Prescription Medications and Abnormal Heart Rhythms: Understanding Genetics of Drug-Induced Long QT Syndrome

by

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Dedication

To my beloved Willi(s), my cherished family, and my dedicated mentor, whose support have been my anchor throughout this journey.

In memory of Luisita & Jose, and in honor to those who have tragically experienced sudden cardiac death.

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Abstract

Drug-induced long QT syndrome (diLQTS) is a serious side effect of many medications, which can lead to sudden cardiac death. While some clinical risk factors for diLQTS have been identified, they do not fully explain the risk, making diLQTS largely unpredictable. Previous evidence shows that both common (minor allele frequency [MAF] >1%) and uncommon ([MAF] <1%) genetic variants are associated with diLQTS risk. However, limitations of those previous studies, such as small sample size, lack of replication, and/or assessment in real-world clinical practice, prevent the clinical utility of genomics. The objective of this dissertation research was to investigate the associations between individual uncommon candidate genetic variants (*KCNE1*-D85N, *KCNE2*-I57T, and *SCN5A*-G615E) and a polygenic risk score (PRS) of common variants (as published by Strauss et al.) with diLQTS risk in a large observational case-control in a real-world clinical practice setting. A retrospective case-control study was conducted utilizing patients from Michigan Genomics Initiative (MGI) treated with high-risk QT-prolonging drugs and with an electrocardiogram (ECG) measurement during drug exposure. The primary outcome was an exaggerated prolongation of the QTc interval (>60 ms change from baseline and/or >500 ms absolute value) after exposure to a high-risk QT-prolonging drug, while the secondary outcome was a composite of *torsades de pointes* (TdP), any ventricular arrhythmia, or sudden cardiac death. The study found that *KCNE1*-D85N was significantly associated with diLQTS (adjusted odds ratio=2.24 [95% CI=1.35-3.58] p=0.001) in 6,083 self-reported white patients treated with 27 different high-risk QT-prolonging medications. However, due to low minor allele

frequencies, there was insufficient power to analyze the associations of *KCNE2*-I57T and *SCN5A*-G615E with diLQTS. The study also validated a PRS for diLQTS published by Strauss et al. demonstrating its association with diLQTS in self-identified white patients. We lacked the statistical power to detect the PRS as a risk factor for diLQTS in African-American and Asian patients, since the sample sizes for those race groups were smaller than the white patients. Despite the association of *KCNE1*-D85N and the PRS with diLQTS in white patients, there was no association found between these genetic variants and the PRS with a composite secondary outcome comprised of history of TdP, any ventricular arrhythmias, and/or sudden cardiac death documented during the drug treatment period. A recursive partitioning data mining approach, Classification and Regression Trees (CART) was employed to investigate interactions among common and uncommon genetic variants and clinical factors. Among white patients, we identified a gene-gene interaction between rs3857067 and rs12025136, whereas among African patients, none of the genetic variants were associated with the risk of diLQTS. In conclusion, our study, in conjugation with multiple previous *in vitro* and *in vivo* studies, highlights the importance of considering *KCNE1*-D85N as a risk factor for diLQTS in future clinical practice guidelines. Implementing the PRS in routine clinical practice could help identify patients at high risk, allowing for proactive measures to prevent this life-threatening adverse drug reaction. The pharmacogenetic interaction between rs3857067 and rs12025136 should be validated in another study with adequate statistical power, using traditional statistical methods.

Chapter 1 Introduction

1.1 The Problem of drug-induced QT prolongation

Drug-induced long QT syndrome (diLQTS) is an adverse effect of over 150 FDA-approved drugs, including antiarrhythmics, antibiotics, antipsychotics, and antidepressants. (CredibleMeds, 2013) It is characterized by prolongation of the corrected QT interval (QTc) on an electrocardiogram (ECG). QT prolongation can lead to a serious ventricular arrhythmia called *torsades de pointes* (TdP), which can cause sudden cardiac death.(Drew et al., 2010; Roden, 2004) diLQTS has also been associated with increased duration of hospital stay, and critically ill patients had 3 times the odds for all-cause in-hospital mortality with diLQTS.(Pickham et al., 2012) diLQTS creates a challenge for healthcare providers to prescribe known QT-prolonging medications and for pharmaceutical scientists evaluating new drug candidates. diLQTS is a major reason why several drugs have failed to gain FDA approval or have been withdrawn or restricted by the FDA. (Roden, 2004) Examples of drugs that have failed to gain FDA approval or had FDA approval withdrawn or restricted because of diLQTS include terfenadine (Seldane®; a non-sedating anti-histamine) and cisapride (Propulsid®; a gastrointestinal prokinetic agent). (Roden, 2004)

Estimating the annual incidence of diLQTS in the population is difficult due to the requirement of an ECG during an episode for accurate confirmation of QT prolongation. Nonetheless, it is estimated that diLQTS leading to TdP occurs at a rate of approximately 3.2 cases per million per year.(Priori et al., 2015) Additionally, studies have estimated that between

5% and 7% of reported cases of ventricular tachycardia (VT), ventricular fibrillation (VF), or sudden cardiac death (SCD) may actually be attributed to diLQTS.(Molokhia et al., 2008) Moreover, assessing the mortality associated with TdP presents its own challenges, as the condition may go unrecognized if a patient dies before an ECG can be obtained. A population-based case-control study conducted in the Netherlands revealed a threefold greater risk of all-cause mortality among patients treated with noncardiac drugs associated with QT prolongation, suggesting that these drugs contribute to over 15,000 deaths annually in the United States and Europe. (Straus et al., 2005)

Established risk factors for diLQTS include female sex, hypokalemia, hypomagnesemia, congestive heart failure, high drug concentrations (e.g., from drug overdoses or drug–drug interactions) and others. (Roden, 2004) Despite these recognized clinical risk factors, only some individuals develop concerning QTc prolongation with drug challenge. Some individuals still have unpredictable, exaggerated responses to QT-prolonging drugs. These exaggerated responses to QT-prolonging drugs can occur even in individuals with a baseline QTc interval within the normal range.(Patel, Yan, & Antzelevitch, 2010) Therefore, there is a critical need to identify additional risk factors of diLQTS, to avoid serious arrhythmias and sudden death in patients.

Research is emerging for genetic risk factors of diLQTS (Strauss et al., 2017), which would have tremendous potential to improve both clinical practice and drug development. Genetic screening for patients at high risk for diLQTS could prevent unnecessary cardiac events from the treatment with QT-prolonging drugs, and it could prevent the unnecessary withdrawal of drugs from development or the market. Repolarization of the cardiac action potential plays a critical role in QT interval duration, and studies of candidate genes involved in cardiac repolarization have identified individual, uncommon genetic variants (minor allele frequency

[MAF] <1%) that are associated with diLQTS. (Niemeijer, van den Berg, Eijgelsheim, Rijnbeek, & Stricker, 2015) Further, genome-wide association studies (GWAS) have identified multiple other common genetic variants (MAF >1%), from a variety of genes with unclear mechanisms, associated with QT interval duration. (Arking et al., 2014) A polygenic score combining those common genetic variants was also associated with risk of diLQTS. (Strauss et al., 2017) However, major knowledge gaps remain. Limitations of those previous studies, such as small sample size, lack of replication, and/or assessment in real-world clinical practice, prevent the clinical utility of genomics. Moreover, interactions among these common and uncommon genetic variants has not yet been investigated, and evidence suggests that both common and uncommon genetic variants can play a role in diLQTS risk. (Roden, Glazer, & Kroncke, 2018)

1.2 Overall Objective

The overall objective of this research was to determine the associations of individual, uncommon candidate genetic variants and the polygenic score of common variants with diLQTS risk in a large, observational case-control in real-world clinical practice. My central hypothesis is that both the individual, uncommon variants, and the polygenic score of common variants, will be associated with diLQTS risk.

1.3 Specific Aims

Aim 1: Determine the association of individual, uncommon candidate genetic variants with diLQTS. *Approach*: Previously identified, (Niemeijer et al., 2015) uncommon candidate genetic variants known to affect function and/or expression of genes involved in repolarization of the cardiac action potential will be tested using logistic regression. *Hypothesis*:

Individual, uncommon candidate genetic variants affecting the function and/or expression of genes involved in repolarization of the cardiac action potential will be associated with diLQTS.

Aim 2: Determine the association of a polygenic score of common variants with diLQTS risk. *Approach:* A previously identified polygenic score, which combines multiple common variants associated with QT interval in GWAS,(Strauss et al., 2017) will be tested using logistic regression. *Hypothesis***:** Polygenic score will be associated with higher risk of diLQTS.

Aim 3: Explore interactions among uncommon and common genetic variants and clinical factors for predicting diLQTS risk. *Approach*: The classification and regression tree (CART) (Krzywinski & Altman, 2017; Witten, Frank, Hall, & Pal, 2016) will be used to discover novel interactions among uncommon and common genetic variants and clinical variables. This aim is independent of aims 1 & 2 because data mining methods can detect interactions in the absence of strong independent effects being assessed in Aims 1 & 2. *Hypothesis***:** CART will discover novel interactions among uncommon and common genetic variants and clinical factors for predicting diLQTS risk.

The expected outcome of this research is the identification of individual, uncommon genetic variants and a polygenic score associated with diLQTS risk. This research will have a major impact because genetic screening could prevent unnecessary cardiac events and deaths in patients with high risk for diLQTS, and it could help prevent the unnecessary withdrawal or restriction of FDA approval for drugs.

1.4 Strengths and Limitations of Previous Research

Previous pharmacogenetic studies have identified individual genetic variants and a polygenic score associated with diLQTS, (Hideki Itoh et al., 2016; Kaab et al., 2012; Nishio et al., 2009; Spellmann et al., 2018; Strauss et al., 2017) but there are four major limitations of the

previous research: 1) small sample sizes, 2) lack of replication, 3) limited assessment in real-world clinical practice, and 4) the combined effects of uncommon and common genetic variants have not been assessed. As far as small samples sizes, the largest candidate gene study of diLQTS was conducted with 176 cases of drug-induced TdP and 207 controls that experienced <50 milliseconds increase in QTc interval after initiation of therapy with QT-prolonging drugs.(Kaab et al., 2012) This research will overcome the limitation of small sample sizes by investigating the genetics of diLQTS in ~6,000 patients.

Common variants (MAF >5%), such as those most often discovered by genome-wide association studies (GWAS), have been associated with diLQTS. GWAS hits for diLQTS were found in and around a variety of types of genes with other functions (e.g., solute transport, intracellular signaling, and the cytoskeleton) (Aberg et al., 2012; Floyd et al., 2018; Volpi et al., 2009; Watanabe et al., 2017) but not in ion channels such as those involved with the cardiac action potential (except for one whole-exome sequencing study that found an association with a *KCNE1* variant) (Weeke et al., 2014). However, none of these novel variants from GWAS have been validated in an independent dataset. This research will overcome the limitation of lack of replication by testing previously identified candidate variants and polygenic score in a large, independent dataset.

A recent polygenic risk score study for diLQTS was developed using 61 common genetic variants from a GWAS of baseline QTc (Arking et al., 2014). Three QT-prolonging drugs (dofetilide, quinidine and ranolazine) were tested in a clinical trial of 22 healthy white volunteers. The genetic risk score explained approximately 30% of the variability in diLQTS. (Strauss et al., 2017) However, the study was limited by a small sample size (N=22), and as opposed to the complex patients seen in real-world clinical practice (i.e., with multiple

comorbidities and concomitant medications), the study was conducted in healthy volunteers. Patients with underlying cardiovascular diseases may experience variation in QT response greater than in healthy individuals.(Al-Khatib, LaPointe, Kramer, & Califf, 2003) This research will overcome the limitation of limited evidence in real-world clinical practice by testing these genetic variants in real-world clinical data from a large healthcare system.

Previous evidence shows that both common and uncommon genetic variants are associated with diLQTS risk. (Roden et al., 2018) However, the combined effects of uncommon and common genetic variants is unknown. Several studies have shown that gene-gene interactions are fundamentally important in complex cardiovascular, non-Mendelian traits such as atrial fibrillation.(Huang et al., 2015) However, previous studies did not assess potential interactions among the uncommon and common genetic variants associated with diLQTS. This research will overcome this limitation by using an advanced data mining method to explore interactions among uncommon and common genetic variants and clinical factors associated with diLQTS.

1.5 How the Proposed Project Will Improve Current Scientific & Clinical Knowledge

This research will improve current scientific & clinical knowledge in three main ways. First, by testing variants in a much larger sample size, we will have more confidence that these variants are true risk factors of diLQTS. Second, as failed replication is a pervasive problem in genetic association research, (Hirschhorn, Lohmueller, Byrne, & Hirschhorn, 2002) testing these variants in an independent dataset will give us further confidence that previous associations were not false positives. Third, testing these variants in real-world patient data from clinical practice, as opposed to a highly controlled clinical trial with healthy volunteers, will improve the applicability of these results to real-world clinical practice. Testing these genetic variants and

polygenic score in a large, real-world clinical practice dataset, will continue progress towards clinical utility.

1.6 How the Field Will Change When the Aims are Achieved

This continuum of research would lead to a shift in the current clinical practice paradigm from non-genomic-guided therapy with QT-prolonging drugs to precision drug therapy based on the patient's individual genetic profile. Therefore, this project addresses a highly relevant clinical knowledge gap by determining the association of uncommon and common genetic variants with diLQTS, bringing this research closer to clinical implementation. This research is the *next, necessary step* toward a clinical trial of precision interventions for preventing diLQTS. We expect to validate and discover novel genetic risk factors for diLQTS. Thus a future clinical trial would compare clinical outcomes such as ventricular arrhythmias, TdP and SCD of patients randomized to the current standard of care (i.e., non-genomic guided therapy with QT-prolonging drugs) to genomic-guided therapy with QT-prolonging drugs (i.e., closer clinical monitoring and/or alternative drug therapies in patients with high genetic risk).

Chapter 2 The Genetics of Drug-Induced QT Prolongation: Evaluating the Evidence for Pharmacodynamic Variants

In this chapter, the results from my literature review will be provided. These results were published with my mentor (A. I. Lopez-Medina, Chahal, & Luzum, 2022). My role was conceptualization, development of data collection, data management and analyses, interpretation of results, and lead manuscript writing. The journal specifically notes “Express permission is not required for this purpose, authors are able to re-use or adapt their article for use in their thesis/dissertation, provided a suitable acknowledgement to the original publication is included (see "Acknowledgement" section below). Authors may also deposit their thesis/dissertation in an online, institutional repository if required by their institution.” In the Acknowledgement section, the journal notes: “The acknowledgement line should state the following information: Adapted from Future Oncol. (2007) 3(5), 569-574 with permission of Future Medicine Ltd.” (Future Medicine)

2.1 Background

Long QT syndrome (LQTS) is characterized by prolongation of the QT interval on an electrocardiogram (ECG), and it can lead to serious ventricular arrhythmias including *torsades de pointe*s (TdP). TdP can cause syncope with or without serious injury, degenerate to ventricular fibrillation and lead to sudden cardiac death (SCD) (Roden, 2004). The normal duration of the QTc interval (corrected for heart rate) is highly debated (Zhang, Post, Blasco-Colmenares, et al., 2011; Zhang, Post, Dalal, et al., 2011), but according to AHA/ACC/HRS

guidelines, QTc values ≥450 ms in men and ≥460 ms in women are designated as prolonged QTc (Rautaharju et al., 2009). Higher QTc values, such as >460 ms in men and >470 ms in women, are also used in clinical practice to distinguish LQTS (Schwartz & Ackerman, 2013). Each 10-ms increase in the QTc is associated with a 5 to 7% exponential increase in the risk of TdP (Drew et al., 2010). QTc intervals ≥500 ms significantly increase the risk for cardiac events – including syncope, aborted cardiac arrest or SCD – by tenfold (Sauer et al., 2007). LQTS can be congenital (i.e., caused by genetics) or acquired (e.g., caused by drugs, electrolyte changes, fever and autonomic tone). Drug-induced long QT syndrome (diLQTS) is an adverse effect of many US FDA-approved drugs, including antiarrhythmics, antibiotics, antipsychotics and antidepressants. (CredibleMeds, 2013) diLQTS is also a major reason why several drugs have been withdrawn from the market, have restricted use on the market or fail to reach the market (Roden, 2004). A famous example is terfenadine (marketed as Seldane), the first nonsedating antihistamine for the treatment of allergic rhinitis. Terfenadine was removed from the market in the 1990s due to its propensity to produce QTc interval prolongation and/or TdP when co-prescribed with CYP3A4 inhibitors. (Fermini & Fossa, 2003)

Established risk factors for diLQTS include female sex, hypokalemia, hypomagnesemia, congestive heart failure, high drug concentrations (e.g., from drug overdoses or drug–drug interactions) and others. (Roden, 2004) Despite these recognized clinical risk factors, only some individuals develop concerning QTc prolongation with drug challenge, which suggests an underlying genetic predisposition. Research is emerging for genetic risk factors of diLQTS, which would have tremendous potential to improve both clinical practice and drug development. Genetic screening for patients at high risk for diLQTS could prevent unnecessary cardiac events from the treatment with QT-prolonging drugs, and it could prevent the unnecessary withdrawal

of drugs from development or the market. Therefore, the overall purpose of this review was to evaluate and summarize the current body of literature on genetic risk factors for diLQTS, with a focus on variants related to pharmacodynamics. To set the stage for this literature review, we start with some background information on the basics of the cardiac action potential (AP) and defining and distinguishing diLQTS.

2.2 Genes involved in the QT interval.

The cardiac action potential (AP) – combined with excitation–contraction coupling – causes the heart to contract. The AP is a brief change in the potential difference across the membrane of a cardiac cell, which is caused by the flow of ions into and out of the cell via ion channels. At rest, the intracellular voltage of cardiac cells is negative. Depolarization occurs when the intracellular voltage becomes positive, whereas repolarization is when the intracellular voltage returns to resting negative potential. Repolarization requires a delicate balance of multiple inward and outward currents through different cardiac ion channels encoded by different genes (Figure 2.1).

The *KCNQ1* gene encodes for the pore–potassium voltage-gated channel subunit of the Kv7.1 channel, which is involved in the repolarization phase of the cardiac AP. Kv7.1 assembles with KCNE1 to form a channel complex constituting the slow component of the delayed rectifier current IKs. The KCNE1 is a short and integral membrane peptide that modulates the gating kinetics of the channel and enhances its stability. Another accessory subunit is MiRP1 encoded by the *KCNE2* gene, which modulates the activity of several ion channels including the potassium channel, Kv11.1 (Schmitt, Grunnet, & Olesen, 2014). Kv11.1 encoded by the *KCNH2* (also termed HERG), conducts the rapid component of the delayed rectifier potassium current, IKr. Another potassium channel involved in the repolarization phase of the cardiac AP is the Kir

2.1 encoded by the *KCNJ2* gene. Kir 2.1 conducts the inwardly rectifying potassium current (IK1), which is essential for stabilizing the resting membrane potential (Dhamoon & Jalife, 2005).

Depolarizing currents such as sodium and calcium are also regulated by different cardiac ion channels encoded by different genes. The *SCN5A* gene encodes the α-subunit of the cardiac sodium channel (Nav1.5), and it is responsible for fast depolarization upstroke of the cardiac AP(Aronsen, Swift, & Sejersted, 2013). The Nav1.5 channels open and inactivate quickly after the initiation of the AP, but sometimes a late sodium current (INa) is generated. Late sodium currents increase the duration of the AP. The *CACNA1C* gene encodes the alpha subunit of a voltage-dependent L-type calcium channel (Cav1.2), which is essential in the plateau phase (phase 2) of the AP, as well as the process of excitation–contraction coupling (Estes et al., 2019).

2.3 What is drug-induced LQTS?

diLQTS is a disorder of delayed repolarization of the cardiac AP caused by drugs (Roden, 2019). The most common mechanism through which QT-prolonging drugs delay cardiac repolarization is by inhibiting the rapid inward current (IKr) from the Kv11.1 potassium channel. The Kv11.1 channel is uniquely susceptible to inhibition by drugs for three main reasons: the inner cavity of the Kv11.1 channel is larger than other ion channels (del Camino, Holmgren, Liu, & Yellen, 2000); the inner cavity contains two aromatic residues that can bind aromatic drugs (Mitcheson, Chen, Lin, Culberson, & Sanguinetti, 2000); and the inner cavity contains four deep hydrophobic pockets (Wang & MacKinnon, 2017). Other, less common mechanisms of diLQTS have been discovered as well, such as drug-induced decreases in the expression of potassium channels on the cell surface (Ficker et al., 2004) and inhibition of phosphoinositide 3-kinase (PI3K) alpha. The inhibition of this kinase has been found to promote diLQTS in a mechanism

that results in enhanced inward late sodium current during the plateau phase of the AP (Roden, 2019).

In most individuals, the drug-induced repolarization delay only results in small, and otherwise harmless, increases in the QTc interval. However, some individuals have exaggerated responses to QT-prolonging drugs, such as extreme prolongation of the QT interval (>500 ms) and TdP. These exaggerated responses to QT-prolonging drugs can occur even in individuals with a baseline QTc interval within the normal range. This is thought to be due to a reduced 'repolarization reserve' in some individuals (Roden, 1998)– that is, some individuals lack redundancy in the mechanisms for repolarization to compensate for inhibition of the rapid inward current from Kv11.1. Repolarization reserve could be reduced by a number of factors, such as hormones (hence the increased risk for diLQTS in women), electrolyte imbalances, disease processes, sympathetic tone (Gallacher et al., 2007; Lengyel et al., 2004; Volders et al., 1999) or genetic variants (the subject of this review).

Given that repolarization depends on a delicate balance of inward and outward currents from cardiac ion channels, genetic variants affecting cardiac ion channels could be expected to affect the risk of diLQTS in a non drug-specific manner (Kaab et al., 2012). This is in contrast to genetic variants in pharmacokinetic genes, which would only increase risk for the specific drugs affected by the specific metabolic enzymes or transporters. This review focused on this concept of repolarization reserve and hence pharmacodynamic variants. For more information on pharmacokinetic variants associated with diLQTS, readers are referred elsewhere (Niemeijer et al., 2015).

2.4 Drug-induced LQTS vs congenital LQTS

Although the clinical presentation of cLQTS and diLQTS are similar (e.g., syncope, cardiac arrest, TdP or sudden cardiac death), Table 2.1 summarizes the differences between cLQTS and diLQTS. Most individuals with cLQTS exhibit longer than normal QTc intervals in the absence of any exposure to a QT-prolonging drug (or other identifiable risk factor) (H. Itoh et al., 2016). It was previously hypothesized that diLQTS is actually a 'forme fruste' or low penetrance cLQTS. Forme fruste means that patients with suspected diLQTS are actually carrying a cLQTS mutation, but they do not present clinically with cLQTS until they have an exposure to a QT-prolonging drug (Moss & Schwartz, 1982). However, more recent research has shown that cLQTS mutations have only been identified in a minority of diLQTS cases (~10–20%) (H. Itoh et al., 2016). Therefore, other genetic variants, with much smaller effect sizes, and not variants known to cause monogenic cLQTS, must play a role in diLQTS.

The *KCNQ1* gene encodes for the pore–potassium voltage-gated channel subunit of the Kv7.1 channel, which is involved in the repolarization phase of the cardiac AP. Kv7.1 assembles with KCNE1 to form a channel complex constituting the slow component of the delayed rectifier current IKs. The KCNE1 is a short and integral membrane peptide that modulates the gating kinetics of the channel and enhances its stability. Another accessory subunit is MiRP1 encoded by the *KCNE2* gene, which modulates the activity of several ion channels including the potassium channel, Kv11.1 (Schmitt et al., 2014). Kv11.1 encoded by the *KCNH2* (also termed HERG), conducts the rapid component of the delayed rectifier potassium current, IKr. Another potassium channel involved in the repolarization phase of the cardiac AP is the Kir 2.1 encoded by the *KCNJ2* gene. Kir 2.1 conducts the inwardly rectifying potassium current (IK1), which is essential for stabilizing the resting membrane potential (Dhamoon & Jalife, 2005).

The genetic architectures of cLQTS and diLQTS are complex (Roden et al., 2018). Most cases of cLQTS can be tied to rare mutations in genes for, or related to, cardiac ion channels. Three genes definitively cause typical cLQTS: KCNQ1, *KCNH2* and *SCN5A*; four more genes have strong or definitive evidence for causing atypical types of cLQTS: *CALM1, CALM2, CALM3* and *TRDN* (Adler et al., 2020). Several other genes have also been reported as causative for cLQTS, but the evidence is limited or disputed (Adler et al., 2020). Our understanding of the genetic architecture of diLQTS is even less advanced than cLQTS. Variants associated with diLQTS could be present in the cLQTS genes listed above, but they could also be present in pharmacokinetic genes and other types of genes (Niemeijer et al., 2015). Typically, only rare variants (mutations with minor allele frequencies <1%) are causative for cLQTS (which only affects approximately 1:2000 individuals), whereas less rare variants (minor allele frequencies: 1–5%) may be modifiers of cLQTS or cause diLQTS. Common variants (minor allele frequencies >5%), such as those discovered by genome-wide association studies (GWAS), are too common to cause cLQTS (Adler et al., 2020) or be strong modifiers of diLQTS. However, they may be weak modifiers of diLQTS, which are tolerated and thus lead to higher frequency in the population.

2.5 Pharmacogenetic evidence search & Evaluation.

Pharmacogenetic studies of diLQTS were identified in PubMed, Google Scholar and the GWAS catalog (Buniello et al., 2019) through April 2021 by combining the following search terms: ‘drug-induced QT prolongation’, ‘acquired QT prolongation’,‘torsades de pointes’, ‘variant’, ‘polymorphism’, ‘pharmacogenetics’, ‘pharmacogenomics’ and ‘arrhythmias’. Studies were also identified from the reference lists of identified articles. Studies were limited to full-length articles and those published in English. Studies were included if they contained

pharmacodynamic genes (e.g., genes for or related to cardiac ion channels) or if they were a GWAS, and if a relationship to drug exposure was stated. For variants identified in clinical studies, the literature was also searched for experimental evidence of functional effects of the variant (e.g., *in vitro, ex vivo* and animal models) in either the presence or absence of drug. All studies were initially reviewed by the first author, with secondary review and consensus by all authors.

A list of all of the genetic variants associated with diLQTS in the identified studies was created. Our goal was to evaluate the evidence for each of these genetic variants as consistently and as unbiased as possible. Given that a standardized method for evidence evaluation has not yet been established in this area, the strength of evidence for each variant was evaluated using a novel semiquantitative scoring system. This scoring system was modified from a previous approach used for cLQTS (Adler et al., 2020), and for Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines (Caudle et al., 2014). Each genetic variant was given 1 point for each of the following six criteria: presence of experimental evidence supporting functional effects of the variant; associated with SCD in a clinical study; associated with small changes in QTc (<50 ms and/or QTc <500 ms) in a clinical study; the variant was independently replicated (i.e., the association was reported in more than one study or dataset); the variant was identified in five or more total cases of diLQTS. The genetic variant was given 2 points if the clinical outcome with which it was associated was TdP and/or marked QTc prolongation (>500 ms). Case report studies were not given a point for the clinical outcome criteria, but they were counted in the criteria 'Variant was identified in five or more total cases of diLQTS'. The number of points was cumulative for the total number of studies meeting those criteria. For example, if a variant was identified in two clinical studies for an association with sudden cardiac

death, then the variant would receive 2 points for sudden cardiac death. On the basis of the total number of points, the strength of the evidence for each variant was classified as the following: definitive (>14 points), strong (11–13 points), moderate (7–10 points) and limited (<6 points).

Sixty studies met the search criteria, and the studies are summarized and listed chronologically in Table 2.4. Among those 60 studies, 112 genetic variants were reported to be associated with diLQTS. The number of variants associated with diLQTS per gene is displayed in Figure 2.2 (only the genes with at least five variants are displayed). The strength of evidence for the variants with definitive, strong and moderate evidence is calculated in Table 2.2 – that is, the number of points for each variant in each evidence category and the supporting citations. The strength of evidence for the remaining variants with limited evidence is calculated in Table 2.5. The variants with definitive and strong evidence are described in more detail below. Variants with moderate and limited strength of evidence are described in more detail in the supplementary material. The goal in the narrative review that follows was to provide a comprehensive overview of all of the evidence for the particular variant, but it is important to note that certain types of evidence were weighed differently in the actual evidence scoring. For example, case reports are mentioned in the narrative text, but they were given little weight in the actual evidence scoring. The distribution of the evidence scores is displayed in Figure 2.3. Number of variants associated with drug induced long QT syndrome by gene. Only genes with > 5 variants are displayed in the figure. Only one variant had definitive evidence for an association with diLQTS (*KCNE1* D85N rs1805128), and one variant had strong evidence for an association with diLQTS (*KCNE2* T8A rs2234916). Four variants had moderate strength of evidence (*SCN5A* L1825P rs79299226, *SCN5A* G615E rs12720452, *KCNE1* D76N rs74315445 and *KCNE2* I57T rs74315448). The rest of the 106 variants only had limited strength of evidence. The minor allele

frequencies of the six variants associated with diLQTS with definitive, strong and moderate evidence are summarized in Table 2.3 by populations in the gnomAD database (Karczewski et al., 2020).

2.6 Variant with definitive evidence: *KCNE1*-D85N (rs1805128)

2.6.1 KCNE1-D85N evidence overview

The substitution of G to A at codon 253 in *KCNE1* causes a change in amino acid in the C terminal of the beta subunit (D85N, Figure 2.4). The C terminal domain of KCNE1 contributes to the modulation of KCNQ1 by physically interacting with the C terminal of KCNQ1 (Kang et al., 2008; Zheng et al., 2010). Therefore, D85N can lead to changes in the biophysical properties of KCNQ1. The minor allele frequency (MAF) of *KCNE1*-D85N in gnomAD ranges from 0.1% in South Asians to 2.5% in Ashkenazi Jewish (Table 2.3). The evidence supporting D85N (rs1805128) as a risk factor for diLQTS comes from five *in vitro* studies supporting functional effects (Du, El Harchi, Zhang, & Hancox, 2013; Nishio et al., 2009; Nof et al., 2011; Sakata et al., 2014; Westenskow, Splawski, Timothy, Keating, & Sanguinetti, 2004), two case reports(Lin et al., 2012; Marstrand, Christensen, Bartels, & Theilade, 2018), three candidate gene clinical case–control studies (Kaab et al., 2012; Martinez-Matilla et al., 2019; Paulussen et al., 2004) and a whole-exome sequencing study (Weeke et al., 2014).

2.6.2 KCNE1-D85N experimental evidence

Experimental evidence supporting functional effects of the *KCNE1*-D85N in the absence of drug was first published by Westenskow *et al.* in 2004 (Westenskow et al., 2004) and then Nishio *et al.* in 2009 (Nishio et al., 2009). Westenskow *et al.* characterized the effect of D85N on IKs currents using a heterologous expression system with *Xenopus* oocytes. The D85N

reduced IKs by about half compared with wild-type KCNQ1 channels (Westenskow et al., 2004). The same effect was observed by Nishio *et al*., however in their biophysical assay performed in mammalian cell lines (Chinese Hamster Ovary [CHO] cells), D85N was found to exert significant loss-of-function effects on both *KCNQ1*- and *KCNH2*-encoded channel currents (Nishio et al., 2009). These results were replicated by Nof *et al.* in 2011(Nof et al., 2011) in transformed human kidney cell lines (TSA201 cells). Homozygous co-expression of the D85N with Kv11.1 reduced IKr by 85%, whereas heterozygous reduced the current by 52%. However, no variation in IKs current amplitude was observed (Nof et al., 2011). Studies examining the effect of D85N on IKs have yielded variable results with both reduced current amplitude (Nishio et al., 2009; Sakata et al., 2014) and no change in amplitude (Nielsen et al., 2007; Nof et al., 2011). Therefore, it seems that D85N exerts effects through modulation of IKr and probably on IKs. More recent evidence by Sakata *et al.* also supports effects of D85N on *KCNE1* through an additional mechanism: protein expression (Sakata et al., 2014). *KCNE1-D85N* expression on the cell membrane in fibroblast cell lines derived from monkey kidney tissue (COS 7 cells) was 20% lower than the wild-type, and the variant was rapidly degraded compared with the wild-type when treated with a proteasome inhibitor (Sakata et al., 2014).

The influence of D85N on the Kv11.1 channel in the presence of some drugs linked to diLQTS has been also evaluated *in vitro*. Du *et al.* (Du et al., 2013) studied the Kv11.1 currents in the presence of quinidine, clarithromycin and cisapride when co-expressed with D85N. The Kv11.1 current blocking potency of quinidine was similar between wild-type and D85N. However, the inhibitory potency of clarithromycin and cisapride was enhanced for the variant compared to wild-type. This study demonstrates that D85N may increase susceptibility to

diLQTS by direct modulation of the sensitivity of the Kv11.1 channel to drug inhibition. However, this may be the case for some Kv11.1 blocking drugs but not all (Du et al., 2013).

2.6.3 KCNE1-D85N clinical evidence

Paulussen *et al.* (Paulussen et al., 2004) were the first to report D85N as a predisposing factor for diLQTS in patients. They screened *KCNE1*, *KCNE2*, *KCNH2*, *KCNQ1* and *SCN5A* for mutations in 32 cases of drug-induced TdP and 32 healthy controls. The D85N polymorphism was found in two patients that experienced TdP after administration of sotalol and quinidine but not in the control subjects (Paulussen et al., 2004). D85N was next reported as a risk factor for diLQTS in another case–control study published by Kaab *et al.* in 2012 (Kaab et al., 2012), who performed a multicenter, large candidate gene study. They screened 1424 tag SNPs in 18 genes associated with ion channels and arrhythmia in 176 cases of drug-induced TdP (Kaab et al., 2012). The D85N was present in 8.6% of patients that experienced TdP after the administration of QT-prolonging drugs and in 2.9% subjects that experienced <50 ms increase in QTc interval after initiation of therapy with QT-prolonging drugs (n = 207). D85N was also found in 1.8% of control subjects of European descent from the general population (n = 837). These results were replicated in a different database with 57 diLQTS cases and 211 healthy population controls in the same publication. The variant allele was present in 3.5% of cases and in 1.4% of controls (Kaab et al., 2012). In the same year, an independent clinical case report was published in a child with the variant D85N, who experienced extreme prolongation of QT interval and TdP after administration of sodium channel blockers for paroxysmal atrial tachycardia (Lin et al., 2012). Two years after Kaab *et al.* study and the clinical case report, a whole-exome sequencing study involving 65 Caucasian patients that experienced TdP and exaggerated QT interval

prolongation (≥600 ms) after initiation with QT-prolonging drugs was published (Weeke et al., 2014). The D85N variant was found in higher frequency in subjects that experienced diLQTS (9.2%) compared with 148 control subjects that experienced <50 ms QTc interval change after drug exposure (0 %) and 515 population controls with European ancestry (3.1% total variants in *KCNE1*) (Weeke et al., 2014). *KCNE1* was one of only two genes in that study in which the burden of rare variants was significantly higher in the diLQTS patients in whole-exome analyses. In 2018, another clinical case involving D85N was reported by Marstrand *et al.* (Marstrand et al., 2018). A female with D85N developed TdP and QTc interval prolongation (640 ms) after citalopram administration (Marstrand et al., 2018). The D85N variant was also found associated with SCD in a recent study published by Martinez-Mantilla *et al.* in 2019 (Martinez-Matilla et al., 2019). A total of 32 patients suspected of having drug-induced channelopathy or sudden unexplained death after drug administration were genotyped by massive parallel sequencing. The D85N was identified in one of the patients that experienced SCD (Martinez-Matilla et al., 2019).

Several other candidate gene studies have assessed *KCNE1* variants in diLQTS, but unlike the aforementioned studies, they did not identify D85N in cases (Aberg et al., 2012; Avery et al., 2014; Behr et al., 2013; Chevalier et al., 2001; Corponi et al., 2019; Itoh et al., 2009; Makita et al., 2002; Roberts et al., 2017; Spellmann et al., 2018; Zerdazi et al., 2019). A possible explanation for this discrepancy is the small samples sizes. The numbers of cases in most of the negative studies were small ($n \leq 30$), except for the study by Corponi *et al.* (Corponi et al., 2019), which sequenced 77 cases of drug-induced QTc interval prolongation. Given the allele frequency of *KCNE1* D85N is approximately 1–2% in the general population and ~8% in diLQTS cases, D85N may not be present in every diLQTS case. The outcome measured in the studies may also explain differences in results. Some of the negative studies (e.g., most of the

GWAS) examined baseline QT or change in QTc (even when changes were small and still within the normal range)(Aberg et al., 2012; Avery et al., 2014; Behr et al., 2013; Floyd et al., 2018; Volpi et al., 2009). Therefore, D85N may not associate with small drug-induced changes in QTc. Regardless of these negative studies, D85N was identified in the only whole-exome sequencing study that examined drug-induced TdP (Weeke et al., 2014), in two clinical case reports (Lin et al., 2012; Marstrand et al., 2018) and in three independent clinical candidate gene studies of drug-induced arrhythmias as described earlier. Its functional effects were also supported by experiments in five publications. Therefore, the overall level of evidence for this variant was defined as definitive.

2.7 Variant with strong evidence: KCNE2-T8A (rs2234916)

2.7.1 KCNE2-T8A evidence overview

T8A occurs in an extracellular domain of MiRP1 (Figure 2.4). The minor allele frequency of *KCNE2*-T8A (rs2234916) is highest in European individuals (0.006) and the lowest in East Asian individuals (<0.000; Table 2.3). The T8A was found to be associated with diLQTS in five candidate gene clinical studies (Abbott et al., 1999; Paulussen et al., 2004; Roberts et al., 2017; Sanchez et al., 2016; Sesti et al., 2000), and two of those studies also had experimental evidence supporting functional effects (Abbott et al., 1999; Sesti et al., 2000). The *KCNE2* gene was also sequenced in several clinical studies, but T8A was not found in any of the diLQTS cases (Chevalier et al., 2001; Itoh et al., 2009; Kaab et al., 2012; Makita et al., 2002). This may be due to the same reasons as described earlier for *KCNE1*-D85N (i.e., small sample sizes and differences in the outcome measured).

