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

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Objectives The aim of this study was to test the hypothesis that rare variants are associated with drug-induced long QT interval syndrome (diLQTS) and torsades de pointes.

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

Methods We performed whole-exome sequencing on 65 diLQTS patients and 148 drug-exposed control subjects of European descent. We used rare variant analyses (variable threshold and sequence kernel association test) and gene-set analyses to identify genes enriched with rare amino acid coding (AAC) variants associated with diLQTS. Significant associations were reanalyzed by comparing diLQTS patients with 515 ethnically matched control subjects from the National Heart, Lung, and Blood Grand Opportunity Exome Sequencing Project.

Results Rare variants in 7 genes were enriched in the diLQTS patients according to the sequence kernel association test or variable threshold compared with drug-exposed controls ($p < 0.001$). Of these, we replicated the diLQTS associations for *KCNE1* and *ACN9* using 515 Exome Sequencing Project control subjects ($p < 0.05$). A total of 37% of the diLQTS patients also had 1 or more rare AAC variants compared with 21% of control subjects ($p = 0.009$), in a pre-defined set of 7 congenital long QT interval syndrome (cLQTS) genes encoding potassium channels or channel modulators (*KCNE1*, *KCNE2*, *KCNH2*, *KCNJ2*, *KCNJ5*, *KCNQ1*, *AKAP9*).

Conclusions By combining whole-exome sequencing with aggregated rare variant analyses, we implicate rare variants in *KCNE1* and *ACN9* as risk factors for diLQTS. Moreover, diLQTS patients 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. (J Am Coll Cardiol 2014;63:1430–7) © 2014 by the American College of Cardiology Foundation

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Long QT interval 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 LQTS (diLQTS) have known arrhythmogenic effects and are predominantly used in arrhythmia therapy. However, nonantiarrhythmic drugs such as methadone, terfenadine, and haloperidol have also been associated with diLQTS, and this has led to high-profile restrictions and withdrawals (2).

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The diLQTS phenocopies many of the clinical features of the congenital LQTS (cLQTS), which are 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 interval–prolonging agent quinidine (4). These observations, coupled with the widely held view that adverse drug reactions (ADRs) 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 as many as 36% to 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 the genetic predisposition to diLQTS using 1,424 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 subjects in whom diLQTS developed and 2 sets of control subjects: 148 drug-exposed control subjects and 515 ethnically matched population control subjects 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 PATIENTS. Patients of self-reported European-American (EA) ancestry were selected from a cohort of patients seen at Vanderbilt University Medical Center who presented with TdP or exaggerated QT interval prolongation (≥600 ms that decreased to <480 ms on drug discontinuation) secondary to commonly prescribed drugs including antiarrhythmic and antipsychotic drugs. In total, 67 patients met this definition (53 with TdP and 14 with exaggerated QT interval prolongation); principal component analysis confirmed EA ancestry in 65 of 67 patients (Online Figs. 1 and 2) (see the Online Appendix for details). Clinical data were extracted manually from hospital charts by physician review.

DRUG-EXPOSED CONTROL SUBJECTS. Drug-exposed control subjects were identified from 845 adults studied after initiation of QT interval–prolonging antiarrhythmic therapy. From this group (17), we selected 148 Caucasian subjects with a baseline corrected QT interval (QTc) ≤470 ms, no on-drug QTc interval >495 ms, and maximal QTc interval change <50 ms on drug.

ESP CONTROL SUBJECTS. We selected 515 Caucasian subjects determined to be unrelated by identity by descent from the National Heart, Lung, and Blood Institute Grand Opportunity ESP to serve as a second set of control subjects (16). The ESP control subjects were drawn from subjects with a high body mass index, chronic obstructive pulmonary disease control, low low-density lipoprotein, 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 Online Appendix.

Association testing. We performed unadjusted and adjusted (age, sex, and first and second principal components) SNP-based association analyses using the Fisher exact and exact logistic regression, respectively; a Bonferroni-adjusted p value $<6.39 \times 10^{-7}$ was considered to be statistically significant on a genome-wide level (78,204 AAC variants [missense, nonsynonymous, or frameshift] were identified, of which 59,977 [76.6%] had a minor allele frequency [MAF] <5%).

