ORIGINAL RESEARCH

To Modify or Not to Modify: Allele-Specific Effects of 3'UTR-*KCNQ1* Single Nucleotide Polymorphisms on Clinical Phenotype in a Long QT 1 Founder Population Segregating a Dominant-Negative Mutation

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BACKGROUND: There are conflicting reports with regard to the allele-specific gene suppression effects of single nucleotide polymorphisms (SNPs) in the 3'untranslated region (3'UTR) of the *KCNQ1* gene in long QT syndrome type 1 (LQT1) populations. Here we assess the allele-specific effects of 3 previously published 3'UTR-*KCNQ1's* SNPs in a LQT1 founder population segregating a dominant-negative mutation.

METHODS AND RESULTS: Bidirectional sequencing of the *KCNQ1's* 3'UTR was performed in the p.Y111C founder population (n=232, 147 genotype positive), with a minor allele frequency of 0.1 for SNP1 (rs2519184) and 0.6 for linked SNP2 (rs8234) and SNP3 (rs107980). Allelic phase was assessed in trios aided by haplotype data, revealing a high prevalence of derived SNP2/3 *in cis* with p.Y111C (89%). Allele-specific association analyses, corrected using a relatedness matrix, were performed between 3'UTR-*KCNQ1* SNP genotypes and clinical phenotypes. SNP1 *in trans* was associated with a significantly higher proportion of symptomatic phenotype compared with no derived SNP1 allele *in trans* (58% versus 32%, corrected *P*=0.027). SNP2/3 *in cis* was associated with a significantly lower proportion of symptomatic phenotype compared with no derived SNP2/3 allele *in cis* (32% versus 69%, corrected *P*=0.010).

CONCLUSIONS: Allele-specific modifying effects on symptomatic phenotype of 3'UTR-*KCNQ1* SNPs rs2519184, rs8234, and rs107980 were seen in a LQT1 founder population segregating a dominant-negative mutation. The high prevalence of suppressive 3'UTR-*KCNQ1* SNPs segregating with the founder mutation could contribute to the previously documented low incidence of cardiac events in heterozygous carriers of the p.Y111C *KCNQ1* mutation.

Key Words: arrhythmia and electrophysiology ■ molecular cardiology

The phenotype of monogenetic disorders is often influenced by environmental and genetic factors beyond the primary putative pathogenic sequence variant, adding to the challenge of clinical management and risk stratification.[1] Modifier genes are thought to underlie some of the phenotypic heterogeneity seen among carriers of identical pathogenic sequence variants in the familial long QT syndrome (LQTS).[2] The most common LQTS subtype, LQTS type 1 (LQT1), is caused by heterozygous loss-of-function variants in the gene encoding potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) gene.[3] In 2012, significant modifying effects by single nucleotide polymorphisms (SNPs) in the 3'untranslated region (3'UTR) of the *KCNQ1* gene

CLINICAL PERSPECTIVE

What Is New?

- Variants in the 3'untranslated region of the gene encoding potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) gene (3'untranslated region [3'UTR]-*KCNQ1*) may explain some of the phenotypic variability in patients with long QT syndrome type 1 (LQT1) via allele-specific modifying effects.
- The allele-specific gene suppression effects of 3'UTR-*KCNQ1* variants have been proposed to ameliorate or exacerbate clinical phenotype depending on whether the *KCNQ1* gene with the LQT1 mutation or the healthy *KCNQ1* gene on the opposite allele is suppressed.
- Here, 3'UTR-*KCNQ1* variants were confirmed to be associated with symptomatic phenotype in an allele-specific manner in a LQT1 founder population segregating a dominant-negative mutation (p.Y111C). Protective effects were seen when 3'UTR-*KCNQ1* variants were located on the same allele as the LQT1 mutation, while suppressive variants located on the healthy allele were associated with a higher risk for cardiac events in the LQT1 founder population.

What Are the Clinical Implications?

- For patients with LQT1 with a dominant-negative mutation, allele-specific analysis of 3'UTR-*KCNQ1* variants could contribute towards patient-specific risk stratification.

Nonstandard Abbreviations and Acronyms

3'UTR	3'untranslated region
KCNQ1	gene encoding potassium voltage-gated channel subfamily Q member 1
LQT1	long QT syndrome type 1
LQTS	long QT syndrome

were reported in 2 heterogeneous LQT1 cohorts.[4] The authors presented luciferase assay data on how 3'UTR-*KCNQ1* SNPs suppressed gene expression of the allele they resided on, in vitro.[4] Thus, SNPs *in trans* with the LQT1-causing variant would be expected to exacerbate the disease phenotype, while SNPs *in cis* would be expected to ameliorate the disease phenotype; this hypothesis is largely, but not entirely, supported by the clinical data.[4] In 2016, the results were in part reproduced in a large cohort including 3 LQT1 founder populations (Finn-G589D, Finn-IVS7-2A>G, and SA-A341V), where derived SNPs *in cis* were associated with shorter QTc

and reduced cardiac events when analyzing the cohort as a whole.[5] However, when population-specific LQT1 variants were controlled for, ameliorating effects of SNPs *in cis* were no longer significant, leading the authors to conclude that 3'UTR-*KCNQ1* SNPs were not acting as genetic modifiers in their LQT1 study population.[5] The publication was followed by a discussion by the authors of both studies, published as letters to the editor in 2016.[6,7] Possible confounding factors raised by Amin et al included the lack of SNPs *in cis* in the Finn-IVS7-2A>G and SA-A341V populations, and that haploinsufficiency causing mutations (p.G589D and IVS7-2A>G) would be less affected by gene suppression effects, as compared with mutations causing dominant-negative effects (such as p.A341V).[6]

In the context of modifier genes, LQTS founder populations sharing mutations identical by descent constitute potent research models.[2] We have previously reported on a Swedish LQT1 founder population associated with an unexpectedly benign phenotype,[8,9] in spite of segregating the p.Y111C mutation that conveys dominant-negative effects in vitro.[10,11] Here we assess the allele-specific modifying effects of 3 previously published 3'UTR-*KCNQ1* SNPs on clinical phenotype in the Y111C/*KCNQ1* founder population.