2.7.2 KCNE2-T8A experimental evidence

In 1999, Abbott *et al.* (Abbott et al., 1999) were the first to report experimental evidence supporting functional effects of T8A. They evaluated the biophysical changes of the Kv11.1 channels when co-expressed with T8A in CHO cells. Their *in vitro* experiments showed that channels containing the T8A variant had decreased voltage dependence for activation, opening more readily upon depolarization compared to wild-type. They also assessed the effects of T8A on quinidine, a potent blocker of IKr and IKs, but they did not find significantly different quinidine sensitivity between wild-type and variant channels *in vitro* (Abbott et al., 1999). However, quinidine has other mechanisms of action, such as effects on sodium channels in Purkinje fibers and the transient outward potassium current (Ito) (Sharma, Hashmi, & Chakraborty, 2021), that were not tested in this experiment. One year after the Abbott *et al.* study was published, Sesti *et al.* (Sesti et al., 2000) characterized the effect of T8A on Kv11.1 channels using a heterologous expression system with transient transfection of CHO cells. T8A only had a small effect on channel function in the absence of drug exposure (15% reduction on density currents), but in the presence of trimethoprim/sulfamethoxazole (TMP/SMX), channels with the T8A variant were approximately twice as sensitive to blockade than wild-type channels (Sesti et al., 2000).

2.7.3 KCNE2-T8A clinical evidence

Regarding clinical studies, Abbott *et al.* (Abbott et al., 1999) were the first to report T8A as a predisposing factor for diLQTS in patients. They screened *KCNE2* for mutations in 250 arrhythmia patients who were negative for known cLQTS mutations. Twenty of the arrhythmia patients had diLQTS, whereas the remaining 230 had inherited or sporadic arrhythmias. They also assessed allele frequencies in a control population of 1010 individuals. T8A was found in

one patient with quinidine-induced arrhythmia, one patient with inherited or sporadic arrhythmia and 16 controls (1.6% minor allele frequency) (Abbott et al., 1999). A year later, Sesti *et al.* (Sesti et al., 2000) published their study of 98 cases of diLQTS. They screened *KCNE2,* and T8A was identified in one patient with long QT interval induced by trimethoprim/sulfamethoxazole (TMP/SMX). These clinical findings are consistent with the *in vitro* findings, in which patients carrying T8A exhibit normal QT intervals at baseline, but exaggerated responses to QT-prolonging drugs.

Paulussen *et al.* in 2004 (Paulussen et al., 2004) and Roberts *et al.* in 2017 (Roberts et al., 2017) also published studies supporting T8A as a predisposing factor for diLQTS, as opposed to causative for cLQTS. Paulussen et al., sequenced *KCNE1, KCNE2, KCNH2, KCNQ1* and *SCN5A* in 32 cases of drug-induced TdP and 32 healthy controls. T8A was identified in one case (culprit drug: amiodarone) but not the controls (Paulussen et al., 2004). Roberts *et al.* evaluated 48 arrhythmia cases with suspected pathogenic *KCNE2* mutations from inherited arrhythmia clinics, the Rochester LQTS registry and published case reports. On the basis of the relatively common allele frequencies, absence of genotype–phenotype segregation and presence of other risk factors, they concluded that *KCNE2* variants, including T8A, are actually susceptibility variants for diLQTS instead of causative for cLQTS (Roberts et al., 2017). More recently, Sanchez *et al.* (Sanchez et al., 2016) reported T8A in a case of SCD after drug administration. Sanchez *et al.* sequenced 55 genes associated with SCD in 789 subjects (<50 years of age) who died suddenly. After histological analysis, it was found that 364 of 789 cases (46%) has cardiac origin. T8A was identified in one subject with diLQTS (culprit drug: unknown) (Sanchez et al., 2016).

2.8 Discussion

To our knowledge, this is the first review that comprehensively collects and rates the strength of evidence for pharmacodynamic genetic variants as risk factors for diLQTS. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology published standards and guidelines for the interpretation of sequence variants (Richards et al., 2015). However, those guidelines avoid common genetic variants (Giudicessi, Roden, Wilde, & Ackerman, 2018), and as the results of our literature review shows, some genetic variants associated with diLQTS are indeed common. Moreover, those guidelines focus on pathogenicity of genes and individual variants with respect to Mendelian disease, but this has not been applied to drug response. A total of 112 variants for diLQTS were identified in the literature. Only one variant had definitive evidence (*KCNE1*-D85N), one had strong (*KCNE2*-T8A), four had moderate (*SCN5A*-L1825P, *SCN5A*-G615E, *KCNE1*-D76N and *KCNE2*-I57T) and the remaining 106 had limited evidence. All the variants with the highest levels of evidence are in genes for ion channels. This is probably because the majority of studies published thus far specifically focused on ion channels as candidate genes. However, recent evidence has shown that that majority of candidate genes studied in pharmacogenetics are not significant in pharmacogenomic GWAS (Linskey, Linskey, McLeod, & Luzum, 2021). Thus there is a strong possibility that other types variants, that have nothing to do with ion channel function, may also predispose to diLQTS.

KCNE2-T8A had clinical evidence (including studies with clinical outcomes like drug-induced TdP or marked QT prolongation), experimental evidence, and it also has been replicated in independent studies. It was close to our definition of a definitive level of evidence, but the main difference in the evidence for this variant and *KCNE1*-D85N is that D85N had much more

experimental evidence. Therefore, the level of evidence for *KCNE2*-T8A could progress to the definitive level in the near future if more functional studies are published supporting it. *SCN5A*-L1825P, *SCN5A*-G615E, *KCNE1*-D76N and *KCNE2*-I57T were classified as a moderate level of evidence. All four of these variants have experimental evidence and clinical evidence (including studies with clinical outcomes). The type of evidence that was missing for all four of these variants is that they have not been identified in at least five total cases of diLQTS. Therefore, the strength of the evidence for these variants would improve if they are identified in more cases of diLQTS in the future. However, that seems unlikely for the *SCN5A* variants, as the *SCN5A* variants are rare (frequency <0.00001 in most populations). The *KCNE2*-I57T variant is not as rare, and thus evidence may emerge more quickly for that variant in the future, and thus result in reclassification. For the remaining 106 variants with only limited evidence, a large proportion of those variants came from the two same studies (H. Itoh et al., 2016; Ramirez et al., 2013). The types of evidence that are most commonly missing for these variants are a lack of independent replication and/or experimental evidence for functional validation. Thus, such evidence would be needed for any of those variants to progress toward clinical validity in the future.

The ultimate goal for these genetic variants would be clinical pharmacogenetic testing to prevent diLQTS. A one-time genetic test could potentially provide benefit for a patient's lifetime. The implementation of a preemptive pharmacogenetic test at a single health system was estimated to prevent nearly 400 adverse drug events in a 5-year period (Schildcrout et al., 2012). However, it is important to keep in mind that other factors are involved in diLQTS and increase the QTc interval change, such as the aforementioned clinical risk factors. Many barriers to the clinical implementation of pharmacogenetic testing have been identified, such as the lack of reimbursement, clinical decision support, education and definitive evidence (Klein, Parvez, &

Shin, 2017; Luzum et al., 2017). However, based on this literature review, at least one of those barriers has been overcome for *KCNE1*-D85N and *KCNE2*-T8A because they have strong evidence. The level of evidence required for the clinical implementation of pharmacogenetic testing is widely debated (Khoury, 2010; Luzum & Cheung, 2018; Luzum et al., 2021; Pirmohamed & Hughes, 2013; Relling, Altman, Goetz, & Evans, 2010). Some argue that a randomized controlled trial demonstrating the clinical utility of the pharmacogenetic test is the minimum level of evidence required (Koch, van Schaik, van Gelder, & Mathijssen, 2013; Nissen, 2011). However, diLQTS is relatively rare and potentially fatal, and *KCNE1*-D85N and *KCNE2*-T8A are not common. Therefore, a randomized controlled trial evaluating the clinical utility of testing *KCNE1*-D85N or *KCNE2*-T8A for the prevention of diLQTS is neither ethical nor feasible. On the basis of the current amount of evidence, in patients whose *KCNE1*-D85N or *KCNE2*-T8A genotype is already known and will be prescribed a QT-prolonging drug, we believe that consideration of these variants as a risk factor is at least justified. How therapy should be modified in carriers of these variants is not yet known. Currently, there are no clinical practice guidelines or FDA recommendations regarding the clinical use of these variants. Perhaps that level of clinical guidance is now needed. In the absence of clinical guidelines, closer clinical monitoring with an electrocardiogram in known *KCNE1*-D85N or *KCNE2*-T8A carriers that will be treated with high-risk QT-prolonging drugs may at least be an option. Crediblemeds.org currently lists 41 drugs on the market in the US with known risk of TdP and 115 with possible risk of TdP. It is unclear at this time which drugs or drug classes would warrant genetic screening for *KCNE1*-D85N and *KCNE2*-T8A. In patients whose *KCNE1*-D85N or *KCNE2*-T8A genotype is not already known, testing may also be justified, but guidelines are needed to address that question as well.

2.8.1 Limitations

This literature review focused on pharmacodynamic variants, and thus our evidence evaluation did not include other types of variants, such as pharmacokinetic variants. We modified the approach used by others for genetic associations with arrhythmia (i.e., Adler *et al.* for cLQTS (Adler et al., 2020), Hosseini *et al.* for Brugada syndrome (Hosseini et al., 2018), Walsh *et al.* for catecholaminergic polymorphic ventricular tachycardia and short QT syndrome (Walsh et al., 2022) and CPIC guidelines for our semiquantitative evidence grading, but there is not yet an established standard approach of evidence evaluation for diLQTS. Thus, our scoring system is a nonvalidated tool for evaluating the strength of evidence of each individual variant, and an assessment of this method has not been completed. Point cutoffs for the definitions of 'definitive', 'strong', 'moderate' and 'limited' evidence are obviously judgment calls and assertions, and the interpretation of the strength of evidence is dependent on the particular stakeholder (Luzum et al., 2021). However, this is the first literature review to attempt to evaluate the evidence for diLQTS genetic variants in a more systematic way, and future work should refine this approach by analyzing the data by meta-analysis. A limitation of our approach is that it applied the same evaluation methods for both rare and common genetic variants, which would require different study designs. Another limitation is that we relied on each publication's distinction between diLQTS versus cLQTS that was exacerbated by a drug exposure. We attempted to overcome this by limiting our literature search to studies that specifically mentioned a drug exposure, but it is challenging to distinguish cLQTS from diLQTS because drug exposure is the most common cause of events in patients with putative cLQTS. There may also be underreporting of risk variants in the published literature as authors may be dissuaded from writing case report studies due to low impact. Our approach also focused on the effects of

individual variants. Thus, it is not surprising that the variants with the strongest evidence were not common because common variants are not likely to have large effect sizes individually. Indeed, our literature search included GWAS of diLQTS, but they did not find common variants with large effect sizes individually. Strauss *et al.* found that a polygenic score derived from GWAS of baseline QT interval had a significant association with drug-induced QT prolongation and TdP (Strauss et al., 2017). That study demonstrates that common variants can play a cumulative role in diLQTS, and our approach focusing on individual variant effects would not have captured that more complex genetic architecture.

2.9 Conclusion

In conclusion, our understanding of the genetic risk factors for diLQTS is still in very early stages. The *KCNE1*-D85N polymorphism is the only variant at this time with definitive evidence for an association with diLQTS, *KCNE2*-T8A had strong evidence and a few more variants had moderate levels of evidence. Future research should focus on the contribution of both rare and common variants to diLQTS, as this area of research has tremendous potential. Advancing our understanding of the genetics underlying diLQTS could not only improve individual patients' outcomes, but it could also help to avoid the inappropriate withdrawal and regulation of drugs on the market.

2.10 Figures

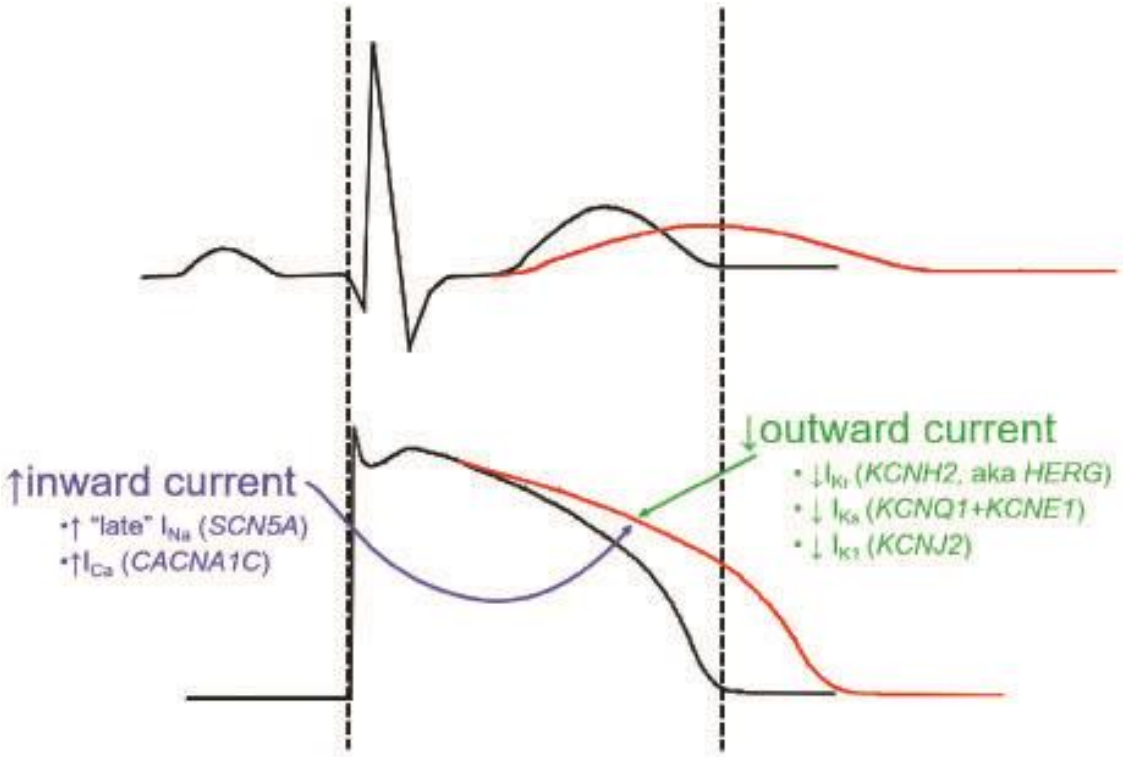

Figure 2.1: The normal QT interval (black, top; delineated by the dashed lines) is a rough indicator of the duration of action potentials in the ventricle (bottom). When the QT is prolonged (red), action potentials in at least some cells in the ventricle must be prolonged, and this can arise from increased inward current through sodium or potassium channels or decreased outward current through potassium channels. The genes encoding these key channels are also shown. Reproduced with permission from (Roden, 2019).

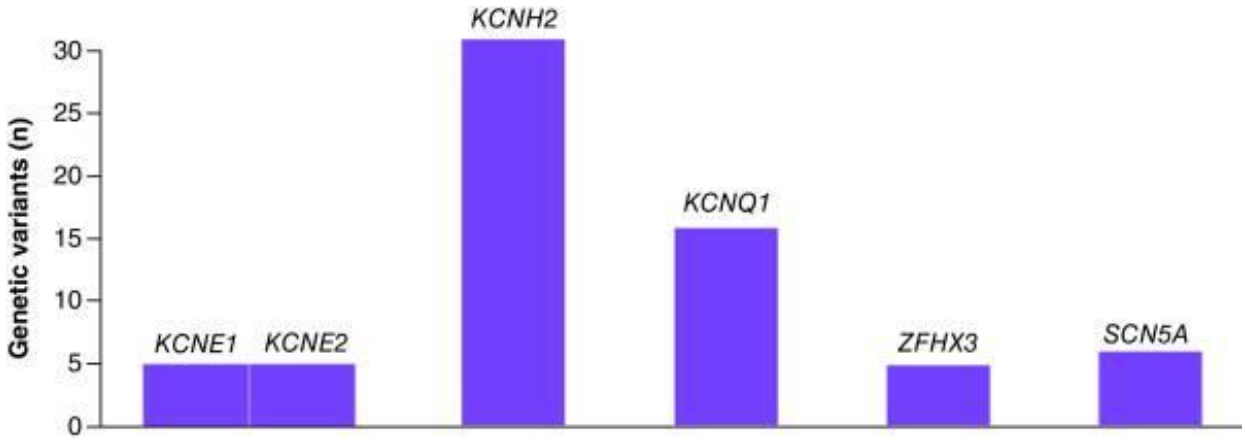

Figure 2.2: Number of variants associated with drug induced long QT syndrome by gene. Only genes with $\geq$ 5 variants are displayed in the figure.

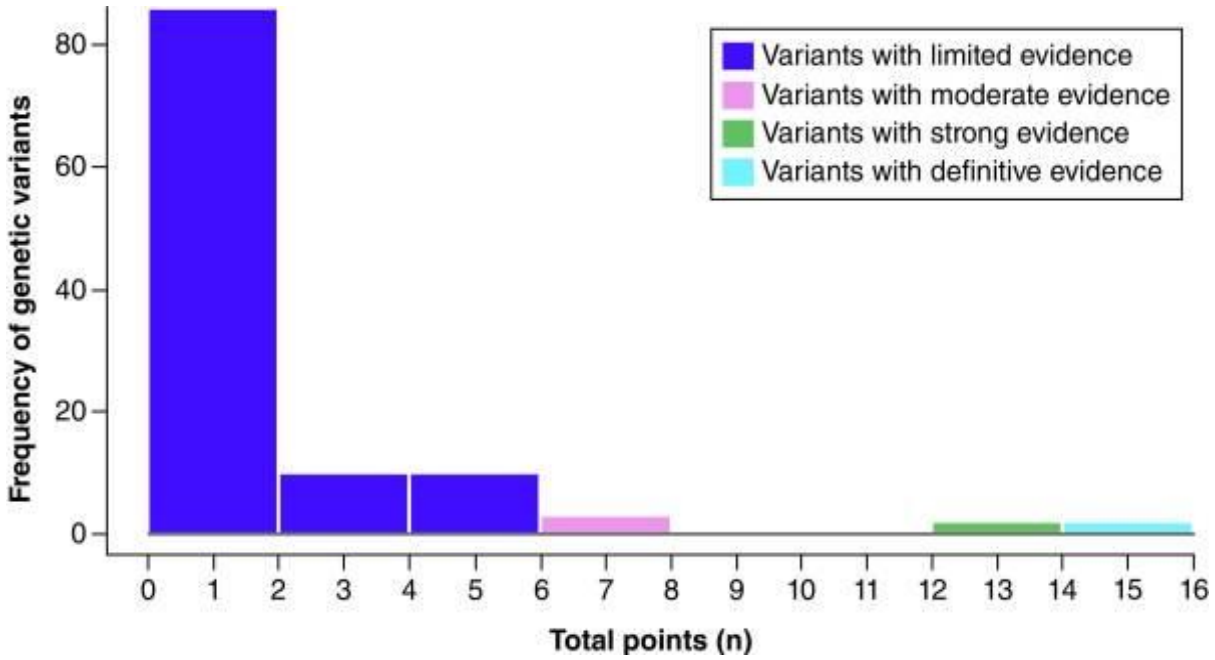

Figure 2.3: The distribution of the evidence scores for the pharmacodynamic variants associated with drug-induced long QT syndrome. Definitive (≥14 points), Strong (11-13 points), Moderate (7–10 points), and Limited (≤ 6 points).

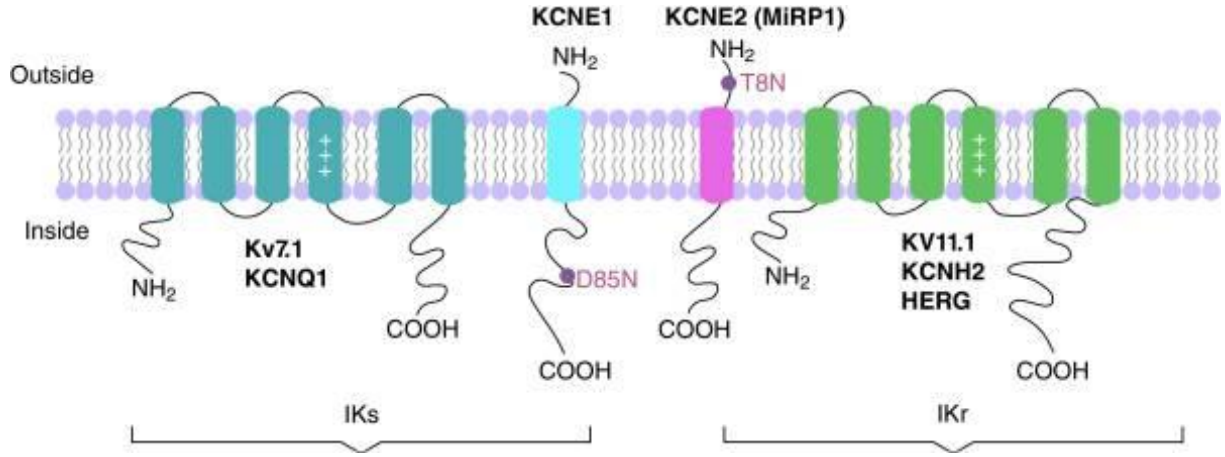

Figure 2.4: Schematic representation of Kv7.1(KCNQ1)/KCNE1 and Kv11.1(KCNH2) /KCNE2 channels complex. Kv7.1 and Kv11.1 are made of six membrane-spanning segments S1–S6 and intracellular N- and C-terminal domains. KCNE1 and KCNE2 are β subunits that interacts with Kv7.1 and Kv11.1 respectively, regulating their biophysical properties. D85N occurs in the C terminal of KCNE1 while T8A occurs in the extracellular domain of KCNE2.

2.11 Tables

Table 2.1: Comparison between congenital long QT syndrome (cLQTS) and drug-induced long QT syndrome (diLQTS).

Characteristic	**cLQTS**	**diLQTS**
Baseline QT (in the absence of a culprit drug)	Usually above normal range (i.e., QT >450 ms in men and >470 in women)	Usually within normal range
Post-exposure QT (in the presence of a culprit drug)	Prolonged	Prolonged
Clinical presentation	Syncope, cardiac arrest, TdP, or sudden cardiac death	Syncope, cardiac arrest, TdP, or sudden cardiac death
Genes	3 definitive genes for typical cLQTS: *KCNQ1*, *KCNH2*, *SCN5A*; 4 definitive genes for atypical cLQTS: *CALM1*, *CALM2*, *CALM3*, *TRDN* (Adler et al., 2020)	Variant may or may not be in a cLQTS gene, and it could also be pharmacokinetic and other types of genes (13).
Variant minor allele frequency (MAF)	<1% (population prevalence 1:2000) (Adler et al., 2020)	< or > 1% (population prevalence difficult to estimate because need drug exposure)

Table 2.2 Evaluation of the strength of evidence for the variants with definitive, strong and moderate evidence for association with diLQTS. Definitive (≥14 points), Strong (11-13 points), Moderate (7-10 points)

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
KCNE1 D85N rs1805128	✓✓ (Nishio et al., 2009) ✓ (Sakata et al., 2014) ✓✓ (Du et al., 2013) ✓ (Nof et al., 2011) ✓ (Westenskow et al., 2004)	✓ (Martinez-Matilla et al., 2019)		✓✓ (Paulussen et al., 2004) ✓✓ (Kaab et al., 2012) ✓✓ (Weeke et al., 2014)	✓ (Kaab et al., 2012) (Paulussen et al., 2004) (Weeke et al., 2014) (Martinez-Matilla et al., 2019)	✓	**16 Definitive**
KCNE2 T8A rs16991652	✓ (Abbott et al., 1999) ✓✓ (Sesti et al., 2000)	✓ (Sanchez et al., 2016)	✓ (Abbott et al., 1999)	✓✓ (Paulussen et al., 2004) ✓✓ (Sesti et al., 2000) ✓✓ (Roberts et al., 2017)	✓ (Paulussen et al., 2004) (Sesti et al., 2000) (Roberts et al., 2017) (Abbott et al., 1999) (Sanchez et al., 2016)	✓	**13 Strong**
KCNE1 D76N rs74315445	✓ (Splawski, Tristani-Firouzi, Lehmann, Sanguinetti, & Keating, 1997) ✓✓			✓✓ (Weeke et al., 2014)			**8 Moderate**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
	(Du et al., 2013) ✓ (Sesti & Goldstein, 1998) ✓ (Bianchi et al., 1999) ✓ (Hoppe, Marbán, & Johns, 2001)						
KCNE2 I57T rs74315448	✓ (Abbott et al., 1999) ✓ (Sesti et al., 2000) ✓ (Tinel, Diochot, Borsotto, Lazdunski, & Barhanin, 2000)		✓ (Hideki Itoh et al., 2016)	✓✓ (Sesti et al., 2000)	✓ (Sesti et al., 2000) (Hideki Itoh et al., 2016)		**7 Moderate**
SCN5A G615E rs12720452	✓ (Albert et al., 2008)	✓ (Sanchez et al., 2016)		✓✓ (P. Yang et al., 2002) ✓✓ (Ramirez et al., 2013)	✓ (P. Yang et al., 2002) (Ramirez et al., 2013) (Sanchez et al., 2016)		**7 Moderate**
SCN5A L1825P rs79299226	✓ (K. Liu, Yang, Viswanathan, & Roden, 2005) ✓ (Makita et al., 2002)		✓ (Hideki Itoh et al., 2016)	✓✓ (Itoh et al., 2009)	✓ (Hideki Itoh et al., 2016) (Itoh et al., 2009)		**7 Moderate**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
	✓ (Itoh et al., 2009)						

Table 2.3 Minor allele frequencies of variants associated with diLQTS with definitive, strong, and moderate evidence by population in the gnomAD database. (Karczewski et al., 2020)

Variant	Total	African	Ashkenazi Jewish	East Asian	European (Finnish)	European (non-Finnish)	Latino	Other	South Asian
Definitive Evidence									
KCNE1 D85N rs1805128	0.009324	0.002164	0.025370	0.005565	0.016880	0.012230	0.002737	0.008993	0.001404
Strong Evidence									
KCNE2 T8A rs2234916	0.003746	0.001004	0.001736	0.000	0.003583	0.006041	0.002540	0.003881	0.000916
Moderate Evidence									
SCN5A L1825P rs79299226	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
SCN5A G615E rs12720452	0.0002901	0.0001694	0.000	0.000	0.00004659	0.0005178	0.0001103	0.0001772	0.0000533
KCNE1 D76N rs74315445	0.0000671	0.0000407	0.000	0.0000512	0.000	0.0001084	0.0000222	0.000	0.0000633
KCNE2 I57T rs74315448	0.0009615	0.0002403	0.001254	0.000	0.000	0.001045	0.001891	0.003044	0.000942

2.13 Appendix

Table 2.4 Summary of studies on genetic risk factors for diLQTS

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
(Chouabe et al., 1997)	- In vitro study Co expression of KvLQT1 mutations or the wild type with *KCNE1* currents in COS cells.	-	-	-	-	-The ***KCNQ1* R555C +** *KCNE1* formed a functional channel, however the variant accelerated the deactivation state and induced a positive voltage shift of the channel activation compared to wild type.
(Donger et al., 1997)	-Candidate gene -Case-control -Single center	-20 families with the Romano-Ward syndrome -200 chromosomes from unrelated control subjects -French ancestry	Syncope or TdP, QT_c>460 ms, or QT_c>440 ms associated with bradycardia or abnormal T-wave pattern	*KCNQ1*	disopyramide -meflaquine -diuretics -terfenadine	-3 of the 20 families (44 carriers) had the ***KCNQ1* R555C** that was absent in the control group. -5 subjects from those families had syncope (disopyramide, meflaquine and diuretics), and 2 subjects died suddenly one of them treated with terfenadine. -The QT intervals of the R555C carriers were often borderline or even normal (<440 ms)
(Splawski et al., 1997)	- In vitro study Co expression of *KCNE1 mutations* or the wild-type with KCNQ1 in *Xenopus* oocytes -Single center	-	-	-	-	-***KCNE1* D76N** reduced IKs by shifting the voltage dependence of activation and accelerating channel deactivation.
(Zhou, Gong, Epstein, & January, 1998)	- In vitro study Co expression of *KCNH2* G628S, and V822M or	-	-	-	-	- ***KCNH2* V822M** caused defects in the transport of HERG channels to the cell surface membrane.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
	the wild-type in HEK 293 cells. -Single center					- ***KCNH2*** **G628S** did not produce functional channels
(Sesti & Goldstein, 1998)	- In vitro study Co expression of *KCNE1 mutants* or the wild-type and KCNQ1 in *Xenopus* oocytes or Chinese hamster ovary cells -Single center	-	-	-	-	- ***KCNE1*** **D76N** caused a decrease in IKs currents and diminished open probability of the KCNQ1 channel compared to wild type.
(Abbott et al., 1999)	-Candidate gene -Case-control & in vitro -Single center	-20 cases of drug induced arrhythmia. -230 patients with inherited or sporadic arrhythmias without mutations in known arrhythmia genes (*KCNQ1*, *KCNH2*, *SCN5A* and *KCNE1*). Ancestry not specified. -1,010 controls. Ancestry not specified.	-arrhythmia	-*KCNE2*	-Drugs were only specified in the 2 cases for which a mutation was identified (clarithromycin and quinidine)	-One in 20 cases had ***KCNE2*** **Q9E** (clarithromycin) that was not found in controls. -One in 20 cases had ***KCNE2*** **T8A** (quinidine), and 1 with inherited/ sporadic arrhythmia and 16 controls. **- One** of the 230 patients with inherited/sporadic arrhythmias had *KCNE2* I57T, however baseline QTc was prolonged (QTc = 470 ms) and there is not evidence the patient was on any medication. <u>In vitro</u>: - ***KCNE2*** **Q9E** increased the voltage dependence of channel activation compared to wild type and also increased the sensitivity of the channel to clarithromycin blockage. - ***KCNE2*** **M54T** increased by3-fold the

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
						rate of closing of the channel compared to wild type. - ***KCNE2* I57T** decrease K^+ flux. - ***KCNE2* T8A** decreased voltage dependence for activation, however T8A did not change the sensitivity of the channel to quinidine blockage.
(Bianchi et al., 1999)	-In vitro study Co expression of wild-type *KCNE1 and KCNE1* mutations with KCNQ1 in *Xenopus* oocytes, HERG and HEK 293 cells -Single center	-	-	-	-	-***KCNE1* D76N** suppressed KCNQ1 (IKs) and KCNH2 (IKr) currents markedly.
(Napolitano et al., 2000)	-Candidate gene -Case-report -Single center	-1 Patient had a prolonged QTc (595 msec) associated with major T wave abnormalities. After discontinuation of culprit drug QTc returned to 430msec. Ancestry unknown. Family pedigree was done in 2 sons	-QT Prolongation and cardiac arrest	-*KCNQ1* -*KCNH2* -*KCNEI* -*SCN5A*	-cisapride	-QTc was normal in the three family members. The patient and her 2 sons were heterozygous for the ***KCNQ1* Y315C**

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		and 1 grandchild.				
(Sesti et al., 2000)	-Candidate gene -Case-control -Single center	-98 patients -1,010 controls from Abbott *et al*, 1999 study. -Caucasian ancestry	-Prolongation of the QT interval (>600 msec) or TdP	-*KCNE2*	-procainamide -oxatomide -quinidine -trimethoprim/sulfamethoxazole	-One in 98 had ***KCNE2* M54T** (procainamide) that was not found in controls. - One in 98 had ***KCNE2* I57T** (oxatomide) that was not found in controls. -One in 98 had ***KCNE2* A116V** (quinidine) that was not found in controls. -One in 20 cases had ***KCNE2* T8A** (TMP/SMX) that was also found in 16 controls from previous study.
(Choquet et al., 2000)	-In vitro study Co expression of *KCNQ1* R243H or the wild type with *KCNQ1* and *KCNE1* in COS cells -Single center	-	-	-	-	***-KCNQ1* R243H** induced a positive voltage shift of the channel activation when co-expressed with *KCNE1*.
(Tinel et al., 2000)	-In vitro study Co expression of *KCNE2* I57T, Q9E or the wild type with *KCNQ1* and KCNE2 in COS cells -Single center	-	-	-	-	-The ***KCNE2* I57T** subunit decreases the rate of activation of the *KCNQ1* current; accelerates the deactivation process. - The ***KCNE2* Q9E** exert a greater inhibition on *KCNQ1* current than wild-type.
(Chevalier et al., 2001)	-Candidate gene -Case-control -Single center	-16 patients with acquired LQTS in intensive care unit	- TdP ± lengthening of the QTc >600 ms.	-*KCNQ*1 -*KCNH2* -*KCNE*1 -*KCNE*2	-sotalol -amiodarone	- One of 16 was heterozygous for ***KCNH2* R328C** that was not found on controls. -The variant reduced the K currents from

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		-100 chromosomes from unrelated control subjects -Ancestry not specified				*HERG* channel in COS7-cells.
(Hoppe et al., 2001)	-In vitro study Co expression of *KCNH2* and *KCNE1* mutants or wild type injected in adenoviral vectors in guinea pig myocardium -Single center	-	-	-	-	***-KCNE1* D76N** prolonged the QT interval in guinea pigs in vivo. It also suppressed IKs currents by 80% and prolonged overall action potential duration by almost 2-fold, which was associated with frequent early after depolarization.
(P. Yang et al., 2002)	-Candidate gene -Case-control & in vitro -Multicenter	-92 cases. Ancestry not specified. -67 controls receiving a QT-prolonging anti-arrhythmic without marked QT prolongation. Ancestry not specified. -71 randomly selected unrelated individuals (48 white and 23 black) -90 anonymous	-TdP ± marked QT prolongation to >600 ms	*-KCNQ1* *-KCNH2* *-SCN5A*	-anti-arrhythmics -quinidine -sotalol -non-anti-arrhythmics	-Five different mutations were identified in 5 cases that were not identified in any of the controls: ***KCNQ1* R583C** (dofetilide), ***KCNH2* R784W** (amiodarone), and ***SCN5A* G615E** (quinidine), **L618F** (quinidine), **F1250L (**sotalol). -Four polymorphisms were identified in cases that were also identified in controls with similar allele frequencies: *KCNQ1* P448R, *KCNH2* K897T, *SCN5A* R34C and H558R

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		individuals from a public database. Ancestry unknown.				
(Makita et al., 2002)	-Candidate gene -Case report & in vitro -Single center	-1 patient presented with syncope and normal QTc= 435 ms and developed QTc prolongation 731msec and TdP after cisapride. QT returned to drug 417 msec after drug discontinuation. Ancestry unknown	-TdP and QT prolongation (480ms)	-*SCN5A* -*KCNQ1* -*KCNH2* -*KCNE1* -*KCNE2*	-cisapride	-The subject had the ***SCN5A* L1825P** -Cisapride did not change the kinetics or the amplitude of the sodium current in either wild-type or L1825P.
(Splawski et al., 2002)	-Candidate gene study -Case-control, in vitro & computational simulation -Single center	-1 African American patient with nonfamilial cardiac arrhythmias with idiopathic dilated cardiomyopathy and hypokalemia developed prolongation of QTc and TdP after amiodarone. -468 control	-TdP and prolongation of QTc (460ms) - Arrhythmia included, syncope, aborted sudden death, medication or bradycardia associated with QTc prolongation, and documented ventricular tachyarrhythmias.	- *SCN5A*	-amiodarone	- Patient had the ***SCN5A S*1102Y**. Eleven members of 23 members had S1102Y and all of them had experienced QT prolongation and/or a history of syncope. - The *SCN5A S*1102Y allele frequency in the general population was 10.1% (95/936); 19.2% in West Africans and Caribbean's (90/468); 13.2% (27/205) in African Americans and in 1 of 123 Hispanics. It was not found in control groups of Caucasians and Asians.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		group of West Africans and Caribbeans - 205 control group of African Americans controls -511 control group of Caucasians -578 control group of Asians -123 control group of Hispanics Case-control with arrhythmia or at high risk for arrhythmia: -23 African American patients with arrhythmia or at high risk for arrhythmia -100 healthy African Americans controls				Case-control with arrhythmia or at high risk for arrhythmia: -Thirteen of 23 cases had the *SCN5A S*1102Y variant (11 heterozygous and 2 were homozygous), that was found in 13 of 100 controls (No controls were homozygous) - In vitro studies revealed, that the *S*1102Y variant produces changes in the biophysical properties of the sodium channel compared to wild-type. However, computational simulation predicted a prolongation of the action potential and an increase in early afterdepolarizations after a blockage of IKr.
(Sun et al., 2004)	-Candidate gene	-7 of 105 subjects from two	-TdP	-*KCNH2*	-dofetilide	-Two in 7 cases had ***KCNH2* 1047L** (one homozygous and the

