**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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**ABSTRACT**

**BACKGROUND:** Long QT syndrome (LQTS) 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 (UTR) 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 three SNPs (rs2519184, rs8234, and rs10798) were genotyped in a total of 747 *KCNQ1* mutation carriers with either 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 three mutation groups. The different SNP haplotypes were neither associated with heart rate corrected QT-interval duration (QTc) nor cardiac events in any of the three 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.

**CONCLUSION:** 3’UTR 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.

**KEYWORDS:** disease modifier genes; genetics; *KCNQ1*; long QT syndrome; untranslated region.

**INTRODUCTION**

The long QT syndrome (LQTS), probably the best known genetic disorder causing life-threatening arrhythmias1-3, has become a useful paradigm to study sudden cardiac death in the young4. An intriguing feature of LQTS is its incomplete penetrance5 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 increasing7-11 or decreasing12 arrhythmic risk and/or QT interval duration.

In 2012, significant interest was generated by a publication by Amin and colleagues13, who reported that single nucleotide polymorphisms (SNPs) in the 3`untranslated region (UTR) of *KCNQ1*, the gene responsible for LQT12, 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 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 reported that 3 SNPs (rs2519184, rs8234 and rs10798) located in the UTR of the gene did modulate *KCNQ1* expression in an allele-specific manner13. 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; 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 was made in 168 LQT1 patients with 33 different mutations from 41 different families followed in two leading referral centers13.

With the simple aim to verify and reproduce the modifying role of 3’UTR SNPs, we studied three large and well characterized LQT1 founder populations14-18. By being homogenous for the disease-causing mutation, which is the major determinant of risk, these populations are an ideal model to test the role of modifying factors6. Our results, entirely unanticipated, were clearly differing from those of Amin et al; however, they provide some novel insights which might prove useful for the study of modifier genes.

**METHODS**

**Study population**

Overall, we studied three independent LQT1 founder populations: one from South Africa14-16 and two from Finland17,18, 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 three founder populations were classified as either symptomatic or asymptomatic according to a previous experience of cardiac events (CE). 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 (SCD). 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. Based on these criteria we enrolled 142 SA-A341V patients, 535 Finn-G589D and 70 Finn-IVS7-2A>G (c.1129-2A>G) mutation-carriers. The mutations were named according to the *KCNQ1* reference sequence NM\_000218.2.

**Genetic analysis**

The 3`UTR SNPs (rs2519184, rs8234 and rs10798) of *KCNQ1* were characterized through PCR and direct sequencing using primers 5'-CTTCCTGAGGGGAGACAGAGC-3' and 5'-GGAACCAAGGTGAGAGCAGTG-3' for the Finnish population to be able to genotype all three variants with a single primer pair, while the South African population was genotyped using the same primers reported by Amin et al13. Investigation of the family trees allowed the identification of the haplotype in *cis* position with the disease-causing mutation for each LQT1 mutation carrier. The complete structure and size of pedigrees and strong LD among SNPs permitted a precise haplotype determination without the need of computational methods of inference.

**Statistical methods**

Normal distribution of continuous variables was assessed by one-sample Kolmogorov Smitnov test. Normal continuous variables are presented as mean ± standard error (SE) and categorical variables 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 mutation carriers (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 ORs 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 gender 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 carried out using the statistical software SPSS Statistics, version 21 (IBM Co, Armonk, NY) and R, version 3.2.119 and its packages *kinship2,*20 *coxme*21 and *lme4*22.

**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 two 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 severity15; indeed, 70% of the patients were symptomatic.

**3`UTR 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 (MAF) in the specific founder populations, we called “derived alleles” the alleles of the three SNPs (A-G-G, respectively) that in Amin’s study 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 three *KCNQ1* SNPs, within each of the three study populations, genotype and allele frequencies are reported in Table 2. Of note, while 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 two latter SNPs were in complete linkage disequilibrium in all MCs included in the study. The SNPs were analyzed separately and as haplotypes in order 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 2 (panels A and B), no significant association between QTc and any of the 3`UTR SNPs was observed. Similarly, the allele-specific haplotype analysis of the three SNPs showed similar mean QTc values across all the represented haplotype combinations (Figure 2, panel C).

