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Confirmation of associations between ion channel gene SNPs and QTc interval duration in healthy subjects

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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, whereas 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 twofold 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.

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Introduction

The cardiac ventricular repolarization 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 and medications⁴), but heritability studies have suggested that genetic factors are also involved in the control of cardiac repolarization 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

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Table 1 SNPs in cardiac ion channel genes associated with QTc length in healthy Caucasian populations

Gene	SNP region	Coding	SNP database	Alleles	cDNA position nomenclature	Genome position ^a	Initial association studies	Association results
KCNQ1	Intron 1	NC	rs757092	A>G	—	Chr11:2455754	Pfeuffer <i>et al</i> , 2005	G>longest QTc ^b
KCNH2	Exon 11	K897T	rs1805123	A>C	2690	Chr7:150276467	Bezzina <i>et al</i> , 2003 Pfeuffer <i>et al</i> , 2005 Gouas <i>et al</i> , 2005	C>shortest QTc
	3'UTR		rs3815459	G>A	—	Chr7:150275327	Pfeuffer <i>et al</i> , 2005	A>longest QTc ^b
SCN5A	Intron 9	NC	—	C>A	IVS9-3 (1141-3)	—	Gouas <i>et al</i> , 2005	A>longest QTc
	Exon 12	H558R	rs1805124	A>G	1673	Chr3:38620424	Aydin <i>et al</i> , 2005 Gouas <i>et al</i> , 2005	G>longest QTc
	Intron 24	NC		G>A	IVS24+116	—	Aydin <i>et al</i> , 2005	A>longest QTc ^d
	Exon 28	D1819D	rs1805126	C>T	5457	Chr3:38567410	Gouas <i>et al</i> , 2005	T>shortest QTc
KCNE1	Intron 2	NC	—	G>A	IVS2-128	chr21:34743980	Aydin <i>et al</i> , 2005	G>longest QTc ^c
	Intron 2	NC	rs2236609	C>T	IVS2-129	chr21:34743981	present study	no association
	Exon 3	G38S	rs1805127	G>A	112	chr21:34743691	Friender <i>et al</i> , 2005 Gouas <i>et al</i> , 2005 Akyol <i>et al</i> , 2007	A>longest QTc no association
KCNE2	Exon 2	T8A	rs2234916	A>G	22	chr21:34664669	Aydin <i>et al</i> , 2005	G>shortest QTc ^c

^adb SNP build 126.

NC, noncoding.

All these SNPs have been studied in the D.E.S.I.R population (Gouas *et al*, 2005 or present study).^bAssociations confirmed in the present study.^cAssociations not confirmed.^dAssociation of the allele A with the shortest QTc was found in this study.

characterized 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 (Data from an Epidemiological Study on the Insulin Resistance syndrome) 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 3 years 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). A total of 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 and 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 described previously.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, Foster City, USA) and genotypes analyzed using SEQSCAPE™ version 2.5 software (Applied Biosystems). Primers used to generate PCR products and hybridization probes used for F.R.E.T are given in Supplementary Table 1.

Statistical analysis

Deviation from the Hardy–Weinberg equilibrium was tested by χ^2 analysis with 1 df, 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 as 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 $D' = D/D_{\max}$ or $-D/D_{\min}$.¹⁹ 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, and *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

Table 2 Genotype counts and allele frequency of the polymorphisms in the shortest and longest QTc groups and odds ratio (95% CI) for longest QTc associated with one copy of minor allele (adjusted for age and sex)

Gene	SNP	Genotype	Shortest QTc	Longest QTc
KCNQ1	rs757092	AA	95	67
		AG	84	105
		GG	20	26
		Minor allele frequency	0.312	0.397
		P-value for HWE	0.82	0.13
		OR (95% CI)	1.48 (1.10–2.01), $P=0.011$	
KCNH2	rs3815459	GG	139	113
		GA	50	72
		AA	9	11
		Minor allele frequency	0.172	0.240
		P-value for HWE	0.11	0.92
		OR (95% CI)	1.48 (1.05–2.10), $P=0.026$	
SCN5A	IVS24+116 G/A	GG	142	159
		GA	48	37
		AA	8	2
		Minor allele frequency	0.162	0.104
		P-value for HWE	0.14	0.93
		OR (95% CI)	0.61 (0.40–0.92), $P=0.018$	
KCNE1	IVS2-128 G>A	GG	192	189
		GA	8	7
		Minor allele frequency	0.020	0.018
		P-value for HWE	0.77	0.80
		OR (95% CI)	0.89 (0.32–2.48), $P=0.83$	
	rs2236609	CC	66	64
		CT	95	99
		TT	37	33
		Minor allele frequency	0.427	0.421
		P-value for HWE	0.79	0.61
		OR (95% CI)	0.98 (0.74–1.30), $P=0.89$	
KCNE2	rs2234916	AA	196	196
		AG	2	2
		Minor allele frequency	0.005	0.005
		P-value for HWE	0.94	0.94
		OR (95% CI)	0.99 (0.14–7.22), $P=1.00$	

Table 3 The estimated *KCNE1* haplotype frequencies in the group with the shortest and the group with the longest QTc intervals and odds ratios for the haplotypes

	rs2236609	rs1805127 (G38S)	Shortest QTc (N = 392 alleles)	Longest QTc (N = 396 alleles)	OR (95% CI) ^a
H1	C	G	0.517	0.468	ref
H2	T	G	0.119	0.152	1.30 (0.86–1.97), $P=0.21$
H3	C	A	0.056	0.111	1.97 (1.11–3.48), $P=0.02$
H4	T	A	0.308	0.269	0.97 (0.69–1.37), $P=0.87$

 $P=0.081$ for the global association.^aOdds ratios with 95% confidence intervals (95% CI) were calculated with reference to the most common haplotype H1.

