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Novel Rare Variants in Congenital Cardiac Arrhythmia Genes are Frequent in Drug-induced Torsades de Pointes

Andrea H. Ramirez, M.D., M.S.[1], **Christian M. Shaffer, M.S.**[2], **Jessica T. Delaney, M.D.**[1], **David P. Sexton, M.S.**[2], **Shawn E. Levy, Ph.D.**[3,4], **Mark J. Rieder, Ph.D.**[5], **Deborah A. Nickerson, Ph.D.**[5], **Alfred L. George Jr., M.D.**[1,6], and **Dan M. Roden, M.D.**[1,6]

[1]Department of Medicine, Vanderbilt University, Nashville, TN AHR, JTD, ALG, DMR

[2]Center for Human Genetics Research, Vanderbilt University, Nashville, TN CMS, DPS

[3]Department of Biomedical Informatics, Vanderbilt University, Nashville, TN SEL

[4]HudsonAlpha Institute for Biotechnology, Huntsville, AL SEL

[5]Department of Genome Sciences, University of Washington, Seattle, WA MJR, DAN

[6]Department of Pharmacology, Vanderbilt University, Nashville, TN ALG, DMR

Abstract

Marked prolongation of the QT interval and polymorphic ventricular tachycardia following medication (drug-induced long QT syndrome, diLQTS) is a severe adverse drug reaction (ADR) that phenocopies congenital long QT syndrome (cLQTS) and one of the leading causes for drug withdrawal and relabeling. We evaluated the frequency of rare non-synonymous variants in genes contributing to the maintenance of heart rhythm in cases of diLQTS using targeted capture coupled to next generation sequencing. Eleven of 31 diLQTS subjects (36%) carried a novel missense mutation in genes with known congenital arrhythmia associations or a known cLQTS mutation. In the 26 Caucasian subjects, 23% carried a highly conserved rare variant predicted to be deleterious to protein function in these genes compared with only 2-4% in public databases ($p < 0.003$). We conclude that rare variation in genes responsible for congenital arrhythmia syndromes is frequent in diLQTS. Our findings demonstrate that diLQTS is a pharmacogenomic syndrome predisposed by rare genetic variants.

Keywords

pharmacogenomics; sudden cardiac death; adverse drug reaction; next generation sequencing

Author for Correspondence: Dan M. Roden, M.D. 2215B Garland Ave, 1275 MRB IV Nashville, TN 37232-0575 USA, Tel: (615) 322-0667, Fax: (615) 343-2325.

Conflict of Interest

Drs. George and Roden have received royalties for a U.S. Letters Patent No. 6,458,542, issued October 1, 2002 for “Method of Screening for Susceptibility to Drug-Induced Cardiac Arrhythmia”.

INTRODUCTION

Adverse drug reactions (ADRs) are common and have been implicated as frequent causes of morbidity and mortality. Marked prolongation of the QT interval and polymorphic ventricular tachycardia following medication (drug-induced long QT syndrome, diLQTS) is a severe ADR that phenocopies congenital long QT syndrome (cLQTS).(1-3) Prolongation of the QT interval and the resultant polymorphic ventricular tachycardia termed torsades de pointes (TdP) can precipitate ventricular fibrillation and sudden cardiac death and is one of the leading causes for drug withdrawal and relabeling.(4) The incidence of diLQTS is estimated at 1-5% of patients receiving antiarrhythmic therapy with QT interval prolonging antiarrhythmic drugs.(5-7) This important ADR is also recognized, albeit at a much lower frequency, with a wide range of “non-cardiovascular” therapies, including antibiotics, antipsychotics, and methadone.(8) Inhibition of a key repolarizing potassium current (termed I_{Kr}) is the common mechanism across drug classes.(9)

Rare and common genetic variation is now well-recognized as one source of variable response to drug therapy,(10,11) including variable susceptibility to ADRs.(12-14) Genome wide association studies (GWAS) in large populations have described common loci influencing QT interval duration in the absence of medications.(15,16) Studies using GWAS for diLQTS have involved far fewer subjects and preliminary data have not consistently implicated loci with high degrees of statistical confidence.(17,18) One missense variant in the *KCNE1* gene, D85N, has been observed more frequently in cases with diLQTS.(19)

To date, mutations in 13 genes encoding ion channel pore-forming proteins or function-modifying subunits(20) have been identified in cLQTS, revealing striking incomplete penetrance in some families.(21) Mutations in ion channel and associated genes have also been implicated in other congenital arrhythmia syndromes, such as catecholaminergic polymorphic ventricular tachycardia (CPVT), which is also characterized by incomplete penetrance and by susceptibility to serious arrhythmias. Previous studies have used Sanger sequencing to screen diLQTS subjects for mutations in cLQTS genes. These analyses have been confined to less than five most commonly identified disease genes, and have identified possible causative variants in 10-20% of subjects,(22,23) with one recent study from Japan having identified mutations in 8/20 (40%) of diLQTS cases.(24)

