Single nucleotide polymorphisms in arrhythmia genes modify the risk of cardiac events and sudden death in long QT syndrome

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BACKGROUND Disease-modifying single nucleotide polymorphisms (SNPs) can help explain incomplete penetrance and variable expressivity in congenital long QT syndrome (LQTS) by altering susceptibility to arrhythmias.

OBJECTIVE The purpose of this study was to assess multiple arrhythmia SNPs (in 16 genes) in a distinct cohort of LQTS patients to identify modifier SNPs influencing the risk of sudden death.

METHODS This study included 273 patients with LQTS from the New Zealand Cardiac Inherited Disease Registry (154 long QT type 1, 96 long QT type 2, and 23 long QT type 3), including 31 patients who had experienced death or resuscitated sudden cardiac death (RSCD). Patients were genotyped for 29 SNPs and tested for associations with clinical events and QTc length. Caucasian (n = 220) and Pacific Islander/New Zealand Maori (n = 53) ethnic groups were analyzed separately. This subgroup of Polynesian ancestry has not been previously studied for LQTS in either presentation or outcome.

RESULTS In Caucasians, four SNPs at two risk loci (*NOS1AP*: rs12143842 and rs16847548; and *KCNQ1:* rs10798 and rs8234) were significantly associated with clinical events after correction for multiple testing. Patients homozygous for the risk allele of rs12143842 had an increased risk of death/RSCD [hazard ratio 10.15, 95% confidence interval (2.38, 43.34), q = 0.045). Several other SNPs showed trends toward association with QTc length and clinical events.

CONCLUSION This study demonstrates that SNPs in *NOS1AP* and *KCNQ1* are associated with an increased risk of cardiac events in LQTS patients, with the hazard ratio suggesting they have significant potential in clinical risk stratification.

KEYWORDS Long QT syndrome; Genetics; Single nucleotide polymorphisms; Arrhythmia; Sudden cardiac death

ABBREVIATIONS **CI** = confidence interval; **ECG** = electrocardiogram; **GWAS** = genome wide association study; **HR** = hazard ratio; **LQT1** = long QT syndrome type 1; **LQT2** = long QT syndrome type 2; **LQT3** = long QT syndrome type 3; **LQTS** = long QT syndrome; **NZ** = New Zealand; **OR** = odds ratio; **QTc** = corrected QT interval; **RSCD** = resuscitated sudden cardiac death; **SCD** = sudden cardiac death; **SNP** = single nucleotide polymorphism

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Introduction

Long QT syndrome (LQTS) was the first inherited arrhythmia syndrome to have its genetic basis revealed and has served as a paradigm for other arrhythmia syndromes.[1] The QT interval plays an integral part in risk stratification of patients with LQTS, with further prognostic information provided by gender, age, prior cardiac events, genotype, and the location and effect of pathogenic mutations in protein structures.[2] Congenital LQTS displays incomplete penetrance and variable expressivity even within families; the penetrance of mutations can range from 25% to 100%.[2] This variation may indicate the presence of significant environmental factors or genetic modifiers such as single nucleotide polymorphisms (SNPs), acting either independent of, or in concert with, the LQTS-causing mutation.

Several genome-wide association studies (GWAS) have found SNPs associated with the QT interval and other measures of cardiac conduction in the general population or in specific populations such as patients with coronary

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artery disease.[3] Other GWAS or candidate gene studies have identified associations between particular SNPs and the risk of cardiac arrest or sudden cardiac death (SCD).[4]

In addition to the type and location of the LQTS-causing mutation, both synonymous SNPs (those not causing a change in amino acid) and nonsynonymous SNPs (those causing a change in amino acid) can modify the severity of the LQTS phenotype. These SNPs may have a modest effect individually, but the coexistence of several risk alleles could unmask an LQTS phenotype that was subclinical in unaffected carrier relatives.

SNPs in the *NOS1AP* gene have recently been investigated in patients with LQTS, after they were found to influence electrocardiographic (ECG) parameters and the risk of cardiac arrest in the general population.[5–7] These studies have suggested that *NOS1AP* SNPs modulate symptom severity and have emphasized the need for replication in independent populations. The study reported here investigates these as part of a wider spectrum of alleles at 29 SNPs (in 16 genes) with disease severity in a cohort of New Zealand (NZ) LQTS patients.

