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Exome Sequencing Implicates an Increased Burden of Rare Potassium Channel Variants in the Risk of Drug Induced Long QT Syndrome

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Abstract

Objectives—To test the hypothesis that rare variants are associated with Drug-induced long QT syndrome (diLQTS) and torsade de pointes (TdP).

Background—diLQTS is associated with the potentially fatal arrhythmia TdP. The contribution of rare genetic variants to the underlying genetic framework predisposing diLQTS has not been systematically examined.

Methods—We performed whole exome sequencing (WES) on 65 diLQTS cases and 148 drug-exposed controls of European descent. We employed rare variant analyses (variable threshold [VT] and sequence kernel association test [SKAT]) and gene-set analyses to identify genes enriched with rare amino-acid coding (AAC) variants associated with diLQTS. Significant

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Conflicts of interest: Drs. Roden and George hold U.S. Letters Patent No. 6,458,542, issued October 1, 2002 for “Method of Screening for Susceptibility to Drug-Induced Cardiac Arrhythmia”, focusing on the D85N variant. Both have received past royalties. Dr. George reports additional funding to his institution by Gilead Sciences to study proprietary compounds, but unrelated to the present study. No other authors report conflicts of interest.

associations were reanalyzed by comparing diLQTS cases to 515 ethnically matched controls from the NHLBI GO Exome Sequencing Project (ESP).

Results—Rare variants in 7 genes were enriched in the diLQTS cases according to SKAT or VT compared to drug exposed controls ($p<0.001$). Of these, we replicated the diLQTS associations for *KCNE1* and *ACN9* using 515 ESP controls ($p<0.05$). A total of 37% of the diLQTS cases also had ≥1 rare AAC variant, as compared to 21% of controls ($p=0.009$), in a predefined set of seven congenital LQTS (cLQTS) genes encoding potassium channels or channel modulators (*KCNE1,KCNE2,KCNH2,KCNJ2, KCNJ5,KCNQ1,AKAP9)*.

Conclusions—By combining WES with aggregated rare variant analyses, we implicate rare variants in *KCNE1* and *ACN9* as risk factors for diLQTS. Moreover, diLQTS cases were more burdened by rare AAC variants in cLQTS genes encoding potassium channel modulators, supporting the idea that multiple rare variants, notably across cLQTS genes, predispose to diLQTS.

Keywords

exome; torsade des pointes; long QT syndrome; genetics, adverse drug event

Introduction

Long QT syndrome (LQTS) is a congenital or acquired (usually drug-induced) change in ventricular repolarization that can evoke the life-threatening ventricular arrhythmia torsades de pointes (TdP) (1). Most drugs associated with drug induced long QT syndrome (diLQTS) have known arrhythmogenic effects and are predominantly used in arrhythmia therapy. However, non-antiarrhythmic drugs such as methadone, terfenadine, and haloperidol have also been associated with diLQTS and this has led to high profile restrictions and withdrawals (2).

The diLQTS phenocopies many of the clinical features of the congenital LQTS (cLQTS), which is caused by mutations in genes encoding ion channels or their regulatory proteins (3). In addition, first degree relatives of patients with diLQTS have been reported to display exaggerated responses to challenge with the QT prolonging agent quinidine (4). These observations, coupled with the widely-held view that adverse drug reactions (ADR) include a genomic component (5,6), have led to searches for DNA variants predisposing to diLQTS (7-9). Mutations in cLQTS disease genes have been reported in up to 36-40% of diLQTS patients (10,11), consistent with the notion that drug challenge may expose the congenital form of the syndrome (12). In a recent study interrogating genetic predisposition to diLQTS using 1424 tag single nucleotide polymorphisms (SNPs) in 18 genes, the *KCNE1* missense polymorphism resulting in D85N conferred an odds ratio of 9.0 for diLQTS (7). A genome-wide association study has not identified strong associations between common polymorphisms and diLQTS (13).

