Original Article

Genetic Modifiers for the Long-QT Syndrome
How Important Is the Role of Variants in the 3′ Untranslated Region of *KCNQ1*?

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Background—Long-QT syndrome is an inherited cardiac channelopathy characterized by delayed repolarization, risk of life-threatening arrhythmia, and significant clinical variability even within families. Three single-nucleotide polymorphisms (SNPs) in the 3′ untranslated region of *KCNQ1* were recently suggested to be associated with suppressed gene expression and hence decreased disease severity when located on the same haplotype with a disease-causing *KCNQ1* mutation. We sought to replicate this finding in a larger and a genetically more homogeneous population of *KCNQ1* mutation carriers.

Methods and Results—The 3 SNPs (rs2519184, rs8234, and rs10798) were genotyped in a total of 747 *KCNQ1* mutation carriers with A341V, G589D, or IVS7-2A>G mutation. The SNP haplotypes were assigned based on family trees. The SNP allele frequencies and clinical severity differed between the 3 mutation groups. The different SNP haplotypes were neither associated with heart rate–corrected QT interval duration (QTc) nor cardiac events in any of the 3 mutation groups. When the mutation groups were combined, the derived SNP haplotype of rs8234 and rs10798 located on the same haplotype with the mutation was associated with a shorter QTc interval (*P*<0.05) and a reduced occurrence of cardiac events (*P*<0.01), consistent with the previous finding. However, when the population-specific mutation was controlled for, both associations were no longer evident.

Conclusions—3′ Untranslated region SNPs are not acting as genetic modifiers in a large group of LQT1 patients. The confounding effect of merging a genetically and clinically heterogeneous group of patients needs to be taken into account when studying disease modifiers. (***Circ Cardiovasc Genet***. 2016;9:330-339. DOI: 10.1161/CIRCGENETICS.116.001419.)

Key Words: arrhythmias ■ genes, modifier ■ KCNQ1 potassium channel ■ long-QT syndrome ■ untranslated region

The long-QT syndrome (LQTS), probably the best-known genetic disorder causing life-threatening arrhythmias,[1–3] has become a useful paradigm to study sudden cardiac death in the young.[4] An intriguing feature of LQTS is its incomplete penetrance[5] and variable expressivity which are commonly observed even among members of the same family, all carriers of the same mutation. There is a consensus that this variability reflects, to a large extent, the presence of additional genetic factors usually referred to as modifier genes.[6] The search for modifier genes has already met with success and has identified several modifiers which, while not being disease causing, contribute to the modulation of the clinical phenotype by either increasing[7–11] or decreasing[12] arrhythmic risk and QT interval duration.

Clinical Perspective on p 339

In 2012, significant interest was generated by a publication by Amin et al,[13] who reported that single-nucleotide polymorphisms (SNPs) in the 3′ untranslated region (UTR) of *KCNQ1*, the gene responsible for LQT1,[2] were associated with a modulation of QT duration and arrhythmic risk for LQT1 patients. The 3′ UTR is, in every gene, a region downstream its coding

DOI: 10.1161/CIRCGENETICS.116.001419

exons, which becomes part of the transcript (mRNA) but is not translated into protein. Figure 1 shows the structure of the KCNQ1 mRNA and the role of the UTR in post-transcriptional regulation of gene expression. Amin et al[13] reported that 3 SNPs (rs2519184, rs8234, and rs10798) located in the UTR of the gene did modulate *KCNQ1* expression in an allele-specific manner. Specifically, rs2519184 A, rs8234 G, and rs10798 G and their haplotype combinations would suppress the expression of the translated normal or LQT1-mutation–containing allele located on the same homologous chromosome (*cis* configuration). As a consequence, the presence of this UTR genetic variation in *cis* to the LQT1-causing allele would be associated with shorter QTc and fewer cardiac events (CEs); conversely, the opposite location, in *trans* configuration to the LQT1-mutation–containing allele and therefore in *cis* with the normal allele, would be associated with a more severe LQTS phenotype. This intriguing observation on the role of 3′ UTR SNPs in influencing both QT and cardiac events (CEs) was made in 168 LQT1 patients with 33 different mutations from 41 different families followed in 2 leading referral centers.[13]

With the simple aim to verify and reproduce the modifying role of 3′ UTR SNPs, we studied 3 large and well-characterized LQT1 founder populations.[14–19] These populations represent an ideal model to test the role of modifying factors[6] because they are homogenous for the disease-causing founder mutation, which is the major determinant of risk. Our results, entirely unanticipated, were clearly differing from those of Amin et al[13] and provide novel insights that might prove useful for the study of modifier genes.

Methods

Study Population

Overall, we studied 3 independent LQT1 founder populations: one from South Africa[14–16] and 2 from Finland[17–19], all previously described in detail. Each one includes a large number of LQTS individuals, clustered in families all related to a single ancestor and, thereby, carrying the same disease-causing mutation. LQT1 genotype-positive individuals from the 3 founder populations were classified as either symptomatic or asymptomatic according to a previous experience of CEs. CEs were syncope (fainting spells with temporary but complete loss of consciousness), aborted cardiac arrest (requiring resuscitation), documented life-threatening arrhythmias, and sudden cardiac death. To enter the study, the mutation carriers had to have DNA available, a clearly defined symptomatic/asymptomatic status, and an ECG. Patients taking any known QT-prolonging medication at the moment of ECG recording and patients known to be compound or double heterozygous for LQTS mutations were excluded. However, the presence of other mutations was not systematically screened for. A detailed family tree was also required for the ascertainment of phase in the families. On the basis of these

criteria, we enrolled 142 SA-A341V patients, 535 Finn-G589D, and 70 Finn-IVS7-2A>G (c.1129-2A>G) mutation carriers (MCs). The mutations were named according to the *KCNQ1* reference sequence NM_000218.2.

All probands and family members provided written informed consent for clinical and genetic testing. Protocols were approved by the ethical review boards of Helsinki University Hospital, Tygerberg Hospital of Stellenbosch University, the University of Pavia and the Vanderbilt University. Approved consent forms were provided in Finnish, English, or Afrikaans as appropriate.