Methods

Study population

The study population (n = 273) was derived from patients enrolled in the NZ Cardiac Inherited Diseases Registry.[8] This is a consent-based clinical registry that includes both living patients referred by pediatric or cardiology services and also victims of unexplained sudden death who have been referred by pathologists or the coroner (following collection of a DNA sample at autopsy).

Included in this analysis were both symptomatic and asymptomatic patients with LQTS-causing mutations in the *KCNQ1*, *KCNH2*, and *SCN5A* genes. Patients with compound mutations in LQTS-susceptibility genes were excluded from this analysis. Ethnicity was obtained from NZ Health Information Service records, which are self-reported, and analyses were divided into two ethnic groups: Caucasians and Pacific Islanders/NZ Maori.

Clinical data

Covariate data included age, gender, ethnicity, QTc, location of the LQTS-causing mutation, and cardiac events. A 12-lead ECG was performed on all live patients. All ECGs were measured manually by cardiologists who specialized in electrophysiology and were familiar with LQTS, and were checked by the senior author. The QT interval was measured in leads II and V_5, and the Bazett formula was used to correct for heart rate ($QTc = QT/\sqrt{RR}$), with the longest interval taken as representative. Cardiac events were captured in the registry database as part of routine clinical follow-up. In this study, resuscitated sudden cardiac death (RSCD) was defined as a cardiac arrest requiring resuscitation by cardiopulmonary resuscitation or external defibrillation.

SNP selection

Thirty-four SNPs were initially selected from GWAS or candidate gene studies identified during a literature search. Selected SNPs had been shown to affect the QT interval or QRS duration in healthy populations (17 SNPs), QT interval or cardiac events in patients with ion channelopathies (11 SNPs), or susceptibility to SCD or cardiac arrest in population studies (6 SNPs). Two SNP loci, rs37062 (*NDRG4*) and rs2519184 (*KCNQ1*), failed during genotyping. Three SNP loci, rs1805128 (*KCNQ1*), rs2234916 (*KCNE2*), and rs41263144 (*SCN5A*), were genotyped but excluded from analyses because the minor allele frequency occurred <0.05. Therefore, the final analyses comprised 29 SNPs from 16 genes (*ATP1B1, CASQ2, CDKN1A, CXADR, GJA1, GPC5, KCNE1, KCNE5, KCNH2, KCNJ2, KCNQ1, NOS1AP, PLN, RNF207, SCN5A, SCN10A*) (Online Supplementary Table 1).

Genotyping

Genomic DNA was isolated from whole blood samples using the Gentra Puregene DNA Extraction kit according to the manufacturer's instructions (Qiagen Pty Ltd, Dusseldorf, Germany) and diluted from stocks to a concentration of 10 ng/μL. Primers were designed using the online mySequenom assay designer suite software (https://www.mysequenom.com). Genotyping was undertaken in multiplex using Sequenom MassARRAY iPLEX assays (Sequenom, San Diego, USA), using mass spectrometry to resolve allele-specific single base extensions (MALDI-TOF).[9]

Statistical analysis

SNP alleles were tested for association with QTc length (as a continuous variable, and dichotomized as ≥ 500 ms or <500 ms, an established threshold where ≥ 500 ms indicates high risk[10]) and cardiac events: SCD/RSCD and "any cardiac event" (SCD/RSCD plus arrhythmic syncope). Follow-up time was defined as the period from birth to either the first event or the study end date if remaining event-free (median 27.12 years, range 0.01–91.95 years).

Multivariable Cox proportional hazards models were used to evaluate predictors of SCD/RSCD, and linear or logistic regression was used to test for associations between each SNP allele and QTc length or "any cardiac event." All models were adjusted for age and LQTS subtype (LQT1 [type 1], LQT2 [type 2], and LQT3 [type 3], corresponding to the *KCNQ1*, *KCNH2,* or *SCN5A* gene, respectively) and for QTc ≥ 500 ms (in the case of cardiac events). Gender was not found to be statistically significant and so was not included in the final models for cardiac events.

The SNP alleles were tested using an additive model, but if there were ≤ 15 patients in any one genotype group then the SNP alleles were tested using a dominant model. Six SNP loci fell into this category: rs10919071 (*ATP1B1*), rs12567209 (*NOS1AP*), rs1805123 (*KCNH2*), rs2242802 (*KCNE1*), rs3864180 (*GPC5*), and rs9398652 (*GJA1*).