Whole exome sequencing (WES) has led to the successful discovery of an associated underlying genomic framework for monogenic diseases including cLQTS (14). These approaches have also been successfully applied to complex traits, for example in the

identification of variants in *DCTN4* modifying pseudomonas infection susceptibility in patients with cystic fibrosis (15). In this study, we used WES to test the hypothesis that rare variants predispose to diLQTS. We report here the results of WES in 65 individuals who developed diLQTS and 2 sets of controls: 148 drug exposed controls and 515 ethnically matched population controls from the Exome Sequencing Project (ESP) (16). We analyzed all amino acid coding (AAC) variants and also focused on genes in which mutations are known to cause cLQTS or other arrhythmia syndromes.

Methods

Study Cohort

diLQTS cases—Cases of self-reported European American (EA) ancestry were selected from a cohort of individuals seen at Vanderbilt University Medical Center who presented with TdP or exaggerated QT interval prolongation (≥600ms that reverted to <480ms upon drug discontinuation) secondary to commonly prescribed drugs including anti-arrhythmics and antipsychotics. In total, 67 individuals met this definition (53 with TdP and 14 with exaggerated QT interval prolongation); principal component analysis confirmed EA ancestry in 65/67 cases (Supplemental Figures 1 and 2). See the supplement for details. Clinical data were extracted manually from hospital charts by physician review.

Drug exposed controls—Drug exposed controls were identified from 845 adults studied after initiation of QT-prolonging antiarrhythmic therapy. From this group (17), we selected 148 Caucasians with baseline QTc≤470msec, no on-drug QTc interval >495 msec, and maximum QTc change <50 msec on drug.

ESP controls—We selected 515 Caucasian subjects, determined to be unrelated by identity by descent (IBD), from the National Heart, Lung and Blood Institute Grand Opportunity Exome Sequencing Project (NHLBI GO ESP) to serve as a second control set (16). The ESP controls were drawn from subjects with high body mass index, chronic obstructive pulmonary disease control, low low-density lipoprotein (LDL), and a set of deeply phenotyped reference samples.

Whole Exome Sequencing

The full methods on library construction and preparation, exome capture, mapping and variant calling, and quality control are described in the supplement

Association testing—We performed an unadjusted and adjusted (age, sex, first and second principal component) SNP based association analyses using Fisher's exact and exact logistic regression, respectively; a Bonferroni adjusted $P<6.39\times10^{-7}$ was considered to be statistically significant on a genome-wide level (78,204 AAC variants [missense, non-synonymous, or frame-shift] were identified, of which 59,977 [76.6%] had a MAF<5%).

We used unidirectional (variable threshold [VT]) (18) and bi-directional (sequence kernel association test [SKAT])(19) rare variant aggregate approaches to test for gene-level associations between the diLQTS cases and the respective control groups. A Bonferroni adjusted $p<3.39\times10^{-6}$ was considered to be statistically significant on a genome-wide level

for both VT and SKAT (14,746 genes harbored an AAC variant). See supplement for details.

Given that our initial analysis approach is likely to be underpowered (Supplemental Figure 3.A and B), we employed an alternative analysis approach where we selected the genes with the strongest associations identified from both VT and SKAT ($p<0.001$) when comparing the diLQTS cases and drug-exposed controls. We then attempted to replicate those associations using the diLQTS cases vs. 515 population controls from the ESP using both VT and SKAT aggregate analyses ($p<0.05$); this use of two control populations, one drug-exposed and one drawn from a general population, is similar to that we previously employed in a candidate diLQTS analysis (7). We only considered genes to be significantly associated with diLQTS if they: i) passed the $p<0.001$ cut-off in both types of rare variant analyses (VT and SKAT) comparing diLQTS cases and drug-exposed controls); and ii) replicated using the diLQTS cases vs. ESP population controls.

