AKAP9 Is a Genetic Modifier of Congenital Long-QT Syndrome Type 1

Carin P. de Villiers, PhD; Lize van der Merwe, PhD; Lia Crotti, MD, PhD; Althea Goosen, RHN; Alfred L. George, Jr, MD; Peter J. Schwartz, MD; Paul A. Brink, MD; Johanna C. Moolman-Smook, PhD; Valerie A. Corfield, PhD

Background—Long-QT syndrome (LQTS), a cardiac arrhythmia disorder with variable phenotype, often results in devastating outcomes, including sudden cardiac death. Variable expression, independently from the primary disease-causing mutation, can partly be explained by genetic modifiers. This study investigates variants in a known LQTS-causative gene, *AKAP9*, for potential LQTS-type 1–modifying effects.

Methods and Results—Members of a South African LQTS-type 1 founder population (181 noncarriers and 168 mutation carriers) carrying the identical-by-descent *KCNQ1* p.Ala341Val (A341V) mutation were evaluated for modifying effects of *AKAP9* variants on heart rate–corrected QT interval (QTc), cardiac events, and disease severity. Tag single nucleotide polymorphisms in *AKAP9* rs11772585, rs7808587, rs2282972, and rs2961024 (order, 5′-3′positive strand) were genotyped. Associations between phenotypic traits and alleles, genotypes, and haplotypes were statistically assessed, adjusting for the degree of relatedness and confounding variables. The rs2961024 GG genotype, always represented by CGCG haplotype homozygotes, revealed an age-dependent heart rate–corrected QT interval increase (1% per additional 10 years) irrespective of A341V mutation status (P=0.006). The rs11772585 T allele, found uniquely in the TACT haplotype, more than doubled (218%) the risk of cardiac events (P=0.002) in the presence of A341V; additionally, it increased disease severity (P=0.025). The rs7808587 GG genotype was associated with a 74% increase in cardiac event risk (P=0.046), whereas the rs2282972 T allele, predominantly represented by the CATT haplotype, decreased risk by 53% (P=0.001).

Conclusions—*AKAP9* has been identified as an LQTS-type 1–modifying gene. Variants investigated altered heart rate–corrected QT interval irrespective of mutation status, as well as cardiac event risk, and disease severity, in mutation carriers. **(*Circ Cardiovasc Genet*. 2014;7:599-606.)**

Key Words: *AKAP9* ■ arrhythmia ■ *KCNQ1* ■ long-QT syndrome

The long-QT syndrome (LQTS), a hereditary cardiac arrhythmia disorder resulting from abnormal ventricular repolarization, is characterized by a prolonged QT interval on the surface ECG and the occurrence of cardiac events, including syncope, cardiac arrest, and sudden death.[1] This syndrome is genetically heterogeneous and hundreds of mutations have been identified in different LQTS-susceptibility genes.[2,3] The particular gene harboring the disease-causing mutation influences the clinical course of the syndrome and may affect specific triggers of cardiac events.[4,5]

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However, despite much progress having been made in identifying LQTS disease-causing genes and risk factors, the syndrome is associated with a high degree of unexplained phenotypic variability, often in families with the same primary disease-causing mutation.[6–8] This is seen in the wide range of heart rate–corrected QT interval duration (QTc), the incidence of cardiac events (syncope, cardiac arrest, and sudden death), age at which the first event occurs, as well as the number and severity of the events experienced, with the most feared being sudden cardiac death. Furthermore, reports of low penetrance in certain LQTS mutation carriers make it difficult to predict clinical outcomes.[7] This variability suggests that additional genetic or environmental factors or a combination of both are at play. Consequently, recent focus has shifted toward identifying genes, or more specifically genetic variants, that, although not directly disease-causative, may contribute to the resulting phenotype.[9–12]

The present study investigates potential modifying effects of the *AKAP9* gene. This gene encodes, among other isoforms, the

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From the Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Medical Research Council (MRC) Centre for Molecular and Cellular Biology, (C.P.d.V., L.v.d.M., J.C.M.-S., V.A.C.), and Department of Internal Medicine (A.G., P.A.B.), Stellenbosch University, Stellenbosch, South Africa; Department of Statistics, University of Western Cape, Bellville, South Africa (L.v.d.M.); IRCCS Istituto Auxologico Italiano, Center for Cardiac Arrhythmias of Genetic Origin and Laboratory of Cardiovascular Genetics, Milan, Italy (L.C., P.J.S.); Department of Molecular Medicine, University of Pavia, Pavia, Italy (L.C.); Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany (L.C.); and Departments of Medicine and Pharmacology, and Institute for Integrative Genomics, Vanderbilt University, Nashville, TN (A.L.G.).

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Correspondence to Carin de Villiers, PhD, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Medical Research Council (MRC) Centre for Molecular and Cellular Biology, Room 4036, Teaching Block, University of Stellenbosch, Tygerberg campus, Francie van Zyl Dr, PO Box 19063, Tygerberg 7505, South Africa. E-mail cpgreen@sun.ac.za

A-kinase anchor protein (AKAP), yotiao. Yotiao forms a macromolecular complex with voltage-gated potassium channel α-subunits, Kv7.1 (also known as KCNQ1), and its associated β-subunits (KCNE1), that are responsible for the slowly activating delayed-rectifier K^+ current, I_{Ks}.[13–15] This AKAP isoform interacts with and enables the phosphorylation of KCNQ1.[13] However, not only does it directly bind with and assist the phosphorylation of KCNQ1, but it is also itself phosphorylated and facilitates the conversion of phosphorylation-induced changes into altered channel activity.[16,17] It is, therefore, clear that yotiao is crucial in the regulation of the KCNQ1-KCNE1 channel, and it comes as no surprise that a mutation (S1579L) discovered in this AKAP isoform results in an LQTS phenotype (LQT11).[18]

It is likely that *AKAP9*, in addition to its identified role in disease causation, acts as a disease modifier. Here we test this hypothesis in members of a South African LQTS founder population of Western European origin.[6,19] Mutation carriers in these families harbor an identical-by-descent *KCNQ1* disease-causing mutation, p.Ala341Val (NM_000218.2:c.1022C>T), hereafter referred to as A341V, yet their disease expression varies considerably, providing an ideal population in which to evaluate additional modifying factors.

