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ORIGINAL ARTICLE

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Single nucleotide polymorphism map of five long-QT genes

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Abstract We screened a white population for single nucleotide polymorphisms (SNPs) in five long QT syndrome genes, namely, KCNQ1 (LQT1), HERG (LQT2), SCN5A (LQT3), KCNE1 (LQT5), and KCNE2 (LQT6). We found 35 SNPs, 10 of which have not been previously described. Ten SNPs were in KCNE1, six in HERG, eight in KCNQ1, four in KCNE2, and seven in SCN5A. Four SNPs were associated with QTc interval in our 141 subjects, one in KCNE1, one in KCNE2, and two in SCN5A. Two of these SNPs have not been described. We conclude that these five long QT syndrome genes contain common variants, some of which are associated with QTc interval in normal persons. We suggest that analysis of these SNPs in a much larger cohort would enable establishment of common haplotypes that are associated with QTc. These haplotypes could facilitate prediction of arrhythmia risk in the general population

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Keywords Single nucleotide polymorphism · QT interval · Sudden cardiac death

Abbreviation *SNP:* Single-nucleotide polymorphism

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Introduction

Cardiovascular disease is the most common cause of death worldwide, and about one-half of cardiac deaths are sudden. Sudden cardiac deaths are attributed to disturbances in cardiac rhythm, almost invariably ventricular tachycardia leading to fibrillation. Ventricular tachycardia is associated with prolongation of the QT interval on electrocardiography [1]. Advances in molecular genetics have provided us with a number of genes that when mutated result in long QT syndromes and risk for sudden cardiac death [2]. These genes code for ion channels, or rather their subunits, and the mutations alter the orderly progression from depolarization to, and including, repolarization. Numerous long QT genes have been elucidated [3]. Rare mutations have been detected in congenital long QT syndrome families that result in the prolongation of the QT interval. Patients with mutations in these genes profit from an implanted defibrillator or from medication. However, persons without a mutation in a QT syndrome gene may develop prolongations in the QT interval as a

response to electrolyte disturbances or medications and develop malignant forms of ventricular tachycardia [4]. The propensity to such developments is also influenced by genetic variance. In an earlier study in normal monozygotic and dizygotic twin subjects and their parents we showed that several components of the electrocardiogram including the QT interval are linked to the loci of long QT syndrome genes [5]. This finding suggests that common variants in disease genes influence the QT interval in the general population. Variants at various QT gene loci can conceivably act in concert. A single-nucleotide polymorphism (SNP) occurs about every 1,000 bp in the genome. It is conceivable that such SNPs affect the expression of the genes by altering the regulation or the amino acid structure of the protein or by some as yet to be elucidated mechanism. We screened five long QT genes in normal subjects for SNPs. We found 35 common SNPs and suggest that these SNPs might be useful in testing variation in QT genes in the general population.

Methods

For subjects we selected DNA from our twin cohort that consists of 94 pairs of monozygotic twins and 47 pairs of dizygotic twins. Since the monozygotic twins are identical, only one subject from each pair was genotyped. The subjects were recruited by advertisement. All were healthy, ingesting no medication, and had no inherited forms of cardiovascular or metabolic disease. All had normal electrocardiograms that had been computerized. Our internal review board approved the studies and written informed consent was obtained from all subjects. Standard 12-lead electrocardiography was performed (Cardiovits CS-100, Schiller, Baar, Switzerland). The duration of the QTc and RR intervals were measured in lead II. The QT interval corrected for heart rate (QTc) was determined according to Bazett's formula as outlined elsewhere [5].