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 (Fig. 3), and in the South African A341V population (Fig. 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 haplotype shared between the 2 Finnish population, no significant differences were observed.

These findings were confirmed when the analysis was carried out using a linear mixed effects regression framework, while taking into account a random family effect and adjusting for gender (Table 1S).

***KCNQ1* 3`UTR SNPs and cardiac events**

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 (panels D, E and F) show the allele-specific effects of the individual SNPs and their allele-specific haplotype with regard to the occurrence of cardiac events. 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 gender and QTc (Table 2S).

**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, Table 3) and the occurrence of cardiac events (Figure 5, Table 4) in the large (n=747) group including all three 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 to those with the ancestral allele in trans to the mutated allele (Figure 5, panels A and D). On the other hand, some interesting findings emerged from the comparison of the SNPs rs8234 and rs10798 across subtypes defined by genotype and by haplotype combinations (Figure 5, panels B and E). Compared to 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 ms and 462 ± 3 ms vs 473 ± 3 ms, p<0.05). This was consistent with the results reported by Amin et al13. 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% vs 36%, p<0.01). The same QTc pattern and prevalence of symptoms were confirmed in the allele-specific haplotype analysis (Figure 5, panel C and F).

However, when the linear and logistic regression mixed models based on the combined data from all the three 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 three 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 three populations was not appropriately controlled for.

**DISCUSSION**

The main finding of the present study was our inability to confirm the report of Amin et al13. 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 three 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 al13. 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 two 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 be22, it should come as no surprise that the search for modifier genes for LQTS pioneered the field and has already met with some significant success. Most of the “modifiers” identified so far increase risk7-11, but also protective ones have been identified12. 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 al13, 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 translation23,24.

Amin et al13 showed that three 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 to 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 non-mutated allele, implying that the mutated allele was more expressed than the non-mutated 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 two major referral centres.

**The long way to replication**

Initially, the rather simple idea of our two research groups was to confirm this exciting finding in our well characterized founder populations14-18 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 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 (i.e., 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 two Finnish founder populations were replicating the findings by Amin et al13.

We then merged together these three populations, to allow us to deal with over 700 patients, and finally a statistically significant result emerged and, encouragingly, it was in the same direction observed by Amin et al13. 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 al13.

**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 two studies. Clearly, the first major difference lies in the number of subjects under study (747 in ours vs 168) and in the number of disease-causing mutations (3 in ours vs 33). This points to a different robustness of the statistical significance for any difference observed in the two studies.

Another very important point is the correction for the disease-causing mutation. In the absence of such a correction and without a sub-analysis in each family, we would have reached the same conclusions as Amin et al13. 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, we cannot exclude that this confounding effect may have been diluted in the study of Amin et al.13 given the greater number of disease-causing mutations.

In both studies, ours and the one by Amin et al13, the derived alleles/haplotypes were analyzed in relation with the mutation being in cis or in trans. However, to understand if 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 expected to be higher than the expression of the normal allele resulting in a decreased number of functional IKs potassium channels. However, this was not the case in the study by Amin et al13. Indeed, when all cases were merged together these two sub-groups of patients had a very 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. As 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 Amin’s et al study13 not all of the three SNPs were decreasing significantly the expression of the allele in cis (i.e. for rs2519184 the p value is 0.072) and, furthermore, the combination of the three 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 very well for the South African population which features a very severe mutation that is very well expressed (the ancestral GAA haplotype is in cis with the mutation) and it is very 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 other hand, this explanation does not apply to the two 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 mutation carriers.However, we cannot exclude the possibility that in these three founder populations there may be other genetic modifier haplotypes, not yet known, that might render the effects of the 3'UTR SNPs less marked.

**Conclusion**

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 favour spurious associations and incorrect conclusions.

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**DISCLOSURES**

Conflict of Interest: None.