Genetic Analysis

The 3′ UTR SNPs (rs2519184, rs8234, and rs10798) of *KCNQ1* were characterized through PCR and direct sequencing using primers 5′-CTTCCTGAGGGGGAGACAGAGC-3′ and 5′-GGAACCAAGGTGAGAGCAGTG-3′ for the Finnish population to be able to genotype all 3 variants with a single primer pair, whereas the South African population was genotyped using the same primers reported by Amin et al.[13] Investigation of the family trees allowed the identification of the haplotype in *cis* position with the disease-causing mutation for each LQT1 mutation carrier (MC). The complete structure and sizes of pedigrees and strong linkage disequilibrium (LD) among SNPs permitted a precise haplotype determination without the need of computational methods of inference.

Statistical Methods

Normally distributed QTc values are summarized as mean±SE, and categorical variables are presented as absolute and relative frequencies. Their comparison across groups defined by their *KCNQ1* genotype and 3′ UTR SNP haplotype arrangements was performed by univariate and multivariate analysis. For QTc, linear mixed effects regression models were used, whereas for comparing the clinical status—expressed as the prevalence of symptomatic MCs—mixed effects logistic regression models were performed. In both models, we controlled for the family relatedness among individuals. The R/lmekin function used for the linear mixed effects models was able to fit models with random family effects through a specific kinship matrix created ad hoc for the correlation among family members. The R/lme4 package managed the variance–covariance matrices of the random family effects via Maximum likelihood estimation. We have considered the observed haplotype configuration of the subjects as a categorical variable. Beta coefficients (and derived odds ratios in the logistic model), precision, and statistical significance were estimated comparing presence of each alternative haplotype configuration with respect to the presence of the haplotype configuration GAA/GAA (reference category). Furthermore, regression models were also adjusted for sex- and population-specific mutations (included as a categorical variable, reference category SA-A341V mutation). Two-sided *P* values <0.05 were considered statistically significant. Calculations were performed using the statistical software SPSS Statistics, version 21 (IBM Co, Armonk, NY) and R, version 3.2.1[20] and its packages kinship2,[21] coxme,[22] and lme4[23].

Given the sample sizes, the study was adequately powered to detect effects similar to those previously published by Amin et al.[13]
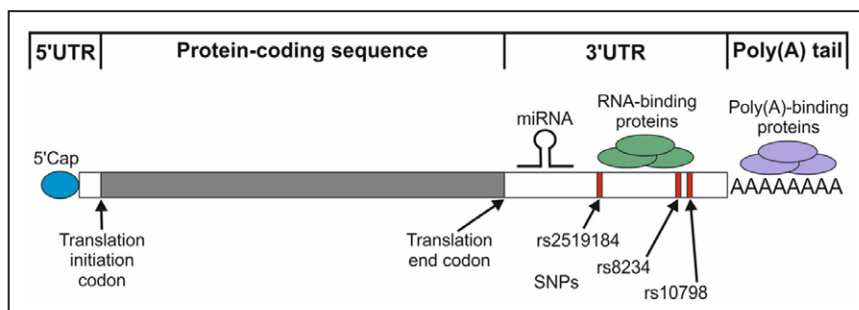

Figure 1. Schematic representation of the KCNQ1 mRNA structure and illustration of some post-transcriptional regulatory elements in eukaryotes. RNA-binding proteins or microRNAs (miRNA) may interact with specific sequence elements located in the untranslated regions (UTR), and this interaction may be modulated by sequence variants such as SNPs.

Table 1.   Clinical Characteristics of the Study Populations

	Founder Populations			
	SA-A341V	Finn-G589D	Finn-IVS7-2A>G	All
Mutation carriers, n	142	535	70	747
Female sex, n (%)	80 (56)	306 (57)	43 (61)	429 (57)
Families, n	22	77	16	115
Symptomatic, n (%)*	100 (70)	58 (11)	7 (10)	165 (22)
QTc, mean±SE, ms†	485±3.6	459±1.5	466±3.5	465±1.4

Data are absolute (n) and relative (%) frequencies or mean±SE. QTc, indicates heart rate–corrected QT interval.

*P<0.001, χ^2=238.

†P<0.001, ANOVA, F=30.8.

Results

The clinical and demographic features of the entire study population, grouped according to the type of the founder mutation, are shown in Table 1.

The 2 Finnish LQT1 founder populations, carrying the G589D and IVS7-2A>G mutation on *KCNQ1*, contributed the majority of MCs (n=535 and n=70, from 77 and 16 families, respectively). They were both characterized by a reasonably benign clinical course, as only 10% of the affected individuals experienced cardiac symptoms, and their mean QTc was only moderately prolonged (459±1.5 and 466±3.5 ms, respectively). Conversely, the South African LQT1-A341V population (n=142 MCs) was characterized by markedly prolonged mean QTc (485±3.6 ms) and by striking clinical severity[15]; indeed, 70% of the patients were symptomatic.

3′Untranslated Region SNPs

The SNPs under investigation were rs2519184, rs8234, and rs10798, all located in the 3′ UTR region of *KCNQ1*[13]. For the purpose of our study, and irrespective of their actual minor allele frequency in the specific founder populations, we called derived alleles the alleles of the 3 SNPs (A-G-G, respectively)

that in the study by Amin et al were suggested to functionally decrease the expression of the allele in *cis*. The wild-type alleles (G-A-A, respectively) were termed ancestral alleles in accordance with the study of Amin et al. For each of the 3 *KCNQ1* SNPs, within each of the 3 study populations, genotype and allele frequencies are reported in Table 2. Of note, although the derived allele frequencies were comparable between populations for the SNP rs2519184 (0.056, 0.05, 0.049 in Finn-G589D, Finn-IVS7-2A>G, and SA-A341V, respectively), they significantly differed for the SNPs rs8234 and rs10798 (0.56, 0.19, 0.14, respectively; Table 2). These 2 latter SNPs were in complete linkage disequilibrium in all MCs included in the study. The SNPs were analyzed separately and as haplotypes to closely replicate the methods of Amin et al.

KCNQ1 3′UTR SNPs and QTc

Among the 535 carriers of the G589D mutation, we tested the potential effect on QTc of the A allele of rs2519184, the G allele of rs8234, and the G allele of rs10798, depending on their specific location in *trans* or in *cis* position with respect to the *KCNQ1* mutation. As shown in Figure 2A and 2B, no significant association between QTc and any of the 3′ UTR SNPs was observed. Similarly, the allele-specific haplotype analysis of the 3 SNPs showed similar mean QTc values across all the represented haplotype combinations (Figure 2C).