Human DNA for SNP screening was amplified with fluorescence-labeled artificial nonhuman sequences and was detected with single-strand conformation polymorphism capillary electrophoreses as described by Aydin et al. [6]. The (first) specific amplifications were carried out in a 15-µl reaction volume, 166 nM of both specific forward and reverse primer, for all intron/exons boundaries of KCNQ1 (LQT1), HERG (LQT2), SCN5A (LQT3), KCNE1 (LQT5), and KCNE2 (LQT6) genes. The (second) universal (for labeling) amplification were carried out in a 15-µl reaction volume, 166 nM of both universal FAM-labeled forward and NED-labeled reverse primer. Both PCR reaction mixes contained: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 250 mM of each deoxyribonucleoside triphosphate (dATP, dCTP, dGTP, and dTTP from Pharmacia), 30 ng genomic DNA or 0.5 µl specific PCR product and 0.6 U Ampli-Taq Gold DNA Polymerase (Applied Biosystems). The amplification for the specific PCR was conducted at 95°C for 10 min, then 35 cycles at 95°C for 15 s and 55 –62.5°C for 15 s and 72°C for 30 s followed by 1 cycle at 72°C for 10 min. The amplification for the universal PCR was conducted at 95°C for 10 min, then 35 cycles at 95°C for 15 s and 58°C for 15 s and 72°C for 30 s followed by 1 cycle at 72°C for 10 min. GC-rich templates were amplified with the original ThermalAce DNA Polymerase Kit from Invitrogen with the original protocol. The PCR amplifications were performed in a 9700 Thermocycler (Applied Biosystems).

Of the universal-labeled PCR product 3 µl was mixed with 10.5 µl Formamide (Applied Biosystems) and 0.5 NaOH (0.3 N). The mixture was denatured at 95°C for 3 min and then rapidly cooled on ice for 2 min. The FAM- and NED-labeled single-strand conformation polymorphism product was separated on the ABI Prism 3100 Capillary electrophoresis genetic analyzer (Applied Biosystems). The samples were electroinjected at 15 kV for 10 s to a 36-cm-long capillary (Applied Biosystems) filled with a filtered polymer containing 5% GeneScan polymer and 10% Glycerol in 1× Tris-borate-EDTA buffer. Electrophoresis was at 13 kV at temperature of 30°C. The signals and resulting data were analyzed for peak color and peak pattern using Collection software V.3.7 and the GeneScan Analysis software V.3.7. Genotype/allele frequencies were determined with the SNaPshot Multiplex Kit (Applied Biosystems) with the original protocol and the specific PCR products as template. Oligonucleotides used for amplification and SNaPshot Multiplex reaction are available on our website (http://www.fvk-berlin.de/fvkweb/fvkindex.html).

The association between genotypes of an SNP and QTc interval was tested with a polygenic model [7]. This model was implemented in an unpublished program. Mean values are modeled according to genotype groups and covariates such as age and sex. The genetic relationship between twins is reflected in the covariances. Restricting the model to equal mean values for all genotypes allowed us to test the significance of association. For the data in our study the model contains five mean components and three variance components. The covariates were sex and age. The estimated mean components were μ_{11}, μ_{12}, and μ_{22} for the three genotypes, μ_{sex} (male or female) to distinguish between the different gender offsets and μ_{age} as age regression coefficient. For example, a 30-year-old woman with a heterozygous genotype has the expected QTc interval $\mu_{12} + \mu_{male} + 30\ \mu_{age}$. As variance components we estimated the covariance between monozygotic twins, the covariance between dizygotic twins, and the remaining environment variance. The maximum of the log-likelihood was estimated one time under the full model (five mean components, three variance components) and one time under the restriction $\mu_{11}=\mu_{12}=\mu_{22}$ (all genotypes are equal). With these two values a log-likelihood ratio test with two degrees of freedom was conducted.

Results

We found 35 SNPs distributed in different loci of five long QT genes. Altogether 27,091 bp were examined for the presence of SNPs in five long QT genes over the intron/exons boundaries. Table 1 presents the genes, the SNPs, the SNP position, the SNP name from the database (when available) and the allele frequencies. According to the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov), Celera (http://www.celera.com), and the inherited arrhythmias database (http://pc4.fsm.it:81/cardmoc), we found that 10 of 35 SNPs were unknown. When the number of SNPs is considered in relation to the scanned range, we found an average of one SNP per 775 bp of human genomic DNA in this normal white population. Figure 1 shows a schematic structure of KCNQ1 (LQT1), HERG (LQT2), SCN5A (LQT3), KCNE1 (LQT5), and KCNE2 (LQT6) and the mapped SNPs. For those in the solid figures, a significant association was found. Thirteen of the 35 SNP were in the coding range. The remaining 22 SNPs were located in introns (17 SNPs) or in 5′- and 3′- untranslated regions (5 SNPs). The minor allele frequency ranged between 50% for SNP no. 9 and 7 and 1% for SNP nos. 3, 4, 6, 17, 19, and 28.