Materials and Methods

Founder Population Subjects

The study population consisted of 23 South African LQTS founder families analyzed in the present investigation, who carry the same *KCNQ1* LQTS-causative mutation, A341V.[6,19] All participants in the study provided written informed consent, and the Stellenbosch University's Faculty of Medicine and Health Sciences Institutional Review Board approved this project (2000/C077). Participants provided demographic information and history of disease (personal and family). Further clinical data collected from clinical records included the timing and type of symptoms experienced, treatment, and ECG recordings. LQTS A341V mutation status was determined for all participants who provided blood. Exon screening of the *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* genes in the A341V founder family probands revealed a second mutation/variant in only 3 of the probands (*KCNE1*: D91E, *KCNH2*: R328C,[20] and *KCNE1*:D85N unpublished data). No statistical correction for the presence of these variants was made because a small number of individuals carried them and there is a lack of functional and clinical information on their effects in the study founder population and LQTS families in general.[20] Genomic DNA was extracted from peripheral blood leucocytes using a previously described method.[21] Clinical information available on the study participants and the number of individuals genotyped per category is summarized in Table 1. The number of individuals included in different stages of the analyses is shown in Figure 1.

ECG Analysis

Of the 349 participants for whom ≥1 single nucleotide polymorphism (SNP) was successfully genotyped in this study, baseline resting ECGs recorded in the absence of β-blocker therapy were available for 273 individuals, of whom 137 were mutation carriers (Figure 1). The 12-lead ECG was analyzed by an investigator experienced with LQTS to ascertain baseline heart rate, RR intervals, and QT interval duration. The QT interval, recorded in the absence of β-blocker therapy, was corrected for heart rate using the Bazett formula and ranged between 397 and 687 ms for the mutation carrier group.[22]

Cardiac Events

Cardiac events recorded included syncope, aborted cardiac arrest (requiring resuscitation), or sudden cardiac death. Mutation carriers were considered symptomatic if they had experienced ≥1 of the above mentioned cardiac events irrespective of age. Mutation carriers were classified as asymptomatic if they were >20 years and had never experienced one of these events. This cutoff value was chosen because a first cardiac event in this LQTS founder population almost invariably occurred before the age of 20 years and rarely after the age of 20 years.[6]

Table 1. Long-QT Syndrome Founder Population Data Summary for Individuals in Whom At Least One SNP was Genotyped

At Least One SNP Genotyped*	n	Noncarriers (n=181)	Mutation Carriers (n=168)
Sex, male (%)	349	95 (52)	75 (45)
BB, yes/total (%)	234	5/68 (7)	97/166 (58)
Age, y, at which ECG was analyzed, mean (SD)	273	34 (20.2)	34.9 (22.9)
BB starting age, y, mean (SD)	95†	32.3 (22.5)	17.2 (17.7)
QTc, median (IQR)	273	401 (383–418)	483 (463–512)
Cardiac events, yes (%)	349	0 (0)	122 (73)
Age first cardiac event, median (range)	115	N/A	6 (2–22)
Age last followed up about CE, median (IQR)	161	N/A	40 (20–57)

BB indicates β-blocker therapy; CE, cardiac events; IQR, interquantile range; QTc, heart rate–corrected QT interval; and SNP, single nucleotide polymorphism.

*All SNPs were genotyped in 176 noncarriers and 162 mutation carriers.

†There were 2 additional individuals for whom it was known that BB therapy was started after the age of 20 y. However, the age at which the treatment started was unknown.

The time to first event analysis was performed on 114 mutation carriers of whom 88 were symptomatic and 26 were asymptomatic. Mutation carriers who had never experienced cardiac events but were on β-blocker therapy before 20 years of age were excluded (n=17), as the treatment could have prevented cardiac events from occurring. Furthermore, symptomatic individuals who were on β-blocker therapy before their first cardiac event were excluded (n=7), as the age for their first cardiac event may have been different in the absence of β-blocker therapy. Finally, mutation carriers lacking required information for statistical modeling (eg, ECG and cardiac event information) did not form part of the analysis (n=30).

The severity of the disorder was evaluated using the score given in Table 2. This analysis included 97 mutation carriers who had never been on β-blocker therapy or only started taking β-blocker treatment after the age of 20 years. In this off β-blocker therapy group, all individuals, except for 2, were above the age of 20 years. One could already be classified in the most severe category by the age of 9 years, and the other, last followed up at the age of 16 years, was already symptomatic (had experienced 1 syncopal episode).The number of individuals present in each severity category 0 to 3 was 27, 31, 25, and 14, respectively. Individuals on β-blocker therapy before the age of 20 years were investigated separately. That group consisted of 43 individuals, with 26 below the age of 20 years (individuals per category 0–3: 13, 9, 7, and 14, respectively).