When the allele-specific effects on QTc of the SNPs rs2519184, rs8234, and rs10798 were evaluated in the second, smaller Finnish founder population IVS7-2A>G (Figure 3), and in the South African A341V population (Figure 4), again no significant differences in mean QTc were observed. Accordingly, no modifying effect on QTc emerged from allele-specific haplotype analyses. For the few haplotypes shared between the 2 Finnish populations, no significant differences were observed.

These findings were confirmed when the analysis was performed using a linear mixed effects regression framework, while taking into account a random family effect and adjusting for sex (Table I in the Data Supplement).

Table 2.   *KCNQ1* 3′ UTR SNPs in the Study Populations

SNP ID	Alleles (Derived Allele in Bold)	Genotype	Finn-G589D (n=535)		Finn-IVS7-2A>G (n=70)		SA-A341V (n=142)	
			n (%)	Derived Allele Frequency	n (%)	Derived Allele Frequency	n (%)	Derived Allele Frequency
rs2519184	G/**A**	GG	475 (89)	0.056	63 (90)	0.05	128 (90)	0.049
		GA	60 (11)		7 (10)		14 (10)	
		AA	…		…		…	
rs8234	A/**G**	AA	93 (17)	0.56	43 (61)	0.19	101 (71)	0.14
		AG	285 (53)		27 (39)		41 (29)	
		GG	157 (29)		…		…	
rs10798	A/**G**	AA	93 (17)	0.56	43 (61)	0.19	101 (71)	0.14
		AG	285 (53)		27 (39)		41 (29)	
		GG	157 (29)		…		…	

Data are absolute (n) and relative (%) frequencies. Rounded percentages may not total 100. SNP indicates single-nucleotide polymorphism; and UTR, untranslated region.
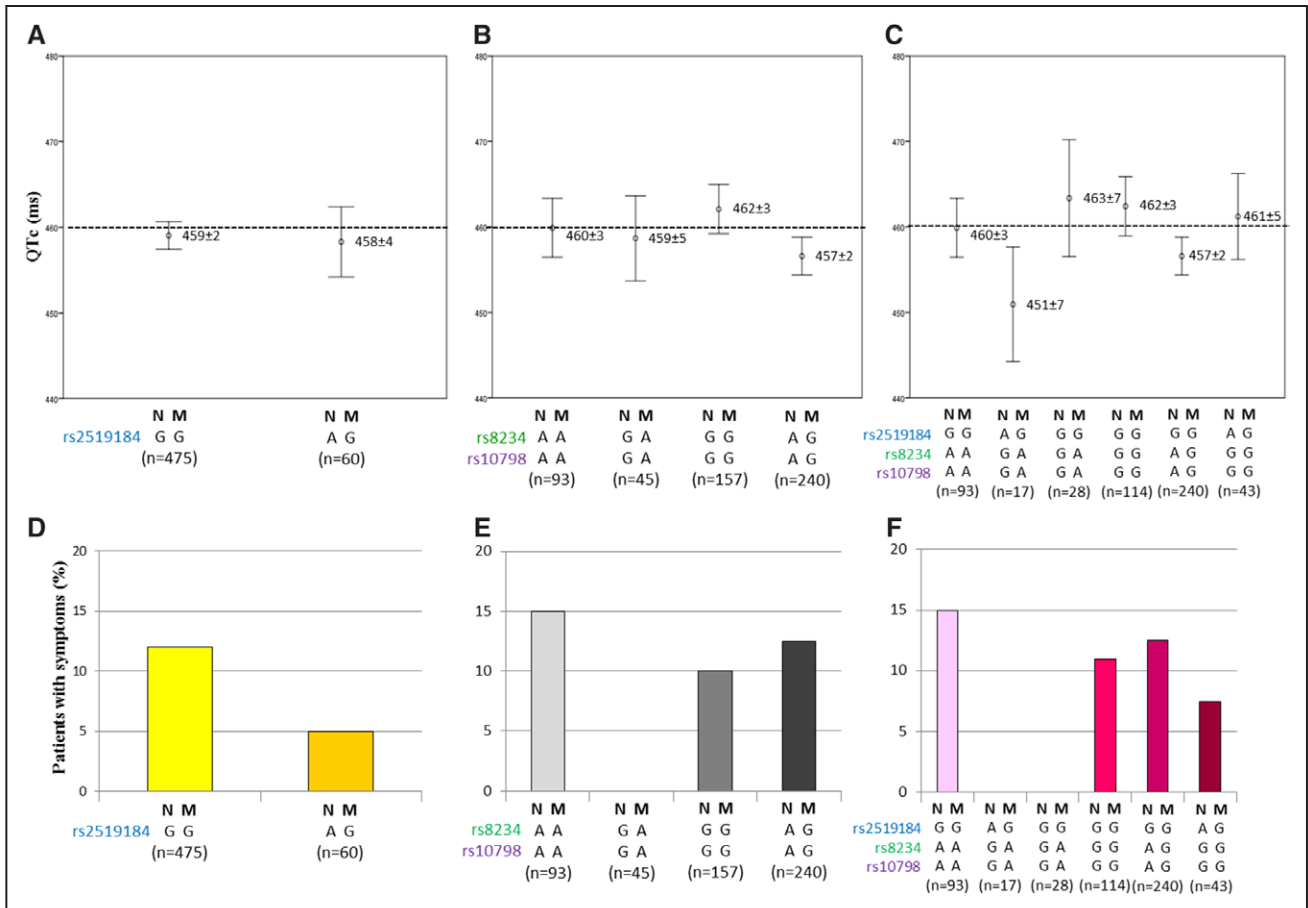
Figure 2. Allele-specific effects of SNPs rs2519184, rs8234, and rs10798 on QTc (**A–C**) and on symptoms (**D–F**) in the study populations of 535 Finnish carriers of the G589D mutation. Effects of single-nucleotide polymorphism (SNP) rs2519184 are displayed in (**A**) and (**D**); effects of SNPs rs8234 and rs10798 in linkage disequilibrium are displayed in (**B**) and (**E**); allele-specific haplotype analyses of the 3 SNPs are displayed in (**C**) and (**F**). For each SNP nucleotide can be either a G (guanine) or an A (adenosine) and can be in *cis* with the wild-type (N) or mutated allele (M). Dotted line is the mean QTc of all individuals within mutation subgroups. Numbers below genotype are group size. Data are mean±SE (bars) for QTc or percentages for symptoms. Values are from descriptive statistics. *P* values are taken from the multivariable models described in Tables 3 and 4.