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
	-Case-control, in vitro & computational simulation -Multicenter (DIAMOND study)	populations: CHF and recent MI associated with left ventricular dysfunction, developed TdP after dofetilide administration. All patients were Danish.				other was heterozygous for the change). These patients had a longer baseline QTc interval than those who did not have TdP. - Five in 98 cases who had not experienced TdP were heterozygous for the 1047L variant. -No significant difference in the QTc values between the patients carrying the SNP and those without the SNP. - In vitro studies shows that the variant leads to a functional impairment of the *KCNH2* channel.
(Hayashi et al., 2004)	-In vitro study Co expression of *KCNH2* M124T or wild-type in *Xenopus* oocytes -Single center					- ***KCNH2* M124T** decrease the amplitude of the HERG current
(Paulussen et al., 2004)	-Candidate gene -Case-control -Multicenter	-32 subjects that showed QTc prolongation >440 ms and TdP after drug exposure. Caucasian Ancestry -32 control, healthy, Caucasian	-TdP	-*KCNE1* -*KCNE2* -*KCNH2* -*KCNQ1* -*SCN5A*	-amiodarone -cisapride -clarithromycin -sotalol -quinidine	-One in 32 had ***KCNE2* T8A** (amiodarone) that was not found in controls. -One in 32 had ***KCNH2* P347S** (cisapride and clarithromycin) that was not found in controls. - Two in 32 cases had ***KCNE1* D85N** (Sotalol and Quinidine) that was not found in controls. -Three synonymous mutation: ***KCNE1* S28S, *KCNQ1* T377T, *KCNQ1* G628G** and one intronic variation **IVS16-6C-T**, were found in one patient but not in the controls.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
(Westenskow et al., 2004)	- In vitro study Co expression of *KCNE1* D85N or the wild-type with *KCNQ1* in *Xenopus* oocytes -Single center	-	-	-	-	-***KCNE1 D85N*** reduced IKs by about half compared to wild-type.
(K. Liu et al., 2005)	- In vitro study -Single center		-	*SCN5A*	-cisapride	-The ***SCN5A* L1825P** displayed a reduction in cell surface expression of the mutant. -Cisapride rescued by 30% the misprocessing, resulting in an increase of the sodium current
(Thomas et al., 2005)	-In vitro study Co expression of KCNQ1-F339 del with KCNE1 in oocytes -Single center	-	-	-	-	-***KCNQ1 F339 del***. in the presence and absence of *KCNE1* causes a reduction of the current compared to the mutant of the *KCNQ1* subunit.
(Lehtonen et al., 2007)	-Candidate gene -Case reports -Single center	-16 unrelated cases with drug induced TdP without history of LQTS -No control -Finish Ancestry	-TdP	-*KCNQ1*-IVS7 -*KCNH2* -*KCNH2*	-amiodarone -sotalol	- Two of 16 had ***KCNQ1* G589D** (amiodarone and sotalol) - One of 16 had ***KCNH2* L552S** (sotalol).
(Ohno et al., 2007)	-In vitro study Co expression of *KCNE1* A8Vor the wild-type with *KCNQ1* and	-	-	-	-	-***KCNE1* A8V** co-expressed with *KCNH2* reduced IKr compared to wild-type.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
	KCNH2 in COS7 cells. -Single center					
(Albert et al., 2008)	-In vitro study. Co expression of SCN5A A572F, A572D, G615E, W1205C or the wild-type with *KCNQ1* in *Xenopus* oocytes	-	-	-	-	- The expression of ***SCN5A* G615E**, A572F, A572D, and W1205C caused a faster recovery from inactivation than the wild type.
(Sakaguchi et al., 2008)	-In vitro study. Coexpression of *KCNH2* A614V or the wild-type in CHO cells-Single center	-	-	-	-	- ***KCNH2* A614V** caused 70% reduction in current density.
(Itoh et al., 2009)	-Candidate gene study -Case-control, in vitro study & computational simulation. -Multicenter	-20 subjects that experienced TdP after drug administration. None of them had a family history of long-QT syndrome. -176 subjects with cLQTS. -220 chromosomes from non-cLQTS and non-diLQTS subjects. -Ancestry unknown	- TdP with marked QT prolongation (>600ms).	-*KCNQ1* -*KCNH2* -*SCN5A* -*KCNE1* -*KCNE2* -*KCNJ2*	-disopyramide, -pirmenol -aprindine -methylenedioxymethamphetamine -cisapride -erythromycin -hydroxydine -probucol	- The age at first cardiac event in subjects with diLQTS was significantly older compared with cLQTS. -The QT_c interval in cLQTS subjects was significantly larger than in diLQTS subjects -The Schwartz score was significantly lower in diLQTS than in cLQTS. -The incidence of mutations was higher in patients with TdP induced by nonantiarrhythmic drugs than by antiarrhythmic drugs - One in 20 cases had ***KCNH2* H492Y** (disopyramide) that was not found in controls - One in 20 cases had ***KCNQ1* R243H** (aprindine) that was not found in controls

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
						-One in 20 cases had ***KCNH2* S706F** (methylenedioxymethamphetamine) that was not found in controls -One in 20 cases had ***SCN5A* L1825P** (cisapride) that was not found in controls - One in 20 cases had ***KCNH2* D342V** (erythromycin) that was not found in controls - One in 20 cases had ***KCNQ1* R231C** (probucol) that was not found in controls - One in 20 cases had ***KCNH2* D614V** (hydroxydine) that was not found in controls - One in 20 cases had ***KCNH2* M756V** (pirmenol) that was not found in controls. -In vitro studies revealed that a change in drug sensitivity was not involved in causing the TdP in the subjects.
(van Noord et al., 2009)	-Candidate gene -Prospective population-based cohort -Single center	-7565 Netherlands participants taking calcium channel blocker.	-Change in QTc	-NOS1AP	-calcium channel blockers	-The **rs10494366 GG** showed significantly more QTc prolongation than users with the TT genotype after administration with verapamil. Similar results observed with the **rs10918594** - Isradipine with the GG genotype showed more QTc prolongation than the TT genotype, however, the SNP rs10494366 did not modify the effect on QTc interval on a large scale. Similar results observed with the rs10918594.
(Nishio et al., 2009)	-Candidate gene	Heterologous expression	N/A	-*KCNE1*	N/A	-***KCNE1* D85N** induced loss of function on both

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
	-Case control & in vitro -Single center	system with a mammalian cell line (CHO cells) - 21 cells that have been transfected with *KCNQ1* and WT *KCNE1* - 25 cells that have been transfected with KCNQ1 and *D85N KCNE1*. -23 cells that were transfected with *KCNH2* and WT *KCNE1* -20 cells that were transfected with *KCNH2* and *D85N KCNE1*.				*KCNQ1* and *KCNH2* channel currents.
(Volpi et al., 2009)	-GWAS -Phase 3 RCT of 14 days of treatment with iloperidone. -Single center	-218 patients with schizophrenia administered iloperidone (16 Asian, 69 white, 91 black or African American and 7 patients of other	-Change in QTc	-GWAS	-iloperidone	-Six SNPs were identified: *CERKL* (rs993648), *SLCO3A1* (rs3924426), *BRUNOL4* (rs4799915), *NRG3* (rs4933824), *NUBPL* (rs7142881) and *PALLD* genes (rs17054392)

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		ethnic origins).				
(Eldstrom et al., 2010)	-In vitro study *Co expression KCNQ1* V215M with *KCNE1* in mammalian cells -Single center					-*KCNQ1* **V215M** induced a positive shift of the activation and accelerated deactivation of the channel compared with the wild-type.
(Qurashi, Schuler, & Janicki, 2011)	-Candidate gene -Post-hoc analysis of existing perioperative genomic database -Single center	-132 surgical patients who had received either granisetron or dolasetron as part of their general anesthesia plan. European ancestry	-Change in QTc	-*NOS1AP*	-granisetron -dolasetron	-The ***NOS1AP* rs10494366** was found to be associated with the QTc intervals before and after drug exposure.
(Nof et al., 2011)	-In vitro study. Co expression of *KCNE1* D85N or the wild-type with *KCNH2 and KCNQ1* in TSA201 cells -Single center	-	-	-	-	-Homozygous *KCNE1*-**D85N** reduced *IKr* by 85%, whereas heterozygous reduced the current by 52%. - *KCNE1*-D85N produced no change in IKs
(Chen et al., 2011)	-In vitro study Co expression of *KCNQ1* S277L with KCNE1 in CHO cells -Single center	-	-	-	-	-***KCNQ1* S277L** reduced current density and the surface expression of the mutant.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
(Aidery et al., 2011)	-In vitro study Co expression of KCNQ1 S277L with KCNE1 in *Xenopus* oocyte -Single center					The ***KCNQ1* S277L** decrease currents and caused a shift of the voltage-dependence of activation compared to wild type.
(Aberg et al., 2012)	-Candidate gene & GWAS - Randomized trial (all patients were treated) -Multicenter	-738 schizophrenia patients without prior QTc prolongation treated with 1 of 5 different anti-psychotic drugs -57% white/European American -29% black/African American -14% other or multiple ancestries	-change in QTc	-GWAS -Candidate genes: *-ATP1B1* *-BRUNOL4* *-CERKL* *-KCNE1* *-KCNH2* *-KCNJ2* *-KCNQ1* *-LIG3* *-LITAF* *-NOS1AP* *-NRG3* *-NUBPL* *-RNF207* *-SCN5A* -multi-gene locus 1 *(PLN* and *SLC35F1)* -multi-gene locus 2 *(CNOT1 GINS3 NDRG4* and *SETD6)*	-olanzapine perphenazine -quetiapine -risperidone -ziprasidone	-GWAS: ***SLC22A23* rs4959235** reached genome-wide significance for QTc change with quetiapine -Candidate genes: Gene-based enrichment analysis showed enrichment for ***NOS1AP*** (140 SNPs) and ***NUBPL*** (39 SNPs)
(Lin et al., 2012)	-Candidate gene -Case-report -Single center	-1 child experienced QT prolongation (QTc ≥ 580 ms) and TdP after administration of sodium channel blockers for paroxysmal atrial	-QT prolongation and TdP	-Not stated	- procainamide - disopyramide	- The ***KCNE1* D85N** was found in the child and in his father.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		tachycardia. Japanese ancestry				
(Kaab et al., 2012)	-Candidate gene -Case-control -Multicenter	-176 diLQTS -207 drug-exposed controls with, <50 msec increase in QTc interval and no QTc interval exceeding 500 msec during drug treatment. -837 general population Validation for D85N: -57 diLQTS -211 controls -European ancestry	-TdP -	-*AKAP9* -*ANK2* -*CACNA1C* -*NOS1AP* -*CASQ2* -*CAV3* -*FKBP1B* -*GPD1L* -*KCNE1* -*KCNE2* -*KCNH2* -*KCNJ2* -*KCNQ1* -*RYR2* -*SCN1B* -*SCN4B* -*SCN4A* -*SCN5A*	-amiodarone - sotalol -quinidine - antipsychotics -antibiotics	-The strongest effect size was detected for ***KCNE1* D85N** with an allele frequency of 8.6% in diLQTS, 2.9% in drug- exposed controls and 1.8% population-based controls. -In the validation cohort, ***KCNE1* D85N** was present in 3.5% of diLQTS cases, and in 1.4% of controls.
(Jamshidi et al., 2012)	-Candidate gene -Case-control -Multicenter	- 58 Caucasian patients who had experienced an arrhythmic event associated with diLQTS. -87 Caucasian controls n	-TdP, ventricular fibrillation and/or cardiac arrest associated with QTc interval prolongation; and QTc interval prolongation with a	-*NOS1AP*	-amiodarone -sotalol	-The **rs10800397** was associated with diLQTS (cases 37.0%, controls 14.4%) in amiodarone users. -The **rs10919035** was associated with diLQTS (cases 27.8%, controls 7.1%) in amiodarone users. -<u>Replication cohort:</u> The SNP was associated with diLQTS (26.8% cases and

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		o history of drug induced arrhythmia. *Replication cohort for rs10919035:* 28 amiodarone-diLQTS cases European descent and 105 European ancestry drug-exposed controls who did not develop qualifying arrhythmias, had <50 msec increase in QTc interval on drug exposure, and no QTc interval exceeding 500 msec during drug treatment. -68 European ancestry, healthy volunteers from the general population and challenged with ibutilide	history consistent with cardiac syncope. After withdrawal of the culprit drug resolution of the QT prolongation were required -Replication cohort for rs10919035: TdP during treatment with amiodarone			16.5% in drug-challenged controls). -Meta-analysis of both studies revealed an OR of 2.81 for each T allele

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		-1480 individuals were tested for association.				
(Behr et al., 2013)	-GWAS -Case-control -Multicenter	-264 drug induced TdP cases. European Ancestry -470 control subjects who did not display any QTc 500 msec or any increase in QT >50 msec after the administration of the same drugs -4880 general population control European Ancestry -Subjects with cLQTS that experienced diLQTS were not excluded.	- TdP and QT prolongation	-GWAS	-ibutilide -sotalol -dofetilide -quinidine	No SNP reached genome wide significance. The variant with the lowest p-value was **rs2276314**, ($p = 3\times10^{-7}$)
(Ramirez et al., 2013)	Candidate gene Case-control Multicenter	31 cases (26 Caucasian, 3 Asian, and 2 African-American) 60 Caucasian controls	TdP	79 genes important for regulating heart rhythm	amiodarone azithromycin disopyramide dofetilide encainide bretyllium ganciclovir sirolimus metoclopramide quinidine	Eleven of 31 diLQTS subjects (36%) carried a novel missense mutation in genes associated with congenital arrhythmia. 23% from the 26 Caucasian subjects carried a rare variant predicted to be deleterious to protein

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		from 1000 Genomes 1,351 Caucasian controls from the NHLBI Exome Sequencing Project			procainamide sotalol trimethoprim/ sulfamethoxazole	function in these genes compared with only 2-4% in public databases (P<0.003).
(Du et al., 2013)	-In vitro study. Co expression of *KCNE1* D85N, A8V, D76N or the wild-type on HEK 293 cells -Single center	-	-	-	Quinidine cisapride clarithromycin	- **KCNE1 A8V, D76N, and D85N** reduced the amplitude of HERG *currents compared to wild-type.* - The HERG *current* was similar between wild type and the three *KCNE1* variants after quinidine, but it was reduced in presence of clarithromycin and cisapride in the *KCNE1* variants.
(L. Liu et al., 2013)	-In vitro study. Co expression of *KCNH2* T473P or the wild-type in CHO-K1 cells -Single center	-	-	-	-	- ***KCNH2* T473P** generated no HERG current. Western blot analysis showed that T473P protein is incompletely processed and does not traffic correctly.
(Kamei et al., 2014)	-Candidate gene -Case-control -Single center	10 autopsy samples from patients who had suffered sudden and unexpected death during medication therapy. Subjects were not previous diagnosed	-Sudden and unexpected death	-*KCNQ1* -*KCNH2*	-psychotropic drugs	- Six in 10 had ***KCNQ1* G643S** (60%), that was found in 11% in both control groups.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		with any cardiac disease. Japanese ancestry -100 control patients with no diagnosis of LQTS. Japanese ancestry - 281 unrelated individuals with no medical history of cardiac disease. Japanese ancestry				
(Sakata et al., 2014)	-In vitro study. Co expression of *KCNE1* D85N or the wild-type COS7 cells with KCNQ1 to form K+ channels. -Single center	-	-	-	-verapamil -amiodarone	-The protein level of ***KCNE1* D85N** was lower than that of the wild-type. - IKs was much smaller in **D85N** than in wild-type. -Verapamil increased the protein level of **D85N**, decreased its ubiquitination, slowed its degradation, and enhanced *KCNQ1/KCNE1* D85N channel currents. -Pretreatment with amiodarone abolished the effects of verapamil.
(Weeke et al., 2014)	-Whole exome sequencing & Candidate gene analysis -Case-control -Single center	-65 diLQTS patients European ancestry -148 drug-exposed control subjects with QTc ≤470 ms, no on-	-TdP ± QT interval prolongation ≥600 msec, that decreased to <480 msec on drug discontinuation	-Whole exome & Candidate genes: *-ACNA1C* *-CAV3* *-KCNE1* *-KCNE2* *-KCNH2* *-KCNJ2* *-KCNJ5* *-KCNQ1* *-SCN4B*	antiarrhythmics antipsychotics	-Six in 65 had ***KCNE1* D85N** and one had ***KCNE1* D76N** -Nine in 65 had *ACN9* **F53L** and one had *ACN9* **T83I**, whereas 4 drug-exposed control subjects were carriers of F53L. -Thirty-seven percent of diLQTS patients had 1 or more rare amino acid coding variants

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		drug QTc interval > 495 ms, and maximal QTc interval change <50 ms on drug. European descent - 515 ethnically matched control subjects.		-*SCN5A* -*SNTA1* -*KCNQ1* -*KCNH2* -*KCNJ2* -*CACNA1C* -*CACNB2* -*GPDL1* -*KCNE3* -*SCN1B* -*SCN3B* -*SCN5A* -*CASQ2* -*RYR2*		compared with 21% of control in a pre-defined set of 7 cLQTS genes.
(Avery et al., 2014)	meta-analysis of 10 cohorts with GWAS Multicenter	33,781 participants of European descent	Change in QT	GWAS	thiazide diuretics tri/tetracyclic antidepressants sulfonylureas hypoglycemic agents	No genome-wide significant cross-sectional interactions were detected for any of the four drug classes. ($P<5.0 \times 10^{-8}$)
(Untereker et al., 2015)	Candidate gene study Retrospective cohort Single center	59 patients taking Amitriptyline/Nortriptyline. Ancestry unknown patients taking venlafaxine. Ancestry unknown	Change in QTc	*NOS1AP*	amitriptyline nortriptyline	The rs10494366 had no association with QTc. The rs12143842 risk alleles CT and TT had lower QTc intervals in amitriptyline group.
(Choi, Wang, Thomas, & Pitt, 2016)	-Candidate gene -Case report & In vitro -Single center	-1 patient with no family history of sudden cardiac death or LQTS suffered a witnessed cardiac arrest while exercising	QTc change and cardiac arrest	30 genes (Not specified)	amitriptyline pseudoephedrine famotidine	The patient had ***SNTA1* E409Q** which is not present in normal population databases. In vitro study: The variant increased late INa in HEK293T cells and in cardiomyocytes compared to wild type and the A390V variant.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		. Ancestry not stated				
(Sanchez et al., 2016)	-Candidate gene - Cohort analysis -Unicenter	789 subjects (≤ 50 years of age) who died suddenly. Excluding cases involved in violent death and drug overdose	-Sudden cardiac death	*55* genes associated with higher risk of Sudden cardiac death	Unknown	-The ***KCNE2* T8A** and ***SCN5A* G615E** were found in 2 cases that experience diLQTS.
(H. Itoh et al., 2016)	Candidate gene Cohort analysis Multicenter	188 subjects with acquired LQTS. Subjects were designated: "true aLQTS" (QTc within normal limits) "unmasked cLQTS" (all others) 2379 members of 1010 genotyped cLQTS families.	TdP, ventricular tachycardia, pre-syncope, syncope, cardiac arrest, ventricular fibrillation, and change in the QTc (QTc≥480 ms) after drug administration.	*KCNQ1* *KCNH2* *SCN5A* *KCNE1* *KCNE2*	Unknown	QTc of aLQTS was shorter (453 ± 39 ms) than in cLQTS (478 ± 46 ms) and longer than in controls (406 ± 26 ms). *KCNQ1* mutations were less frequent than *KCNH2 in aLQTS than cLQTS.* It was identified 47 disease-causing mutations in aLQTS subjects
(Watanabe et al., 2017)	Candidate gene Cohort Single center	109 inpatients with schizophrenia treated with antipsychotics at the time of the study. Patients with	-Change in QTc	DNA Sequencing: *ATP1B1* rs10919071 *KCNH2* rs2968863 *KCNJ2* rs17779747 *KCNQ1* rs12296050 *LITAF* rs8049607 *NDRG4* rs7188697	olanzapine risperidone quetiapine levomepromazine - aripiprazole perospirone chlorpromazine haloperidol bromperidol zotepine fluphenazine sulpiride	The ***PLN*rs11970286 and *NDRG4* rs7188697** were found in patients with the longest QTc. The increased numbers of risk alleles were the major predictors of QTc prolongation.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		family history of congenital QT syndrome were excluded. Ancestry unknown		*NOS1AP* rs12143842 *PLN* rs11970286 *RNF207* rs846111 *SCN5A* rs11129795		
(Fabbri et al., 2017)	Candidate gene study Prospective study, cross-sectional and Randomized trial (CATIE) Multicenter	Patients diagnosed with mood or psychotic disorder: 145 cross-sectional sample, 68 prospective sample, 515 randomized sample from CATIE, a multi-center, double-blind study that evaluate the effectiveness between first- and second-generation antipsychotics	Prospective sample: baseline/follow-up changes in ECG. cross-sectional sample, ECG parameters were compared among drugs with different risk profile. ECG change at baseline and at the end of each phase (1, 2, and 3).	*CACNA1C*	psychotropics	-*CACNA1C* rs1006737 was associated with lower QTc interval.
(Salem et al., 2017)	GWAS Prospective study Multicenter	489 healthy individuals (discovery cohort) 495 participated in the replication cohort. European or North	Change in QTc baseline	GWAS	Sotalol	No association found.

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		African ancestry				
(Strauss et al., 2017)	Polygenic score Randomized, double-blind, placebo-controlled, cross-over trial study of healthy subjects. Case-control analysis	22 subjects without a family history of cardiovascular disease or unexplained sudden cardiac death. Subjects also had to have a baseline QTc <450 ms for men and 470 ms for women. 17 white, 4 black and 1 Asian	Change in QTc & TdP	Polygenic score of 61 SNPs from GWAS of QT interval	dofetilide quinidine ranolazine	In white subjects, genetic score explained 30% of the variability of QT in response to dofetilide, 23% in response to quinidine and 27% in response to ranolazine In African American, there was a correlation between baseline QTc and response to dofetilide, but not for quinidine or ranolazine. Increasing genetic QT risk score was associated with increased risk of drug-induced TdP (12.1%).
(Roberts et al., 2017)	Candidate gene Case series Multicenter	48 individuals from 28 families possessing a rare *KCNE2* variant classified as likely pathogenic or pathogenic. -Probands of 7 families had LQTS phenotype secondary to QT-prolonging stressor.	TdP, QT prolongation >500 msec, cardiac arrest, syncope	*KCNE1, KCNE2 KCNH2 KCNQ1 SCN5A*	amiodarone Hydroxyzine fluoxetine desipramine	-Three probands had the ***KCNE2 T8A*.** One of them developed TdP (amiodarone) and the second one syncope (Hydroxyzine and Fluoxetine)
(Noordam et al., 2017)	GWAS	21 different cohorts of	Change of QTc. Participants	GWAS	tri/tetracyclic antidepressants (TCA)	*The TGFBR3* **rs2291477** was found to be associated with

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
	Cohort analysis & Metanalysis Multicenter	three ancestral populations: European (n=45,706 ; n=1,417 TCA users), African (n=10,235 ; n=296 TCA users) and Hispanic/ Latino (n=13,808 ; n=147 TCA users)	with atrial fibrillation, heart failure or a QRS duration ≥120 ms, a pacemaker, and/or second or third degree atrioventricular block were excluded			TCAs and QTc length in the Hispanic cohorts.
(Marstrand et al., 2018)	Candidate gene Case-report Single center	A female Danish patient experienced QTc >600ms at admission. At the following day she developed TdP. After discontinuation of citalopram the QTc returned to normal range.	QTc>600ms and TdP	Unknown	citalopram	The subject was heterozygote carrier of the ***KCNE1*** **D85N.**
(Nagasawa et al., 2018)	Candidate gene Case-control Single center	-10 cases of sudden death involving methamphetamines use. None of the participants had heart conditions -10 cases involving new	sudden death	*KCNQ1* *KCNH2*	methampheta mines new psychotropic substances.	The ***KCNQ1*** **G643S** was found in one patient of the 10 in the methamphetamine group and six in the 10 of new psychotropic substances group**.** This SNP was found in 11% of the control group (N=100)

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		psychotropic substances 100 control patients with no diagnosis of LQTS (Same Kamei *et al.*,2014)				
(Spellmann et al., 2018)	Candidate gene study Randomized, double-blind parallel-group trial Single center	199 patients with schizophrenia. All patients were Caucasian. Patients were assigned to monotherapy with an antipsychotic for 5 weeks.	Change in QTc	DNA sequencing: KCNH2 K897T KCNQ1 rs10798 rs757092 LOC105378779 rs10458561 LOC101927066 rs16895513 NOS1AP rs12143842 NUBPL rs7142881 SCN5A H558R	risperidone quetiapine olanzapine amisulpride aripiprazole haloperidol	No associations found
(Floyd et al., 2018)	GWAS & Meta-analysis Ancestry-specific meta-analyses of 11 cohorts. Multicenter	71, 857 patients taking sulfonylurea. Ancestry: European, African American, and Hispanic/ Latino	Change in QT, JT, and QRS intervals -	GWAS	sulfonylureas	The intronic variants **rs9966832** and **rs830233** reached genome-wide significance for change in the QT interval.
(Corponi et al., 2019)	Candidate gene Cohort analysis Multicenter	77 patients with mood or psychotic disorders being treated with antidepressants and	Change in QT	*ABCB1* *ACN9* *BRUNOL4* *CERKL* *KCNE1* *KCNH2* *NOS1AP* *NUBPL*	psychotropics	No association was found

Article	Study Design	Subjects	Endpoint(s)	Gene(s)	Drug(s)	Summary of Results
		antipsychotics. Ancestry unknown				
(Zerdazi et al., 2019)	Candidate gene Cohort analysis Single center	154 patients in current methadone daily dose. No patient reported previous history of syncope, TdP or LQTS. Ancestry: European and African American	Change in QTc	*KCNE1* *KCNQ1* *KCNH2* *NOS1A* *SCN5A*	methadone	The ***KCNE1*** **rs11911509** (only homozygotes) was associated with QT interval prolongation. -The **rs11911509** was found to have interaction between methadone dosage and QTc interval.
(Martinez-Matilla et al., 2019)	- Candidate gene - Cohort analysis -Multicenter	2 patients suspected of having drug-induced channelopathy or sudden unexplained death	TdP, borderline QTc interval, long QTc interval, Brugada Syndrome and sudden cardiac death	96 genes associated with risk of Sudden cardiac death	Unknown	-**The** *TTN* **Asn4539*,** *MYBPC3* **V1125 M** were found in two patients with suspected diLQTS and *KCNH2* **D982V** in one of sudden death. - *KCNE1* **D85N** was identified in one patient who also had the variant *PKP2* **P472R**

Table 2.5 Evaluation of the strength of evidence for variants associated with diLQTS.

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
ACN9 F53L rs62624461				✓✓ (Weeke et al., 2014)		✓	**3 Limited**
ACN9 T83I rs34146273				✓✓ (Weeke et al., 2014)			**2 Limited**
AKAP6 V839A rs533577792				✓✓ (Ramirez et al., 2013)			**2 Limited**
AKAP7 Q112R –				✓✓ (Ramirez et al., 2013)			**2 Limited**
AKAP9 Q3531E rs767137372				✓✓ (Ramirez et al., 2013)			**2 Limited**
APLP2 R504L rs541781240				✓✓ (Ramirez et al., 2013)			**2 Limited**
ATP2A2 R504L –				✓✓ (Ramirez et al., 2013)			**2 Limited**
BRUNOL4 rs4799915			✓ (Volpi et al., 2009)				**1 Limited**
CACNA1C A1733V rs201049603				✓✓ (Ramirez et al., 2013)			**2 Limited**
CACNB2 I170V rs1174211196				✓✓ (Ramirez et al., 2013)			**2 Limited**
CACNB2 M1V rs746311834				✓✓ (Ramirez et al., 2013)			**2 Limited**
CALR D418G –				✓✓ (Ramirez et al., 2013)			**2 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
CAV3 T78M rs72546668				✓✓ (Ramirez et al., 2013)			**2 Limited**
CERKL rs993648			✓ (Volpi et al., 2009)				**1 Limited**
GPD1L V249M rs148464224				✓✓ (Ramirez et al., 2013)			**2 Limited**
IVS16-6C-T intronic				✓✓ (Paulussen et al., 2004)			**2 Limited**
Intergenic rs9966832			✓ (Floyd et al., 2018)				**1 Limited**
Intergenic rs830233			✓ (Floyd et al., 2018)				**1 Limited**
JPH2 T286A rs144022614				✓✓ (Ramirez et al., 2013)			**2 Limited**
JPH2 V345L rs748233107				✓✓ (Ramirez et al., 2013)			**2 Limited**
JPH3 R656W rs201403180				✓✓ (Ramirez et al., 2013)			**2 Limited**
KCND3 R566C rs139901716				✓✓ (Ramirez et al., 2013)			**2 Limited**
KCNE1 A8V rs199473348	✓✓ (Du et al., 2013) ✓ (Ohno et al., 2007)		✓ (Hideki Itoh et al., 2016)				**4 Limited**
KCNE1 S28S				✓✓ (Paulussen et al., 2004)			**2 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
KCNE1 intronic rs11911509			✓ (Zerdazi et al., 2019)				**1 Limited**
KCNE2 A116V rs199473367				✓✓ (Sesti et al., 2000)			**2 Limited**
KCNE2 M54T rs74315447	✓ (Abbott et al., 1999)			✓✓ (Sesti et al., 2000)			**3 Limited**
KCNE2 Q9E rs16991652	✓✓ (Abbott et al., 1999) ✓ (Tinel et al., 2000)		✓ (Abbott et al., 1999)				**4 Limited**
KCNN3 F315L rs1283882852				✓✓ (Ramirez et al., 2013)			**2 Limited**
KCNH2 1047L rs36210421				✓✓ (Sun et al., 2004)			**2 Limited**
KCNH2 A913V rs77331749			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 A1116V rs199473032			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 A490T rs28928905			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 A614V rs199472944	✓ (Sakaguchi et al., 2008)		✓ (Hideki Itoh et al., 2016)				**2 Limited**
KCNH2 D111V rs199472860			✓				**1 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
			(Hideki Itoh et al., 2016)				
KCNH2 D982V –			✓ (Martinez-Matilla et al., 2019)				**1 Limited**
KCNH2 D342V rs199472889	✓ (Itoh et al., 2009)		✓ (Hideki Itoh et al., 2016)	✓✓ (Itoh et al., 2009)	✓ (Itoh et al., 2009) (Hideki Itoh et al., 2016)		**5 Limited**
KCNH2 D614V rs199472944				✓✓ (Itoh et al., 2009)			**2 Limited**
KCNH2 G628S rs121912507	✓ (Zhou et al., 1998)		✓ (Hideki Itoh et al., 2016)				**2 Limited**
KCNH2 H492Y rs199472910	✓ (Itoh et al., 2009)		✓ (Hideki Itoh et al., 2016)	✓✓ (Itoh et al., 2009)	✓ (Itoh et al., 2009) (Hideki Itoh et al., 2016)		**5 Limited**
KCNH2 E971K –			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 L552S rs199472918				✓✓ (Lehtonen et al., 2007)			**2 Limited**
KCNH2 L559F rs794728374			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 L776P –			✓				**2 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
			(Hideki Itoh et al., 2016)				
KCNH2 M756V rs199473534	✓ (Itoh et al., 2009)		✓ (Hideki Itoh et al., 2016)	✓✓ (Itoh et al., 2009)	✓ (Itoh et al., 2009) (Hideki Itoh et al., 2016)		**5 Limited**
KCNH2 M124T rs199472862	✓✓ (Hayashi et al., 2004)		✓ (Hideki Itoh et al., 2016)				**3 Limited**
KCNH2 N633I rs199472961			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 P347S rs138776684				✓✓ (Paulussen et al., 2004)			**2 Limited**
KCNH2 P846T rs199473006			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 P114S rs199472861			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 R328C rs199473505				✓✓ (Chevalier et al., 2001)			**2 Limited**
KCNH2 R784W rs12720441	✓ (P. Yang et al., 2002)			✓✓ (P. Yang et al., 2002) ✓✓ (Ramirez et al., 2013)	✓ (P. Yang et al., 2002) (Ramirez et al., 2013)		**6 Limited**
KCNH2 R948S rs121912514			✓				**1 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
			(Hideki Itoh et al., 2016)				
KCNH2 R1033W rs199473021				✓✓ (Ramirez et al., 2013)			**2 Limited**
KCNH2 R744del –			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNH2 S706F rs199472985	✓ (Itoh et al., 2009)		✓ (Hideki Itoh et al., 2016)	✓✓ (Itoh et al., 2009)	✓ (Itoh et al., 2009) (Hideki Itoh et al., 2016)		**5 Limited**
KCNH2 T473P rs199473512	✓ (L. Liu et al., 2013)		✓ (Hideki Itoh et al., 2016)				**2 Limited**
KCNH2 T74R rs199473422			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNQ1 R555C rs120074185	✓ (Chouabe et al., 1997)		✓ (Hideki Itoh et al., 2016)	✓✓ (Donger et al., 1997)	✓ (Donger et al., 1997; Hideki Itoh et al., 2016)	✓	**6 Limited**
KCNH2 V822M rs121912506	✓ (Zhou et al., 1998)		✓ (Hideki Itoh et al., 2016)				**2 Limited**
KCNQ1 A344spl –			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNQ1 F339del –	✓ (Thomas et al., 2005)		✓				**2 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
			(Hideki Itoh et al., 2016)				
KCNQ1 G589D rs120074190				✓✓ (Lehtonen et al., 2007)			**2 Limited**
KCNQ1 G643S rs1800172		✓ (Kamei et al., 2014) ✓ (Nagasawa et al., 2018)				✓	**3 Limited**
KCNQ1 G628G –				✓✓ (Paulussen et al., 2004)			**2 Limited**
KCNQ1 G269S rs120074193			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNQ1 G272V –			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNQ1 IVS9+1 G>A –			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNQ1 R231C rs199473457	✓ (Itoh et al., 2009)		✓ (Hideki Itoh et al., 2016)	✓✓ (Itoh et al., 2009)	✓ (Hideki Itoh et al., 2016) (Itoh et al., 2009)		**5 Limited**
KCNQ1 R243H rs120074196	✓ (Chouabe et al., 2000) ✓ (Itoh et al., 2009)		✓ (Hideki Itoh et al., 2016)	✓✓ (Itoh et al., 2009)	✓ (Hideki Itoh et al., 2016) (Itoh et al., 2009)		**6 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
KCNQ1 R259C rs199472719			✓ (Hideki Itoh et al., 2016)				**1 Limited**
KCNQ1 R583C rs17221854	✓ (P. Yang et al., 2002)			✓✓ (P. Yang et al., 2002)			**3 Limited**
KCNQ1 T377T –				✓✓ (Paulussen et al., 2004)			**2 Limited**
KCNQ1 S277L rs199472730	✓ (Aidery et al., 2011) ✓ (Chen et al., 2011)		✓ (Hideki Itoh et al., 2016)				**3 Limited**
KCNQ1 V215M rs17215479	✓ (Eldstrom et al., 2010)		✓ (Hideki Itoh et al., 2016)				**2 Limited**
KCNQ1 Y315N rs1554894448			✓ (Hideki Itoh et al., 2016)				**1 Limited**
MYBPC3 V1125 M –			✓ (Martinez-Matilla et al., 2019)				**1 Limited**
NDRG4 rs7188697			✓ (Watanabe et al., 2017)				**1 Limited**
*NOS1AP** rs10494366			✓ (Quraishi et al., 2011) ✓				**2 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
			(van Noord et al., 2009)				
NOS1AP rs10918594			✓ (van Noord et al., 2009)				**1 Limited**
NOS1AP rs10800397				✓✓ (Jamshidi et al., 2012)			**2 Limited**
NOS1AP rs10919035				✓✓ (Jamshidi et al., 2012)	✓ (Jamshidi et al., 2012)		**3 Limited**
NRG3 rs4933824			✓ (Volpi et al., 2009)				**1 Limited**
NUBPL rs7142881			✓ (Volpi et al., 2009)				**1 Limited**
PALLD rs17054392			✓ (Volpi et al., 2009)				**1 Limited**
PLN rs11970286			✓ (Watanabe et al., 2017)				**1 Limited**
PPP2R3A F1000L _				✓✓ (Ramirez et al., 2013)			**2 Limited**
RYR2 E4361Q rs794728795				✓✓ (Ramirez et al., 2013)			**2 Limited**
RYR2 L2607P _				✓✓ (Ramirez et al., 2013)			**2 Limited**
RYR2 L555V rs769320446				✓✓ (Ramirez et al., 2013)			**2 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
SCN5A F1250L rs45589741				✓✓ (P. Yang et al., 2002)			**2 Limited**
SCN5A L618F rs45488304				✓✓ (P. Yang et al., 2002)			**2 Limited**
*SCN5A S*1102Y rs7626962				✓✓ (Splawski et al., 2002)		✓	**3 Limited**
SCN5A R225Q –			✓ (Hideki Itoh et al., 2016)				**1 Limited**
SNTA1 E409Q rs786205850	✓ (Choi et al., 2016)						**1 Limited**
SNTA1 T147N rs141724500				✓✓ (Ramirez et al., 2013)			**2 Limited**
SLCO3A1 rs3924426			✓ (Volpi et al., 2009)				**1 Limited**
SLC22A23 rs4959235			✓ (Aberg et al., 2012)				**1 Limited**
TTN Asn4539*			✓ (Martinez-Matilla et al., 2019)				**1 Limited**
TGFBR3 rs2291477			✓ (Noordam et al., 2017)				**1 Limited**
ZFHX3 L741F				✓✓ (Ramirez et al., 2013)			**2 Limited**
ZFHX3 K3689E rs149908041				✓✓ (Ramirez et al., 2013)			**2 Limited**

Variant	Experimental evidence supporting functional effects	Clinical Evidence			Independent replication	Variant identified in ≥ 5 total cases	Total of Points and Overall Strength of Evidence
		Clinical outcome sudden cardiac death	Clinical outcome change in baseline QTc <50ms and/or QTc <500ms	Clinical outcome TdP and/or marked QTc prolongation >500 ms			
ZFHX3 G117S rs201151237				✓✓ (Ramirez et al., 2013)			**2 Limited**
ZFHX3 H3611T rs771413197				✓✓ (Ramirez et al., 2013)			**2 Limited**
ZFHX3 T3640M rs1273193703				✓✓ (Ramirez et al., 2013)			**2 Limited**

2.13.1 Variants with Moderate Evidence

2.13.1.1 SCN5A L1825P (rs79299226)

The L1825P (rs79299226) occurs in the C terminal of the sodium channel ($Na_v1.5$). The minor allele frequency of L1825P is too rare to be found in public databases**,** but *L1825P* was found to be associated with diLQTS in two candidate gene studies (Hideki Itoh et al., 2016; Itoh et al., 2009), one of those with experimental evidence supporting its functional effect (Itoh et al., 2009), and two additional *in vitro* studies (Itoh et al., 2009; K. Liu et al., 2005; Makita et al., 2002).The *SCN5A* gene was also assessed in several other clinical studies, but L1825P was not found in any of the diLQTS cases (Aberg et al., 2012; Napolitano et al., 2000; Paulussen et al., 2004; Ramirez et al., 2013; Roberts et al., 2017; Splawski et al., 2002; Weeke et al., 2014; P. Yang et al., 2002; Zerdazi et al., 2019).

