Genetic Cardiac Arrhythmias in the Young:
From Population Trends to Cellular Mechanisms

by

Thomas Michael Roston

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Medicine
University of Alberta

Abstract

Arrhythmic heart disease in the young often has a hereditary genetic basis and may carry a lifelong burden of increased morbidity and mortality. The presenting rhythm can be atrial and/or ventricular origin, and both the age of onset and associated risk of sudden cardiac death vary markedly. This is influenced in part by the genetic driver(s) of disease. Atrial fibrillation is one of the most malignant atrial arrhythmias that can negatively impact quality of life and lead to an increased risk of stroke, but generally not sudden death. Although ample research efforts have been dedicated to understanding the different facets of the more common acquired atrial fibrillation in older adults, relatively little is known about atrial fibrillation presenting in young patients with no known risk factors for acquired atrial fibrillation. The latter is thought to represent a potentially genetic arrhythmia condition. In contrast, ventricular-predominant arrhythmia syndromes classically presenting <40 years of age consist mainly of inherited conditions that can cause sudden death. These syndromes may be under-recognized due to their low prevalence, often non-specific and initially innocuous presentations, and concealed nature despite standard clinical testing. Once recognized and diagnosed, which may include cascade family screening, these patients can be treated by targeting the molecular mechanisms of the underlying ion channel dysfunction. There is increasing recognition that the mechanisms of genetic atrial and ventricular arrhythmias in the young are overlapping, making their combined study logical.

This dissertation includes six retrospective studies and one systematic review and meta-analysis that collectively examine the incidence, disease mechanisms, and therapeutic outcomes of potentially genetic arrhythmia conditions in the young. First, we report a population study investigating the incidence of atrial fibrillation/flutter in young Canadians. We show a strong sex-predilection to lone AF in young males, but worse outcomes in affected females. By developing a

rigorous definition of lone/idiopathic atrial fibrillation, we conclude that this population-level approach may be an effective methodology to identify young AF patients who may benefit from genetic discovery. Next, the focus turns to a population-level study of syncope in the young, as this is a very common symptom in genetic arrhythmia syndromes. Amongst 11,488 children with syncope presenting for emergency care, there was a low rate of hospitalization (2%) but a high burden of comorbidities and likelihood of re-presentation. Cardiac conditions were common, but mortality was extremely low (one potentially cardiac death amongst 11,488 syncopal patients at 1-year). These findings suggest that although syncope can be the sentinel symptom of a genetic arrhythmia syndrome, this population approach using administrative coding is likely too non-specific to be useful in identifying those rare children predisposed to sudden death.

Finally, using a large international registry cohort, we examine a rare form of genetic ventricular-predominant arrhythmia caused by cardiac ryanodine receptor dysfunction, called catecholaminergic polymorphic ventricular tachycardia. We show that implantable cardioverter defibrillators are paradoxically associated with increased harm, chronotropic incompetence during exercise is a risk factor for arrhythmic events, and homology mapping is a useful tool to predict RyR2 variant pathogenicity in this disorder. Through deeper phenotypic analysis of the registry, we further identify one of the first cases of a novel genetic arrhythmia syndrome related to the ryanodine receptor, now being termed cardiac ryanodine receptor release deficiency syndrome.

Collectively, the studies comprising this dissertation provide a comprehensive overview of the methodologies needed to address key question in this diverse and challenging population. Future efforts should focus on leveraging the centralized nature of the single-payer healthcare system in Canada to better link population level cardiac outcomes to deeply phenotyped and genotyped arrhythmia registry cohorts.

Preface

This dissertation includes seven original research studies that are under final preparation for or already published in peer reviewed journals. Minor adjustments to the wording, length and formatting of published materials were undertaken throughout this document to minimize redundancy and comply with University style standards. I am a first author on all abstract presentations and published manuscripts, and at least one of my two co-supervisors are senior authors on each study (Drs. P. Kaul & S. Sanatani). All publishers provided written permission to reproduce these works for the dissertation (Appendix A). Ethical approval for the retrospective and prospective CPVT Registries was obtained from UBC C&W Research Ethics Board (H12-01622 & H14-00301) and approval for the population health work was obtained from the University of Alberta (Pro00072777). Since Chapter 4 was a systematic review and meta-analysis of published data, the project did not require ethics approval. All works were completed during my tenure as a Master's/Doctoral student. Several statisticians, scientists and clinicians contributed to the studies as described here.

Chapters 2 and 3 are population health studies. For both studies, I conceived the research questions, directed the needed analyses, interpreted the results, and wrote the first draft and all subsequent drafts of the manuscript. For Chapter 3, which is now published, I also corresponded with journal editors, and undertook the requested revisions. Statistical support was provided by Dr. Sunjidatul Islam (Chapter 2) and Dr. Dat Tran (Chapter 3). All other co-authors contributed substantially by refining the research questions, interpreting the results, critically appraising and editing the manuscripts and/or supervising the projects. Chapters 2 and 3 in abstract form were presented at the Canadian Cardiovascular Congress in October 2019, and October 2017, respectively. Bibliographic details for Chapter 2, which will be submitted for publication shortly, are as follows:

- *Roston TM,* Islam S,* Hawkins N, Laksman ZW, Krahn AD, Sandhu R, Kaul P. Age- and Sex-Based Trends in Lone Atrial Fibrillation: A Population Study. Under preparation for submission to publisher. *shared first author*

A version of Chapter 3 is published as an original research manuscript as follows:

- *Roston TM, Tran DT, Sanatani S, Sandhu R, Sheldon R, Kaul P. A Population-Based Study of Syncope in the Young. Can J Cardiol 2018; 34(2):195-201.*

Chapter 4 is a systematic review and meta-analysis of observational data. I conceived the research question, completed the initial systematic review, undertook the analyses, wrote the first draft of the manuscript, corresponded with journal editors, and undertook the requested revisions. All other co-authors contributed substantially by refining the research question, interpreting the results, appraising and editing the manuscript and/or supervising the project. Two co-authors (Dr. K. Jones and Ms. F. Perry) further contributed by performing an additional database search and four co-authors helped to verify select patient-level data (Drs. J.M. Bos, M.J. Ackerman, K.V.V. Lieve and P.J. Schwartz). A version of Chapter 4 is published as follows:

- *Roston TM,* Jones K,* Hawkins NM, Bos JM, Schwartz PJ, Perry F, Ackerman MJ, Laksman ZWM, Kaul P, Lieve KVV, Atallah J, Krahn AD, Sanatani S. Implantable cardioverter defibrillator use in catecholaminergic polymorphic ventricular tachycardia: A systematic review. Heart Rhythm 2018; 15(12):1791-1799. *shared first author*

Chapters 5, 6, 7 and 8 are observational studies of the Pediatric and Congenital Electrophysiology Society CPVT Registry population. All studies have been presented in abstract form at various local, national and/or international meetings. For Chapter 7, I received an Early Career Investigator Award at the 2017 American Heart Association Scientific Sessions, and best podium presentation award at the 2018 UBC Cardiac Sciences Research Day. For Chapter 8, I received the Paul Man Award in Translational Medicine at the 2016 University of Alberta Department of Medicine Research Day, and the John H. Dirks Award for best overall research project at the 2016 UBC Internal Medicine Research Day. For both studies, I conceived the research questions, created the data entry tool, communicated with contributing centres, directed the analyses, interpreted the results, wrote the first manuscript drafts, corresponded with journal editors, and addressed reviewer comments. For Chapter 5, I contributed similarly, with the exception that Dr. S. Franciosi performed the statistical analyses and revised the manuscript I initially drafted prior to publisher acceptance. For Chapter 6, Dr. J. Potts provided statistical support. The molecular modeling described in Chapters 6-8 was performed by the Van Petegem Lab (predominantly post doctoral fellows Drs. Z. Yuchi and O. Haji-Ghassemi). For Chapter 8, the functional HEK293 studies were performed by the Chen Lab (predominantly PhD student Ms. W. Guo). All other co-authors contributed to data entry, supervision of the analyses, and/or critical appraisal of the initial manuscripts as site investigators/research managers in the Pediatric and

Congenital Electrophysiology Society network, principal investigators in the Registry, or principal investigators of basic science laboratories. Versions of Chapters 5-8 are published as follows:

- *Franciosi S,* Roston TM,* Perry F, Knollman BC, Kannankeril PJ, Sanatani S. Chronotropic Incompetence as a Risk Predictor in Children and Young Adults with Catecholaminergic Polymorphic Ventricular Tachycardia. J Cardiovasc Electrophysiol 2019 Oct;30(10): 1923-1929. *shared first author*
- *Roston TM, Yuchi Z, Kannankeril PJ, Hathaway J, Vinocur J, Etheridge SP, Potts JE, Maginot K, Salerno JC, Cohen M, Hamilton RM, Pflaumer A, Mohammed S, Kimlicka L, Kanter RJ, LaPage MJ, Collins KK, Gebauer RA, Temple JD, Batra AS, Erickson C, Miszczak-Knecht M, Kubus P, Bar-Cohen Y, Kantoch M, Thomas VC, Hessling G, Anderson C, Young ML, Choi SHJ, Cabrera Ortega M, Lau YR, Johnsrude CL, Fournier A, van Petegem F, Sanatani S. The clinical and genetic spectrum of catecholaminergic polymorphic ventricular tachycardia: findings from an international multicenter registry. Europace 2018; 20(3):541-547.*
- *Roston TM,* Haji-Ghassemi O,* LaPage MJ, Batra AS, Bar-Cohen Y, Anderson C, Lau YR, Maginot K, Gebauer RA, Etheridge SP, Potts JE, Van Petegem F, Sanatani S. Catecholaminergic polymorphic ventricular tachycardia patients with multiple genetic variants in the PACES CPVT Registry. PLoS One 2018;13(11): e0205925. *shared first author*
- *Roston TM, Guo W, Krahn AD, Wang R, Van Petegem F, Sanatani S,† Chen SRW, † Lehman A.† A novel RYR2 loss-of-function mutation (I4855M) is associated with left ventricular non-compaction and atypical catecholaminergic polymorphic ventricular tachycardia. J Electrocardiol 2017;50 (2):227-233. †Shared senior author*

Finally, select components, either paraphrased or reproduced, appearing in Chapter 1 have been published in a review article. I drafted the original material for this published work, with contribution and supervision by Drs. F. Van Petegem and S. Sanatani.

- *Roston TM, Van Petegem F, Sanatani S. Catecholaminergic polymorphic ventricular tachycardia: a model for genotype-specific therapy. Curr Opin Cardiol 2017; 32 (1): 78-85.*

Dedication

To my Mom, whose love has encouraged me to pursue my full potential in life

Acknowledgements

I owe a debt of gratitude to my supervisors, Dr. Shubhayan Sanatani, Division of Cardiology, BC Children's Hospital and the University of British Columbia, and Dr. Padma Kaul, School of Public Health and Department of Medicine, University of Alberta. Both were immeasurably supportive when I proposed that they jointly supervise this PhD across two universities. Their patience and flexibility as I travelled between Vancouver and Edmonton over the first several years of the program enabled my success as a graduate student. I am also grateful to Dr. Andrew Krahn, Division of Cardiology, University of British Columbia, and Dr. Joseph Atallah, Division of Pediatric Cardiology, University of Alberta, who similarly embraced the creativity of this venture as dedicated committee members. I also thank those who graciously volunteered as examiners, the exemplary University of Alberta professors who taught me so much, the Faculty of Graduate Studies and Research staff members, my clinical mentors who encouraged me to pursue formal research training, and my family for their unwavering support.

The scientific works that comprise this dissertation were supported by several individuals, societies and foundations. The CPVT studies would not have been possible without the Pediatric and Congenital Electrophysiology Society, nor without the expertise of Dr. Wayne Chen, University of Calgary, and Dr. Filip Van Petegem, University of British Columbia, and their lab members. I further thank Dr. Dat Tran and Dr. Sunjidatul Islam, School of Public Health, University of Alberta for their statistical support. I acknowledge several funding sources: Heart and Stroke Foundation (Sanatani, Krahn), Rare Disease Foundation (Sanatani, Roston), Canadian Institutes of Health Research (Van Petegem, Krahn), Canadian Arrhythmia Network (Kaul), Canadian "Rare Diseases: Models & Mechanisms" Network (Chen), and E-Rare Joint Transnational Call for Proposals 2015 "Improving Diagnosis and Treatment of Catecholaminergic Polymorphic Ventricular Tachycardia: Integrating Clinical and Basic Science" (Sanatani, Wilde). The Queen Elizabeth II Scholarship contributed to my graduate student stipend.

Finally, I thank the patients and families who entrust us with their medical care and volunteer as research participants. Their tragic, and all-too-similar stories of early-onset arrhythmia and sudden unexpected death have continuously motivated this journey and reminded me of the true purpose of biomedical research. I endeavour to always put their needs first throughout my career.