We performed an analysis to seek for association between variants and QTc interval. We found four SNPs that showed a promising association. The results of these SNPs are shown separately in Fig. 2. For the four SNPs the values of the mean components that were calculated by our maximum likelihood estimation are shown in

Table 1 Characteristics of found and mapped single-nucleotide polymorphism(s) (*SNPs*) in a normal white population. Given are different properties of the SNPs found in five different long QT syndrome genes (KCNQ1, HERG, SCN5A, KCNE1, KCNE2). The nomenclature for the SNP position was used according to den Dunnen and Antonarakis [22]. The SNP frequencies were determined using 188 DNA samples (94 pairs of monozygotic twins, 47 pairs of dizygotic twins; since the monozygotic twins are identical, only one subject from each pair was genotyped). (NC noncoding, *HW* Hardy Weinberg distribution, Nsyn nonsynonymous, *iad* inherited arrhythmias database, *PIC* polymorphism information content

SNP no.	Gene name	SNP region	Base	Coding	SNP position	SNP database	HW χ^{2a}	PIC	SNP frequency
1	KCNE1	Intron2	T/C	NC	IVS2–129 T>C	rs2236609	2.79	0.37	0.41
2	KCNE1	Intron2	G/A	NC	IVS2–128 G>A	–	0.05	0.03	0.02
3	KCNE1	Intron2	T/C	NC	IVS2–59 T>C	rs2236608	0.03	0.03	0.01
4	KCNE1	Exon3	G/A	Syn S28S	C.84 G>A	iad	0	0.01	0.01
5	KCNE1	Exon3	A/G	Nsyn G38S	C.112 A>G	rs1805127	0.76	0.36	0.38
6	KCNE1	Exon3	G/A	Nsyn D85 N	C.253 G>A	rs1805128	0.02	0.02	0.01
7	KCNE1	3′UTR	A/G	NC	C*124 A>G	rs2070357	2.14	0.37	0.5
8	KCNE1	3′UTR	A/G	NC	C*132 A>G	–	0.17	0.06	0.03
9	KCNE1	3′UTR	C/T	NC	C*456 C>T	rs2070356	1.75	0.37	0.50
10	KCNE1	3′UTR	G/C	NC	C*480 G>C	–	0.59	0.1	0.05
11	HERG	Exon6	C/T	Syn I489I	C.1467 C>T	rs740952	0.38	0.26	0.19
12	HERG	Exon6	C/T	Syn F513F	C.1539 C>T	rs1805120	0.51	0.26	0.19
13	HERG	Exon8	T/C	Syn T652T	C.1956 T>C	rs10226664	0.32	0.37	0.43
14	HERG	Intron8	G/C	NC	IVS8+39 G>A	rs2072412	1.52	0.32	0.28
15	HERG	Intron8	G/A	NC	IVS8+40 G>A	rs2072413	1.52	0.32	0.28
16	HERG	Exon11	A/C	Nsyn K897T	C.2690 A>C	rs1805123	0.08	0.3	0.25
17	KCNQ1	Exon11	G/C	Nsyn S484T	C.1451 G>C	–	0	0.01	0.01
18	KCNQ1	Intron11	A/G	NC	IVS11+46 A>G	rs760419	2.95	0.37	0.44
19	KCNQ1	Intron11	C/G	NC	IVS11+50 C>G	–	0	0.01	0.01
20	KCNQ1	Intron12	T/C	NC	IVS12+14 T>C	iad	0.33	0.18	0.11
21	KCNQ1	Exon13	G/A	Syn S546S	C.1638 G>A	rs1057128	0.05	0.27	0.20
22	KCNQ1	Intron13	G/A	NC	IVS13+36 G>A	rs163150	1.14	0.34	0.32
23	KCNQ1	Intron14	T/C	NC	IVS14+43 T>C	rs81204	4.68	0.28	0.22
24	KCNQ1	Intron15	G/T	NC	IVS15+32 G>T	iad	1.22	0.13	0.07
25	KCNE2	5′-UTR	G/A	NC	C-77 G>A	–	1.31	0.13	0.08
26	KCNE2	Intron1	C/T	NC	IVS1–44 C>T	rs9305548	0	0.25	0.18
27	KCNE2	Intron1	A/G	NC	IVS1–16 A>G	–	0.48	0.09	0.05
28	KCNE2	Exon2	A/G	Nsyn T8A	C.22A>G	rs2234916	0.22	0.02	0.01
29	SCN5A	Exon2	G/A	Syn A29A	C.87 G>A	rs6599230	2.03	0.24	0.17
30	SCN5A	Intron9	C/A	NC	IVS9–3 C>A	–	0.58	0.21	0.14
31	SCN5A	Intron10	G/A	NC	IVS10–24 G>A	rs7428779	2.65	0.22	0.15
32	SCN5A	Exon12	A/G	Nsyn H558R	C.1673 A>G	rs1805124	1.1	0.27	0.20
33	SCN5A	Exon17	G/A	Syn E1061E	C.3183 G>A	rs7430407	2.2	0.19	0.12
34	SCN5A	Intron24	T/C	NC	IVS24+53 T>C	–	0.33	0.1	0.05
35	SCN5A	Intron24	G/A	NC	IVS24+116 G>A	–	4.17	0.19	0.12