Experimental evidence supporting functional effects of the *SCN5A*-L1825P was first published by Makita et al 2002 (Makita et al., 2002). Makita et al characterized the effect of *L1825P* on sodium currents expressed in TSA-201 cells. Their *in vitro* experiments showed that channels containing the *L1825P* variant causes a reduction in the sodium current density and a negative shift of steady-state inactivation. This change in the gating properties of the channels result in greater reduction in sodium channel availability during excitation compared to wild type. After the exposure with cisapride, no change was observed in L1825P mutant sodium current, meaning that the increase in the QT interval induced by cisapride may be mediated by mechanisms other than direct effects on cardiac sodium channels (Makita et al., 2002). Indeed, three years later, Liu et al. (K. Liu et al., 2005) reported that cisapride partially restored the surface expression of the L1825P channels, which were depleted at baseline compared to wild-type. The increase of the expression of sodium channels after cisapride may be the cause of the augmentation of the late inward sodium current that leads to QT interval prolongation in patients with the variant. (K. Liu et al., 2005) The effect of L1825P on ventricular APs was also evaluated by Itoh et al, 2009 (Itoh et al., 2009) using a computer simulation model. The variant showed a mild increase in magnitude of the AP duration compared to wild type.(Itoh et al., 2009) A clinical case report was the first to report *L1825P* in a case of cisapride-induced TdP (Makita et al., 2002). This SNP caused a slight prolongation in the QT interval at baseline (QTc = 480 ms), but a very long QT interval (QTc =731 ms) after the exposure to cisapride. After 6 days without the drug, the QT interval returned to baseline values (QTc= 417 ms). (Makita et al., 2002) Itoh et al., in 2009 also published a candidate gene clinical study supporting L1825P as a predisposing factor for diLQTS (Itoh et al., 2009). *KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2* and *KCNJ2* were sequenced in 20 subjects that experienced TdP after drug administration and

220 chromosomes from controls. L1825P was identified in 1 case (culprit drugs: cisapride and pirmenol), but not in controls. (Itoh et al., 2009) In 2016, Itoh et al (Hideki Itoh et al., 2016) also published a study supporting L1825P as a predisposing factor for acquired QT prolongation. They sequenced *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* genes in a cohort of 188 subjects diagnosed with acquired QT prolongation. L1825P was one of the mutations found in the cohort. (Hideki Itoh et al., 2016) However, only 81 of those 188 subjects experienced QT prolongation after culprit drug administration. The authors did not specify if L1825P was found in the diLQTS cases versus other acquired forms of LQTS.

2.13.1.2 SCN5A G615E (rs12720452)

Another variant in *SCN5A* associated with diLQTS is G615E (rs12720452). G615E occurs in the intracellular domains of the α-subunits DI-II linker of the sodium channel. The minor allele frequency of G615E is highest in European individuals (0.0006) and lowest in East Asian individuals and Ashkenazi Jewish (<0.000; Table 3). G615E has been found to be associated with diLQTS in three candidate gene studies (Ramirez et al., 2013; Sanchez et al., 2016; P. Yang et al., 2002) and in one experimental study supporting its functional effects (Albert et al., 2008). The *SCN5A* gene was also assessed in several other clinical studies, but G615E was not found in any of the diLQTS cases. (Itoh et al., 2009; Kaab et al., 2012; Makita et al., 2002; Napolitano et al., 2000; Paulussen et al., 2004; Sanchez et al., 2016; Spellmann et al., 2018; Splawski et al., 2002; Watanabe et al., 2017; Zerdazi et al., 2019).

Discrepancies in experimental studies supporting the functional effect of G615E have been observed in the literature. Yang et al, 2002 (P. Yang et al., 2002) were the first to evaluate the effect of G615E on the sodium current in the absence of drug. Their *in vitro* experiments showed that channels containing G615E did not alter the amplitude or the voltage dependence of

the activation/inactivation of the sodium channel in mammalian expression vectors. The authors concluded that G615E played no role in the diLQTS phenotype. (P. Yang et al., 2002) However, years later it was found that the G615E variant in Xenopus oocytes changes the channel activity by reducing the recovery times from fast inactivation without altering the magnitude of these currents. (Albert et al., 2008) This alteration in the channel gating has been previously reported to cause marked changes in the cardiac AP duration leading to an increase in the QT interval. (Abriel et al., 2001)

As far as clinical studies, Yang et al, (P. Yang et al., 2002) were the first to report G615E in a case of quinidine-induced arrhythmia. They screened *KCNQ1, KCNH2, SCN5A* in 92 patients that displayed marked QT prolongation from normal values to >600 ms and/or TdP after culprit drug (P. Yang et al., 2002). They also assessed allele frequencies in three control groups: 67 patients receiving QT prolonging drugs without marked QT prolongation; 71 randomly selected unrelated individuals; and 90 anonymous individuals from a public database. G615E was found in 1 patient with quinidine-induced arrhythmia but not in controls.

Ramirez et al in 2013 (Ramirez et al., 2013) and Sanchez et al 2016 (Sanchez et al., 2016) also found G615E as a predisposing factor for drug-induced LQTS in candidate gene clinical studies. Ramirez et al sequenced 79 genes important for regulating heart rhythm in 31 subjects that experienced TdP after culprit drugs. They also assessed allele frequencies in a control population of 60 Caucasian individuals and in 1,351 individuals from the general population. G615E was found in 1 patient with quinidine and sotalol-induced arrhythmia but not in controls (Ramirez et al., 2013). Sanchez et al screened 55 genes associated with higher risk of SCD in 789 subjects ($\leq$ 50 years of age) who died suddenly. G615E was identified in 1 case that experienced drug-induced arrhythmia (Sanchez et al., 2016).

2.13.1.3 KCNE1-D76N (rs74315445)

Another variant of the *KCNE1* gene has been associated with diLQTS, but with a moderate level of evidence. The D76N (rs74315445) occurs in the C terminal of KCNE1. The minor allele frequency of *KCNE1*-D76N is highest in European individuals (0.01%) and lowest in Ashkenazi Jewish, European (Finish) and other individuals (Table 3). The D76N was found to be associated with diLQTS in a whole exome sequencing study, and five studies with experimental evidence supporting functional effects (Bianchi et al., 1999; Du et al., 2013; Hoppe et al., 2001; Sesti & Goldstein, 1998; Splawski et al., 1997). Experimental evidence supporting functional effects of the *D76N* was first published by Splawski et al, 1997 (Splawski et al., 1997). Splawski et al evaluated the biophysical properties of KCNQ1 channels when co-expressed with KCNE1 mutations in *Xenopus* oocytes. D76N reduced IKs by shifting the voltage dependence of activation and accelerating channel deactivation. These results were also observed a year later by Sesti et al, 1998 (Sesti & Goldstein, 1998). D76N caused a decrease in IKs currents and diminished open probability of the KCNQ1 channel compared to wild type in *Xenopus* oocytes and *CHO cells* (Sesti & Goldstein, 1998). A year later, Bianchi et al. (Bianchi et al., 1999) characterized the effect of D76N on KCNQ1 channels using *Xenopus* oocytes, HERG and HEK 293 cells. They showed that channels containing the D76N variant had decreased not just IKs currents but IKr currents. (Bianchi et al., 1999) These results were replicated two years later by Hoppe et al (Hoppe et al., 2001). The co-expression of *KCNE1* D76N or the wild type with *KCNH2* in adenoviral vectors in guinea pig myocardium showed that D76N prolonged the QT interval in vivo and it also suppressed IKs currents by 80%. D76N also prolonged the overall action potential duration by almost 2-fold, which was associated with frequent early after depolarization. (Hoppe et al., 2001) The influence of D76N on the KCNH2

channel in the presence of some drugs linked to diLQTS has been also evaluated in vitro. Du et al. 2013 (Du et al., 2013) studied the KCNH2 currents in the presence of quinidine, clarithromycin and cisapride when co-expressed with D76N. The Kv11.1 *current* blocking potency of quinidine was similar between wild type and D76N. However, the inhibitory potency of clarithromycin and cisapride was enhanced for the variant compared to wild type. This study demonstrates that D76N may increase susceptibility to diLQTS by direct modulation of the sensitivity of the KCNH2 channel to drug inhibition. (Du et al., 2013)

In a whole-exome sequencing study, D76N has been found as a predisposing factor for diLQTS. This study involved 65 Caucasian patients that experienced TdP and exaggerated QT interval prolongation (≥600 ms) after initiation with QT prolonging drugs was published. (Weeke et al., 2014) The D76N variant was found in higher frequency in subjects that experienced diLQTS compared to 148 control subjects that experienced <50 ms QTc interval change after drug exposure and 515 population controls with European ancestry (Weeke et al., 2014). *KCNE1* was one of only 2 genes in that study in which the burden of rare variants was significantly higher in the diLQTS patients in whole-exome analyses.

2.13.1.4 KCNE2-I57T (rs74315448)

Another variant of the *KCNE2* gene has been associated with diLQTS, but with a moderate level of evidence. The I57T (rs74315448) occurs in the transmembrane segment of the accessory β-subunit. The minor allele frequency of *KCNE2*-I57T (rs74315448) ranges from 0% in East Asian and European Finish individuals to 0.1% in Latinos (Table 3). The I57T was found to be associated with diLQTS in two candidate gene clinical studies (Hideki Itoh et al., 2016; Sesti et al., 2000), and four studies with experimental evidence supporting functional effects (Abbott et al., 1999; McCrossan, Roepke, Lewis, Panaghie, & Abbott, 2009; Sesti et al., 2000;

Tinel et al., 2000). The *KCNE2* gene was also assessed in several other clinical studies, but I57T was not found in any of the diLQTS cases.(Chevalier et al., 2001; Itoh et al., 2009; Kaab et al., 2012; Makita et al., 2002; Paulussen et al., 2004; Sanchez et al., 2016; Weeke et al., 2014). Experimental evidence supporting functional effects of the *KCNE2*-I57T in the absence of drug was first published by Abbott et al 1999 (Abbott et al., 1999) and then Sesti et al, 2000 (Sesti et al., 2000). Abbott et al evaluated the biophysical changes of the hERG channels when co-expressed with I57T. The variant diminished K^+ flux compared to wild type channels in *CHO cells* (Abbott et al., 1999). *A year later,* Sesti et al, (Sesti et al., 2000) characterized the effect of I57T on hERG channels using a heterologous expression system with transient transfection of CHO cells. They showed that channels containing the I57T variant had decreased IKr currents (Sesti et al., 2000), consistent with the Abbott et al *in vitro* results. These results were replicated in the same year by Tinel et al in 2000 (Tinel et al., 2000). The co-expression of *KCNE2* I57T or the wild type with *KCNQ1* and *KCNE2* in COS cells showed that I57T also reduced the K^+ current carried by the *KCNQ1* channel (Tinel et al., 2000). By reducing both IKr and IKs currents, the I57T is likely to participate in the reduction of the cardiac 'repolarization reserve'(Roden, 1998), thereby predisposing carriers to TdP after administration of QT prolonging drugs.

In candidate gene clinical studies, I57T has been found as a predisposing factor for diLQTS. In the Sesti et al, 2000 (Sesti et al., 2000) study previously described, I57T was found in a patient with a prolonged QT interval induced by the histamine H1 receptor antagonist oxatomide (N=98), that was not found in controls (N=1010) (Sesti et al., 2000). I57T was also one of the variants found in the cohort of 188 subjects diagnosed with acquired QT prolongation

in the study published by Itoh et al in 2016. (Hideki Itoh et al., 2016) However, it was not specified if I57T was found in the diLQTS cases, as opposed to other causes of acquired LQTS.

2.13.2 Variants with Limited Evidence

We identified 106 additional variants that have been associated with diLQTS in clinical studies, albeit with limited strength of evidence (Supplemental Table 2). The type of evidence that is most commonly missing for these variants are a lack of replication and/or experimental evidence for functional validation. A large proportion of the variants with limited evidence come from only two studies. Twenty eight of these variants came from the study by Ramirez et al in 2013 (Ramirez et al., 2013). A strength of the Ramirez et al study is that it focused on a clinical outcome: drug-induced TdP (31 cases). The Ramirez et al study was also one of the most comprehensive studies, in that it screened 79 genes important for regulating heart rhythm. Therefore the Ramirez et al study could have identified so many novel variants for diLQTS because it was so comprehensive, and those novel variants just have not been replicated and/or functionally validated yet.

Thirty-six of the variants with limited evidence come from the study by Itoh et al in 2016 (Hideki Itoh et al., 2016). That study screened five of the cLQTS genes in 188 patients with acquired LQTS. Patients were symptomatic (TdP, syncope, cardiac arrest, or ventricular fibrillation) or had marked QTc prolongation. The sample size for that study was relatively large, and thus it may have had more power to identify novel variants compared to most other studies that had smaller sample sizes. Therefore like the study by Ramirez et al, those novel variants just may not have been replicated and/or functionally validated yet. Notably, the study by Itoh et al focused on acquired LQTS and not specifically drug-induced LQTS (although 68% of the cases had drug exposure).

Chapter 3 Genetic Risk Factors for Drug-Induced Long QT Syndrome: Findings from a Large Real-World Case-Control Study.

In this chapter, the results from Aim 1 are presented. These results were published with my mentor in (Ana I. Lopez-Medina et al., 2024). My role was development of data collection, data management and analyses, interpretation of results, lead manuscript writing. The journal specifically notes “Express permission is not required for this purpose, authors are able to re-use or adapt their article for use in their thesis/dissertation, provided a suitable acknowledgement to the original publication is included (see "Acknowledgement" section below). Authors may also deposit their thesis/dissertation in an online, institutional repository if required by their institution.” In the Acknowledgement section, the journal notes: “The acknowledgement line should state the following information: Adapted from Future Oncol. (2007) 3(5), 569-574 with permission of Future Medicine Ltd.” (Future Medicine)

3.1 Background

The QT interval on an electrocardiogram (ECG) measures the time period it takes the ventricles to complete their electrical excitation spanning between the depolarization and repolarization phases of its cardiomyocytes action potentials.(Drew et al., 2010; Roden, 2004) QT interval depends on heart rate and is often corrected (QTc) with various possible methods,(Desai, Li, Desta, Malik, & Flockhart, 2003) with the Bazett method being the most commonly used in clinical practice.(Luo, Michler, Johnston, & Macfarlane, 2004) Normal QTc

interval ranges are approximately 350 to 450 milliseconds (msec) for adult males and 360 to 460 msec for adult females.(Patel et al., 2010) QTc intervals ≥ 500 msec increase the risk of experiencing cardiac events, such as syncope, aborted cardiac arrest, *torsades de pointes* (TdP), or sudden cardiac death, by tenfold. (Goldenberg et al., 2011) QT prolongation can be either congenital (cLQTS) or acquired. cLQTS occur in approximately 1 in 2,000 individuals, and it is caused by specific genetic mutations in ion channels that are involved in the depolarization and repolarization phases of the ventricular action potential.(Adler et al., 2020) Acquired long QT syndrome is primarly caused by several commonly used FDA-approved drugs, including antiarrhythmics, antibiotics, antipsychotics, and antidepressants.(CredibleMeds, 2013) Drug-induced long QT syndrome (diLQTS), characterized by delayed cardiac repolarization due to drugs, primarily results from the inhibition of the rapid potassium inward current (I_{Kr}) from the Kv11.1 potassium channel.(Roden, 2019) This susceptibility is attributed to the larger inner cavity,(del Camino et al., 2000) aromatic residues,(Mitcheson et al., 2000) and hydrophobic pockets (Wang & MacKinnon, 2017) in the Kv11.1 channel. dILQTS is a potentially life-threatening adverse reaction that can ultimately lead to TdP. diLQTS has also been associated with significantly longer hospital stay (11.5 vs. 5.5 days), and tripled risk of all-cause in-hospital mortality among critically ill patients. (Pickham et al., 2012) Although clinical risk factors for diLQTS have been identified, such as female sex, hypokalemia, hypomagnesemia, and congestive heart failure,(Roden, 2004) they do not explain all risks for diLQTS.(Khatib, Sabir, Omari, Pepper, & Tayebjee, 2021) Therefore, there is a critical need to identify additional risk factors of diLQTS, so that serious arrhythmias and sudden death can be prevented in patients.

Current evidence strongly suggests that genetics plays a role in the risk of diLQTS.(A. I. Lopez-Medina et al., 2022; Niemeijer et al., 2015; Strauss et al., 2017) A family study revealed

significantly higher risk of diLQTS in first-degree relatives of patients with cLQTS compared to the general population.(Kannankeril et al., 2005) Despite the shared clinical manifestations of cLQTS and diLQTS, recent research indicates that cLQTS mutations are identified in only a minority of diLQTS cases (approximately 10–20%). (H. Itoh et al., 2016) This suggests that other genetic variants, characterized by smaller effect sizes and not associated with monogenic cLQTS, likely contribute to the occurrence of diLQTS. According to a recent position statement by the European Society of Cardiology, the heritability of QT interval duration in the general population is about 35%, (Magavern et al., 2022) but the heritability estimate of diLQTS remains unknown. Candidate gene studies have primarily focused on variants affecting cardiac ion channels (e.g., *KCNE1*-D85N, *KCNE2*-T8A, *SCN5A*-L1825P, *SCN5A*-G615E, *KCNE2*-I57T, *KCNE1*-D76N), and several uncommon variants (minor allele frequency (MAF) <5%) have been associated with diLQTS.(A. I. Lopez-Medina et al., 2022; Niemeijer et al., 2015) However, these previous studies had several limitations that hampered the clinical utility of the pharmacogenetics of diLQTS, such as small sample size, lack of replication, and/or assessment in real-world clinical settings. Therefore, this study aims to address the aforementioned limitations by assessing the independent association of candidate genetic variants in cardiac ion channels with the risk of diLQTS in a large observational case-control study in real-world clinical settings.

3.2 Materials & Methods

3.2.1 Study design

This is a single-center, retrospective case-control study using clinical and genomic data from the Michigan Genomics Initiative (MGI).(Zawistowski et al., 2023) MGI is the genomic biobank that integrates whole genome array & imputed genotype data with electronic health

records (EHR) at the University of Michigan Health System (also called "Michigan Medicine"). MGI participants are primarily recruited while awaiting a diagnostic or interventional procedure either at a preoperative appointment or on the day of their operative procedure at Michigan Medicine. Eligible patients aged 18 years or older who were prescribed at least one dose of a high-risk QT-prolonging drug (see "Drug Exposure" section below for more details) between March 1st , 2001 and September 30th, 2022 were selected from MGI for this study. QTc data was available starting in 2012. Patients were excluded from the study if they: (i) were diagnosed with congenital long QT syndrome (cLQTS); (ii) had any QTc $\geq$ 500 msec before any treatment with a high-risk QT-prolonging drug; (iii) were diagnosed with left bundle branch block; and/or (iv) used a pacemaker. The study was carried out in accordance with the Declaration of Helsinki and was approved by the local Institutional Review Board with a waiver of informed consent.

3.2.2 Subject Selection

Patients included in the study were selected from Michigan Medicine's EHR with MGI genetic data. (Figure 3.1) The initial eligibility criteria involved individuals who had been prescribed at least one dose of a high-risk QT-prolonging drug (Table 3.1) during the study period. Subsequently, we refined the patient sample by excluding those without any ECG measurements. Two ECGs, i.e., both before and during a prescription with a high-risk drug, was not a requirement for inclusion in the study. A single ECG during the prescription for a high-risk drug was sufficient for inclusion in the study, since QTc >500 msec is an established risk factor for severe arrythmias. Moreover, most patients did not have an ECG measured prior to a prescription for a high-risk drug. Pediatric patients were excluded from the study. Given our focus on diLQTS, we also excluded patients with a QTc > 500 msec before any treatment with a high-risk QT-prolonging drug, with a diagnosis of congenital long QT syndrome, or patients who

were not prescribed a QTc-prolonging drug at the time of their maximum QTc values (sometimes the maximum QTc was measured during gaps in time between prescriptions for QT prolonging drugs). Additionally, considering the impact of pacemakers and left bundle branch block on QT interval measurement accuracy, we also excluded patients with these conditions from our sample. In order to ensure independence among the participants, pairwise genetic relatedness was also considered during patient selection. Patients with close relatives within the sample (kinship coefficients greater than 0.125, i.e., first or second-degree relatives) were randomly selected from each related pair and excluded. Additionally, 59 patients were also excluded because, even though they were genotyped by MGI, their genotype data did not meet quality control standards. The genotype data for the three candidate variants analyzed: *KCNE1*-D85N, *KCNE2*-I57T, and *SCN5A*-G615E (see the "Candidate variant selection" section for detail) were checked across all racial groups. However, these variants were not found in any non-white race groups. Consequently, these three variants were exclusively analyzed within the white patient subgroup in our sample, comprising a total of 6,083 patients.

3.2.3 Data Collection

Patients were initially identified using the DataDirect system, (Data Office for Clinical & Translational Research) a self-serve tool that enables access to discrete clinical data, such as demographics, anthropometry, vital signs, diagnoses, procedures, exams, medications (ordered and administered), and labs (ordered and results). To ensure compliance with exclusion criteria, the Electronic Medical Record Search Engine (EMERSE) was utilized to search through clinical notes (dictated or typed) for terms, (Hanauer, Mei, Law, Khanna, & Zheng, 2015) from cardiology to pathology. Data collection focused around two points in time: the start date for the first prescription for a QT prolonging drug (Table 3.1), and the index date was defined as the

date of the highest QTc value (Bazett) measured during any high-risk QT prolonging drug prescription. Baseline refers to the time period prior to the start of any prescription for a QT prolonging drug. Medications were collected within a 1-year window around the index date. Age was determined at the index date. The identification of comorbidities relied on ICD-9 and 10 diagnosis codes and lab values, subsequently calculated using Elixhauser and Charlson scoring systems, as previously described. (Wasey, 2017)

3.2.4 Drug exposure

A list of high-risk QT-prolonging drugs sourced from the evidence-based list compilation in CredibleMeds,(CredibleMeds, 2013) a federally-funded, expert-curated, nonprofit website dedicated to foster safe medication practices. CredibleMeds classifies medications into conditional, possible, or known risk for TdP**.** To prioritize drugs with the highest level of evidence of association with TdP risk, we selected only those categorized as known risk for TdP**.** The high-risk QT-prolonging drugs list, detailed in Table 3.1, guided patient selection and subsequent analysis. Medication records were collected regardless of route of administration, formulation, dose, or frequency. Therefore, exposure to high-risk QT-prolonging drugs was defined as the presence or absence of any listed medication in Table 3.1 within the patient's medication list. Additionally, drug-drug interactions with these QT-prolonging drugs were defined as concurrent and systemic use of either cytochrome P450/p-glycoprotein (CYP/p-gp) inhibitors or inducers known major interaction potential, as outlined by Micromedex®. (Merative Micromedex)

3.2.5 Outcomes

The primary outcome was the presence of QTc prolongation, defined as a change of >60 msec from the baseline QTc and/or an absolute QTc value ≥500 msec while patients were prescribed any high-risk QT-prolonging drugs. Controls had a maximum QTc values <500 msec during their prescription(s) for QT prolonging drug(s). The QTc interval measurements were automatically recorded using a computer-based, FDA-approved electrocardiogram (ECG) system (General Electric [GE] MUSE™ Cardiology Information System),(Sorajja, Bhakta, Scott, Altemose, & Srivathsan, 2010) which is the standard system utilized in routine clinical practice at Michigan Medicine. The QT intervals measurements were obtained from an average of across the 12-leads of the ECG,(Sorajja et al., 2010) with the primary outcome corrected for heart rate using the Bazett formula ($QTc = QT/\sqrt{RR}$). However, recognizing that Bazett's formula tends to overestimate QT duration, (Chiladakis et al., 2012) sensitivity analyses were also conducted using two additional QT correction methods: Fridericia ($QTc = QT / RR^{1/3}$) (FRIDERICIA, 1920) and Framingham ($QTc = QT + 0.154 * (1 - RR)$).(Sagie, Larson, Goldberg, Bengtson, & Levy, 1992) Additionally, if the QRS complex exceeded 120 msec, further QTc correction was performed using QTc=QTc-(QRS-100), in line with established literature.(Lester, Paglialunga, & Johnson, 2019)

3.2.6 Candidate variant selection

A semi-quantitative scoring system to evaluate the strength of evidence for pharmacodynamic genetic variants as risk factors for diLQTS (i.e., characterized as limited, moderate, strong, or definitive evidence) was established.(A. I. Lopez-Medina et al., 2022) Among the evaluated variants, one displayed definitive evidence (*KCNE1*-D85N*)*, one exhibited strong evidence (*KCNE2*-T8A), while four showcased moderate evidence (*SCN5A*-L1825P, *SCN5A*-G615E, *KCNE2*-I57T, *KCNE1*-D76N). The rest of the 107 genetic variants had limited

evidence. For our study, we specifically selected the genetic variants with at least moderate strength of evidence for association with diLQTS, covered by MGI genotyping or imputation, totaling three: *KCNE1*-D85N, *KCNE2*-I57T, and *SCN5A*-G615E.

3.2.7 Genomic data

Genomic data were made available by the MGI, as previously published.(Zawistowski et al., 2023) Briefly, all genotyping was performed at the UM Advanced Genomics Core lab with standard quality checks,(Zajac et al., 2019) and using Illumina Infinium CoreExome v12.1 bead arrays® (Illumina, San Diego, CA). Quality checks were routinely conducted in batches of genotyped samples, leading to samples exclusions from the study based on the following criteria: participant withdrawal, genotype-inferred sex mismatch or missing self-reported sex, atypical sex chromosomal aberration, high kinship coefficient (≥0.45) with a different sample, sample-level call-rate below 99%, technical duplicates or twin sample with higher call rate, contamination level exceeding 2.5%, call rate on any individual chromosome ≤95%, or sample processed in a flagged DNA extraction batch due to technical issue. Imputation was performed using the world-famous Michigan Imputation Server (Michigan Imputation Services)with the Haplotype Reference Consortium r1.1 (HRC) (McCarthy et al., 2016) as the reference panel. Standard post-imputation filters were applied to remove poorly imputed variants ($r^2 < 0.3$ and MAF < 0.01%) to ensure a high-quality dataset. The genotypes for all three candidate genetic variants analyzed were imputed. Pairwise genetic relatedness among all patients in the sample was analyzed using Kinship-based INference for GWAS (KING) v2.1.3.(Manichaikul et al., 2010) Patients with close relatives within the sample, with kinship coefficients greater than 0.125 (i.e., first or second degree relatives), were randomly selected from each related pair and excluded from the sample.

3.2.8 Statistical analysis

Patients were stratified into two groups based on the primary outcome: without prolonged QTc and with prolonged QTc. Categorical variables were represented as counts and percentages and compared between subgroups using the χ^2 test (or Fisher's exact test, when necessary). Continuous variables distribution was assessed using the Kolmogorov-Smirnov test and visual inspection of distribution plots. Normally distributed continuous variables were represented as mean ± standard deviation (SD) and compared between groups using the Student's t-test. Non-normally distributed continuous variables were represented as median ± interquartile range (IQR) and compared between groups using the Mann-Whitney U Test.

Univariable logistic regression models were used to assess the independent association of clinical variables with prolonged QTc presence. Variables showing significance ($p<0.05$) between the groups were considered as clinical predictors, and were included in logistic regression models as covariates for genetic variants. A propensity score was calculated for each patient using all variables with $p<0.05$.(D'Agostino, 1998) Matching without prolonged and prolonged QTc groups 1:1 by propensity score formed a new propensity-matched sample. The MatchIt package for RStudio was used to calculate propensity scores and match patients. Subsequently, multivariable logistic regression models were used to assess the association of each of the three genetic variants with diLQTS risk. Odds ratios (OR) and respective 95% confidence intervals (95% CI) were calculated for the unmatched sample in unadjusted and propensity score adjusted models (Models 1 and 3), and for the propensity-matched sample in unadjusted models (Model 2). Model 3 was considered the primary results due to its superior statistical power compared to Model 2. Unlike Model 1, Model 3 incorporated adjustments with clinical covariates. In consideration of the low MAF for each variant, the three candidate genetic

variants was determined *a priori* to be tested using the dominant genetic model (i.e., major allele homozygotes *versus* heterozygotes + minor allele homozygotes). To minimize potential population stratification, self-identified race groups were analyzed separately. A Bonferroni-corrected p-value of 0.0167 (0.05÷3) for each candidate genetic variant was *a priori* set as the threshold for statistically significance. Given this Bonferroni-corrected alpha, total sample size available for analysis (n = 6,083), event rate defined by the Bazett QT correction method, and MAF observed for each variant Table 3.2, this analysis had 80% power to detect odds ratios of 2.21, 9.03 and 44.85 for *KCNE1*-D85N, *KCNE2*-I57T and *SCN5A*-G615E, respectively. Furthermore, an exploratory analysis was conducted based on specific drug class and individual drugs, if at least 500 patients were treated with any of the high-risk QT-prolonging drugs. All statistical analyses were performed using R version 4.2.2.

3.3 Results

3.3.1 Baseline characteristics

A total of 6,989 eligible patients who met the study inclusion/exclusion criteria (Figure 3.1), 6,083 (87.0%) self-reported to be White, 565 (8.1%) African American, 111 (1.6%) Asian, 46 (0.7%) American Indian or Alaska Native, 2 (0.03%) Native Hawaiian or Other Pacific Islander, and 182 (2.6%) unknown race. Genotype data for all three candidate variants, *KCNE1*-D85N, *KCNE2*-I57T, and *SCN5A*-G615E, were checked across all racial groups, but none of these variants were found in any of the non-white race groups. This finding is consistent with the reported MAF in gnomAD, where these three variants are most frequent among European populations and rare in non-European populations. Consequently, these three variants were exclusively analyzed within the white patient subgroup in our study. The genotype and allele

frequencies for these three genetic variants among white patients are shown in Table 3.2. All genotype frequencies were in Hardy-Weinberg equilibrium with *p*-values >0.05, and all of the allele frequencies were similar to those previously reported for Europeans in gnomAD. The primary outcome of QTc prolongation occurred in 12.0% of all patients, with a prevalence of 11.4% in women and 12.8% in men (p = 0.103 for sex-based difference). The clinical baseline characteristics of both groups are shown in Table 3.3**.** Overall, before propensity score matching, patients with prolonged QTc exhibited a significantly higher prevalence of electrolyte disturbances (hypokalemia, defined as potassium<3.5 mEq/L and hypocalcemia, defined as calcium <8.5mg/dL), renal (chronic kidney disease), liver and cardiovascular conditions (congestive heart failure, coronary artery syndrome, hypertension, peripheral vascular disease, arrhythmias and history of myocardial infarction and stroke), chronic obstructive pulmonary disease, and diabetes mellitus compared to patients without prolonged QTc. Moreover, patients with prolonged QTc had a significantly higher prevalence of history of alcohol consumption, use of loop diuretics, digoxin and beta-blockers, than those without prolonged QTc. Elixhauser and Charlson comorbidities scores were also significantly higher in patients with prolonged QTc than those without prolonged QTc. In contrast, patients without prolonged QTc had a higher prevalence of concomitant usage of over two QTc prolonging drugs, compared to those with prolonged QTc. There were no significant differences observed in any of the other variables assessed such as age, sex, body mass index, hypothyroidism, or cancer. For the subset of patients that had baseline ECG data available (N=433, 7% of total sample size), the median number of days [interquarile range] between the baseline QTc and the index date was 610 [1011] and 844 [1458] for those without QTc and with prolonged QTc, respectively (p=0.519). The time between the start date of the QT prolonging drug and the index date was also similar between the

two groups (Median [IQR]: 1622 [1395 for those without QTc prolongation and 1607 [1457] for those with QTc prolongation; p=0.964). For the purpose of matching and adjustment, age was dichotomized at 68 years old, due to this age has been previously associated as an independent risk factor for diLQTS in hospitalized patients.(Tisdale et al., 2013) After applying a 1:1 propensity score matching, 733 patients were included in each group, effectively eliminating all of the significant differences in the clinical baseline characteristics. In addition, no significant differences were found in baseline clinical characteristics between carriers and non-carriers of the three candidate genetic variants (except for age and use of digoxin, which were significantly higher in the carriers of *SCN5A*-G165E. Additionally, non carriers of *KCNE1*-D85N exhibited a significantly higher age compared to carriers Table 3.5.

3.3.2 Drug exposure

Out of the forty drugs marketed in the US that are classified as known risk for TdP by CredibleMeds, twenty-seven (67.5%) were prescribed in our sample Table 3.4. Dofetilide, dronedarone, sotalol, and papaverine were prescribed significantly more frequently on the index date for those who had prolonged QTc than those without prolonged QTc. In contrast, azithromycin, ciprofloxacin, propofol, and ondansetron were prescribed significantly more frequently for those without prolonged QTc than those with prolonged QTc.

3.3.3 Associations of candidate genetic variants with the risk of diLQTS

The association of *KCNE1*-D85N (rs1805128) and *KCNE2*-I57T (rs7415448) with the risk of diLQTS, as defined by Bazett's QT correction method is displayed in Figure 3.2. *KCNE1*-D85N met the Bonferroni-corrected level of statistical significance ($p < 0.0167$) in all models Model 1 ($p = 0.006$), Model 2 ($p=0.009$) and Model 3 ($p = 0.001$). The odds ratios for *KCNE1*-

D85N were 1.9, 2.9, and 2.2 in Models 1, 2, and 3, respectively. *KCNE2*-I57T did not meet the Bonferroni-corrected level of statistical significance in any of the models using the Bazett QTc correction method ($p > 0.1$ in all 3 models). The odds ratios for *KCNE2*-I57T were 2.4, 1.5, and 1.5 in Models 1, 2, and 3, respectively. Only 4 total patients carried the *SCN5A*-G615E (rs12720452) variant, and none of the carriers had prolonged QTc. Thus, *SCN5A*-G615E was not included in Figure 3.2. However, complete regression results for all 3 variants are displayed in Table 3.6.

Since Bazett's formula for correcting QT interval tends to overestimate the QT duration, sensitivity analyses were also performed using the Fridericia and Framingham QT correction methods. The findings are shown in Table 3.7. Regardless of the QT correction method, none of the 4 *SCN5A*-G615E carriers were in the prolonged QTc group. Therefore, the results for *SCN5A*-G615E were similar across the 3 QTc correction methods. However, the results for *KCNE1*-D85N and *KCNE2* I57T slightly differed among the 3 QTc correction methods. *KCNE1*-D85N did not meet the Bonferroni-corrected level of statistical significance in any of the three models with Fridericia QT correction method or Model 1 or Model 2 with the Framingham correction method ($p \geq 0.04$). However, *KCNE1*-D85N and diLQTS risk was still statistically significant in Model 3 with the Framingham QT correction method (OR = 2.25 (; 95% CI = 1.11-4.16; $p = 0.015$). In contrast, even though *KCNE2*-I57T was not statistically significant in any of the models using the Bazett correction method, *KCNE2* I57T was statistically significant in Model 1 with the Fridericia QT correction method (OR = 6.53; 95% CI = 1.74-20.80; $p = 0.002$)and Models 1 and 3 with the Framingham QT correction method (*KCNE2* I57T in Model 3 with Framingham: OR = 6.70; 95% CI = 1.51-24.85; $p = 0.006$).

Given that *KCNE1*-D85N was the most frequent of the 3 variants, we also conducted exploratory analyses for *KCNE1*-D85N by specific drug class and individual drugs if at least 500 patients were treated with any of the individual drugs. The findings are shown detailed in Figure 3.3. Overall, all p-values exceeded 0.3 across various individual drugs (ondasentron, azithromycin, propofol, ciprofloxacin) and drug classes (antiarrhythmics, antibiotics, antidepressants/antipsychotics, antifungals). Although these subgroup analyses are underpowered, these results suggest that *KCNE1*-D85N does not affect risk differently among different drugs/drug classes.

3.4 Discussion

To the best of our knowledge, this is the largest study that investigated the association of candidate genetic variants with the risk of diLQTS. The largest previous candidate gene studies with a similar endpoint all had <100 cases.(Itoh et al., 2009; Sesti et al., 2000; P. Yang et al., 2002) We analyzed 3 candidate genetic variants with moderate-to-high level of prior evidence for their association with diLQTS in our previous literature review and had genotype data available in our study: *KCNE1*-D85N, *KCNE2*-I57T and *SCN5A*-G615E. (A. I. Lopez-Medina et al., 2022) Our results further support *KCNE1*-D85N as significant risk variant for diLQTS. The study had insufficient power to provide precise estimates for *KCNE2*-I57T, and *SCN5A*-G615E.

3.4.1 KCNE1-D85N and diLQTS

KCNE1 encodes the beta subunit of voltage-gated potassium channels, a pivotal player in modulating the alpha subunit of the cardiac channel encoded by KCNQ1. Together, KCNQ1 and KCNE1 form a complex that generates the slowly activating potassium current, also known as I_{Ks}. This current, in conjunction with the rapidly activating potassium current (I_{Kr}), results in

cardiac repolarization. D85N leads to a substitution of asparagine with aspartic acid in the C-terminal of the beta subunit. When examined in heterologous expression models, *KCNE1*-D85N abolished up to 50% of KCNQ1-encoded currents in the absence of drugs.(Westenskow et al., 2004) Additionally, *in vitro* studies have found that the cell membrane expression of *KCNE1*-D85N is 20% lower than that of the wild-type. (Sakata et al., 2014) While there is no direct effect between D85N and drugs, the susceptibility to diLQTS in D85N carriers may be attributed to a diminished "repolarization reserve". Repolarization reserve refers to a lack of redundancy in mechanisms compensating for the inhibition of Kv11.1.(Roden, 1998) These *in vitro* findings have been translated in both clinical candidate gene association studies (Kaab et al., 2012; Martinez-Matilla et al., 2019; Paulussen et al., 2004) and a whole-exome sequencing study,(Weeke et al., 2014) thereby confirming the association between *KCNE1*-D85N and diLQTS. On the other hand, several other candidate gene studies did not identify thee association between the D85N variant and diLQTS cases.(Aberg et al., 2012; Avery et al., 2014; Behr et al., 2013; Chevalier et al., 2001; Corponi et al., 2019; Itoh et al., 2009; Makita et al., 2002; Roberts et al., 2017; Spellmann et al., 2018; Zerdazi et al., 2019) This lack of association may be attributed to the small sample size of cases in the negative studies (largest sample size consisting of only 77 cases) and the low MAF of this variant (only ~1% in Europeans). Consequently, it is plausible that these studies might be underpowered and have introduced type 2 errors. By analyzing this variant in a much larger sample size herein, we heighten the confidence that the association between *KCNE1*-D85N and diLQTS is not either spurious or a false-positive, especially after meeting the Bonferroni corrected level of significance. Moreover, an added strength of our investigation lies in utilizing authentic patient data from real-world clinical settings, diverging from the highly controlled clinical trial in a previous study.(Aberg et al.,

2012) This improvement markedly reinforces the relevance and applicability of our findings in real-world clinical settings.