Multiple testing was accounted for by using a false discovery rate. This rate used the "q value" package in R,[11] with a significance threshold (q value) of 0.10. This corresponds to an expectation that up to 10% of declared discoveries are false.[12] Analyses were carried out with either SAS version 9.3 (SAS Institute, Cary, NC) or R programming language version 2.15.3.

A random effects model to adjust for the family structures could not be developed because of the large number of single-member families (n = 40). However, the differences in genotype frequencies between singletons and multimember families were tested using a χ^2 test and there were no significant differences for any SNP alleles. This lack of difference indicates there is no enrichment for particular alleles due to family clustering.

Results

Clinical characteristics, QTc interval, and patient outcome

The clinical characteristics of 273 patients with LQTS (220 Caucasian and 53 Maori/Pacific Islander) from 93 families are listed in Table 1. Fifty-eight percent of the patients were female and 56%, 35%, and 8% had mutations in the *KCNQ1*, *KCNH2,* and *SCN5A* gene, respectively, with 70 different mutations in total. Ten patients (4%) experienced SCD, 21 (8%) experienced RSCD (5 requiring cardiopulmonary resuscitation and 16 requiring external defibrillation), and 58 patients (21%) experienced syncope.

Patients with QTc ≥500 ms (n = 85) were significantly more likely to have suffered a cardiac event [odds ratio (OR) 2.98, 95% confidence interval (CI) (1.76, 5.03), $P < .001$] or SCD/RSCD [OR 3.84, 95% CI (1.69, 8.71), $P = .001$] over the follow-up period. The risk of "any cardiac event" was particularly apparent in patients with the longest QTc intervals, with QTc ≥550 ms (n = 27) associated with a 13.42-fold increase in risk [95% CI (4.51, 39.92), $P < .001$], compared with other patients.

Long QT syndrome sub-type and age were significant predictors of SCD/RSCD but not "all cardiac events" combined. Patients with LQT1 or LQT2 were less likely to have experienced SCD/RSCD than those with LQT3 [hazard ratio (HR) 0.13, 95% CI (0.05, 0.32), $P < .001$; and HR 0.18, 95% CI (0.07, 0.46), $P < .001$, respectively]. Increasing age was associated with a decreasing risk of SCD/RSCD [HR per year increasing age = 0.92, 95% CI (0.89, 0.95), $P < .001$]. Overall, the effect of gender on outcome was not statistically significant in this cohort. This remained so within LQT subtypes (LQT1/2/3) and when split by age before and after adolescence (<15 years and ≥15 years); however, group sizes were small and may not be sufficiently powered to show this relationship.

Modifier SNPs: Caucasian patient group

The genotype frequencies for the 29 candidate modifier SNPs are reported in Online Supplementary Table 1. None of the alleles deviated from Hardy-Weinberg equilibrium.

Clinical events

In Caucasian patients, four SNPs at two risk loci (rs12143842 and rs16847548 in *NOS1AP*; rs10798 and rs8234 in *KCNQ1*) were significantly associated with clinical events, after correction for multiple testing. Patients homozygous for the risk alleles in the *NOS1AP* gene (rs12143842 or rs16847548) were at significantly increased risk of SCD/RSCD (Table 2 and Figure 1) or "any cardiac event" (Table 3 and Supplementary Table 2). Similarly, patients with at least one copy of the minor allele for rs10798 or rs8234 in the 3′ untranslated region of the *KCNQ1* gene had a significantly increased risk of "any cardiac event" (Table 3). In a subanalysis by long QT type, this association remained in LQT1 patients [rs10798 OR = 2.21, 95% CI (1.16, 4.21), $P = .016$; and rs8234 OR = 2.32, 95% CI (1.20, 4.47), $P = .010$, though q values were not significant]. The association was not proven in LQT2 or LQT3 patients [rs10798 OR = 1.97, 95% CI (0.98, 3.96),

Table 1 Demographics of study patients by long QT syndrome subtype.