Here we test the hypothesis that rare variants in arrhythmia genes contribute to risk for diLQTS with a comprehensive analysis of rare non-synonymous variants across a large set of genes involved in the maintenance of heart rhythm in cases of diLQTS using targeted capture coupled to next generation sequencing and compare frequencies to publicly available databases. Elucidating predictors of ADRs could lead to safer use of currently available drugs and enable development of newer drugs with decreased potential for toxicity.

MATERIALS AND METHODS

Study Subject Ascertainment

Drug-associated TdP was diagnosed in patients receiving a recognized culprit drug who developed the typical electrocardiographic features, including QT prolongation or deformity, pause-dependent onset, and polymorphic ventricular tachycardia lasting >10 beats in the 150 to 240 beats/min range.(8) More rapid polymorphic ventricular tachycardia was classified as ventricular fibrillation, and such patients were not included. Most cases included are from Vanderbilt University Medical Center; in all cases, electrocardiographic documentation of the event and of the inciting drug was required. A blood sample was obtained from each patient for extraction of DNA from lymphocytes. For Vanderbilt patients, informed consent using a method approved by the Institutional Review Board was obtained. For non-Vanderbilt patients, local Human Subjects approval was obtained.

High-throughput Genotyping

A set of 79 genes important for regulating heart rhythm (the “Rhythmonome”(25)) was targeted (Supplementary Table 1) and included known cLQTS genes, other genes associated with congenital arrhythmia syndromes (e.g. *RYR2*,(26) *GPD1L*(27)), genes encoding known or suspected partners of disease gene proteins (e.g. *KCNEx, SCNxB*), and genes identified in genome-wide studies as modulators of normal QT intervals (e.g. *NOS1AP*). A custom Nimblegen array was designed to capture exon and flanking sequences of the 79 genes, totaling 260 kb of targeted DNA. A barcoding approach using unique 7bp sequences to multiplex four samples in a single lane was developed and implemented. Single end 36bp plus 7bp barcode reads with four samples per lane were generated on an Illumina Genome Analyzer II.

Barcodes of 7bp were stripped from the short reads using a custom Perl script. Short read sequences were aligned to the hg18 reference genome with BWA(28). The Genome Analysis Toolkit (GATK)(29) base quality score recalibration, indel realignment, duplicate removal, SNP calling and genotyping were performed simultaneously across all 31 samples using standard hard filtering parameters.(30)

Data from 1000 Genomes low coverage genome sequencing pilot project was downloaded for 60 CEU subjects.(31) A program written in C++ was used to extract variants in the targeted region of interest.

The NHLBI GO Exome Sequencing Project (ESP) (http://snp.gs.washington.edu/EVS/) provided allele frequencies for variants detected in the regions of interest in 1351 individuals of Caucasian ancestry. Individual genotypes were not available due to confidentiality constraints.

Variants from all three sources were annotated using the Seattle Seq Annotation tool,(32) novelty ascertained using KAVIAR,(33) conservation scores determined using PhastCons(34) and GERP,(35) and in silico prediction of function determined using PolyPhen2(36) and SIFT.(37) A database for storage was created using MySQL and named ‘Variation’.

Sanger sequencing

Confirmatory genotyping was performed using Sanger sequencing for all novel rare missense variants and those conserved and deleterious in high priority arrhythmia genes passing all filters or failing one filter. Polymerase chain reaction (PCR) primers were designed using the NCBI Primer Blast program to eliminate non-specific targets. Amplicons were sequenced in one direction using an Applied Biosystems 3730 sequencer. If a variant was identified, the sequencing reaction was repeated with the opposite primer for confirmation.

Statistical Analysis

The two tailed heteroscedastic t test was used to compare variants per subject in the 1000 Genomes data and diLQTS case data. The two-tailed Fisher's exact test was used for all 2×2 table comparisons. Statistical calculations were performed using Stata.

RESULTS

Custom capture and high throughput sequencing

We designed and utilized a custom exon capture strategy targeting 79 genes related to heart rhythm, previously dubbed the "Rhythmonome"(25) (Supplementary Table 1) including the 13 genes previously implicated as disease genes in cLQTS, as well as 9 other genes related to familial arrhythmia syndromes congenital short QT syndrome (SQTS), Brugada syndrome (BrS), and CPVT. Capture of exons and flanking sequences totaling 260kb was successful in 31 of 33 subjects with diLQTS (Supplementary Table 2). High quality next generation sequence was generated with average read depth of 27x across the targeted region. Additional alignment metrics are reported in Supplementary Table 3.