3.4.2 KCNE2- I57T and diLQTS

The *KCNE2* gene, very similar to *KCNE1*, encodes the beta subunit of voltage-gated potassium channels including the hERG channel, which generate IKr.(Schmitt et al., 2014) The I57T variant occurs in the transmembrane segment of the accessory β-subunit, and can decrease I_{Kr} by 34%.(Sesti et al., 2000) Previous candidate gene (Hideki Itoh et al., 2016; Sesti et al., 2000) and *in vitro* studies supporting functional effects (Abbott et al., 1999; McCrossan et al., 2009; Sesti et al., 2000; Tinel et al., 2000) have found *KCNE2*-I57T to be significantly associated with diLQTS. However, there are other studies that have yielded contradictory findings regarding this variant. (Chevalier et al., 2001; Itoh et al., 2009; Kaab et al., 2012; Makita et al., 2002; Paulussen et al., 2004; Sanchez et al., 2016; Weeke et al., 2014) In our study, *KCNE2*-I57T did not meet the Bonferroni-corrected level of statistical significance in any of the models using the Bazett QTc correction method. However, some of the sensitivity analyses using the Fridericia and Framingham QTc correction methods were statistically significant. These discrepancies arising from our findings for the I57T variant could be explained by the decrease in statistical power due to its lower allele frequency (~0.1% MAF in Europeans). In contrast to the D85N variant, the occurrence of the I57T variant was notably rarer, approximately 9.5 times less frequent within our study. Therefore, whether or not the I57T variant is a definitive risk factor for diLQTS remains unclear. To determine the ultimate implication of this variant in diLQTS, it is imperative to perform further investigations encompassing sample sizes that exceed that of our current study.

3.4.3 SCN5A-G615E and diLQTS

SCN5A encodes the α-subunit of the cardiac sodium channel, crucial for the rapid depolarization upstroke of the cardiac action potential.(Aronsen et al., 2013) The G615E occurs in the intracellular domains of the sodium channel α-subunits. However, there have been conflicting findings regarding its functional impact in experimental studies.(Albert et al., 2008; P. Yang et al., 2002) While one study in *Xenopus* oocytes indicated that G615E reduced recovery times from fast inactivation, affecting cardiac sodium channel activity,(Albert et al., 2008) a contradictory study by Yang *et al*. revealed that this variant did not alter the amplitude or voltage dependence of sodium channel activation/inactivation in mammalian expression vectors, suggesting it played no role in the diLQTS phenotype.(P. Yang et al., 2002) Several earlier candidate gene association studies reported an association of G615E with diLQTS.(Ramirez et al., 2013; Sanchez et al., 2016; P. Yang et al., 2002) However, in various other candidate gene association studies, G615E was not found in any diLQTS cases,(Itoh et al., 2009; Kaab et al., 2012; Makita et al., 2002; Napolitano et al., 2000; Paulussen et al., 2004; Sanchez et al., 2016; Spellmann et al., 2018; Splawski et al., 2002; Watanabe et al., 2017; Zerdazi et al., 2019) possibly due to its rarity (~0.05% MAF in Europeans). Previous pharmacogenomic analysis indicated that rare genetic variants might have much larger effect sizes than common ones.(Ramsey et al., 2012) Therefore, while a larger expected effect size for G615E might compensate for lower statistical power due to its very low MAF, our study's power estimate demonstrated that even a large sample size like ours was severely underpowered to detect a significant association for G615E. Approximately 14,654 patients are needed detect an OR of 10 with 80% power. Thus, more studies are needed to confirm the association of *SCN5A*-G615E with diLQTS risk. Despite the very small number of carriers, it is worth noting that none of the four G615E carriers in our study exhibited prolonged QT.

3.4.4 Clinical Implication

The ultimate goal concerning *KCNE1*-D85N, involves its integration into clinical pharmacogenetic testing to prevent diLQTS. Currently, there is a lack of clinical practice guidelines or recommendations from authorities like the US Food and Drug Administration and Clinical Pharmacogenetics Implementation Consortium regarding the management of carriers of these variants. A recent position statement by the European Society of Cardiology emphasized the relevance of personalized treatment and risk stratification for diLQTS,(Magavern et al., 2022) but also stressed the need for comprehensive validation studies to effectively translate *KCNE1*-D85N into clinical practice. Conducting randomized clinical trials to showcase the clinical utility of pharmacogenetic testing for these variants is probably not feasible due to their low MAF.(Luzum et al., 2021) Despite this, the wealth of evidence from comprehensive studies aligned with our large-scale investigation suggest that *KCNE1*-D85N warrant formal evaluation for publication in a clinical practice guideline, and possibly for clinical implementation. The recent, landmark clinical trial published by the Ubiquitous Pharmacogenomics Consortium demonstrated the feasibility and efficacy of a pharmacogenetic testing panel in reducing the risk of severe adverse drug reactions,(Swen et al., 2023) indicating that pharmacogenomic panel incorporating D85N would be feasible with minimal upfront costs, and lifelong applicable for the patient. This approach is suggested to minimize prescription complications and to improve health care with substantial cost-benefit perspective.

3.4.5 Limitations

This study has several limitations that require careful consideration. Primarily, it was a retrospective observational study conducted within a single health system, possibly restricting the generalizability of our findings. Our reliance on comorbidity data extracted from the EHR

using ICD-9 and ICD-10 codes is known to be not entirely accurate. Patients were categorized based on self-identified race groups instead of ancestry, although there is a very high agreement between self-identified white patients in MGI and European ancestry.(Zawistowski et al., 2023) MGI participants are predominantly recruited before or during surgical procedures; as a result, our sample is not a random representation of patients at Michigan Medicine, potentially introducing selection biases. Additionally, our study was focused solely on drugs posing a high risk of TdP, whereas there exist over 100 drugs with a conditional risk for TdP, (CredibleMeds, 2013) which were not included in our analysis. Furthermore, the data collected regarding patients' medication lists encompassed only prescribed medications, and assessing adherence to these prescriptions directly was not feasible. Our analysis only considered pharmacodynamic variants with at least moderate evidence of association with diLQTS; thus excluding the potential contribution of other pharmacodynamic variants with lower levels of prior evidence as well as pharmacokinetic variants, which might also influence diLQTS risk.(A. I. Lopez-Medina et al., 2022)

While Bazett's formula has been widely adopted in clinical practice and is preferred for long QT syndrome types 1 and 2 (Dahlberg, Diamant, Gilljam, Rydberg, & Bergfeldt, 2021), our study recognizes its tendency to overestimate the number of patients with potentially dangerous QTc prolongation. Notably, the Fridericia and Framingham correction formulae have demonstrated superior rate correction and a better estimate of 30-day and 1-year mortality (Vandenberk et al., 2016). The robustness of our findings is evident in the consistency of associations for *KCNE1-D85N* in Model 3 across the three QT correction methods, as shown in Supplemental Table 3. The results in Model 3 of the Framingham study with Bazett further reinforces the reliability of our conclusions. However, it is worth noting that although the p-value

for D85N with Fridericia in Model 3 did not achieve statistical significance, the odds ration still exceeded 1. While the result did not reach conventional significance levels, the proximity to significance suggests that *KCNE1*-D85N, when using Fridericia, could potentially be a risk factor for diLQTS. Finally, QT prolongation, the focus of our study, serves as a surrogate measure for more severe clinical outcomes, such as TdP and sudden cardiac death.

3.5 Conclusion

This is the largest study of candidate genetic variants in cardiac ion channels associated with the risk of diLQTS to date. *KCNE1*-D85N significantly associated with diLQTS risk, while the study was underpowered to determine associations for *KCNE2*-I57T *and SCN5A*-G615E with diLQTS. Incorporating these findings into a clinical pharmacogenomic program may translate to preventing adverse events.

3.6 Figures

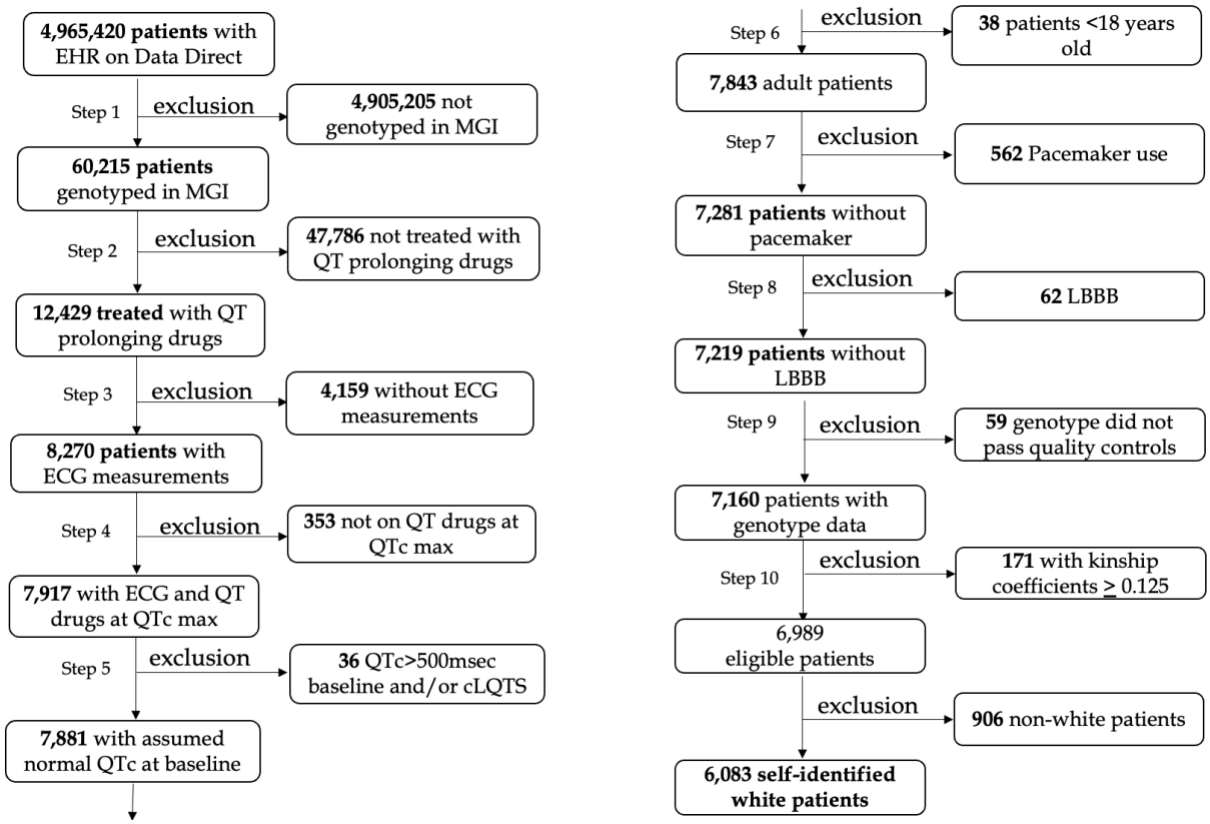

Figure 3.1: Flow chart with patient selection. EHR: Electronic Health Record; MGI: Michigan Genomics Initiative; ECG: Electrocardiogram; cLQTS: congenital long QT syndrome; LBBB: left bundle branch block.

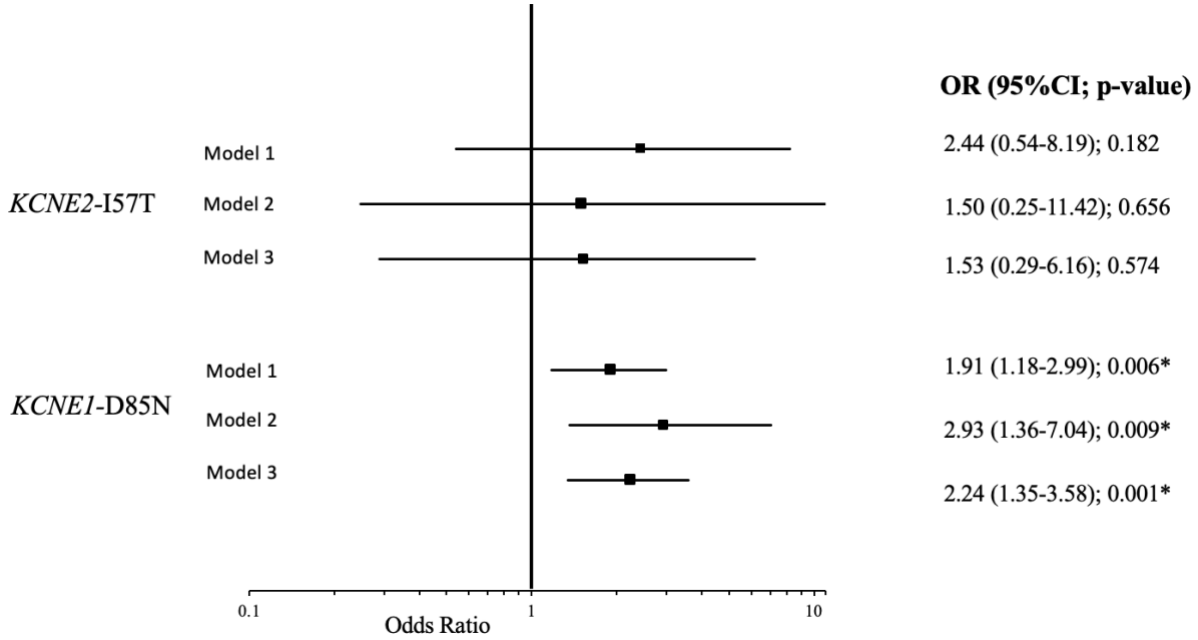

Figure 3.2: Forest plot of the logistic regression models assessing the association of candidate genetic variants with diLQTS in white patients (dominant genetic model). Model 1: Unmatched sample with unadjusted model (total n = 6,083). Model 2: Propensity-matched sample with unadjusted model (total n = 1,466). Model 3: Unmatched sample with model adjusted for propensity score (total n = 6,083). *p<0.0167 (Bonferroni correction for multiple comparisons) The squares represent the odds ratio of the individual candidate gene variant in the three models and the horizontal lines indicate the 95% confidence interval.

3.7 Tables

Table 3.1: List of high-risk QT-prolonging drugs with a known risk of TdP on the U.S. market from CredibleMeds.

Antiarrhythmics Quinidine Sotalol Dofetilide Disopyramide Flecainide Amiodarone Ibutilide Dronedarone Procainamide	**Antibiotics** Clarithromycin Azithromycin Levofloxacin Erythromycin Moxifloxacin Ciprofloxacin	**Antipsychotics** Haloperidol Droperidol Chlorpromazine Pimozine Thioridazine **Antidepressants** Citalopram Escitalopram	**Antifungals** Fluconazole Pentamidine **Cholinesterase inhibitor** Donepezil **Opioid agonist** Methadone
Phosphodiesterase inhibitor Cilostazol Anagrelide	Antimalarial Chloroquine Hydroxychloroquine Anesthetic Propofol Sevoflurane Cocaine	Anti-cancer Oxaliplatin Vandetanib Arsenic trioxide Cesium Chloride Mobocertinib	Vasodilator Papaverine Antiemetics Ondansetron

Table 3.2: Allele frequency, genotype frequency, and Hardy-Weinberg equilibrium (HWE) assessment of the candidate genetic variants for diLQTS among self-reported white patients.

SNPs	**Allele frequency in non-Finnish European population in gnomAD**	**Allele frequency observed in sample**	**Genotype frequencies, n (%)**	**HWE (p-value)**
SCN5A G615E rs12720452	0.0005178	0.000329	CC: 6079 (99.9) CT: 4 (0.1) TT: 0 (0.00)	0.980
KCNE2 I57T rs74315448	0.001045	0.000986	TT: 6071 (99.8) TC: 12 (0.2) CC: 0 (0.00)	0.938
KCNE1 D85N rs1805128	0.01223	0.009206	CC: 5971 (98.1) CT: 112 (1.9) TT: 0 (0.00)	0.469

Table 3.3:Baseline characteristics of patients without prolonged QTc and prolonged QTc overall and by propensity score matched sample of self-reported white patients. BMI: Body mass index; CHF: congestive heart failure; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CrCl: creatinine clearance; DM: diabetes mellitus; HTN: hypertension; MI: myocardial infarction; PVD: peripheral vascular disease. Bolded p-values < 0.05.

Characteristics	**Unmatched sample**		**p-value**	**Propensity-matched sample**		**p-value**
	Without prolonged QTc	**With prolonged QTc**		**Without prolonged QTc**	**With prolonged QTc**	
Patients, n (%)	5,350 (88.0)	733 (12.0)	__	733 (50.0)	733 (50.0)	__
Aged 68 years or older, n (%)	2,967 (55.5)	403 (55.0)	0.838	297 (40.5)	263 (35.9)	0.076
Female, n (%)	2,985 (55.8)	385 (52.5)	0.103	373 (50.9)	385 (52.5)	0.565
Hypokalemia, n (%)	325 (6.1)	103 (14.1)	**<0.001**	86 (11.7)	103 (14.1)	0.212
Hypocalcemia, n (%)	1,005 (18.8)	298 (40.7)	**<0.001**	317 (43.2)	298 (40.7)	0.341
Hypomagnesemia, n (%)	1,059 (19.8)	128 (17.5)	0.149	155 (21.1)	128 (17.5)	0.085
BMI (kg/m^2), mean (SD)	30.3 (7.5)	29.9 (7.9)	0.343	30.7 (7.5)	29.9 (7.9)	0.070
CHF, n (%)	369 (6.9)	148 (20.2)	**<0.001**	137 (18.7)	148 (20.2)	0.509
HTN, n (%)	3,010 (56.3)	495 (67.5)	**<0.001**	509 (69.4)	495 (67.5)	0.465
CAD, n (%)	2,279 (42.6)	387 (52.8)	**<0.001**	393 (53.6)	387 (52.8)	0.794
Hypothyroidism, n (%)	754 (14.1)	105 (14.3)	0.911	119 (16.2)	105 (14.3)	0.345
History of MI, n (%)	374 (7.0)	96 (13.1)	**<0.001**	91 (12.4)	96 (13.1)	0.754
DM, n (%)	1,097 (20.5)	222 (30.3)	**<0.001**	234 (31.9)	222 (30.3)	0.535
DM complicated, n (%)	353 (6.6)	87 (11.9)	**<0.001**	91 (12.4)	87 (11.9)	0.810
History of Stroke, n (%)	529 (9.9)	121 (16.5)	**<0.001**	121 (16.5)	121 (16.5)	1.000
CKD, n (%)	707 (13.2)	184 (25.1)	**<0.001**	202 (27.6)	184 (25.1)	0.313
Severe liver disease, n (%)	82 (1.5)	30 (4.1)	**<0.001**	35 (4.8)	30 (4.1)	0.612
Liver disease, n (%)	598 (11.2)	115 (15.7)	**0.001**	134 (18.3)	115 (15.7)	0.211
COPD, n (%)	429 (8.0)	96 (13.1)	**<0.001**	97 (13.2)	96 (13.1)	1.000
Cancer, n (%)	385 (7.2)	51 (7.0)	0.887	53 (7.3)	51 (7.0)	0.925
PVD, n (%)	458 (8.6)	113 (15.4)	**<0.001**	118 (16.1)	113 (15.4)	0.774
Arrhythmia, n (%)	974 (18.2)	223 (30.4)	**<0.001**	209 (28.5)	223 (30.4)	0.456
History of alcohol consumption, n (%)	189 (3.5)	47 (6.4)	**<0.001**	44 (6.0)	47 (6.4)	0.829
Loop Diuretic, n (%)	659 (12.3)	261 (35.6)	**<0.001**	254 (34.7)	261 (35.6)	0.743
Digoxin, n (%)	21 (0.4)	16 (2.2)	**<0.001**	15 (2.0)	16 (2.2)	1.000
Beta-blockers, n (%)	1,453 (27.2)	341 (46.5)	**<0.001**	350 (47.7)	341 (46.5)	0.676
≥2 QT prolonging drugs, n (%)	2,771 (51.8)	272 (37.1)	**<0.001**	274 (37.4)	272 (37.1)	0.957
Elixhauser score (IQR)	7.0 (13.0)	11.0 (14.0)	**<0.001**	12 (14.0)	11 (14.0)	0.547
Charlson score (IQR)	3 (5.0)	4 (5.0)	**<0.001**	5 (5)	4 (5)	0.727
Days between start date of QT prolonging drug and maximum QTc value (IQR)	1622 (1395)	1607 (1457)	0.964	1739 (1368)	1607 (1457)	0.108

Table 3.4: List of high-risk QT-prolonging drugs prescribed on the index date in our patient sample and their frequency between groups.

Drug class	**Drugs, n (%)**	**Without prolonged QTc n = 5,350 (88.0%)**	**With prolonged QTc n = 733 (12.0%)**	**p-value**
Antiarrhythmics	Disopyramide	7 (0.1)	0 (0.0)	0.604
	Dofetilide	14 (0.2)	24 (2.6)	<0.001
	Dronedarone	2 (0.0)	3 (0.3)	0.021
	Flecainide	40 (0.7)	10 (1.1)	0.212
	Quinidine	0 (0.0)	1 (0.1)	0.137
	Sotalol	39 (0.7)	21(2.3)	<0.001
	Procainamide	2 (0.0)	1 (0.1)	0.357
Antibiotics	Azithromycin	556 (9.5)	26 (2.8)	<0.001
	Ciprofloxacin	607 (10.4)	54 (5.8)	<0.001
	Clarithromycin	4 (0.1)	1 (0.1)	0.521
	Levofloxacin	290 (5.0)	34 (3.7)	0.102
	Moxifloxacin	11 (0.2)	1 (0.1)	1.000
	Erythromycin	11 (0.2)	3 (0.3)	0.426
Antidepressants	Citalopram	414 (7.1)	43 (4.6)	0.007
	Escitalopram	382 (6.5)	49 (5.3)	0.167
Cholinesterase inhibitor	Donepezil	46 (0.8)	10 (1.1)	0.332
Opioid agonist	Methadone	54 (0.9)	10 (1.1)	0.587
Antipsychotics	Haloperidol	66 (1.1)	12 (1.3)	0.787
	Chlorpromazine	9 (0.2)	2 (0.2)	0.655
Antifungals	Fluconazole	388 (6.7)	58(6.3)	0.712
	Pentamidine	2 (0.0)	2 (0.2)	0.093
Antimalarial	Hydroxychloroquine	173 (3)	33 (3.6)	0.378
Anesthetic	Propofol	2,263 (38.8)	275 (29.7)	<0.001
Antiemetic	Ondansetron	4315 (74.0)	537 (58.0)	<0.001
Oncology	Oxaliplatin	16 (0.3)	2 (0.2)	1.000
Vasodilator	Papaverine	38 (0.7)	21 (2.3)	<0.001
Phosphodiesterase Inhibitor	Cilostazol	32 (0.5)	2 (0.2)	0.312

3.8 Appendix

Table 3.5: Baseline characteristics among candidate genetic variants between carriers and non-carriers. BMI: Body mass index; CHF: congestive heart failure; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CrCl: creatinine clearance; DM: diabetes mellitus; HTN: hypertension; MI: myocardial infarction; PVD: peripheral vascular disease. Bolded p-values < 0.05.

Characteristics	***SCN5A* G615E**			***KCNE2* I57T**			***KCNE1* D85N**		
	Carrier rs12720452 (N=4)	**No Carrier rs12720452 (N=6,079)**	**p-value**	**Carrier rs74315448 (N=12)**	**No Carrier rs74315448 N (=6,071)**	**p-value**	**Carrier rs1805128 N (=112)**	**No Carrier rs1805128 (N=5,971)**	**p-value**
Age≥68, n (%)	4 (100.00)	2038 (33.5)	**0.013**	2 (16.7)	2040 (33.6)	0.359	23 (20.5)	2019 (33.8)	**0.004**
Female, n (%)	2 (50.0)	3368 (55.4)	1.000	4 (33.3)	3366 (55.4)	0.151	58 (51.8)	3312 (55.5)	0.496
Hypokalemia, n (%)	1 (25.0)	427 (7.0)	0.253	0 (0.0)	428 (7.0)	1.000	5 (4.5)	423 (7.0)	0.353
Hypocalcemia, n (%)	2 (50.0)	1301 (21.4)	0.203	3 (25)	1300 (21.4)	0.728	27 (24.1)	1276 (21.4)	0.560
Hypomagnesemia, n (%)	0 (0.0)	1187 (19.5)	1.000	2 (16.7)	1185 (19)	1.000	18 (16.1)	1169 (19.6)	0.419
BMI	30.7 (4.8)	30.2 (7.6)	0.872	29.3 (7.0)	30.2 (7.6)	0.647	30.2 (7.6)	31.4 (8.2)	0.160
CKD, n (%)	0 (0.0)	891 (14.7)	1.000	1 (8.3)	890 (14.7)	1.000	20 (17.9)	871 (14.6)	0.404
CHF, n (%)	0 (0.0)	517 (8.5)	1.000	1 (8.3)	516 (8.5)	1.000	11 (9.8)	506 (8.5)	0.737
HTN, n (%)	3 (75.0)	3502 (57.6)	0.642	6 (50.0)	3499 (57.6)	0.771	60 (53.6)	3445 (57.7)	0.436
CAD, n (%)	1 (25.0)	2665 (43.8)	0.636	6 (50.0)	2660 (43.8)	0.774	43 (38.4)	2623 (43.9)	0.283
MI, n (%)	1 (25.0)	469 (7.7)	0.275	0 (0.0)	470 (7.7)	0.617	9 (8.0)	461 (7.7)	0.858
Stroke, n (%)	2 (50.0)	648 (10.7)	0.059	2 (16.7)	648 (10.7)	0.372	11 (9.8)	639 (10.7)	0.885
Liver disease, n (%)	0 (0.0)	713 (11.7)	1.000	2 (16.7)	711 (11.7)	0.643	16 (14.3)	697 (11.7)	0.482
Severe Liver Disease, n (%)	0 (0.0)	112 (1.8)	1.000	1 (8.3)	111 (1.8)	0.200	1 (0.9)	111 (1.9)	0.725
DM, n (%)	1 (25.0)	1318 (21.7)	1.000	3 (25.0)	1316 (21.7)	0.730	25 (22.3)	1294 (21.7)	0.960
DM complicated, n (%)	0 (0.0)	440 (7.2)	1.000	2 (16.7)	438 (7.2)	0.214	9 (8)	431 (7.2)	0.712
Hypothyroidism, n (%)	0 (0.0)	859 (14.1)	1.000	3 (25.0)	856 (14.1)	0.234	845 (14.2)	14 (12.5)	0.719
COPD, n (%)	0 (0.0)	525 (8.6)	1.000	0 (0.0)	525 (8.6)	0.616	4 (3.6)	521 (8.7)	0.079
Cancer, n (%)	0 (0.0)	436 (7.2)	1.000	0 (0.0)	436 (7.2)	1.000	6 (5.4)	430 (7.2)	0.580

PVD, n (%)	0 (0.0)	571 (9.4)	1.000	2 (16.7)	569 (9.4)	0.313	13 (11.6)	558 (9.3)	0.516
History of alcohol consumption, n (%)	0 (0.0)	236 (3.9)	1.000	1 (8.3)	235 (3.9)	0.378	6 (5.4)	230 (3.9)	0.451
Loop Diuretic, n (%)	1 (25.0)	919 (15.1)	0.481	3 (25.0)	917 (15.1)	0.408	17 (15.2)	903 (15.1)	1.000
Digoxin, n (%)	1 (25.0)	36 (0.6)	**0.048**	0 (0.0)	37 (0.6)	1.000	0 (0.0)	37 (1.2)	1.000
Beta blockers, n (%)	2 (50.0)	1792 (29.5)	0.595	6 (50.0)	1788 (29.5)	0.213	32 (28.6)	1762 (29.5)	0.912
≥2 QT-prolonging drugs, n (%)	2 (50.0)	3041 (50.0)	1.000	3.0 (25.0)	3040 (50.1)	0.092	60 (53.6)	2983 (50.0)	0.508
Elixhauser score (IQR)	7.2 (5.1)	8.4 (9.3)	0.905	11.6 (15.4)	8.4 (9.3)	0.991	8.4 (9.3)	7.8 (9.9)	0.2448
Charlson score (IQR)	4.5 (7.2)	3.0 (5.0)	0.869	3.0 (6.0)	3.0 (5.0)	0.837	3.0 (5.0)	2.0 (5.0)	0.2391

Table 3.6: Multivariable logistic regression models assessing the association of candidate genetic variants with diLQTS in self-reported white patients (dominant genetic model) using the Bazett QTc correction method. Model 1: Unmatched sample unadjusted model (total n = 6,083). Model 2: Propensity-matched sample unadjusted model (total n = 1,466). Model 3: Unmatched sample propensity score-adjusted model (total n = 6,083). *p<0.017 (Bonferroni correction for multiple comparisons)

Genetic variants	**OR (95%CI; p-value)**	**Normal QTc, n (%)**	**Prolonged QTc, n (%)**	**χ^2/Fisher Exact test p-value**
***SCN5A* G615E rs12720452**				
Model 1	9.36e-06 (N/A-2.01e+08); 0.965	4 (0.1)	0 (0.0)	1.000
Model 2	3.48e-06 (N/A-1.93e+23); 0.969			
Model 3	6.10e-06 (N/A-4.16e+07); 0.963			
***KCNE2* I57T rs74315448**				
Model 1	2.44 (0.54-8.19); 0.182	9 (0.2)	3 (0.4)	0.168
Model 2	1.50 (0.25-11.43); 0.656			
Model 3	1.53 (0.29-6.15); 0.574			
***KCNE1* D85N rs1805128**				
Model 1	1.91 (1.18-2.99); 0.006*	89 (1.7)	23 (3.1)	0.008*****
Model 2	2.93 (1.35-7.04); 0.009*			
Model 3	2.24 (1.35-3.58); 0.001*****			

Table 3.7: Multivariable logistic regression models assessing the association of candidate genetic variants with diLQTS in self-reported white patients (dominant genetic model) using the Fridericia and Framingham QTc correction methods. **Model 1:** Unmatched sample with unadjusted model (total n = 6,083). **Model 2:** Propensity-matched sample with unadjusted model (total n = 1,466). **Model 3:** Unmatched sample with propensity score-adjusted model (total n = 6,083). *p<0.017 (Bonferroni correction for multiple comparisons)

Genetic variants	OR (95%CI; p-value)	Normal QTc, n (%)	Prolonged QTc, n (%)	χ^2/Fisher Exact test p-value
Fridericia QTc correction method				
***SCN5A* G615E rs12720452**				
Model 1	1.66e-05 (N/A -3.57e+08); 0.967			
Model 2	9.61e-06 (N/A-5.33e+23); 0.972	4 (0.1)	0 (0)	1.000
Model 3	1.14e-05 (N/A-1.01e+08); 0.965			
***KCNE2* I57T rs74315448**				
Model 1	6.53 (1.74-20.80); 0.002*	8 (0.1)	4 (0.9)	0.008*
Model 2	4.16 (0.69-31.69); 0.119			
Model 3	5.00 (1.13-18.61); 0.022			
***KCNE1* D85N rs1805128**				
Model 1	1.57 (0.81-2.76); 0.145	100 (1.8)	12 (2.8)	0.139
Model 2	1.76 (0.82-3.63); 0.128			
Model 3	1.87 (0.95-3.37); 0.052			
Framingham QTc correction method				
***SCN5A* G615E rs12720452**				
Model 1	2.15e-05 (N/A -4.61e+08); 0.968			
Model 2	1.32e-05 (N/A-7.34e+23); 0.972	4 (0.1)	0 (0)	1.000
Model 3	1.52e-05 (N/A-1.49e+08); 0.966			
***KCNE2* I57T rs74315448**				
Model 1	8.46 (2.25-27.01); 0.001*	8 (0.1)	4 (1.2)	0.003*
Model 2	5.75 (0.95-43.77); 0.056			
Model 3	6.70 (1.51 -24.85); 0.006*			
***KCNE1* D85N rs1805128**				
Model 1	1.85 (0.93-3.33); 0.056	101 (1.7)	11 (3.2)	0.061
Model 2	2.13 (0.97-4.42); 0.047			
Model 3	2.25 (1.11-4.16); 0.015*			

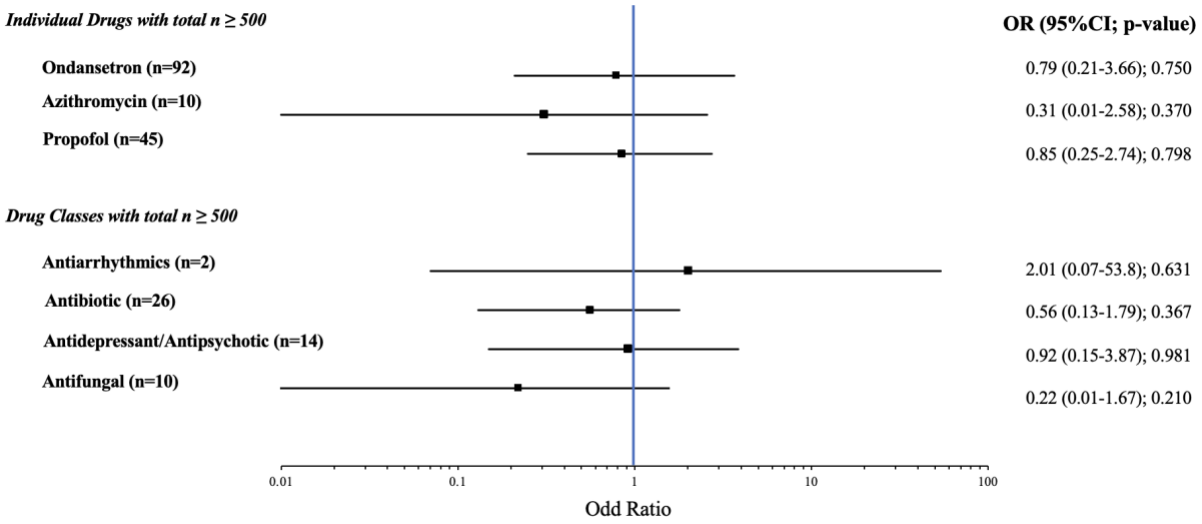

Figure 3.3:Forest plot of the logistic regression unmatched model adjusted for propensity score assessing the association of *KCNE1*-D85N by specific drug class and individual drugs if at least 500 total patients were treated with any of the individual drugs or drug classes. Sample sizes next to the drug names are the number of D85N carriers treated with that particular drug or drug class. The squares represent the odds ratio of the individual drug and drug classes and the horizontal lines indicate the 95% confidence interval

Chapter 4 Association of a Polygenic Risk Score for Drug-Induced Long QT Syndrome: Independent Validation in a Real-World Patient Population

4.1 Background

Since the 1960s, the medical community has acknowledged that certain drugs can unexpectedly and significantly prolong the QT interval in an electrocardiogram; (Selzer & Wray, 1964) a condition known as drug-induced long QT syndrome (diLQTS). This adverse drug reaction is currently associated with over 150 FDA-approved medications.(CredibleMeds, 2013; Woosley, Black, Heise, & Romero, 2018) QT interval prolongation signifies a delay in the ventricular repolarization of the cardiac action potential. This delay has the potential to trigger a dangerous cardiac arrhythmia known as *torsades de pointes* (TdP), and in severe cases, can even result in sudden cardiac death. (Roden, 2004) Despite identifying several clinical risk factors for diLQTS, such as congestive heart failure, hypokalemia, and female sex, accurately predicting its occurrence remains challenging.

Recent evidence has highlighted the role of genetics as an underlying risk factor for diLQTS.(A. I. Lopez-Medina et al., 2022) Some rare variants in the genes for cardiac ion channels can cause significant QT prolongation in the absence of any drug exposure, i.e., congenital long QT syndrome (cLQTS), (Adler et al., 2020) or exposure to QT-prolonging drugs can sometimes reveal patients with previously undiagnosed cLQTS.(H. Itoh et al., 2016) In addition to these rare genetic variants, common genetic variants, usually characterized by a minor allele frequency (MAF) > 1% in humans, may also influence susceptibility to diLQTS in the general population without causing cLQTS. (Arking et al., 2014; Newton-Cheh et al., 2009;

Pfeufer et al., 2009; Roden et al., 2018) While each common variant only slightly increases the QT interval (~1 to 3 msec per allele), their collective impact can amplify diLQTS risk significantly. (Strauss et al., 2017) The aggregation of the cumulative risk attributed to multiple common genetic variants can be quantified through a polygenic risk score (PRS). The American Heart Association recently published a scientific statement affirming that PRSs have the potential to enhance the predictive accuracy for a spectrum of cardiovascular conditions, including atrial fibrillation, coronary artery disease, venous thrombosis, hypercholesterolemia, and type 2 diabetes. (O'Sullivan et al., 2022)

Strauss et al (Strauss et al., 2017) developed a PRS for diLQTS by leveraging 61 common genetic variants identified from a genome-wide association study (GWAS) of baseline QT intervals (corrected for heart rate = QTc). The effectiveness of this PRS was assessed in a small clinical trial enrolling 22 healthy volunteers (77% white race) who were administered three QT-prolonging drugs (dofetilide, quinidine, and ranolazine). The PRS significantly associated with diLQTS from all three drugs, and it explained approximately 30% of the individual variability in diLQTS. (Strauss et al., 2017) The PRS also significantly associated with the risk of drug-induced TdP in 216 cases vs. 771 ancestry-matched controls with European descent ($p = 1.3 \times 10^{-7}$). However, to our knowledge, this PRS for predicting risk of diLQTS has not yet been independently validated by another research team. Thus, this study aimed to assess the association of the diLQTS PRS published by Strauss et al in a large, independent case-control study.

4.2 Methods

4.2.1 Study design

This is a single-center, retrospective observational case-control study that used clinical and genomic data from the Michigan Genomics Initiative (MGI) at the University of Michigan (UM) Health System. (Zawistowski et al., 2023) The study included adult patients who were prescribed at least one high-risk QT-prolonging drug (CredibleMeds, 2013) between October 1st, 2012 and September 30th, 2022. Patients with (i) cLQTS, (ii) pre-existing high QTc levels (QTc ≥500 msec prior to treatment with a high-risk QT prolonging drug), (iii) left bundle branch block, or (iv) pacemaker use were excluded. The primary outcome was exaggerated QTc prolongation, defined as either a change of >60 msec from the baseline QTc or an absolute QTc value ≥500 msec during a prescription for any high-risk QT-prolonging drug. The QTc was adjusted for heart rate using Bazett correction method. All clinical data, including laboratories, comorbidities, and concomitant medications were collected as close as possible to the index date, defined as the date of the highest QTc value (Bazett) during any high-risk QT prolonging drug prescription. The study was carried out in accordance with the Declaration of Helsinki and was approved by the local Institutional Review Board with a waiver of informed consent.