METHODS

The study included 232 individuals (147 genotype positive and 85 genotype negative) from a Swedish LQT1 founder population segregating p.Y111C (*KCNQ1* c.332A>G).[8,9] As previously described, genotype-positive status (ie, carrying the familial pathogenic sequence variant p.Y111C) and genotype-negative status (ie, not carrying the familial pathogenic sequence variant p.Y111C) were ascertained in the p.Y111C founder population by molecular genetic testing, performed within the clinical setting.[12] All the genotype positives were heterozygous for the p.Y111C mutation. The founder status of the LQT1 population has been previously confirmed by genealogical studies and microsatellite marker analysis.[8,9]

All participants signed an informed consent, and the study was approved by the Regional Ethical Committee in Umeå, Umeå University, Sweden (Dnr 05–127 M). The data that support the findings of this study are available from the corresponding author upon reasonable request.

Data on clinical characteristics, last updated in August 2021, were obtained from medical records and questionnaires.[9,12] QT interval duration was measured in standard 12-lead ECGs recorded at 50 mm/s, obtained from medical records, when available recorded in absence of β-blocker therapy. As previously described, ECGs were duplicated, coded, and assessed on 2 separate occasions by 1 observer.[12] QT intervals

were measured manually, preferably in lead II as a mean of 3 consecutive QT intervals, and corrected for heart rate by Bazett's formula (QT/√R-R), using the mean of the R-R intervals preceding the measured beats. The mean of the 2 QTc values was used in the subsequent analyses.[12] Using age- and sex-specific cut-offs based on the age at ECG, (ie, children [1–15 years], males [>15 years], and females [>15 years]), the QTc values were designated as prolonged or normal/borderline.[13] Cardiac events were defined as transient but complete loss of consciousness, documented ventricular tachycardia/ventricular fibrillation, aborted cardiac arrest requiring resuscitation, or sudden cardiac death.

Allele-Specific Analysis of 3'UTR-*KCNQ1* SNPs

All study participants were genotyped for 3 previously published noncoding SNPs located in the 3'UTR of the *KCNQ1* gene: SNP1–rs2519184 (G/A), SNP2–rs8234 (A/G), SNP3– rs107980 (A/G), with major (ancestral) followed by minor (derived) alleles in parentheses. Bi-directional sequencing of the entire 3'UTR of the *KCNQ1* gene was performed, using an ABI 3500xL Dx Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the following forward and reverse primers: 5′-GGCACCTTCCCTTCTCTGG-3′; 5′-ACCACCATGCCAGTGATGTC-3′; 5′-CACAGCCTGC ACTTGGG-3′; 5′-CAGGGCTCCTCTCCAGC-3′; 5′-CAGT CTCACCATTTCCCCAG-3′; 5′-GCCCAGAACAGGAGCG AC-3′. Sequences were evaluated using the Sequencer software version 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). The rs-numbers for denoting the SNPs were obtained from the dbSNP database (www.ncbi. nlm.nih.gov/projects/SNP). Ancestral (major) and derived (minor) alleles were denoted as "A" and "a", respectively (AA: major homozygote, aa: minor homozygote, and Aa: heterozygote). Allelic phase analysis for 3'UTR- *KCNQ1* SNPS (ie, whether SNPs were located on the same allele as the mutation [*in cis*] or on the allele from the nonaffected parent [*in trans*]), was performed by trio analysis, aided by available microsatellite marker-derived haplotype data.[8] In text, tables, and figures, the allele-specific data were consistently presented as if the p.Y111C mutation was residing on the left allele, and minor/major alleles hence described from left to right as *in cis* (aA) and *in trans* (Aa). Because *in cis* gene suppression effects are expected to be greater than *in trans* gene suppression effects for a dominant-negative mutation,[4] combined genotypes including both *in cis* and *in trans* SNPs (aa) were coded as *in cis* for association testing purposes.

Statistical Analysis

In text, tables, and figures, continuous variables were presented as mean±SD (except when comparing the study population to previously published LQT1 cohorts,

then mean±SEM was used), and categorical variables were presented as n (%).

Association testing between 3'UTR- *KCNQ1* SNP genotypes and categorical traits age- and sex-adjusted QTc (prolonged=1, normal/borderline=0) and clinical phenotype (symptomatic=1, asymptomatic=0) was performed using the χ^2 test. Correction for type 1 errors in allelic association testing was performed for each trait using a relatedness matrix.[14,15] Trait-specific relatedness correction factors were generated by calculating the relative relatedness between all individuals in the study population with and without the trait being tested for.[14,15] The relatedness matrix was based on previously published detailed pedigrees for the p.Y111C founder population, extrapolated from comprehensive genealogy and microsatellite marker data.[8,9] An overview of the p.Y111C founder population pedigree, with the study population indicated by filled symbols, is presented in Figure 1. The relatedness matrix was generated by custom-made programming, in accordance with the methods as described by Bourgain[14] and Choi,[15] using Python (https://docs.python.org/3). A relatedness-corrected *P*<0.05 level of statistical significance was used for all analyses.[14,15]

GraphPad Prism 8.4.3 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com) was used for descriptive analyses and graphs. The R Project for Statistical Computing was used for verifying the relatedness matrix and printing the founder population pedigree (https://www.r-project.org). Inkscape Project, 2020 (available at: https://inkscape.org) was used to generate composite images. The first author (A.W.) had full access to all the data in the study and takes responsibility for its integrity and the data analysis.