AKAP9 Genotyping

TaqMan Validated SNP Genotyping Assays (Applied Biosystems, Foster City, CA) were used to genotype all DNA samples for the intronic variants rs11772585, rs7808587, rs2282972, and rs2961024 (http://www.ncbi.nlm.nih.gov/SNP). These SNPs were selected to ensure entire gene coverage, including 2 kb on either side, using Tagger analysis (http://www.broadinstitute.org/mpg/tagger), applying default settings with an r^2 cutoff value of 0.8 and a minor allele frequency of 0.2 (common SNPs).[23] The HapMap Genome Browser release No. 24 (phase 1 and 2) full data set was used, and information

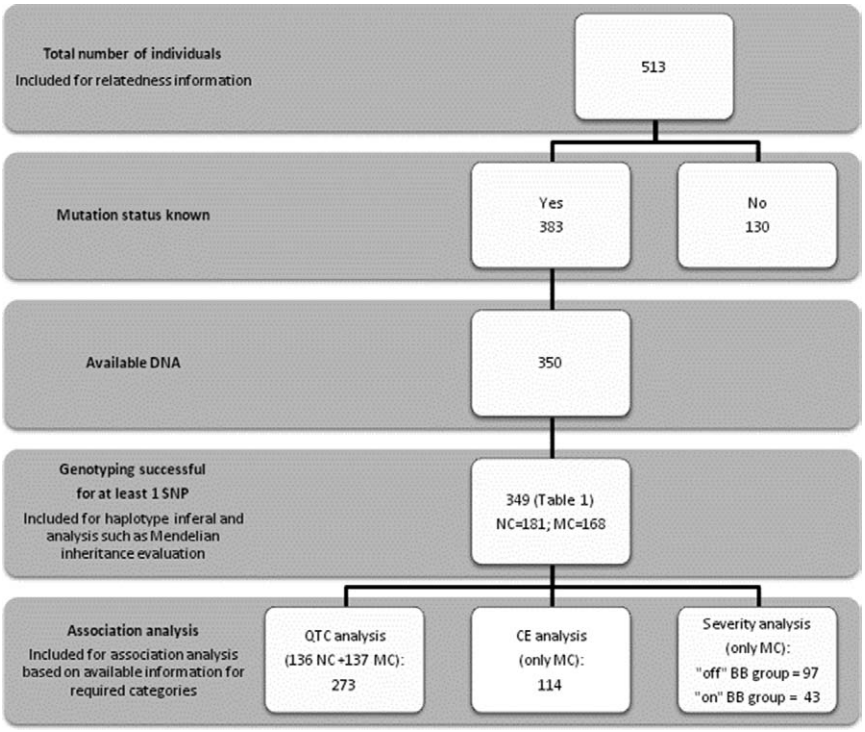

Figure 1. Number of individuals entered into analyses based on available information. BB indicates β-blocker treatment; CE, cardiac event; MC, mutation carriers; NC, noncarriers; QTc, heart rate–corrected QT interval; and SNP, single nucleotide polymorphism.

pertaining to the Centre d'Etude du Polymorphisme Humain (CEPH) HapMap population (Utah residents with ancestry from northern and western Europe) was selected.

Polymerase chain reactions were set up in 384-well plates, each reaction consisted of 20 ng of genomic DNA, 2.5 μL ABI TaqMan Universal Polymerase Chain Reaction Master Mix, 0.25 μL TaqMan primer and probe dye mix, and 1.25 μL DNase-free sterile water. Amplifications took place using the following cycle: 2 minutes at 50°C, 10 minutes at 95°C, followed by 40 cycles of 15 s at 92°C, and 1 minutes 30 s at 60°C. Allele discrimination was achieved by analyzing polymerase chain reaction–amplified products with the ABI Prism 7900HT Sequence Detection System SDS version 2.3 software (autocaller confidence level=95%). All allelic and genotype data given for the chosen SNPs corresponded to the positive DNA strand relative to the reference sequence.

Statistical Analysis

SNP Analysis

Pedstats v 6.11[24] was used to check Mendelian inheritance, as well as to perform exact tests Hardy–Weinberg Equilibrium among selected maximally informative but unrelated individuals from the founder population. Haploview version 4.2[25] was used to estimate the minor allele frequencies, as well as the linkage disequilibrium (LD) measure D'. As Haploview does not always select the same group of maximally informative but unrelated individuals for estimating LD, the analysis was repeated 100× and median values are reported. R, a language and environment for statistical computing, freely available from www.R-project.org and R packages Coxme (Terry Therneau, 2012. coxme: Mixed-Effects Cox Models. R package version 2.2–3. http://CRAN.R-project.org/package=coxme) and kinship2 (Terry Therneau, Elizabeth Atkinson, Jason Sinnwell, Martha Matsumoto, Daniel Schaid and Shannon McDonnell, 2012. kinship2: Pedigree functions) were used for statistical analysis.

Table 2. Long-QT Syndrome Severity Score Used to Evaluate Effects on Disease Severity

Score Value	Satisfying Condition
0	No cardiac events
1	1–3 syncope episodes
2	More than 3 syncope episodes; no cardiac arrest or sudden cardiac death
3	Cardiac arrest or sudden cardiac death

Traits

The traits investigated in this study were QTc duration, a severity score (Table 2), and the time until the first cardiac event (age). As this founder population contains many affected individuals, the QTc duration data were not normally distributed; it was positively skewed because of some large values. Because the mean and SD are not appropriate statistical measures in this case, terms of the median and interquantile range were described. Furthermore, linear statistical modeling required a log-transformation to approximate symmetry before analysis, ensuring the validity of results and conclusions. Kaplan–Meier curves are used to illustrate the age at first cardiac event.