We used unidirectional (variable threshold [VT]) (18) and bidirectional (sequence kernel association test [SKAT]) (19) rare variant aggregate approaches to test for gene-level associations between the diLQTS patients and the respective control groups. A Bonferroni-adjusted p value $<3.39 \times 10^{-6}$ was considered to be statistically significant on a genome-wide level for both VT and SKAT (14,746 genes

Abbreviations and Acronyms

AAC = amino acid coding
ADR = adverse drug event(s)
cLQTS = congenital long QT interval syndrome
diLQTS = drug-induced long QT interval syndrome
EA = European-American
ESP = Exome Sequencing Project
I_{Kr} = cardiac delayed rectifier potassium current
I_{Ks} = cardiac slow delayed potassium rectifier current
LQTS = long QT interval syndrome
MAF = minor allele frequency
QTc = corrected QT interval
SKAT = sequence kernel association test
SNP = single nucleotide polymorphism
TdP = torsades de pointes
VT = variable threshold
WES = whole exome sequencing

harbored an AAC variant) (see the Online Appendix for details).

Given that our initial analysis approach is likely to be underpowered (Online Figs. 3A and 3B), we used an alternative analysis approach in which we selected the genes with the strongest associations identified from both VT and SKAT ($p < 0.001$) when comparing the diLQTS patients and drug-exposed control group. We then attempted to replicate those associations using the diLQTS patients versus 515 population control subjects from the ESP using both VT and SKAT aggregate analyses ($p < 0.05$); this use of 2 control populations, 1 drug-exposed and 1 drawn from a general population, is similar to those we previously used in a candidate diLQTS analysis (7). We only considered genes to be significantly associated with diLQTS if they: 1) passed the $p < 0.001$ cutoff in both types of rare variant analyses (VT and SKAT) comparing diLQTS patients and drug-exposed control subjects); and 2) replicated using the diLQTS patients versus ESP population control subjects.

Candidate gene and gene-set analyses. A priori, we selected a total of 20 genes with a strong level of evidence of being associated with congenital arrhythmia syndromes ([cLQTSs: *AKAP9*, *ANK2*, *CACNA1C*, *CAV3*, *KCNE1*, *KCNE2*, *KCNH2*, *KCNJ2*, *KCNJ5*, *KCNQ1*, *SCN4B*, *SCN5A*, *SNTA1*; short QT interval syndrome: *KCNQ1*, *KCNH2*, *KCNJ2*; Brugada syndrome: *CACNA1C*, *CACNB2*, *GPDL1*, *KCNE3*, *SCN1B*, *SCN3B*, *SCN5A*; catecholaminergic polymorphic ventricular tachycardia (CPVT): *CASQ2*, *RYR2*) (Online Table 2). Previous studies (8) implicated the D85N *KCNE1* variant with diLQTS (1.2% MAF in 4,300 EA subjects in the ESP); accordingly, we included variants with an MAF <1.5% and defined these as rare. We calculated the proportions of diLQTS patients, drug-exposed control subjects, and ESP control subjects who harbored 1 or more rare variants in each of these genes considered individually. The Fisher exact test was applied to detect differences in rare variant burden between diLQTS patients and drug-exposed control subjects (adjusted for multiple comparisons [$n = 20$], $p < 0.0025$ was considered statistically significant). For genes significantly enriched with rare variants among the diLQTS patients versus drug-exposed control subjects, we proceeded to the diLQTS patients versus ESP control subjects comparison. Last, we hypothesized that diLQTS patients are more burdened by rare variants in the 3 well-defined gene sets described previously 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 patients, drug-exposed control subjects, and ESP control subjects who harbored 1 or more rare variants in the genes listed earlier. A p value <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 patients and the drug-exposed control subjects and replicated these associations using the ESP control subjects.