RESULTS

The study population included 232 individuals from the p.Y111C founder population, 147 genotype positive and 85 genotype negative. The clinical characteristics of the study population, stratified by age at ECG, sex, and genotype, are summarized in Table 1. The proportion of individuals with a prolonged QTc (defined as >460 milliseconds in children up to 15 years of age, >450 milliseconds for males, and >470 milliseconds for females),[13] was 79% (72%–85%) in genotype positives and 8% (5%–9%) in genotype negatives (Table 1).

Among genotype-positive individuals (mean age at last update 47±23 years), 53/147 (36%) had a clinical history of at least 1 cardiac event.

Allele-Specific Analyses of 3'UTR-*KCNQ1* SNPs

Three 3'UTR-*KCNQ1* SNPs with previously documented allele-specific repressive properties[4] (rs2519184, rs8234,
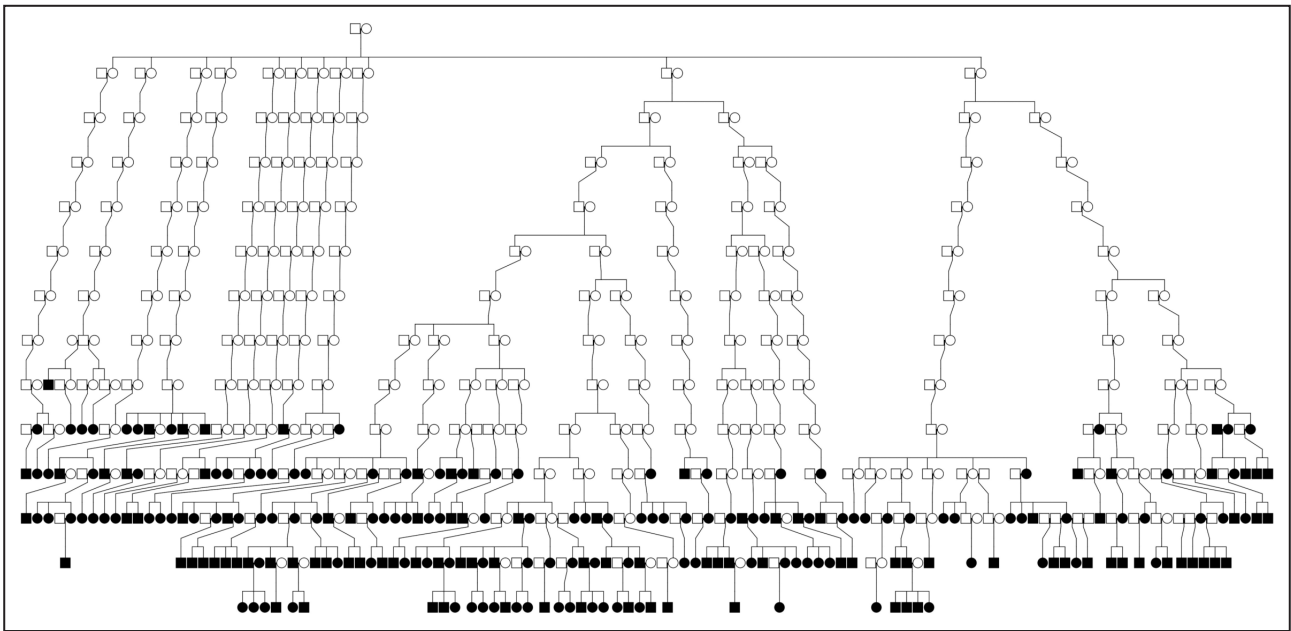
Figure 1. Overview of the p.Y111C/*KCNQ1* founder population pedigree.
The founder pedigree, based on previous genealogical and microsatellite marker analyses,[8,9] describes the relatedness of the study population (n=232, filled symbols), including 147 genotype-positive and 85 genotype-negative individuals. Circle: female. Square: male. Filled symbol: individual genotyped for 3'UTR-*KCNQ1* single nucleotide polymorphisms rs2519184, rs8234, and rs107980. 3'UTR indicates 3'untranslated region; and *KCNQ1*, gene encoding potassium voltage-gated channel subfamily Q member 1.

and rs10798) were analyzed in the p.Y111C founder population. The minor allele frequency for SNP1 (rs2519184) was 0.08 in genotype positives and 0.11 in genotype negatives. SNP2 (rs8234) and SNP3 (rs10798) were in complete linkage disequilibrium and had a minor allele frequency of 0.62 in genotype positives and 0.61 in genotype negatives (Table 2).

Allelic phase analysis for the 3 3'UTR-*KCNQ1* SNPs (ie, whether a derived allele resided on the same [*in cis*] or opposite [*in trans*] allele as p.Y111C), was performed in genotype-positive individuals, using trio analysis and relatedness data from previously established pedigrees.[8,9] For SNP1, a derived allele *in trans* was seen in 24/147 (16%) of genotype positives while 123/147 (84%) had no derived alleles. For SNP2/3, two derived

alleles *in cis* (with or without concomitant derived alleles *in trans*) were seen in 131/147 (89%) of genotype positives. Detailed results from the allelic phase analysis are presented in Figure 2.