KCNQ1 3′UTR SNPs and CEs

We then evaluated in each founder population the potential effect of the derived A-G-G alleles on the clinical status of *KCNQ1* MCs. Figures 2, 3, and 4, D through F, show the allele-specific effects of the individual SNPs and their allele-specific haplotype with regard to the occurrence of CEs. As previously observed for the QT interval, no significant differences in the prevalence of symptomatic MCs were observed according to the location of the A-G-G alleles in cis or trans to the mutation and also the trend was inconsistently variable across the genetic subgroups. These results were reproduced by a mixed effect logistic regression model, taking into account the clustering of individuals in families and adjusting for sex and QTc (Table II in the Data Supplement).

Effect of the 3′ UTR SNPs on QTc and Symptoms in the Pooled South African and Finnish Populations

As a next, and final step, we re-evaluated the potential effect of the allele-specific location of the SNPs rs2519184, rs8234, and rs10798, and of their haplotype combinations, on QTc (Figure 5 and Table 3) and the occurrence of CEs (Figure 5

and Table 4) in the large (n=747) group including all 3 study populations, irrespective of their own basal clinical severity and genetic structure. Among the individuals with the derived allele of rs2519184 (A) in *trans* to the mutated allele, both the QTc and the prevalence of symptoms was almost identical compared with those with the ancestral allele in trans to the mutated allele (Figure 5A and 5D). On the contrary, some interesting findings emerged from the comparison of the SNPs rs8234 and rs10798 across subtypes defined by genotype and by haplotype combinations (Figure 5B and 5E). Compared with the reference group, represented by homozygous carriers of A-A alleles, when the derived G-G alleles of rs8234 and rs10798 were in cis to the *KCNQ1* mutated allele, QTc was significantly shorter (457±2 and 462±3 versus 473±3 ms; *P*<0.05). This was consistent with the results reported by Amin et al.[13] Furthermore, a corresponding significant reduction in the prevalence of symptomatic MCs was observed when the derived G-G alleles of the SNPs rs8234 and rs10798 were located in *cis* to the mutation (10% and 12% versus 36%; *P*<0.01). The same QTc pattern and prevalence of symptoms were confirmed in the allele-specific haplotype analysis (Figure 5C and 5F).
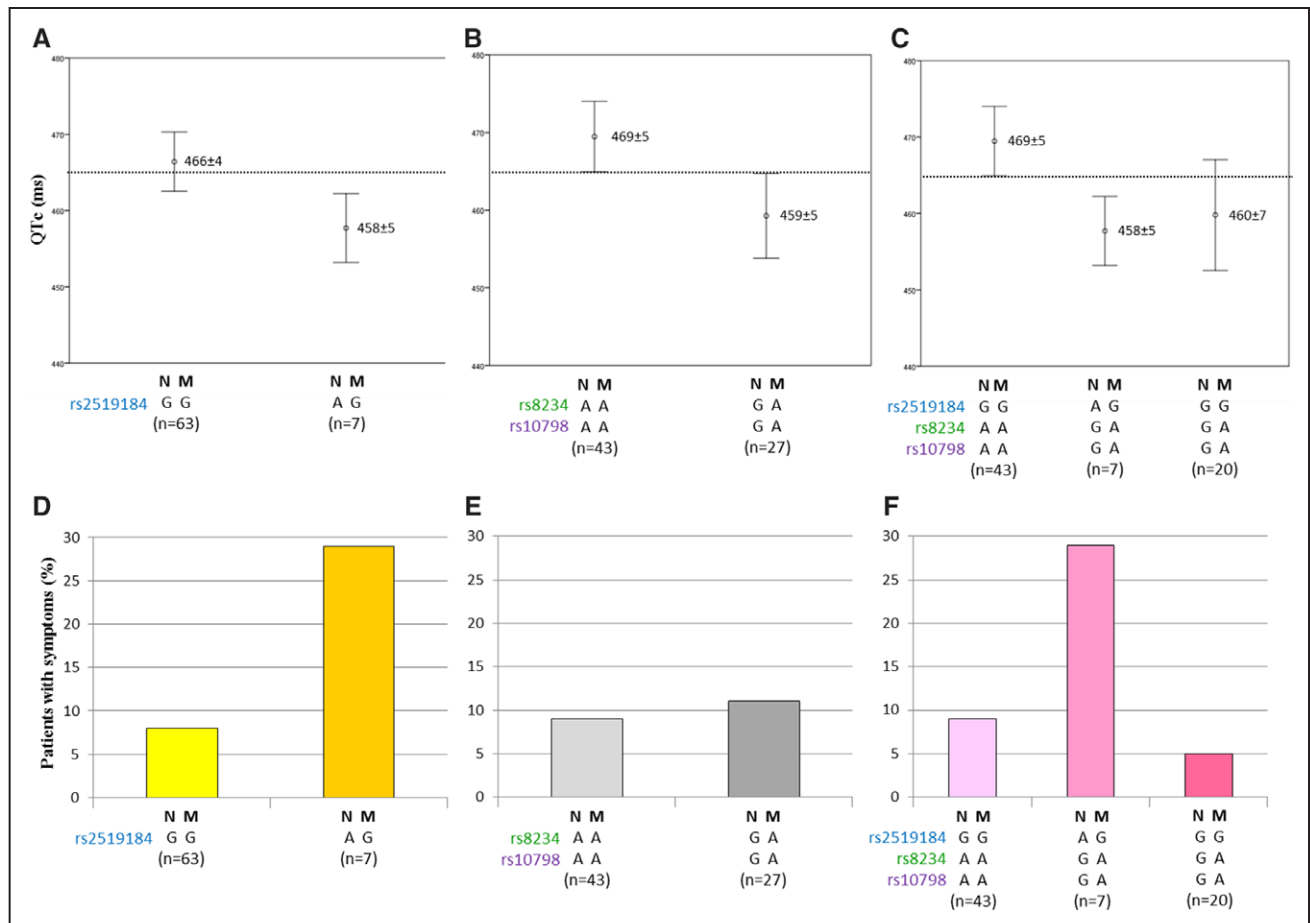
Figure 3. Allele-specific effects of single-nucleotide polymorphisms (SNPs) rs2519184, rs8234, and rs10798 on QTc (**A–C**) and on symptoms (**D–F**) in the study populations of 70 Finnish carriers of the IVS7-2A>G mutation. The scheme is the same used for Figure 2, but the numbers refer to the Fin IVS7-2A>G founder population. Data are mean±SE for QTc or percentages for symptomatology.