Linear mixed-effects models were used for QTc duration and the severity score, whereas mixed-effects Cox regression models were used for the analysis of time to first cardiac event. All models were adjusted for sex as a fixed effect and for the degree of relationship between each pair of individuals in the study (using a per individual random effect with kinship coefficients), as well as a per family uncorrelated random effect. The linear models were further adjusted for age and the QTc model also for the presence or absence of the A341V mutation as a fixed effect. Therefore, significant QTc analysis associations were representative of the change in QTc irrespective of mutation status. The mixed-effects Cox regression models for the time to first cardiac event analysis were additionally adjusted for QTc. Effect estimates from the models are reported as percentage change (not milliseconds) for QTc because of the back-transformation (exponentiation) of the logarithms, change in score for severity and hazard ratio (HR) for experiencing a cardiac event.

As an indication of precision, 95% confidence intervals (CIs) are also provided.

Genetic Association

For single SNPs, the genotype effect was tested by splitting it into an additive term (number of minor alleles) and a heterozygote flag (yes/no) in all models. This is equivalent to testing a genotype effect on 2 degrees of freedom. If either of the components was significant, the corresponding

genetic model, additive, dominant, or recessive on the minor allele, was tested. The result from the best fitting model is reported.

Simwalk version 2.91[26] was used to infer the most probable maternal and paternal haplotype configuration for individuals with the necessary genotype and phase information in the pedigree. Haplotypes with an estimated frequencies larger than 0.01 were analyzed. An additive (without heterozygote flag) model of association was fitted for individual haplotypes, as described for SNP evaluations, thereby comparing each haplotype to all others in each model. Additionally, the interaction of each possible confounder (age, sex, and presence or absence of A341V) was tested with all genetic variables (alleles and haplotypes) on QTc. Furthermore, the interaction of sex was tested with each genetic variable on the risk of first cardiac event.

In this study, no correction for multiple testing was performed. Correcting for multiple testing can be overly stringent in family-based association studies. Furthermore, a Bonferroni correction assumes independence between the different tests performed and is, therefore, not appropriate when analyzing different SNPs in LD.[27]

Results corresponding to *P* values lower than 5% are described as significant and reported. A flow diagram of the statistical methods applied is provided in the Data Supplement.

Results

Of the 350 individuals, for whom DNA was available, only 1 individual was not successfully genotyped for ≥1 of the SNPs (Figure 1; Table 1). Furthermore, all 4 SNPs analyzed were in Hardy–Weinberg Equilibrium for the selected subset of unrelated individuals. Minor allele frequencies varied from 0.10 (T allele) for rs11772585 to 0.46 (G allele) for rs7808587. The number of individuals successfully genotyped for the different SNPs and allele and haplotype frequencies are given in Table 3. Because of strong LD between the SNPs and because this study was conducted with related individuals, haplotype frequencies varied from the expected if assuming linkage equilibrium. The LD analysis, in the unrelated subgroups, revealed a D' value of 1 for all SNP pairs investigated, for all 100 runs. A D' value of 1 indicates that the 2 markers being tested are in complete (not perfect) LD,[28] with ≥1 two-marker haplotype having a frequency of 0, as was the case for all SNP pairs investigated in the present study.

Each SNP was evaluated for association with all traits, and the significant results are tabulated in Table 4, as well as discussed briefly below.

QTc Analysis

Initial investigation of the data revealed a highly significant correlation of sex (P<0.001) with QTc. Women had an estimated 5.6% longer QTc than males. Additionally, the presence of A341V was correlated with QTc (P<0.001). Mutation carriers had an estimated 21.4% longer QTc than noncarriers. QTc heritability was estimated at 31% (55% unadjusted for A341V).

A significant interaction between age and rs2961024 on QTc (P=0.006), after adjusting for sex and mutation status, was detected. Individuals with the TT genotype for this SNP showed a 1% (95% CI, 0.2–1.3) shorter QTc with every additional 10 years of life, whereas for each minor G allele carried in genotypes GT and GG, this age effect increased by 1% (95% CI, 0.3–1.6) over the same time period compared with the TT genotype. The net result was no significant effect for GT and a 1% increase for the GG genotype. Figure 2 indicates that this increase compounded over several years. The curves commenced at the highest QTc for a female mutation carrier (Figure 2A) but dropped for male mutation carrier (Figure 2B) and noncarrier estimates (Figures 2C and 2D) because of the difference in baseline QTc values. However, the relative position of the genotype curves to each other remained the same.

As an example, a 10-year-old individual with the GG genotype and a QTc of 450 ms would have an increase of 4.5 ms over the next 10 years. The 1% increase over the following 10 years would then be calculated from a QTc of 454.5 ms. Continuing this process, the effect of this risk genotype from 10 to 40 years of age would be an increase of 13.6 ms (QTc 40 years≈463.6 ms). However, as the millisecond change depends on the QTc of the individual and the QTc values for mutation carriers in this founder population ranged between 397 and 687 ms (mean age=34.9), the actual change in milliseconds, increase or decrease, between mutation carriers is expected to be extremely variable.