Variant calling and annotation

The targeted region contained 6,267 variants across all subjects, with variant counts by genotype, subject, and ethnicity presented in Supplementary Table 4. Of the identified variants, 633 were in unique locations and annotations for function and novelty are given in Supplementary Table 5. Novelty was determined using KAVIAR, including annotation of dbSNP132 and 1000 Genomes Pilot and Phase 1 data.(33) We used Sanger sequencing to confirm all missense variants in the 22 congenital arrhythmia genes and novel, highly conserved or deleterious variants in the other 57 genes (Supplementary Table 6). There were 32 novel missense variants, of which 26 were highly conserved or predicted to be deleterious to protein function and 11 that occurred in the 22 congenital arrhythmia syndrome genes. Three variants had been previously reported in cLQTS, and each was a heterozygous substitution. The novel mutations in the 22 congenital arrhythmia genes and the novel, highly conserved, or deleterious variants in the remaining 57 Rhythmonome genes are reported in Table 1. In addition to three previously described mutations associated with cLQTS, we confirmed missense variants across the whole set of 79 genes in 20 of 31 subjects (64.5%, Table 2). Those in congenital arrhythmia syndrome genes occurred in 11 of 31 subjects (36%).

Comparison to publicly available data

To estimate the prevalence of similar novel rare variants in the general population detected with next generation sequencing, data from the subset of 26 Caucasian diLQTS subjects were compared to those from the pilot phase of the 1000 Genomes project obtained from 60 Caucasian subjects as well as exome sequence data from 1351 Caucasian individuals provided by the ESP. For this comparison only the most conserved and deleterious variants were considered.

In the 1000 Genomes pilot data with average read depth 2-4x, the same 260kb targeted region contained 21 novel, rare, missense variants, of which only 1 (a homozygous RYR2 variant) was predicted to be deleterious and in a highly conserved region. No mutations previously associated with cLQTS were observed in these 60 subjects. While a similar percentage of variants were novel and missense in both the 1000 Genomes data and the diLQTS data (3.1% vs. 5.0%, respectively; $p = 0.76$), fewer total variants per subject were identified in the 1000 Genomes data than in the diLQTS cases (166 vs. 269.2, respectively; $p < 0.0001$).

Data from 1351 Caucasian individuals provided by the ESP were obtained in genomic intervals overlapping the targeted region with average read depth of 83x. In this data set, only minor allele frequency, rather than individual genotype, is reported, and therefore allele and carrier frequencies cannot be directly compared. Of the 2216 exomic variants discovered, 710 were novel missense alleles, of which 33 were at sites conserved across species and were predicted to be deleterious in the 22 congenital arrhythmia genes. In this set, 14 previously detected cLQTS mutations were also found. A comparison of both the 1000 Genomes data and ESP data to the 26 Caucasian diLQTS variant carriers is shown in Table 3. More than 23% of Caucasian diLQTS subjects carry a previously identified cLQTS mutation or a novel, missense, conserved, deleterious variant, while less than 2% of subjects in 1000 Genomes carry a similar variant ($p = 0.0027$). In ESP, while genotypes are not provided, assuming each subject only carried one minor allele, and all were heterozygous, less than 4% of subjects carried such variants ($p = 0.0003$).

DISCUSSION

Our data support the hypothesis that rare variants in congenital arrhythmia syndrome genes contribute to diLQTS susceptibility given significantly higher occurrence in cases of diLQTS compared to general populations.

Severe ADEs such as diLQTS are challenging to study, as phased drug development and safety monitoring ensure such reactions are rare. Further, precise definition of the phenotype and ascertainment of cases may be difficult.(38) The collection of subjects studied here is valuable given its careful characterization and documentation of the most severe, life-threatening consequence of prolongation of the QT interval, torsades de pointes, in time course with exposure to a culprit medication and without obvious other clinical cause. While the offending agents across these subjects are diverse, the common mechanism of inhibition of a key repolarizing potassium current makes collective analysis appropriate.(9) As sample size grows, subset drug-specific analysis may be possible.

