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## A Large Candidate Gene Survey Identifies the *KCNE1* D85N Polymorphism as a Possible Modulator of Drug-Induced Torsades de Pointes

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## Abstract

**Background**—Drug-induced long QT syndrome (diLQTS) is an adverse drug effect that has an important impact on drug use, development, and regulation. Here, we tested the hypothesis that common variants in key genes controlling cardiac electrical properties modify the risk of diLQTS.

**Methods and Results**—In a case-control setting, we included 176 patients of European descent from North America and Europe with diLQTS, defined as documented *torsades de pointes* during treatment with a QT prolonging drug. Control samples were obtained from 207 patients of European ancestry who displayed <50 msec QT lengthening during initiation of therapy with a QT-prolonging drug, and 837 controls from the population based KORA study. Subjects were successfully genotyped at 1,424 single nucleotide polymorphisms (SNPs) in 18 candidate genes including 1,386 SNPs tagging common haplotype blocks, and 38 non-synonymous ion channel gene SNPs. For validation we used a set of cases (n=57) and population-based controls of European descent. The SNP *KCNE1* D85N (rs1805128), known to modulate an important potassium current in the heart, predicted diLQTS with an odds ratio of 9.0 (95% confidence interval: 3.5–22.9). The variant allele was present in 8.6% of cases, 2.9% of drug-exposed controls, and 1.8% of population controls. In the validation cohort the variant allele was present in 3.5% of cases, and in 1.4% of controls.

**Conclusions**—This high-density candidate SNP approach identified a key potassium channel susceptibility allele that may be associated with the rare adverse drug reaction *torsades de pointes*.

## Keywords

candidate genes; death, sudden; SNP; torsade de pointes; adverse drug events

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Unexpected marked prolongation of the QT interval after initiation of drug therapy, associated with risk of a morphologically distinct pattern of polymorphic ventricular tachycardia (*torsades de pointes*) has been recognized since the 1960s.<sup>1–3</sup> The most common class of drugs causing drug-induced long QT syndrome (diLQTS) is the membrane-active antiarrhythmic drug category, with an incidence approaching 8% in some reports.<sup>1–3</sup> However, diLQTS can occur, albeit more rarely, during treatment with various drugs including many that are not prescribed for cardiovascular indications.<sup>1–3</sup> DiLQTS has been a major cause for drug relabeling and withdrawal, creating obstacles for drug development and uncertainties during drug approval and regulatory processes.<sup>1–3</sup>

Clinical studies have identified risk factors for diLQTS such as hypokalemia<sup>4–6</sup> or female sex,<sup>7</sup> but the phenomenon remains unpredictable in a given individual. Moreover, the congenital long QT syndromes, usually caused by mutations in ion channel or associated genes, are characterized by the same pattern of *torsades de pointes*. Since the congenital LQTS may have variable penetrance,<sup>8</sup> and some mutation carriers may display normal QT intervals, this finding has led to the hypothesis that the drug-induced “acquired” LQT syndromes are conditions predisposed by heritable variants in QT-controlling genes. Indeed, screening the common disease genes associated with the congenital syndrome has identified mutations in 5–15% of subjects with diLQTS.<sup>9–14</sup> One recent study reported congenital LQTS mutations in 8/20 (40%) Japanese cases.<sup>15</sup>

A second hypothesis is that common genetic variants modulate diLQTS risk, and small studies have identified such variants.<sup>12,14</sup> In addition, one study reported that the extent of QT prolongation among first degree relatives of subjects with diLQTS was greater than that seen in first degree relatives of subjects who tolerated QT prolonging drugs without relevant QT prolongation.<sup>16</sup> In the present study, we tested the second hypothesis, using a large cohort of cases drawn from multiple collaborating sites, and interrogating common variants across ion channel and other high priority candidate genes.

## Methods

### Subject sample

The 176 diLQTS patients that constituted the case cohort, defined as documented *torsades de pointes* during treatment with a drug having known QT prolonging potential, were from North America or Europe, and all were of self-identified European descent. The diagnosis was ascertained by cardiologists who specialize in clinical electrophysiology at 7 participating centers. The case definition required documented *torsades de pointes*, associated with any reversible QT prolongation during the drug exposure. Covariates sought included age, sex, self-reported ethnicity, arterial hypertension, history of atrial fibrillation, serum creatinine, and culprit drug, as well as information serum potassium concentrations (with particular emphasis on hypokalemia) at the time of the index arrhythmia. Written informed consent to participate in the study including the collection and use of DNA samples for genetic analysis was obtained with Institutional Review Board (IRB) approval at each center.

The drug-exposed controls consisted of 207 self-identified European ancestry subjects derived from a clinical study at Vanderbilt University Medical Center, under appropriate IRB-approved protocols. The study used electronic medical record-based surveillance to identify in-patients in whom QT prolonging antiarrhythmics were being initiated. Patients consenting to participate (91% of those approached) agreed to allow collection of clinical data, including drug-associated QT interval duration changes, as well as DNA samples. For this study, the drug-exposed control cohort was defined by the absence of qualifying arrhythmias, <50 msec increase in QTc interval using Bazett’s formula for heart rate correction, and no QTc interval exceeding 500 msec during drug treatment.