Table 3. Minor Allele and Haplotype Frequencies in the South African Long-QT Syndrome Founder Population

	rs11772585	rs7808587	rs2282972	rs2961024
Number genotyped	346	345	345	342
Minor allele	T	G	T	G
MAF	0.10	0.46	0.44	0.29
Haplotypes				
HF=0.443	C	A	T	T
HF=0.282	C	G	C	G
HF=0.171	C	G	C	T
HF=0.098	T	A	C	T

HF indicates haplotype frequency; and MAF, minor allele frequency.

Table 4. Long-QT Syndrome Modifying Effects of *AKAP9* SNPs and Haplotypes

Modifying Effect	SNP/Haplotype*	*P* Value	Effect Size	Effect Description
QTc, % change per 10 y	rs2961024	0.006	1%	per G ↑ vs TT
	CGCG	0.012	1%	per CGCG ↑ vs 0:CGCG
CE, % change in risk	rs11772585	0.002	118%	CT ↑ vs CC
	rs7808587	0.046	74%	GG ↑ vs AA and AG
	rs2282972	0.001	53%	CT and TT ↓ vs CC
	TACT	0.006	100%	1:TACT ↑ vs 0:TACT
	CATT	0.022	35%	per CATT ↓ vs 0:CATT
Severity, change in score	rs11772585	0.025	0.58	CT ↑ vs CC
	TACT	0.032	0.54	1:TACT ↑vs 0:TACT

↑ indicates an increase; ↓, decrease; 0:, haplotype absent; 1:, 1 copy of the haplotype present; CE, cardiac events, QTc, heart rate–corrected QT interval; and SNP, single nucleotide polymorphism.

*Haplotype order: rs11772585, rs7808587, rs2282972, rs2961024

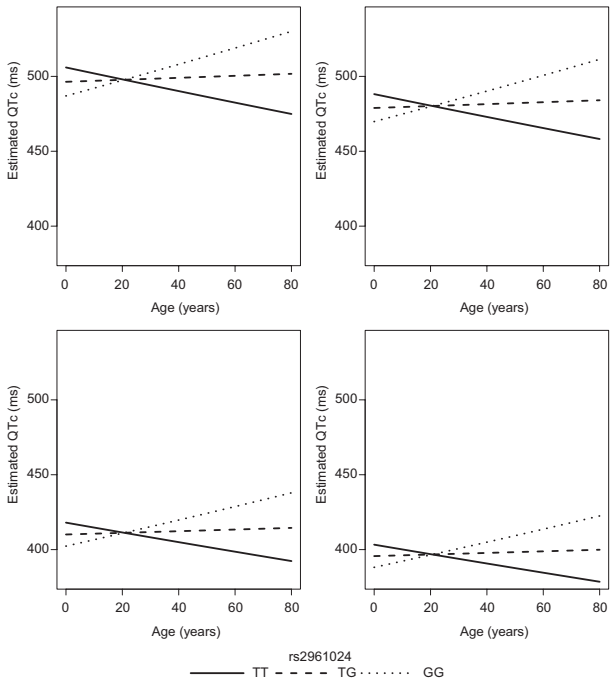

Figure 2. Age-dependent change in QTc for rs2961024 genotypes by sex and A341V mutation status. Modelled curves indicating the 1% per 10-year increase with each G allele relative to the TT genotype for a female mutation carrier (**A**), male mutation carrier (**B**), female noncarrier (**C**), and male noncarrier (**D**). The net result was no significant effect for GT and a 1% increase for the GG genotype. The curves were identical for the different categories; however, they start at different baseline QTc values. QTc indicates heart rate–corrected QT interval.

Interestingly, all individuals in the founder population who had the GG genotype at rs2961024 were also homozyotes for each of the other 3 SNPs investigated, that is they all had 2 copies of the CGCG (order: rs11772585, rs7808587, rs2282972, and rs2961024; frequency=0.282) haplotype. Consistent with this observation, and with the above results, individuals with 2 copies of the CGCG haplotype had an average QTc 1% (95% CI, 0.2–1.4) longer per 10-year increase in age (P=0.012), whereas those carrying 1 copy of CGCG showed no significant effect on QTc duration, and in individuals without a copy of this haplotype, there was an average 1% (95% CI, 0.2–1.3) shorter QTc duration per 10-year increase in age. However, given the small number of CATG observations, it is impossible to discern whether the effect of rs2961024 is restricted to the CGCG haplotype.

Interestingly, after adjusting for A341V and sex, rs2961024 was also significantly associated with QTc (P=0.014) independent of age, with the GG genotype associated with longer QTc relative to the TT and GT genotypes. Furthermore, including an adjustment for age yielded the same result, with the presence of the GG genotype associated with a 5% longer QTc (95% CI, 1.0–9.1) compared with TT and GT. However, no statistically significant additive allelic or haplotype associations with QTc were observed, given these adjustments.

Therefore, after adjusting for age, sex, and A341V, both rs2961024 and the interaction between age and rs2961024 are significant predictors of QTc. The QTc increases with age, and this observation is progressively more pronounced with each additional G allele compared with the TT genotype; even ignoring age, the average QTc is higher. Therefore, the age-dependent effect can be considered the best description of the observed association between rs2961024 and QTc.

Cardiac Events Analysis

The risk of experiencing a cardiac event was significantly greater in participants with a longer QTc duration for all models investigated (P=0.000035), consistent with previously reported associations in this founder population.[6] The significant effects described below are independent of QTc.

The *AKAP9* SNP, rs11772585, was associated with an increased risk of cardiac events (P=0.002). For this SNP, no TT genotypes were observed, yet having the T allele within the CT genotype more than doubled the risk of having a cardiac event (HR=2.18; 95% CI, 1.33–3.60). Figure 3A illustrates the 118% greater probability of a cardiac event. Haplotype analysis supported this result (Table 4).