Additionally, studies of ADEs must also discriminate between risk factors (including genetic variants) predisposing to the disease that was the indication for the therapeutic agent and the adverse drug reaction itself. For example, several subjects carry mutations in *ZFHX3*, a transcription factor that has been implicated in susceptibility to atrial fibrillation,(39) a common indication for QT-prolonging antiarrhythmics. These novel rare variants may predispose to atrial fibrillation itself, or increase susceptibility of individuals to diLQTS with exposure to I_{Kr}-blocking drugs, though defining any causal relationships will require functional investigation.(40) Limiting the comparison to the 22 genes know to contribute to congenital repolarization disease alterable by drug challenge is the conservative approach we adopted here.

This analysis makes use of publicly available data as population controls. While the clinical characteristics of these subjects are unknown, the rare nature of these drug reaction outcomes makes occurrence in these samples unlikely. As next generation sequencing data increases exponentially, reuse of such data across studies will become increasingly important. Given variability between sequencing technologies, comparisons such as the variants per subject reported in Table 3 and average read depth are important to understand the coverage and technical merit of platforms being evaluated. In this case, average read depth of ESP data was far greater (83x) while average read depth of the 1000 Genomes data (2-4x) was far less than the case subjects (27x). Alignment and variant calling pipelines also differ, while currently most data is made public in the final variant format such as .vcf, additional studies are needed to evaluate the possible utility and privacy implications of publicly releasing more basic data at the read level for uniform direct comparisons.

Collapsing variants across genes is necessary as these rare, novel mutations are expected to be private to a family, and direct comparison of individual variant frequencies such as done in genome-wide association studies is not possible. Focusing analysis on the variants in the most evolutionarily conserved locations as well as those predicted to be damaging *in silico* also distinguishes these variants as more likely deleterious beyond background variation. Despite finding a potential explanation for the diLQTS outcome in more cases than controls, many cases did not carry such a variant. Future direction includes expanding this hypothesis to drug-specific pharmacokinetic and pharmacodynamics genes as variants in such pathways may have caused supra-therapeutic concentrations of medications.

Drug-induced LQTS is particularly important to drug discovery and development, as many therapeutics are stopped in development due to this severe adverse event. The variants discovered here and this approach to characterizing predisposing variants may be of use in both retrospective analyses of adverse events in trials as well as eventually prospective screening of trial subjects prior to participation. The rare nature of this variation also implies genotyping a specific variant may only be appropriate for screening within a family. These observations may extend to investigation of other complex diseases and adverse drug reactions.

Our data finding rare variants in cases of diLQTS in excess of population controls support the idea diLQTS is a pharmacogenomic syndrome predisposed by rare genetic variation. The

development of genomic approaches to identify individuals at high risk for severe ADRs may allow prediction of safe, targeted therapy and improve drug safety.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Novel,(26) rare, missense variants confirmed by Sanger sequencing.

Gene	Association	chr	position	Amino Acid Change	Protein position	Conserved*	Predicted deleterious†
KCNH2	cLQT2□	7	150275404	ARG,TRP	1033/1160	no	yes
CACNA1C	cLQT8□	12	2658977	ALA,VAL	1733/2139	yes	na
AKAP9	cLQT11□	7	91565028	GLN,GLU	3531/3908	yes	no
SNTA1	cLQT12□	20	31490364	THR,ASN	147/506	yes	yes
KCND3	Brugada	1	112121241	ARG,CYS	566/656	yes	no
GPD1L	Brugada	3	32175498	VAL,MET	249/352	yes	yes
RYR2	CPVT‡	1	235699065	LEU,VAL	555/4968	yes	yes
RYR2	CPVT‡	1	235881420	LEU,PRO	2607/4968	yes	yes
RYR2	CPVT‡	1	236014716	GLU,GLN	4361/4968	yes	no
CACNB2	cSQT¥	10	18469672	MET,VAL	1/623	yes	no
CACNB2	cSQT¥	10	18843173	ILE,VAL	170/606	yes	no
KCNN3		1	153061273	PHE,LEU	315/732	yes	no
PPP2R3A		3	137303609	PHE,LEU	1000/1151	yes	no
AKAP7		6	131528023	GLN,ARG	112/327	yes	no
APLP2		11	129505198	ARG,LEU	504/764	yes	yes
ATP2A2		12	109248584	SER,CYS	184/998	yes	yes
AKAP6		14	32138413	VAL,ALA	839/2320	yes	no
ZFHX3		16	71378611	LYS,GLU	3689/3704	yes	no
ZFHX3		16	71378757	THR,MET	3640/3704	yes	na
ZFHX3		16	71378845	HIS,TYR	3611/3704	yes	na
ZFHX3		16	71549323	LEU,PHE	741/3704	yes	na
ZFHX3		16	71551197	GLY,SER	117/3704	yes	na
JPH3		16	86281433	ARG,TRP	656/749	yes	no
CALR		19	12912058	ASP,GLY	165/418	yes	yes
JPH2		20	42221808	VAL,LEU	345/697	yes	no
JPH2		20	42221985	THR,ALA	286/697	yes	yes

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* GERP ≥ 4 or PhastCons ≥ 0.9

† Polyphen2 'probably damaging' or SIFT 'DAMAGING'

‡ Catecholaminergic Polymorphic Ventricular Tachycardia

□ Congential Long QT Syndrome

¥ Congenital Short QT Syndrome

Table 2

Characteristics of 20 subjects with confirmed novel* rare variants and previously associated cLQTS variants.