However, when the linear and logistic regression mixed models based on the combined data from all the 3 populations were also controlled for the population-specific LQT1 mutation, all the previously reported genotype–phenotype associations disappeared, the only factor remaining significantly associated with both QTc and clinical status being the mutation itself (Tables 3 and 4). Therefore, and key for the interpretation of the entire study, the differences among the 3 study populations in terms of both clinical severity and 3′ UTR SNPs allele/haplotype frequencies were likely to cause spurious associations when the confounding effect of merging the 3 populations was not appropriately controlled for.

Discussion

The main finding of the present study was our inability to confirm the report of Amin et al.[13] That study had raised major interest because of the exciting concept that the allele-specific location of certain SNPs in the 3′ UTR region of the *KCNQ1* gene could carry opposite effects depending on whether they were in *cis* or *trans* to the mutation in terms of arrhythmic risk and QT interval duration for LQT1 patients. Our data, collected in 3 large LQT1 founder populations with a different risk profile, suggest that when we observed differences, they

were the consequence of a spurious association caused by the population-specific mutations and, without proving it, raise the possibility that a similar problem might have influenced the conclusion by Amin et al.[13] The present results indicate that there is no evidence, at this time, that SNPs in the 3′ UTR region of the *KCNQ1* gene, act as modifier genes for all LQT1 patients. At the same time, our analyses provide concepts that might prove useful to avoid interpretative errors in the study of modifier genes.

Modifier Genes in LQTS

The major clinical interest for modifier genes goes beyond the important basic principles related to how genetic variants (probably relatively common) modify the effect of a disease-causing mutation. Indeed, this interest originates by a simple, but by no means trivial, question that faces every clinical cardiologist dealing with inherited arrhythmogenic disorders: why is it that of 2 siblings with the same mutation, one dies suddenly at an early age and the other goes through life without symptoms?

As LQTS undeniably represents the best example of how tight can the relationship between genotype and phenotype be[24], it should come as no surprise that the search for modifier genes for LQTS pioneered the field and has already met with
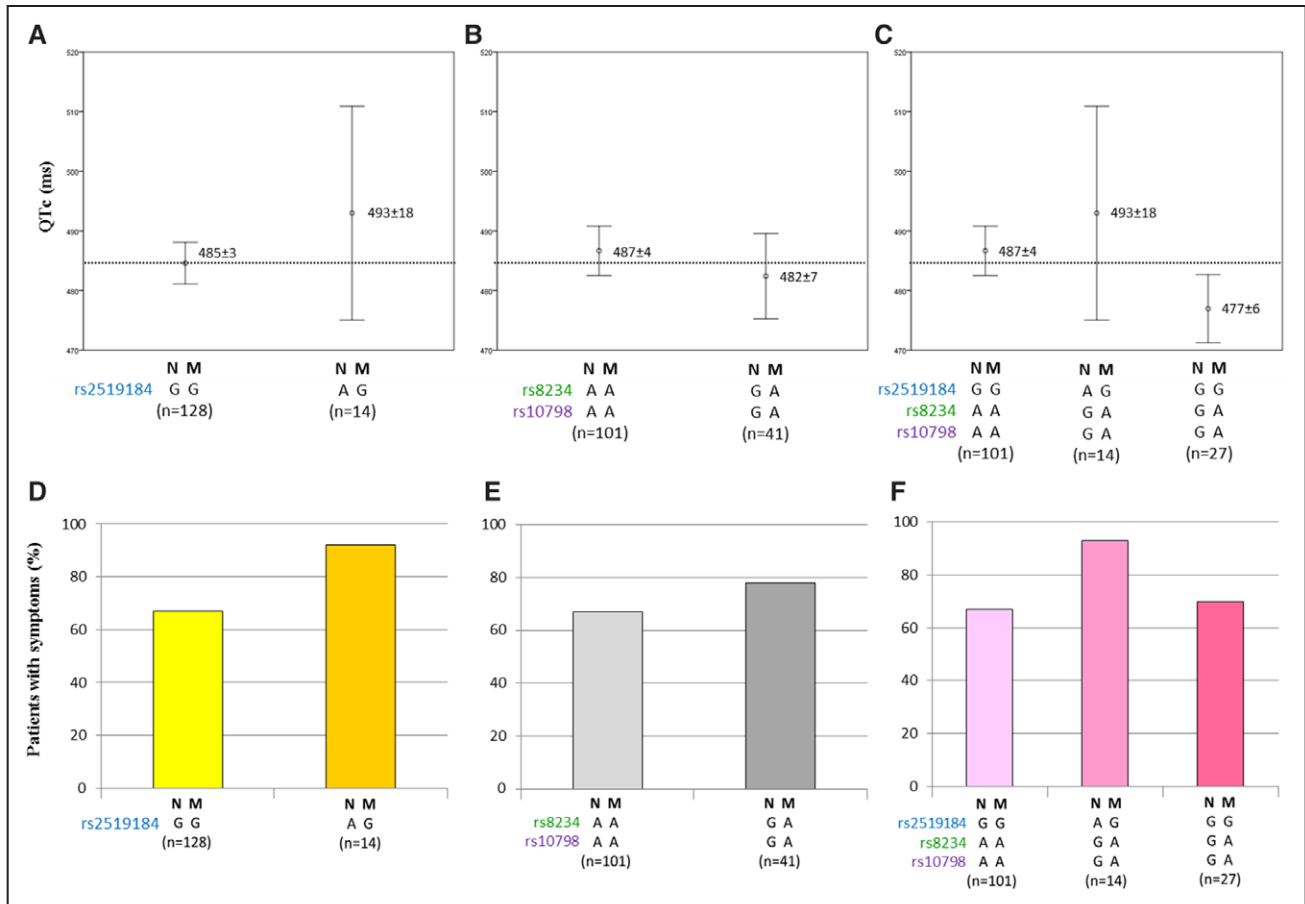
Figure 4. Allele-specific effects of SNPs rs2519184, rs8234, and rs10798 on QTc (**A–C**) and on symptoms (**D–F**) in the study population of 142 South African carriers of the A341V mutation. The scheme is the same used for Figures 2 and 3, but the numbers refer to the SA-A341V founder population. Data are mean±SE for QTc or percentages for symptoms.

