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Population-based association studies have identified several polymorphic variants in genes encoding ion channel subunits associated with the electrocardiographic heart-rate corrected QT (QTc) length in healthy populations of Caucasian origin (*KCNH2* rs1805123 (K897T) and rs3815459, *SCN5A* rs1805126 (D1819D), 1141-3 C>A, rs1805124 (H558R), and IVS24+116 G>A, *KCNQ1* rs757092, *KCNE1* IVS2-128 G>A and rs1805127 (G38S), and *KCNE2* rs2234916 (T8A). However, few of these results have been replicated in independent populations. We tested the association of SNPs *KCNQ1* rs757092, *KCNH2* rs3815459, *SCN5A* IVS24+116 G>A, *KCNE1* IVS2-128 G>A and *KCNE2* rs2234916 with QTc length in two groups of 200 subjects presenting the shortest and the longest QTc from a cohort of 2008 healthy subjects. All polymorphisms were in Hardy-Weinberg equilibrium in both groups. The minor allele *SCN5A* IVS24+116 A was more frequent in the group of subjects with the shortest QTc, while the minor alleles *KCNQ1* rs757092 G and *KCNH2* rs3815459 A were more frequent in the group with the longest QTc. There was no significant difference for *KCNE1* IVS2-128 G>A and *KCNE2* rs2234916 between the two groups. Haplotype analysis showed a 2-fold increased risk of QTc lengthening for carriers of the haplotype combining alleles C and A of the two common *KCNE1* SNPs, IVS2-129 C>T (rs2236609) and rs1805127 (G38S), respectively. In conclusion, our study confirms the reported associations between QTc length and *KCNQ1* rs757092 and *KCNH2* rs3815459.

Introduction

The cardiac ventricular repolarisation process, which is measured by the QT interval length on the electrocardiogram (ECG), is known to be influenced by various parameters (heart rate[1], age[2], sex[3], medications[4]) but heritability studies have suggested that genetic factors are also involved in the control of cardiac repolarisation at the population level[5-8]. In each individual, the activity and expression levels of cardiac ion channels establish a subtle equilibrium between depolarizing and repolarizing currents determining the action potential duration of ventricular cardiomyocytes. Mutations identified in ion channel encoding genes are responsible for familial ventricular arrhythmia disorders with high risk of sudden death, such as the short and long QT syndromes characterised by shortened or prolonged QT intervals, respectively[9-12].

Several groups have undertaken genetic association studies to identify QTc-associated polymorphisms in ion channel encoding genes in healthy subjects[13-18]. Our group and others showed that the T897 allele (K897T, rs1805123) in *KCNH2* (LQT2), encoding the α-subunit of the voltage-gated I_{Kr} channel, and the R558 allele (H558R, rs1805124) in *SCN5A* (LQT3), encoding the cardiac voltage-gated sodium channel $Na_v1.5$, have a shortening[14,16,18] and a prolonging[15,16] influence on the QTc interval, respectively, in independent populations of French and German origin. Five other polymorphisms, *SCN5A* IVS24+116 G>A, *KCNE1* IVS2-128 G>A, *KCNE2* rs2234916 (T8A), *KCNQ1* rs757092, *KCNH2* rs3815459, have also been associated with QTc length in German populations[15,18] (Table 1), but these associations have never been confirmed in a population of different origin. We thus studied these five polymorphisms in our French D.E.S.I.R population.

Materials and methods

Study population

Participants in the study Data from an Epidemiological Study on the Insulin Resistance syndrome (D.E.S.I.R) 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. Briefly, 3478 subjects underwent a 12-lead resting ECG at three year intervals (D0 and D3). QT intervals were measured in lead II (ms) by Cardionics® software and corrected for heart rate by use of the

Fridericia formula (QTc). 1470 subjects were excluded on the basis of a known or detected cardiac pathology, diabetes, treated or untreated high blood pressure, medication known to prolong the QT interval, and a difference ≥ 30 ms in QTc between D0 and D3. The features (mean age, mean heart rate, mean QTc interval, QTc range) of the 200 selected subjects (100 men and 100 women) with the shortest age-adjusted mean QTc, and of the 200 selected subjects (100 men and 100 women) with the longest age-adjusted mean QTc intervals, from the 2008 healthy D.E.S.I.R subpopulation, have been previously described[16].