Statistical analyses. All analyses were performed using PLINK/SEQ (http://atgu.mgh.harvard.edu/plinkseq/), SAS version 9.2 (SAS Institute Inc., Cary, North Carolina), and R: A Language and Environment for statistical Computing (http://R-project.org).

Ethics. Approval of the study of diLQTS and QT Star Panel Study (QTSPS) subjects was obtained from the Vanderbilt Institutional Review Board, and written informed consent was obtained from each study participant prior to enrollment. Each subject in the National Heart, Lung, and Blood Institute Grand Opportunity ESP also provided consent.

Results

We analyzed DNA from 65 diLQTS patients and 148 drug-exposed control subjects of EA ancestry. Table 1 lists the clinical characteristics of the diLQTS patients and the drug-exposed control subjects, including the index drug responsible for causing diLQTS. No summary level

Table 1 Baseline Characteristics of Study Subjects

	Patients	Drug-Exposed Control Subjects*
N	65	148
Female	45 (69.2)	66 (44.6)
Age, yrs		
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
QTc, 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)	—
Noncardiac§	19 (29.2)	—
History		
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)

Values are n (%) or median (interquartile range). *For drug-exposed control subjects, index drug identifies the drug with which they were challenged. †Seventeen patients had taken either 2 (n = 16) or 3 (n = 1) index drugs. ‡Cardiac other includes encainide, mexiletine, lidocaine, bretylium, and ibutilide. §Noncardiac includes hydroxyzine, nortriptyline, sertraline, escitalopram, venlafaxine, trazadone, promethazine, methadone, trifluorperazine, prochlorperazine, tizanidine, fluoroquinolone, co-trimoxazole, sevoflurane, fluconazole, levofloxacin, metoclopramide, and clarithromycin.

QTc = corrected QT interval.

phenotypic information was available for the 515 ESP control subjects.

Association testing. INDIVIDUAL VARIANTS. Online Figure 4 depicts the QQ plot of the AAC single variant–based association analyses. Online Tables 4 and 5 present 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 ($p < 6.39 \times 10^{-7}$). The *KCNE1* D85N (rs1805128) variant conferred odds ratios of 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 ($p < 3.39 \times 10^{-6}$). 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 patients versus drug-exposed control subjects ($p < 0.001$). However, only 2 genes, *ACN9* and *KCNE1*, were also identified in both VT and SKAT at a significance level of $p < 0.05$ using diLQTS patients versus 515 ESP control subjects (Table 2).

The association in *KCNE1* was driven by 2 rare missense SNPs (D85N [rs1805128], n = 6, and D76N [rs74315445], n = 1) present in 7 of the diLQTS patients (10.7%). By contrast, only 1 missense variant (R98Q [rs150454912]) in *KCNE1* was identified among the drug-exposed control subjects (0.7%), and 16 were identified among the ESP control subjects (3.1%). *KCNE1* D85N has previously been implicated as a risk allele for diLQTS in a candidate gene study of 176 subjects that included 27 of the 65 samples also studied here (7). To ensure that our results were not driven by overlapping samples from a previous study, we performed subanalyses on the nonoverlapping 38 diLQTS patients versus the 148 drug–exposed control subjects, which confirmed our initial findings (3 of 38 vs. 0 of 148, $p = 0.008$). In silico assessment of a rare variant function using PolyPhen-2 and SIFT (sorting tolerant from intolerant) 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 patients were carriers of F53L and 1 was a carrier of T83I, whereas 4 drug-exposed control subjects were carriers of 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 (Online Fig. 5). In addition, in silico assessment of a rare variant function in *ACN9* using PolyPhen-2 and SIFT indicates that the F53L variant is more deleterious than the T83I variant (Online Table 6). The F53L variant conferred odds ratios of 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 patients harboring a rare AAC variant in *KCNE1* compared with the drug-exposed control and ESP control subjects ($p = 0.001$ and 0.009, respectively). No other individual congenital arrhythmia gene displayed statistically significant enrichment among the diLQTS patients compared with the drug-exposed control subjects (Table 3).