We also used control subjects from the general population. These were recruited from the population-based KORA Study (Cooperative Health Research in the Region of Augsburg),<sup>17</sup> and were all of European descent. In brief, 4856 subjects in ten subsets of equal size, stratified according to sex and age, underwent a survey in 1994–95 (KORA S3), and again in 2004–2005 (KORA F3). From among the KORA population, we included 837 randomly selected subjects as controls, maintaining the original sex-distribution. The study was approved by the ethics committee of the Bavarian Medical Association.

Finally, we used cases with diLQTS from the Drug Induced Arrhythmia Risk Evaluation (DARE) Study, who met the inclusion criteria above, for a validation cohort.<sup>18</sup> These validation cases were compared to healthy population controls recruited by DARE from the primary care practices of cases to achieve geographical matches, aiming for a 2:1 to 4:1 ratio of controls to cases. Controls were excluded if there was any prior history of ventricular arrhythmia, drug-induced arrhythmia, syncope or congenital long QT syndrome. All validation control subjects underwent digital 12 lead ECGs and were excluded if there was significant abnormality.

## Genotyping

Eighteen candidate genes were studied. These included genes reported to be disease-causing in cardiac arrhythmia syndromes, functionally important subunits of these genes, and genes significantly associated with the QT interval in the general population based on the results of genome wide association studies (Table 1).<sup>19,20</sup>

Haplotype tagging SNPs (tagSNPs) were selected to systematically analyze each gene using Tagger<sup>21</sup> based on HapMap<sup>22</sup> data release #20 / phase II using the March 2006 NCBI B35 genome assembly and dbSNP build 125 data. The following criteria were applied for tagSNP selection: HapMap CEU population, pairwise only tagging with  $r^2 \geq 0.8$  and a minor allele frequency (MAF)  $\geq 10\%$ . To account for genetic variation in regions surrounding each gene, we considered up- and downstream genetic data in the tagging procedure. Included regions were defined by linkage-disequilibrium (LD), blocks as previously reported.<sup>23</sup> We included at least 50 kb of both up- and downstream information. A total of 1,386 SNPs were selected for genotyping using this method. In addition, we included all 38 non-synonymous SNPs with minor allele frequencies  $\geq 1\%$  previously reported in the 18 candidate genes. A summary of all 1,424 SNPs chosen for study is presented in Table 1, and detailed information including a complete SNP-list is presented in Supplemental Table 1.

Genotyping of all cases, drug-exposed controls, and KORA population-based controls was performed using a custom Illumina GoldenGate assay (Illumina OPA GS0007558) according to the manufacturer's recommendations using the Illumina Beadstation 500GX (Illumina Inc., San Diego, USA) in a single laboratory (Genome Analysis Center, Helmholtz Zentrum München, Germany). The Illumina BeadStudio software clustering algorithm was used for initial data analysis. Thereafter, intensity plots of all variants were examined individually and manual genotype calling was performed if necessary.

Monomorphic SNPs or those with a minor allele frequency  $< 1\%$ , and SNPs, as well as samples with call rates  $< 90\%$ , were excluded from the analysis. A total of 70 SNPs were removed prior to analysis. Hardy Weinberg Equilibrium (HWE) was calculated for the remaining SNPs, and deviations from HWE expectations were noted but not excluded from analyses (Supplemental Table 1). After filtering, the overall genotyping call rate was 97.1%, and a total of 1,354 SNPs had sufficient quality for analysis. Details of SNPs as well as their clustering relative to the 18 selected genes can be found in Table 1. Clusterplots for SNPs selected for replication are shown in Supplemental Figure 1.

For validation of rs1805128, 57 additional diLQTS cases from the DARE cohort and 211 controls were genotyped for *KCNE1* D85N (rs1805128) using standard direct Sanger sequencing of the PCR amplicon in both directions. We also used the DARE set to examine whether an initial finding with rs7295250 would replicate. For this experiment, samples were genotyped using the Illumina Infinium Human CVD 50K Bead Array<sup>24</sup> analyzed with the Illumina platform 500GX (the Biomics Centre, St. George's University of London). Of 138 individuals, two had  $> 10\%$  missing genotypes and were excluded. Another three control

subjects were excluded having an overall genotype pattern suggestive of non-European descent despite self-report. Thereafter, 47 cases and 86 controls were available with 100% genotyping call rate for rs7295250.

### Statistical analysis

All subjects included in the study were of European descent. We attempted the STRUCTURE<sup>25,26</sup> and EIGENSTRAT<sup>27</sup> methods to account for population stratification, but no relevant stratification was identified. In particular, the first five principal components were nonsignificant.

All tests of association were performed using PLINK.<sup>28</sup> Each SNP was tested for association to diLQTS between cases and controls using logistic regression adjusted for age and sex in two different analyses: diLQTS cases vs. drug-exposed controls, and diLQTS cases vs. population controls. In addition, we attempted sex-stratified analyses adjusted for age in each analysis group. Bonferroni correction for multiple comparisons would have required an uncorrected p-value of  $3 \times 10^{-5}$ . Given our sample size and the fact that ours presumably represents already one of the largest collections of diLQTS cases, we applied less stringent criteria for identifying SNPs to be evaluated by replication. We planned to take forward to the DARE follow-up set any SNP with a P value of  $<0.01$  for both drug-drug exposed and drugcontrol population comparisons.