Table of Contents

List of Tables

List of Figures

List of abbreviations

AAD	anti-arrhythmic drug
ACMG	American College of Genetics and Genomics
ATC	anatomic therapeutic chemical
ARVC	arrhythmogenic right ventricular cardiomyopathy
ASCVD	atherosclerotic cardiovascular disease
AF	atrial fibrillation/flutter
AV	atrioventricular
BP	blood pressure
BrS	Brugada syndrome
CASQ2	calsequestrin-2
RyR2	cardiac Ryanodine Receptor
CPVT	catecholaminergic polymorphic ventricular tachycardia
CCI	Canadian Classification of Health Interventions
CI	chronotropic incompetence
CRDS	Cardiac Ryanodine receptor release deficiency syndrome
cryoEM	cryo-electron micrograph
ECG	electrocardiogram
ED	emergency department
EST	exercise stress test
ExAC	Exome Aggregation Consortium
FY	fiscal year
GOF	gain-of-function
gnomAD	Genome Aggregation Database
GWAS	genome wide association study
HR	heart rate
IAS	inherited arrhythmia syndrome
ICD	implantable cardioverter defibrillator
ICD-10	international classification of diseases, 10th revision
ICD-9	international classification of diseases, 9th revision
IQR	interquartile range
LCSD	left cardiac sympathetic denervation
LVNC	left ventricular non-compaction
LQTS	long QT syndrome
LOF	loss-of-function
MA-PHR	maximal predicted heart rate
METS	metabolic equivalents
NSVT	non-sustained ventricular tachycardia
OAT	optimal anti-arrhythmic therapy

OAC	oral anticoagulation
P/LP	pathogenic/likely pathogenic
PACES	Pediatric and Congenital Electrophysiology Society
PFD	pore forming domain
PRISMA	preferred reporting items for systematic reviews and meta-analyses
pVSD	pseudo-voltage sensing domain
SR	sarcoplasmic reticulum
SNP	single nucleotide polymorphism
sol2	solenoid region
SEM	standard error of the mean
S-ICD	subcutaneous implantable cardioverter defibrillator
SCA	sudden cardiac arrest
SCD	sudden cardiac death
SUD	sudden unexpected death
TaF	thumb and finger domain
TIA	transient ischemic attack
VUS	variant of uncertain significance
VA	ventricular arrhythmia
VAS	ventricular arrhythmia score
VE	ventricular ectopy
VF	ventricular fibrillation
VPB	ventricular premature beats
VT	ventricular tachycardia
WT	wild type
WPW	Wolff-Parkinson White syndrome

PART I:
INTRODUCTON AND LITERATURE REVIEW

Chapter 1: Epidemiology and Genetics of Arrhythmias in the Young

1.1 Arrhythmias and Genomics at the Population Level

Cardiovascular disease may affect ~2% of young people <40 years old[1] and often leads to lifelong morbidity and early mortality. However, because the consequences of traditional cardiovascular risk factors, like diabetes, hypertension and hypercholesterolemia, take decades to accrue, the clinical phenotype, genetic architecture and underlying pathophysiology of heart disease in the young is unique. To date, awareness campaigns have largely focused on atherosclerotic cardiovascular disease (ASCVD) of mid- to later-onset, and have not yet focused on heart disease in young Canadians. The most common cardiac conditions manifesting early in life include: (1) structural congenital heart disease, which often requires early surgical correction/palliation, (2) non-ischemic cardiomyopathy, which can occur as an isolated condition or accompany a syndrome, and (3) inherited arrhythmia syndromes (IAS), which are genetic and potentially lethal primary electrical disorders related to ion channel dysfunction. The latter two categories can be especially devastating because warning signs of heart disease are often absent or overlooked until a dramatic event occurs, like sudden unexpected death (SUD).[2] These tragedies and losses have substantial repercussions on the family and on society.[3] Additionally, their genetic underpinnings may have cardiovascular health and reproductive planning implications for the grieving family.[2] Over the past three decades, the genetics of arrhythmias in the young have been decoded at an exponential rate, which has included the discovery of both monogenic and polygenic disease contributors.[4-6] However, knowledge translation from the molecular laboratory to the wider young population has lagged, with controversy relating to genetic causation, molecular-based risk stratification, disease incidence, and the value of widespread screening and effective population-level interventions.[3] This dissertation follows a stepwise progression. Firstly, it examines the broad population-level trends in syncope and atrial fibrillation in the young, which may indirectly lead to new ways of identifying individuals with genetic susceptibility to arrhythmia. It then progresses to the clinical and genotypic characteristics of a rare IAS manifesting in childhood, called catecholaminergic polymorphic ventricular tachycardia (CPVT), and finally concludes with the discovery of a new form of early-onset IAS, which is now known as cardiac ryanodine receptor (RyR2) calcium release deficiency syndrome (CRDS). The designs of these studies were intentionally varied to encompass the research methodology needed to better characterize and comprehend genetic electrophysiologic heart disease in the young. This approach also led to the opportunistic study of new arrhythmia syndromes and mechanisms.

1.1.1 Population trends of arrhythmia in the young

There are three major anatomical locations that are prone to arrhythmias: the atria (upper chambers), the ventricles (lower and main systemic pumping chambers) and the atrioventricular (AV) node, which electrically connects the atria and ventricles. Atrial arrhythmias can cause palpitations, pre-syncope/syncope and fatigue, and sometimes stroke. However, they have a less sinister course than ventricular arrhythmias largely because of the atrioventricular (AV) node, which slows the conduction of dangerously fast rhythms from the atria to the ventricles. The AV node itself and surrounding tissues can also be an arrhythmia source. Atrial fibrillation/flutter (AF) is the most common form of arrhythmia in adults, defined by rapid propagation of disorganized electrical activity in the atria, which can lead to stasis, thrombus formation and systemic embolism.[7] Conversely, when arrhythmias arise in the ventricle, they can be life-threatening because no mechanism exists to slow propagation, potentially leading to lethal ventricular fibrillation (VF).[4] Standard therapies therefore differ between the two arrhythmias. For AF, treatment includes ventricular rate control with an AV nodal blocker (eg. beta-blocker or Ca^{2+}-channel blocker), stroke/systemic embolism prevention with an oral anticoagulant (OAC), and possibly rhythm control with an anti-arrhythmic drug (AAD), electrical cardioversion and/or catheter ablation.[7] Ventricular arrhythmias are usually managed with beta-blockers, AADs, catheter ablation and possibly coronary artery revascularization.[8] Implantable cardioverter defibrillators (ICD) are an important SUD prevention strategy.[8] At a population-level, some data exist on both atrial and ventricular arrhythmias, but studies have rarely focused on incidence and outcomes in young people.

1.1.1.1 Atrial arrhythmias

Population-level studies on atrial arrhythmias in the young are largely derived from larger cohorts comprising the general AF population. The Framingham Heart Study provides the longest duration of follow-up for AF, with the latest 50-year trends showing a gradual quadrupling in incidence.[9] In the modern era, age-adjusted incidence was 14.36 per 1,000 in males and 8.55 per 1,000 females.[9] Both stroke and mortality have decreased over time, likely related to contemporary therapy for co-morbid conditions and OAC for stroke prevention. Risk factors for the development of AF, like uncontrolled hypertension, diabetes, and heart failure, have all been decreasing over time, although their effect-size on AF risk has remained stable.[9] The observed increase in AF has

been largely driven by new cases with advancing age, whereas AF incidence in the young has appeared relatively stable, suggesting genetic factors as being a major driver. Consistent with this observation, parental AF was one of the strongest risk predictors for AF in offspring within the Framingham cohort.[10] In another large population study, AF in the young was rare with 19.3% of cases occurring at <65 years, which again, was largely unaffected by the era of diagnosis.[11] While incidence was projected to rise overall in this study, it was most pronounced in men and in the elderly. These non-modifiable factors consistently appear to play an important role in AF.[11]

When AF occurs in the young, and in the absence of recognized risk factors for AF, it is commonly referred to as "lone," or "idiopathic" AF, as will be discussed in Section 1.2.4. This distinction has relevance because the natural history and genetic implications of lone AF differ markedly from acquired forms of AF. Population studies focusing on lone AF are scarce and pre-date the modern era. In 1985, the first report from the Framingham study on lone AF, simplistically defined as AF developing in the absence of ASCVD, heart failure, rheumatic valve disease and/or hypertension, was published.[12] No standardized set of investigations were undertaken to exclude these comorbidities, and hypertension was liberally defined as >160/95 mmHg. Furthermore, elderly patients were included in the lone AF definition. Among the 0.8% of subjects who developed lone AF (median age 70 years), stroke occurred 4-times more commonly compared to controls. In contrast, subsequent population data from Olmsted County showed a low risk of stroke and death in lone AF.[13] However, the study definitions were more conservative, with additional exclusion criteria for the lone AF group, including hyperthyroidism, chronic obstructive pulmonary disease, age >60 years, milder hypertension (>140/90 mm Hg), life-shortening non-cardiovascular disease, or the co-existence of trauma, surgery or acute medical illness. Using this definition, the lone AF population was much younger (mean age 44 years), survival at 15 years was more favourable at 94%, and the incidence of stroke was lower (0.35 events per 100 person-years). The discrepancies between the studies can be attributed to the younger age and hypertension criteria applied to the Olmsted County-based population cohort. Collectively, these two studies provided evidence for stroke prevention strategies based on age and hypertension. While the concept of lone AF as a unique pathophysiological entity remains unproven, the distinctions between AF endophenotypes continues to have clinical relevance, as lone AF is consistently recognized as a more benign, earlier onset form of the disease.[14] Although now

antiquated, the Olmsted County study provided important insights into lone AF potentially driven by genetic factors.

There are also other forms of atrial arrhythmia in the young. Accessory pathways between the atria and ventricles, and supraventricular tachycardias, caused by increased automaticity or re-entry via the AV node, are particularly common. Overt accessory pathways defined by pre-excitation on rest ECG occur in 100-300 young people per 100,000 (<40 years old), and are associated with a mildly increased risk of SCD.[15] Recent epidemiological data suggest a supraventricular tachycardia incidence of 73 per 100,000, with advanced age, female sex, and a variety of cardiovascular comorbidities being risk factors.[16] Because atrial arrhythmias not classified as AF are often improperly coded, generally have curative therapies and are not linked to substantial morbidity or genetic factors, this dissertation focuses on AF and ventricular arrhythmias.

1.1.1.2 Ventricular arrhythmias, syncope and sudden cardiac death

Ventricular arrhythmias are classified based on their morphology – either monomorphic or polymorphic. Monomorphic arrhythmias tend to be "scar-mediated," whereby pre-existing structural damage creates micro re-entry circuits that propagate ventricular tachycardia (VT). The rate and stability of the arrhythmia is pre-determined by the electrical properties within the circuit.[4] Conversely, polymorphic arrhythmias typically develop due to a complex interplay of triggered activity, alterations in conduction and refractoriness, and are the dominant morphology in IAS.[4] Polymorphic VT is usually less stable and can quickly degenerate into generally lethal VF, although this is a risk in monomorphic VT as well. While these ventricular arrhythmias are most commonly a complication of obstructive ASCVD, VT/VF leading to syncope and SCD are some of the most feared manifestations of congenital heart disease, genetic cardiomyopathy and IAS.

Population-level estimates of SCD incidence in the young (<35 years) are conflicting, likely owing to geographic loculation of certain high-risk genetic conditions, like hypertrophic and arrhythmogenic cardiomyopathy.[17] Regions with high rates of immigration may provide the best estimates of the worldwide burden of SCD and VT/VF in the young. Data from the United Kingdom report an incidence of 1.8 per 100,000 – with ASCVD, cardiomyopathy and ventricular arrhythmia being the most common culprits.[18] A recent population-level analysis of young United States residents showed an SCD incidence of 1.32 per 100,000, which was slightly lower than a

previous estimate of 2.28 per 100,000 Americans.[19] However, the latter study included cases of sudden infant death syndrome, which inflates the incidence. Overall, the risk and causes of SCD varied by age in that study. Younger patients were less likely to die, but when SCD occurred, congenital heart disease was most often implicated. Those in the 26 to 34 year-old age bracket were most likely to suffer SCD, usually due to ASCVD. Overall, a range of 1-5 SCDs in the young per 100,000 is a generally accepted contemporary estimate,[20-22] with genetic heart disease dominating in the early years and ASCVD emerging as a major cause after 30 years of age. Figure 1.1 depicts the proportionate culprit causes of SCD in the young.[23]

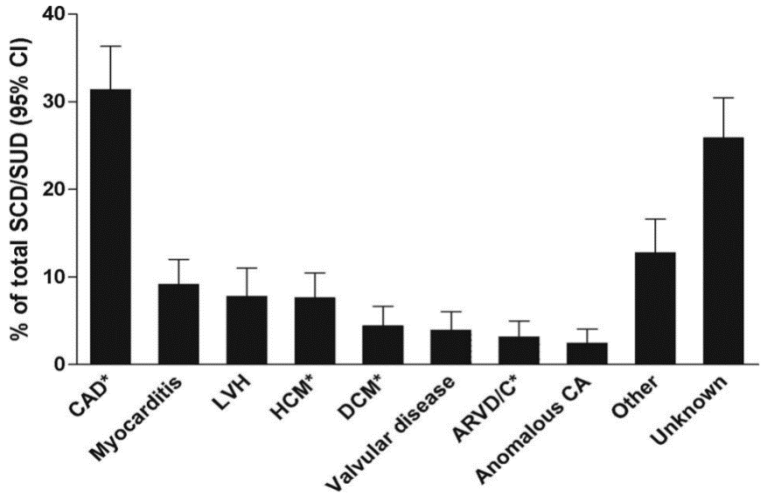


Figure 1.1 Causes of SCD in the young (<40 years old) based on a review of histopathological series (reproduced with permission from Van der Werf et al., *Circ Arrhythm Electrophysiol.,* 2010).[3]

Syncope, defined as a brief loss of consciousness due to transient and rapidly resolving cerebral hypoperfusion, is very common, occurring in >35% of the population at some point in life.[24] While it can rarely be a manifestation of VT/VF from genetic heart disease, it is most often due to autonomic nervous system dysfunction, termed vasovagal syncope. This type of syncope generally has a benign course, but its dramatic appearing nature can be alarming to patients, families and physicians, leading to considerable healthcare utilization.[25] Vasovagal syncope can usually be diagnosed based on a thorough history and physical exam, and in the absence of features suggestive of arrhythmic syncope, no testing or pharmacologic treatment is recommended.[26] Contemporary estimates of the incidence of syncope, and syncope-related hospitalizations are highly varied, with a bimodal peak affecting the young (<20 years) and old (>80 years).[24, 27, 28] In the Framingham cohort, the incidence was 6.2 per 1,000 patient-years,[28] however, in the centralized Danish medical system, the incidence of hospitalization was markedly higher at 17.2 per 1,000 patient-years.[27] While the threshold to admit patients and perform additional testing vary based on the local medicolegal landscape and healthcare infrastructure, dramatic differences in incidence and outcome estimates reflect the challenging nature of studying this problem, which is

highly heterogeneous and common. Overall, the incidence of serious cardiac pathology, like IAS, as a driver of syncope in the young is low, but relatively few datasets are confined to children. Better establishing the outcomes after syncope in young Canadians, including risk for SCD and VT/VF, can help to shape public health policy and practice guidelines. This is a primary aim of Chapter 3.