[a] 95% confidence interval

Table 2. We estimated an offset of the QTc interval for each SNP genotype. These estimations range from 347 ms for SNP no. 2 (KCNE1) with the genotype AG to 403 ms for SNP no. 28 (KCNE2) with the genotype AG. However, we only had seven individuals with this genotype. Additionally, we obtained expected values for the gender-related differences in the QTc intervals. In our sample women had on average 18 ms longer QTc intervals than men. The age regression coefficient showed that the increase in QTc intervals amounts 0.3 ms to 0.4 ms per year, independently of sex and genotype. Based on these estimates a 30-year-old woman with genotype GA in SNP no. 32 (SCNA5) has an expected QTc interval of $364+18.1+30\times0.42=394.7$ ms. If she has the genotype GG in the same SNP, we expected a QTc interval of $382+18.1+ 30\times0.42=412.7$ ms.

We report 35 SNPs from five long QT genes that were analyzed in 188 normal subjects. Ten SNPs in our normal subjects have not been described previously. Four SNPs were promising in terms of a suggestive odds ratio or an association with QT interval in these normal subjects. We suggest that these SNPs will be useful for much larger studies to test the notion that SNPs and haplotypes from normal individuals can predict QT interval in the general population. We believe that our SNP catalog can provide a resource for more comprehensive family and case-control studies in the normal population to establish haplotype patterns in multiple genes that could potentially predict risk of sudden death in putatively normal persons.

In our earlier linkage study on long QT loci [5] we found significant linkage of QTc with LQT1. In our current study we found no evidence for association with QT interval for the gene residing at this locus. There are two possible explanations for this finding. Either a different gene in close proximity to LQT1 is the actual cause of the linkage phenomenon. Alternatively, other as yet undiscovered SNPs in LQT1 are of functional relevance. Given the known relevance of LQT1 for the long QT