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**LEGENDS FOR FIGURES**

**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.

**Figure 2**

Allele-specific effects of SNPs rs2519184, rs8234 and rs10798 on QTc (A,B,C) and on symptoms (D, E, F) in the study populations of 535 Finnish carriers of the G589D mutation. Effects of 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 three 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 table 3 and 4.

**Figure 3**

Allele-specific effects of SNPs rs2519184, rs8234 and rs10798 on QTc (A,B,C) and on symptoms (D, E, 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.

**Figure 4**

Allele-specific effects of SNPs rs2519184, rs8234 and rs10798 on QTc (A,B,C) and on symptoms (D, E, F) in the study populations 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.

**Figure 5**

Allele–specific effects of SNPs rs2519184, rs8234 and rs10798 on QTc (A,B,C) and on symptomatology (D, E, F) in the pooled population of Finnish and South African mutation carriers (total population: 747 MCs). The scheme is the same used for figures 2-4, but the numbers refer to all mutation-carriers in the three founder populations. Data are mean ± SE for QTc or percentages for symptomatology.

P-values are taken from the multivariable models described in Table 3 and 4.

\*p <0.05; \*\* p<0.01; \*\*\*p<0.001

**Table 1. Clinical characteristics of the study populations**

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|  | **FOUNDER POPULATIONS** | | |  |
|  | **SA-A341V** | **Finn-G589D** | **Finn-IVS7-2A>G** | **All** |
|
| Mutation carriers, n | 142 | 535 | 70 | 747 |
| Female, 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± Standard Error

QTc = heart rate corrected QT-interval\* p<0.001, Chi-square =238

\*\* p<0.001, ANOVA, F =30.8

**Table 2. *KCNQ1* 3’UTR SNPs in the study populations**

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|  |  |  |  | |  | |  | |
| **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 to 100

**Table 3. Estimated effect of 3’UTR observed haplotypes on QTc, using 747 individuals from the pooled Finnish and South-African population (admixture). Linear mixed effect regression was adjusted by sex and, in a separate regression model, also by LQT1 disease-causing mutation (SA-A341V as reference category, Finn-G589D, Finn-IVS7-2A>G). Regression coefficient (Beta), standard error (SE) and p-value (P) are shown.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Admixture population** |  |  | **Admixture, adjusted by mutation** |  |  |
|  | **Beta** | **SE** | **P** | **Beta** | **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 (F vs M)** | 21.02 | 2.53 | <2.0x10-16 | 21.27 | 2.50 | <2.0x10-16 |
| **Mutation type** |  |  |  |  |  |  |
| **Finn-G589D**  **vs SA-A341V** |  |  |  | -28.01 | 5.05 | 3.0x10-8 |
| **Finn-IVS7-2A>G**  **vs SA-A341V** |  |  |  | -20.82 | 6.04 | 5.7x10-4 |

**Table 4. Estimated effect of 3’UTR observed haplotypes on symptomatic clinical status, using 747 individuals from the pooled Finnish and South-African population (admixture). 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 reference category, Finn-G589D, Finn-IVS7-2A>G). Odds ratio (OR), 95% confidence intervals (95% CI) and p-value (P) are shown.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Admixture population** |  |  | **Admixture adjusted by mutation** |  |  |
| **Haplotypes,**  **Reference category: GAA/GAA** | OR | 95% CI | P | OR | 95% CI | P |
| **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 (F vs M)** | 1.33 | 0.83-2.13 | 0.23 | 1.10 | 0.71-1.79 | 0.61 |
| **QTc (ms)** | 1.02 | 1.01-1.03 | 5.9x10-08 | 1.01 | 1.00-1.02 | 1.14x10-5 |
| **Mutation type** |  |  |  |  |  |  |
| **Finn-G589D**  **vs SA-A341V** |  |  |  | 0.06 | 0.03-0.11 | <2.0x10-16 |
| **Finn-IVS7-2A>G**  **vs SA-A341V** |  |  |  | 0.05 | 0.02-0.13 | 7.47x10-11 |