The validation stage associations in the DARE cohort for rs1805128 and rs7295250 were assessed among cases and controls only assuming an additive genetic model adjusted for age and sex.

### Results

The clinical characteristics of the cohorts are presented in Table 2. Among the 176 diLQTS cases, 128 (73%) were female, and the mean age was  $63 \pm 17$  years. The single most common drug associated with *torsades de pointes* was sotalol ( $n=52$ ); other common culprit antiarrhythmic drugs included quinidine ( $n=18$ ) and amiodarone ( $n=17$ ). Cumulatively, antiarrhythmics ( $n=107$ ), antipsychotics ( $n=14$ ) and antibiotics ( $n=12$ ) were associated with diLQTS in 133 (76%) of the cases. In 28 cases, combinations of two or more culprit drugs were implicated.

Serum potassium was available in 119 cases at the time of the event and was below the laboratory specific reference range in 34 (19.3%) cases. Resting heart rate was different between cases ( $70.0 \pm 17.4$  /min), drug-exposed controls ( $80.7 \pm 23.9$  /min) and population controls ( $69.0 \pm 11.2$  /min). Atrial fibrillation was more prevalent in the drug-exposed control group (88.9%) compared to the population control group (1.7%) and to the diLQTS cases (53.4%). Quantitatively analyzable 12-lead resting ECG measurements were available in 135 cases, 205 drug-exposed controls, and 824 population controls. ECG measurements were included prior to the event or at steady state post event and after culprit drug withdrawal. Baseline QT intervals as well as corrected QT intervals using multiple formulae was prolonged at baseline in cases compared to both control groups.

Single SNP tests of association assuming an additive genetic model were performed for 1,354 variants across all 18 genes of interest between diLQTS cases and drug-exposed controls (Figure 1, **black symbols**). The association results for all SNPs at a significance threshold of  $p \leq 0.01$  are shown in Table 3, and the results for all associations are shown in Supplemental Table 1. All tests of association used a logistic regression model adjusted for age and sex. SNP rs7295250, a variant on chromosome 12 in the genomic region of the L-type calcium channel gene *CACNA1C*, was associated with the smallest p-value among all

tests of association. This variant conferred an odds ratio of 1.88 (95% confidence interval (CI) 1.30 – 2.71,  $p=7.62 \times 10^{-4}$ ). The strongest effect size within this dataset was detected for rs1805128, which is a non-synonymous coding (missense) SNP in exon 1 of the cardiac ion channel gene *KCNE1*. This variant leads to an amino acid substitution from aspartic acid to asparagine at position 85 of the translated protein (Asp 85 Asn; D85N). The variant was significantly associated with diLQTS with an odds ratio of 9.92 (95% CI 2.36 – 41.80,  $p=1.77 \times 10^{-3}$ ). The SNP showed a marked difference in allele frequency between diLQTS cases and drug-exposed controls: 8.6% vs. 2.9%.

In parallel, we performed an analysis comparing diLQTS cases with population control subjects (Figure 1, **grey symbols**). Tests of association results with  $p \leq 0.01$  are shown in Table 3 and the complete results are shown in Supplemental Table 1. The most significantly associated variant at this stage was the previously detected rs1805128 / D85N, with an odds ratio of 8.88 (95% CI 3.26 – 24.17,  $p=1.95 \times 10^{-5}$ ). The difference in allele frequencies between cases and population-based controls was 8.6% versus 1.8%. SNP rs7295250 showed weaker, but significant association in the analysis versus population controls subjects: odds ratio 1.56 (95% CI 1.18 – 2.07,  $p=1.77 \times 10^{-3}$ ). Other SNPs showed strong associations, but were not (or not as significantly) associated in the parallel analysis comparing diLQTS cases and drug-exposed controls.

To validate the association between diLQTS and SNPs rs1805128 / D85N and rs7295250, we assessed the relationship in the independent DARE sample consisting of 57 diLQTS cases and 211 population-based, matched control subjects. Clinical characteristics of this validation sample are provided in Table 2. Cases displayed a higher proportion of cardiovascular disease than controls, and hypokalemia at the time of the index arrhythmia was identified in 25% of those cases for whom admission potassium levels were available. Amiodarone (51%) and sotalol (26%) were the two most common culprit drugs. Six patients (11%) were receiving diuretics, usually in combination with another drug. In 12 patients (21%) more than one culprit drug was implicated.

The effect of rs1805128 / D85N in the validation analysis showed a trend in the same direction as observed in the discovery analysis (Table 3). We identified 1 homozygote and 2 heterozygotes among diLQTS cases, and 6 heterozygotes among controls. The minor allele frequencies of cases and controls in this dataset were 3.5% and 1.4%, respectively, which translated into an odds ratio of 1.5 (95% CI 0.35 – 6.57,  $p=0.58$ ).

In an analysis of rs1805128 stratified by sex, we found similar effect estimates and significance levels for both men and women suggesting no major effect modification by sex. In each subgroup, the results were comparable to the main age- and sex-adjusted analysis (data not shown). SNP rs7295250 failed to replicate in the DARE cohort with a non-significant effect estimate in the opposite direction (Table 3).