Candidate gene and gene-set analyses—*A priori* we selected 20 genes with strong level of evidence of being associated with congenital arrhythmia syndromes ([cLQTS]: AKAP9,ANK2,CACNA1C,CAV3,KCNE1,KCNE2,KCNH2, KCNJ2,KCNJ5,KCNQ1,SCN4B,SCN5A,SNTA1; short-QT syndrome[SQTS]: *KCNQ1,KCNH2,KCNJ2;* Brugada syndrome[BrS]: *CACNA1C,CACNB2,GPDL1,KCNE3,SCN1B,SCN3B,SCN5A*; catecholaminergic polymorphic ventricular tachycardia[CPVT]: *CASQ2,RYR2*) (Supplemental Table 2). Previous studies (8) implicated the D85N *KCNE1* variant with diLQTS (MAF 1.2% in 4300 EAs in the ESP); accordingly, we included variants with a MAF<1.5% and defined these as rare. We calculated the proportions of diLQTS cases, drug exposed controls, and ESP controls who harbored one or more rare variants in each of these genes considered individually. Fisher's exact test was applied to detect differences in rare variant burden between diLQTS cases and drug exposed controls (adjusted for multiple comparisons ($n=20$), $p<0.0025$ was considered statistically significant). For genes that were significantly enriched with rare variants among the diLQTS cases vs. drug-exposed controls, we proceeded to the diLQTS cases vs. ESP controls comparison. Lastly, we hypothesized that diLQTS cases are more burdened by rare variants in the 3 well defined gene sets described above consisting of the 20 congenital arrhythmia syndrome genes, the subset of 13 cLQTS genes, and the subset of 7 cLQTS genes encoding potassium channel pore-forming subunits or modulators (*AKAP9,KCNE1,KCNE2,KCNH2,KCNJ2,KCNJ5,KCNQ1)*. In these analyses, we calculated the proportion of diLQTS cases, drug exposed controls, and ESP controls that harbored one or more rare variants in genes listed above. A p-value $p<0.017$ adjusted for multiple comparisons ($n=3$), was considered statistically significant. We selected genes that passed p-values adjusted for multiple comparisons when comparing the diLQTS cases vs. drug-exposed controls and replicated these associations using the ESP controls.

Statistical analyses

All analyses were performed using PLINK/SEQ (http://atgu.mgh.harvard.edu/plinkseq/) and SAS, version 9.2 (SAS institute Inc., Cary, NC, USA), and R: A language and environment for statistical computing (http://R-project.org).

Ethics

Approval for the study of diLQTS and QTSPS subjects was obtained from the Vanderbilt Institutional Review Board (IRB) and written informed consent was obtained from each study participant prior to enrollment. Each subject in the NHLBI GO ESP also provided consent.

Results

We analyzed DNA from 65 diLQTS cases and 148 drug exposed controls of European American ancestry. Table 1 lists the clinical characteristics of the diLQTS cases and the drug exposed controls, including the index drug responsible for causing diLQTS. No summary level phenotypic information was available for the 515 ESP controls.

Association testing

Individual variants—Supplemental Figure 4 depicts the QQ plot of the AAC single variant based association analyses. Supplemental Table 4 and 5 presents the strongest associations for the single marker association analysis of AAC variants in adjusted and unadjusted association models, respectively. No single AAC variant reached the Bonferroni adjusted genome-wide significance threshold ($P<6.39\times10^{-7}$). The *KCNE1* D85N (rs1805128) variant conferred an odds ratio (OR) =30.8 (p=0.0008) and 13.6 (p=0.019) for inducing diLQTS, in the unadjusted and adjusted association analyses respectively.

Gene level associations—We identified rare AAC variants in a total of 14,746 genes. No gene reached the Bonferroni adjusted genome-wide significance threshold ($p<3.39\times10^{-}6$). In the alternate rare variant gene level analyses approach, VT and SKAT identified 5 and 4 genes (7 different genes in total), respectively, that were enriched with rare variants in the diLQTS cases vs. drug exposed controls (p<0.001). However, only two genes, *ACN9* and *KCNE1*, were also identified in both VT and SKAT at a significance level of p<0.05 using diLQTS cases vs. 515 ESP controls (Table 2).