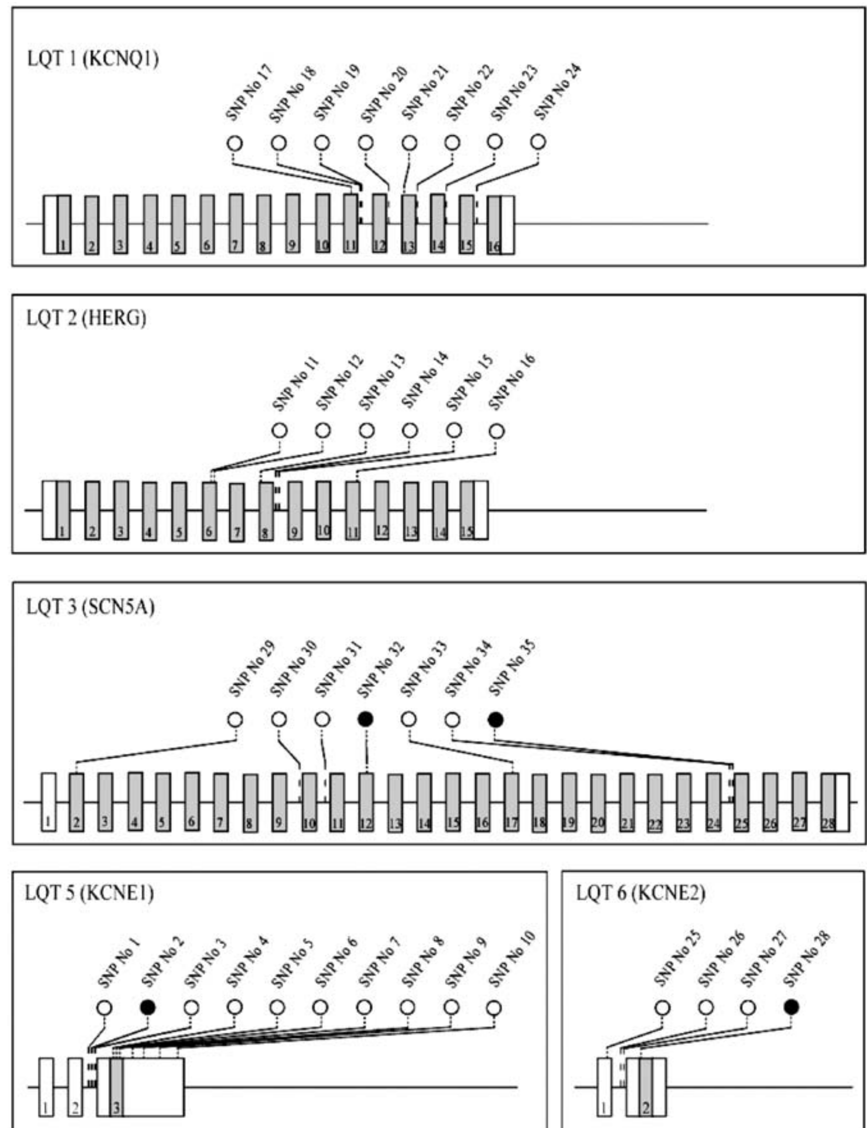

Fig. 1 Schematic structure of the encoding genes causing long QT syndrome and an overview about found and physically mapped single-nucleotide polymorphisms (*SNPs*). *Gray numbered squares* Coding exons; *white squares* untranslated 5′ and 3′ regions. *White circle* Found and mapped SNPs in the white population. *Filled circles* SNPs that are significantly associated whit the long QTc-interval

syndrome the later hypothesis is more likely. Regulatory elements outside the currently screened genomic regions may harbor those genetic variations. We should also point out that the numbers of subjects in this study were small. It is conceivable that we do not have sufficient statistical power to find weaker associations.

Contrary to the finding of no association with significant linkage in LQT1, our current study yielded evidence for association of LQT3 and LQT5 despite the fact that no linkage to those regions was found in our earlier study. There are a number of methodological reasons for this. In general the power of association studies is greater than that of linkage [8]. Furthermore, in our current study our sample of monozygotic twins was included, although they yielded no information for linkage. By increasing the sample size we could further increase the power. The relevant gene in the LQT4 region has only recently been cloned and published [9]. This gene, together with LQT7, will be screened in a future project.