## Discussion

In this study, we tested the hypothesis that common genetic variants modulate the risk for diLQTS. We observed that the *KCNE1* variant rs1805128, resulting in D85N, confers substantially increased risk for drug-induced *torsades de pointes*. The key resources that allowed us to identify this variant as a risk allele were the accumulation of a large set of cases through an international collaboration, large control sets of subjects with drug response phenotypes, and availability of population controls.

The D85N variant has been implicated previously as a modulator of QT interval<sup>19,29,30</sup> and T wave morphology.<sup>31</sup> In addition, D85N had been described as a rare variant in a small study of 32 diLQTS cases.<sup>12</sup> One recent study reported that co-expression in heterologous

cells of *KCNE1*-D85N with genes encoding potassium channel pore-forming subunits (*KCNQ1* and *KCNH2*) impaired  $I_{Kr}$  and  $I_{Ks}$ , two key repolarizing potassium currents, and suggested it contributed to the variable penetrance of the congenital long QT syndrome.<sup>32</sup>

Life-threatening adverse drug reactions are rare by their nature, since high-risk drugs are not generally widely prescribed. Further, many such adverse reactions occur in an unpredictable fashion, and sudden death due to this mechanism may not be recognized clinically. Thus, the impact of culprit drugs on the balance between risk and benefit of drug therapy in an individual patient has not been predictable *a priori*. Contemporary genomic approaches to analyze human phenotypes, such as common diseases, rely on discovery and replication cohorts, often accumulating tens of thousands of subjects to validate the predictive power of variants with odds ratios  $<2$ . Accumulating such large numbers of cases is by definition impossible for rare adverse drug effects.<sup>33,34</sup> Hence, the expectation with the use of either an intensive candidate gene interrogation or even a genome-wide approach is that common contributory variants can be identified only if they confer relatively high odds ratios. Further, demonstration of biologic plausibility, as in this study, bolsters an argument that a variant identified in this fashion contributes to the phenotype under study. Other examples of rare adverse drug effects analyzed using genomic approaches include statin-related myopathy<sup>35</sup> and abacavir-related skin reactions.<sup>36</sup> In both cases, individual genetic variants with strong effects on risk and with biologic plausibility arguments have been identified.

We studied cases of diLQTS caused by a wide range of drugs and drug classes, although the majority was antiarrhythmic-related. Atrial fibrillation was most prevalent in the drug-exposed control group compared to the population control group and to the diLQTS cases. The reason for this distribution pattern was the fact that many diLQTS cases and drug-exposed controls received culprit drugs for atrial fibrillation treatment. It is widely accepted that culprit drugs all share a common proximate mechanism, which is inhibition of the rapid component of the cardiac delayed rectifier potassium current,  $I_{Kr}$ .<sup>1-3</sup> This common mechanism justifies the inclusion in this analysis of multiple culprit drugs. High concentrations of offending drugs are also clinical risk factors for diLQTS, although mechanisms producing such elevations are drug-specific. Thus the analysis we report did not consider variability in drug concentrations. While the phenotype we studied here required documented torsades de pointes, this arrhythmia has the potential to degenerate to ventricular fibrillation, and thus cause sudden cardiac death. Indeed, use of QT-prolonging antipsychotics<sup>37</sup> and of other high potency  $I_{Kr}$  blockers<sup>38</sup> has been associated with an increased risk of SCD.

Electrophysiological experiments using a range of approaches, from whole hearts<sup>39-41</sup> to single mammalian cells<sup>42</sup> to computational simulations,<sup>43</sup> support the concept that variability in the extent to which  $I_{Kr}$  block prolongs QT interval is determined not only by this specific current but also by multiple other currents whose integrated activity generates normal cardiac repolarization. A framework for analyzing diLQTS susceptibility suggests that multiple mechanisms control normal cardiac repolarization. Thus, loss of a single component in this system is unlikely to cause marked QT derangement; this redundancy has been referred to as “repolarization reserve”.<sup>44</sup> As a consequence, individuals with a reduced repolarization reserve, due to common genetic variants or other risk factors such as sex, may display entirely normal QT intervals until drug challenge eliminates the remaining reserve, resulting in marked QT prolongation and torsades de pointes. Recent studies have implicated a second key repolarizing current,  $I_{Ks}$ , as a major contributor to repolarization reserve.<sup>41-43</sup> Our finding, that a variant in *KCNE1* is a risk factor for diLQTS, is entirely consistent with this view, and supports the idea of using such a “system” approach<sup>45</sup> to analyze the physiologic underpinnings of other rare, previously unpredictable adverse drug effects. Our diLQTS cases presented with significantly prolonged QTc measurements compared to

controls. We attribute this finding in part to the fact that many ECGs in cases unavoidably were collected when the patients were not entirely off the QT prolonging drugs. Our findings demonstrate the power both of multi-institutional collaborations to study rare adverse drug effects, and of contemporary genomic approaches as a first step to definitive studies to directly test the value of “pre-prescription genotyping” to optimize drug therapy. Further studies will be required to determine whether pre-prescription genotyping at this site can reduce the incidence of diLQTS.