The association in *KCNE1* was driven by two rare missense SNPs (D85N [rs1805128], (n=6) and D76N [rs74315445], (n=1)) present in 7 of the diLQTS cases (10.7%). By contrast, only 1 missense variant (R98Q, [rs150454912]) in *KCNE1* was identified among the drug exposed controls (0.7%) and 16 were identified among the ESP controls (3.1%). *KCNE1* D85N has previously been implicated as a risk allele for diLQTS in a candidate gene study of 176 cases that included 27/65 the samples also studied here (7). To ensure that our results were not driven by overlapping samples from a previous study, we performed a sub-analyses on the non-overlapping 38 diLQTS vs. the 148 drug-drug exposed controls which confirmed our initial findings (3/38 vs. 0/148, p=0.008). *In-silico* assessment of rare variant function using PolyPhen2and SIFT suggests that the variants identified in KCNE1 (D85N, D76N, and R98Q) are deleterious. Two rare heterozygous missense SNPs in *ACN9* (F53L, [rs62624461] and T83I, [rs34146273]) drove the association for this gene; 9 diLQTS cases were carriers for F53L and 1 was a carrier for T83I, while 4 drug exposed controls were carriers for F53L. Based on data from the Encyclopedia of DNA Elements (ENCODE) consortium, the F53L variant is situated in a region of high regulatory activity whereas less

regulatory activity was identified for the T83I variant (Supplemental Figure 5). In addition, *in silico* assessment of rare variant function in *ACN9* using PolyPhen2 and SIFT indicates that the F53L variant is more deleterious than the T83I variant (Supplemental Table 6). The F53L variant conferred an OR = 8.5 and 6.0 for inducing diLQTS in adjusted and unadjusted SNP based association models, respectively (p=0.001).

Candidate gene analyses—We identified a 16 and 3.5-fold enrichment among diLQTS cases harboring a rare AAC variant in *KCNE1,* compared with the drug exposed controls and ESP controls (p=0.001 and 0.009, respectively). No other individual congenital arrhythmia gene displayed statistically significant enrichment among the diLQTS cases compared to the drug exposed controls (Table 3).

We also examined the proportion of individuals that harbored ≥1 rare variant in predefined gene sets. Cases with diLQTS had a ~2-fold enrichment of rare AAC variants in potassium channel or channel modulating protein encoding genes, compared to the drug exposed- and ESP controls (p=0.009 and p=0.013, respectively) (Table 3). To evaluate whether this association was independent of *KCNE1* we performed a subset analysis of potassium channel pore-forming subunits or modulatory proteins but excluding *KCNE1* which confirmed our initial findings compared to drug exposed controls and the ESP controls (p=0.032 and 0.009, respectively). No enrichment of rare AAC variants among diLQTS cases was seen in other gene set tests of congenital arrhythmia genes or cLQTS genes (Table 3).

Discussion

In the present study we used whole exome sequencing to test the hypothesis that rare variants are associated with the risk of developing diLQTS. Using rare variant aggregation approaches, variants in 7 genes were associated with diLQTS susceptibility. However, only two genes, *KCNE1* and *ACN9,* were consistently associated with diLQTS across different aggregate analyses with different control populations. We also found a greater burden of AAC variants in cLQTS potassium channel or modulating protein coding genes among diLQTS cases as compared to drug exposed- and ESP controls.