some significant success. Most of the modifiers identified to date increase risk[7–11] but also protective ones have been identified.[12] To know whether or not a LQTS patient also carries identified modifiers allows the competent clinician to modulate the management in a more or less aggressive way, to tailor the specific risk for the individual patient.

This is why the report by Amin et al,[13] pointing to a new mechanism by which genetic variants could modify the effect of a mutation, has generated such a large interest. It also explains why we tried to confirm this finding in founder populations carrying the same disease-causing mutation.

An Intriguing Hypothesis

Only a small part of the DNA (< 2%) encodes proteins while the function of the more prevalent noncoding part is still largely unknown. Intronic regions that are transcribed but not translated into proteins, located upstream and downstream a given gene, are known, respectively, as 5′ and 3′ UTR. They have a regulatory role in gene expression, especially in the control of mRNA stability and translation.[25,26]

Amin et al[13] showed that 3 SNPs in the 3′ UTR of *KCNQ1* and specifically, rs2519184 A, rs8234 G, and rs10798 G (derived alleles) induce a lower expression of the *KCNQ1* allele in *cis*, compared with rs2519184 G, rs8234 A, and rs10798 A (ancestral alleles). They demonstrated this regulatory role

experimentally by testing the luciferase activity in neonatal rat cardiomyocytes and H10 cells transfected with plasmids containing the different 3′ UTR SNPs and their haplotype combinations. They tested these experimental results at clinical level and found indeed that when the derived SNP alleles were in *cis* with the nonmutated allele, implying that the mutated allele was more expressed than the nonmutated one, the patients had a longer QTc and an increased arrhythmic risk. By contrast, when the derived alleles were in *cis* with the mutated allele, implying that the mutated allele would be less expressed than the wild type, the clinical manifestations were less severe. These data were based on 168 LQT1 subjects from 41 different families with 33 mutations evaluated in 2 major referral centers.

Long Way to Replication

Initially, the rather simple idea of our 2 research groups was to confirm this exciting finding in our well-characterized founder populations[14–19] because of the possibility that, by avoiding the inherent variability caused by different mutations, we might have come up with even more impressive results because of the high power provided by the large number of carriers of the same mutation.

In the *KCNQ1* IVS7-2A>G (Finland) and the *KCNQ1* A341V (South Africa) populations, the G-A-A haplotype was
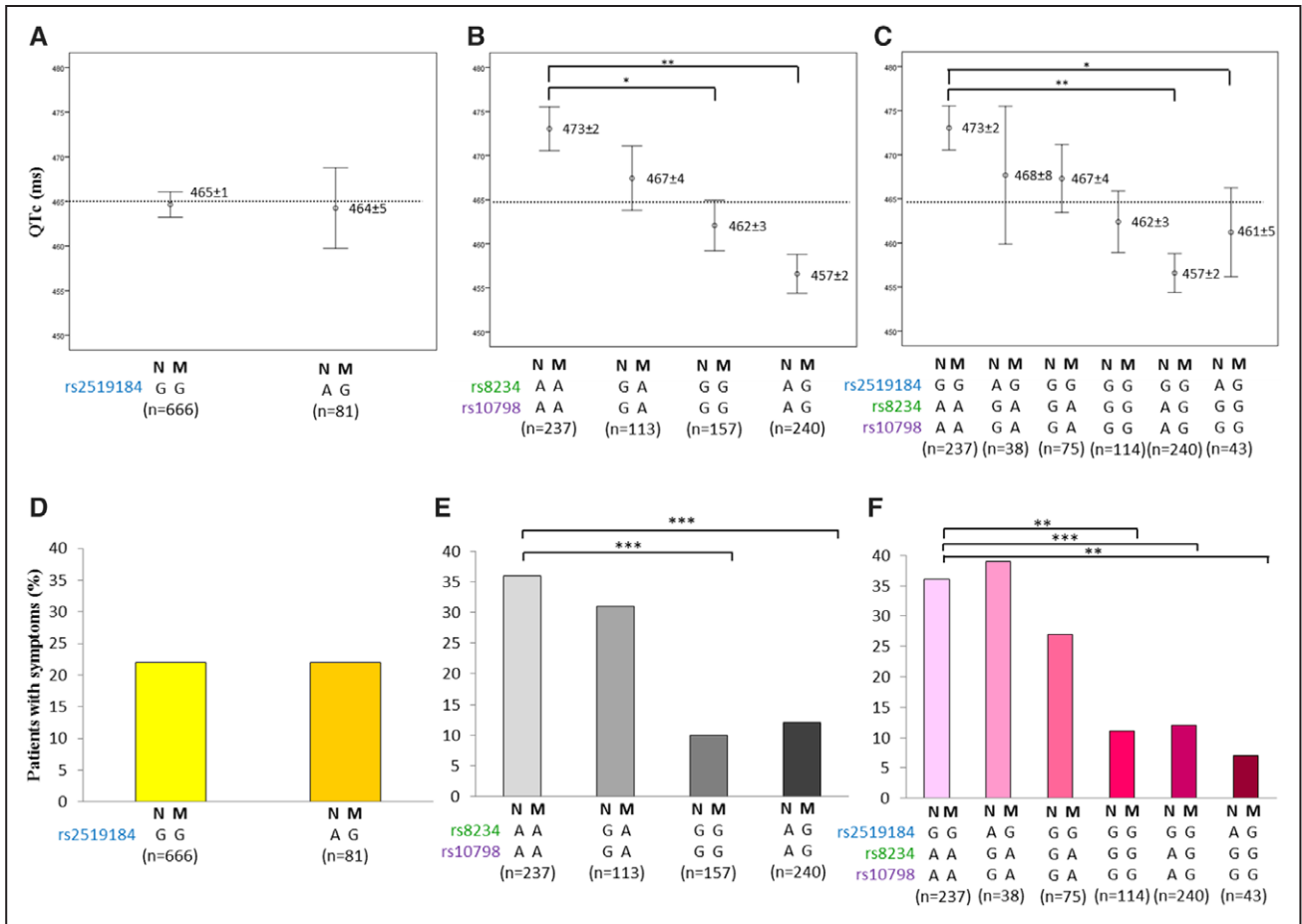
Figure 5. Allele-specific effects of SNPs rs2519184, rs8234, and rs10798 on QTc (**A–C**) and on symptomatology (**D–F**) in the pooled population of Finnish and South African mutation carriers (total population: 747 mutation carriers [MCs]). The scheme is the same used for Figures 2 through 4, but the numbers refer to all mutation carriers in the 3 founder populations. Data are mean±SE for QTc or percentages for symptomatology. *P* values are taken from the multivariable models described in Tables 3 and 4. *P<0.05; **P<0.01; ***P<0.001.