	Total	KCNQ1	KCNH2	SCN5A
n (%)	273	154 (56)	96 (35)	23 (8)
Families (n)	93	53	28	12
Female [n (%)]	157 (58)	93 (60)	55 (57)	9 (39)
Age [median (IQR)]	30 (16, 50)	32 (19, 52)	30 (15, 47)	22 (8, 32)
Ethnicity [n (%)]				
Caucasian	220 (80)	133 (86)	71 (73)	17 (74)
Maori	46 (17)	21 (14)	21 (22)	5 (22)
Pacific Islander	7 (3)	0 (0)	5 (5)	1 (4)
Most significant clinical event				
Sudden death	10 (4)	3 (2)	4 (4)	3 (13)
Resuscitated SCD	21 (8)	10 (6)	6 (6)	5 (22)
Syncope	58 (21)	37 (24)	18 (19)	2 (9)
None	183 (67)	104 (68)	68 (71)	13 (57)
ECG				
QTc [median (IQR)]	481 (461, 514)	480 (465, 510)	489 (460, 516)	475 (440, 520)
QTc ≥500 ms (%)	85/250 (34)	45/142 (32)	31/85 (37)	9/23 (39)
QTc ≥550 ms (%)	27/250 (11)	13/142 (9)	13/85 (15)	1/23 (4)

ECG = electrocardiogram; IQR = interquartile range; QTc = corrected QT interval; SCD = sudden cardiac death.

Table 2 SNPs with significant associations in a Cox proportional hazards model of death/resuscitated sudden cardiac death in the Caucasian patient group

Gene	SNP	Genotype	Event/no event* (n)	Hazard ratio	*P* value	q value
NOS1AP	rs12143842	CC	4/106	1		
		TC	9/65	3.93 (1.18, 13.13)	.026	0.234
		TT	6/17	10.15 (2.38, 43.34)	.002	0.045
NOS1AP	rs16847548	TT	7/79	1		
		CT	11/96	3.22 (1.09, 9.55)	.035	0.263
		CC	4/23	8.57 (2.30, 31.9)	.001	0.045
NOS1AP	rs4657139	TT	5/69	1		
		AT	7/104	1.04 (0.29, 3.77)	.946	0.954
		AA	9/27	5.73 (1.56, 21.21)	.009	0.101
NOS1AP	rs10494366	TT	4/63	1		
		GT	9/106	1.26 (0.37, 4.27)	.712	0.953
		GG	8/30	4.07 (0.25, 15.77)	.043	0.276

SNP = single nucleotide polymorphism.
*Event/no event equals the number of patients in each genotype group who have/have not experienced resuscitated sudden cardiac death or death.

$P = .057$; and rs8234 OR = 1.99, 95% CI (0.99, 3.99), $P = .052$].

Two other SNPs in the *NOS1AP* gene (rs4657139 and rs10494366) showed a trend toward association with "any cardiac event" ($P < .05$), but these did not remain significant after correction with the false discovery rate (Table 3).

QTc length

There were trends indicating that two SNPs (rs3864180 in *GPC5* and rs4074536 in *CASQ2*) may be associated with QT interval length (Table 3). The A allele of rs3864180 (*GPC5* gene) was associated with having a QTc ≥500 ms [OR = 1.62, 95% CI (1.01, 2.61), $P = .049$], whereas the C allele of rs4074536 (*CASQ2*) decreased the likelihood of having a QTc ≥500 ms [OR = 0.60, 95% CI (0.37, 0.98), $P = .041$; Table 3]. However, neither remained significant after correction with the false discovery rate.

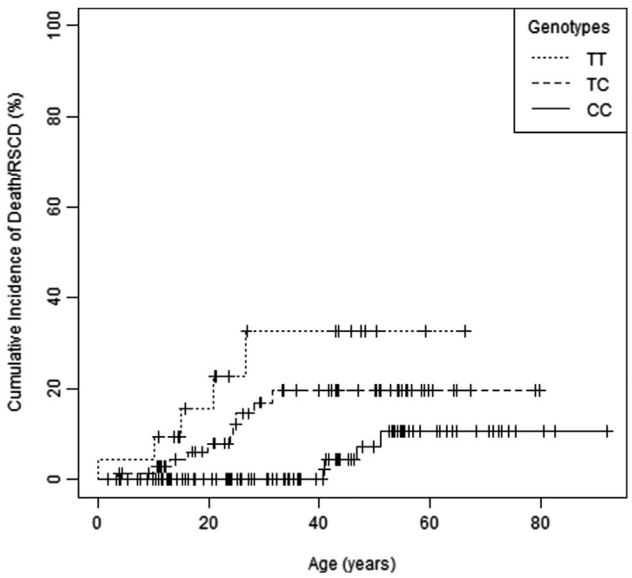

Figure 1 Kaplan-Meier survival analysis showing the cumulative incidence of death or resuscitated sudden cardiac death (RSCD) in the different genotype groups for the *NOS1AP* polymorphism rs12143842 ($P = .009$).