## Limitations and conclusions

Restricting our study to participants of European descent does not allow us to make any conclusion with regard to other ethnicities. Although we did not detect relevant population stratification in our cohort, diLQTS cases were of European descent, but all population controls were Germans only. Current standards in genetic association studies generally require rigorous standards regarding adjustment for multiple comparisons and independent replication. As outlined above, the rarity of our phenotype prevented us from collecting higher numbers of participants to meet this standard. However, the clinical importance of the phenotype justified the acceptance of a trade-off in requirements. In addition, we aimed to minimize false-positive results by using two control groups different in nature, and an independent validation step, which showed consistent but not significant results. As particularly the sample size of the case group was small, we attribute this finding to a limitation of statistical power. In addition to the sample size, also the low frequency of the D85N risk allele is of importance when interpreting the nonsignificant validation. However, the D85N variant is known to modulate potassium current and prolong repolarization. Data attributing risk to this variant in our study and previous work is creating a portfolio of evidence that supports the idea of genotyping subjects at risk prior to drug exposure. At present, such an option might be still distant, however, will become more interesting once further supporting replication evidence is available. How much evidence is required to act on a specific variant in this fashion remains controversial, and is likely to evolve as genotyping costs and very large databases evolve.<sup>46</sup> We submit that the clarity of our findings would have benefitted from additionally adjusted models including ECG measurements like the QTc interval, or other clinical covariates like potassium levels or concomitant diseases. Due to an excess of missing data in our case samples, we were restricted to a limited adjustment as presented. Future investigations will be required to gain further insight regarding the effect of other factors on the association between D85N and diLQTS.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

These data have been deposited at Pharmacogenetics and Pharmacogenomics Knowledge Base / PS209206 (pharmgkb.org), and are being deposited at dbGaP, The database of Genotypes and Phenotypes (<http://www.ncbi.nlm.nih.gov/gap>).

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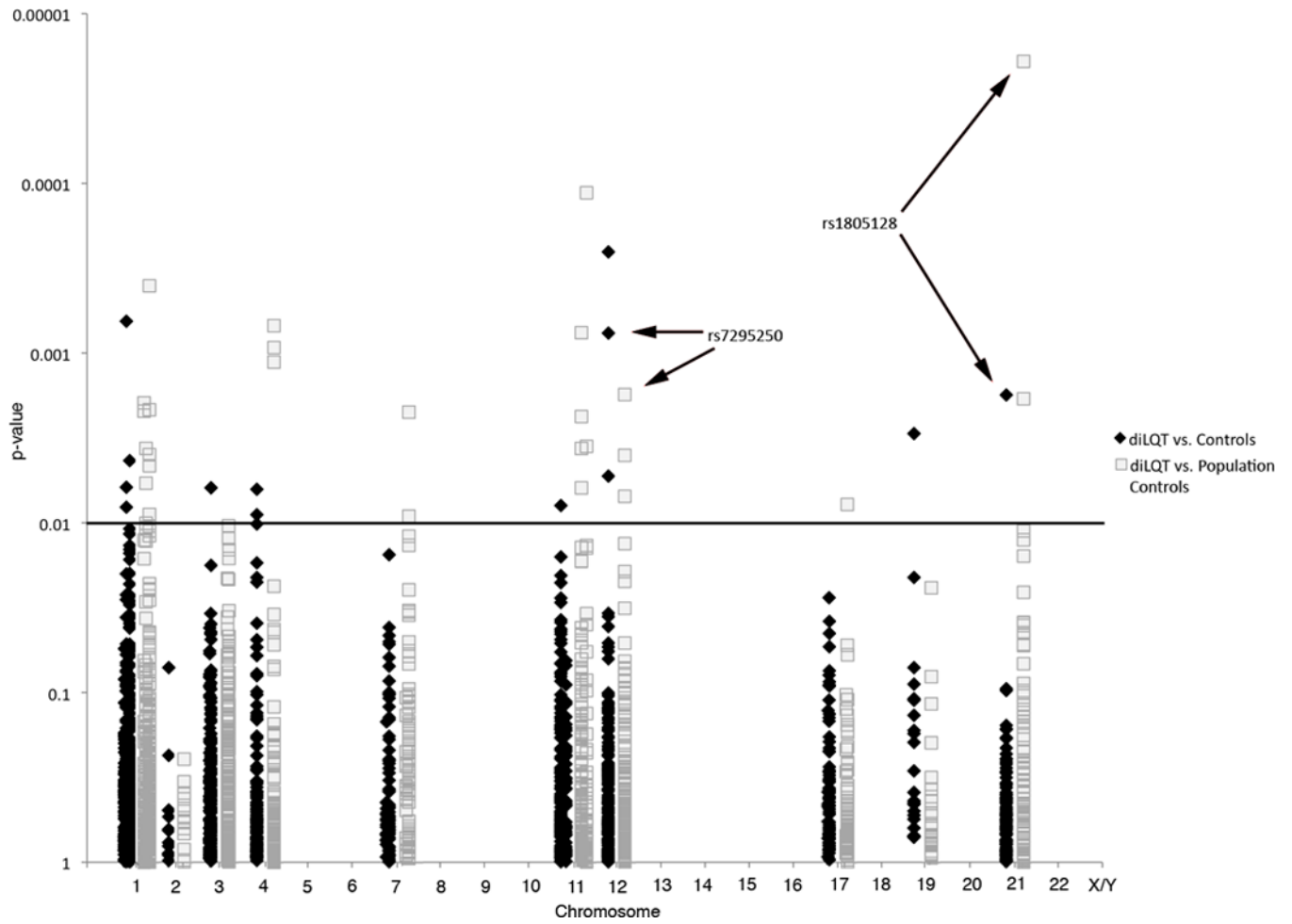
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**Figure 1.** Manhattan-plot illustrating the genotyping results.  $-\log_{10}$  p-values plotted against the SNPs' chromosomal location. diLQTS cases vs. drug-exposed controls in black; diLQTS cases vs. population controls in gray. The horizontal line indicates threshold of  $p=0.01$  for SNP replication. Arrows mark associations with  $p \leq 0.01$  in both comparisons.