We are clearly not the first to study SNPs in long QT genes. Ackerman et al. [10] focused on nonsynonymous SNPs in four long QT genes. They were specifically interested in ethnic diversity. Four of their nonsynonymous SNPs in KCNE1 (SNP nos. 5 and 6), HERG (SNP no. 16), and KCNE2 (SNP no. 28) are also reported here. Ackerman et al. [10] did not record electrocardiography, and QTc was not measured in their subjects. The SNP no. 28 in KCNE2 was found to cause a novel QT interval prolongation in response to sulfamethoxasole. Sesti et al. [11] found this SNP in 1.6% of their population. We found the SNP in 1% of our subjects. Paulussen et al. [12] focused on patients with drug-induced long QT syndrome. They found three SNPs causing missense mutations in KCNE1, KCNE2, and HERG. We found the former two SNPs in our normal subjects (SNP no. 6 and 28), but not the missense-causing SNP in HERG. The group then studied 32 patients and 32 control subjects who were screened for SNPs. They found 25 SNPs in the cases and identified 18

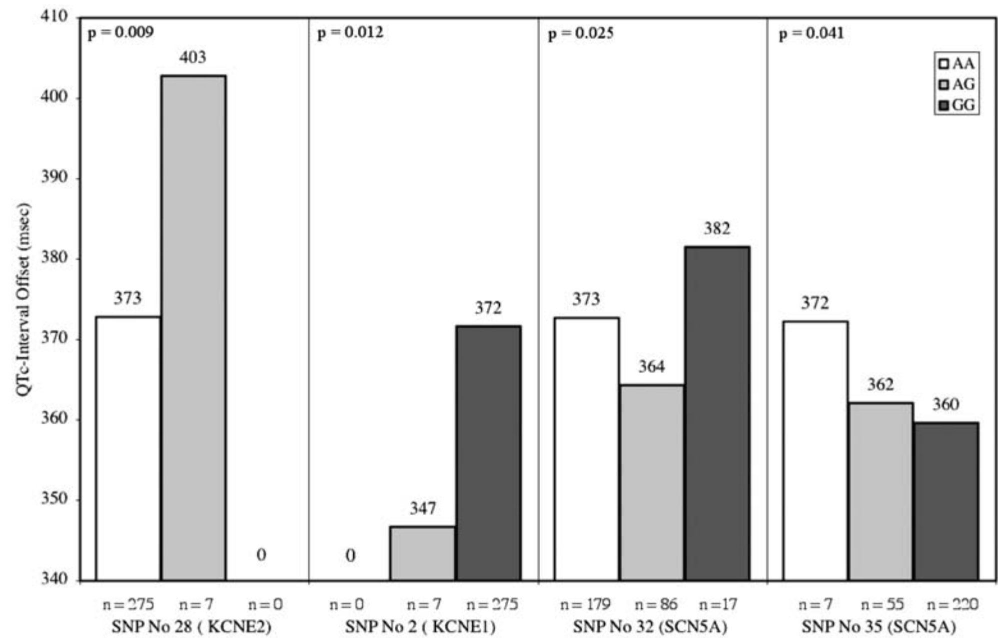

Fig. 2 Estimated genotypic QTc Offsets. *Numbers under each bar graph* Genotyped samples for the SNP nos. 28, 2, 32, and 35 (compare with Table 1)

Table 2 Estimated parameter by the application of the polygenic model

SNP no.	Genotype			Offset for female sex	Age regression coefficient
	AA	GA	GG		
28 (KCNE2)	373	403	–	18.7	0.31
2 (KCNE1)	–	347	372	18.4	0.39
32 (SCN5A)	373	364	382	18.1	0.42
35 (SCN5A)	372	362	360	18.5	0.42

of these SNPs in controls. Yang et al. [13] also studied SNPs in patients with drug-induced long QT syndrome. They found SNPs in HERG and SCN5A. However, no difference in allele frequency was found when they compared cases to controls. We also found the SNPs in our subjects (SNP nos. 16 and 32).

Bezzina et al. [14] focused on the nonsynonymous SNP C2690 A>C in HERG, also reported here (SNP no. 16). They found that the homozygous C/C genotype led to a shorter QTc than the other two genotypes. This association was found only in women. Our current study included no sex stratification due to power limitations. Fernandez et al. [15] studied two SNPs in HERG. They used site-directed mutagenesis and patch clamp physiology to study the effects of their mutations. One of these SNPs appeared in our normal subjects (SNP no. 13). Viswananthan et al. [16] focused on SCN5A. They described two SNPs that occurred in a person with symptomatic bradycardia and conduction defects. The individual belonged to a small family that was then studied. Interestingly, one of these SNPs, the nonsynonymous variant H558R also occurred in our normal subjects and was associated with QTc, as shown in Fig. 2. The homozygous (G/G) form had a QTc of 382 ms in our normal subjects.