The SNPs at *NOS1AP* and *KCNQ1* shown to increase the risk of cardiac events in this cohort (rs12143842, rs16847548, rs10798, and rs8234) were not independent predictors of QTc length.

Modifier SNPs: Maori/Pacific Islander patient group

There were marked differences in SNP frequencies between Caucasians and the Maori/Pacific Islander group (indicated by asterisks, Online Supplementary Table 1). For example, the R1193Q polymorphism in *SCN5A* (rs41261344) was excluded from statistical analyses among Caucasians because the minor allele frequency was <0.05; however, 9 of 53 (17%) of those in the Maori/Pacific Islander ethnic group carried R1193Q along with their primary LQTS-causing mutation.

In the Maori/Pacific Islander patient group, several SNPs were associated with QT interval length, although none remained significant after correction for multiple testing (Online Supplementary Table 3). Risk alleles of rs846111 (*RNF207*), rs8234, and rs10798 (*KNCQ1*) showed a trend for association (before correction for multiple testing) with a decreasing QTc, and rs3815459 (*KNCH2*) with an increasing QTc. No SNPs showed significant associations with cardiac events in this patient group.

Discussion

We studied the association of 29 candidate modifier SNPs in arrhythmia genes with disease severity in a distinct cohort of patients with LQTS. The chief finding confirms the role of SNPs at two risk loci, *NOS1AP* and *KCNQ1,* as predictors of cardiac events and sudden death, independent of traditional risk factors such as QTc length, age, and mutation locus.

Several other SNPs showed trends toward associations with clinical events or QTc length, but these were no longer significant after correction for multiple testing. These signals will require evaluation in other cohorts.

LQT subtype was an independent predictor of death/RSCD in this cohort, and patients with LQT1 or LQT2 were at a lower risk of death/RSCD than patients with LQT3. The LQT3 patient group was small (n = 23), and the mean age at

Table 3 SNPs with significant associations with "any cardiac event" and with the likelihood of having QTc ≥500 ms in the Caucasian patient group

			Any event			QTc ≥500 ms		
Gene	SNP	Tested allele	OR (95% CI)	*P* value	q value	OR (95% CI)	*P* value	q value
KCNQ1	rs8234	G	2.27 (1.40–3.70)	.001	0.015	0.70 (0.44–1.12)	.140	0.629
KCNQ1	rs10798	G	2.21 (1.36–3.58)	.001	0.015	0.72 (0.46–1.15)	.169	0.629
NOS1AP	rs12143842	T	1.89 (1.18–3.00)	.008	0.077	0.75 (0.47–1.19)	.217	0.629
NOS1AP	rs16847548	C	1.86 (1.15–2.99)	.012	0.087	0.85 (0.53–1.37)	.505	0.748
NOS1AP	rs10494366	G	1.59 (1.01–2.52)	.049	0.237	0.64 (0.41–1.01)	.058	0.441
NOS1AP	rs4657139	A	1.60 (1.02–2.53)	.045	0.237	0.75 (0.48–1.18)	.216	0.629
CASQ2	rs4074536	C	1.03 (0.65–1.64)	.892	0.988	0.60 (0.37–0.98)	.041	0.441
GPC5	rs3864180	A	0.79 (0.49–1.28)	.340	0.865	1.62 (1.01–2.61)	.049	0.441

CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.

which events occurred was younger in this group (13 years vs 26 and 17 years for LQT1 and LQT2 patients, respectively), which may account for the striking difference. Reports from the International LQTS Registry examining this end-point in various age groups showed that LQTS subtype was not an independent predictor of outcome, with other clinical risk factors such as gender and QTc duration taking precedence.[13–15]