We found that rare AAC variation in genes encoding potassium channel modulators were associated with diLQTS. A common action of most drugs associated with diLQTS is to inhibit the rapid component of the cardiac delayed rectifying potassium current (I_{Kr}), encoded by *KCNH2* (3,7). Net drug effects on repolarization are thought to be mediated by direct repolarizing-prolonging effects in I_{Kr} (or perhaps other currents)(20), as well as by effects on other currents flowing during repolarization; thus, for example, marked QT prolongation seems to be less of a liability with I_{Kr} blockers that also inhibit inward sodium or calcium currents (e.g. verapamil, amiodarone). Hence, the extent to which an I_{Kr} blocking drug causes diLQTS may reflect both the extent of I_{Kr} block itself, as well as the interplay of multiple depolarizing and repolarizing ion currents; the concept that multiple ionic currents ordinarily provide a buffer against excessive QT prolongation has been termed repolarization reserve (12). This concept further suggests that multiple genetic or acquired lesions in these currents may remain asymptomatic until an I_{Kr} blocking drug exposes them,

a situation of “reduced repolarization reserve”. In particular, multiple studies have implicated reduction of function in I_{Ks}, another important repolarizing current, as an important contributor to reduced repolarization reserve (21). This represents a specific example of the more general “two-hit” hypothesis wherein the combination of a genetic variant impairing I_{Ks} and additional risk factors (in the diLQTS case, a selective I_{Kr} blocker) increases the risk for diLQTS.

Utilizing an agnostic rare variant approach we identified two genes associated with diLQTS: *KCNE1* and *ACN9* (Table 2). *KCNE1* has previously been associated with T-wave morphology, QT interval, and the LQT5 form of cLQTS (7,22,23). Indeed, one previous study in a Japanese cohort has implicated the D85N variant as a cause of the congenital long QT syndrome, although its frequency in our control populations suggests it is unlikely to provoke the full-blown congenital LQTS in the absence of other QT prolonging influences such as other genetic variants or drugs (24). *KCNE1* encodes a trans-membrane protein whose co-assembly with the product of *KCNQ1* is required to recapitulate $I_{Ks.}$(25). Highlighting the importance of *KCNE1* in the setting of diLQTS was the proportion of diLQTS cases burdened by rare AAC variants in *KCNE1* compared with both control groups (11% [7/65] vs.2.5% 8[17/663], respectively [p=0.0039]). The ability to identify *KCNE1* with the methods applied in the present study lends support to the notion of reduced repolarization reserve via decreased I_{Ks} being critical for the predisposition to develop diLQTS, as outlined above (12). We also replicate here the D85N signal we and others previously reported in a large candidate gene study evaluating risk for diLQTS; this finding thus further reinforces the status of D85N as a risk allele in this setting and suggests that studies to determine if avoiding QT prolonging drugs in patients known to carry this variant reduces diLQTS. In comparison, little is known about the human homolog of *ACN9* and how it may contribute to diLQTS. *ACN9* is believed to be involved in gluconeogenesis(26) and is required for the assimilation of ethanol or acetate into carbohydrate and has also been identified as a correlate of alcohol dependence (27). While functional studies are paramount in order to truly determine the suggested link between *ACN9* and diLQTS, but beyond the scope of the present paper, it is noteworthy that the biggest driver in the *ACN9* signal (F53L) is in a region of considerable regulatory activity which could be important in the setting of diLQTS. In comparison, little regulatory activity is identified in the vicinity of T83I.

Among the 65 diLQTS cases, two variants drove the *KCNE1* association, D76N and D85N, and D76N has previously been associated with the congenital form of the syndrome. However, while D85N has previously been associated with diLQTS, D76N has not (7). The D76N variant is a known suppressor of currents KvLQT1 and HERG (encoded by *KCNQ1* and *KCNH2*, respectively) and was one of four rare *KCNE1* variants characterized by Bianchi et al., three of which were functionally important (28). The enrichment of rare variants among patients with LQTS(29) and among diLQTS patients(30) compared to healthy individuals supports the notion of diLQTS being a cLQTS phenocopy via pharmacologically or genetically mediated perturbations of cardiac repolarization.

