorphisms was performed. They generated six common haplotypes, which were similarly distributed between the two groups.

KCNE1 polymorphisms

The known SNPs, 112 G>A and 253 G>A, leading to the amino-acid changes G38S and D85N, respectively, were analyzed. Genotype frequencies did not deviate from the Hardy–Weinberg equilibrium in either group. The two polymorphisms were in weak LD ($D' = -0.46$) (Table B, web supplement).

Allele and genotype frequencies of the 112 G>A SNP (G38S) did not differ significantly between the two groups (Table 3). By contrast, the A allele of the 253 G>A SNP (D85N) was significantly more frequent in the longest than in the shortest QTc group (0.008 in the shortest QTc *vs* 0.030 in the longest QTc group, $P=0.016$). No homozygote 253 A/A was identified in this study population. The OR [95% CI] for longest QTc associated with carriage of minor A allele was 4.21 [1.17–15.16], $P=0.028$) (Table 3). Frequencies of the four possible haplotypes were not estimated owing to the low frequency of the 253 A allele.

Table 3 Frequency of *KCNE1* gene polymorphisms in the shortest and longest QTc groups and odds ratio (95% CI) for longest QTc associated with genotype

	QTc	
Polymorphism	*Shortest, N=200*	*Longest, N=198*
112 G>A (G38S)		
GG	81 (40.5%)	74 (37.4%)
GA	93 (46.5%)	97 (49.0%)
AA	26 (13.0%)	27 (13.6%)
Allele frequencies	0.637/0.363	0.619/0.381
OR [95% CI][a]	1.15 [0.77–1.72], $P=0.50$	
253 G>A (D85N)		
GG	197 (98.5%)	186 (93.9%)
GA	3 (1.5%)	12 (6.1%)
AA	0 (0.0%)	0 (0.0%)
Allele frequencies	0.992/0.008	0.970/0.030***
OR [95% CI][a]	4.21 [1.17–15.16], $P=0.028$	

Nucleotide numbering starts from the ATG start codon (Genbank accession M26685).
[a]Carriers of the minor allele opposed to homozygotes for the frequent allele; ***$P=0.016$.

KCNH2 2690 A>C polymorphism

The *KCNH2* 2690 A>C SNP, leading to the K897T amino-acid change, had previously been reported in Finnish (frequency of 0.16[21,32]), German (≈0.24[22]), American (0.14[15]) and Japanese (0.02[33]) populations. The minor allele frequency *KCNH2* 2690 C in the French population was 0.27, close to that of the German population.

Genotype frequencies were in the Hardy–Weinberg equilibrium in the two groups.

The minor allele 2690 C was significantly more frequent in the group with the shortest QTc interval (0.31 in the shortest QTc *vs* 0.22 in the longest QTc group, $P=0.0065$) and was associated with a lower risk of QTc interval prolongation (OR=0.64 [0.46–0.88], $P=0.0055$) (Table 4). This effect was seen in males as well as females (OR=0.70 [0.45–1.08]) for males, OR=0.57 [0.36–0.91] for females, $P=0.50$.

SCN5A polymorphisms

In this study, four *SCN5A* SNPs were analyzed: 1141-3 C>A in intron 9, 1673 A>G in exon 12, leading to the amino-acid change H558R, 5457 C>T in exon 28 leading to the

Table 4 Frequency of *KCNH2* gene polymorphism in the shortest and longest QTc groups and odds ratio (95% CI) for longest QTc associated with genotype

	QTc	
Polymorphism	*Shortest N = 200*	*Longest N = 198*
2690 A>C (K897T)		
AA	99 (49.5%)	119 (60.1%)
AC	77 (38.5%)	71 (35.9%)
CC	24 (12.0%)	8 (4.0%)
Allele frequencies	0.687/0.313	0.780/0.220***
OR [95% CI][a]	0.64 [0.46–0.88], $P=0.0055$	

Nucleotide numbering starts from the ATG start codon (Genbank accession U04270).
[a]Carriers of the minor allele opposed to homozygotes for the frequent allele; ***$P=0.0065$.

synonymous polymorphism D1819D, and −92 C>A in the core promoter of the gene.