The proportion of genotype positives with age- and sex-specific prolonged QTc, stratified by 3'UTR-*KCNQ1* SNP genotype, is presented in Figure 2A. For SNP1, the proportion of individuals with a prolonged QTc was the same among those with no derived allele and those with a derived allele *in trans* (79%). For SNP2/3, the proportion of prolonged QTc was lowest among genotype positives with derived SNP2/3 alleles *in cis*, 101/131 (77%). Among genotype positives with no derived SNP2/3 alleles, 9/10 (90%) had a prolonged QTc, while all genotype positives with

Table 1. Clinical Characteristics in 232 Individuals From the p.Y111C Founder Population, Stratified by Genotype, Sex, and Age at ECG

	All	Children <15 y	Males ≥15 y	Females ≥15 y
Genotype positive (n, row %)	147	37 (25)	43 (29)	67 (46)
QTc, ms	483±34	479±35	476±43	490±25
Prolonged QTc* (n, %)	116 (78)	27 (73)	31 (72)	57 (85)
Age at ECG, y	32±22	4.2±4.0	35±15	46±18
Genotype negative (n, row %)	85	21 (25)	32 (38)	32 (38)
QTc, ms	433±24	431±25	422±21	444±22
Prolonged QTc* (n, %)	7 (8)	1 (5)	3 (9)	3 (9)
Age at ECG, y	35±27	4.3±4.7	38±17	46±19

Continuous variables are presented as mean±SD.
*Defined as >460 milliseconds in children up to 15 years of age, >450 milliseconds for males, and >470 milliseconds for females.[13]

Table 2. Frequency of 3'UTR-KCNQ1 Sequence Variants With Reported Modifying Properties Analyzed in a Y111C/KCNQ1 Founder Population

Status	Sequence variant	Alleles (A/a)	Genotype	N (%)	MAF
Genotype positive (n=147)					
	rs2519184	G/A	GG AG AA	123 (84) 24 (16) 0	0.082
	rs8234	A/G	AA AG GG	10 (7) 92 (63) 45 (30)	0.619*
	rs107980	A/G	AA AG GG	10 (7) 92 (63) 45 (30)	0.619*
Genotype negative (n=85)					
	rs2519184	G/A	GG AG AA	67 (79) 18 (21) 0	0.106
	rs8234	A/G	AA AG GG	42 (49) 34 (40) 9 (11)	0.612*
	rs107980	A/G	AA AG GG	42 (49) 34 (40) 9 (11)	0.612*

A/a indicates ancestral (A) and derived (a) alleles; 3'UTR, 3'untranslated region; and MAF, minor allele frequency (derived allele).

*Sequence variants rs8234 and rs107980 were in complete linkage disequilibrium.

derived SNP2/3 alleles *in trans* and no derived alleles *in cis* had a prolonged QTc, 6/6 (100%). There was no significant difference in the proportion of individuals with an age- and sex-adjusted prolonged QTc between 3'UTR-*KCNQ1* SNP genotypes (SNP2/3 *in cis* versus no SNP2/3 *in cis*, P=0.123 using χ^2 test [test statistic 2.376]), P=0.102 following correction for relatedness (correction factor 1.124, corrected test statistic 2.670).

The proportion of genotype positives with a history of cardiac events, 53/147 (36%), stratified by 3'UTR-*KCNQ1* SNP genotype, is presented in Figure 2B. For SNP1 (rs2519184), the proportion of individuals with symptomatic phenotype was significantly higher among those with a derived allele *in trans* (SNP1 *in trans*, 14/24 [58%] versus no derived alleles in SNP1, 39/123 (32%), P=0.013 using χ^2 test (test statistic 6.175), P=0.027 following correction for relatedness [correction factor 0.794, corrected test statistic 4.905]).

For SNP2/3, 4 genotypes were present among genotype positives: no derived variants (AA, n=10, proportion symptomatic 60%), derived variant *in cis* (aA, n=85, proportion symptomatic 28%), homozygous-derived variants (aa, n=46, proportion symptomatic 39%), and a derived variant *in trans* (Aa, n=6, proportion symptomatic 83%). The proportion of individuals with a symptomatic phenotype was significantly lower among those with a derived allele *in cis* (SNP2/3 *in cis*, 42/131 [32%] versus no SNP2/3 *in cis*, 11/16 [69%], P=0.004 using χ^2 test [test statistic 8.325], P=0.010 following correction for relatedness [correction factor 0.794, corrected test statistic 6.613]).

In p.Y111C proband families with at least 10 genotype positives with complete data on 3'UTR-*KCNQ1* SNP1-3 genotype and clinical characteristics (n=3), we present the data in pedigree form (Figure 3A through C).

A comparison of frequency and allelic phase of 3'UTR-KCNQ1-derived variants and clinical presentation between the Swedish p.Y111C population and previously published LQT1 cohorts, including dominant-negative, mixed genotype, and haploinsufficiency LQT1 study populations, is presented in Table 3.

DISCUSSION

Here we present allele-specific data on 3 previously published 3'UTR-*KCNQ1* sequence variants (rs2519184, rs8234, and rs107980)[4] and their association with clinical phenotype in a founder population segregating a dominant-negative mutation, Y111C-*KCNQ1*.