While early-onset syncope, VT/VF and SCD are issues of widescale importance, the opportunities to link national administrative databases to specific cardiac conditions is limited at present. A major factor contributing to this challenge is that the accuracy of administrative diagnostic coding is often poor. For example, hypertrophic cardiomyopathy, the most common form of life-threatening genetic heart disease in the young, is commonly misclassified.[29] This may be because left ventricular *hypertrophy* sounds similar to and can be a phenocopy of hypertrophic cardiomyopathy. Likewise, acquired forms of channelopathy, like drug-induced QT prolongation, are easily confused with congenital long QT syndrome (LQTS), a condition that will be addressed in Section 1.2.1. In contrast, for some rare cardiac conditions with names and mechanisms that do not overlap with acquired forms of heart disease, like Wolff-Parkinson-White syndrome (WPW), administrative datasets have helped to inform incidence and risk of SCD and syncope.[15] Unfortunately, for the IAS conditions, outlined in Section 1.2, the true incidence of each IAS type derived from administrative data is largely unknown. Therefore, additional efforts relying on population-level genomics and international clinical registries are needed to address these gaps.

1.1.2 Population-level genomic datasets

Genetic discoveries beginning in the late 1980s and progressing into the modern era have redefined our ability to diagnose, risk stratify and accurately screen young patients with suspected IAS. A major advancement during this genomic era occurred in the early 2000s, with the successful completion of the human genome project. Since then, research-based and clinical testing have evolved to consider the entire genome in unexplained human disease. The cost of this process has exponentially declined, which has availed whole genome sequencing to all of medicine and much of society.[30] Nowadays, several repositories of whole genome data on hundreds of thousands of people are now readily available for analysis, which can provide insights into disease prevalence, define clinical risk and differentiate benign from pathological genetic variation.

1.1.2.1 Open access control populations

There are several important genomic control populations available for public use. In the setting of IAS, these allow for a genomic comparison between study subjects and those sequenced for unrelated reasons. The largest of these is the Genome Aggregation Database (gnomAD), which is an open-access online repository of over 125,000 exome sequences and over 15,000 whole genome sequences.[31] These were collected from research subjects of large-scale sequencing projects undertaken for reasons unrelated to the development of gnomAD. The database has grown rapidly in the past several years, with its predecessor, the Exome Aggregation Consortium (ExAC) Browser, being half the size of gnomAD.[32] Amongst numerous data fields, these repositories report minor allele frequencies for all the IAS genes, which allows for researchers to compare the frequency of a rare variant in their study population to these large control populations. This process helps to adjudicate the pathogenicity of rare variants identified in IAS patients, as discussed in Section 1.3 and undertaken in Chapters 7 and 8.

A limitation of these datasets is that they are de-linked from the medical record. This means that genotype-phenotype correlations cannot be determined for the variants found in gnomAD. In contrast, the UK Biobank is a longitudinal population study that collects both genotypic and phenotypic data on human volunteers, including questionnaires and tests of their cardiovascular health (Figure 1.2).[33, 34] UK Biobank has now released some of its genetic data, which will eventually include genomic assays of 820,967 single nucleotide polymorphisms (SNP). This will allow for the creation and validation of polygenic risk scores for a range of diseases, including cardiovascular disease, which will be reviewed at length in Section 1.1.2.2. A similar initiative is being led by the Geisinger Health

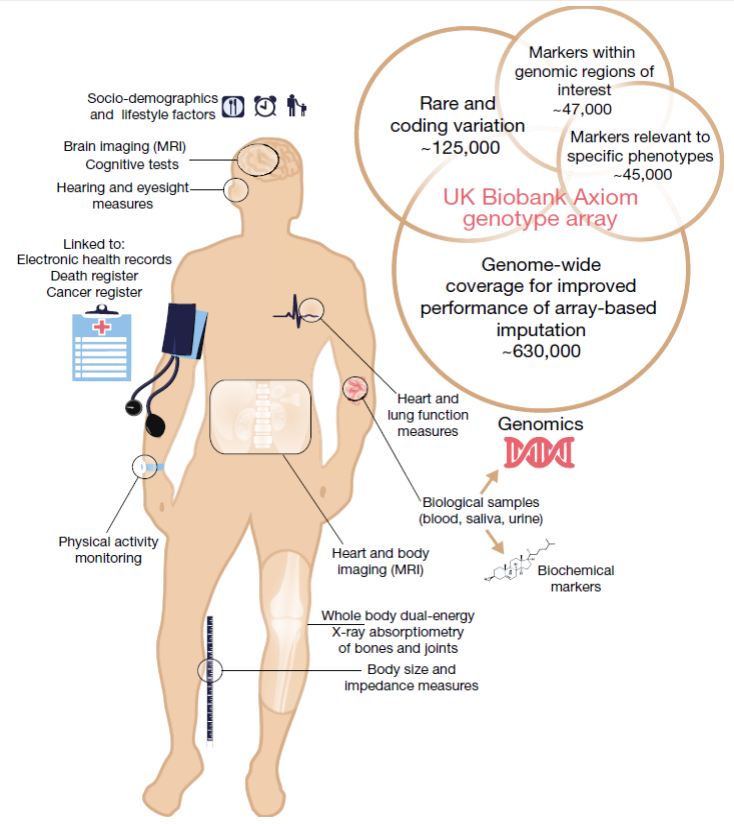


Figure 1.2. Deep phenotyping and genotyping in the UK Biobank (reproduced with permission from Bycroft et al, *Nature,* 2018).[34]

system in Pennsylvania, which involves performing whole genome sequencing in all patients as a component of routine clinical practice.[35] This "genome-first" method allows researchers and clinicians to reverse the standard diagnostic approach to IAS, whereby the genetic information drives the need for IAS assessment.[36] Such an approach has two potential benefits: (1) individuals at genetic risk of SCD due to latent IAS are diagnosed and treated pre-emptively, and (2) a greater understanding of the penetrance, expressivity and clinical risk related to the traditional IAS genes. Both opportunities may benefit the IAS population considerably, as the presently available observational data are limited by a selection bias towards clinically overt disease.[37] Emerging data from Geisinger are showing that rare, potentially damaging IAS variants are not infrequently identified in apparently normal patients,[38, 39] and that they can predict the risk of developing a phenotype, potentially under certain environmental and acquired circumstances, as described by Cerrone et al (Figure 1.3).[40] These large scale sequencing projects will provide population-level genetic and clinical data that will be transformative in understanding and preventing AF, syncope and SUD in the young.

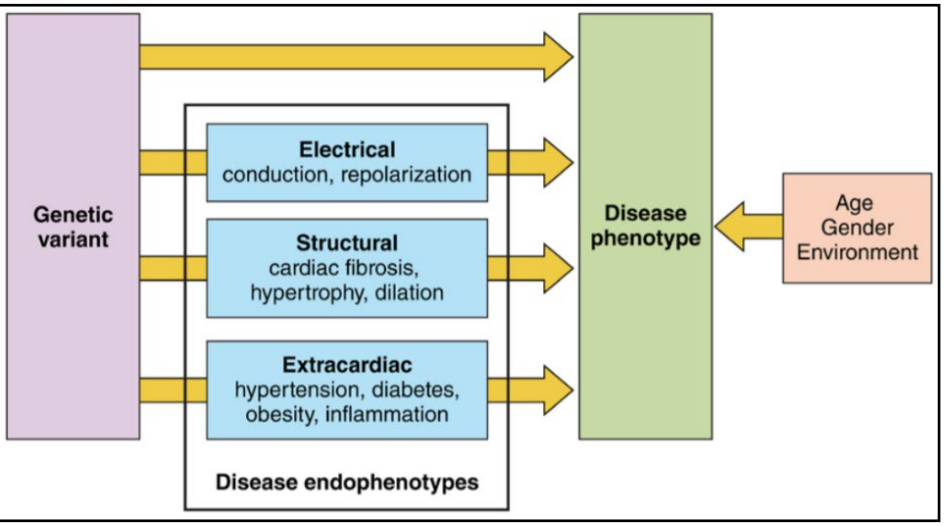


Figure 1.3. Expression of a genetic variant may require the development of additional modifiable and non-modifiable risk factors (reproduced with permission from Cerrone et al., *Circulation,* 2019).[40]

1.1.2.2 Genome wide associations and polygenic risk

Common diseases can also be investigated at a population genomic level. Genome wide association studies (GWAS) aimed at identifying the many common loci that contribute to a disease have informed the interplay between genetic and environmental factors leading to arrhythmia. Recently, a gene that was first identified using GWAS in 2008 for beta-thalassemia was successfully corrected using gene-editing technology.[41] GWAS is based on a few basic principles. The first is that common diseases (eg. diabetes, ASCVD, hypertension) are more likely

to occur in a genetically susceptible person, especially when lifestyle/environmental stressors are present. The genetic factors driving these conditions are likely "polygenic" in nature, meaning that a single gene, or even several genes, responsible for such a common disease is unlikely.[40] The second is that every human harbours SNPs that individually have very small effect sizes on developing a given disease. However, if an individual person carries enough "damaging" SNPs, they cross a phenotypic threshold that manifests as a clinically identifiable disease (Figure 1.4).[40] By amassing thousands to hundreds of thousands of patients with a disease, researchers can begin to find these important SNPs. These can then be weighted and combined to create a disease susceptibility risk score (ie. polygenic risk score) for any partially hereditary condition. These technologies are mainly used for common diseases where it is simpler to recruit subjects. In AF, GWAS have laid the groundwork for the discovery of many susceptibility loci, including genes now believed to underlie lone AF.[42, 43] However, in uncommon disorders, like the IAS, it is very difficult to amass a population of adequate size to perform a GWAS. Despite feasibility issues, international efforts are underway to use these technologies in the setting IAS,[44, 45] but are not yet robust enough to change practice.

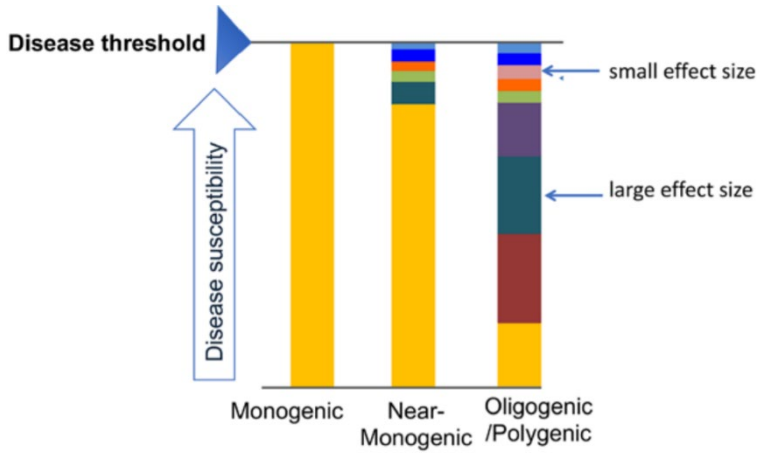


Figure 1.4. Proposed model that accounts for complex inheritance of genetic disease (reproduced with permission from Cerrone et al., *Circulation*, 2019).[40]

1.2 Inherited Arrhythmia Syndromes

IAS, also known as the cardiac ion channelopathies, are a group of life-threatening, genetically-mediated disorders that can manifest as syncope and sudden cardiac arrest (SCA) due to VT/VF. While rare, they are a leading cause of SUD in young people without structural heart disease.[46] Emerging evidence also suggests that atrial arrhythmias in the young, particularly AF, can be an atrial-specific form of IAS.[47, 48] The most well-recognized forms of channelopathy are caused by genetic mutations in the ion channel genes that encode proteins which gate Na^+, K^+ and Ca^{2+} current during myocardial depolarization and repolarization.[4] These include, but are not limited to LQTS, CPVT and Brugada syndrome (BrS). In up to half of unexplained SCAs, the diagnosis

remains as idiopathic VF despite extensive work-up, whereas the smallest proportion of victims will have incredibly rare conditions, like short QT syndrome and early repolarization syndrome.[2, 46] In this section, the three most common forms of IAS are reviewed, and the concept of an atrial-specific IAS is presented.