The minor allele frequencies were 0.20 for 1141-3 A, 0.24 for 1673 G, 0.31 for 5457 T, and 0.003 (two out of 780 alleles) for −92 A. This latest SNP was less represented in our population than in the previous sample of 71 subjects of mixed ethnicity ($N=71$, 4.2%).[34] Owing to its low frequency, it was not studied further.

The three frequent SNPs were in the Hardy–Weinberg equilibrium in the two groups.

The 1141-3 C>A and 1673 A>G SNPs were in strong association, with only 8.3% of recombinant subjects. These two polymorphisms were in weak LD with the 5457 C>T polymorphism (Table C, web supplement). The minor alleles 1141-3 A and 1673 G were more frequent in the group with the longest QTc interval, with an OR [95% CI] of 1.66 [1.18–2.35], $P=0.0039$ associated with carriage of allele 1141-3 A and 1.5 [1.1–2.1], $P=0.01$ associated with carriage of allele 1673 G (Table 5). Conversely, the minor allele 5457 T was more frequent in the group with the shortest QTc interval and associated with a lower risk of QTc interval prolongation (OR = 0.65 [0.48–0.89], $P=0.0063$) (Table 5).

A haplotype analysis combining the 1141-3 C>A SNP and the 5457 C>T polymorphisms was performed. The 1673 A>G polymorphism was not included in the haplotype analysis because of its high concordance with the 1141-3 C>A. There was a globally significant difference in haplotype distribution between the two groups ($P=0.002$) (Table 6). We examined whether the effect of each polymorphism had an independent effect on risk by testing the interaction between the two alleles, but this did not reach significance ($P=0.65$), indicating that the two polymorphisms acted independently from each other.

Interaction between polymorphisms among the four genes

Potential interactions between polymorphisms of the different genes were explored in a systematic way. An interaction was detected between the *KCNH2* 2690 A>C SNP and the *KCNQ1* 2031 + 932 A>G polymorphism. Actually, the effect of the *KCNH2* 2690 C allele previously observed appeared to be confined to homozygotes for the major *KCNQ1* 2031 + 932 A allele (OR = 0.33 [0.18–0.60], $P=0.0003$), whereas the effect was not significant in carriers of the *KCNQ1* 2031 + 932 G allele (OR = 1.18 [0.67–2.05]) (test of homogeneity of ORs, $P=0.004$).

Table 5 Frequency of *SCN5A* gene polymorphisms in the shortest and longest QTc groups and odds ratio (95% CI) for longest QTc associated with genotype

	QTc	
Polymorphism	*Shortest N = 200*	*Longest N = 198*
1141-3 C>A		
CC	139 (69.5%)	117 (59.1%)
CA	57 (28.5%)	64 (32.3%)
AA	4 (2.0%)	17 (8.6%)
Allele frequencies	0.837/0.163	0.753/0.247*
OR [95% CI][a]	1.66 [1.18–2.35], $P=0.0039$	
1673 A>G (H558R)		
AA	126 (63.0%)	106 (53.5%)
AG	66 (33.0%)	71 (35.9%)
GG	8 (4.0%)	21 (10.6%)
Allele frequencies	0.795/0.205	0.715/0.285**
OR [95% CI][a]	1.52 [1.11–2.10], $P=0.010$	
5457 C>T (D1819D)		
CC	77 (38.5%)	109 (55.0%)
CT	103 (51.5%)	71 (35.9%)
TT	20 (10.0%)	18 (9.1%)
Allele frequencies	0.642/0.358	0.730/0.270***
OR [95% CI][a]	0.65 [0.48–0.89], $P=0.0063$	

Nucleotide numbering starts from the ATG start codon (Genbank accession NM000335).
[a]Carriers of the minor allele opposed to homozygotes for the frequent allele; *$P=0.0057$, **$P=0.0210$, ***$P=0.0032$.