Derived 3'UTR-*KCNQ1* SNPs rs2519184 (A), rs8234 (G), and rs10798 (G) have been shown by in vitro luciferase assay to suppress *KCNQ1* expression in an allele-specific manner, which would be expected to shift the balance between mutated and wild-type subunits towards a >50% reduction of Kv7.1 function when these variants occur *in trans*, and a <50% reduction of Kv7.1 function when these variants occur *in cis*.[4] In the p.Y111C population, having 3'UTR-*KCNQ1* SNP1 *in trans* (16% of genotype positives) is associated with a significantly increased probability for symptomatic phenotype (58% versus 32% for those with no derived SNP1), while having 3'UTR-*KCNQ1* SNP2/3 *in cis*
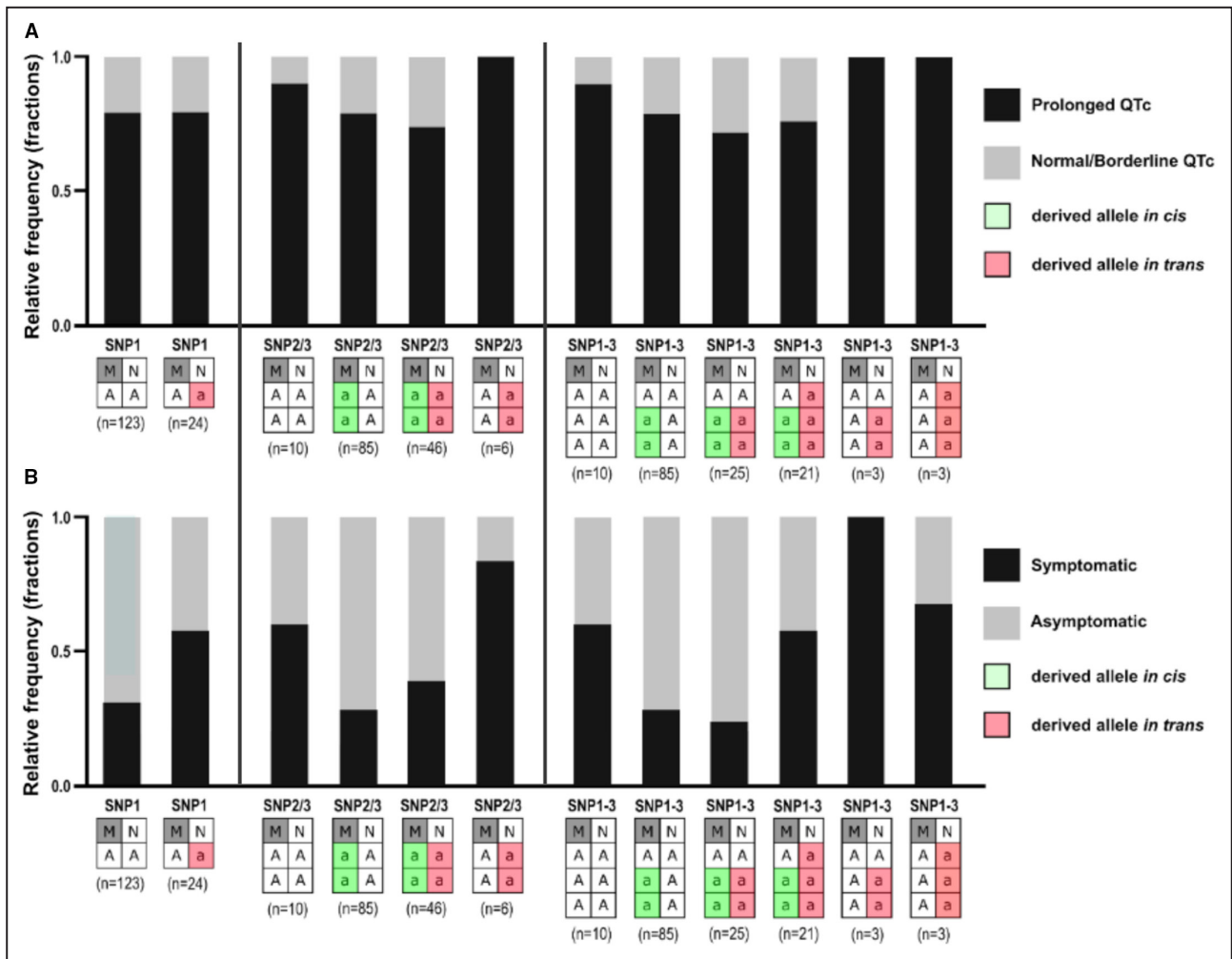
Figure 2. The proportion of p.Y111C/*KCNQ1* genotype-positive individuals with prolonged QTc and symptomatic phenotype, stratified by 3'UTR-*KCNQ1* single nucleotide polymorphism (SNP) genotype and allelic phase.