While previous studies have identified single loci or SNPs strongly predictive of some ADRs (5,6), this does not appear to be the case with diLQTS. Our data support the idea that the fundamental lesion leading to diLQTS susceptibility is initially subclinical changes in

the physiology of cardiac repolarization and that this can arise from rare genetic variation in multiple components of that system and is not restricted to a single SNP or gene (i.e. genetic variation in clusters of genes can give rise to the same phenotype) (31). In keeping with such a systems approach, we examined rare AAC variants in three well defined gene sets/networks(32). While we did not see a greater burden of rare variants among diLQTS cases in two of the three gene sets, 37% (24/65) of patients with diLQTS harbored ≥1 rare AAC variant in cLQTS potassium genes and their modulators compared to 21% (142/663) in the control groups, reinforcing the importance of viewing disease phenotypes in the context of a systems approach; importantly, this enrichment was observed even when *KCNE1* was omitted from the analysis.

While an enrichment of rare AAC variants in cLQTS potassium channel pore-forming or modulator genes was identified among diLQTS cases, the majority of diLQTS cases (~63%) was not burdened by rare AAC variants in these genes, suggesting that other mechanisms outside the coding regions of cLQTS associated potassium channels affect diLQTS susceptibility. In support of this idea, several studies have implicated non-coding variants in *NOS1AP* and in *KCNQ1* as modulators of penetrance of the in the congenital LQTS(33,34) and in amiodarone-related diLQTS (35). Among diLQTS cases, the cLQTS gene harboring the greatest proportion of rare AAC variants was *AKAP9*, encoding a *KCNQ1* partner required to transduce beta adrenergic receptor activation of I_{Ks}(36). A mutation in *AKAP9* in the *KCNQ1* binding domain reduces the interaction between *KCNQ1* and *AKAP9* blunting physiologic adrenergic-mediated increase in I_{Ks} thereby generating the LQT11 phenotype (37).

Recent findings by Crotti et al. have identified rare de novo variants in genes encoding calmodulin (CALM1 and CALM2) associated with long-QT syndrome and recurrent cardiac arrests (14). Among the diLQTS cases and the drug exposed controls, no rare AAC variants in the three calmodulin genes were identified.

Limitations

One limitation of the present study relates to the rarity of the phenotype in question which translates into a small case group and thus limited study power (Supplemental Figures 3.A and B). While a single variant association testing approach may prove useful in the setting of common variants, it performs less well in a rare variant setting (e.g. MAF=1-2%), although effect sizes and associated MAF are estimates of the true values. In comparison, gene collapsing tests performed better although limited study power remains an issue. In addition, to limit effects associated with population stratification which may lead to spurious associations, we only included populations of the same ancestry which reduced the sample size even further, and limits the extent to which our findings can be applied to other ancestries. Another limitation relates to the lack of phenotypic information for the ESP controls which may have affected our findings. However, as diLQTS/TdP is a rare ADR, the effects of this ascertainment bias on our study findings are likely to be small. While a large well-phenotyped reference population with individual level information would is provide a better comparison, such large publically available datasets are not yet available. We adopted the approach of dual comparisons, cases versus drug-exposed controls and cases versus

population controls, to mitigate this issue. Other limitations that warrant consideration includes the distribution of causative alleles throughout the genome (i.e. not confined to single genes) may also have affected our findings. Thus, a traditional single marker association approach was unsuccessful for implicating disease loci related to diLQTS. In contrast, aggregated rare variant analyses, an emerging standard in rare variant analyses of complex disease, identified two genes, of which one has a clear biological significance associated with diLQTS. While rare variants may be associated with large effect sizes, functional characterization of each variant is would be a useful approach to help establish such causation, including *ACN9*.