4.2.2 Data Collection

Patients were initially identified using the DataDirect system (Data Office for Clinical & Translational Research), a tool that enables access to various clinical data in the UM Health System electronic health records. This included demographics, vital signs, diagnoses, procedures, medications, and lab results. The Electronic Medical Record Search Engine (EMERSE) (Hanauer et al., 2015) was used to screen clinical notes for specific terms to ensure compliance with exclusion criteria. The entry date started with the first prescription of any high-risk QT prolonging drug, while the index date was determined by the highest QTc value during

such a prescription. Controls had a maximum QTc value below 500 msec. Data collection extended until September 30, 2022. Baseline QTc interval, comorbidities, and concurrent medications were gathered within a 6-month window around the index date. Comorbidities were identified through ICD-9 and 10 codes and lab values, with Elixhauser and Charlson scoring systems applied. (Wasey, 2017)

4.2.3 Drug exposure

The list of high-risk QT-prolonging drugs was obtained from CredibleMeds, (Woosley et al., 2018) a federally-funded website focusing on safe medication practices. CredibleMeds categorizes drugs based on their risk for Torsades de Pointes (TdP) as conditional, possible, or known. For this study, only drugs categorized as known risk for TdP were considered. The identified high-risk drugs, were used for patient selection and subsequent analysis. Medication records were collected without restrictions on administration route, formulation, dose, or frequency. Exposure to high-risk QT-prolonging drugs was defined by the presence or absence of any high risk QT prolonging drug within the patient's medication history. Additionally, drug-drug interactions were determined by concurrent and systemic use of cytochrome P450/p-glycoprotein (CYP/p-gp) inhibitors or inducers with known major interaction potential with the specific QT prolonging drug prescribed, as specified by Micromedex®.(Merative Micromedex)

4.2.4 Outcomes

The primary outcome was the occurrence of exaggerated QTc prolongation, defined as a change exceeding 60 msec from the baseline QTc and/or an absolute QTc value equal to or greater than 500 msec, during the prescription of any high-risk QT-prolonging drug. QTc

interval measurements were automatically recorded using an FDA-approved electrocardiogram (ECG) system (General Electric [GE] MUSE™ Cardiology Information System), the standard system in routine clinical practice at the UM Health System. The QT intervals were derived from an average across the 12 leads of the ECG, and the primary outcome was adjusted for heart rate using the Bazett formula ($QTc = QT/\sqrt{RR}$).

4.2.5 Genomic data

The MGI provides access to genomic data, as detailed elsewhere. (Zawistowski et al., 2023) Briefly, genotyping was conducted at the UM Advanced Genomics Core lab, adhering to standard quality assessment measures, (Zajac et al., 2019) and using Illumina Infinium CoreExome genotyping arrays. Imputation was performed using the Michigan Imputation Server (Michigan Imputation Services) employing the Trans-Omics for Precision Medicine (TOPMed) panel as the reference panel. Rigorous post-imputation filters were applied to eliminate inadequately imputed variants (those with $r^2 < 0.3$ and MAF < 0.01%), resulting in a high-quality dataset. Genetic relatedness between all patients in the study was assessed through Kinship-based INference for GWAS (KING) v2.1.3. (Manichaikul et al., 2010) To ensure independence in the sample, individuals closely related, denoted by kinship coefficients exceeding 0.125 (indicating first or second-degree relatives), were randomly chosen from each related pair and subsequently excluded.

The MGI quantified ancestry in patients using the ADMIXTURE software, merging genotypes from approximately 160,000 quality-controlled sites with a Human Genome Diversity Project and continental population labels used as reference.

4.2.6 Polygenic score calculation

Strauss *et al*. (Strauss et al., 2017) developed different scores for European and African ancestry. The European PRS score was calculated by using the allelic effects estimated from a GWAS of 100,000 individuals of European ancestry.(Arking et al., 2014) The African American PRS was calculated by the allelic effects estimated of an African American GWAS of 13,105 individuals.(Smith et al., 2012) Due to the absence of an Asian PRS, we applied the European score to the Asians because the European score was developed with a larger sample size than the African score.

The polygenic risk score was calculated for each patient as previously described by Strauss et al. (Strauss et al., 2017) Briefly, the score was determined by considering the weighted effect size of each genetic variant on the QTc interval multiplied by the number of "QT raising" alleles. For example, rs16857031 – a C (major allele)/G (minor allele) variant – where the G being the "QTc raising" allele associated with a 2.36 msec longer QT interval per allele copy. In the scenario of an individual being homozygous for the "QT raising allele" (GG), its contribution to the score equates to 4.72 msec (2.36*2). For heterozygous individuals (CG), the contribution stands at 2.36 msec (2.36*1), while for those homozygous for CC, the contribution is 0 msec (2.36*0). This iterative process was applied to all 61 and 60 variants for European and African American respectively, with the cumulative contributions determining the overall polygenic score.

4.2.7 Statistical analysis

Patients were categorized into two groups, normal and prolonged, based on their QTc intervals as defined earlier. Categorical variables were compared using χ^2 or Fisher's exact test. The normality of continuous variables were evaluated using the Kolmogorov-Smirnov test. For

normally distributed variables, means ± SD were used, while non-normally distributed variables were represented as median ± IQR. Univariable logistic regression models were employed to identify potential confounders by assessing the association of clinical predictors with prolonged QTc.

Given the presence of several significantly different covariates between the two groups at the index date, a propensity score was calculated for each patient utilizing all variables with a p-value < 0.05.(D'Agostino, 1998) These propensity scores were then employed to establish a 1:1 matching ratio between normal and prolonged QTc patients, resulting in the formation of a novel propensity-matched sample without significant differences in clinical characteristics at the index date. The association of PRS with the risk of diLQTS was assessed using 4 logistic regression models: the unmatched sample in unadjusted models (Model 1), propensity-matched sample in unadjusted models (Model 2), and unmatched sample adjusted for propensity score (Model 3), and a specific model for self-identified Asians adjusted for only 2 covariates ("history of stroke" and "Charlson score") that were significantly different between the two groups at the index date (Model 4).

In order to develop a simple cutoff to differentiate low-risk from high-risk PRS patients, we determined a cutoff for each racial subgroup (Figure 4.3). This cutoff was determined by assessing the association between every 5-unit increase in the PRS and diLQTS using the unmatched sample with model adjusted for propensity score (Model 3). Then, the PRS was analyzed 2 ways: 1) as a continuous variable (i.e., the odds ratio for diLQTS risk for every 1 unit increase in PRS) and 2) as a categorical variable (i.e., the odds ratio for diLQTS risk for high-risk group defined by the specific high-risk cutoff determined for each race group in (Figure 4.3).

All statistical analyses were performed using R version 4.2.2. A $p < 0.05$ in Model 2 was *a priori* defined as statistically significant for the White and African-American patients because Model 2 used the largest sample size available but also adjusted for multiple clinical covariates via the propensity score. A $p < 0.05$ was considered statistically significant for Model 4 for the Asian patients. A sensitivity analysis categorizing patient groups by >90% continental ancestry (European, African, West Asian, Central/South Asian, and East Asian) instead of race was also performed to evaluate consistency of the findings in race vs. ancestry patient groups.

4.3 Results

A total of 6,989 eligible patients who met the study inclusion/exclusion criteria (Figure 3.1), 6,083 (87.0%) self-reported to be White, 565 (8.1%) African American, 111 (1.6%) Asian, 46 (0.7%) American Indian or Alaska Native, 2 (0.03%) Native Hawaiian or Other Pacific Islander, and 182 (2.6%) unknown race. Given the small sample sizes of patients in the American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, and unknown race groups, the PRS was only calculated and analyzed within the White, African American and Asian patient subgroups.

The primary outcome of exaggerated QT prolongation occurred in 12.0% of White, 12.2% of African American and 8.2% of Asian patients. The clinical characteristics at the index date of the three groups are shown in Table 4.1**.** In white patients, before propensity score matching, patients with prolonged QTc exhibited a significantly higher prevalence of electrolyte disturbances (hypokalemia, defined as potassium<3.5 mEq/L and hypocalcemia, defined as calcium <8.5mg/dL), renal (chronic kidney disease), liver and cardiovascular conditions (congestive heart failure, coronary artery syndrome, hypertension, peripheral vascular disease, arrhythmias and

history of myocardial infarction and stroke), chronic obstructive pulmonary disease, and diabetes mellitus compared to patients with a normal QTc. Moreover, patients with prolonged QTc had a significantly higher prevalence of history of alcohol consumption, use of loop diuretics, digoxin and beta-blockers, than their normal QTc counterparts. Elixhauser and Charlson comorbidities scores were also significantly higher in patients with prolonged QTc than in their normal QTc counterparts. There were no significant differences observed in any of the other variables assessed such as age, sex, body mass index, hypothyroidism, or cancer. After applying a 1:1 propensity score matching, 731 patients were included in each group, effectively eliminating all of the significant differences in the clinical baseline characteristics (Table 4.2). In addition, no significant differences were found in baseline clinical characteristics between low and high risk in the PRS.

In African Americans, before propensity score matching, patients with prolonged QTc exhibited a significantly higher rates of hypocalcemia, diabetes mellitus, renal (chronic kidney disease), liver and cardiovascular conditions such as congestive heart failure, coronary artery syndrome, hypertension, peripheral vascular disease and arrhythmias, compared to patients with a normal QTc. Moreover, patients with prolonged QTc had a significantly higher prevalence of use of loop diuretics and beta-blockers, than their normal QTc counterparts. Elixhauser and Charlson comorbidities scores were also significantly higher in patients with prolonged QTc than in their normal QTc counterparts. There were no significant differences observed in any of the other variables assessed such as age, sex, hypokalemia, hypomagnesemia, body mass index, hypothyroidism, history of myocardial infarction or stroke, COPD, cancer, history of alcohol consumption and the use of digoxin and more than 2 concomitant use of QT prolonging drugs. After applying 1:1 propensity score matching, 69 patients were included in each group,

effectively eliminating all of the significant differences (except for BMI, higher in the high-risk group) in the clinical baseline characteristics (Table 4.2). In addition, some significant differences were found in baseline clinical characteristics between African-Americans with low and high risk PRS. The low-risk PRS group exhibited higher hypokalemia, CKD and hypertension (Table 4.5).

In Asians, patients with prolonged QTc exhibited a significantly higher history of stroke and Charlson score compared to patients with a normal QTc (Table 4.2). There were no significant differences observed in any of the other variables assessed. In addition, no significant differences were found in baseline clinical characteristics between the Asian patients with low and high-risk PRS (except for hypertension and CKD, which were significantly higher in the high-risk group; (Table 4.5). Given that only 9 of the Asian patients had prolonged QTc, we were unable to perform 1:1 propensity matching for the Asian group of patients. This is why the results for the Asian patients are only presented for Model 1 (unmatched and unadjusted sample) and Model 4 (adjusted for only 2 covariates: "history of stroke" and "Charlson score") and not for Model 2 (propensity-matched sample unadjusted for covariates).

4.3.1 Drug exposure

Out of the forty drugs marketed in the US that are classified as known risk for TdP by CredibleMeds, twenty-seven (67.5%) were prescribed in white, twenty-two in African American (55.0%) and sixteen (40.0%) in the Asian group (Table 4.4). In white patients, Dofetilide, dronedarone, sotalol, and papaverine were prescribed significantly more frequently on the index date for those who had prolonged QTc than those who had normal QTc. In contrast, azithromycin, ciprofloxacin, propofol, and ondansetron were prescribed significantly more frequently for those who had normal QTc than those who had prolonged QTc. In African

Americans, sotalol was prescribed significantly more frequently on the index date for those who had prolonged QTc than those who had normal QTc. In contrast, ciprofloxacin was prescribed significantly more frequently for those who had normal QTc than those who had prolonged QTc. In Asians, there were not significant differences in the frequency of prescribed drugs on the index date for both groups.

4.3.2 Associations of PRS with the risk of diLQTS

In white patients, the mean ± standard deviation of the PRS was 86.8 ± 7.1, with minimum and maximum values of 62.4 and 114.4, respectively. The association of the PRS as a continuous variable with the risk of diLQTS is shown in Table 4.3**.** The statistical significance of the PRS remained consistent across all three regression models, regardless of whether the sample was propensity matched or not and covariate adjusted or not. The normal distribution of the PRS for patients with normal and prolonged QTc can be observed in Figure 4.1. The distributions of PRS for patients with normal and prolonged QTc largely overlap, but the distribution of the PRS for the patients with prolonged QTc exhibit higher PRS values. Figure 4.2 shows the association of the PRS scaled by low and high-risk cutoffs with diLQTS. The statistical significance of the PRS remained consistent across all three regression models in the white patient group. The odds ratios ranged from 1.44 to 1.88 for the low vs. high-risk cutoff across all three models. These findings were similar when analyzed in a subgroup of patients with >90% European ancestry (data not shown).

In the African American group, the mean ± standard deviation of the PRS was -36.8 ± 4.1, with minimum and maximum values of -47.0 and -21.8, respectively. While there is an overlap in the normal distribution of the PRS among patients with normal and prolonged QTc,

those with prolonged QTc tend to display slightly elevated PRS values, albeit to a minimal extent (Figure 4.1). The PRS was not statistically significant in any of the three regression models ($p > 0.05$ in all 3 models; Table 4.3) or scaled by low and high risk cutoff (Figure 4.2). However, the lack of statistical significance could be due to the small sample size, as the odds ratios ranged from 1.58 to 2.18 across for the low vs. high-risk cutoff all three models, which were similar odds ratios compared to the larger sized White patient group. These findings were similar when analyzed in a subgroup of patients with >90% African ancestry (data not shown).

In the Asian patients, the mean ± standard deviation of the PRS was 90.6 ± 4.8, with minimum and maximum values of 78.5 and 104.2, respectively. Similar to the white and African-American groups, there was a large amount of overlap in the distributions of the PRS in the Asian patients with normal and prolonged QTc (Figure 4.1), but the distribution appears to be shifted to higher values in the patients with prolonged QTc. The PRS was not statistically significant in Model 1 ($p = 0.081$), however when adjusted by covariates, the PRS reached statistical significance. ($p= 0.049$ in Model 4). However, the statistical significance of the PRS was lost across the two regression models when it was scaled by low and high risk cutoff. The odds ratios ranged from 2.82 to 3.21 for the low vs. high-risk cutoff across all models (Figure 4.2).

4.4 Discussion

To the best of our knowledge, this is the first independent study validating the diLQTS PRS initially proposed by Strauss *et al.* [8] within a large real-world patient case-control. Our results unequivocally demonstrated the association of this PRS with diLQTS in self-identified white patients. Our findings also suggest that the PRS is a risk factor for diLQTS in African-American and Asian patients as well, but the sample sizes for those race groups were smaller than the white

patients. Our research also extends the prior research by encompassing patients from a real-world clinical setting with diverse comorbidities and many different high-risk QT prolonging drugs.

The association between this PRS and diLQTS underscores the clinical relevance of these genetic variants in diLQTS. Many individual variants, including those located at the *NOS1AP* locus and within various ion channels such as *SCN5A, KCNH2, KCNE1, KCNJ2,* have previously been associated with an elevated risk of diLQTS.(A. I. Lopez-Medina et al., 2022) While these variants individually exhibit minor effects on baseline QT intervals (e.g., 1–3 ms) in GWAS (Arking et al., 2014), their cumulative impact suggests the PRS's potential to effectively predict diLQTS.

PRS offer substantial potential in assessing individual risk and guiding preventive strategies for cardiovascular diseases, including arrhythmias. (Börschel et al., 2021; O'Sullivan et al., 2022) By having a diLQTS PRS, patients in the general population without cLQTS can gain a quantitative gauge of their individual risk in developing diLQTS when exposed to high-risk QT prolonging drugs independent of other risk factors. A small, proof-of-concept randomized clinical trial demonstrated the promising clinical utility of PRS in patients with cardiovascular disease.(Kullo et al., 2016) Patients that received their PRS for coronary heart disease in addition to their clinical risk score for coronary heart disease had significantly lower LDL cholesterol levels and significantly improved drug prescribing compared to the patients that received their clinical risk scores without their PRS. This independent validation study of the diLQTS PRS initially published by Strauss et al takes the next steps towards clinical utility for this PRS as well.

This study has limitations to note. It has a retrospective observational design within a single health system, potentially limiting the generalizability and applicability of our findings to other

health systems. Another limitation is the absence of an Asian PRS, thus we relied in the European PRS to calculate the Asian PRS. Comorbidity data was extracted from the EHR employing ICD-9 and ICD-10 codes, both acknowledged to have inaccuracies. Moreover, our analysis revolved around prescribed medications in patients' lists, without a direct assessment of adherence to these prescriptions. Lastly, it is important to acknowledge that QT prolongation serves as a surrogate indicator for more serious clinical outcomes such as TdP and sudden cardiac death.

4.5 Conclusion

In conclusion, a PRS for diLQTS independently validated in a large patient case-control from a real-world clinical setting. Its robust association was replicated regardless of the covariate adjustment method used in a large group of white patients. The PRS was not associated with diLQTS in smaller groups of African-American and Asian patients. Implementing these findings into clinical practice holds the promise of preemptively identifying patients at high risk and preventing the occurrence of this potentially life-threatening adverse drug reaction.

4.6 Figures

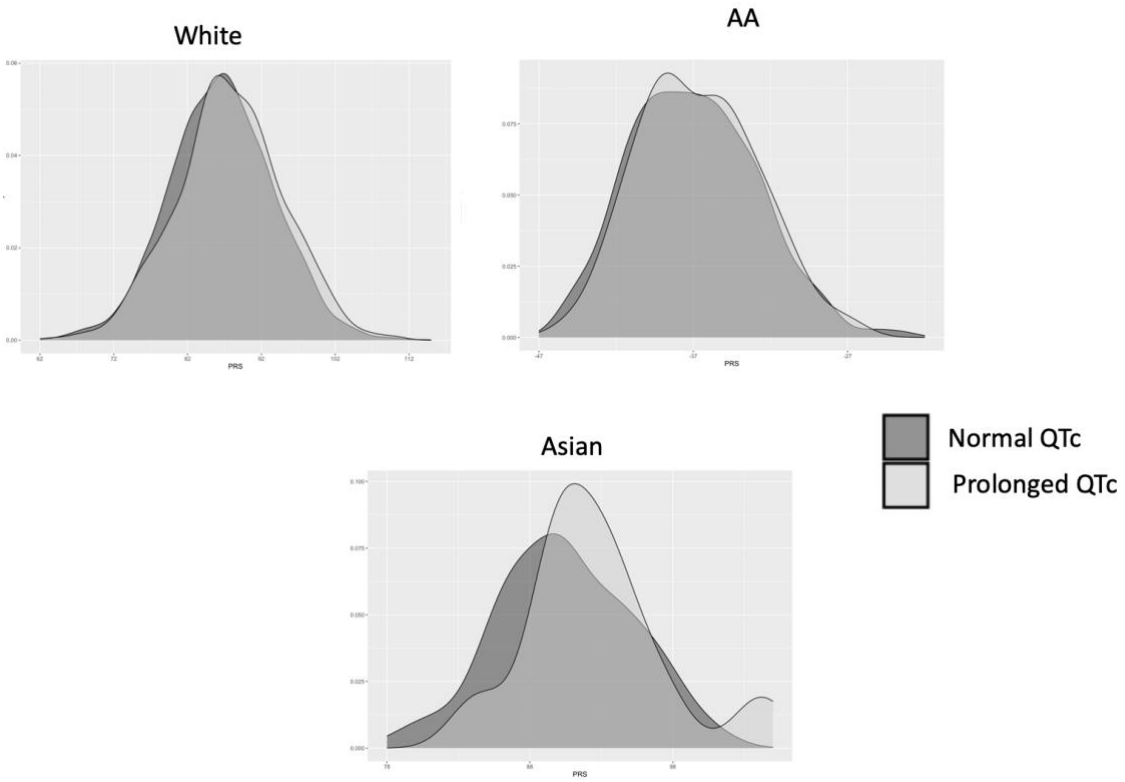

Figure 4.1 Normal distribution of the PRS for normal QTc and prolonged QTc groups of self-reported white, African American and Asian patients.

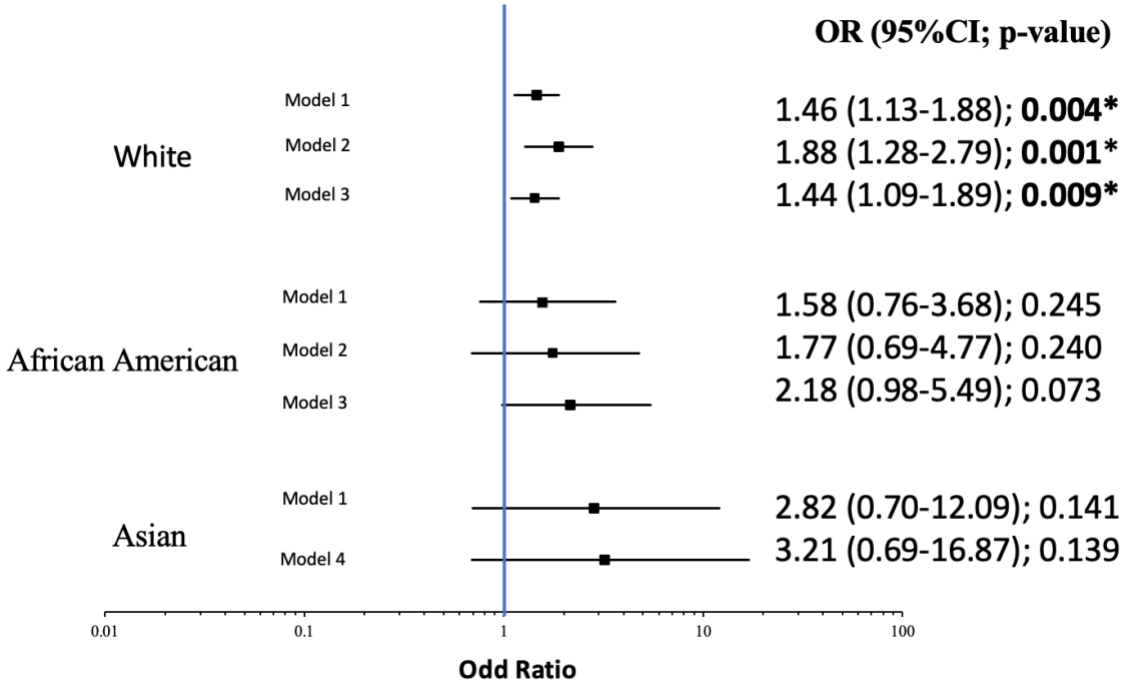

Figure 4.2 Multivariable logistic regression models assessing the association of the low vs. high polygenic risk score cutoffs with diLQTS in white, African American and Asian patients. **Model 1:** Unmatched sample with unadjusted model. **Model 2:** Propensity-matched sample with unadjusted model. **Model 3:** Unmatched sample with model adjusted for propensity score. **Model 4**: Unmatched sample adjusted by the covariates "history of stroke" and "Charlson score". *p<0.05.

4.7 Tables

Table 4.1 Baseline characteristics of the normal QTc vs. prolonged QTc groups within self-reported White, African American and Asian patients. BMI: Body mass index; CHF: congestive heart failure; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CrCl: creatinine clearance; DM: diabetes mellitus; HTN: hypertension; MI: myocardial infarction; PVD: peripheral vascular disease. Bolded p-values < 0.05.

Characteristics	**White Unmatched sample**			**African American Unmatched sample**			**Asian Unmatched sample**		
	Normal QTc	**Prolonged QTc**	**p-value**	**Normal QTc**	**Prolonged QTc**	**p-value**	**Normal QTc**	**Prolonged QTc**	**p-value**
Patients, n (%)	5,341 (88.0)	731 (12.0)	__	489 (87.6)	69 (12.3)	__	101 (91.8)	9 (8.2)	__
Aged 68 years or older, n (%)	1,119 (21.0)	165 (22.6)	0.338	41 (8.4)	10 (14.5)	0.116	16 (15.8)	1 (11.1)	1.000
Female, n (%)	2,981 (55.8)	384 (52.5)	0.102	329 (66.9)	45 (65.2)	0.892	58 (57.4)	2 (22.2)	0.076
Hypokalemia, n (%)	325 (6.1)	103 (14.1)	**<0.001**	35 (7.1)	6 (8.7)	0.621	5 (5.0)	1 (11.1)	0.408
Hypocalcemia, n (%)	1,002 (18.8)	297 (40.6)	**<0.001**	61 (12.4)	27 (3.1)	**<0.001**	21 (20.8)	2 (22.2)	1.000
Hypomagnesemia, n (%)	1,061 (19.9)	127 (17.4)	0.123	63 (12.8)	9 (13.0)	1.000	14 (13.9)	1 (11.1)	1.000
BMI (kg/m^2), mean (SD)	30.3 (7.5)	29.9 (7.9)	0.247	32.5 (8.4)	33.9 (8.9)	0.235	26.5 (5.4)	24.6 (2.9)	0.129
CHF, n (%)	369 (6.9)	148 (20.2)	**<0.001**	43 (8.7)	20 (29.0)	**<0.001**	7 (6.9)	0 (0.0)	1.000
HTN, n (%)	3,006 (56.3)	493 (67.4)	**<0.001**	312 (63.4)	54 (78.3)	**0.022**	52 (51.5)	7 (77.8)	0.172
CAD, n (%)	2,276 (42.6)	385 (52.7)	**<0.001**	185 (37.6)	40 (58.0)	**0.002**	42 (41.6)	4 (44.4)	1.000
Hypothyroidism, n (%)	754 (14.1)	104 (14.2)	0.981	30 (6.1)	11 (15.9)	**0.010**	14 (13.9)	1 (11.1)	1.000
History of MI, n (%)	373 (7.0)	95 (13.0)	**<0.001**	35 (7.1)	9 (13.0)	0.095	6 (5.9)	0 (0.0)	1.000
DM, n (%)	1,095 (20.5)	222 (30.4)	**<0.001**	129 (26.2)	21 (30.4)	0.551	27 (26.7)	0 (0.0)	0.109
DM complicated, n (%)	353 (6.6)	87 (11.9)	**<0.001**	45 (9.1)	20 (29.0)	**<0.001**	10 (9.9)	2 (22.2)	0.254
History of Stroke, n (%)	529 (9.9)	119 (16.3)	**<0.001**	56 (11.4)	11 (15.9)	0.319	8 (7.9)	3 (33.3)	**0.045**
CKD, n (%)	704 (13.2)	184 (25.2)	**<0.001**	78 (15.9)	32 (46.4)	**<0.001**	12 (11.9)	1 (11.1)	1.000
Severe liver disease, n (%)	82 (1.5)	30 (4.1)	**<0.001**	2 (0.4)	1 (1.4)	0.326	1 (1.0)	0 (0.0)	1.000
Liver disease, n (%)	598 (11.2)	115 (15.7)	**0.001**	38 (7.7)	17 (24.6)	**<0.001**	9 (8.9)	1 (11.1)	0.590
COPD, n (%)	427 (8.0)	95 (13.0)	**<0.001**	30 (6.1)	6 (8.7)	0.428	6 (5.9)	0 (0.0)	1.000

Cancer, n (%)	383 (7.2)	51 (7.0)	0.922	25 (5.1)	5 (7.2)	0.399	9 (8.9)	0 (0.0)	1.000
PVD, n (%)	457 (8.6)	113 (15.5)	**<0.001**	24 (4.9)	11 (15.9)	**0.002**	3 (3.0)	1 (11.1)	0.293
Arrhythmia, n (%)	972 (18.2)	223 (30.5)	**<0.001**	57 (11.6)	21 (30.4)	**<0.001**	14 (13.9)	2 (22.2)	0.616
History of alcohol consumption, n (%)	189 (3.5)	47 (6.4)	**<0.001**	16 (3.3)	4 (5.8)	0.292	0 (0.0)	0 (0.0)	-
Loop Diuretic, n (%)	655 (12.3)	260 (35.6)	**<0.001**	52 (10.6)	19 (27.5)	**<0.001**	9(8.9)	1 (11.1)	0.590
Digoxin, n (%)	21 (0.4)	16 (2.2)	**<0.001**	3 (0.6)	2 (2.9)	0.117	1 (1.0)	0 (0.0)	1.000
Beta blockers, n (%)	1,448 (27.1)	340 (46.5)	**<0.001**	129 (26.2)	30 (43.5)	**0.005**	28 (27.7)	2 (22.2)	1.000
$\geq$2 QT prolonging drugs, n (%)	2,765 (51.8)	271 (37.1)	**<0.001**	234 (47.6)	36 (52.2)	0.555	47 (46.5)	3 (33.3)	0.507
Elixhauser score (IQR)	7 (13.0)	11.0 (13.5)	**<0.001**	3 (10.0)	9 (15)	**<0.001**	4 (12.0)	12 (6.0)	0.056
Charlson score (IQR)	3 (5.0)	4 (5.0)	**<0.001**	2 (3.0)	5 (5.0)	**<0.001**	2 (6.0)	7 (6.0)	**0.033**

Table 4.2 Characteristics at the index date of the normal QTc vs. prolonged QTc groups after propensity score matching in self-reported white patients and African American patients.

Characteristics	**White propensity-matched sample**		**p-value**	**African American propensity-matched sample**		**p-value**
	Normal QTc	**Prolonged QTc**		**Normal QTc**	**Prolonged QTc**	
Patients, n (%)	731 (50.0)	731 (50.0)	__	69 (50.0)	69 (50.0)	__
Aged 68 years or older, n (%)	172 (23.5)	165 (22.6)	0.709	7 (10.1)	10 (14.5)	0.606
Female, n (%)	370 (50.6)	384 (52.5)	0.496	33 (47.8)	45 (65.2)	0.059
Hypokalemia, n (%)	107 (14.6)	103 (14.1)	0.823	5 (7.2)	6 (8.7)	1.000
Hypocalcemia, n (%)	311 (42.5)	297 (40.6)	0.490	27 (39.1)	27 (39.1)	1.000
Hypomagnesemia, n (%)	136 (18.6)	127 (17.4)	0.085	8 (11.6)	9 (13.0)	1.000
BMI (kg/m^2), mean (SD)	30.3 (7.6)	29.9 (7.8)	0.325	30.7 (7.5)	33.9 (8.9)	**0.032**
CHF, n (%)	137 (18.7)	148 (20.2)	0.509	22 (31.9)	20 (29.0)	0.853
HTN, n (%)	494 (67.6)	493 (67.4)	1.000	58 (84.1)	54 (78.3)	0.514
CAD, n (%)	391 (53.5)	385 (52.7)	0.793	37 (53.6)	40 (58.0)	0.732
Hypothyroidism, n (%)	113 (15.5)	104 (14.2)	0.556	8 (11.6)	11 (15.9)	0.621
History of MI, n (%)	101 (13.8)	95 (13.0)	0.701	12 (17.4)	9 (13.0)	0.636
DM, n (%)	235 (32.1)	222 (30.4)	0.498	20 (29.0)	21 (30.4)	1.000
DM complicated, n (%)	81 (11.1)	87 (11.9)	0.682	18 (26.1)	20 (29.0)	0.849
History of Stroke, n (%)	110 (15.0)	119 (16.3)	0.565	11 (15.9)	11 (15.9)	1.000
CKD, n (%)	182 (24.9)	184 (25.2)	0.952	34 (49.3)	32 (46.4)	0.865
Severe liver disease, n (%)	25 (3.4)	30 (4.1)	0.582	1 (1.4)	1 (1.4)	1.000
Liver disease, n (%)	112 (15.3)	115 (15.7)	0.885	13 (18.8)	17 (24.6)	0.536
COPD, n (%)	99 (13.5)	95 (13.0)	0.817	6 (8.7)	6 (8.7)	1.000
Cancer, n (%)	53 (7.3)	51 (7.0)	0.925	2 (2.9)	5 (7.2)	0.441
PVD, n (%)	121 (16.6)	113 (15.5)	0.618	8 (11.6)	11 (15.9)	0.621
Arrhythmia, n (%)	232 (31.7)	223 (30.5)	0.651	19 (27.5)	21 (30.4)	0.851
History of alcohol consumption, n (%)	47 (6.4)	47 (6.4)	1.000	2 (2.9)	4 (5.8)	0.681
Loop Diuretic, n (%)	246 (33.7)	260 (35.6)	0.475	16 (23.2)	19 (27.5)	0.696
Digoxin, n (%)	13 (1.8)	16 (2.2)	0.708	1 (1.4)	2 (2.9)	1.000
Beta blockers, n (%)	359 (49.1)	340 (46.5)	0.346	29 (42.0)	30 (43.5)	1.000
$\geq$2 QT prolonging drugs, n (%)	290 (39.7)	271 (37.1)	0.333	28 (40.6)	36 (52.2)	0.232
Elixhauser score (IQR)	12 (14.0)	11 (13.5)	0.612	11.0 (13.0)	9.0 (15.0)	0.709
Charlson score (IQR)	5 (5)	4 (5)	0.629	4.0 (4.0)	5.0 (5.0)	0.534

Table 4.3 Multivariable logistic regression models assessing the association of the polygenic risk score with diLQTS in white, African American and Asian patients. Model 1: Unmatched sample with unadjusted model. Model 2: Propensity-matched sample with unadjusted model. Model 3: Unmatched sample with model adjusted for propensity score. Model 4: Unmatched sample adjusted by the covariates "history of stroke" and "Charlson score". *p<0.05.

Race Groups & Models	OR (95%CI; p-value)
White	
Model 1 (n = 6,083)	1.02 (1.01-1.03); **0.0001***
Model 2 (n = 1,462)	1.02 (1.01-1.04); **0.0005***
Model 3 (n = 6,083)	1.02 (1.00-1.03); **0.0003***
African American	
Model 1 (n = 558)	1.02 (0.96-1.09); 0.411
Model 2 (n = 138)	1.08 (1.00-1.18); 0.055
Model 3 (n = 558)	1.04 (0.97-1.10); 0.252
Asian	
Model 1 (n = 110)	1.13 (0.98-1.32); 0.081
Model 4 (n = 110)	1.19 (1.01-1.45); 0.049

4.8 Appendix

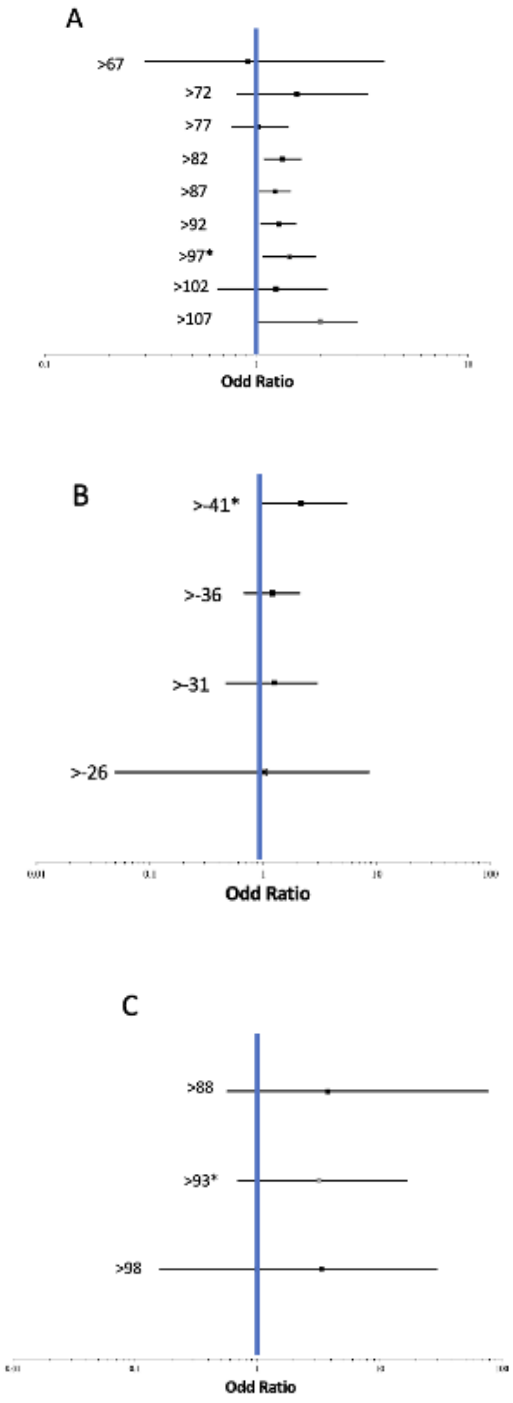

Figure 4.3 Forest plot of the logistic regression model of the unmatched sample with model adjusted for propensity score (Model 3) aiming to ascertain the optimal threshold distinguishing low and high-risk PRS. The figure illustrates the association between a 5-unit increase in PRS and the incidence of diLQTS in self-identified A) white B) black C) Asian patients. * Threshold value determined for each race group.

Table 4.4 List of high-risk QT-prolonging drugs prescribed on the index date in self-reported white, African American and Asian and their frequency between normal and prolonged QTc group.