A, Prolonged QTc was defined based on age- and sex-specific cut-offs.[17] **B**, Symptomatic phenotype was defined as having had at least 1 cardiac event. For SNP1 (rs2519184), all genotype-positive (n=147) had either no derived variants (AA, n=123, proportion prolonged QTc 79%, symptomatic 32%) or a derived variant *in trans* (Aa, n=24, proportion prolonged QTc 79%, symptomatic 58%). The proportion of individuals with a symptomatic phenotype was significantly higher among those with a derived allele *in trans* (SNP1 *in trans* versus no derived SNP1 allele, P=0.027 by relatedness corrected χ^2 test). SNP2 (rs8234) and SNP3 (rs107980) were in complete linkage disequilibrium. For SNP2/3, 4 combinations were present in the genotype-positive study population: no derived variants (AA, n=10, proportion prolonged QTc 90%, symptomatic 60%), derived variant *in cis* (aA, n=85, proportion prolonged QTc 79%, symptomatic 28%), homozygous-derived variants (aa, n=46, proportion prolonged QTc 74%, symptomatic 39%), and a derived variant *in trans* (Aa, n=6, proportion prolonged QTc 100%, symptomatic 83%). The proportion of individuals with a symptomatic phenotype was significantly lower among those with a derived allele *in cis* (SNP2/3 *in cis* versus no SNP2/3 *in cis*, P=0.010 by relatedness-corrected χ^2 test). When combining the data from SNP1-3, 6 combinations were present: AA, AA, n=10, proportion prolonged QTc 90%, symptomatic 60%; AA, aA, n=85, proportion prolonged QTc 79%, symptomatic 28%; AA, aa, n=25, proportion prolonged QTc 72%, symptomatic 24%; Aa, aa, n=21, proportion prolonged QTc 76%, symptomatic 67%; AA, Aa, n=3, proportion prolonged QTc 100%, symptomatic 100%; and Aa, Aa, n=3, proportion prolonged QTc 100%, symptomatic 67%. 3'UTR indicates 3'untranslated region.

(89% of genotype positives) is associated with significant protective effects (32% symptomatic versus 69% for those with no derived SNP2/3 *in cis*).

3'UTR-*KCNQ1* SNPs have previously been identified as modifiers of clinical phenotype in 2 LQT1 cohorts (combined n=168) and a LQTS cohort study (n=220, the majority LQT1).[4,16] In the study by Amin et al, having a derived variant *in trans* in any of the

3'UTR-*KCNQ1* SNPs was significantly associated with marked QTc prolongation, and derived rs8234 and rs107980 variants *in trans* significantly associated with symptomatic phenotype.[4] In the study by Earle et al, rs8234 and rs107980 were independently associated with risk for cardiac events (no allelic-phase analysis was performed).[16] However, in the study by Crotti et al, neither rs2519184 nor rs8234 and rs107980 were
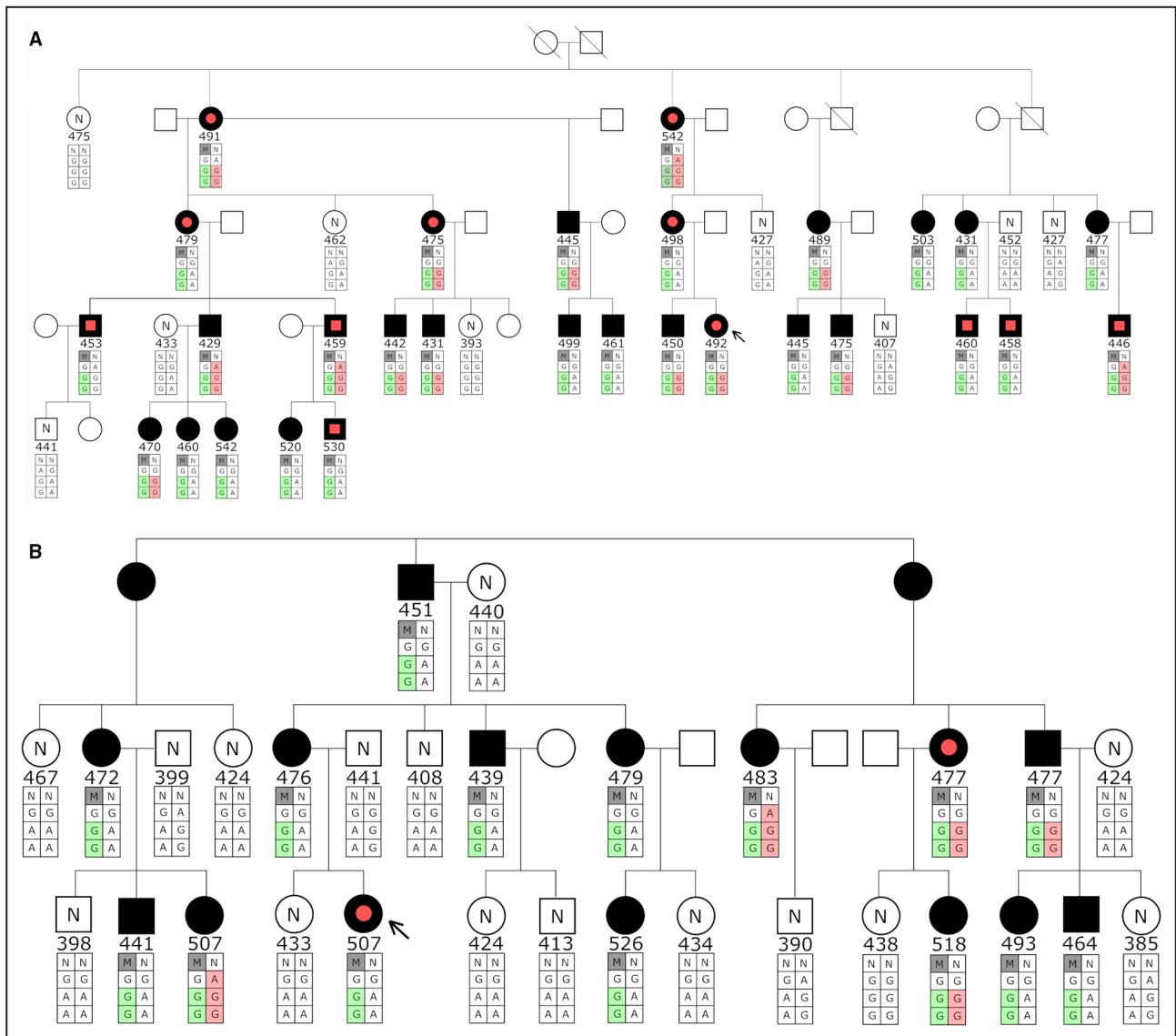
Figure 3. **Pedigrees illustrating p.Y111C families with at least 10 genotype-positive individuals with complete data on clinical phenotype, 3'UTR-*KCNQ1* single nucleotide polymorphism (SNP) genotype, and allelic phase.**
A, 29 genotype-positive and 9 genotype-negative individuals with complete data. **B**, 15 genotype-positive and 15 genotype-negative individuals with complete data. **C**, 10 genotype-positive and 5 genotype-negative individuals with complete data. Arrow: proband. Circle: female. Square: male. Black filled circle/square: genotype positive. Red filled circle/square: experience of cardiac event(s). N inside circle/square: genotype negative. Empty symbol: not tested. Number under each symbol: QTc. Rows of squares: First row: M–allele where the p.Y111C sequence variant (mutation) resides, N–normal allele; Second row: SNP1 (rs2519184); Third row: SNP2 (rs8234); Fourth row: SNP3 (rs107980). Ancestral alleles for SNP1-3: GAA. Derived alleles for SNP1-3: AGG. Green: derived allele *in cis* (on the same allele as the mutation). Red: derived allele *in trans* (on the opposite allele as the mutation). In genotype negatives no *in cis/in trans* markers are used because both alleles are normal. 3'UTR indicates 3'untranslated region; and *KCNQ1*, gene encoding potassium voltage-gated channel subfamily Q member 1.