Another *AKAP9* SNP, rs7808587, was associated with a recessive model (P=0.046), with the GG genotype increasing the cardiac event risk by 74% (HR=1.74; 95% CI, 1.01–3.01) relative to the AG and AA genotypes. Figure 3B illustrates this increased probability of a cardiac event.

Finally, rs2282972 was associated with a dominant model (P=0.001), with the CT and TT genotypes reducing the cardiac event risk by 53% (HR=0.47; 95% CI, 0.29–0.74) relative to the CC genotype. Figure 3C illustrates this reduced probability of a cardiac event.

Consistent with the above result, CATT, the predominant haplotype containing the rs2282972 T allele, was found to be associated with a decrease of 35% (HR=0.65; 95% CI, 0.45–0.94) per haplotype copy (P=0.022).

Severity Score

The effect of any of these SNPs on the severity of the disease was investigated using the score given in Table 2. In agreement with the other results, rs11772585 also affected disease severity for the off β-blocker therapy group. The T allele was significantly associated (P=0.025) with a higher score by a value of 0.58 (95% CI, 0.07–1.08). Haplotype analysis supported this result (Table 4). The separately investigated smaller group consisting of individuals on treatment did not show any statistically significant results.

Discussion

The present study demonstrates that yotiao, the protein encoded by the *AKAP9* gene, is a modifier of the clinical phenotype of LQTS. This finding is important given that individuals with LQTS present with symptoms that can vary considerably, irrespective of their carrying the same disease-causal mutation, as demonstrated by the families in this study.[6–8] In this founder population, *KCNQ1* A341V mutation carriers have a broad range of QT intervals and they may be symptomatic or asymptomatic, depending on the occurrence of cardiac events.[6] Worldwide, the A341V mutation is associated with a severe form of the disease, with the likelihood of cardiac events occurring more frequently and earlier in affected individuals relative to other *KCNQ1* mutations.[29] However, LQT1 individuals >20 years of age who, despite not having treatment,

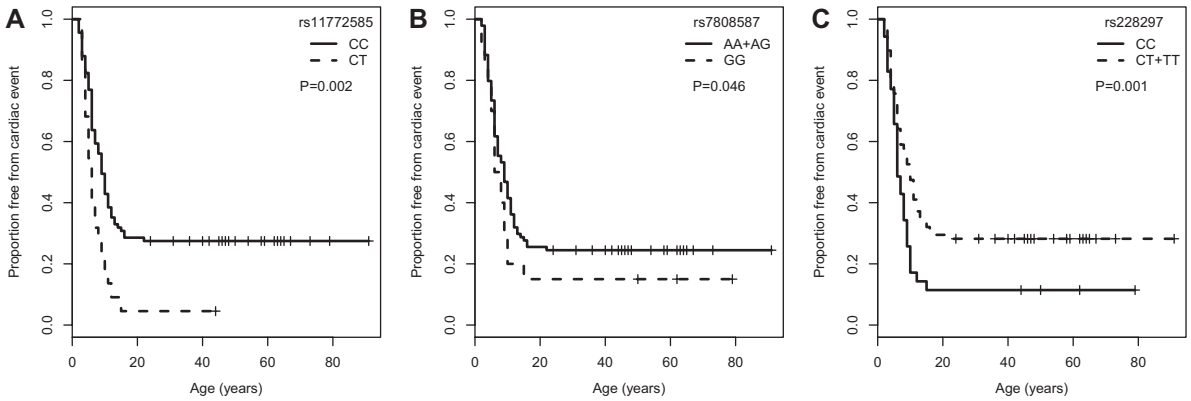

Figure 3. Genotype-specific risk of cardiac events for *AKAP9*. Kaplan–Meier cumulative event-free survival of rs11772585, rs7808587, and rs2282972 genotypes.

are asymptomatic seldom become symptomatic afterward.[5,29] Accordingly, mutation carriers identified after this age rarely started treatment, explaining the low β-blocker treatment percentage for the study population in Table 1.

Despite the severe phenotype of the A341V mutation, it has been shown that it only has a modest dominant negative effect on the wild-type channel.[6] The channel subunits encoded by the mutated allele because of their association with other subunits to form the complete ion channel complex, negatively influence the function of the wild-type subunits and thereby the performance of the channel. Heijman et al[30] showed that the severity conferred by this mutation can, in part, be explained by the reduced phosphorylation of KCNQ1. Yotiao plays an important role in this phosphorylation process, as well as in downstream changes in channel activity as a consequence of this phosphorylation.[13,17] Therefore, it could be proposed that variants in the *AKAP9* gene that result in altered yotiao expression, structure, or function are likely to influence channel properties and could explain some of the severity and phenotypic differences observed. As several *KCNQ1* mutations, including A341V, suppress cAMP or protein kinase A–mediated channel regulation,[30,31] it is possible that modifying effects by *AKAP9* variants are acting on the wild-type channels.

This investigation strengthens the notion that yotiao is indeed involved in conferring some of the severity and phenotypic variability. *AKAP9* variants in this study were shown to alter the QTc interval, as well as the risk and severity of cardiac events, in the South African A341V founder population. As these findings were observed in a population with a specific genetic background, additional work is required to verify that the same effect is observed in other population groups, as well as for different types of LQTS. Furthermore, as these variants are located in the intronic regions of the gene, they themselves are unlikely to be the direct cause of the modifying effects, and variants in LD with them require further investigation. However, this work clearly indicates involvement of the *AKAP9* gene region as an LQTS modifier.