Table 6 Comparison of the main haplotype frequencies of the *SCN5A* gene in the shortest and longest QTc groups

Haplotypes		*Frequency*		
1141-3 C>A	*5457 C>T*	*Shortest*	*Longest*	*P-value*
C	C	0.515	0.528	Reference
C	T	0.323	0.224	0.020
A	C	0.128	0.202	0.087
A	T	0.034	0.046	0.580

Global difference in haplotype frequencies between the shortest and longest QTc groups: $P=0.002$.

Discussion

While gender and age influence QT duration, a genetic determination of the QT length has been suggested.[7] Several studies have focused on SNPs located in genes responsible for the LQTS as potential determinants of cardiac repolarization duration. Indeed, the cardiac AP is orchestrated by potassium and sodium ion channel subunits encoded by the *KCNQ1* (LQT1), *KCNE1* (LQT5), *KCNH2* (LQT2), *KCNE2* (LQT6) and *SCN5A* (LQT3) genes, in which rare mutations lead to QTc prolongation associated with ventricular arrhythmias. The first SNPs found associated with the QTc length in healthy subjects were 2690 A>C (K897T) of *KCNH2*[21,22,32] and, more recently, SNPs in *KCNQ1*, *KCNE1*, *KCNE2* and *SCN5A*.[23,35]

While these studies determined the impact of SNPs on QT length as a quantitative trait, we adopted an original approach by opposing two contrasted groups of healthy untreated subjects: 200 subjects presenting the shortest and 198 subjects presenting the longest QTc interval length, selected from a cohort of 2008 healthy subjects. We then compared the allele, genotype and haplotype frequencies of 10 *KCNQ1*, two *KCNE1*, four *SCN5A* and one *KCNH2* SNPs between the two groups and searched for potential interactions between them.

KCNQ1 and *KCNE1* polymorphisms

We screened the entire coding sequence, exon/intron boundaries and the 3′UTR region of the *KCNQ1* gene and identified six new rare SNPs (<0.10) (459 G>T (T153 T), 477+20 A>G, 918+13 C>T, 984 C>T (I328I), 1514+7 G>T and 1514+14 G>T), and two more frequent ones (>0.10) (477+80 insGG, 2031+479 G>A).

Busjahn *et al*[7] initially suggested that the LQT1 locus, containing *KCNQ1*, may represent a quantitative trait locus (QTL) for the QTc phenotype in the normal population. However, in our study, no *KCNQ1* SNPs, alone or in combination, demonstrated an association with the QTc length. In a recent study, Aydin and *al*[23] also failed to identify *KCNQ1* SNPs associated with QTc length in the normal monozygotic and dizygotic twins previously studied by Busjahn. The discrepancy between results from linkage and association studies suggests that a different gene in the vicinity of *KCNQ1* might be at the origin of the linkage previously detected in the region. Another explanation might be that there exists in *KCNQ1* an undetected functional SNP not in complete LD with the SNPs genotyped in the present study as well as in Aydin's report.[23] Besides, in a recent report analyzing 81 SNPs spreading over the entire *KCNQ1* gene, several haplotype blocks were identified in weak LD one with each other, and a rare G allele (rs757092 A>G) in a high LD haplotype block in intron 1 was associated with a QTc prolongation in the study population.[35] The 477+80 insGG polymorphism is located 50 kb downstream from the rs757092 A>G SNP in the genomic sequence of *KCNQ1*. They may be in weak LD in our population and would explain why we failed to detect any *KCNQ1* SNP effect on the QTc length.