Polymorphism typing

Sample genotypes were determined using PCR and sequencing for the *KCNH2* rs3815459 G>A, *KCNE1* IVS2-128 G>A, *KCNE1* rs2236609 (IVS2-129 C>T), *KCNE1* rs1805127 (G38S) and *KCNE2* rs2234916 (T8A) polymorphisms, and fluorescence resonance energy transfer (F.R.E.T) and probe melting curves for the *KCNQ1* rs757092 A>G, *SCN5A* IVS24+116 G>A, *SCN5A* IVS9-3 C>A and rs1805126, *KCNH2* rs1805123 (K897T), as described previously[16]. The sequencing reactions were run on an ABI 3100 sequencer (Applied Biosystems) and genotypes analysed using SEQSCAPE™ v2.5 software (Applied Biosystems). Primers used to generate PCR products and hybridization probes used for F.R.E.T are given in Supplementary online Table 1.

Statistical analysis

Deviation from the Hardy-Weinberg equilibrium was tested by χ^2 analysis with 1 degree of freedom, separately, in the shortest and longest QTc groups. Allele frequencies were estimated from genotype frequencies by gene counting. The study compared the longest and the shortest QTc groups. The odds ratio (OR) [95% CI] for longest QTc associated with one copy of the minor allele was estimated from logistic regression analysis, adjusted for age and sex. 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. Estimation of the pairwise linkage disequilibrium (LD) coefficients between polymorphisms was expressed in terms of D'=D/Dmax or -D/Dmin[19]. In order to estimate Haplotype frequencies, we combined the polymorphisms studied here with the polymorphisms previously genotyped[16], when they were not in almost complete concordance and had

a frequency >0.02. In *KCNQ1*, polymorphisms located upstream exon 15 were in weak LD, generating multiple rare haplotypes[16]. Thus, haplotype study for *KCNQ1* polymorphisms combining the two SNPs in intron 1 and the SNPs located upstream exon 15 was not possible. Haplotype analyses were performed by use of a maximum likelihood model implemented in the THESIAS program (http://genecanvas.ecgene.net)[20]. A *P*-value < 0.05 was considered statistically significant. All computations were carried out with SAS software (SAS Institute, Cary, NC, USA).

Results

Association analysis of individual SNPs

Five SNPs were genotyped in the 200 subjects with the shortest QTc and the 200 subjects with the longest QTc from a population of 2008 healthy subjects: *KCNQ1* rs757092, *KCNH2* rs3815459, *SCN5A* IVS24+116 G>A, *KCNE1* IVS2-128 G>A, *KCNE2* rs2234916 (T8A). Genotype frequencies did not deviate from the Hardy-Weinberg equilibrium, in either group, for any of the studied SNPs.

The minor allele G of *KCNQ1* rs757092 and A of *KCNH2* rs3815459 were significantly more frequent in the longest than in the shortest QTc group (0.397 vs 0.312 for rs757092, 0.240 vs 0.172 for rs3815459). The OR [95% CI] for the longest QTc associated with carrying the minor allele was 1.48 [1.10-2.01], *P*=0.011 for rs757092 and 1.48 [1.05-2.10], *P*=0.026 for rs3815459, respectively (Table 2).

Conversely, the minor allele A of *SCN5A* IVS24+116 was significantly more frequent in the shortest than in the longest QTc group (0.162 vs 0.104), with OR [95% CI] for the longest QTc associated with carrying the minor allele of 0.61 [0.40-0.92], *P*=0.018 (Table 2).

KCNE1 IVS2-128 G>A and *KCNE2* rs2234916 A>G variations were rare in our population (0.019 and 0.005, respectively) and there was no significant association between QTc length and these polymorphisms. A more common *KCNE1* polymorphism, 5' adjacent to IVS2-128 G>A, named IVS2-129 C>T (rs2236609), was also genotyped. Allele frequency did not differ significantly between the shortest and longest QTc groups (0.427 vs 0.421, respectively) (Table 2).

Haplotype analysis

Haplotypes were constructed for rs1805123 A>C (K897T) and rs3815459 G>A within *KCNH2,* IVS9-3 C>A, IVS24+116 G>A and rs1805126 (D1819D) within *SCN5A*, and rs2236609 and rs1805127 (G38S) within *KCNE1*.

KCNH2 rs1805123 (K897T) and rs3815459 were in moderate LD (D'= -0.60). Haplotype analysis confirmed the shortened QTc-associated effect observed for rs1805123 and the prolonged QTc-associated effect observed for rs3815459 and showed that their respective effects were independent of each other (data not shown, test for interaction between the two alleles p=0.76).