Most other genetic modifying effects reported have been associated with already known LQTS genes. Variants associated with QTc and sudden cardiac death have been described for *KCNQ1*, *KCNH2*, *SCN5A*, *ANK2*, *KCNE1*, *KCNE2*, and *KCNJ2* (LQT1-7).[11,32–35] Additionally, recent genome-wide association studies have revealed a few novel QTc-associated loci, including the nitric oxide synthase 1 adaptor protein gene (*NOS1AP*).[34–36] *NOS1AP* has since been repeatedly associated with QTc interval, LQTS disease severity, and sudden cardiac death risk,[37–40] with significant modifying effects reported for the same founder family described in the present study.[9]

AKAP9 Variant Associated With QTc Duration

The heritability of the QT interval is reported to range between 25% and 55% (latter value from present study), with many common variants examined to date not explaining much more than 7% of the total QTc variation.[34,41] Furthermore, 31% of the estimated heritability for the South African A341V founder population could not be explained by the A341V mutation, age, or sex (this study).

The present study identified an age-dependent additive allelic association between the rs2961024 SNP and QTc (Table 4; Figure 2). This SNP was identified as a modifier for QTc irrespective of mutation status in this specific founder population. Older individuals, with 2 copies of the minor allele (GG genotype), displayed a longer QTc. The opposite was true for individual carrying 2 copies of the major allele. Pfeufer et al also report age-specific effects on QT interval when looking at the gene encoding the RING finger protein 207, *RNF207*, as well as that encoding the cardiac phospholamban protein, *PLN*.[35] However, in their investigation, each minor allele of the particular SNP investigated contributed toward a larger QT-prolonging effect (rs846111) or a lower QT-shortening effect (rs12210810) in younger individuals.

Although, in this study, an association between rs2961024 and QTc was observed both with and without considering the age-dependent effect, other investigations, not evaluating interactions with specific confounding variables, could be missing specific variable-dependent QTc associations. Additionally, studies using additive allelic models could miss only dominant and recessive effects. This highlights the importance of evaluating different genetic inheritance models and interactions of genotypes with confounding variables on clinical outcomes. Many previously reported common variants associated with QT interval only alter the variability by small percentages, emphasizing the relevance of considering multiple models for detection.[41]

AKAP9 Variants Associated With Modifying Risk and Severity of Cardiac Events

The *AKAP9* gene not only alters QTc duration but also influences the risk and severity of cardiac events, after controlling for QTc. However, different variants were associated with cardiac event risk and severity than the one shown to alter QTc interval duration (Table 4). The extent of the phenotypic variability in LQTS, as well as the existence of mutation carriers with normal QTc intervals that experience cardiac events, suggests that there are ≥1 or more different variants involved in modifying cardiac events and severity to QT interval duration.[6–8,42] Furthermore, as yotiao-dependent regulation of the KCNQ1-KCNE1 channel is so versatile, in targeting many proteins to the channel complex and in ensuring not only efficient KCNQ1 phosphorylation but also the subsequent channel response, it is not hard to imagine that different variants in its encoding gene could result in slightly different phenotypic expression.[13,17,43,44] Interestingly, Kao et al also report a few *NOS1AP* variants affecting the QT interval but not sudden cardiac death.[45] Work dedicated to understanding the different mechanisms responsible for these observations requires further attention.

Three *AKAP9* SNPs were significantly associated with cardiac event risk, and 1 of these was also associated with disease severity. The rs11772585 minor allele (T) was significantly associated with both the risk of having a cardiac event and increasing the severity of the disease. The presence of this allele more than doubled the risk of a cardiac event. These results were supported by the haplotype analysis (Table 4). The rs7808587 GG genotype was associated with a 74% increase in cardiac event risk, whereas the rs2282972 minor allele (T) revealed a protective effect, reducing the risk of an event by 53%. A protective effect associated with another LQTS-causative gene variant, *KCNQ1* rs2074238, has also recently been reported, with the relevant allele (T allele) modifying both the risk of symptoms and QTc duration.[46]

Conclusions

Variants in several arrhythmia-related genes have been reviewed and their contributions toward QTc interval and sudden cardiac death described in different populations.[11,12,41] However, the LQTS gene, *AKAP9*, encoding a prominent protein in the regulation of the KCNQ1-KCNE1 channel, has not previously been described as a potential modifier. Results evaluated here clearly demonstrate that this gene contributes to LQTS phenotypic variability. However, as these SNPs are located in the intronic regions of this gene, it remains to be seen which functional or regulatory variants in LD with these SNPs are responsible for these modifying effects. Furthermore, it would be interesting to see whether similar effects can be replicated in other populations. Importantly, these findings provide insight into the role that *AKAP9* plays, not only as an LQTS-causal gene, but also as a phenotypic modifier.The identification of the mutation-specific risk associated with a growing number of modifier genes epitomizes the evolution in the understanding of LQTS, a disease which increasingly represents the clearest example of how tightly connected the relationship between genotype and phenotype can be and how this is progressively impacting on clinical management.[29,47]

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Disclosures

None.