1.2.1 Long QT syndrome

LQTS is the most common cardiac ion channelopathy (1:2000).[2] It was originally described over 60 years ago by two physicians who recognized the syndromic constellation of QT interval prolongation, polymorphic VT, and sensorineural hearing loss, due to expression of mutant K^+ channels in the inner ear (Figure 1.5).[49] These rare and especially severe cases of LQTS are caused by homozygous or biallelic mutations in K^+ or Na^+ channel genes, whereas most LQTS patients have heterozygous mutations and manifest a milder, but still life-threatening, isolated arrhythmic phenotype. The diagnosis is primarily made on a rest and stress electrocardiogram (ECG), which show QT interval prolongation due to delayed myocardial repolarization.[2] Among the >17 genetic forms of LQTS, three predominate, each with their unique phenotypic manifestations.[5] For LQTS1, gain-of-function (GOF) mutations in *KCNQ1*, encoding $K_V7.1$ K^+ channel, predispose to adrenergic-related or swimming-related events. In LQTS2, caused by *KCNH2* encoded $K_v11.1$ K^+ channel GOF mutations, the arrhythmic triggers are often sudden loud noises and the post-partum period in women. Conversely, in LQTS3, related to *SCN5A* encoded $Na_v1.5$ Na^+ channel GOF mutations, most events occur at rest or during sleep. AF is more common in LQTS patients, especially LQTS2, suggesting co-expression of channel defects in both atrial and ventricular myocytes. In most circumstances, a beta-blocker is well-tolerated and effective treatment for LQTS.[2] Rarely, additional AADs or procedures, like a left cardiac sympathetic denervation (LCSD) or an implantable cardioverter defibrillator (ICD) are required, and genotype helps to guide these decisions. As the importance of family screening and

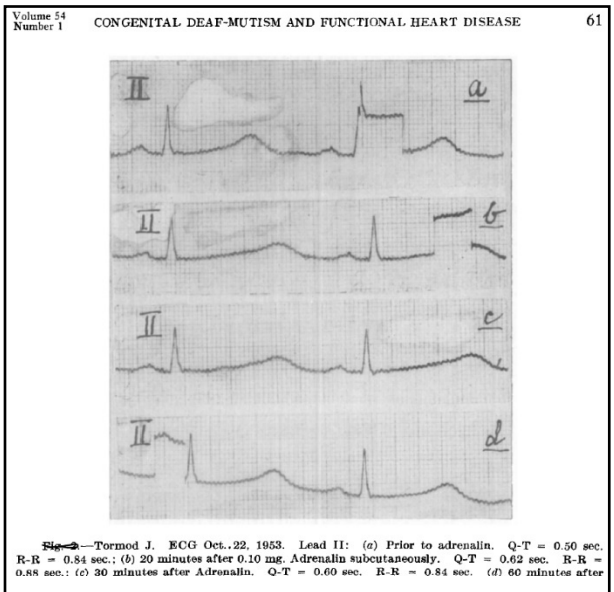


Figure 1.5. The first known ECG tracings of severe LQTS leading to congenital deafness, with adrenaline provocation lengthening the corrected QT interval to >620 ms (reproduced with permission from Jervell & Lange-Nielsen, *Am Heart J*, 1957).[49]

access to genetic testing have improved, a greater proportion of LQTS patients are identified prior to the development of symptoms, allowing for prophylactic lifestyle changes and treatment to be initiated.

1.2.2 Brugada syndrome

BrS is a rare and poorly understood condition related to Na^+ current that predisposes to right precordial lead ST elevation, syncope and SCA. Original accounts can be traced to various Asian folklore, which had described a familial syndrome of SUD during sleep in young, previously healthy males, prior to its clinical and molecular characterization.[50] While phenotypic expression can be variable, it is strongly associated with male sex, febrile illness, and pharmacologic Na^+-channel antagonism.[50] Like in other forms of IAS, BrS patients are at much higher risk of developing AF.[51] The only well-established genetic cause of BrS is *SCN5A* LOF variants,[52] with most genotype-elusive cases likely being polygenic.[45] Despite a multitude of studies, including several large multi-national registries, the predictors of SCA in BrS are unclear. Quinidine and the ICD are treatment options.[53]

1.2.3 Catecholaminergic polymorphic ventricular tachycardia

CPVT is a channelopathy that leads to polymorphic or bidirectional ventricular arrhythmias and atrial arrhythmias during exertion or emotional stress (Figure 1.6).[54, 55] Although clinically reported as early as 1960,[55] it was not until 2001 that Priori et al implicated *RYR2* GOF mutations in CPVT, which encodes the human cardiac ryanodine receptor (RyR2).[56] RyR2 is the largest ion channel in the human genome with a complex homotetrameric structure and is crucial to the fundamental physiologic process of Ca^{2+}-induced Ca^{2+}-release.[57] There are other very rare forms of biallelic CPVT, the most recognized of which is related to *CASQ2*-coded Calsequestrin-2, a protein also involved in Ca^{2+}-induced Ca^{2+}-release.[2, 5] The resting ECG is usually normal in CPVT, although low resting heart rates are

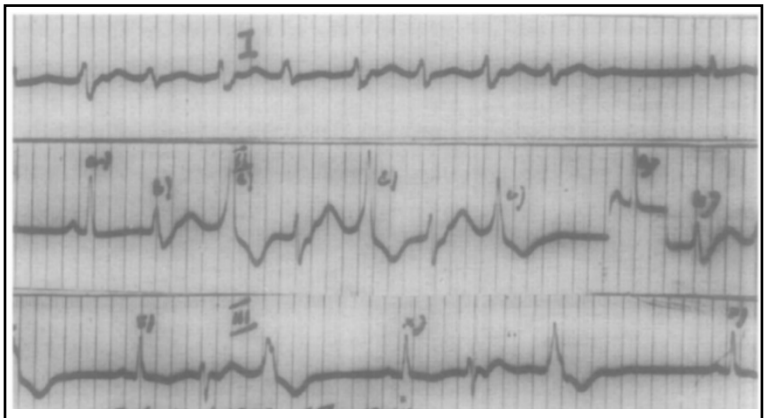


Figure 1.6. Oldest known ECG tracing of probable CPVT showing multi-focal VPBs in a 12 year-old female with syncope and a normal QT interval. She later died suddenly during exercise at 19 years of age (reproduced with permission from Berg, *Am Heart J*, 1960).[55]

common.[2] The diagnosis is made using stress ECG showing a characteristic escalating pattern of initially bidirectional/polymorphic ventricular premature beats (VPB) at a heart rate around 100-120 beats per minute, to more complex arrhythmias with further exertion, including VT. Classically, CPVT is regarded as the most severe of the channelopathies, with a median age of symptom onset of ~10 years and a very high risk of cardiac arrest at presentation (~25%).[58] The cornerstones of therapy are a beta-blocker, an ICD, and avoidance of adrenergic triggers (exercise and heightened emotion).[2] Flecainide and LCSD are effective ancillary treatments for those with breakthrough arrhythmias despite beta-blocker.[2] Because of the high burden of SCA/SCD in CPVT, the ICD has been widely used, including a primary prevention implant rate exceeding 50% in children.[58] However, anecdotal data have suggested that ICDs are associated with poor outcomes in CPVT, including inappropriate shocks for atrial arrhythmias and appropriate shocks for stable VT that lead to faster and more unstable forms of polymorphic VT/VF.[58, 59] The role of the ICD in CPVT remains poorly understood, in part because genetic and clinical risk stratification is virtually non-existent. Further knowledge pertaining to CPVT risk stratification and ICD outcomes is urgently needed. Chapters 4-7 describe advances in these areas.

1.2.4 Idiopathic/lone atrial fibrillation

As discussed in Section 1.1.1, for most patients, AF is a *secondary* manifestation of another chronic stressor, like hypertension, cardiac ischemia, heart failure and/or thyroid disease. While these and other environmental factors drive AF, it is also highly heritable, indicating that genetic susceptibility plays a role in its pathogenesis.[10] Over the past decade, genetic predictors have been described, which apply most to young patients and those without traditional AF triggers. When AF occurs in this setting, it is often called "lone" or "idiopathic" AF, although these terms are inconsistently defined, making it nearly impossible to properly characterize and understand the disorder

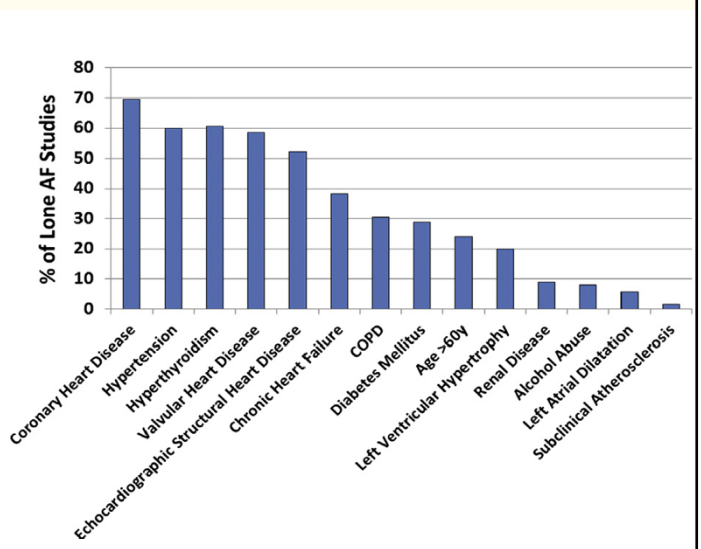


Figure 1.7. Variable inclusion of secondary factors in the definition of lone/idiopathic AF in 125 studies (reproduced with permission from Wyse et al, *J Am Coll Cardiol*., 2014).

(Figure 1.7).[14] For the purposes of simplicity and to reflect the emerging recognition that some forms of AF are genetic in origin, lone AF should be used when the condition develops in the absence of an identifiable environmental or comorbid medical trigger. The distinction between lone and secondary AF is important, as lone AF is more frequently hereditary, leads to a greater burden of symptoms, and manifests at an earlier age. Unfortunately, rhythm control strategies often do not result in lasting remission,[60] especially for lone AF in the young. Additionally, little is known about lone AF as a distinct endophenotype because AF has been traditionally studied as one disease entity, whereby all forms of AF are grouped together and analyzed. Consequently, genomic studies of large populations have failed to replicate the role of some candidate genes.

The genetic architecture of lone AF may be informed by other types of inherited heart disease. Recently, the gene encoding Titin, a sarcomeric protein, and most common cause of inherited dilated cardiomyopathy was implicated in AF.[61, 62] Similarly, lone AF also appears to have a shared genetic basis involving Na^+, K^+, and Ca^{2+} channels with the traditional IAS.[14, 43] The frequent co-existence of atrial arrhythmias in the traditional IAS further supports this connection.[51, 58, 63] In fact, the first gene linked to autosomal dominant lone AF in a family was *KCNQ1*,[47] the cause of LQTS1, and numerous ion channel variants have since been implicated in AF.[43] Similarly, short QT syndrome causes both SCD and AF, with mainly K^+ channel genes having been implicated.[53] These observations suggest that some forms of lone AF represent an isolated genetically-based atrial channelopathy. Chapter 2 will address the contemporary burden of lone AF in young patients to better establish its prevalence and therapeutic outcomes, and determine whether this may be an effective methodology to identify those who may benefit from genetic discovery.

1.3 A "Bench to Bedside to Population" Approach to Genetic Arrhythmia

Knowledge translation in heart disease in the young is encumbered by its relative rarity, frequent miscoding/misdiagnosis in administrative datasets and lack of deep phenotype linkage in large repositories of genomic information. Furthermore, the rapid evolution in sequencing and experimental techniques and the changing phenotypic definitions of diseases have made it difficult to integrate genomics into routine clinical care for young patients. Aside from LQTS,[2] none of the channelopathies have robust genotype-phenotype correlations and the incidence of IAS and its population-level manifestations remain contested. To address these limitations, the methodology

for identifying a new gene in the setting of IAS, for testing the pathophysiological impact of a variant, and for adjudicating its pathogenicity have become better established in recent years.

1.3.1 Gene discovery

The pursuit of novel genetic causes for arrhythmia have undergone substantial methodological and technical changes over the past 30 years. Initially, researchers were limited by an incomplete knowledge of the genome, and instead relied upon linkage analysis studies within large families. This technique involved the arduous process pursuing Sanger sequencing of all available family members to identify shared genetic changes in phenotype positive subjects that were absent in familial controls.[64] A major limitation to this approach was the lack of understanding about the organization and location of genes within the genome – thus, for many conditions, only select loci could be attributed to a disease. With enough persistence and luck, distinct genes could be identified using this technique, which accounted for several major IAS discoveries of the 1990s, including the genetic basis for LQTS and BrS.[65-67] Once the genome was fully elucidated, researchers could determine where genes responsible for specific physiological processes were located, leading to a surge in gene discovery efforts. This "candidate gene approach," whereby the physiological processes felt to account for a syndrome were adjudicated through sequencing, was pursued with incredible vigour, and a multitude of rare genetic variants were linked to thousands of pathophysiological processes.[64] In retrospect, this overly simplistic approach failed to consider the many modifying and contributing genes that may underlie a disease, was often rooted only in biological plausibility and did not consider the marked genetic heterogeneity present even in genes felt to be highly conserved across species.[64] The latter limitation was an issue because variants were deemed "rare," and therefore likely disease-causing, when they were absent in ~500 controls. Nowadays, based on population-level genomic efforts, as previously discussed in Section 1.1.2, we know that all humans harbour hundreds of ultra-rare variants (ie. present in <1 in 100,000 persons) across the genome which do not result in overt disease.[31, 32] Thus, while the candidate gene approach identified many potentially relevant IAS genes, it also implicated rare variants in various IAS which were likely not the cause of disease. The latest efforts related to whole genome sequencing have further produced genes associated with IAS, but often suffer from the same limitations. Ultimately, an unbiased adjudication of the entire genome in large kindred, which is subsequently compared to hundreds of thousands of controls and is recapitulated by predictive *in vitro* and *in vivo* modeling, represents the most robust methodology at present.