Age	Gender	Ethnicity	Offending Drug	Baseline QTc	Previously observed cLQTS variants	Novel congenital arrhythmia gene variants	Novel remaining rhythmonome gene variants
75	Male	Caucasian	**amiodarone**	465	*KCNH2* Arg784Trp		
60	Male	Caucasian	**quinidine**	320	*CAV3* Thr78Met	*GPD1L* Val249Met	
75	Male	Caucasian	**quinidine and sotalol**	428	*SCN5A* Gly615Glu		*JPH3* Arg656Trp
18	Female	Caucasian	**metoclopramide**	431		*AKAP9* Gln3531Glu	
80	Female	Caucasian	**trimethoprim-sulfamethoxazole**	na		*CACNB2* Met1Val	
68	Male	Caucasian	**sotalol**	na		*KCND3 Arg566Cys*	
39	Female	Caucasian	**encainide and bretyllium**	394		*RYR2* Glu4361Gln	
60	Male	Caucasian	**dofetilide**	436		*CACNA1C* Ala1733Val	*CALR* Asp418Gly
54	Male	African American	**ganciclovir and sirolimus**	490		*CACNB2* Ile170Val *SNTA1* Thr147Asn	*ZFHX3* Gly117Ser
72	Female	Caucasian	**sotalol**	399		*KCNH2* Arg1033Trp *RYR2* Leu555Val	*PPP2R3A* Phe1000Leu ZFHX3 His3611Tyr
59	Male	Caucasian	**dofetilide**	436		*RYR2* Leu2607Pro	*ZFHX3* Thr3640Met
60	Female	Asian	**disopyramide**	440			*AKAP6* Val839Ala
78	Male	Caucasian	**azithromycin**	398			*AKAP7* Gln112Arg
44	Female	Caucasian	**sotalol and quinidine**	na			*APLP2* Arg504Leu
63	Female	Asian	**disopyramide**	438			*ATP2A2* Arg504Leu
73	Female	Caucasian	**quinidine and procainamide**	420			*JPH2* Thr286Ala
67	Male	Caucasian	**quinidine**	399			*JPH2* Val345Leu
73	Female	Caucasian	**quinidine**	431			*KCNN3* Phe315Leu
66	Female	Asian	**disopyramide**	383			*ZFHX3* Leu741Phe
75	Male	Caucasian	**quinidine**	440			*ZFHX3* Lys3689Glu

*Novel means not previously reported in KAVIAR(26) including dbSNP132 and 1000Genomes Pilots and Phase 1

Table 3

Comparison of gene variants in diLQTS Caucasians and controls

	diLQTS Caucasians n = 26	1000 Genomes CEU n = 60	ESP Caucasians n = 1351
Total variants	5168	9974	na
Average variants per subject	198.8	166.2	na
Variants in unique locations	528	424	2216
Variants in unique locations per subject	20.3	7.1	na
Novel* variants	44 (8.3%)	73 (17.2%)	1326 (59.8%)
Missense or Nonsense variants	146 (27.7%)	84 (19.8%)	1043 (47.1%)
Novel, missense or nonsense variants	25 (4.7%)	21 (5.0%)	710 (32.0%)
Novel, missense or nonsense, conserved, predicted deleterious all 79 genes	9 (1.7%)	1 (0.2%)	118 (5.3%)
Novel, missense or nonsense, conserved, predicted deleterious in 22 congenital genes	4 (0.8%)	1 (0.2%)	33 (1.5%)
Previously detected cLQTS mutations	3	0	14
Subjects with cLQTS or Novel, missense or nonsense, conserved, predicted deleterious in 22 congenital arrhythmia genes	6 (23.1%)†	1 (1.7%)†	47‡ (3.5%)†

*Novel means not previously reported in KAVIAR(26) including dbSNP132 and 1000Genomes Pilots and Phase 1

†Percent is subjects with a variant

‡Because individual genotypes are not reported, this assumes all congenital arrhythmia gene mutations and novel mutations occurred in different subjects and all were heterozygotes