Table 1

## Candidate gene based SNP selection

Gene	chr	Gene position	Tagged region	tag SNPs	ns SNPs
<i>AKAP9 Isoform 1</i>	7	91,214,843–91,384,640	91,104,000–91,700,000	16	4
<i>ANK 2 Isoform 1</i>	4	114,328,474–114,662,489	114,160,000–114,690,000	114	4
<i>CACNA1C</i>	12	2,032,725–2,672,368	1,900,000–2,820,000	173	3
<i>NOS1AP</i>	1	158,771,239–159,071,471	158,680,000–159,170,000	130	-
<i>CASQ2</i>	1	115,954,685–116,023,312	115,810,000–116,130,000	66	4
<i>CAV3</i>	3	8,750,496–8,763,450	8,700,000–8,840,000	62	1
<i>FKBP1B</i>	2	24,184,279–24,198,199	24,134,278–24,248,198	13	-
<i>GPD1L</i>	3	32,123,148–32,185,205	31,980,000–32,230,000	50	-
<i>KCNE1</i>	21	34,740,858–34,805,483	34,640,000–34,930,000	68	3
<i>KCNE2</i>	21	34,658,193–34,665,310	34,640,000–34,930,000	28	-
<i>KCNH2 / HERG</i>	7	150,079,697–150,112,662	149,930,000–150,200,000	65	2
<i>KCNJ2</i>	17	65,677,271–65,687,776	65,610,000–65,765,000	42	-
<i>KCNQ1</i>	11	2,422,797–2,826,915	2,260,000–2,900,000	150	1
<i>RYR2</i>	1	233,531,743–234,323,329	233,427,218–234,427,217	232	8
<i>SCN1B</i>	19	40,213,374–40,223,192	40,140,000–40,270,000	24	-
<i>SCN4B</i>	11	117,509,302–117,528,745	117,350,000–117,620,000	47	-
<i>SCN4A</i>	17	59,369,646–59,404,010	59,220,000–59,520,000	24	1
<i>SCN5A</i>	3	38,564,557–38,666,167	33,843,000–38,790,000	82	7
<b>Subtotal</b>				<b>1386</b>	<b>38</b>
<b>Total</b>					<b>1424</b>

Basic information on the 18 genes under investigation and the number of investigated SNPs. All data based on human genome built hg 17, NCBI built 35. chr – chromosome; tag SNP – haplotype tagging SNP; ns SNP – non-synonymous coding SNP (for detailed information per SNP, see Supplemental Table 1).

**Table 2**

Baseline characteristics of the study sample

	dILQTS cases (n=176)	drug-exposed controls (n=207)	p-value (dILQTS cases vs. controls)	Population controls (n=837)	p-value (dILQTS cases vs. population controls)	DARE dILQTS cases (n=57)*	DARE population controls (n=211)*	p-value (DARE cases vs. controls)
<b>Clinical characteristics</b>								
Male sex, n (%)	48 (27.3%)	137 (66.2%)	<0.001	417 (49.8%)	<0.001	19 (33.3%)	101 (47.9%)	0.053
Age (at event) (years)	63.0 ± 16.7	61.4 ± 14.0	0.32	60.7 ± 10.2	0.10	63.4 ± 14.2	68.6 ± 11.3	0.005
Arterial Hypertension n (%)	82 (46.6%)	124 (59.9%)	0.73	485 (57.9%)	0.39	33 (64.7%)	87 (42.7%)	0.007
Atrial fibrillation, n (%)	94 (53.4%)	184 (88.9%)	<0.001	14 (1.7)	<0.001	28 (49.0%)	-	-
Hypokalemia at event n (%)	34 (19.3%)	-	-	-	-	12 (21.0%)	-	-
<b>ECG characteristics</b>								
QT interval, ms	442.7 ± 91.8	381.1 ± 47.2	<0.001	403.2 ± 30.4	<0.001	416.8 ± 51.7	407.8 ± 29.4	0.107
RR interval, ms	910.8 ± 228.7	792.5 ± 212.1	<0.001	964.7 ± 165.2	0.009	900.0 ± 230.0	950.0 ± 150.0	0.048
QTc Bazett, ms	468.3 ± 77.2	433.8 ± 31.9	<0.001	413.0 ± 23.4	<0.001	444.7 ± 25.3	420.2 ± 20.1	<0.001
QTc Fridericia, ms	458.1 ± 77.0	414.6 ± 28.7	<0.001	409.3 ± 20.1	<0.001	434.4 ± 26.2	416.7 ± 33.6	0.001
QTc Framingham, ms	448.8 ± 75.6	409.8 ± 27.7	<0.001	403.6 ± 19.6	<0.001	432.8 ± 25.5	415.5 ± 34.3	0.001
QTc RAS, ms	447.1 ± 73.8	411.2 ± 27.4	<0.001	405.4 ± 19.9	<0.001	-	-	-