SCN5A IVS24+116 G>A was in tight LD with rs1805126 (D1819D) (D'= +0.97) and in moderate LD with IVS9-3 C>A (D'= -0.63). The minor allele IVS24+116/A belongs to a single haplotype combining IVS9-3 /C and 5457/T. Thus, as no haplotype effect had previously been detected between 1141-3 and 5457[16], no additional information on haplotypes was provided by IVS24+116.

KCNE1 rs2236609 and rs1805127 were in moderate LD (D'= +0.61). Haplotype analysis (Table 3) showed that haplotype combining alleles rs2236609 C and rs1805127 A (H3) was more frequent in the group with the longest QTc intervals, with OR [95% CI] for the longest QTc associated with carrying one copy of haplotype (H3) of 1.97 [1.11-3.48], *P*=0.020, with reference to the most frequent haplotype (H1).

Discussion

Twin- or family-based genome-wide linkage analyses have identified three QTc duration influencing genomic loci on chromosomes 11p (LQT1)[7], 4q (LQT4)[7] and 3p[7,21]. Polymorphic variants that may influence QTc duration in healthy populations of Caucasian origin have been assessed in genes encoding ion channel subunits. Some minor alleles of these polymorphisms were described as either associated with a shortening or a lengthening of QTc[14,16,18,21]. However, few of these results have been replicated in independent populations. In this study, we tested SNPs *KCNQ1* rs757092 A>G, *KCNH2* rs3815459 G>A, *SCN5A* IVS24+116 G>A, *KCNE1* IVS2-128 G>A and *KCNE2* rs2234916 A>G for association with QTc length in our population. Correction for multiple testing was not required since a small number of

polymorphisms was studied and we intended to verify the previously observed associations.

We found that the alleles *KCNQ1* rs757092 G and *KCNH2* rs3815459 A were more frequent in the group of subjects with the longest QTc, which confirm the initial association described in a German population[18].

We found that allele *SCN5A* IVS24+116 A was more frequent in the group of subjects with the shortest QTc. In the initial study, Aydin et *al* (2005) reported an association between this polymorphism and QTc length in a normal German population of twins and showed that subjects with *SCN5A* IVS24+116 A/A genotype had longer QTc than subjects with *SCN5A* IVS24+116 G/G[15]. Additional studies in large series are needed to elucidate whether or not this polymorphism is associated with QTc length.

The alleles *KCNE1* IVS2-128 A and *KCNE2* rs2234916 G were very rare and the allele frequency was not significantly different between the two groups for any of them. Nevertheless, haplotype analysis of two common *KCNE1* SNPs (rs2236609 and rs1805127 (G38S)) showed a 2-fold increased risk of QTc lengthening when carrying the haplotype combining alleles rs2236609 C and rs1805127 A. In our previous study, there was no evidence that SNP rs1805127 was associated with QTc length[16], as recently reported in a large population sample[17], while Friedlander and al. reported its association with the QTc interval in men[22]. In this latter study, variance-component linkage analysis revealed weak evidence of linkage of *KCNE1* SNPs with QTc interval while family-based association analysis demonstrated a significant association between SNP rs1805127 and QTc interval, suggesting that SNP rs1805127 could have a modest effect and act as a susceptibility locus on the QTc trait. This modest effect could be detected only by haplotype analyses in our population. Further association studies with *KCNE1* markers would enable the role of *KCNE1* on QTc length to be settled.

In *SCN5A,* IVS24+116 in intron 24 and rs1805126 (D1819D) in exon 28 were in nearly complete positive LD in our population, contributing to the same trend of association of their alleles with the shortest QTc[16]. Both these polymorphisms were in weak LD with IVS9-3 C>A SNP which was associated with prolonged QTc in our previous study[16]. In *KCNH2,* rs1805123 (K897T) and rs3815459 were in moderate negative LD and showed independent and opposite effects on QTc length. High-resolution LD mapping studies of these genes should enable the identification of

haplotype blocks and relevant tagging SNPs for genetic association studies. Further investigations are needed to identify the functional variants that could explain the opposite and independent effects observed for some SNPs lying in the same gene.

In summary, our study replicated the associations reported for SNPs rs757092 in *KCNQ1* and rs3815459 in *KCNH2*, reported association of allele A of IVS24+116 G>A in *SCN5A* with the shortest QTc, and failed to replicate the associations of IVS2-128 G>A in *KCNE1* and rs2234916 (T8A) in *KCNE2*. It is possible that these conflicting results could be resolved with further investigations using larger sample sizes.

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