significantly associated with QTc or cardiac events when the LQT1 founder populations were analyzed separately (Finn-G589D n=535, Finn-ISV7-2>A n=70, SA-A341V n=142).[5] While the Crotti et al study included a large number of genotype-positive participants (n=747), it included relatively few that carried a 3'UTR-*KCNQ1* SNP *in cis*.[5] Specifically, the Finn-ISV7-2>A population (n=70, 39% with a 3'UTR-*KCNQ1* SNP *in trans*) and the SA-A341V population (n=142, 29%

with a 3'UTR-*KCNQ1* SNP *in trans*) had not a single individual with a derived allele *in cis* in SNP1-3, precluding further analysis of the impact of *in cis* variants in those populations.[5] In the large Finn-G589D population (n=535), in spite of a high frequency of derived 3'UTR-*KCNQ1* variants (74% *in cis* and 8% *in trans*), no significant allele-specific modifying effects of cardiac phenotype were seen.[5] This finding suggests that 3'UTR-*KCNQ1* SNP1-3 do not significantly affect
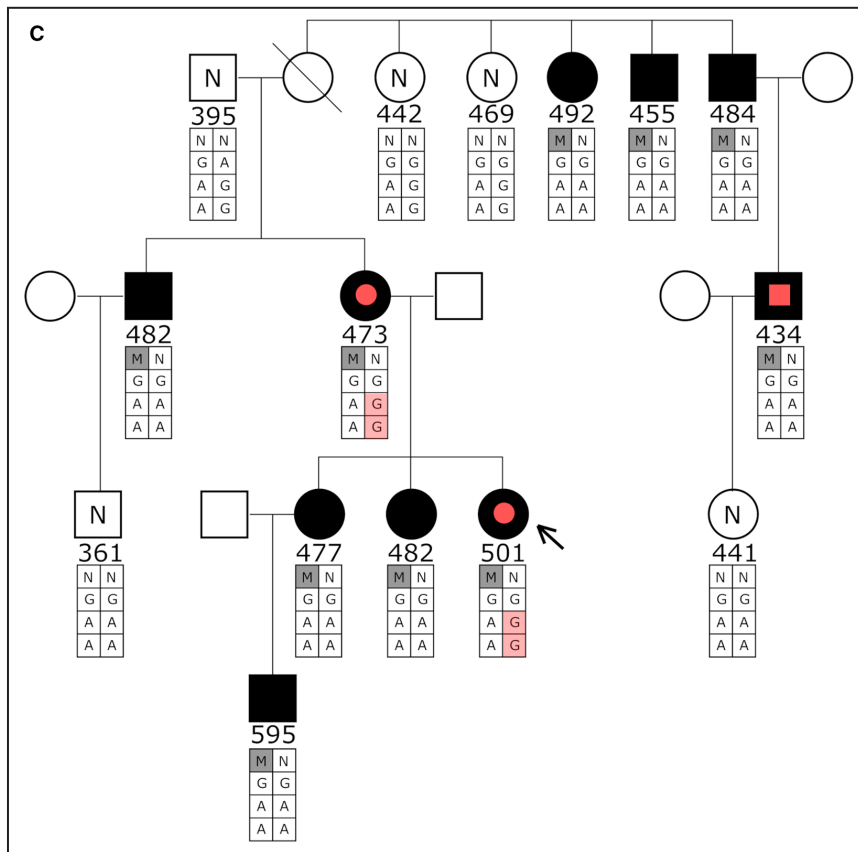
Figure 3. Continued

phenotypic heterogeneity in patients carrying mild LQT1 haploinsufficiency mutations, such as p.G589D.