The table provides baseline clinical characteristics and ECG measures prior to the event or at steady state after the event after culprit drug withdrawal. QT corrections: QTc Bazett =  $QT / RR^{1/2}$ ; QTc Fridericia =  $QT / RR^{1/3}$ ; QTc Framingham =  $QT + 0.154 * (1 - RR)$ . Continuous parameters are expressed as mean ± standard deviation and are compared using a two-sample t-test. Discrete variables are displayed as frequency and percentage, and are compared between groups using the Fisher's Exact Test.

\* The DARE sub-sample of 47 cases and 86 controls was a subset of the entire DARE cohort showing similar demographic characteristics, and is not presented separately.

**Table 3**  
SNPs with significant associations between diLQTS cases to controls or population controls

SNP	chr	Gene	Coded Allele	Non-coded Allele	MAF	Function	diLQT cases (n=176) vs. drug-exposed controls (n=207)		diLQT cases (n=176) vs. population controls (n=837)		DARE cases (n=57) vs. DARE controls (n=211)	
							OR(95%CI)	p-value	OR(95%CI)	p-value	OR(95%CI)	p-value
rs2238018	12	CACNA1C	C	T	0.20	Intron	2.22(1.45-3.41)	2.53×10 <sup>-04</sup>	1.32(0.97-1.79)	7.94×10 <sup>-02</sup>		
rs164124	1	CAPON	A	G	0.33	Intron	0.55(0.39-0.78)	6.49×10 <sup>-04</sup>	0.8(0.6-1.06)	1.16×10 <sup>-01</sup>		
<b>rs7295250</b>	<b>12</b>	<b>CACNA1C</b>	<b>C</b>	<b>T</b>	<b>0.22</b>	<b>Intron</b>	<b>1.88(1.3-2.71)</b>	<b>7.62×10<sup>-04</sup></b>	<b>1.56(1.18-2.07)</b>	<b>1.77×10<sup>-03</sup></b>	<b>0.95(0.54-1.67)</b>	<b>8.7×10<sup>-1</sup></b>
<b>rs1805128</b>	<b>21</b>	<b>KCNE1</b>	<b>A</b>	<b>G</b>	<b>0.02</b>	<b>D85N</b>	<b>9.92(2.36-41.8)</b>	<b>1.77×10<sup>-03</sup></b>	<b>8.88(3.26-24.17)</b>	<b>1.95×10<sup>-05</sup></b>	<b>1.51(0.35-6.57)</b>	<b>5.8×10<sup>-1</sup></b>
rs8103371	19	SCN1B	C	T	0.12		2.06(1.28-3.32)	2.98×10 <sup>-03</sup>	1.53(1.06-2.21)	2.43×10 <sup>-02</sup>		
rs2249287	1	RYR2	C	T	0.21	Intron	0.54(0.36-0.83)	4.30×10 <sup>-03</sup>	0.67(0.47-0.96)	2.86×10 <sup>-02</sup>		
rs2238017	12	CACNA1C	A	G	0.35	Intron	1.62(1.16-2.28)	5.31×10 <sup>-03</sup>	1.37(1.05-1.78)	2.22×10 <sup>-02</sup>		
rs10800465	1	CAPON	C	T	0.26	Intron	0.6(0.42-0.87)	6.17×10 <sup>-03</sup>	0.83(0.62-1.12)	2.28×10 <sup>-01</sup>		
rs7427106	3	SCN5A	C	G	0.13	Intron	1.98(1.21-3.22)	6.22×10 <sup>-03</sup>	1.13(0.79-1.61)	4.95×10 <sup>-01</sup>		
rs1395112	4	ANK2	A	G	0.34		0.62(0.44-0.87)	6.35×10 <sup>-03</sup>	0.72(0.54-0.96)	2.38×10 <sup>-02</sup>		
rs10741726	11	KCNQ1	G	T	0.45	Intron	1.55(1.12-2.15)	7.91×10 <sup>-03</sup>	1.18(0.9-1.55)	2.22×10 <sup>-01</sup>		
rs12026931	1	CAPON	C	T	0.08	Intron	2.36(1.25-4.45)	8.09×10 <sup>-03</sup>	1.28(0.81-2.02)	2.96×10 <sup>-01</sup>		
rs10155204	4	ANK2	C	T	0.13		0.48(0.27-0.83)	8.96×10 <sup>-03</sup>	0.62(0.39-0.99)	4.43×10 <sup>-02</sup>		
rs10216051	7	KCNH2	A	G	0.37		1.49(1.08-2.05)	1.54×10 <sup>-02</sup>	1.51(1.16-1.96)	2.25×10 <sup>-03</sup>		
rs1032913	4	ANK2	C	T	0.21		0.61(0.4-0.91)	1.72×10 <sup>-02</sup>	0.55(0.38-0.79)	1.14×10 <sup>-03</sup>		
rs3766884	1	RYR2	C	T	0.18	Intron	1.59(1.07-2.38)	2.30×10 <sup>-02</sup>	1.78(1.29-2.45)	4.06×10 <sup>-04</sup>		
rs16928297	11	KCNQ1	G	T	0.23	Intron	1.52(1.05-2.21)	2.78×10 <sup>-02</sup>	1.69(1.25-2.3)	7.66×10 <sup>-04</sup>		
rs596502	1	RYR2	C	T	0.21	Intron	1.5(1.03-2.17)	3.51×10 <sup>-02</sup>	1.54(1.14-2.08)	4.65×10 <sup>-03</sup>		
rs11062288	12	CACNA1C	C	T	0.27	Intron	1.46(1.03-2.09)	3.57×10 <sup>-02</sup>	1.53(1.15-2.04)	4.04×10 <sup>-03</sup>		
rs1532876	4	ANK2	C	G	0.15		0.57(0.33-0.97)	3.91×10 <sup>-02</sup>	0.46(0.3-0.72)	6.97×10 <sup>-04</sup>		
rs3733617	4	ANK2	C	T	0.05	P2835S	1.97(1-3.88)	4.89×10 <sup>-02</sup>	2.6(1.48-4.59)	9.38×10 <sup>-04</sup>		
rs689264	11	SCN4B	C	T	0.08	Intron	1.68(0.96-2.92)	6.93×10 <sup>-02</sup>	2.38(1.53-3.7)	1.15×10 <sup>-04</sup>		
rs16847548	1	CAPON	C	T	0.22	Promoter	1.41(0.97-2.06)	7.57×10 <sup>-02</sup>	1.55(1.14-2.12)	5.88×10 <sup>-03</sup>		
rs757086	11	KCNQ1	C	T	0.08	Intron	1.39(0.96-2)	7.78×10 <sup>-02</sup>	1.59(1.18-2.14)	2.39×10 <sup>-03</sup>		