We also hypothesized that allelic variants in the regulatory subunit of the I_{Ks} channel, minK encoded by *KCNE1*, could influence the channel function, and as a consequence, the length of the QTc interval. We studied two known nonsynonymous polymorphisms, G38S[23,36] and D85N.[19,23,37] Although the G38 allele was shown to be associated with atrial fibrillation,[36] none of the G38S alleles was associated with the QTc length in our French population of healthy subjects. In contrast, the rare N85 allele was four times more frequent in the group with the longest QTc interval than in the group with the shortest QTc.

Wei *et al*[19] described the N85 allele as a risk factor of drug-induced LQT syndrome. In this study, no variation in I_{Ks} current amplitude was observed when N85-minK was coexpressed with wild-type (WT) KvLQT1 in CHO cells, the channels activated more slowly and deactivated to a greater extent after a long diastolic pause, a latent defect that may be unmasked by drug block and render individuals susceptible to acquired LQTS.

Recently, the N85 variant was reported in some LQTS patients presenting longer QTc intervals and a higher incidence of cardiac arrhythmia compared to family members carrying only a LQT syndrome mutation. In this study, a marked reduction of I_{Ks} current was observed when the N85-minK variant was coexpressed with the WT KvLQT1 channel in *Xenopus* oocytes.[38]

Whatever the discrepancy about the KvLQT1/N85-minK channel properties expressed in heterologous expression systems, there is evidence that the *KCNE1* N85 allele modifies the I_{Ks} channel function leading to a prolongation of the QTc interval length, which means that D85N would be a 'functional' polymorphism. Our association study is in accordance with these studies since 6% (12/198) of healthy subjects with a QTc interval in the upper limit (394–433 ms) were carriers of the N85 allele compared to 1.5% (3/200) of healthy subjects with shorter QTc interval (<365 ms).

Recent association studies reported the rare allele IVS2-128 A and rs727957 T associated with the prolongation of the QTc length in healthy Caucasian subjects.[23,35] The former is located in intron 2 and the latter in a haplotype block ending 20 kb upstream of the *KCNE1* gene. The functional nature of these SNPs is undetermined but these data indicate that the *KCNE1* gene may act as a QTL for the cardiac ventricular repolarization in healthy subjects. Thus, these SNPs represent good candidates to explore the penetrance variability in LQT syndrome patients.

The *KCNH2* K897T polymorphism

We found that the 2690 C allele, leading to a threonine residue at position 897 of the HERG protein, was more prevalent in the group with the shortest QTc interval. This

result is in accordance with previous association studies.[22,35] Bezzina *et al*[22] showed that female 2690 C/C homozygotes had shorter QTc intervals in comparison to female heterozygotes and 2690 A/A homozygotes. Moreover, they showed by *in vitro* studies that HERG-T897 channels hastened cardiac repolarization compared to HERG-K897 channels. By contrast, an earlier association study in a Finnish population showed opposite results: female heterozygotes or 2690 C/C homozygotes had longer QTc intervals than females 2690 A/A homozygotes.[32] This discrepancy may reflect either the occurrence of additional population-specific functional polymorphisms, influencing the effect of the K897T polymorphism, or the limitations of association studies.

Our results not only confirmed a hastened cardiac repolarization in both male and female carriers of the 2690 C allele but also suggested that this effect may be dependent on a *KCNQ1* polymorphism located in the 3′UTR part of the gene (2031 + 932 A > G).

Interestingly, I_{Kr}, underlined by the HERG channels, and I_{Ks}, underlined by KvLQT1/minK channels, were shown to interact both at a functional and a physical level. Indeed, when I_{Kr} is reduced, the Ap is prolonged, causing I_{Ks} activation to increase so as to prevent excessive repolarization delays,[39,40] and KvLQT1 was found to associate physically with HERG in both CHO cells and native cardiomyocytes, and to increase membrane localization of HERG in CHO cells.[41]