Drug Class	Drug Name	White			African American			Asian		
		Normal QTc N=5345	Prolonged QTc N= 731	p-value	Normal QTc N=489	Prolonged QTc N=69	p-value	Normal QTc N=101	Prolonged QTc N=9	p-value
Antiarrhythmics	Disopyramide	5 (0.1)	0 (0.0)	1.000	1 (0.2)	0 (0.0)	1.000	-	-	-
	Dofetilide	13 (0.2)	22 (3.0)	<**0.001**	0 (0.0)	1 (1.4)	0.124	-	-	-
	Dronedarone	2 (0.0)	2 (0.3)	0.073	-	-	-	-	-	-
	Flecainide	38 (0.7)	6 (0.8)	0.646	1 (0.2)	0 (0.0)	1.000	-	-	-
	Quinidine	0 (0.0)	1 (0.1)	0.120	-	-	-	-	-	-
	Sotalol	28 (0.5)	17 (2.3)	<**0.001**	1 (0.2)	2 (2.9)	**0.042**	0 (0.0)	1 (11.1)	0.082
	Procainamide	2 (0.0)	1 (0.1)	0.319	-	-	-	-	-	-
Antibiotics	Azithromycin	517 (9.7)	26 (3.6)	<**0.001**	57 (11.7)	5 (7.2)	0.411	12 (11.9)	0 (0.0)	0.593
	Ciprofloxacin	562 (10.5)	50 (6.8)	**0.002**	40 (8.2)	11 (5.9)	**0.045**	5 (5.0)	2 (22.2)	0.101
	Clarithromycin	4 (0.1)	1 (0.1)	0.473	1 (0.2)	0 (0.0)	1.000	1 (1.0)	0 (0.0)	1.000
	Levofloxacin	270 (5.1)	32 (4.4)	0.487	16 (3.3)	2 (2.9)	1.000	3 (3.0)	0 (0.0)	1.000
	Moxifloxacin	11 (0.2)	0 (0.0)	0.381	1 (0.2)	0 (0.0)	1.000	1 (1.0)	0 (0.0)	1.000
	Erythromycin	11 (0.2)	3 (0.4)	0.233	1 (0.2)	0 (0.0)	1.000	-	-	-
Antidepressants	Citalopram	394 (7.4)	37 (5.1)	**0.027**	9 (1.8)	2 (2.9)	0.634	6 (5.9)	0 (0.0)	1.000
	Escitalopram	358 (6.7)	42 (5.7)	0.371	25 (5.1)	1 (1.4)	0.233	9 (8.9)	1 (11.1)	0.590
Cholinesterase inhibitor	Donepezil	41 (0.8)	8 (1.1)	0.374	2 (0.4)	0 (0.0)	1.000	-	-	-
Opioid agonist	Methadone	52 (1.0)	9 (1.2)	0.550	4 (0.8)	1 (1.4)	0.484	1 (1.0)	0 (0.0)	1.000
Antipsychotics	Haloperidol	64 (1.2)	10 (1.4)	0.718	8 (1.6)	2 (2.9)	0.356	1 (1.0)	0 (0.0)	1.000
	Chlorpromazine	8 (0.1)	2 (0.3)	0.343	0 (0.0)	1 (1.4)	0.124	1 (1.0)	0 (0.0)	1.000
Antifungals	Fluconazole	366 (6.8)	54 (7.4)	0.644	51 (10.4)	3 (4.3)	0.130	9 (8.9)	1 (11.1)	0.590
	Pentamidine	2 (0.0)	2 (0.3)	0.073	-	-	-	-	-	-
Antimalarial	Hydroxychloroquine	164 (3.1)	30 (4.1)	0.167	33 (6.7)	3 (4.3)	0.604	2 (2.0)	1 (11.1)	0.228
Anesthetic	Propofol	2,108 (39.4)	237 (32.4)	<**0.001**	158 (32.3)	24 (34.8)	0.785	34 (33.7)	2 (22.2)	0.715
Antiemetic	Ondansetron	4,042 (75.6)	472 (64.6)	<**0.001**	385 (78.7)	47 (68.1)	0.069	74 (73.3)	5 (55.6)	0.266
Oncology	Oxaliplatin	16 (0.3)	2 (0.3)	1.000	-	-	-	-	-	-
Vasodilator	Papaverine	36 (0.7)	19 (2.6)	<**0.001**	2 (0.4)	2 (2.9)	0.077	2 (2.0)	0 (0)	1.000
Phosphodiesterase Inhibitor	Cilostazol	30 (0.6)	2 (0.3)	0.421	4 (0.8)	0 (0.0)	1.000	-	-	-

Table 4.5 Clinical characteristics between low and high risk PRS among self-identified white, African American and Asian. BMI: Body mass index; CHF: congestive heart failure; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; HTN: hypertension; MI: myocardial infarction; PVD: peripheral vascular disease. Bolded p-values indicate $p < 0.05$.

Characteristics	**White**			**African American**			**Asian**		
	Low Risk (N=5602)	**High Risk (N= 474)**	**p-value**	**Low Risk (N=92)**	**High Risk (N=466)**	**p-value**	**Low Risk (N=74)**	**High Risk (N= (36)**	**p-value**
Age≥68, n (%)	1184 (21.1)	100 (21.1)	1.000	9 (9.8)	42 (9.0)	0.843	12 (16.2)	5 (13.9)	1.000
Female, n (%)	3115 (55.6)	254 (53.6)	0.423	63 (68.5)	308 (66.1)	0.748	39 (52.7)	21 (58.3)	0.725
Hypokalemia, n (%)	387 (6.9)	41 (8.6)	0.184	13 (14.1)	28 (6.0)	**0.014**	4 (4.5)	2 (7.0)	0.353
Hypocalcemia, n (%)	1194 (21.3)	105 (22.2)	0.712	15 (16.3)	73 (15.7)	1.000	14 (18.9)	9 (25.0)	0.465
Hypomagnesemia, n (%)	1094 (19.5)	98 (20.7)	0.587	12 (13.0)	60 (12.9)	1.000	9 (12.2)	6 (16.7)	0.560
BMI	30.3 (7.5)	29.9 (7.8)	0.251	32.5 (8.4)	33.9 (8.9)	0.240	25.9 (4.5)	27.2 (6.7)	0.331
CKD, n (%)	813 (14.5)	75 (15.8)	0.479	28 (30.4)	82 (17.6)	**0.007**	5 (6.8)	8 (22.2)	**0.027**
CHF, n (%)	472 (8.4)	45 (9.5)	0.475	16 (17.4)	47 (10.1)	0.065	6 (8.1)	1 (2.8)	0.423
HTN, n (%)	3,231 (57.7)	268 (56.5)	0.666	69 (75.0)	294 (63.1)	**0.038**	34 (45.9)	25 (69.4)	**0.034**
CAD, n (%)	2451 (43.8)	210 (44.3)	0.854	34 (37.0)	191 (41.0)	0.546	32 (43.2)	14 (38.9)	0.819
History of MI, n (%)	431 (7.7)	37 (7.8)	1.000	8 (8.7)	36 (7.7)	0.678	5 (6.8)	1 (2.8)	0.662
History of Stroke, n (%)	592 (10.6)	56 (11.8)	0.443	13 (14.1)	54 (11.6)	0.610	8 (10.8)	3 (8.3)	1.000
Liver disease, n (%)	657 (11.7)	56 (11.8)	1.000	11 (12.0)	41 (8.8)	0.330	8 (10.8)	2 (5.6)	0.493
Severe Liver Disease, n (%)	109 (1.8)	9 (1.9)	0.859	0 (0.0)	3 (0.6)	1.000	1 (1.4)	0 (0.0)	1.000
DM, n (%)	1,221 (21.8)	96 (20.3)	0.469	29 (31.5)	118 (25.3)	0.270	18 (24.3)	9 (25.0)	1.000
DM complicated, n (%)	398 (7.1)	42 (8.9)	0.185	13 (14.1)	52 (11.2)	0.526	9 (12.2)	3 (8.3)	0.748
Hypothyroidism, n (%)	798 (14.2)	60 (12.7)	0.377	6 (6.5)	35 (7.5)	1.000	12 (16.2)	3 (8.3)	0.377
COPD, n (%)	481 (8.6)	41 (8.6)	1.000	5 (5.4)	31 (6.7)	0.818	5 (6.8)	1 (2.8)	0.662
Cancer, n (%)	406 (7.3)	30 (6.3)	0.505	3 (3.3)	27 (5.8)	0.450	6 (8.1)	3 (8.3)	1.000
PVD, n (%)	525 (9.4)	45 (9.5)	0.996	4 (4.3)	31 (6.7)	0.489	3 (4.1)	1 (2.8)	1.000
Arrhythmia, n (%)	1,096 (19.6)	99 (20.9)	0.525	13 (14.1)	65 (13.9)	1.000	11 (14.9)	5 (13.9)	1.000

History of alcohol consumption, n (%)	213 (3.8)	23 (4.9)	0.311	2 (2.2)	18 (3.9)	0.553	0 (0.0)	0 (0.0)	-
Loop Diuretic, n (%)	845 (15.1)	70 (14.8)	0.906	13 (14.1)	58 (12.4)	0.786	5 (6.8)	5 (13.9)	0.291
Digoxin, n (%)	32 (0.6)	5 (1.1)	0.207	1 (1.1)	4 (0.9)	0.595	1 (1.4)	0 (0.0)	1.000
Beta blockers, n (%)	1,646 (29.4)	142 (30.0)	0.833	26 (28.3)	133 (28.5)	1.000	19 (25.7)	11 (30.6)	0.756
≥2 QT-prolonging drugs, n (%)	2,802 (50.0)	234 (49.4)	0.823	44 (47.8)	223 (47.9)	1.000	34 (45.9)	16 (44.4)	1.000
Elixhauser score (IQR)	8.0 (14.0)	7.0 (14.0)	0.297	3.0 (11.5)	3.0 (11.0)	0.966	3.0 (12.0)	4.0 (9.25)	0.591
Charlson score (IQR)	3.0 (5.0)	3.0 (5.0)	0.228	2.0 (4.0)	2.0 (4.0)	0.556	2.0 (6.0)	3.0 (5.0)	0.155

Table 4.6 Multivariable logistic regression models assessing the association of the standardized polygenic risk score with diLQTS in white, black and Asian patients only. Model 1: Unmatched sample with unadjusted model. Model 2: Propensity-matched sample with unadjusted model. Model 3: Unmatched sample with model adjusted for propensity score. Model 4: Unmatched sample adjusted by the covariates "history of stroke" and "Charlson score". *p<0.05.

Genetic variants	**OR (95%CI; p-value)**	**Normal QTc, n (%)**	**Prolonged QTc, n (%)**	**χ^2/Fisher Exact test p-value**
White				
Model 1	1.46 (1.13-1.88); **0.004***			
Model 2	1.88 (1.28-2.79); **0.001***	397 (7.4)	77 (10.5)	**0.004***
Model 3	1.44 (1.09-1.89); **0.009***			
African American				
Model 1	1.58 (0.76-3.68); 0.245			
Model 2	1.77 (0.69-4.77); 0.240	405 (82.8)	61 (88.4)	0.319
Model 3	2.18 (0.98-5.49); 0.073			
Asian				
Model 1	2.82 (0.70-12.09); 0.141			
Model 4	3.21 (0.69-16.87) 0.139	31 (30.7)	5 (55.6)	0.150

Chapter 5 Determining the Association of Uncommon Genetic Variants and a Polygenic Risk Score of Common Variants with Cardiac Events

5.1 Background

Drug-induced QT prolongation (diLQTS) can result in ventricular arrhythmias, including *torsades de Pointes* (TdP), which can either resolve spontaneously or progress to ventricular fibrillation, ultimately leading to sudden cardiac death. (Goldenberg et al., 2011) Estimating the overall incidence of drug-induced TdP in a population is challenging for several reasons. First, the arrhythmia is often transient, and an accurate diagnosis requires an ECG taken during an arrhythmic episode to confirm the presence of TdP. Secondly, the International Classification of Diseases (10th revision) used in electronic health records (EHR) lack a specific code for TdP or excessive QT prolongation. As a result, TdP are typically reported under codes for ventricular tachycardia, ventricular fibrillation, or sudden cardiac death (SCD). One study estimated that between 5% and 7% of reports of ventricular tachycardia, ventricular fibrillation, or sudden cardiac death were, in fact, cases of diLQTS.(Molokhia et al., 2008) Determining the frequency of death due to TdP is also problematic, as if a patient dies before an ECG is obtained, TdP may not be recognized as the cause of sudden death. Despite these challenges, numerous studies have shown that many drugs that prolong the QT interval are associated with a significantly higher risk of TdP. (CredibleMeds, 2013) Although there is no definitive threshold for TdP onset, QTc intervals ≥ 500 msec increase the risk of experiencing cardiac events, such as syncope, aborted

cardiac arrest, TdP, or sudden cardiac death, by tenfold.(Goldenberg et al., 2011) Moreover, for each 10 msec prolongation beyond this threshold, there is an exponential increase of approximately 5% to 7% in the risk of developing TdP.(Sauer et al., 2007) Therefore, QT prolongation remains the best surrogate measure for predicting TdP risk.

In previous chapter #3, we assessed the association of genetic variants with QT prolongation in presence of QT prolonging drugs. However, the association of *KCNE1*-D85N, *KCNE2*-I57T, and *SCN5A*-G615E with cardiac events, such as syncope, aborted cardiac arrest, TdP, or sudden cardiac death, remain unclear, as previous candidate gene studies have been limited by small sample sizes. (Kaab et al., 2012; Paulussen et al., 2004; Weeke et al., 2014) Additionally, the Polygenic Risk Score (PRS) developed by Strauss et al. (Strauss et al., 2017) was found to be significantly associated with the risk of drug-induced TdP in a study involving 216 cases compared to 771 ancestry-matched controls of European descent ($p = 1.3 \times 10\text{-}7$). However, to date, this PRS for predicting the risk of clinical outcomes has not been independently validated by another research team. Therefore, this chapter aimed to evaluate the association of *KCNE1*-D85N, *KCNE2*-I57T, *SCN5A*-G615E and the PRS developed by Strauss et al. with the risk of cardiac events.

5.2 Materials & Methods

5.2.1 General Methods

The study design, subject selection, data collection, drug exposure, candidate variant selection, and genomic data, were described in previous chapter (Chapter #3). Additionally, the calculation of the polygenic risk score (PRS) was previously detailed in Chapter #4.

5.2.2 Outcome

The clinical outcome was defined as a composite of specific clinical diagnoses such as history of TdP, premature ventricular complex (PVC), any ventricular arrhythmias, and/or sudden death. Cases were identified as patients who experienced the clinical outcome documented during the drug treatment period, while controls were patients who did not experience the clinical outcome. ICD-9 and ICD-10 codes were identified using the DataDirect system, (Data Office for Clinical & Translational Research) while the Electronic Medical Record Search Engine (EMERSE) was utilized to search through clinical notes (dictated or typed). (Hanauer et al., 2015) PVC and ventricular arrhythmias were collected based on previous studies algorithms, (Asatryan et al., 2023) using the presence of ICD-9 and ICD- 10 (Table 5.1). TdP were collected from EMERSE and it was queried for the term "torsades de pointes" or "TdP". Sudden cardiac death was identified by the death report in the EHR. Sudden death with cause unknown was considered in the composite clinical outcome.

5.2.3 Data analysis

Patients were stratified into control and cases based on the presence of the composite clinical outcome as defined earlier. Categorical variables were compared using χ^2 or Fisher's exact test. The normality of continuous variables was evaluated using the Kolmogorov-Smirnov test. For normally distributed variables, means ± SD were used, while non-normally distributed variables were represented as median ± IQR. Univariable logistic regression models were employed to

identify potential confounders by assessing the association of clinical predictors with the clinical outcome. Given the presence of several significantly different covariates between the two groups, a propensity score was calculated for each patient utilizing all variables with a p-value < 0.05.(D'Agostino, 1998) These propensity scores were then employed to establish a 1:1, 1:2, 1:3 and 1:4 matching ratio between control and cases, resulting in the formation of 4 novel propensity-matched samples without significant differences in clinical characteristics at the index date. The association of QT prolongation with the risk of the composite clinical outcome was assessed using 6 logistic regression models: the unmatched sample in unadjusted models (Model 1), propensity-matched 1:1 sample in unadjusted models (Model 2), propensity-matched 1:2 sample in unadjusted models (Model 3), propensity-matched 1:3 sample in unadjusted models (Model 4), propensity-matched 1:4 sample in unadjusted models (Model 5), and unmatched sample adjusted for propensity score (Model 6).

The association of *KCNE1*-D85N, *KCNE2*-I57T, *SCN5A*-G615E was also assessed using the 6 logistic regression models described above. A Bonferroni-corrected p-value of 0.0167 (0.05÷3) for each candidate genetic variant was *a priori* set as the threshold for statistically significance. The association of the PRS with the clinical outcome was also assessed using the 6 logistic regression models described above. However, given that only 15 of the African American patients had experience the composite clinical outcome, we were unable to perform 1:1 propensity matching for this group of patients. This is why we had a specific model for self-identified African American adjusted for 7 covariates that were significantly different between cases and control (Model 7). Then, the PRS was analyzed 2 ways: 1) as a continuous variable (i.e., the odds ratio for clinical outcome risk for every 1 unit increase in PRS) and 2) as a categorical variable (i.e., the odds ratio for clinical outcome risk for high-risk group defined by

the specific high-risk cutoff determined for each race group in Chapter 5-Figure 4.2. All statistical analyses were performed using R version 4.2.2. A $p < 0.05$ was considered statistically significant for all the PRS models. A sensitivity analysis categorizing patient groups by >90% continental ancestry (European and African) instead of race was also performed to evaluate consistency of the findings in race vs. ancestry patient groups. Odds ratios (OR) and respective 95% confidence intervals (95% CI) were calculated for all the models.

5.3 Results

5.3.1 Association of diLQTS with the clinical outcome

A total of 6,083 (87.0%) patients self-reported as White, and 558 (8.0%) as African American, met the study's inclusion/exclusion criteria (see Chapter #3,Figure 3.1). The composite clinical outcome occurred in 3.3% of White patients and 2.7% of African American patients. Only one Asian patient experienced the clinical outcome and was therefore excluded from the analysis. The incidence of cases was statistically higher in patients with QT prolongation than in those without QT prolongation in both racial groups (see Table 5.2 and Table 5.4). Additionally, 22.4% of White patients and 40% of African American patients with prolonged QTc experienced the composite clinical outcome, compared to 11.7% of White patients and 11.6% of African American patients with QTc prolongation who did not experience a clinical outcome (Table 5.5). However, the association of QT prolongation with the risk of the composite clinical outcome, as defined by Bazett's QT correction method, was only found to be statistically significant in Model 1 for both racial groups (see Table 5.5). There was no statistical significance of the QT prolongation across Model 2-6 in white patients and Model 7 in African American patients.

5.3.2 Associations of candidate genetic variants with the composite clinical outcome

A total of 6,083 (87.0%) patients self-reported to be White met the study inclusion/exclusion criteria (Chapter #3 Figure 3.1). The genotype and allele frequencies for *KCNE1*-D85N, *KCNE2*-I57T, and S*CN5A*-G615E among white patients are shown in Chapter #3. The clinical baseline characteristics of cases and controls are shown in Table 5.2**.** Overall, before propensity score matching, cases had a higher prevalence of cardiovascular conditions (congestive heart failure, coronary artery syndrome, and history of myocardial infarction) compared to controls. Moreover, cases had a significantly higher prevalence of QT prolongation and the use of loop diuretics, digoxin, and beta-blockers than controls. Additionally, Elixhauser comorbidity scores were significantly higher in cases than in controls. Controls were primarily females compare to cases. There were no significant differences observed in any of the other variables assessed such as age older than 68 years old, body mass index, electrolytes abnormalities, hypothyroidism, hypertension, diabetes, chronic kidney disease, stroke, chronic obstructive pulmonary disease, peripheral vascular disease, history of alcohol consumption, liver disease, cancer, prescribed more than two QT prolonging drugs, or Charlson score. After applying a 1:1, 1:2, 1:3 and 1:4 propensity score matching, all of the significant differences in the clinical baseline characteristics were eliminated. In addition, no significant differences were found in baseline clinical characteristics between carriers and non-carriers of the three candidate genetic variants (except for age and use of digoxin, which were significantly higher in the carriers of SCN5A-G165E. Additionally, non-carriers of *KCNE*1-D85N exhibited a significantly higher age compared to carriers (Chapter 3 Table 3.5.

The association of *KCNE1*-D85N (rs1805128), *KCNE2*-I57T (rs7415448) and *SCN5A*-G615E (rs12720452) with the risk of the composite clinical outcome, as defined by Bazett's QT correction method is displayed in Table 5.6. None of the three genetic variants met the Bonferroni-corrected level of statistical significance ($p < 0.0167$) in any of the models assessed ($p > 0.304$). The odds ratios for *KCNE1*-D85N range from 0.46 to 0.53. The odds ratios for *KCNE2*-I57T range from 2.01 to 3.01. Only 4 total patients carried the *SCN5A*-G615E (rs12720452) variant, and none of the carriers were cases.

5.3.3 Associations of PRS with the risk of cardiac events

The PRS was only analyzed within the 6,083 White (91.0%) and 558 (8.0%) African American, since only one Asian experienced the composite clinical outcome. The clinical characteristics of white patients are shown in Table 5.2 while the clinical characteristics of African American are shown in Table 5.4. In African Americans, cases exhibited a significantly higher rates of congestive heart failure, hypothyroidism, cancer and QT prolongation, compared to controls. Moreover, cases had significantly higher prevalence of use of beta blockers, than controls. Elixhauser and Charlson comorbidities scores were also significantly higher in cases than controls. There were no significant differences observed in any of the other variables assessed. In addition, some significant differences were found in baseline clinical characteristics between African-Americans with low and high risk PRS. The low-risk PRS group exhibited higher hypokalemia, CKD and hypertension. (Chapter 5 Table 4.5**).**

In white patients, the mean ± standard deviation of the PRS was 86.8 ± 7.1, with minimum and maximum values of 62.4 and 114.4, respectively. The association of the PRS as a

continuous variable with the risk of clinical outcome is shown in Table 5.7. There was not statistical significance of the PRS across all three regression models, regardless of whether the sample was propensity matched or not and covariate adjusted or not. The normal distribution of the PRS for cases and control is shown in Figure 5.1. The distributions of PRS for control and cases largely overlap, but the distribution of the PRS for the cases exhibit higher PRS values (Table 5.1). Table 5.8 shows the association of the PRS scaled by low and high-risk cutoffs with the composite clinical outcome. The PRS was not statistically significant in any of the three regression models in the white patient group ($p > 0.05$ in all 3 models). The odds ratios ranged from 1.09 to 1.76 for across all three models. These findings were similar when analyzed in a subgroup of patients with >90% European ancestry (data not shown).

In the African American group, the mean ± standard deviation of the PRS was -36.8 ± 4.1, with minimum and maximum values of -47.0 and -21.8, respectively. The normal distribution of the PRS for cases and control can be observed in Figure 5.1. It shows an overlap in the normal distribution of the PRS among cases and controls. The PRS was not statistically significant in any of the three regression models ($p > 0.05$ in all 3 models; Table 5.7 or scaled by low and high risk cutoff (Table 5.8**)**.

5.4 Discussion

This is the largest study that investigated the association of uncommon genetic variants and a PRS of common variants proposed by Strauss *et al*. [8] with the risk of clinical outcomes, defined by the presence of ventricular arrhythmias and/or sudden death, within a large real-world patient case-control. None of the three genetic variants (*KCNE1* D85N, *KCNE2*-I57T, and *SCN5A*-G615E) and the PRS met the statistical significance for the risk of the composite clinical outcomes during QT prolonging drugs.

There are several reasons that most likely explain the lack of significant association between genetic variants and the PRS with the clinical outcomes in our findings. Firstly, the accuracy of collecting clinical events from the EHR using ICD-9/ICD-10 codes and EMERSE may have been limited, leading to potential underestimation of events. Although these codes are useful for billing purposes, previous studies have highlighted the challenges of using them to accurately capture clinical information.(Molokhia et al., 2008) Additionally, we only have access to the Michigan Medicine EHR. Therefore, if patients experienced these events and sought care at another hospital (typically the nearest facility in an emergency), our study would not have captured those events. This likely results in underestimation of their incidence and prevalence of these clinical events. Moreover, TdP does not have specific ICD-10 codes and is often classified under ventricular arrhythmias, leading also to potential underreporting. Sarganas et al. (Sarganas et al., 2014) found that the reporting rate of TdP for symptomatic patients is 2.5 per million person-years for men and 4.0 per million for women, with 60% attributed to drugs. Additionally, the lack of ECG results for cardiac events occurring outside the hospital may have led to an underestimation of these events.

Secondly, these clinical outcomes are rare, which makes it challenging to clinically capture. ECG recordings during the event are often necessary for an accurate diagnosis.ECG recordings during the event are often necessary for an accurate diagnosis. Some European centers have estimated an annual reporting rate of drug-induced TdP between 0.8 and 1.2 per million person-years (Molokhia et al., 2008), with the frequency of death due to TdP difficult to estimate due to under-recognition before ECG documentation. Unexpected sudden cardiac death is also rare, with an estimated incidence ranging from 50 to 100 per 100,000 individuals per year in Europe and North America.(Fishman et al., 2010)

Lastly, at the molecular level, complexities in drug action and clinical effects can lead to discrepancies.(Roden, 2004) Although QT prolongation is an essential first step in TdP, it is usually not considered sufficient to induce TdP. For instance, while amiodarone prolongs the QT interval in patients with a normal baseline QT, it rarely causes TdP.(Lazzara, 1989) On the other hand, terfenadine, despite causing minimal QT prolongation, was frequently associated with TdP, leading to its withdrawal from the market.(Monahan et al., 1990) This suggests that additional risk factors beyond QT prolongation play a significant role in facilitating drug-induced TdP. Factors such as increased dispersion in the recovery of excitability and the development of early afterdepolarizations may be involved in the proarrhythmic effects leading to TdP.

It is also important to note QT prolongation was not strongly associated with the composite of clinical outcome. The odd ratios in the unadjusted model (Model 1) were 2.1 and 5.8 for white and African American patients respectively. A previous study found that QT prolongation increases risk of cardiac event by 10-fold. (Goldenberg et al., 2011) However, determining the precise degree to which QT prolongation increases the risk of cardiac events is challenging since it has primarily been measured in patients with cLQTS. Furthermore, our

analyses reveled that adjusting for covariates, the association between QT prolongation and the composite clinical outcome ceased to be significant. Patients who experienced the composite clinical outcome had a higher incidence of heart diseases such as congestive heart failure, coronary artery disease, and a history of myocardial infarction compared to the controls. These findings suggest that these comorbidities play a significant role in explaining the composite clinical outcome. This is not surprising since, as previous studies have found that patients with heart failure often exhibit down-regulation of potassium channels (Tsuji et al., 2000) and up-regulation of calcium channels,(Sipido et al., 2000) leading to a prolongation of the action potential. The increase in the duration of the action potential, increases the risk of TdP in patients exposed to a drug that further impairs repolarization.(Roden, 1998) Additionally, ventricular arrhythmias can result from structural heart disease, such as reentry involving scar tissue after a MI.(El-Sherif, Smith, & Evans, 1981). Moreover, the incidence of ventricular arrhythmias in patients with CAD can be as high as 40%. (F. Yang, Turakhia, & Froelicher, 2014)

This study, like chapters 3 and 4, had additional limitations worth noting. It adopts a retrospective observational design within a single health system, which may restrict the generalizability and applicability of our findings to other health systems. Comorbidity data was extracted from the EHR employing ICD-9 and ICD-10 codes, both acknowledged to have inaccuracies. Moreover, our analysis revolved around prescribed medications in patients' lists, without a direct assessment of adherence to these prescriptions. Lastly, we aimed to include syncope in the composite clinical outcome; however, due to significant discrepancies between the syncope ICD-10/ICD-9 codes and terms identified in EMERSE, we have opted to exclude syncope from the composite clinical outcome.

5.5 Conclusion

In conclusion *KCNE1* D85N, *KCNE2*-I57T, and *SCN5A*-G615E and the PRS of genetic common variants were not significantly associated with ventricular arrhythmias and sudden death in patients treated with QT prolonging drugs in a large health system.

5.6 Figures

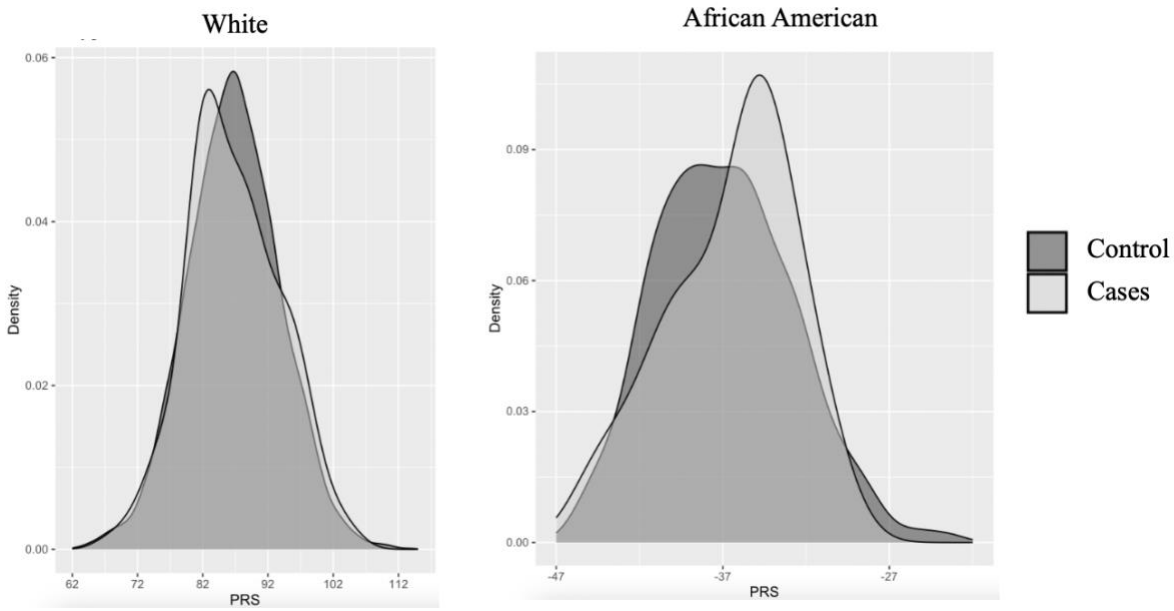

Figure 5.1 Normal distribution of the PRS for cases and controls of self-reported white, African American and Asian patients.

5.7 Tables

Table 5.1 Clinical outcome identification from the electronic health record.

Clinical outcome	Field Names	Data codes	ICD codes
Premature ventricular complex	Premature ventricular depolarization	Diagnoses - main ICD10 Diagnoses – secondary ICD10 Underlying (primary) cause of death: ICD10 Contributory (secondary) cause of death: ICD10	I49.3
Ventricular arrhythmias	Reentry ventricular arrhythmia, Ventricular tachycardia, Ventricular fibrillation and flutter, Cardiac arrest with successful resuscitation, Cardiac arrest, unspecified	Diagnoses - main ICD10 Diagnoses – secondary ICD10 Underlying (primary) cause of death: ICD10 Contributory (secondary) cause of death: ICD10	I47.0, I47.2, I49.0, I46.0, I46.1, I46.9
	Paroxysmal ventricular tachycardia, Ventricular fibrillation and flutter, Cardiac arrest	Diagnoses - main ICD9 Diagnoses - secondary ICD9	427.1, 427.4, 427.5

Table 5.2 Baseline characteristics of control and cases and by propensity score matched sample of self-reported white patients. BMI: Body mass index; CHF: congestive heart failure; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CrCl: creatinine clearance; DM: diabetes mellitus; HTN: hypertension; MI: myocardial infarction; PVD: peripheral vascular disease. Bolded p-values < 0.05.

Characteristics	**Unmatched sample**		**p-value**	**Propensity-matched sample 1:1**		**p-value**
	Control	**Cases**		**Control**	**Cases**	
Patients, n (%)	5,882 (96.7)	201 (3.3)	__	201 (50.0)	201 (50.0)	__
Aged 68 years or older, n (%)	1,967 (33.4)	75 (37.3)	0.286	83 (41.3)	75 (37.3)	0.475
Female, n (%)	3,273 (55.6)	97 (48.3)	**0.046**	103 (51.2)	97 (48.3)	0.618
Hypokalemia, n (%)	418 (7.1)	10 (5.0)	0.307	14 (7.0)	10 (5.0)	0.528
Hypocalcemia, n (%)	1,257 (21.4)	46 (22.9)	0.669	43 (21.4)	46 (22.9)	0.810
Hypomagnesemia, n (%)	1,147 (19.5)	40 (19.9)	0.960	36 (17.9)	40 (19.9)	0.702
BMI (kg/m^2), mean (SD)	30.3 (7.6)	30.7 (7.3)	0.404	30.8 (7.8)	30.7 (7.3)	0.892
CHF, n (%)	457 (7.8)	60 (29.9)	**<0.001**	58 (28.9)	60 (29.9)	0.913
HTN, n (%)	3,379 (57.4)	126 (62.7)	0.160	126 (62.7)	126 (62.7)	1.000
CAD, n (%)	2,557 (43.5)	109 (54.2)	**0.003**	114 (56.7)	109 (54.2)	0.688
Hypothyroidism, n (%)	837 (14.2)	22 (10.9)	0.226	30 (14.9)	22 (10.9)	0.298
History of MI, n (%)	434 (7.4)	36 (17.9)	**<0.001**	27 (13.4)	36 (17.9)	0.272
DM, n (%)	1,265 (21.5)	54 (26.9)	0.084	47 (23.4)	54 (26.9)	0.490
DM complicated, n (%)	426 (7.2)	14 (7.0)	0.991	24 (11.9)	14 (7.0)	0.125
History of Stroke, n (%)	627 (10.7)	23 (11.4)	0.812	31 (15.4)	23 (11.4)	0.306
CKD, n (%)	857 (14.6)	34 (16.9)	0.410	34 (16.9)	34 (16.9)	1.000
Severe liver disease, n (%)	110 (1.9)	2 (1.0)	0.589	5 (2.5)	2 (1.0)	0.449
Liver disease, n (%)	694 (11.8)	19 (9.5)	0.365	29 (14.4)	19 (9.5)	0.166
COPD, n (%)	508 (8.6)	17 (8.5)	1.000	13 (6.5)	17 (8.5)	0.569
Cancer, n (%)	424 (7.2)	12 (6.0)	0.584	15 (7.5)	12 (6.0)	0.680
PVD, n (%)	545 (9.3)	26 (12.9)	0.103	30 (14.9)	26 (12.9)	0.666
History of alcohol consumption, n (%)	229 (3.9)	7 (3.5)	1.000	11 (5.5)	7 (3.5)	0.470
Loop Diuretic, n (%)	869 (14.9)	60 (29.9)	**<0.001**	67 (33.3)	60 (29.9)	0.520
Digoxin, n (%)	30 (0.5)	7 (3.5)	**<0.001**	9 (4.5)	7 (3.5)	0.800
Beta-blockers, n (%)	1,704 (29.0)	90 (44.8)	**<0.001**	98 (48.8)	90 (44.8)	0.484
≥2 QT prolonging drugs, n (%)	2,939 (50.0)	104 (41.7)	0.672	100 (49.8)	104 (51.7)	0.765
QT prolongation, n (%)	688 (11.7)	45 (22.4)	**<0.001**	41(20.4)	45 (22.4)	0.715
Elixhauser score (IQR)	8.0 (14.0)	12.0 (12.0)	**<0.001**	12 (14.0)	12 (12.0)	0.969
Charlson score (IQR)	3 (5.0)	3 (6.0)	0.456	4 (5)	3 (6)	0.056

Table 5.3 Baseline characteristics of propensity score matched sample with 1:2, 1:3 and 1:4 matching ratio of cases and control in self-reported white patients. BMI: Body mass index; CHF: congestive heart failure; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CrCl: creatinine clearance; DM: diabetes mellitus; HTN: hypertension; MI: myocardial infarction; PVD: peripheral vascular disease. Bolded p-values < 0.05.

Characteristics	**Propensity-matched sample 1:2**		**p-value**	**Propensity-matched sample 1:3**		**p-value**	**Propensity-matched sample 1:4**		**p-value**
	Control	**Cases**		**Control**	**Cases**		**Control**	**Cases**	
Patients, n (%)	402 (66.7)	201 (33.3)	__	603 (75.0)	201 (25.0)	__	804 (80.0)	201 (20.0)	_
Aged 68 years or older, n (%)	164 (40.8)	75 (37.3)	0.462	230 (39.1)	75 (37.3)	0.900	309 (38.4)	75 (37.3)	0.833
Female, n (%)	202 (50.2)	97 (48.3)	0.708	304 (50.4)	97 (48.3)	0.654	395 (49.1)	97 (48.3)	0.887
Hypokalemia, n (%)	32 (8.0)	10 (5.0)	0.235	42 (7.0)	10 (5.0)	0.408	57 (7.1)	10 (5.0)	0.359
Hypocalcemia, n (%)	90 (22.4)	46 (22.9)	0.973	137 (22.7)	46 (22.9)	1.000	189 (23.5)	46 (22.9)	0.926
Hypomagnesemia, n (%)	70 (17.4)	40 (19.9)	0.526	111(18.4)	40 (19.9)	0.715	157 (19.5)	40 (19.9)	0.984
BMI (kg/m^2), mean (SD)	29.9 (7.7)	30.7 (7.3)	0.250	30.3 (7.8)	30.7 (7.3)	0.502	30.3 (7.3)	30.7 (7.3)	0.518
CHF, n (%)	111 (27.6)	60 (29.9)	0.632	180 (29.9)	60 (29.9)	1.000	229 (28.5)	60 (29.9)	0.767
HTN, n (%)	266 (66.2)	126 (62.7)	0.450	405 (67.2)	126 (62.7)	0.282	542 (67.4)	126 (62.7)	0.236
CAD, n (%)	220 (54.7)	109 (54.2)	0.977	329 (54.6)	109 (54.2)	1.000	438 (54.5)	109 (54.2)	1.000
Hypothyroidism, n (%)	53 (13.2)	22 (10.9)	0.513	86 (14.3)	22 (10.9)	0.282	113 (14.1)	22 (10.9)	0.298
History of MI, n (%)	66 (16.4)	36 (17.9)	0.730	104 (17.2)	36 (17.9)	0.914	131 (16.3)	36 (17.9)	0.656
DM, n (%)	85 (21.1)	54 (26.9)	0.142	162 (26.9)	54 (26.9)	1.000	231 (28.7)	54 (26.9)	0.662
DM complicated, n (%)	31 (7.7)	14 (7.0)	0.869	50 (8.3)	14 (7.0)	0.652	60 (7.5)	14 (7.0)	0.928
History of Stroke, n (%)	51 (12.7)	23 (11.4)	0.759	76 (12.6)	23 (11.4)	0.757	101 (12.6)	23 (11.4)	0.755
CKD, n (%)	61 (15.2)	34 (16.9)	0.664	105 (17.4)	34 (16.9)	0.957	143 (17.8)	34 (16.9)	0.852
Severe liver disease, n (%)	2 (0.5)	2 (1.0)	0.604	11 (1.8)	2 (1.0)	0.535	11 (1.4)	2 (1.0)	1.000
Liver disease, n (%)	39 (9.7)	19 (9.5)	1.000	59 (9.8)	19 (9.5)	1.000	79 (9.8)	19 (9.5)	0.979
COPD, n (%)	33 (8.2)	17 (8.5)	1.000	59 (9.8)	17 (8.5)	0.676	71 (8.8)	17 (8.5)	0.978
Cancer, n (%)	23 (5.7)	12 (6.0)	1.000	40 (6.7)	12 (6.0)	0.860	57 (7.1)	12 (6.0)	0.679
PVD, n (%)	59 (14.7)	26 (12.9)	0.649	90 (14.9)	26 (12.9)	0.562	119 (14.8)	26 (12.9)	0.575
History of alcohol consumption, n (%)	10 (2.5)	7 (3.5)	0.602	15 (2.5)	7 (3.5)	0.458	21 (2.6)	7 (3.5)	0.477
Loop Diuretic, n (%)	128 (31.8)	60 (29.9)	0.686	175 (29.0)	60 (29.9)	0.893	232 (28.9)	60 (29.9)	0.848
Digoxin, n (%)	13 (3.2)	7 (3.5)	1.000	16 (2.7)	7 (3.5)	0.625	19 (2.4)	7 (3.5)	0.454
Beta-blockers, n (%)	200 (49.8)	90 (44.8)	0.286	280 (46.4)	90 (44.8)	0.744	367 (45.6)	90 (44.8)	0.887
$\geq$2 QT prolonging drugs, n (%)	188 (46.8)	104 (51.7)	0.286	295 (48.9)	104 (51.7)	0.541	393 (48.9)	104 (51.7)	0.518
QT prolongation, n (%)	86 (21.4)	45 (22.4)	0.861	132 (21.9)	45 (22.4)	0.961	173 (21.5)	45 (22.4)	0.863
Elixhauser score (IQR)	12 (14.7)	12 (12.0)	0.588	12 (14.0)	12 (12.0)	0.535	12 (15.0)	12 (12.0)	0.534
Charlson score (IQR)	3.5 (6.0)	3 (6.6)	0.483	3 (6.0)	3 (6.0)	0.391	3 (6.0)	3 (6.0)	0.319

Table 5.4 Baseline characteristics of control and cases on self-reported African American patients. BMI: Body mass index; CHF: congestive heart failure; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CrCl: creatinine clearance; DM: diabetes mellitus; HTN: hypertension; MI: myocardial infarction; PVD: peripheral vascular disease. Bolded p-values < 0.05.