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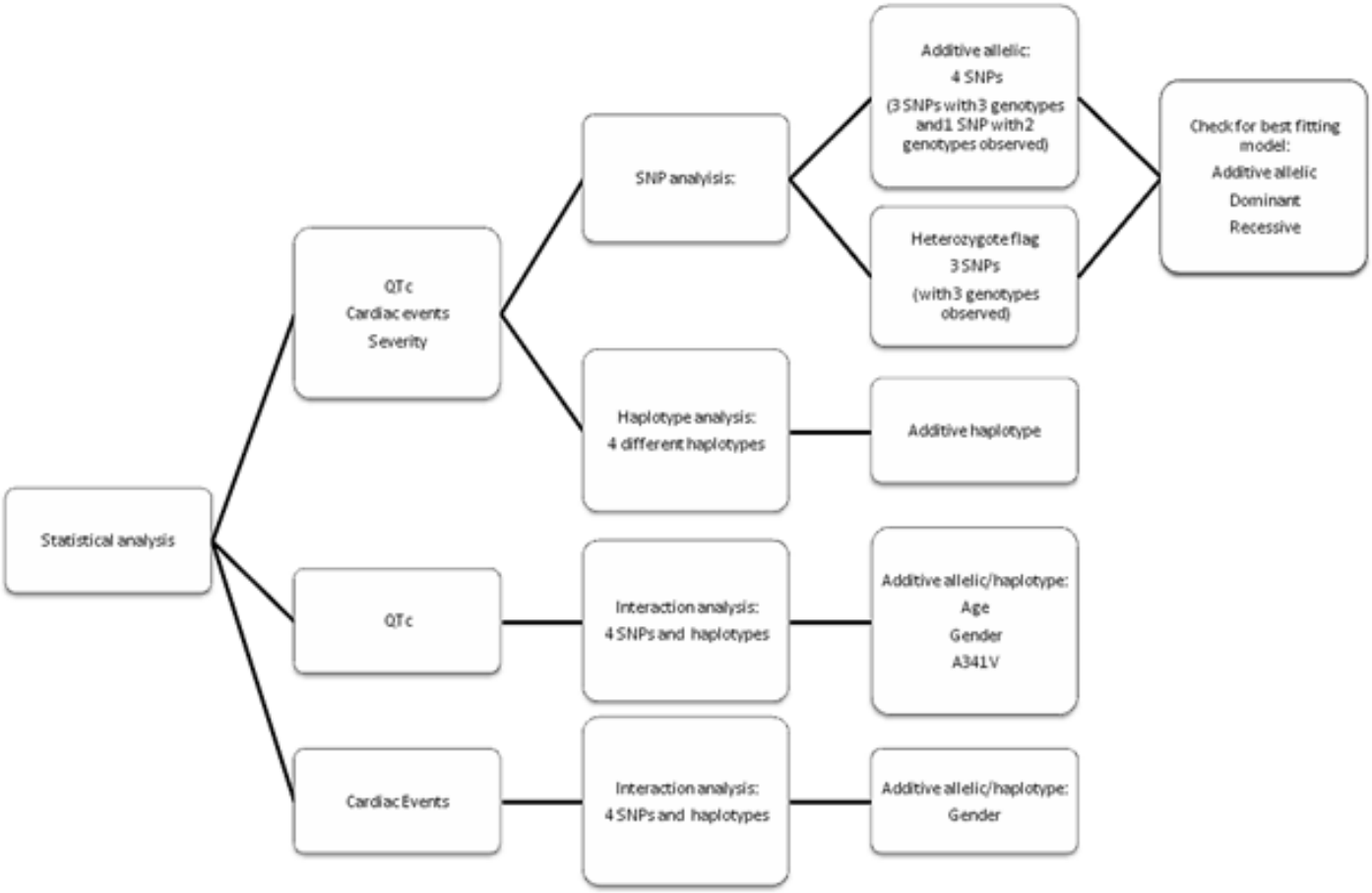
CLINICAL PERSPECTIVE

The long-QT syndrome (LQTS) is a cardiac arrhythmia disorder of genetic origin characterized by a prolonged QT interval and cardiac events, including syncope, cardiac arrest, and sudden death, often affecting young individuals. LQTS has extensive phenotypic variability irrespective of the presence of the identical primary disease-causative mutation, and this striking heterogeneity of the clinical manifestations is not only one of its most puzzling features but also hampers clinical management and risk stratification of family members carrying the same mutation. We investigated the potential of a known LQTS-causal gene, *AKAP9*, to act as a modifier in a large South African LQTS (*KCNQ1* A341V) founder population. A common *AKAP9* variant was shown to alter heart rate–corrected QT interval, whereas 3 others were significantly associated with the risk of cardiac events. Furthermore, 1 of the variants also increased disease severity. These results strengthen the concept that variants in LQTS-causal genes can modify the clinical presentation and implicate *AKAP9*, for the first time, as an LQTS modifier. These *AKAP9* modifying effects, in combination with the growing cluster of other identified LQTS modifiers, represent a step forward toward individual risk stratification clinical management.

SUPPLEMENTAL MATERIAL



Statistical analysis overview: Each outcome was modeled as a function of two genetic terms. The first was additive allelic (counting the number of minor alleles) and the second was a heterozygote flag. After detecting significant associations, we tested whether a dominant or recessive model provided a better fit. We also tested genetic interactions with each of A341V mutation, age and gender on QTc and with gender on cardiac events. Each model was adjusted for appropriate confounders.

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AKAP9 Is a Genetic Modifier of Congenital Long-QT Syndrome Type 1

Carin P. de Villiers, Lize van der Merwe, Lia Crotti, Althea Goosen, Alfred L. George, Jr, Peter J. Schwartz, Paul A. Brink, Johanna C. Moolman-Smook and Valerie A. Corfield

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