We did not study all the available long QT genes, and more genes should be added. We have not yet completed analysis of *ANKB* that codes for ANKRIN-β [9]. Nor have we analyzed KCNJ2 that codes for the I_{Kr} K^+ channel α-subunit [17]. Both these genes were cloned recently and obviously need to be included in a comprehensive analysis. Also missing are two calcium signaling molecules, ryanodine receptor 2 and calsequestrin 2, which may cause catecholaminergic polymorphic ventricular tachycardia [18]. Three mutations in ryanodine receptor 2 and two in calsequestrin 2 have been described. More genes relevant to QT interval will undoubtedly be reported in future studies.

To test the hypothesis that common variants in disease genes can influence the QT phenotype in the normal population would require large numbers of normal persons phenotyped in terms of their electrocardiographic findings. Such a dataset would then allow haplotype estimation and analyses that were not included in this screening study due to power limitations. We have performed similar studies in an analysis of lipid metabolism in normal subjects. The aim of those studies was to find common haplotypes in genes relevant to lipid metabolism to gain inference to the genetic variance in low- and high-density lipoprotein cholesterol. We found that an SNP and subsequent haplotype analysis of chlolesterol ester transfer protein, lipoprotein lipase, hepatic tryglyceride lipase, apoprotein E, and lechithin-cholesterol acyltransferase accounted for more than 40% of the genetic variance in an analysis of 732 persons from 184 German families [19]. We have subsequently expanded this analysis to include 13 genes and now account for more than 70% of the genetic variance for low- and high-density lipoprotein. Moreover, we can explain almost all of the genetic variance in the clinically relevant ratio of low- to high-density lipoprotein [20].

Candidate gene association studies lend themselves for studying complex genetic conditions. A recent study focusing on type 2 diabetes mellitus focused on 15 genes encoding molecules known to primarily influence pancreatic β-cell function [21]. They found that five, namely ABCC8 (sulfonylurea receptor), KCNJ11 (KIR6.2), SLC2A2 (GLUT2), HNF4A (HNF4α), and INS (insulin), significantly altered disease risk. In three genes, the risk allele, haplotype, or both, had a biologically consistent effect on a relevant physiological trait. To obtain these insights the investigators studied 2,134 whites in a case-control study. They subsequently investigated an independent quantitative trait cohort. For diabetes the investigators established the absence of large single-gene effects and the detection of multiple small effects that accentuate the need for the study of large populations.

We suggest that a similar approach is necessary to study QT interval in the normal population. We found in our lipid studies that family investigations are helpful in providing the most information on genetic influences of the SNPs and haplotypes [19, 20]. Subsequently, however, large confirmatory cohorts will be necessary. These cohorts should probably first consist of normal persons to delineate the effect of haplotypes and SNPs and multiple loci on the distribution of QT interval in the general population. Thereafter, when important haplotypes, SNPs, and their interactions are known, populations in which sudden cardiac death is common could be investigated. Large-scale intervention trials of patients with congestive heart failure, in whom sudden cardiac death is common, might be helpful in such investigations. The purpose of our report is to facilitate such investigations. We do not have at our disposal a population with computerized electrocardiograms to pursue this issue further. We hope that with a comprehensive SNP map of at least these five genes, coupled with others, such investigations will become possible.

Finally, we draw attention to possible clinical relevance for characterizing common variants in the long QT genes influencing QT interval. A host of environmental factors influence QT interval [4]. Common examples include macrolide antibiotics and neuroleptic drugs. Women have longer QT intervals than men, and the phase of the menstrual cycle affects QT interval. The pharmaceutical industry is currently required to conduct detailed testing on any product regarding its effect the QT interval in normal persons. We hypothesize that common variants probably influence QT responses to environmental factors in the normal population. Knowing these variants would be of great value in predicting risk and would facilitate identifying candidates for testing. The SNPs presented here will facilitate future investigations.

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