	African American Unmatched sample		
Characteristics	**Control**	**Cases**	**p-value**
Patients, n (%)	543 (97.3)	15 (2.7)	-
Aged 68 years or older, n (%)	49 (9.0)	2 (13.3)	0.639
Female, n (%)	362 (66.7)	9 (60.0)	0.588
Hypokalemia, n (%)	39 (7.2)	2 (13.3)	0.303
Hypocalcemia, n (%)	84 (15.5)	4 (26.7)	0.273
Hypomagnesemia, n (%)	69 (12.7)	3 (20.0)	0.426
BMI (kg/m^2), mean (SD)	32.7 (8.6)	32.0 (6.3)	0.659
CHF, n (%)	56 (10.3)	7 (46.7)	**0.001**
HTN, n (%)	352 (64.8)	11 (73.3)	0.592
CAD, n (%)	216 (39.8)	9 (60.0)	0.180
Hypothyroidism, n (%)	37 (6.8)	4 (26.7)	**0.019**
History of MI, n (%)	41 (7.6)	3 (20.0)	0.107
DM, n (%)	141 (26.0)	6 (40.0)	0.239
DM complicated, n (%)	62 (11.4)	3 (20.0)	0.401
History of Stroke, n (%)	67 (12.3)	0 (0.0)	0.236
CKD, n (%)	104 (19.2)	6 (40.0)	0.090
Severe liver disease, n (%)	2 (0.4)	1 (6.7)	0.079
Liver disease, n (%)	50 (9.2)	2 (13.3)	0.642
COPD, n (%)	35 (6.4)	1 (6.7)	1.000
Cancer, n (%)	26 (4.8)	4 (26.7)	**0.006**
PVD, n (%)	34 (6.3)	1 (6.7)	1.000
History of alcohol consumption, n (%)	20 (3.7)	0 (0.0)	1.000
Loop Diuretic, n (%)	68 (10.6)	3 (27.5)	0.422
Digoxin, n (%)	4 (0.7)	1 (6.7)	0.128
Beta-blockers, n (%)	149 (27.4)	10 (66.7)	**0.002**
$\geq$2 QT prolonging drugs, n (%)	258 (47.5)	9 (60.0)	0.435
QT prolongation, n (%)	63 (11.6)	6 (40.0)	**0.006**
Elixhauser score (IQR)	3 (10.0)	16 (16.5)	**<0.001**
Charlson score (IQR)	2 (3.5)	6 (4.0)	**<0.001**

Table 5.5 Multivariable logistic regression models assessing the association of QT prolongation with the composite clinical outcomes in self-reported white and African American patients (dominant genetic model) using the Bazett QTc correction method. **Model 1:** Unmatched sample unadjusted model. **Model 2:** Propensity-matched 1:1 sample unadjusted model. **Model 3:** Propensity-matched 1:2 sample unadjusted model. **Model 4:** Propensity-matched 1:3 sample unadjusted model. **Model 5:** Propensity-matched 1:4 sample unadjusted model. **Model 6:** Unmatched sample propensity score-adjusted model. **Model 7**: Unmatched sample adjusted by the 7 covariates in self-reported African American patients. *p<0.05

Race Groups & Models	**OR (95%CI; p-value)**	**Prolong QTc, without clinical outcome, n (%)**	**Prolong QTc, with clinical outcome, n (%)**	χ^2/**Fisher Exact test p-value**
White Patients				
Model 1 (n=6,083)	2.18 (1.53-3.04); **7.72e-06***			
Model 2 (n=402)	1.13 (0.70-1.82); 0.627	688 (11.7)	45 (22.4)	**<0.0001***
Model 3 (n=603)	1.06 (0.70-1.59); 0.780			
Model 4 (n=804)	1.03 (0.70-1.50); 0.883			
Model 5 (n=1,005)	1.05 (0.72-1.52); 0.789			
Model 6 (n=6,083)	1.15 (0.76-1.69); 0.497			
African American				
Model 1 (n=598)	5.08 (1.65-14.6); **0.003***	63 (11.6)	6 (40.0)	**0.006***
Model 7 (n=598)	1.09 (0.23-4.21); 0.907			

Table 5.6 Multivariable logistic regression models assessing the association of candidate genetic variants with the composite clinical outcomes in self-reported white patients (dominant genetic model) using the Bazett QTc correction method. **Model 1:** Unmatched sample unadjusted model (total n = 6,083). **Model 2:** Propensity-matched sample unadjusted model. **Model 3:** Propensity-matched 1:2 sample unadjusted model. **Model 4:** Propensity-matched 1:3 sample unadjusted model. **Model 5:** Propensity-matched 1:4 sample unadjusted model. **Model 6:** Unmatched sample propensity score-adjusted model. *p<0.017 (Bonferroni correction for multiple comparisons)

Genetic variants	**OR (95%CI; p-value)**	**Control, n (%)**	**Cases, n (%)**	χ^2/**Fisher Exact test p-value**
***SCN5A* G615E rs12720452**				
Model 1 (n=6,083)	3.75e-05 (N/A-8.05e+08); 0.970	4 (0.1)	0 (0.0)	1.000
Model 2 (n=402)	N/A			
Model 3 (n=603)	2.56e-06 (N/A-6.28e+41); 0.981			
Model 4 (n=804)	3.84e-06 (N/A-9.44e+41); 0.981			
Model 5 (n=1,005)	5.13e-06 (N/A-1.26e+42); 0.982			
Model 6 (n=6,083)	1.42e-05 (N/A-1.02e+17); 0.980			
***KCNE2* I57T rs74315448**				
Model 1 (n=6,083)	2.67 (0.15-13.82); 0.348	11 (0.2)	1 (0.5)	0.332
Model 2 (n=402)	N/A (0.04-25.4); N/A			
Model 3 (n=603)	2.01 (0.08-50.9); 0.623			
Model 4 (n=804)	3.01 (0.12-76.4); 0.437			
Model 5 (n=1,005)	2.01 (0.09-21.0); 0.571			
Model 6 (n=6,083)	2.21 (0.16-12.34); 0.462			
***KCNE1* D85N rs1805128**				
Model 1 (n=6,083)	0.53 (0.09-1.67); 0.372	110 (1.9)	2 (1.0)	0.589
Model 2 (n=402)	0.49 (0.068-2.56); 0.420			
Model 3 (n=603)	0.57 (0.08-2.37); 0.482			
Model 4 (n=804)	0.46 (0.07-1.67); 0.304			
Model 5 (n=1,005)	0.49 (0.08-1.76); 0.351			
Model 6 (n=6,083)	0.52 (0.08-1.66); 0.360			

Table 5.7 Multivariable logistic regression models assessing the association of the polygenic risk score with the composite clinical outcomes in self-identified white and African American patients. **Model 1:** Unmatched sample unadjusted model. **Model 2:** Propensity-matched 1:1 sample unadjusted model. **Model 3:** Propensity-matched 1:2 sample unadjusted model. **Model 4:** Propensity-matched 1:3 sample unadjusted model. **Model 5:** Propensity-matched 1:4 sample unadjusted model. **Model 6:** Unmatched sample propensity score-adjusted model. **Model 7**: Unmatched sample adjusted by the 7 covariates in African American patients. *p<0.05.

Race Groups & Models	OR (95%CI; p-value)
White	
Model 1 (n = 6,083)	1.00 (0.98-1.02); 0.982
Model 2 (n = 402)	1.02 (0.99-1.05); 0.168
Model 3 (n=603)	0.99 (0.97-1.02); 0.773
Model 4 (n=804)	1.00 (0.98-1.02); 0.886
Model 5 (n=1,005)	1.00 (0.98-1.03); 0.791
Model 6 (n = 6,083)	1.00 (0.98-1.02); 0.949
African American	
Model 1 (n = 558)	1.07 (0.95-1.20); 0.218
Model 7 (n = 558)	1.09 (0.96-1.24); 0.172

Table 5.8 Multivariable logistic regression models assessing the association of the standardized polygenic risk score with the composite clinical outcome in white and African American patients. **Model 1:** Unmatched sample unadjusted model. **Model 2:** Propensity-matched 1:1 sample unadjusted model. **Model 3:** Propensity-matched 1:2 sample unadjusted model. **Model 4:** Propensity-matched 1:3 sample unadjusted model. **Model 5:** Propensity-matched 1:4 sample unadjusted model. **Model 6:** Unmatched sample propensity score-adjusted model. **Model 7**: Unmatched sample adjusted by the 7 covariates in African American patients. *p<0.05.

Race Groups & Models	OR (95%CI; p-value)	Control	Cases	p-values
White				
Model 1 (n = 6,083)	1.09 (0.64-1.77); 0.720	457 (7.8)	17 (8.5)	0.823
Model 2 (n = 402)	1.76 (0.80-4.09); 0.168			
Model 3 (n=603)	1.07 (0.57-1.95); 0.833			
Model 4 (n=804)	1.27 (0.68-2.25); 0.432			
Model 5 (n=1,005)	1.26 (0.69-2.17); 0.427			
Model 6 (n = 6,083)	1.09 (0.63-1.78); 0.729			
African American				
Model 1 (n = 558)	1.29 (0.35-8.36); 0.739	453 (83.4)	13 (86.7)	1.000
Model 7 (n = 558)	1.82 (0.39-14.5); 0.501			

Chapter 6 Exploring Interaction Among Genetic Variants and Clinical Factors for Drug-Induced Long QT syndrome: A Data Mining Approach with Classification and Regression Trees

6.1 Background

In the previous chapter #3 and #4, we found that both common and uncommon genetic variants are associated with an increased risk of diLQTS. We assessed these variants as independent risk factors for diLQTS. However, the effect of a single gene on the phenotype may depend on the presence of one or more genes, a phenomenon known as epistasis. Several studies have highlighted the importance of epistasis in complex cardiovascular traits such as atrial fibrillation (Huang et al., 2015) and coronary artery disease.(Musameh et al., 2015) Additionally, gene-environment interactions can also modify the phenotype of cardiovascular conditions. However, detecting epistasis and gene-environmental interactions presents statistical challenges. Traditional parametric statistical methods can identify epistasis and gene-environmental interactions by incorporating multiplicative interaction terms (e.g., SNP1*SNP2) into the model. However, the power of such models diminishes significantly with an increasing number of parameters. Furthermore, traditional regression models are typically limited to testing pairwise interactions due to the exponential increase in model complexity associated with higher-order interactions, leading to substantial challenges with multiple testing.

In the era of artificial intelligence (AI), the capacity to predict outcomes across diverse disciplines has expanded significantly. (Ramesh, Kambhampati, Monson, & Drew, 2004) One

notable advancement lies in the emergence of machine learning, a branch of AI which focuses on the use of data and algorithms that mimic human learning processes, gradually improving its accuracy. Classification and Regression Trees (CART) is a machine learning tool that employed algorithms to autonomously navigate databases for patterns or regularities. Unlike traditional statistical analyses, CART is non-parametric and is free from assumptions. It does not require a pre-defined underlying relationship between the outcome and predictors or imply a cause-and-effect relationships between variables. Instead, it focuses on identifying statistical associations among them (Leclerc et al., 2009). CART inherently evaluates higher-order interactions and considers all genetic inheritance models, resulting in easily interpretable and clinically applicable output models without the need for extensive computations.

CART can be broadly categorized into recursive partitioning, combinatorial, or neural network approaches. Recursive partitioning, particularly tree-based techniques, offers several advantages including the ability to manage a large number of input variables, rapid computation times, suitability for genetic heterogeneity, and the production of easily interpretable final models. This method involves data splitting into increasingly smaller and homogeneous subsets based on researcher-specified criteria (Crichton et al., 1997). Within this process, all predictor variables are assessed at each level to identify the split resulting in the purest nodes (Prasad et al., 2006) according to the machine-learned algorithm. Repercussive partitioning has gained atraction in medicine for risk stratification (Karaolis et al., 2010) and prognosis determination (Lamborn et al., 2004).

In addition to risk quantification, CART serves as a valuable tool for generating new knowledge. Its analytical approach is particularly suitable for uncovering associations between genetic variants and clinical factors with diLQTS risk, some of which may display nonlinear

relationships not easily discernible through conventional regression-based methods. Given the combined impacts of these genetic variants alongside clinical risk factors remains unclear, the objective of this chapter is to evaluate the predictive performance of combining genetic variants and clinical factors in predicting diLQTS risk using CART.

6.2 Methods

The "rpart" command package from R version 4.2.2 was used for generating CART. The binary outcome variable was the presence of QTc prolongation, defined as a change of >60 msec from the baseline QTc and/or an absolute QTc value ≥500 msec while patients were prescribed any high-risk QT-prolonging drugs. The QT intervals measurements were obtained from an average of across the 12-leads of the ECG, (Chapter 2) with the primary outcome corrected for heart rate using the Bazett formula.

A total of 28 clinical and 94 genetic input variables were included in the analysis (Table 6.1). The full dataset was randomly divided into a 70% training dataset and 30% validation dataset. Variable selection was performed in the training dataset and the tree performance was evaluated on the test dataset. An internal validation using 10-fold cross validation was used during the tree building process. Tree accuracy was assessed using area under the receiver operating characteristic curve (AUC ROC) and misclassification rate. Decision trees were also derived in racial strata (self-reported white and African American). Due to the small sample size of Asian, we were not able to construct a decision tree for this race group.

6.3 Results

When recursive partitioning all of the white patients, the variables that stayed in the model for predicting diLQTS were presence of loop diuretic, rs3857067, Stroke, BMI>=40,

female and rs12025136 (Figure 6.1). In presence of loop diuretics, patients with the rs3857067 diplotype TT and a carrier of the alternative allele T of rs12025136, had 68% probability of diLQTS. In absence or in presence of 1 copy of the alternative T in the rs3857067, additional clinical factors such as the presence of a history of stroke and a BMI less than 40, increased the probability of diLQTS to 56%. However, being female further elevated the diLQTS risk to 77% in this group. The ROC AUC was 0.6168 and the misclassification rate was 12.7%.

In African Americans, the variables that remained in the model for predicting diLQTS were hypocalcemia and Elixahouser score (Figure 6.2). Patients with hypocalcemia and Elixhauser score > 19, the risk of diLQTS was 78%. Then AUC was 0.585, and the misclassification rate was 14.2%.

6.4 Discussion

To our knowledge, this is the first report using data mining to detect gene-gene and gene-environmental interactions associated with diLQTS. We analyzed 28 clinical and 94 genetic variants to explore their associations with diLQTS. Among white patients, we identified a gene-gene interaction between rs3857067 and rs12025136, whereas among African American patients, none of the genetic variants were associated with the risk of diLQTS.

The rs3857067 variant is in an intergenic region on chromosome 4, with the nearest gene being SMARCAD1-DT. The rs3857067 has been previously associated with QT prolongation ($p=1 \times 10^{-9}$) in a genome-wide association study (GWAS) involving 70,389 individuals of European ancestry. (Arking et al., 2014) These findings have been validating in a recent study of 500,584 participants from the UK Biobank. The rs3857067 was found to be associated with an increase in QTc duration ($p<0.0007$) and an sudden cardiac death (hazard ratio = 1.23, $p = 0.03$) when considered individually. (Arora et al., 2023) Similarly, the rs12025136 variant, located on

chromosome 1 and nearest to the *NOS1AP* gene, was also identified as an intronic variant associated with QT prolongation in the GWAS of Arking et al., 2014.(Arking et al., 2014) However, this association with diLQTS risk, unlike rs3857067, has not been validated. Both rs3857067 and rs12025136 were included in the polygenic risk score of diLQTS proposed by Strauss et al.(Strauss et al., 2017) However, this is the first time, that interactions between these variants have been identified. It's crucial to understand that data mining methods are not designed for hypothesis testing; rather, they serve to generate hypotheses. For instance, the overarching hypothesis of this study was that CART would uncover novel interactions among both uncommon, common genetic variants and clinical factors to predict the risk of diLQTS. Due to the limited sample sizes in the leaf nodes, the pharmacogenetic interaction between rs3857067 and rs12025136 should be validated in another study with adequate statistical power, using traditional statistical methods. It also needs to be tested in experimental model to determine at what level the interaction is occurring (i.e. transcriptional, translational, functional, or physiological level). When considering African American patients, none of the genetic variants were included in the model.

It is important to highlight that both models had an AUC less than 0.7, which is considered below the threshold for acceptable discrimination between patients with normal and prolonged QTc. This suggests that the interaction between rs3857067 and rs12025136 may not be real. Additionally, *KCNE1*-D85N did not appear in the model, despite its significance in Aim 1 (Chapter #3). Considering these findings, other machine learning methods such as random forest may offer a more effective approach to exploring genetic interactions with diLQTS risk.

6.5 Conclusion

To our knowledge, this is the first report using data mining to detect gene-gene and gene-environmental interactions associated with diLQTS. Among white patients, we identified a gene-gene interaction between rs3857067 and rs12025136, whereas among African patients, none of the genetic variants were found to be associated with the risk of diLQTS. The pharmacogenetic interaction between rs3857067 and rs12025136 should be validated in in another study with adequate statistical power, using traditional statistical methods.

6.6 Figure

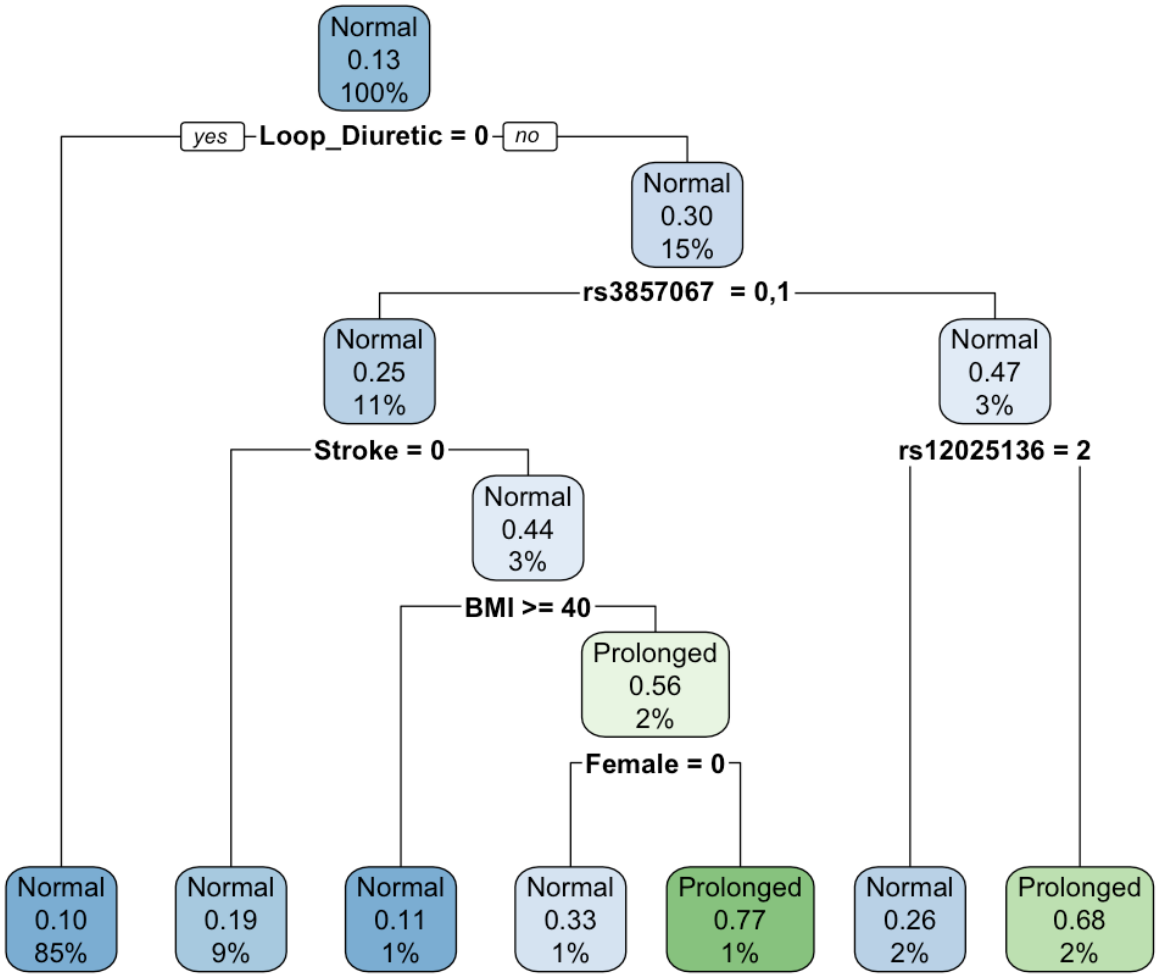

Figure 6.1 Decision tree output from recursive partitioning all white patients (n=6,083) using the CART algorithm in R version 4.2.2 is shown. Progressive binary splits of the data are made, considering all input variables and their values, to best classify white patients according to the binary outcome variable of QT prolongation.

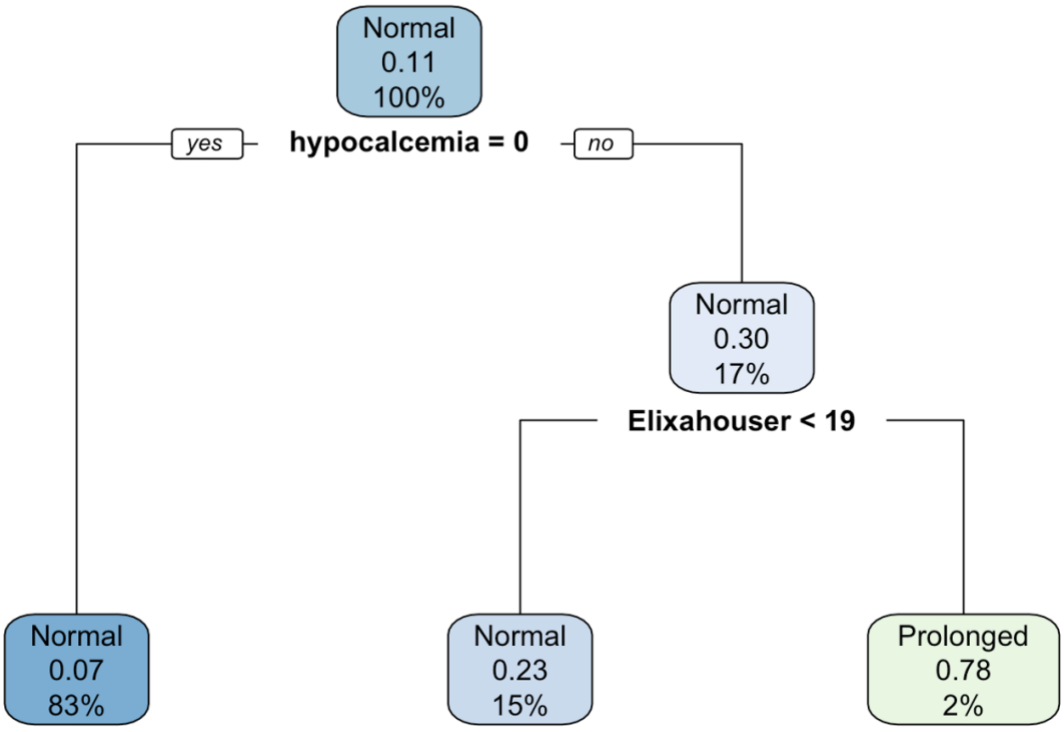

Figure 6.2 Decision tree output from recursive partitioning all African American patients (n=598) using the CART algorithm in R version 4.2.2 is shown. Progressive binary splits of the data are made, considering all input variables and their values, to best classify white patients according to the binary outcome variable of QT prolongation.

6.7 Tables

Table 6.1 Genetic and clinical variables input in the CART model.

SNP	Chr.	Nearest Gene	SNP	Chr.	Nearest Gene	Clinical Variables
rs10919070	1	ATP1B1	rs3105593	15	USP50-TRPM7	Aged 68 years or older
rs11809180	1	ATP1B1	rs246258	16	CNOT1	Female
rs12061601	1	ATP1B1	rs4784934	16	CNOT1	Hypokalemia
rs1983546	1	ATP1B1	rs1296720	16	CREBBP	Hypocalcemia
rs545833	1	ATP1B1	rs12444261	16	LITAF	Hypomagnesemia
rs12025136	1	NOS1AP	rs735951	16	LITAF	BMI (kg/m^2)
rs12143842	1	NOS1AP	rs246185	16	MKL2	CHF
rs164133	1	NOS1AP	rs10775360	17	KCNJ2	HTN
rs16857031	1	NOS1AP	rs1396515	17	KCNJ2	CAD
rs17460657	1	NOS1AP	rs17763769	17	KCNJ2	Hypothyroidism
rs347272	1	NOS1AP	rs236586	17	KCNJ2	History of MI
rs3934467	1	NOS1AP	rs1052536	17	LIG3	DM
rs4656345	1	NOS1AP	rs9892651	17	PRKCA	DM complicated
rs2273042	1	RNF207	rs1805128	21	KCNE1	History of Stroke
rs846111	1	RNF207	rs7626962	3	SCN5A	CKD
rs2298632	1	TCEA3	rs72546668	3	CAV3	Severe liver disease
rs12997023	2	SLC8A1	rs74315448	21	KCNE2	Liver disease
rs6544311	2	SLC8A1	rs74315447	21	KCNE2	COPD
rs938291	2	SP3	rs16991652	21	KCNE2	Cancer
rs295140	2	SPATS2L	rs144022614	20	JPH2	PVD
rs7561149	2	TTN-CCDC141	rs830233	5	-	Arrhythmia
rs17784882	3	C3ORF75	rs201049603	12	CACNA1C	History of alcohol consumption
rs11708996	3	SCN5A-SCN10A	rs138776684	7	KCNH2	Loop Diuretic
rs6793245	3	SCN5A-SCN10A	rs10494366	1	NOS1AP	Digoxin
rs6801957		SCN5A-SCN10A	rs10800397	1	NOS1AP	Beta-blockers
rs9851710	3	SCN5A-SCN10A	rs10919035	1	NOS1AP	≥2 QT prolonging drugs
rs2363719	4	SLC4A4	rs10918594	1	-	Elixhauser score (IQR)
rs3857067	4	SMARCAD1	rs139901716	1	KCND3	Charlson score (IQR)
rs10040989	5	GFRA3	rs77331749	7	KCNH2	
rs7765828	6	GMPR	rs11970286	6	-	

rs10499087	6	SLC35F1-PLN	rs45488304	3	SCN5A	
rs11153730	6	SLC35F1-PLN	rs9966832	18	-	
rs12210733	6	SLC35F1-PLN	rs4799915	18	CELF4	
rs17349133	6	SLC35F1-PLN	rs141724500	20	SNTA1	
rs465226	6	SLC35F1-PLN	rs2291477	1	TGFBR3	
rs9920	7	CAV1	rs12720452	3	SCN5A	
rs1805121	7	KCNH2	rs199473505	7	KCNH2	
rs2072413	7	KCNH2	rs541781240	11	APLP2	
rs1961102	8	AZIN1	rs7142881	14	NUBPL	
rs16936870	8	NCOA2	rs7188697	16	CNOT1	
rs2485376	10	GBF1	rs4933824	10	NRG3	
rs174583	11	FADS2	rs149908041	16	ZFHX3-AS1	
rs2074238	11	KCNQ1	rs1800172	11	KCNQ1	
rs7122937	11	KCNQ1	rs74315445	21	KCNE1	
rs3026445	12	ATP2A2	rs174583	11	FADS2	
rs728926	13	KLF12	rs3924426	15	SLCO3A1	
rs2273905	14	ANKRD9	rs17054392	4	PALLD	
rs993648	2	CERKL				

Chapter 7 Discussion

Drug-induced long QT syndrome (diLQTS) is an adverse effect of over 150 FDA-approved drugs commonly used for a wide range of clinical conditions. It occurs when certain medications (i.e. antibiotics, antifungals, antidepressants) prolong the QT interval in the electrocardiogram (ECG). QT prolongation can predispose individuals to a potentially life-threatening arrhythmia known as torsades de pointes (TdP), which can progress to ventricular fibrillation and sudden cardiac death. (Drew et al., 2010; Roden, 2004) Unfortunately, clinical characteristics do not entirely explain the variability in diLQTS response. Genetics has emerged as a potential missing link that could help predict the risk of diLQTS. Previous evidence shows that both common (minor allele frequency [MAF] >1%) and uncommon ([MAF] <1%) genetic variants are associated with diLQTS risk.(Roden et al., 2018) Therefore, the collective objective of this dissertation research was to determine the associations and interactions of individual, uncommon candidate genetic variants and the polygenic score of common variants with diLQTS risk in a large, observational case-control in real-world clinical practice.

Repolarization of the cardiac action potential is crucial in determining the duration of the QT interval. Studies investigating candidate genes involved in cardiac repolarization have identified individual, uncommon genetic variants associated with diLQTS (Niemeijer et al., 2015) Conversely, genome-wide association studies (GWAS) have uncovered numerous common genetic variants from various genes linked to QT interval duration, with unclear mechanisms. (Arking et al., 2014) To assess the strength of evidence of those genetic variants with diLQTS risk, we developed a novel semiquantitative scoring system, adapted from the

approach used for congenital Long QT Syndrome (cLQTS). We were able to identify 112 variants associated with diLQTS in the literature. Among these, only one variant showed definitive evidence (*KCNE1*-D85N), one had strong evidence (*KCNE2*-T8A), four had moderate evidence (*SCN5A*-L1825P, *SCN5A*-G615E, *KCNE1*-D76N, and *KCNE2*-I57T), and the remaining 106 had limited evidence. However, a notable limitation of previous studies candidate studies was their small sample size.

To overcome the limitation of small sample size from previous pharmacogenomic studies, we investigated the associations of uncommon genetic variants with in a large observational case-control study involving 6,083 self-reported white patients treated with 27 different high-risk QT-prolonging medications, of whom 12.0% had diLQTS. To our knowledge, this study represents the largest candidate gene study to date investigating the association of candidate genetic variants with the risk of diLQTS. Our results further corroborate the significance of *KCNE1*-D85N as a risk variant for diLQTS. However, our study lacked sufficient power to provide precise estimates for *KCNE2*-I57T and *SCN5A*-G615E.

KCNE1-D85N has been found as a risk factor for diLQTS in various studies. *In vitro* studies have provided evidence of functional effects supporting this association (Du et al., 2013; Nishio et al., 2009; Nof et al., 2011; Sakata et al., 2014; Westenskow et al., 2004). Additionally, two case reports (Lin et al., 2012; Marstrand et al., 2018), three candidate gene studies (Kaab et al., 2012; Martinez-Matilla et al., 2019; Paulussen et al., 2004) and a whole-exome sequencing study (Weeke et al., 2014) have supported this association. The susceptibility to diLQTS in individuals carrying D85N may be due to a reduced "repolarization reserve." This term refers to a lack of backup mechanisms compensating for either decrease repolarizing or increase depolarizing currents during the cardiac action potential. (Roden, 2004) Subjects with

reduced repolarization reserve are more likely to develop QT prolongation and TdP when exposed to drugs that blocks the rapid component of the delayed rectifier potassium current (IKr) in the heart. However, there are conflicting findings, with several studies failing to identify an association between the D85N variant and diLQTS cases.(Aberg et al., 2012; Avery et al., 2014; Behr et al., 2013; Chevalier et al., 2001; Corponi et al., 2019; Itoh et al., 2009; Makita et al., 2002; Roberts et al., 2017; Spellmann et al., 2018; Zerdazi et al., 2019) These negative results may be attributed to the small sample sizes in these studies (the largest sample size consisting of only 77 cases) and the low minor allele frequency (MAF) of this variant (approximately 1% in Europeans). As a result, these studies may have been underpowered and could have introduced type 2 errors. By analyzing this variant in a much larger sample size in our study, we enhance the confidence that the association between *KCNE1*-D85N and diLQTS is not a false-positive, especially after meeting the Bonferroni corrected level of significance. Additionally, this dissertation benefits from utilizing authentic patient data from real-world clinical settings, deviating from the highly controlled clinical trial setting in a previous study.(Aberg et al., 2012) This improvement strengthens the relevance and applicability of our findings in real-world clinical practice.

In addition to rare genetic variants, common genetic variants, may also influence susceptibility to diLQTS risk. (Arking et al., 2014; Newton-Cheh et al., 2009; Pfeufer et al., 2009; Roden et al., 2018) Common variants are not likely to have large effect sizes individually, however, their collective impact can magnify diLQTS risk significantly. (Strauss et al., 2017) Strauss et al. developed a PRS of common genetic variants for diLQTS; however, its efficacy was evaluated in a limited clinical trial involving 22 healthy volunteers (77% white race) (Strauss et al., 2017). Additionally, the PRS also was significantly associated with the risk of

drug-induced TdP in 216 cases vs. 771 ancestry-matched controls with European descent ($p = 1.3 \times 10^{-7}$). Nevertheless, this PRS for predicting diLQTS risk has not yet undergone independent validation by another research team. We decided to validate the diLQTS PRS initially proposed by Strauss *et al*. [8] within a large real-world patient case-control. Our results demonstrated the association of this PRS with diLQTS in self-identified white patients. However, we lacked sufficient power to determine the PRS's association with diLQTS risk in African-American and Asian patients due to smaller sample sizes in these racial groups compared to white patients. Our research also extends the prior research by encompassing patients from a real-world clinical setting with diverse comorbidities and many different high-risk QT prolonging drugs. The association between this PRS and diLQTS underscores the clinical relevance of these common genetic variants in diLQTS. Many individual variants, including those located at the *NOS1AP* locus and within various ion channels such as *SCN5A, KCNH2, KCNE1, KCNJ2*, have previously been associated with an elevated risk of diLQTS.(A. I. Lopez-Medina et al., 2022) While these variants individually exhibit minor effects on baseline QT intervals (e.g., 1–3 ms) in GWAS (Arking et al., 2014), their cumulative impact suggests the PRS's potential to effectively predict diLQTS.

This dissertation aimed to assess the relationship between uncommon and common genetic variants and diLQTS, using QTc prolongation as the primary outcome. We also considered a composite secondary outcome comprised of history of TdP, syncope, any ventricular arrhythmias, and/or sudden death documented during the drug treatment period. However, we did not find any association between *KCNE1*-D85N, *KCNE2*-I57T, *SCN5A*-G615E, and the PRS with this composite clinical outcome. Several factors may have contributed to these results. Firstly, the accuracy of collecting clinical events from EHR ICD-9/ICD-10 codes

and EMERSE may have been limited. Previous studies have highlighted the challenges of using these codes to capture clinical information accurately (Horsky, Drucker, & Ramelson, 2017). Secondly, these clinical outcomes are rare making them challenging to clinically capture. Quantifying the association between these rare outcomes and genetics is challenging, as a high sample size is often needed for statistical power, especially for uncommon variants. Lastly, at the molecular level, complexities in drug action and clinical effects can lead to discrepancies. (Roden, 2004) Although QT prolongation is an essential first step in TdP, it is usually not considered sufficient to induce TdP. Factors such as increased dispersion in the recovery of excitability and the development of early afterdepolarizations may be involved in the proarrhythmic effects leading to TdP.

We employed a recursive partitioning data mining approach, CART, to investigate interactions among common, uncommon genetic variants, and clinical factors. Data mining methods offer several advantages over traditional statistical approaches when dealing with numerous variables, as they are essentially assumption and model-free and can handle high-dimensional data. Among white patients, we identified a gene-gene interaction between rs3857067 and rs12025136, whereas among African patients, none of the genetic variants were associated with the risk of diLQTS. The pharmacogenetic interaction between rs3857067 and rs12025136 should be validated in in another study with adequate statistical power, using traditional statistical methods.

This dissertation research has the potential to significantly impact both diLQTS clinical practice and diLQTS genetic research. The ultimate goal concerning *KCNE1*-D85N is its integration into clinical pharmacogenetic testing to prevent diLQTS. The association between *KCNE1*-D85N and diLQTS risk represents a major finding in this research, bringing it closer to

potential clinical application. While conducting randomized clinical trials to demonstrate the clinical utility of pharmacogenetic testing for this variant may not be feasible due to a low minor MAF (Luzum et al., 2021), the amount of evidence from comprehensive studies, aligned with our large-scale investigation, suggests that *KCNE1*-D85N warrants formal evaluation for publication in a clinical practice guideline, and potentially for clinical implementation. A one-time genetic test could potentially provide lifetime benefits for a patient. We also believe that consideration of this variant as a risk factor is justified for patients whose *KCNE1*-D85N genotype is already known and will be prescribed a QT-prolonging drug. This research also impacts the field of diLQTS genetic association research, validating for the first time a PRS for diLQTS and applying advanced analytical methods such as data mining to uncover associations with diLQTS. This independent validation study of the diLQTS PRS initially published by Strauss et al takes the next steps towards clinical utility for this PRS as well. Future studies should focus on determining if preemptive genotyping with the implementation of the PRS prior to prescribing a QT prolonging drug can reduce the incidence of serious adverse events.

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