We also examined the proportion of patients who harbored 1 or more rare variants in pre-defined gene sets. Patients with diLQTS had an approximately 2-fold enrichment of rare AAC variants in potassium channel or channel-modulating protein-encoding genes compared with the drug-exposed and ESP control subjects ($p = 0.009$ 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 with drug-exposed control and the ESP control subjects ($p = 0.032$ and 0.009, respectively). No enrichment of rare AAC variants among diLQTS patients was seen in other gene-set tests of congenital arrhythmia genes or cLQTS genes (Table 3).

Table 2 Genes With Significant Associations Between diLQTS Patients and Drug-Exposed Control or ESP Control Subjects According to Aggregated Rare Variant Analysis

	VT p Values		SKAT p Values	
Gene	diLQTS Patients (N = 65) vs. Drug-Exposed Control Subjects (N = 148)	diLQTS Patients (N = 65) vs. ESP Control Subjects (N = 515)	diLQTS Patients (N = 65) vs. Drug-Exposed Control Subjects (N = 148)	diLQTS Patients (N = 65) vs. ESP Control Subjects (N = 515)
KCNE1	0.0005	0.0033	0.0002	0.005
ACN9	0.0005	0.0006	0.0008	8.96×10^{-5}

Genes that reached a significance level of $p < 0.001$ comparing the diLQTS patients and the drug-exposed control subjects and also replicated using the diLQTS patients versus 515 ESP control subjects ($p < 0.05$) using VT or SKAT.

diLQTS = drug-induced long QT interval syndrome; ESP = Exome Sequencing Project; SKAT = sequence kernel association test; VT = variable threshold.

Discussion

In the present study, we used WES to test the hypothesis that rare variants are associated with the risk of the development diLQTS. Using rare variant aggregation approaches, variants in 7 genes were associated with diLQTS susceptibility. However, only 2 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-encoding genes among diLQTS patients compared with drug-exposed and ESP control subjects.

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Table 3 Proportion of Subjects Harboring 1 or More Rare Amino Acid Changing Variants (Minor Allele Frequency <1.5% in ESP4300 EA) in 20 High-Priority Genes

Gene	diLQTS Patients (N = 65)	Drug-Exposed Control Subjects (N = 148)	Proportional Enrichment (diLQTS Patients/ Drug-Exposed Control Subjects)	p Value	ESP Control Subjects (N = 515)	Proportional Enrichment (diLQTS Patients/ ESP Control Subjects)	Replication p Value
*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)	0.0	
*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

Values are n (%). Four genes with no rare amino acid changing variants (minor allele frequency <1.5% among 4,300 European Americans in the ESP [ESP4300 EA]) in diLQTS patients and drug-exposed control subjects are not listed (*CASQ2*, *KCNJ2*, *KCNJ5*, *SCN3B*). Congenital arrhythmia genes include genes with strong evidence of being associated with congenital long QT interval syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, and short QT interval syndrome. *Adjusted for multiple comparisons (n = 20); $p < 0.0025$ is considered statistically significant. †Adjusted for multiple comparisons (n = 3); $p < 0.017$ 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*.
cLQTS = congenital long QT interval syndrome; other abbreviations as in Table 2.

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 rectifier 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 interval 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 interval 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 the reduction of function in cardiac slow delayed potassium rectifier current (I_{Ks}), another important repolarizing current, as an important contributor to reduced repolarization reserve (21). This represents a specific example of the more general "2-hit" hypothesis in which the combination of a genetic variant impairing I_{Ks} and additional risk factors (in the diLQTS patients, a selective I_{Kr} blocker) increases the risk of diLQTS.