SNP loci with confirmed associations with disease severity

NOS1AP

The SNPs rs12143842 and rs16847548 in the *NOS1AP* gene were associated with a dramatically increased risk of SCD/RSCD in this cohort. The HR was large for both and was greatest for rs12143842 at 10.2. To give a clinical perspective, HRs of this order for RSCD and SCD in the International Registry Data were associated with a history of more than 10 cardiac events before age 18 years (HR = 12) or QTc >550 ms (HR = 10) in adults with LQTS, or syncope in the last 2 years in adolescents (HR = 12).[13,14,16] These SNPs are in strong linkage dysequilibrium ($r^2 = 0.83$), meaning they are likely indicators for the same causal variant, whether it be either of them or another variant also in strong linkage dysequilibrium. SNPs in the *NOS1AP* gene were first identified as modifiers of QT length in a population-based GWAS,[17] with subsequent GWAS confirming this association with multiple independent signals.[18–20] The associations of *NOS1AP* gene variants with an increased risk of SCD have also been reported in two separate population-based cohorts.[4,21]

In previous studies of patients with LQTS, Tomas et al[7] found that among 901 LQTS patients, two *NOS1AP* SNPs (rs16847548 as in the current study, and rs4657139) were associated with QTc prolongation and all cardiac events. Crotti et al[6] found that among 500 subjects with LQT1 (205 gene positive), the same two SNPs, rs16847548 and rs4657139, also were associated with increased risk of symptoms and life-threatening arrhythmias. In a recent preliminary report focusing on LQT2, several SNPs at the *NOS1AP* locus were associated with QT prolongation (including rs16847548) and one with cardiac events.[22] One fine mapping study identified rs12143842 in the 5′ region of the *NOS1AP* gene as the likely causal variant for QT interval increase in a population-based cohort.[23]

NOS1AP encodes carboxyl-terminal PDZ ligand of nitric oxide synthase 1 (neuronal) adaptor protein (CAPON), a regulator of the nitric oxide synthase pathway that was first identified in the brain and later in ventricular myocytes. When overexpressed, CAPON can shorten action potentials by inhibiting L-type calcium channels ($I_{Ca,L}$) and enhancing the rapidly activating delayed rectifier potassium current (I_{Kr}).[24] The *NOS1AP* SNPs with significant associations in this and other cohorts are in noncoding regions of the gene, so alleles may be exerting effects by altering transcription and resulting CAPON levels. The major allele genotypes of the noncoding *NOS1AP* SNPs rs10494366, rs10918594, and rs12039600 have been associated with lower *NOS1AP* RNA levels in cardiac tissue as well as shorter JT intervals (measured from the J point to the end of the T wave) compared with patients with the minor allele genotype.[25,26]

It is intriguing that in our cohort, *NOS1AP* had a distinct association with cardiac events and SCD but not with QTc. Some of this effect may be due to the fact that five of the patients who were deceased never had an ECG because their diagnosis was made after their death by molecular autopsy. Even though *NOS1AP* clearly has an association with QTc prolongation in the general population and in other cohorts, our findings may indicate that the proarrhythmic effect of these SNPs is not entirely linked to lengthening of the QT interval alone. Some patients with LQTS and a normal QTc may experience fatal arrhythmia, as exemplified by the recent finding that the risk of sudden death from C-loop mutations in *KCNQ1* is independent of the QTc.[27]

KCNQ1

The SNPs rs10798 and rs8234, located in the 3′ UTR of the *KNCQ1* gene and in complete linkage dysequilibrium ($R^2 = 1$), are significantly associated with an increased risk of cardiac events in Caucasians. The 3′ UTR of mRNA transcripts encodes *cis*-regulatory binding sites for small noncoding micro-RNAs (miRNAs) that can inhibit gene expression after transcription, and SNPs in this region can affect the binding of these miRNAs. Amin et al[5] showed that

the minor allele of three SNPs in the 3′ UTR of *KCNQ1* (rs2519184, rs8234, and rs10798) could modify symptom prevalence and QTc length in patients with LQT1 by suppressing translation. The inheritance of the minor allele haplotype in *cis* with the LQTS-causing mutation reduced disease severity by decreasing the amount of mutant α-subunits available to form Kv7.1 channels. In contrast, patients with the minor allele haplotype on a normal *KCNQ1* allele had a longer mean QTc and increased arrhythmia susceptibility due to the reduced availability of normal α-subunits.