always in *cis* with the mutation, meaning that the allele with the mutation was normally expressed. The expectation was that when the A-G-G haplotype (the derived haplotype) was on the opposite allele (ie, in trans with the mutation), there would be a lower expression of the wild type and therefore a longer QTc and more severe symptoms. However, this is not what happened, and actually, the trend of QTc duration for the *KCNQ1* IVS7-2A>G was in the opposite direction. The population in which more haplotypes were represented was the *KCNQ1* G589D (Finland) population, but even in this group, there were no differences to support a role of these SNPs as disease modifiers.

We were dumbfounded by these completely negative results. All analyses were repeated several times, but eventually, it became clear that neither the South African nor the 2 Finnish founder populations were replicating the findings by Amin et al.[13]

We then merged together these 3 populations, to allow us to deal with >700 patients, and finally, a statistically significant result emerged, and encouragingly, it was in the same direction observed by Amin et al.[13] However, as soon as the models were controlled for the population-specific LQT1 mutation, it became clear that the association was spurious and simply related to the major effect of the disease-causing

mutation. We were thus forced to conclude that we could not replicate the findings by Amin et al.[13]

Searching for an Explanation
Given these unexpected negative results, we tried to find a reasonable explanation, and as a first step, we started by looking at differences between the 2 studies. Clearly, the first major difference lies in the number of subjects under study (747 in ours versus 168) and in the number of disease-causing mutations (3 in ours versus 33). This points to a different robustness of the statistical significance for any difference observed in the 2 studies.

Another important point is the correction for the disease-causing mutation. In the absence of such a correction and without a subanalysis in each family, we would have reached the same conclusions as Amin et al.[13] Our data demonstrate that when we obtained positive results they were indeed influenced by the strong confounding effect of the specific mutations in our founder populations. However, even if unlikely, we cannot entirely exclude that this confounding effect may have been diluted in the study by Amin et al.[13] given the large number of disease-causing mutations.

In both studies, ours and the one by Amin et al,[13] the derived alleles/haplotypes were analyzed in relation with

Table 3.   Estimated Effect of 3′ UTR Observed Haplotypes on QTc, Using 747 Individuals From the Pooled Finnish and South African Population

	Pooled Population			Pooled Population Adjusted by Mutation		
	β	SE	P	B	SE	P
Haplotypes, reference category: GAA/GAA						
AGG/GAA	−2.17	5.95	0.72	−2.18	5.87	0.71
AGG/GGG	−14.06	6.34	0.027	−0.95	6.63	0.89
GAA/GGG	−12.50	4.06	0.0021	−0.83	4.57	0.86
GGG/GAA	−1.76	4.54	0.70	−1.37	4.48	0.76
GGG/GGG	−8.64	4.61	0.06	3.35	5.08	0.51
Sex (female vs male)	21.02	2.53	$<2.0\times10^{-16}$	21.27	2.50	$<2.0\times10^{-16}$
Mutation type						
Finn-G589D vs SA-A341V				−28.01	5.05	3.0×10^{-8}
Finn-IVS7-2A>G vs SA-A341V				−20.82	6.04	5.7×10^{-4}

Linear mixed effect regression was adjusted by sex and, in a separate regression model, also by LQT1 disease-causing mutation (SA-A341V as the reference category, Finn-G589D, Finn-IVS7-2A>G). Regression coefficient (β), SE, and P value are shown. QTc, indicates heart rate–corrected QT interval; SNP, single-nucleotide polymorphism; and UTR, untranslated region.

the mutation being in *cis* or in *trans*. However, to understand whether the data fit with the hypothesis, one should also consider what is present in the other 3′ UTR region and its relation with the mutation. Indeed, it is the final balance between expression of the mutated and of the wild-type alleles that determines the outcome. As a straightforward example, we should also expect that when the derived haplotype is present in both alleles the QTc should be shorter than when it is present only in the wild-type allele, because in the latter case the expression of the mutated allele is

Table 4.   Estimated Effect of 3′ UTR Observed Haplotypes on Symptomatic Clinical Status, Using 747 Individuals From the Pooled Finnish and South African Population

Haplotypes, reference category: GAA/GAA	Pooled Population			Pooled Population Adjusted by Mutation		
	OR	95% CI	P Value	OR	95% CI	P Value
AGG/GAA	1.81	0.73–4.52	0.2	1.86	0.71–4.85	0.21
AGG/GGG	0.13	0.031–0.53	0.004	0.70	0.18–2.62	0.59
GAA/GGG	0.29	0.14–0.60	0.0009	1.21	0.58–2.50	0.61
GGG/GAA	0.65	0.32–1.35	0.24	0.69	0.33–1.42	0.31
GGG/GGG	0.24	0.10–0.58	0.002	1.11	0.48–2.56	0.81
Sex (female vs male)	1.33	0.83–2.13	0.23	1.10	0.71–1.79	0.61
QTc, ms	1.02	1.01–1.03	5.9×10^{-08}	1.01	1.00–1.02	1.14×10^{-5}
Mutation type						
Finn-G589D vs SA-A341V				0.06	0.03–0.11	$<2.0\times10^{-16}$
Finn-IVS7-2A>G vs SA-A341V				0.05	0.02–0.13	7.47×10^{-11}

Mixed effect logistic regression was adjusted by sex and QTc and, in a separate regression model also by LQT1 disease-causing mutation (SA-A341V as the reference category, Finn-G589D, Finn-IVS7-2A>G). Odds ratio (OR), 95% confidence intervals (95% CI), and P value are shown. QTc, indicates heart rate–corrected QT interval; and UTR, untranslated region.

expected to be higher than the expression of the normal allele resulting in a decreased number of functional I_{Ks} potassium channels. However, this was not the case in the study by Amin et al.[13] Indeed, when all cases were merged together, these 2 subgroups of patients had a similar QTc, and this does not fit with the entire hypothesis and interpretation.