SNP	chr	Gene	Coded Allele	Non-coded Allele	MAF	Function	dilQT cases (n=176) vs. drug-exposed controls (n=207)			dilQT cases (n=176) vs. population controls (n=837)			DARE cases (n=57) vs. DARE controls (n=211)		
							OR(95%CI)	p-value	OR(95%CI)	p-value	OR(95%CI)	p-value	OR(95%CI)	p-value	
rs1786176	11	SCN4B	C	T	0.09		1.53(0.9–2.57)	1.13×10 <sup>-01</sup>	1.82(1.22–2.72)	3.57×10 <sup>-03</sup>					
rs2237898	11	KCNQ1	A	C	0.07	Intron	1.59(0.88–2.85)	1.21×10 <sup>-01</sup>	1.84(1.19–2.86)	6.27×10 <sup>-03</sup>					
rs4817639	21	KCNE2	C	T	0.21		1.31(0.9–1.91)	1.64×10 <sup>-01</sup>	1.63(1.2–2.21)	1.88×10 <sup>-03</sup>					
rs2238092	12	CACNA1C	A	G	0.25	Intron	1.29(0.9–1.86)	1.68×10 <sup>-01</sup>	1.52(1.12–2.06)	7.03×10 <sup>-03</sup>					
rs2819774	1	RYR2	A	T	0.34	Intron	1.22(0.88–1.7)	2.28×10 <sup>-01</sup>	1.48(1.13–1.94)	4.00×10 <sup>-03</sup>					
rs11022922	11	KCNQ1	C	T	0.22	Intron	1.24(0.87–1.78)	2.39×10 <sup>-01</sup>	1.6(1.17–2.19)	3.67×10 <sup>-03</sup>					
rs4427395	1	RYR2	A	G	0.34	Intron	1.17(0.84–1.63)	3.43×10 <sup>-01</sup>	1.54(1.17–2.03)	2.18×10 <sup>-03</sup>					
rs4564142	1	CASQ2	C	T	0.16		1.18(0.78–1.8)	4.38×10 <sup>-01</sup>	1.7(1.21–2.38)	2.22×10 <sup>-03</sup>					
rs7522269	1	CAPON	C	T	0.22	Intron	1.16(0.8–1.66)	4.38×10 <sup>-01</sup>	1.6(1.17–2.2)	3.67×10 <sup>-03</sup>					
rs3860222	1	CASQ2	A	G	0.23		1.15(0.8–1.67)	4.50×10 <sup>-01</sup>	1.63(1.2–2.22)	1.98×10 <sup>-03</sup>					
rs1985961	17	SCN4A	C	T	0.40		1.13(0.82–1.56)	4.68×10 <sup>-01</sup>	1.44(1.1–1.89)	7.85×10 <sup>-03</sup>					
rs2805468	1	RYR2	A	G	0.32	Intron	0.92(0.66–1.3)	6.47×10 <sup>-01</sup>	1.44(1.1–1.89)	8.98×10 <sup>-03</sup>					
rs10240738	7	KCNH2	C	T	0.46	Promoter	1.07(0.78–1.46)	6.66×10 <sup>-01</sup>	1.42(1.09–1.85)	9.2010 <sup>-03</sup>					

The table presents association results for all SNPs that reached a significance level of  $p \leq 0.01$  either comparing dil\_QTS cases vs. drug-exposed controls or dil\_QTS cases vs. population controls. The two SNPs fulfilling these criteria are printed in bold. MAF – minor allele frequency.