Unfortunately, genotyping by Sequenom MassARRAY does not distinguish whether a SNP allele is in *cis* or in *trans* with the LQTS-causing mutation. It seems plausible that the signal of increased risk of cardiac events shown in this cohort is because the majority of the minor alleles are in *trans* to the mutation, resulting in reduced availability of normal α-subunits.

Another SNP in *KCNQ1* (rs2074238) has recently been linked to fewer symptoms and a shorter QTc in patients with LQTS.[28] This SNP is not in linkage dysequilibrium with rs10798 and rs8234 ($r^2 = 0.08$).

SNP loci with suggested associations with disease severity

CASQ2

The C allele of rs4074536, T66A in exon 1 of the *CASQ2* gene was associated with a decreased likelihood of having QTc ≥500 ms (uncorrected $P = .041$). *CASQ2* encodes calsequestrin 2, a protein found in the sarcoplasmic reticulum of cardiac and slow skeletal muscle cells with a primary role of storing Ca^{2+}. Homozygous or compound heterozygous mutations in the *CASQ2* gene have been associated with the cardiac channelopathy catecholaminergic polymorphic ventricular tachycardia. T66A is not thought to cause any functional changes to the protein structure.[29]

GPC5

The A allele of rs3864180, an intronic SNP in the *GPC5* gene, was associated with an increased likelihood of having QTc ≥500 ms (uncorrected $P = .049$). In the Oregon Sudden Unexpected Death Study, the G allele of this SNP was associated with a decreased risk of cardiac arrest and a shortened QT interval.[30] *GPC5* encodes glypican 5, a type of heparan sulfate proteoglycan found in the extracellular matrix and bound to the outer surface of the plasma membrane in the cardiovascular system.[31]

SCN5A

The subgroup of Maori and Pacific Islanders has not been previously studied for LQTS in either presentation or outcome. Although the numbers were too small to show any significant associations after correction for multiple testing, 17% of the patient group carried the R1193Q polymorphism in the *SCN5A* gene along with their primary LQTS-causing mutation. This frequency is similar to that found in a control population of Han Chinese,[32] whereas this polymorphism is found in only 0.2% to 0.3% of Caucasians in the general population.[33,34]

This has similarities to the *SCN5A* S1103Y polymorphism, which is known to subtly modulate arrhythmia risk and is found in approximately 13% of Black Americans but is not generally found in Caucasians.[35]

Study limitations

Clinical data for some variables were not available for a significant proportion of patients in this analysis, precluding multivariable analysis. This included whether patients were treated with beta-blocker drugs; hence, the potential effect of this on cardiac event rates has not been accounted for. Also, because accurate event dates were not available for many syncopal cardiac events, the use of Cox proportional hazards models was restricted to SCD/RSCD data.

This cohort consists of a varied population with different LQTS-causing mutations known to confer varying risks. Although this complicates quantifying the effects of individual SNPs, we tried to adjust for this by including LQTS subtype as a covariate in the multivariable models.

The small number of patients in the Maori/Pacific Islander group meant there was a lack of statistical power to detect significant associations with cardiac events or with QTc length following adjustment for multiple testing.

Some of the members of this cohort are related, with 75 Caucasian and 18 Maori/Pacific Islander families of varying sizes (range 1–13 members). Ideally this would be accounted for during statistical analysis, but this was not possible with the family substructures. McArdle et al[36] undertook simulations of SNP associations in various family structures with and without accounting for this and showed that not adjusting does not bias the point estimate or greatly affect the power to detect SNP effects but may increase type I errors. Acknowledging these limitations, significant results were obtained, even after correction for multiple testing.

Conclusion

This first investigation into the NZ LQTS patient cohort demonstrates that modifier SNPs in *NOS1AP* and *KCNQ1* are associated with an increased risk of SCD and cardiac events in patients with LQTS, with the HR suggesting they have significant potential in clinical risk stratification. The study also identified other SNPs that may be influential and warrant evaluation in other cohorts. Maori and Pacific Islanders have different SNP frequencies from Caucasians, and the way in which these ancestry-specific SNPs modulate clinical phenotypes requires further investigation.

Appendix A

Supplementary data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.hrthm.2013.10.005.

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