Using an agnostic rare variant approach, we identified 2 genes associated with diLQTS: *KCNE1* and *ACN9* (Table 2). *KCNE1* was previously associated with T-wave morphology, QT interval, and the LQT5 form of cLQTS (7,22,23). Indeed, 1 previous study in a Japanese cohort implicated the D85N variant as a cause of the cLQTS, although its frequency in our control populations suggests that it is unlikely to provoke the full-blown congenital LQTS in the absence of other QT interval–prolonging influences such as other genetic variants and drugs (24). *KCNE1* encodes a transmembrane 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 patients burdened by rare AAC variants in *KCNE1* compared with both control groups (11% [7 of 65] vs. 2.5% 8 [17 of 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 the development of diLQTS, as outlined earlier (12). We also replicate here the D85N signal that we and others previously reported in a large candidate gene study evaluating the risk of diLQTS; this finding thus further reinforces the status of D85N as a risk allele in this setting and suggests that studies be performed to determine whether avoiding QT interval–prolonging drugs in patients known to carry this variant reduces diLQTS. By 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). Although functional studies are paramount to truly determine the suggested link between *ACN9* and diLQTS, but are beyond the scope of the present paper, it is noteworthy that the greatest 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 patients, 2 variants drove the *KCNE1* association, D76N and D85N, and D76N has previously been associated with the congenital form of the syndrome. However, although 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 1 of 4 rare *KCNE1* variants characterized by Bianchi et al. (28), 3 of which were functionally important. The enrichment of rare variants among patients with LQTS (29) and among diLQTS patients (30) compared with healthy subjects supports the notion of diLQTS being a cLQTS phenocopy via pharmacologically or genetically mediated perturbations of cardiac repolarization.

Although 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 3 well-defined gene sets/networks (32). Although we did not see a greater burden of rare variants among diLQTS patients in 2 of the 3 gene sets, 37% (24 of 65) of patients with diLQTS harbored 1 or more rare AAC variants in cLQTS potassium genes and their modulators compared with 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.

Although an enrichment of rare AAC variants in cLQTS potassium channel pore-forming or modulator genes was identified among diLQTS patients, the majority of diLQTS patients (~63%) were 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 noncoding variants in *NOS1AP* and in *KCNQ1* as modulators of penetrance in cLQTS (33,34) and in amiodarone-related diLQTS (35). Among diLQTS patients, 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 a physiological adrenergic-mediated increase in I_{Ks}, thereby generating the LQT11 phenotype (37).

Recent findings by Crotti et al. (14) identified rare de novo variants in genes encoding calmodulin (*CALM1* and *CALM2*) associated with LQTS and recurrent cardiac arrests. Among the diLQTS patients and the drug-exposed control subjects, no rare AAC variants in the 3 calmodulin genes were identified.

Study 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 (Online Figs. 3A and 3B). Although 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% to 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 that 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 control subjects, which may have affected our findings. However, as diLQTS/TdP is a rare ADR, the effects of this ascertainment bias in our study findings are likely to be small. Although a large, well-phenotyped reference population with individual level information would provide a better comparison, such large, publicly available datasets are not yet available. We adopted the approach of dual comparisons, patients versus drug-exposed control subjects and patients versus population control subjects, to mitigate this issue. Other limitations that warrant consideration include 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. By contrast, aggregated rare variant analyses, an emerging standard in rare variant analyses of complex disease, identified 2 genes, 1 of which has a clear biological significance associated with diLQTS. Although

rare variants may be associated with large effect sizes, functional characterization of each variant would be a useful approach to help establish such causation, including *ACN9*.

Although diLQTS patients were significantly more burdened by rare AAC variants in potassium channel cLQTS genes compared with the drug-exposed control and the ESP control subjects (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 2-fold (or greater) enrichment among diLQTS-affected patients compared with unaffected subjects does lend support to the identified 2-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- and bidirectional effects (34). However, a lack of independence among 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, because diLQTS patients had a greater burden of AAC variants than the control subjects. The present study not only provides insights into the underlying genetic architecture predisposing to diLQTS, but also lends support to the notion that that multiple rare variants, notably across cLQTS genes, predispose to diLQTS.

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Key Words: adverse drug event ■ exome ■ genetics ■ long QT interval syndrome ■ torsade des pointes.

APPENDIX

For supplemental tables and figures, please see the online version of this article.