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 as 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*¹⁵ 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. 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 twofold 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 whereas Friedlander *et al*²² reported its association with the QTc interval in men. In this latter study, variance-component linkage analysis revealed weak evidence of linkage of *KCNE1* SNPs with QTc interval, whereas 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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References

- 1 Bazett H: An analysis of the time relations of electrocardiograms. *Heart* 1920; **7**: 353–367.
- 2 Reardon M, Malik M: QT interval change with age in an overtly healthy older population. *Clin Cardiol* 1996; **19**: 949–952.
- 3 Yang H, Elko P, LeCarpentier GL *et al*: Sex differences in the rate of cardiac repolarization. *J Electrocardiol* 1994; **27** (Suppl): 72–73.
- 4 Kaab S, Hinterseer M, Nabauer M, Steinbeck G: Sotalol testing unmasks altered repolarization in patients with suspected acquired long-QT-syndrome – a case-control pilot study using i.v. sotalol. *Eur Heart J* 2003; **24**: 649–657.
- 5 Russell MW, Law I, Sholinsky P, Fabsitz RR: Heritability of ECG measurements in adult male twins. *J Electrocardiol* 1998; **30** (Suppl): 64–68.
- 6 Carter N, Snieder H, Jeffery S *et al*: QT interval in twins. *J Hum Hypertens* 2000; **14**: 389–390.
- 7 Busjahn A, Knoblauch H, Faulhaber H-D *et al*: QT interval is linked to 2 long-QT syndrome loci in normal subjects. *Circulation* 1999; **99**: 3161–3164.
- 8 Friedlander Y, Lapidus T, Sinnreich R, Kark JD: Genetic and environmental sources of QT interval variability in Israeli families: the kibbutz settlements family study. *Clin Genet* 1999; **56**: 200–209.
- 9 Bellocq C, van Ginneken AC, Bezzina CR *et al*: Mutation in the *KCNQ1* gene leading to the short QT-interval syndrome. *Circulation* 2004; **109**: 2394–2397.
- 10 Wolpert C, Schimpf R, Giustetto C *et al*: Further insights into the effect of quinidine in short QT syndrome caused by a mutation in *HERG*. *J Cardiovasc Electrophysiol* 2005; **16**: 54–58.
- 11 Priori SG, Pandit SV, Rivolta I *et al*: A novel form of short QT syndrome (SQT3) is caused by a mutation in the *KCNJ2* gene. *Circ Res* 2005; **96**: 800–807.
- 12 Splawski I, Shen J, Timothy K *et al*: Spectrum of mutations in long QT syndrome genes *KVLQT1*, *HERG*, *SCN5A*, *KCNE1*, and *KCNE2*. *Circulation* 2000; **102**: 1178–1185.
- 13 Pietila E, Fodstad H, Niskasaari E *et al*: Association between *HERG* K897T polymorphism and QT interval in middle-aged Finnish women. *J Am Coll Cardiol* 2002; **40**: 511–514.
- 14 Bezzina CR, Verkerk AO, Busjahn A *et al*: A common polymorphism in *KCNH2* (*HERG*) hastens cardiac repolarization. *Cardiovasc Res* 2003; **59**: 27–36.
- 15 Aydin A, Bahring S, Dahm S *et al*: Single nucleotide polymorphism map of five long-QT genes. *J Mol Med* 2005; **83**: 159–165.
- 16 Gouas L, Nicaud V, Berthet M *et al*: Association of *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A* polymorphisms with QTc interval length in a healthy population. *Eur J Hum Genet* 2005; **13**: 1213–1222.

- 17 Akyol M, Jalilzadeh S, Sinner MF *et al*: The common non-synonymous variant G38S of the KCNE1-(minK)-gene is not associated to QT interval in Central European Caucasians: results from the KORA study. *Eur Heart J* 2007; **28**: 305–309.
- 18 Pfeufer A, Jalilzadeh S, Perz S *et al*: Common variants in myocardial ion channel genes modify the QT interval in the general population: results from the KORA study. *Circ Res* 2005; **96**: 693–701.
- 19 Tiret L, Amouyel P, Rakotovao R, Cambien F, Ducimetiere P: Testing for association between disease and linked marker loci: a log-linear-model analysis. *Am J Hum Genet* 1991; **48**: 926–934.
- 20 Tregouet DA, Escolano S, Tiret L, Mallet A, Golmard JL: A new algorithm for haplotype-based association analysis: the Stochastic-EM algorithm. *Ann Hum Genet* 2004; **68**: 165–177.
- 21 Newton-Cheh C, Larson MG, Corey DC *et al*: QT interval is a heritable quantitative trait with evidence of linkage to chromosome 3 in a genome-wide linkage analysis: The Framingham Heart Study. *Heart Rhythm* 2005; **2**: 277–284.
- 22 Friedlander Y, Vatta M, Sotoodehnia N *et al*: Possible association of the human KCNE1 (minK) gene and QT interval in healthy subjects: evidence from association and linkage analyses in Israeli families. *Ann Hum Genet* 2005; **69**: 645–656.

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