In their letter to the editor following the Crotti et al publication, Amin et al noted that the expected effect of allelic suppression would be more pronounced in populations segregating dominant-negative mutations.[6] This assumption was based on the postulated mechanism by which 3'UTR-KCNQ1 SNPs modulate clinical phenotype (ie, by affecting the ratio between normal and mutated subunits available for co-assembly into tetrameric Kv7.1 potassium channels).[4] In the case of haploinsufficiency mutations, these typically lead to truncated/nonfunctional subunits that do not co-assemble with normal subunits.[6] In the case of dominant-negative mutations, the resultant subunits typically co-assemble and cause trafficking defects, whereas a single mutated

Table 3. Frequency and Allelic Phase of 3'UTR-KCNQ1–Derived Variants and Clinical Presentation: Comparison With Previously Published LQT1 Cohorts Including Dominant-Negative, Mixed Genotype, and Haploinsufficiency LQT1 Mutations

Mutation type/effect	Study population	n	MAF SNP1	MAF SNP2/3	No MA n (%)	MA *in cis** n (%)	MA *in trans*[†] n (%)	QTc (ms±SEM)	CE (%)
DN	Swe-Y111C	147	0.08	0.62	10 (7)	131 (89)	6 (4)	483±3	36
DN	SA-A341V[5]	142	0.05	0.14	101 (71)	0	41 (29)	485±4	70
H/DN[‡]	Mayo[4]	84	0.20	0.35	31 (37)	33 (39)	20 (24)	451±4	21
H/DN[‡]	AMC[4]	84	0.04	0.21	52 (62)	7 (8)	25 (30)	446±7	21
H	Finn-G589D[5]	535	0.06	0.56	93 (17)	397 (74)	45 (8)	459±2	11
H	Finn-IVS7-2A>G[5]	70	0.05	0.19	43 (61)	0	27 (39)	466±4	10

3'UTR indicates 3'untranslated region; CE, cardiac event; DN, dominant negative, H- haploinsufficiency; *KCNQ1*, gene encoding potassium voltage-gated channel subfamily Q member 1; LQT1, long QT syndrome type 1; MA, minor allele; MAF, minor allele frequency; SNP, single nucleotide polymorphism; SNP1, rs2519184; SNP2, rs8234; and SNP3- rs107980.

*Any minor allele *in cis* for SNP1-3.

†Any minor allele *in trans*, no minor allele *in cis* for SNP1-3.

‡Cohort containing ≈20% dominant-negative mutations.[4]

subunit may cause sequestration of the tetrameric Kv7.1 channel.[6] Gene suppression *in trans*, and particularly *in cis*, would therefore have a greater effect on the proportion of channels that reach the cell membrane in the case of dominant-negative mutations as compared with haploinsufficiency mutations.[6]

Thus, the assessment of 3'UTR-*KCNQ1* SNP-related effects calls for comparisons between cohorts and populations segregating the same mutation type, specifically dominant-negative *KCNQ1* mutations, such as p.Y111C[8,9] and p.A341V.[17,18] The clinical phenotypes of the p.Y111C and p.A341V populations have been previously described in several publications noting the unusually mild presentation of the p.Y111C population and the unusually severe presentation of the p.A341V population.[9,17] An example of this is the marked difference in the proportion of symptomatic genotype positives in the 3'UTR-*KCNQ1* SNP study populations, 36% for p.Y111C versus 70% for p.A341V. Indeed, in spite of in vitro studies predicting the p.Y111C mutation to result in a 75% loss-of-function,[10,11] it is associated with a low incidence of sudden cardiac death in the p.Y111C founder population, including historically before β-blocker treatment, a finding that has remained largely unexplained.[8,9] Comparing the proportion of genotype positives with derived 3'UTR-*KCNQ1* SNP1-3 *in trans*, 4% of 147 p.Y111C genotype positives had a derived variant *in trans* and none *in cis*, compared with 29% of 142 SA-A341V genotype positives. In our p.Y111C founder population, 100% of genotype positives with a derived variant *in trans* and none *in cis* had an age- and sex-adjusted prolonged QT and 83% had experienced a cardiac event. Comparing the proportion of genotype positives with derived 3'UTR-*KCNQ1* SNP1-3 *in cis*, 89% of 147 p.Y111C genotype positives had derived variants *in cis*, while none of the 142 SA-A341V genotype positives had a derived variant *in cis*. In this study, we found a significant association between *in cis* 3'UTR-*KCNQ1* SNP2/3 genotype and asymptomatic clinical phenotype. The finding of 3'UTR-*KCNQ1*-derived variants segregating *in cis* with the p.Y111C mutation in the founder population could thus contribute to the unexpectedly low frequency of syncope and life-threatening cardiac events documented in p.Y111C founder heterozygotes[8,9] via allele-specific repression effects.[4] As we previously theorized, genetic modifiers segregating within the haplotype block could by such a mechanism convey an overall protective effect to the founder population.[9]

CONCLUSIONS

We found significant allele-specific modifying effects of 3'UTR-*KCNQ1* sequence variants rs2519184, rs8234, and rs107980 on symptomatic phenotype in a LQT1 founder population segregating the dominant-negative mutation p.Y111C. Sequence variant rs2519184 *in trans*

with the p.Y111C mutation (16% of genotype positives) was associated with a significantly increased risk of cardiac events. Sequence variants rs8234 and rs107980 *in cis* with the p.Y111C mutation (89% of genotype positives) were associated with a significantly lowered risk of cardiac events. The high prevalence of sequence variants rs8234 and rs107980 segregating *in cis* with the founder mutation could contribute to the documented low incidence of life-threatening cardiac events in the p.Y111C founder population. Our findings support the potential role of allele-specific analysis of 3'UTR-*KCNQ1* sequence variants in the clinical risk stratification of patients with LQT1 carrying dominant-negative mutations.

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