1.3.2 Molecular adjudication of pathogenicity and mechanism

Once a putative genetic variant is linked to IAS, several molecular techniques can adjudicate its pathogenicity and mechanism. For ion channels, the patch clamp technique has been extensively used to decipher the electrophysiological impact of a variant. For a given ion channel mutant, the change in current or voltage, measured via the action potential, is measured across the mutant membrane and compared to a control channel. The difference detected may represent the electrophysiological mechanism of arrhythmia, such as a loss- or gain-of-function, although the specificity of this technique is lower than originally recognized.[68] Stem cell models are also often used to investigate arrhythmia variants. This technique involves expressing a candidate variant in an embryonic derived or human induced pluripotent derived stem cell model and comparing electrophysiological properties to wild-type. Knock-in animal models may also be used to express candidate mutations. Other options include scoring pathogenicity based on simple amino acid conservation and evolutionary factors, like SIFT[69] and PolyPhen,[70] which are algorithms that can predict whether a variant has a deleterious impact on protein structure. The growing availability of cryo-electron micrograph crystal structures of in tact proteins also allows for more accurate predictions of pathogenicity based on the theoretical biophysical properties of a variant.[71] These techniques are used to adjudicate novel variants in RyR2 described in the studies encompassing Chapters 6-8.

1.3.3 Deep clinical phenotyping

Comprehensive genomic characterization of disease is only as powerful as the available data on clinical phenotype. In many circumstances, although young patients with an arrhythmia may satisfy diagnostic criteria, this may result in seemingly similar, but truly different diseases being grouped together. For example, in AF, it has been shown that while the surface ECG may satisfy basic diagnostic criteria, it cannot differentiate the many structural and electrical substrates for AF.[72, 73] Using magnetic resonance imaging,[74] 3-dimensional echocardiography,[75, 76] and cardiac activation mapping,[77, 78] researchers have shown that distinct endophenotypes of AF exist, which can be used to more accurately subclassify AF patients and tailor therapies. Similarly, in cardiac arrest in the young, the previous standard to satisfy a diagnosis of idiopathic VF was a normal ECG and echocardiogram and an angiogram showing no significant ASCVD. However, specialized testing aimed at specifically provoking the unique mechanisms of IAS reclassified

>50% of idiopathic VF survivors as having an established IAS diagnosis.[46] This "deep phenotyping" technique has been defined as "the precise and comprehensive analysis of phenotypic abnormalities in which the individual components of the phenotype are observed and described" (direct quote, page 1, Robinson et al[79]). Such an approach facilitates more accurate grouping of patients based on common biological pathways and mechanisms of disease, rather than by crude clinical diagnostic criteria.[80] Rare diseases, such as those described in Chapters 4-8, provide the ideal models for integration of deep phenotyping with genomic discovery because therapeutic advances rely heavily on refining biological mechanisms rather than on large trials.[81] Both the UK Biobank and Geisinger "MyCode" community health initiative are using deep phenotyping of the health record to enhance their genomic initiatives.[34, 35] In Chapter 8, we show that deep phenotyping of CPVT registry patients elucidated a novel endophenotype of RyR2-related disease and a new mechanism of arrhythmia.

1.3.4 Application of consensus variant classification

Finally, an important and increasingly emerging problem in the field is variant mis-classification and subsequent re-classification. Owing to the early limitations in gene discovery techniques, like the candidate gene approach, and a lack of healthy control genomes, comprehensive phenotyping and molecular tools to recapitulate the mechanism of disease, many rare variants in the ion channel and cardiac structure genes were misclassified as being disease-causing. We are increasingly realizing that many of these findings were erroneous or failed to account for the polygenic nature of inherited disease. As such, systematic variant (re)classification has become a standard research and clinical practice. The current recommendations for the interpretation of sequence variants has been set by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology,[82] which has advocated for standardized descriptive variant terminology (pathogenic, likely pathogenic, uncertain significance, likely benign and benign), and translational criteria for placing variants into these categories (population, computational, functional, and segregation data). The studies encompassing Chapters 4-8 use the ACMG criteria to adjudicate the pathogenicity of RyR2 variants.

PART II:

POPULATION TRENDS IN ARRHYTHMIAS IN THE YOUNG

Chapter 2: Age- and Sex-Based Trends in Lone Atrial Fibrillation: A Population Study

2.1 Abstract

Background: Unexplained atrial fibrillation (AF) is often termed lone AF, although definitions are highly varied, and existing studies did not systematically exclude patients with newer, secondary AF triggers. We sought to characterize lone AF based on a stricter definition that excludes a wide-range of potential comorbid triggers, and compare it to secondary AF.

Methods: In this population study, lone AF was strictly defined by the lack of any identifiable triggering medical problem. Comparisons by type of AF (lone vs secondary), age of onset and sex were undertaken using Chi-square testing, and freedom from stroke, transient ischemic attack, systemic thromboembolism or death was adjudicated using Kaplan-Meier analysis. Outcomes were assessed using time-dependent Cox regression models.

Results: There were 33,150 incident AF diagnoses, including 1,166 (3.5%) patients with lone AF, 947 (81.2%) of whom were ≤65 years-old. The incidence of lone and secondary AF increased over time. Secondary AF patients were more likely to receive rate and rhythm control drugs ($p<0.0001$), while lone AF patients more often underwent electrical cardioversion ($p<0.0001$). Amongst patients ≤65 years-old with a CHADS65 indication for stroke prevention (n=4,823), 2,522 (52.3%) were prescribed an anticoagulant by 1-year. In lone AF, males were younger at diagnosis vs females (45 vs 58 years, $p<0.001$). However, females with lone AF were more likely to suffer from the primary endpoint at 1- and 3-years vs males (2.7% vs 0.9%, $p=0.024$ and 7.8% vs 2.6%, $p=0.002$, respectively).

Conclusions: While lone AF manifests especially early in life among males, women consistently suffer from poorer outcomes despite receiving similar therapies compared to their male counterparts. Oral anticoagulation uptake is poor in lone AF even when a CHADS65 indication for stroke prevention is present.

2.2 Introduction

Atrial fibrillation and flutter (AF) are common arrhythmias that affect up to 2% of the population and lead to substantial morbidity and mortality, mainly due to a heightened risk of stroke.[83] Acquired, environmental and genetic factors play a role in its pathogenesis,[10, 83] including male sex, advancing age and a family history of AF.[10, 84] In most individuals, AF occurs "secondary" to a major comorbid condition, either cardiac in origin, like coronary artery disease and heart failure, or systemic in nature, like sepsis, thyroid, and lung disorders.[83] However, in a small proportion of predominantly young individuals, AF develops as a primary disorder in the absence of an identifiable trigger, termed idiopathic or "lone" AF.[12, 13, 85, 86] While event-free survival is likely better in lone AF,[84, 85] its definition with respect to age of onset and comorbidity burden has been inconsistent.[14] For example, the Framingham Heart Study of lone AF included patients with hypertension and advanced age,[12] both now considered as strong risk factors for secondary AF.[87] A further inability to account for modern screening for contributing conditions, improvements in catheter ablation techniques, wider use of oral anticoagulation (OAC) and the multitude of emerging secondary AF risk factors continue to confound our understanding of lone AF.[12-14, 86] As such, the incidence, predictors and outcomes of truly lone AF are unknown.

In this study, we aimed to extensively characterize AF in a large population dataset using strict, contemporary definitions of lone and secondary AF, which accounted for a multitude of potentially AF-predisposing comorbidities. As a first step, we compared lone to secondary AF at baseline with respect to age, presenting circumstances and socioeconomic status. Next, we examined therapies and outcomes for lone versus secondary AF. Lastly, we focused on studying previously identified risk factors for AF in the general population, like advancing age and male sex, in the lone AF cohort.

2.3 Methods

Study Population:

All patients with an incident diagnosis of AF between April 1, 2006 and March 31, 2015 (2006-2014 fiscal years) in Alberta, Canada were included. The first diagnosis of AF was identified from inpatient and ambulatory databases using validated[88-90] International Classification of Diseases 9th (ICD9: "427.3") and 10th edition (ICD10: "I48") codes from the primary diagnostic fields during the study period. Prevalent AF cases were excluded, defined as a pre-existing AF diagnosis in any database (inpatient, ambulatory and claims) from 1994 onward to current diagnosis. All patients

with incident AF were categorized into three groups based on the location/setting of the first presentation: outpatient clinic, emergency department, and hospitalization; these groups were mutually exclusive. Since administrative coding to distinguish between atrial fibrillation and atrial flutter is not reliable,[90] these conditions were combined.

Data Sources:

Data were acquired by linking 6 population databases maintained by the Alberta Ministry of Health as reported previously.[91] These included (1) the Ambulatory Care database, which tracks all visits to the 101 emergency departments (ED) in Alberta, and used to identify the cohort; (2) Discharge Abstract Database, that records all admissions to acute care facilities; (3) Physician Claims Database, that tracks all fee-for-service claims for insured health services; (4) Alberta Population Registry and Vital Statistics, that track vital statistics for Alberta inhabitants; and (5) Pharmacy Information Network, that provides all prescriptions filled in Alberta from 2008 onward. Thus, only patients diagnosed from 2008-2014 fiscal years were included in the prescription analysis. Medications were classified using the 2016 Guidelines for Anatomical Therapeutic Chemical Classification and Defined Daily Dose Assignment, 9th Edition.[92] Canadian Classification of Health Interventions (CCI) volume 3[93] was used to identify procedural interventions, which included 1.HH.59 and 1.HZ.59 (cardiac ablation) and 1.HZ.09 (electrical cardioversion) (Supplemental Table 2.1).

Definitions:

Lone AF was diagnosed when incident AF occurred in the absence of (1) predisposing comorbidities 3-years before and 1-year after index AF diagnosis and (2) predisposing acute events present 1-month before and 1-month after index AF diagnosis. AF-predisposing comorbid and acute conditions were identified using ICD9 and ICD10 codes from inpatient, ambulatory and physician office databases and are comprehensively listed in Supplemental Tables 2.2-2.3. In brief, chronic AF-predisposing comorbidities included any thyroid disorder, circulatory system disease, lung disease, diabetes, malignancy and/or obesity diagnosis. Acute AF-predisposing events included infections, major trauma, poisonings/overdoses and/or major surgeries. Surgical procedures were identified using CCI volume 3, where a surgery requiring overnight hospitalization was defined as an acute AF-predisposing event. If any of these aforementioned factors were present within the pre-specified time periods, the case was classified as “secondary

AF," with all the remaining cases considered to be lone AF. Patients were further subdivided by age ≤65 years or >65 years at the time of diagnosis to define young and older AF onset, respectively.

Outcomes:

The primary outcome of interest was a composite endpoint of stroke, TIA, systemic thromboembolism or death within each prespecified AF group at 1- and 3-years of follow-up. Exploratory endpoints were AF re-diagnosis (as a primary diagnosis), and re-presentation for non-cardiovascular causes at 1- and 3-year, as well as the role of sex and age on the development of lone and secondary AF.

Statistical Analysis:

Categorical variables are presented as frequencies with percentages and compared across groups using Chi-square tests. Annual household income and age were presented as median with interquartile range (25^{th}, 75^{th} percentiles) and compared across groups using Mann-Whitney U test. We examined baseline characteristics, outcomes and medication uptake among young AF patients stratified by lone AF and secondary AF as well as among lone AF patients stratified by age group (young: ≤65 years and old: >65 years), and sex (male and female). We also calculated incidence of AF indexed to Alberta population by fiscal year overall, stratified by type of AF (lone and secondary), and by age groups (young and old). To examine the temporal trend in the incidence, we applied Poisson regression models or negative binomial models in case of over dispersion, as appropriate.

In addition, we examined medication uptake and outcomes at 3-years from the diagnosis of AF. For this analysis, we excluded patients who were diagnosed with lone AF after March 31, 2012 in order to provide the same opportunity for 3-year follow up for each patient. Event-free probabilities for primary endpoint and recurrent AF at 3-year were plotted using Kaplan-Meier curves. To assess the effect of various standard medications, i.e. OAC, antiplatelets, atrioventricular nodal (AV) nodal blockers, and anti-arrhythmic drugs (AAD), we used time-dependent Cox regression models with medication as time-dependent covariate. In the case of recurrent AF, bleeding or non-cardiovascular outcomes, death was considered as a competing risk in the model. OAC use was examined by guideline indication among young patients using the

Canadian Cardiovascular Society CHADS65 scoring system.[7] For all analyses, statistical significance was defined as a two-sided p-value of <0.05. Analyses were performed using a software SAS version 9.4 (SAS Institute, Inc., Cary, NC).

2.4 Results

Of 53,059 patients with AF, 33,150 (62.5%) received a new diagnosis of AF during the study period, 1,166 (3.5%) of whom met criteria for lone AF. This latter group was further subdivided by age >65 years (219; 18.8% of lone AF) and ≤65 years (947; 81.2% of lone AF) at diagnosis. Supplemental Figure 2.1 summarizes the study population grouped by age and AF type. The incidence rate of all-cause AF steadily rose on average by 1.7% from 2007 to 2014 fiscal year [rate ratio 1.017 (95% CI: 1.011-1.24); p<0.001] (Figures 2.1-2.2).