Although diLQTS cases were significantly more burdened by rare AAC variants in potassium channel cLQTS genes compared with the drug-exposed controls and the ESP controls, (36.9% vs. 18.9% and 22.1%, respectively) we acknowledge the possibility that the direct genetic effect induced by rare AAC variants in potassium channel cLQTS genes may be small considering that ~20% of controls also carried a rare AAC variant in these genes. However, based on findings from previous case-control studies, a two-fold enrichment (or more) among diLQTS affected individuals compared to unaffected individuals does lend support the identified two-fold times enrichment of rare AAC variants in potassium channel cLQTS genes (7,24). In agreement with the current state of practice, we used multiple tests assuming uni-directional and bi-directional effects (34). However, lack of independence between such variant aggregate tests represents a limitation.

Conclusions

Using next-generation sequencing, we identified rare variants in *KCNE1* (D76N and D85N) and *ACN9* associated with diLQTS. Furthermore, our findings suggest that variation in potassium currents may contribute to the phenotype, as diLQTS cases had a larger burden of AAC variants than the controls. The present study not only provides insights into the underlying genetic architecture predisposing diLQTS, but also lends support to the notion that that multiple rare variants, notably across cLQTS genes, predispose to diLQTS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviation list

diLQTS	drug induced long QT syndrome
WES	whole exome sequencing

VT	variable threshold
SKAT	sequence kernel association test
TdP	torsades de pointes
AAC	amino acid coding
GWAS	genome-wide association study
MAF	Minor allele frequency
SNP	single nucleotide polymorphisms
PCR	polymerase chain reaction

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Table 1

Baseline Characteristics of Study Subjects

	Cases	Drug exposed controls
N	65	148
Sex, female (%)	45 (69.2)	66 (44.6)
Age, median (IQR)		
Overall	58.9 (42.3-73.6)	65.7 (56.1-70.9)
Female	57.3 (42.3-72.6)	65.4 (58.9-73.3)
Male	61.2 (43.9-73.9)	60.7 (54.0-67.9)
Outcome		
Torsades de pointes (%)	51 (78.5)	0
Median QTc value (IQR), ms	634 (602-667)	444 (427-462)
Index Drug (%)*		
Quinidine	16 (24.6)	-
Sotalol	15 (23.1)	132 (89.2)
Dofetilide	8 (12.3)	16 (10.8)
Amiodarone	7 (10.8)	-
Procainamide	4 (6.2)	-
Cardiac other	5 (7.7)	-
Non-cardiac	19 (29.2)	-
History of,n (%)		
Hypertension	17 (26.2)	100 (67.6)
Diabetes	11 (17.9)	31 (21.0)
Ischemic heart disease	20 (30.8)	42 (28.4)
Myocardial infarction	17 (26.2)	37 (25.0)

Interquartile range = IQR; ms = milliseconds; Cardiac other = encainide, mexiletine, lidocaine, bretylium, and ibutilide;

non-cardiac = hydroxyzine, nortriptyline, sertraline, escitalopram, venlafaxine, trazadone, promethazine, methadone, trifluorperazine, prochlorperazine, tizanidine, fluoroquinolone, cotrimoxazole, sevoflurane, fluconazole, levofloxacin, metoclopramide, and clarithromycin. For drug exposed controls, index drug identifies the drug that they were challenged with

*17 cases had taken either 2 (n=16) or 3 (n=1) index drugs.

Table 2

Genes with significant associations between diLQTS cases and drug exposed controls or ESP controls according to aggregated rare variant analysis

Gene	VT p-values		SKAT p-values	
	diLQTS cases (n=65) vs. drug-exposed controls (n=148)	**diLQTS cases (n=65) vs. ESP Controls (n=515)**	**diLQTS cases (n=65) vs. drug-exposed controls (n=148)**	**diLQTS cases (n=65) vs. ESP Controls (n=515)**
KCNE1	0.0005	0.0033	0.0002	0.005
ACN9	0.0005	0.0006	0.0008	8.96E-05