The only *KCNQ1* allele in interaction with the HERG-T897 allele is located in the 3′UTR part of the gene. This *KCNQ1* 2031 + 932 A allele, or a yet unidentified polymorphism LD with it, may lead to more stable transcripts, or to a better subcellular targeting as previously shown for other genes.[42–44] Even if a direct effect on QTc length was not detectable, the *KCNQ1* 2031 + 932 A may potentiate the I_{Kr} current displayed by the HERG-T897 channels.[22]

SCN5A polymorphisms

We studied four *SCN5A* SNPs which have been previously reported: −92 C > A,[34] 1141-3 C > A,[23] 1673 A > G (H558R),[15,33] 5457 C > T (D1819D).[33] Almost completely concordant, the 1141-3 A and 1673 G minor alleles were more frequent in the group with the longest QTc interval, whereas the 5457 T minor allele was more frequent in the group with the shortest QTc length. The effects of the 1141-3 C > A (or the 1673 A > G) and the 5457 C > T SNPs were independent, as shown by the haplotype analysis.

The association of the 1673 A > G SNP, or H558R, with QT length confirms the result recently published by Aydin *et al*.[23] This polymorphism is located in the Na^+ channel I–II interdomain cytoplasmic linker, and two studies have suggested that it could modulate the effect of arrhythmia-causing *SCN5A* mutations.[16,45] However, no difference in Na^+ channel activation and inactivation has been observed between the hH1-H558 and the hH1-R558 clones.[16] It is not possible from our results to disentangle the effect of the H558R polymorphism from that of the 1141-3 C > A SNP because of their tight LD. This latter SNP is located in the acceptor splice site in intron 9 and might affect the splicing. The observed effect may also be due to another unknown polymorphism in LD with these two SNPs.

The minor allele of the 5457 C > T SNP, located in the last exon of the gene (exon 28) and leading to the synonymous D1819D polymorphism, was associated with the shortest QTc length. Interestingly, Aydin *et al*[23] described a SNP located in intron 24 of *SCN5A* (IVS24 + 116 G > A) with the minor allele associated with a shorter QTc. The functional nature of these variants on the sodium channel activity or expression remains elusive at this point. Indeed, gain of function mutations in *SCN5A* were associated with a QTc prolongation in LQTS patients, while loss of function mutations did not produce a QTc shortening in Brugada patients.

One potential limitation of our study was that it was restricted to healthy untreated subjects. We excluded patients taking medications susceptible to prolong the QT interval (antiarrhythmics, neuroleptics, antihistaminics, antihypertensive treatments), as well as subjects with cardiovascular diseases or diabetes. For this reason, extrapolation of our results to the general population might be questionable. Replication of the present results in an independent population would greatly strengthen our findings.

The findings of our study are important for a better understanding of the risk of arrhythmias and sudden death in healthy subjects as well as in patients with cardiovascular diseases. Indeed, a prolonged baseline QT interval was shown to predispose patients with coronary artery disease to sudden death[46] or myocardial infarction[47,48] whereas a short baseline QT interval was associated with reduced mortality in dofetilide-treated patients with moderate to severe heart failure and reduced left ventricular systolic function.[49] Although rare, the SQTS where patients present a drastic decrease of the QTc interval is also linked to life-threatening arrhythmias and sudden death.[12] So *KCNH2* 2690A > C (K897T) and *SCN5A* 5457 C > T SNPs would be expected to be protective factors, while *KCNE1* 253 G > A (D85N), *SCN5A* 1141-3 C > A and 1673 A > G (H558R) would be risk factors for QT prolongation and onset of arrhythmias in patients with LQT syndrome or with other cardiovascular diseases; the contrary would be expected in patients with a SQTS.

In conclusion, our results suggest that genetic polymorphisms located in the *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A* genes might, independently or in interaction, influence the QTc length in healthy Caucasian subjects. These variants might also modulate the effects of ion channel mutations leading to arrhythmias such as LQT and Brugada syndromes and contribute to the phenotypic variability observed in these disorders.

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