Figure 2.1

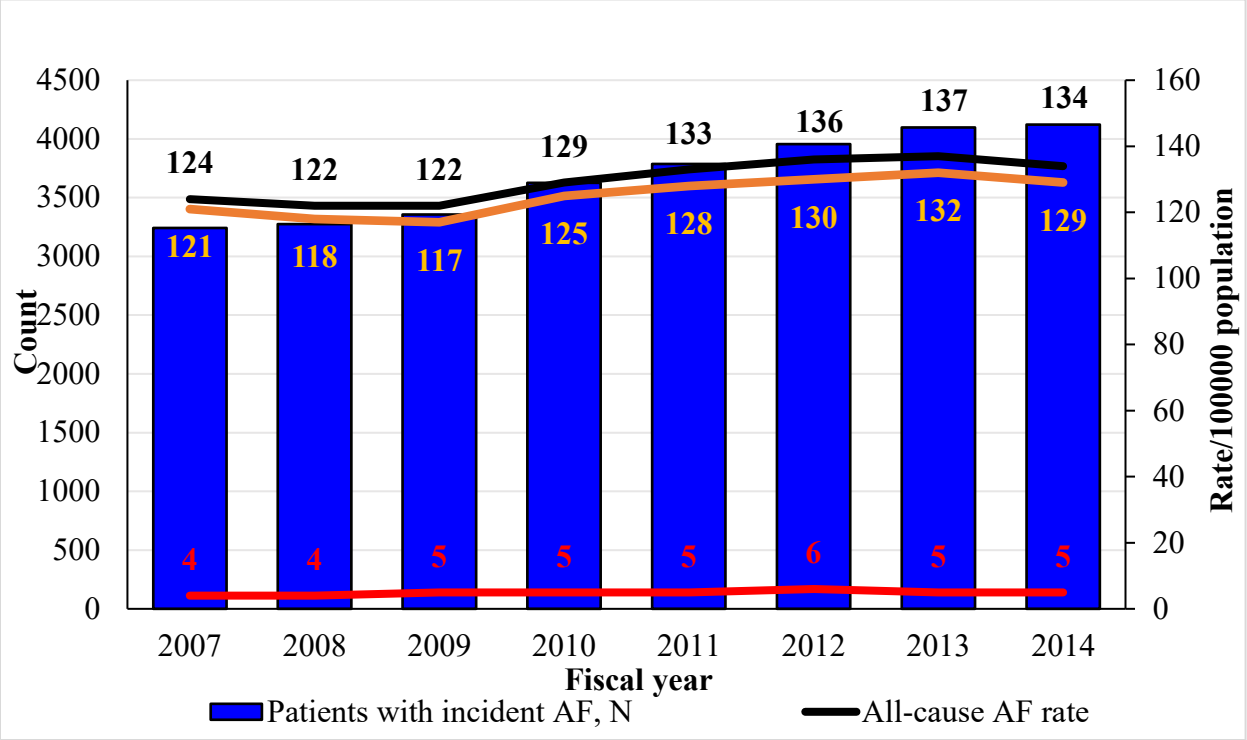


Figure 2.1: Incidence rate of all-cause (lone and secondary) AF indexed to 100,000 Alberta population

Figure 2.2

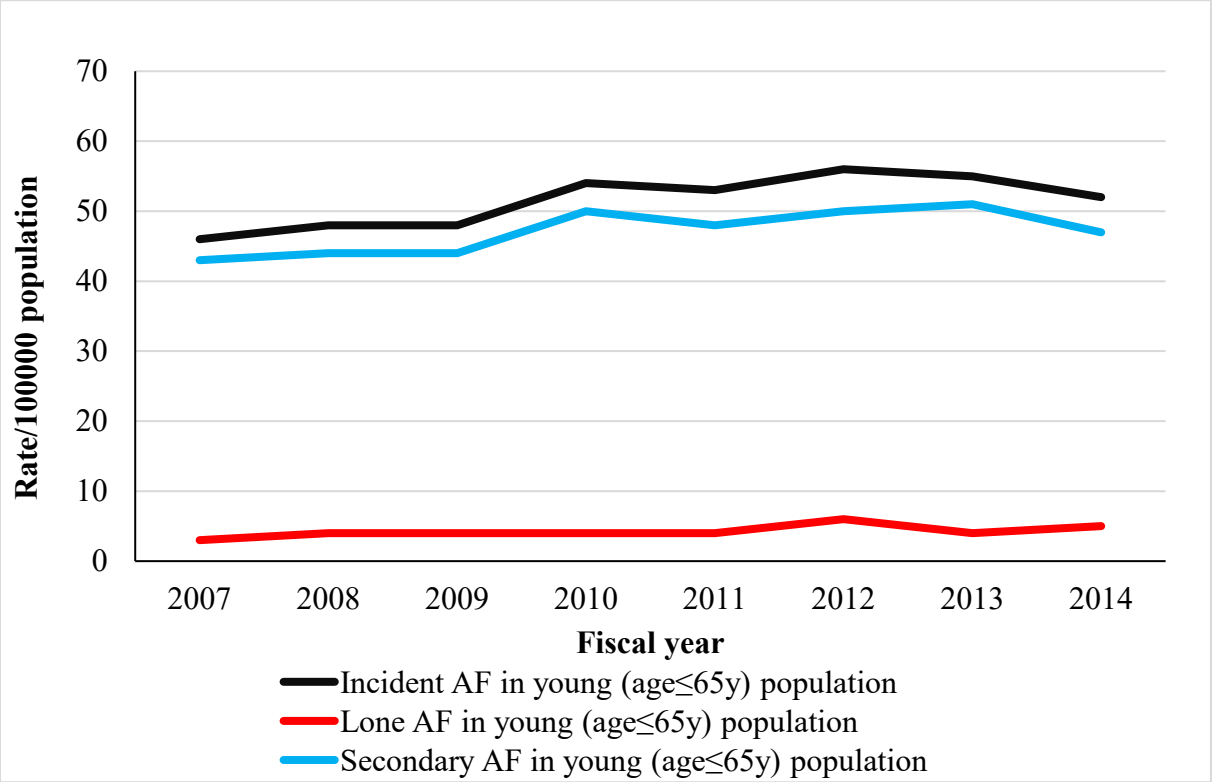


Figure 2.2: Incident rate of AF among young (age≤65 years) population

Lone AF vs. Secondary AF in the Total Study Population:

Lone AF was first compared to secondary AF without subdivision by age group (Table 2.1a). Over the study period, the incidence of both lone AF and secondary AF increased (4.1%; p=0.005 and 1.6%; p<0.001) (Figures 2.1-2.2). At 1- and 3-years, there was a greater risk of meeting all adverse endpoints in secondary AF compared to lone AF (Figures 2.3-2.4, Table 2.1b, and Supplemental Table 2.4a). In lone AF, electrical cardioversion was attempted more frequently, whereas secondary AF patients were more likely to undergo catheter ablation (Table 2.1b).

Table 2.1a

Variable Name	Lone AF	Secondary AF	Total	P-value
Total N	**1166**	**31984**	**33150**	
Age (years) at presentation, median (IQR)	47 (35, 61)	72 (61, 80)	71 (60, 80)	<0.001
Age group (years)				
20-44	521 (44.7)	2088 (6.5)	2609 (7.9)	<0.001
45-54	219 (18.8)	2756 (8.6)	2975 (9.0)	
55-65	207 (17.8)	5418 (16.9)	5625 (17.0)	

66-79	156 (13.4)	12894 (40.3)	13050 (39.4)	
80+	63 (5.4)	8828 (27.6)	8891 (26.8)	
Sex (males)	868 (74.4)	16954 (53.0)	17822 (53.8)	<0.001
Locale of diagnosis				
Outpatient clinic	225 (19.3)	9371 (29.3)	9596 (28.9)	<0.001
ED	820 (70.3)	15335 (47.9)	16155 (48.7)	
Inpatient	121 (10.4)	7278 (22.8)	7399 (22.3)	
Patients' residence				
Rural	214 (18.4)	6053 (18.9)	6267 (18.9)	0.62
Urban	952 (81.6)	25931 (81.1)	26883 (81.1)	
Neighborhood household income (x1000CAD), median (IQR)	75 (68, 96)	73 (66, 90)	73 (66, 90)	0.001

Table 2.1a: Characteristics of new-onset AF patients - lone vs. secondary

Table 2.1b

Variable	Lone AF	Secondary AF	Total	P-value
Catheter ablation within 7-day	1 (0.1)	94 (0.3)	95 (0.3)	0.27
Electrical cardioversion within 7-day	276 (23.7)	3809 (11.9)	4085 (12.3)	<0.001
Catheter ablation within 30-day	1 (0.1)	135 (0.4)	136 (0.4)	0.10
Electrical cardioversion within 30-day	285 (24.4)	4066 (12.7)	4351 (13.1)	<0.001
Catheter ablation within 1-year	4 (0.3)	532 (1.7)	536 (1.6)	<0.001
Electrical cardioversion within 1-year	316 (27.1)	5663 (17.7)	5979 (18.0)	<0.001
1-year outcomes				
Death	12 (1.0)	2624 (8.2)	2636 (8.0)	<0.001
Stroke	2 (0.2)	538 (1.7)	540 (1.6)	<0.001
Stroke/TIA/Embolism	4 (0.3)	806 (2.5)	810 (2.4)	<0.001
Death/Stroke/TIA/Embolism	16 (1.4)	3262 (10.2)	3278 (9.9)	<0.001
Bleeding	5 (0.4)	1523 (4.8)	1528 (4.6)	<0.001
Atrial fibrillation/flutter	187 (16.0)	6640 (20.7)	6827 (20.6)	<0.001
Non CV hospitalization/ED visits	383 (32.9)	16,967 (53.1)	17350 (52.3)	<0.001

Table 2.1b: Treatments and outcomes of new-onset AF – lone vs secondary

Figure 2.3

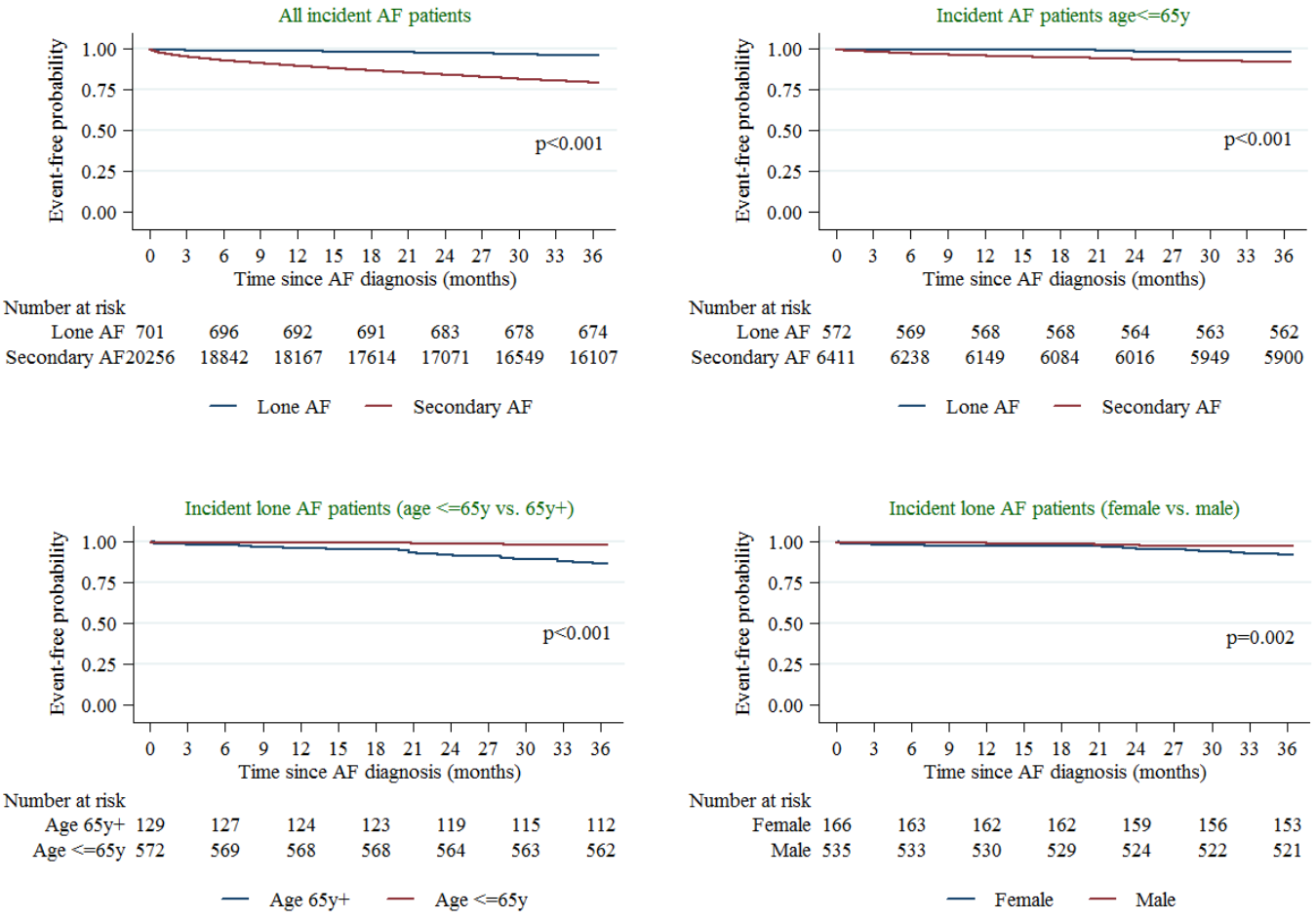


Figure 2.3: Event-free probabilities for composite outcomes (death/stroke/TIA/embolism) at 3-year since the diagnosis of AF