An additional possible explanation comes from the luciferase experiments in neonatal rat cardiomyocytes. Because there is considerable variation in gene expression regulation between different species, rat cardiomyocytes may not be an optimal cell line to study human gene expression. In the study by Amin et al,[13] not all of the 3 SNPs were decreasing significantly the expression of the allele in *cis* (ie, for rs2519184 the *P* value is 0.072), and furthermore, the combination of the 3 derived alleles reached only borderline statistical significance ($P=0.049$).

These mild changes in the expression profile could be the major determinant of the discrepancies observed. A reasonable hypothesis might be that these mild changes are not able to act as genetic modifiers of the arrhythmic risk in all KCNQ1 populations. This explanation would fit well for the South African population which features a severe mutation that is well expressed (the ancestral G-A-A haplotype is in *cis* with the mutation), and it is likely that modest changes in the expression of the wild-type allele would not be able to influence the phenotype of the patients. On the contrary, this explanation does not apply to the 2 Finnish populations, in which the disease-causing mutation is reasonably benign, and in the larger G589D founder population, the mutated allele is in *cis* with the derived GGG haplotype in most of the MCs. However, it is not possible to exclude that in our 3 founder populations, there might be other genetic modifier haplotypes, not yet known, that might decrease the effects of the 3′ UTR SNPs.

Conclusions

Our data indicate that, contrary to the current view, the 3′ UTR SNPs are not acting as genetic modifiers in a large number of genetically homogenous LQT1 patients.

Our analysis also suggests the need for similar studies to beware of minor effects, to require large numbers, to use as much as possible founder populations as discovery cohorts, and especially to consider the confounding effect of the inclusion of various disease-causing mutations which is likely to favor spurious associations and incorrect conclusions.

Acknowledgments

Sources of Funding

Disclosures

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CLINICAL PERSPECTIVE

Modifier genes are clinically important because on one hand they can explain part of the clinical heterogeneity of the long-QT syndrome, present even among family members sharing the same disease-causing mutation, which is the major determinant of risk, whereas on the other, they help refining risk stratification. Several modifier genes have been identified to date and some have been replicated in independent cohorts of LQTS patients, paving the way for their use in clinical practice as patient-specific risk modulators, because they can either increase or decrease arrhythmic risk. Single-nucleotide polymorphisms in the 3′ untranslated regions of *KCNQ1* have been proposed as LQT1-specific modifier, as they were reported to modulate the expression of the *KCNQ1* allele present in *cis*, thereby influencing the degree of expression of the wild-type versus the mutated allele. Given the potential importance of the concept and of the finding, we sought to replicate it in a larger and genetically more homogeneous population of *KCNQ1* mutation carriers, including 747 LQT1 patients from 3 different founder populations and thus carrying only 3 mutations. We could not confirm the previous finding because we could not show any modifying role of these SNPs, indicating that they do not act as genetic modifiers in large numbers of LQT1 patients and therefore cannot be used in clinical practice to improve risk stratification. Furthermore, we showed that lack of correction for the population-specific mutations could produce false-positive results. This type of study requires large numbers and adequate correction for possibly confounding effects.

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SUPPLEMENTAL MATERIAL

Table 1S. Estimated effect of 3'UTR observed haplotypes on QTc, using individuals separately taken from the Finnish and South-African populations. Linear mixed effect regression was adjusted by sex. Regression coefficient (Beta), standard error (SE) and p-value (P) are shown.

	SA-A341V			Finn-G589D			Finn-IVS7-2A>G		
	Beta	SE	P	Beta	SE	P	Beta	SE	P
Haplotypes, Reference GAA/GAA									
AGG/GAA	9.17	11.06	0.41	-6.77	8.63	0.43	-15.33	10.46	0.14
AGG/GGG				0.28	6.55	0.97			
GAA/GGG				0.04	4.61	0.99			
GGG/GAA	-11.52	8.56	0.18	6.15	7.01	0.38	3.16	7.31	0.67
GGG/GGG				4.34	5.08	0.39			
Sex (F vs M)	26.86	6.62	5×10^{-5}	19.69	2.88	$<2\times10^{-16}$	27.22	6.63	$<2\times10^{-16}$

Table 2S. Estimated effect of 3'UTR observed haplotypes on symptomatic clinical status, using individuals separately taken from the Finnish and South-African populations. Mixed effect logistic regression was adjusted by sex and QTc. Odds ratio (OR), 95% confidence intervals (95% CI) and p-value (P) are shown.

Haplotypes, Reference category: GAA/GAA	SA-A341V			Finn-G589D			Finn-IVS7-2A>G		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
AGG/GAA	5.37	0.57-51.31	0.140	-	-	-	7.85	0.78-78.26	0.080
AGG/GGG				0.43	0.11-1.62	0.213			
GAA/GGG				0.76	0.37-1.57	0.457			
GGG/GAA	0.72	0.28-1.88	0.50	-	-	-	0.51	0.05-5.55	0.578
GGG/GGG				0.69	0.30-1.61	0.395			
Sex (F vs M)	1.64	0.71-3.70	0.240	1.00	0.55-1.82	0.990	2.04	0.24-16.67	0.505
QTc (ms)	1.02	1.01-1.03	0.005	1.01	1.01-1.02	8×10^{-4}	1.03	0.99-1.07	0.142

Data are not shown (-) when the GLMM was not converging due to the absence of symptomatic MCs in the corresponding subgroups.