Genes that reached a significance level of p<0.001 comparing the diLQTS cases vs. the drug exposed controls and also replicated using the diLQTS cases vs. 515 ESP controls (p<0.05) using variable threshold (VT) or sequence kernel association tests (SKAT) diLQTS, drug induced long QT syndrome

Table 3

Proportion of individuals harboring ≥1 rare amino acid changing variant (MAF<1.5% in ESP4300 EA) in 20 high priority genes

	diLQTS Cases (n=65)	Drug exposed Controls (n=148)	Proportional enrichment (diLQTS cases / Drug exposed controls)	P	ESP Controls (n=515)	Proportional enrichment (diLQTS cases / ESP controls)	Replication P
AKAP9 *	18 (27.7)	20 (13.5)	2.0	0.019	84 (16.3)	1.7	
ANK2 *	9 (13.8)	14 (10.1)	1.5	0.346	62 (12.0)	1.2	
CACNA1C *	1 (1.5)	7 (0.3)	0.3	0.440	27 (5.2)	0.3	
CACNB2 *	0 (0.0)	1 (0.0)	0.0	1.000	5 (1.0)	0.0	
CAV3 *	1 (1.5)	0 (0.0)	-	0.305	6 (1.2)	1.3	
GPD1L *	2 (3.1)	1 (0.7)	4.6	0.221	4 (0.8)	4.0	
KCNE1 *	7 (10.8)	1 (0.7)	15.9	0.001	16 (3.1)	3.5	0.009
KCNE2 *	2 (3.1)	4 (2.7)	1.1	1.000	10 (1.9)	1.6	
KCNE3 *	0 (0.0)	3 (2.0)	0.0	0.555	9 (1.7)	0.0	
KCNH2 *	4 (6.2)	3 (2.0)	3.0	0.204	9 (1.7)	3.5	
KCNQ1 *	1 (1.5)	1 (0.7)	2.3	0.518	4 (0.8)	2.0	
RYR2 *	3 (4.6)	14 (9.5)	0.5	0.283	35 (6.8)	0.7	
SCN1B *	0 (0.0)	4 (2.7)	0.0	0.316	13 (2.5)	0.0	
SCN4B *	0 (0.0)	2 (1.4)	0.0	1.000	0 (0.0)	0	
SCN5A *	2 (3.1)	10 (6.8)	0.5	0.353	27 (5.2)	0.6	
SNTA1 *	1 (1.5)	1 (0.7)	2.3	0.518	6 (1.2)	1.3	
Collapsed†							
Congenital Arrhythmia genes‡	33 (49.2)	67 (45.9)	1.1	0.55	251 (48.7)	1.0	
cLQTS genes§	30 (44.6)	51 (34.5)	1.3	0.126	203 (39.4)	1.2	
Potassium channel cLQTS genes‖	24 (36.9)	28 (18.9)	2.0	0.009	114 (22.1)	1.7	0.013

cLQTS, congenital long QT syndrome; diLQTS, drug induced long QT syndrome; ESP, exome sequencing project 4 Genes with no rare amino acid changing variants (MAF<1.5% among 4300 European Americans in the ESP [ESP4300 EA]) in diLQTS cases and drug exposed controls are not listed (*CASQ2, KCNJ2, KCNJ5, SCN3B*)

Congenital arrhythmia genes include genes with strong evidence of being associated with congenital long QT syndrome, Brugada syndrome, Catecholaminergic polymorphic ventricular tachycardia, and short QT syndrome

[*] adjusted for multiple comparisons (n=20), a p-value <0.0025 is considered statistically significant

[†] adjusted for multiple comparisons (n=3), a p-value <0.017 is considered statistically significant

[‡] AKAP9, ANK2, CACNB2, CACNA1C, CASQ2, CAV3, GPD1L, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNJ5, KCNQ1, SCN1B, SCN3B, SCN4B, SCN5A, RYR2, SNTA1

[§] AKAP9, ANK2, CACNA1C, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, SCN4B, SCN5A, SNTA1

[//] KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, AKAP