Figure 2.4

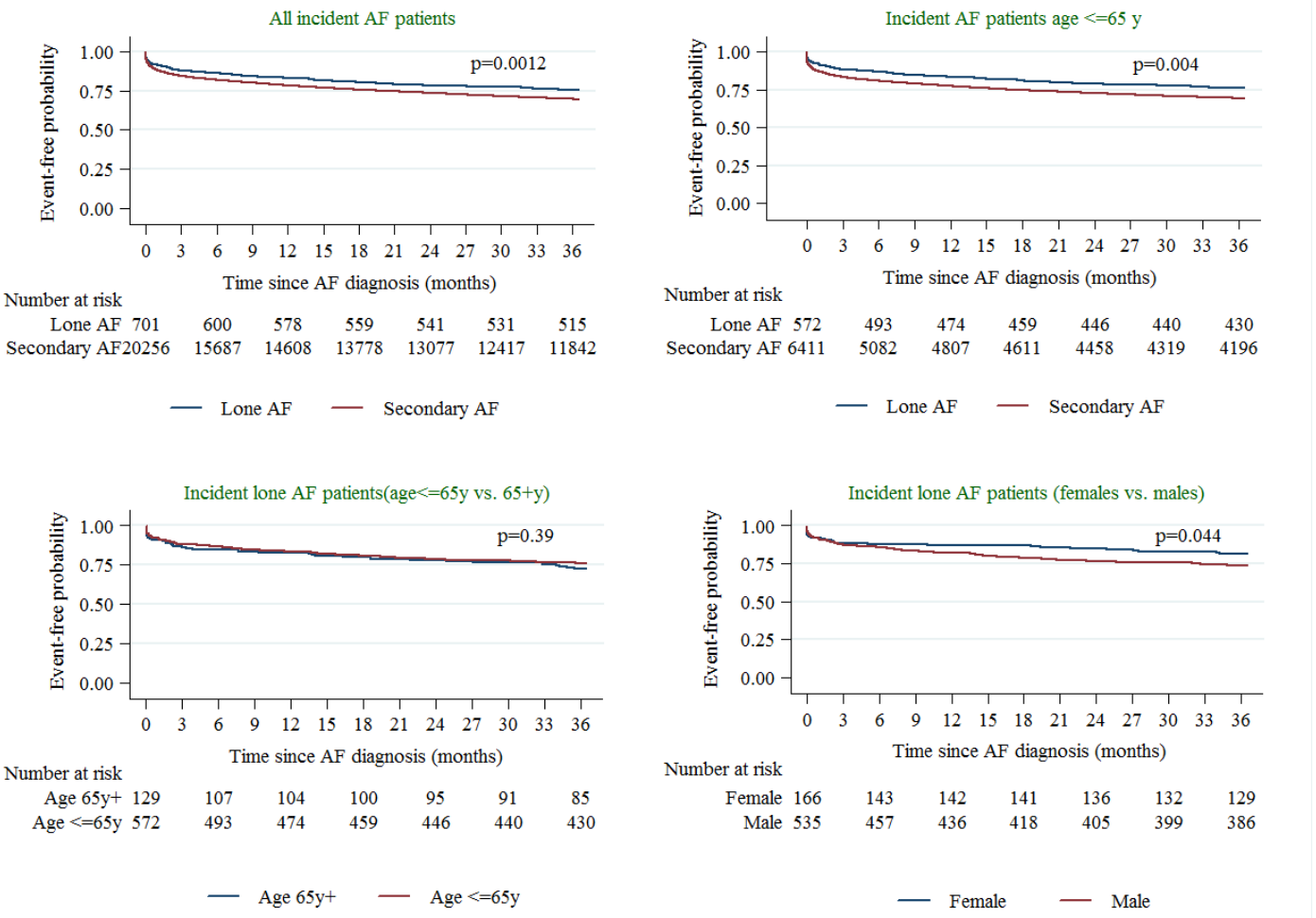


Figure 2.4: Event free probabilities for recurrent AF at 3-year since the diagnosis of AF

Lone AF vs. Secondary AF in the Young:

In the young population (≤65 years at diagnosis), lone AF was compared to secondary AF (Table 2.2a). Age of onset in lone AF (42 years, IQR 23-53) was lower compared to secondary AF (55 years, IQR 47-61) ($p<0.001$). The incidence of AF in the young (≤65 years) rose on average by 2.3% over the study period ($p<0.001$), including increases in both lone (3.9% per year) and secondary AF (2.2% per year) (Figure 2.2). The rise in lone AF diagnoses was 1.8 times greater than in secondary AF, though statistically insignificant ($p=0.30$). The location of diagnosis differed between the two groups (Table 2.2a). At 1- and 3-years, there was a greater risk of meeting all

adverse endpoints in secondary AF compared to lone AF (Table 2.2b and Supplemental Table 2.4b).

Table 2.2a

Variable Name	Lone AF	Secondary AF	Total	P-value
Total N	**947**	**10262**	**11209**	
Age (years) at presentation, median (IQR)	42 (32, 53)	55 (47, 61)	55 (45, 60)	<0.001
Age groups (years)				
20-44	521 (55.0)	2088 (20.3)	2609 (23.3)	<0.001
45-54	219 (23.1)	2756 (26.9)	2975 (26.5)	
55-65	207 (21.9)	5418 (52.8)	5625 (50.2)	
Gender				
Females	198 (20.9)	3639 (35.5)	3837 (34.2)	<0.001
Males	749 (79.1)	6623 (64.5)	7372 (65.8)	
Locale of diagnosis				
Outpatient clinic	161 (17.0)	2804 (27.3)	2965 (26.5)	<0.001
ED	687 (72.5)	5434 (53.0)	6121 (54.6)	
Inpatient	99 (10.5)	2024 (19.7)	2123 (18.9)	
Patients' residence				
Rural	169 (17.8)	1867 (18.2)	2036 (18.2)	0.79
Urban	778 (82.2)	8395 (81.8)	9173 (81.8)	
Neighborhood household income (x1000CAD), median (IQR)	76 (68, 96)	76 (68, 94)	76 (68, 94)	0.94

Table 2.2a: Comparisons of characteristics among young (≤65 years) patients - lone vs. secondary

Table 2.2b

Variable	Lone AF	Secondary AF	Total	P-value
Catheter ablation within 7-day	0 (0.0)	50 (0.5)	50 (0.5)	0.02
Electrical cardioversion within 7-day	253 (26.7)	1870 (18.2)	2123 (18.9)	<0.001
Catheter ablation within 30-day	0 (0.0)	74 (0.7)	74 (0.7)	0.003
Electrical cardioversion within 30-day	260 (27.5)	1974 (19.2)	2234 (19.9)	<0.001
Catheter ablation within 1-year	3 (0.3)	297 (2.9)	300 (2.7)	<0.001
Electrical cardioversion within 1-year	287 (30.3)	2689 (26.2)	2976 (26.6)	0.006
1-year outcomes				
Death	5 (0.5)	293 (2.9)	298 (2.7)	<0.001
Stroke	1 (0.1)	79 (0.8)	80 (0.7)	0.014
Stroke/TIA/Embolism	1 (0.1)	105 (1.0)	106 (1.0)	0.002

Death/Stroke/TIA/Embolism	6 (0.6)	410 (4.0)	416 (3.7)	<0.001
Bleeding	3 (0.3)	265 (2.6)	268 (2.4)	<0.001
AF	158 (16.7)	2269 (22.1)	2427 (21.7)	<0.001
Non-CV hospitalization/ED visits	321 (33.9)	4929 (48.0)	5250 (46.8)	<0.001

Table 2.2b: Cardiac intervention and outcomes among young (≤65 years) patients- lone vs. secondary

Therapies and Outcomes in Lone vs. Secondary AF in the Young

Electrical Cardioversion & Catheter Ablation: Table 2.1b and 2.2b summarize the uptake of catheter ablation and electrical cardioversion at 1-year. In both the older and younger populations, electrical cardioversion was more commonly used for lone AF, whereas catheter ablation was more common in secondary AF.

Oral Anticoagulation: Prescription of OAC was determined at 1-day, 30-days and 1-year post-AF diagnosis in the young (Table 2.3). Within 1-year, secondary AF patients more often received OAC prescriptions (42.1 vs 13.9%; p<0.001). We then examined the effect of medication for stroke/systemic embolism prevention on outcomes among 9,372 young patients who were diagnosed between January 1, 2008 and March 31, 2015. Among these patients, 326 (3.5%) suffered from the composite endpoint of stroke, TIA, systemic thromboembolism or death during the first year of follow-up, 104 (31.9%) of whom received a prescription for OAC prior to the outcome occurring. In these young patients, a lack of OAC prescription was not associated with the risk of meeting this primary endpoint (HR 0.96 (95% CI 0.75-1.21), p=0.70). Among those young AF patients with a guideline-based indication for long-term OAC (n=4,823), 2,522 (52.3%) received an OAC prescription at 1-year, but only 82 of them received OAC prior to the primary endpoint. This was not associated with a reduction in the primary endpoint (HR 1.05 (95% CI 0.83-1.33; p=0.76). In the 14% (n=1,327) of young AF patients who received an antiplatelet agent alone (no OAC), including 46 of 800 (5.75%) with lone AF, antiplatelets were not associated with the primary endpoint (HR 1.13 (95% CI 0.82-1.56); p=0.44). Despite having an OAC indication, 18% (n=885) had an antiplatelet agent only. At 1-year, bleeding occurred in 3 (0.3%) lone AF patients and 282 (2.8%) secondary AF patients (p<0.001). Bleeding was associated with antiplatelet (HR 1.53 (95% 1.03-2.15); p=0.013) and OAC (HR 2.50 (95% CI 1.92-3.26, p<0.001) prescription within the first year.

Table 2.3

Medications	Lone AF	Secondary AF	Total	P-value
Total N	**800**	**8572**	**9372**	
Within 1-day				
Warfarin	20 (2.5)	566 (6.6)	586 (6.3)	<0.001
NOAC	23 (2.9)	291 (3.4)	314 (3.4)	0.43
OAC	43 (5.4)	852 (9.9)	895 (9.5)	<0.001
Antiarrhythmic drug including Sotalol	6 (0.8)	61 (0.7)	67 (0.7)	0.90
Antiplatelet	30 (3.8)	250 (2.9)	280 (3.0)	0.19
Beta blocker	113 (14.1)	1247 (14.5)	1360 (14.5)	0.75
Calcium channel blocker	21 (2.6)	361 (4.2)	382 (4.1)	0.03
Digoxin	2 (0.3)	48 (0.6)	50 (0.5)	0.25
Within 30-day				
Warfarin	40 (5.0)	1718 (20.0)	1758 (18.8)	<0.001
NOAC	38 (4.8)	736 (8.6)	774 (8.3)	0.0002
OAC	78 (9.8)	2411 (28.1)	2489 (26.6)	<0.001
Antiarrhythmic drug including Sotalol	12 (1.5)	383 (4.5)	395 (4.2)	<0.001
Antiplatelet	44 (5.5)	839 (9.8)	883 (9.4)	<0.001
Beta blocker	172 (21.5)	3228 (37.7)	3400 (36.3)	<0.001
Calcium channel blocker	31 (3.9)	1015 (11.8)	1046 (11.2)	<0.001
Digoxin	3 (0.4)	368 (4.3)	371 (4.0)	<0.001
Within 1-year				
Warfarin	58 (7.3)	2589 (30.2)	2647 (28.2)	<0.001
NOAC	59 (7.4)	1340 (15.6)	1399 (14.9)	<0.001
OAC	111 (13.9)	3607 (42.1)	3718 (39.7)	<0.001
Antiarrhythmic drug including Sotalol	48 (6.0)	1158 (13.5)	1206 (12.9)	<0.001
Antiplatelet	58 (7.3)	1551 (18.1)	1609 (17.2)	<0.001
Beta blocker	230 (28.8)	4829 (56.3)	5059 (54.0)	<0.001
Calcium channel blocker	45 (5.6)	1955 (22.8)	2000 (21.3)	<0.001
Digoxin	7 (0.9)	671 (7.8)	678 (7.2)	<0.001

Table 2.3: Medication uptakes among young (≤65y) AF patients - lone vs. secondary

Pharmacologic Rate & Rhythm Control: A rate control strategy using AV nodal blockers was attempted in the minority of young lone AF patients during follow-up (Table 2.3), with most beta-blocker prescriptions occurring in the first 30-days (172 of 230 patients, 74.8%). In young

secondary AF, beta-blockers were used in 37.7% at 30-days (3,228 patients) and 56.3% by 1-year (4,829 patients). All types of rate control drugs were more often used in secondary AF than in lone AF at 30-days and 1-year ($p<0.001$). Lone AF patients were less likely to receive an AAD at all follow-up points examined ($p<0.001$). AAD use was not associated with the primary endpoint (HR 0.71 (95% CI 0.45-1.14), p=0.15), but was associated with AF re-diagnosis (HR 1.57 (95% 1.29-1.92, $p<0.001$)) and non-cardiovascular disease (re)hospitalization and (re)presentation to emergency care (HR 1.18 (95% CI 1.05-1.33), p=0.006)). AV nodal blocker use was associated with the primary endpoint (HR 1.28 (95% CI 1.01-1.61); p=0.038)), AF recurrence (HR 1.70 (95% CI 1.55-1.89), $p<0.001$) and non-cardiovascular disease (re)hospitalization and (re)presentation to emergency care (HR 1.22 (95% CI 1.14-1.30), $p<0.001$). Supplemental Table 2.5a-b summarize the 3-year prescription trends.

Sex-based Comparisons in Lone AF:

Total Lone AF Population: Several differences were found between males and females with lone AF (Table 2.4). Females were older (58 years, IQR 40-70) than males (45 years, IQR 34-57) ($p<0.001$) and more likely to be diagnosed in the outpatient setting vs emergency or inpatient setting ($p<0.001$). There were no sex-based differences in the use of electrical cardioversion or catheter ablation during follow-up (Table 4). Males had a greater likelihood of being free of the primary composite endpoint at 1- and 3-years compared to females (0.9% vs 2.7%; p=0.024 and 2.6% vs 7.8%; p=0.002, respectively). Re-diagnoses of AF was greater at 1-year and 3-years in males than in females (17.6 vs 12.1%, p=0.025 and 26.4 vs 18.1%; p=0.029, respectively). There were no significant differences based on sex for OAC prescription at 1-year (21.3 vs 17.3%; p=0.15) or 3-years (20.2 vs 21.2%; p=0.78). However, females were more likely to receive calcium channel blocker (11.5 vs 6.2%; p=0.006) and digoxin (3.2 vs 0.7%; p=0.003) prescriptions by 1-year. We also performed the same sex-based analyses in younger patients, as summarized in Table 2.5.

Table 2.4

Variable Value	Younger 20-65 years	Older 65+ years	P-value	Females All ages	Males All ages	p-value
Total N	**947**	**219**		**298**	**868**	
Age at presentation, median (IQR)	42 (32, 53)	73 (68, 81)	<0.001	58 (40, 70)	45 (34, 57)	<0.001
Sex						
Females	198 (20.9)	100 (45.7)	<0.001	-	-	
Males	749 (79.1)	119 (54.3)		-	-	
Location of diagnosis						
Outpatient clinic	161 (17.0)	64 (29.2)	<0.001	83 (27.9)	142 (16.4)	<0.001
ED	687 (72.5)	133 (60.7)		187 (62.8)	633 (72.9)	
Inpatient	99 (10.5)	22 (10.0)		28 (9.4)	93 (10.7)	
Residence						
Rural	169 (17.8)	45 (20.5)	0.35	53 (17.8)	161 (18.5)	0.77
Urban	778 (82.2)	174 (79.5)		245 (82.2)	707 (81.5)	
Neighborhood household income (x1000CAD), median (IQR)	76(68, 96)	72(65, 91)	0.033	73(65, 93)	75(68, 96)	0.069
Electrical cardioversion within 7-day	253 (26.7)	23 (10.5)	<0.001	43 (14.4)	233 (26.8)	<0.001
Electrical cardioversion within 30-day	260 (27.5)	25 (11.4)	<0.001	45 (15.1)	240 (27.7)	<0.001
Electrical cardioversion within 1-year	287 (30.3)	29 (13.2)	<0.001	47 (15.8)	269 (31.0)	<0.001
1-year outcome						
Death	5 (0.5)	7 (3.2)	0.003	5 (1.7)	7 (0.8)	0.20
Death/stroke/TIA/Embolism	6 (0.6)	10 (4.6)	<0.001	8 (2.7)	8 (0.9)	0.024
Bleeding	3 (0.3)	2 (0.9)	0.24	2 (0.7)	3 (0.4)	0.61
Atrial fibrillation/flutter	158 (16.7)	29 (13.2)	0.21	35 (11.7)	152 (17.5)	0.019
Non CV hospitalization/ED visits	321 (33.9)	62 (28.3)	0.11	103 (34.6)	280 (32.3)	0.46

Table 2.4: Characteristics of lone AF patients - stratified by age (young vs old) and by sex (females vs males)

Table 2.5

Variable Name	Younger 20-65 years	Older 65+ years	P-value	Females All ages	Males All ages	P-value
Total N	**800**	**183**		**253**	**730**	
Within 1-day						
Warfarin	20 (2.5)	15 (8.2)	0.0002	9 (3.6)	26 (3.6)	0.99
NOAC	23 (2.9)	5 (2.7)	0.92	8 (3.2)	20 (2.7)	0.72
OAC	43 (5.4)	20 (10.9)	0.006	17 (6.7)	46 (6.3)	0.82
Antiarrhythmic drug including Sotalol	6 (0.6)	0	0.24	3 (1.2)	3 (0.4)	0.17
Antiplatelet	30 (3.8)	4 (2.2)	0.30	8 (3.2)	26 (3.6)	0.76
Beta blocker	113 (14.1)	27 (14.8)	0.83	36 (14.2)	104 (14.2)	0.99
Calcium channel blocker	21 (2.6)	9 (4.9)	0.10	10 (4.0)	20 (2.7)	0.33
Digoxin	2 (0.3)	3 (1.6)	0.017	3 (1.2)	2 (0.3)	0.079
Within 3- day						
Warfarin	40 (5.0)	31 (16.9)	<0.001	22 (8.7)	49 (6.7)	0.29
NOAC	38 (4.8)	13 (7.1)	0.19	16 (6.3)	35 (4.8)	0.34
OAC	78 (9.8)	44 (24.0)	<0.001	38 (15.0)	84 (11.5)	0.14
Antiarrhythmic drug including Sotalol	12 (1.5)	1 (0.6)	0.31	5(2.0)	8 (1.1)	0.29
Antiplatelet	44 (5.5)	8 (4.4)	0.54	13 (5.1)	39 (5.3)	0.90
Beta blocker	172 (21.5)	46 (25.1)	0.28	58 (22.9)	160 (21.9)	0.74
Calcium channel blocker	31 (3.9)	17 (9.3)	0.002	15 (5.9)	33 (4.5)	0.37
Digoxin	3 (0.4)	5 (2.7)	0.0014	4 (1.6)	4 (0.5)	0.12
Within 1-year						
Warfarin	58 (7.3)	45 (24.6)	<0.001	33 (13.0)	70 (9.6)	0.12
NOAC	59 (7.4)	27 (14.8)	0.0014	24 (9.5)	62 (8.5)	0.63
OAC	111 (13.9)	69 (37.7)	<0.001	54 (21.3)	126 (17.3)	0.15
Antiarrhythmic drug including Sotalol	48 (6.0)	9(4.9)	0.57	13 (5.1)	44 (6.0)	0.60
Antiplatelet	58 (7.3)	12 (6.6)	0.74	19 (7.5)	51 (7.0)	0.78
Beta blocker	230 (28.8)	68 (37.2)	0.026	80 (31.6)	218 (29.9)	0.60
Calcium channel blocker	45 (5.6)	29 (15.8)	<0.001	29 (11.5)	45 (6.2)	0.006
Digoxin	7 (0.9)	6 (3.3)	0.01	8 (3.2)	5 (0.7)	0.003

Table 2.5: Medication uptake among lone AF patients - by age (young vs old) and by sex (females vs males)

Age-based Comparisons in Lone AF:

We compared patients meeting criteria for lone AF based on young (≤65 years) vs. older age (>65 years) of onset. The earlier-onset group was more likely to be male (79.1 vs 54.3%; p<0.001) and to be diagnosed in the emergency/inpatient setting (p<0.001). For the primary composite endpoint, older lone AF patients had more events than younger patients at 1-year (4.6 vs 0.6%; p<0.001) and 3-years (13.2 vs 1.7%; p<0.001). However, at 1-year and 3-years, there was no significant difference in risk of AF re-diagnosis or non-cardiovascular disease (re)presentation/(re)admission to hospital (Table 2.6). OAC (37.7 vs 13.9%; p<0.001), beta-blocker (372 vs 28.8%;p=0.026), and calcium channel blocker (15.8 vs 5.6%; p<0.001) prescription was greater in older lone AF patients at 1-year.

Table 2.6

Variable Name	Females	Males	P-value
Total N	**198**	**749**	
Age at presentation, median (IQR)	47 (35, 58)	42 (32, 52)	<0.001
Location of diagnosis			
Outpatient clinic	61 (30.8)	100 (13.4)	<0.001
ED	120 (60.6)	567 (75.7)	
Inpatient	17 (8.6)	82 (10.9)	
Residence			
Rural	34 (17.2)	135 (18.0)	0.78
Urban	164 (82.8)	614 (82.0)	
Neighborhood household income (x1000CAD), median (IQR)	76 (66, 97)	76 (68, 96)	0.93
Electrical cardioversion within 30-day	32 (16.2)	228 (30.4)	<0.001
Electrical cardioversion within 1-year	33 (16.7)	254 (33.9)	<0.001
1-year outcome			
Death	2 (1.0)	3 (0.4)	0.29
Death/stroke/TIA/Embolism	3 (1.5)	3 (0.4)	0.079
Bleeding	1 (0.5)	2 (0.3)	0.6
Atrial fibrillation/flutter	22 (11.1)	136 (18.2)	0.018
Non CV hospitalization/ED visits	70 (35.4%)	251 (33.5%)	0.58

Table 2.6: Characteristics and 1-year outcome of young (age≤65y) lone AF patients - stratified by sex

2.5 Discussion

This population study demonstrates that truly lone AF is very rare, especially when older patients are excluded from the definition, and that the prognosis is generally favourable. However, lone AF

patients are not free from adverse outcomes, including a high rate of re-hospitalization and AF re-diagnosis over a short duration of follow-up, which is further complicated by a rising incidence of AF in the general population. The unique challenges faced by young lone AF patients include a highly skewed preponderance towards young males being affected, but significantly worse outcomes in females. In older lone AF patients, there is a concerning trend towards OAC underuse. Collectively, these data suggest that lone AF warrants further study as a unique clinical entity distinct from secondary AF.

Several our findings related to sex and age require additional comment. In keeping with established trends in all-cause AF,[12, 13, 94, 95] the impact of male sex on lone AF susceptibility was highly pronounced, with a 13-year difference in the median age of diagnosis. While previous studies have failed to clearly identify biological explanations for sex differences in AF, elevated endogenous bioavailable testosterone appeared to increase AF risk in the Multi-Ethnic Study of Atherosclerosis, and X-linked recessive factors may play a role in large AF kindred.[96, 97] In our study, we could not rule out lifestyle as an environmental contributor, such as sex-based differences in alcohol intake and smoking.[98, 99] Young males may also be more likely to be classified as secondary AF because a higher incidence of cardiovascular comorbidities compared to females. Despite these associations, when females did develop lone AF, they were more likely to experience a TIA, stroke, systemic thromboembolism or death during follow-up. Recent studies on all-comers with nonvalvular AF have attempted to clarify the controversial role of sex on stroke risk.[100, 101] These newer data show that men and women face a similar risk of stroke and systemic thromboembolism, with the exception being elderly women who are of slightly higher risk.[101] We attempted to account for differences in therapy that could explain our findings. However, women were treated similarly, except for a slightly greater use of digoxin and calcium channel blockers, which are traditionally viewed as second-line rate control agents. We were not able to determine whether physician bias contributed to a greater use of these drugs, or if women were less likely to achieve adequate rate control on standard beta-blocker regimens.

Therapies were difficult to comprehensively adjudicate in this cohort. We attempted to address the interactions between clinical events and guideline-based medication use, cardioversion and catheter ablation. While we observed that in lone AF, AV nodal blockers and AAD were associated with an increase in event risk, this was potentially confounded by the fact that patients with a greater burden of persistent or permanent AF more often receive treatment. Thus, this group

would be expected to have worse outcomes. Similarly, we did not observe an improvement in the primary outcome in patients receiving OAC or antiplatelets for stroke protection. However, this is likely due to the very low event rate and low overall uptake of these therapies in the lone AF group.

We found that the incidence of both lone and secondary AF rose throughout the study period. This may be because of a greater awareness of AF in the contemporary era and more opportunities for subclinical detection, given the widespread use of wearable devices and implantable monitors. It is also possible that metabolic risk factors, like the rise in obesity over time, were incompletely coded and contributed to this trend in lone AF. Importantly, we show that when applying a strict definition to lone AF, including young age of onset, the incidence compared to secondary AF is very low (2.9% of all new AF diagnoses during the study period). This is much lower than historical estimates of lone AF prevalence, broadly reported as accounting for 2-30% of all AF cases.[14] This wide range and greater magnitude is likely due to previously unrecognized secondary AF risk factors, inconsistent definitions of lone AF over time and variable classifications based on persistence of AF.[14]

The incidence and temporal trends in lone AF have increasing clinical relevance. Data are emerging to show that many young patients and families afflicted by early-onset AF often have a predisposing genetic substrate for the condition.[43, 102, 103] In the past year, truncating mutations in the gene encoding Titin have emerged as a strong risk factor for lone AF, especially in the very young.[61, 62, 104] Future opportunities to prevent and detect lone AF early in life may improve outcomes, and lead to tailored therapy,[105] with AF genotype-specific precision therapy studies ongoing (ClinicalTrials.gov Identifiers NCT02347111 and NCT02404415). The present study provides an estimate of the number of AF cases that might benefit from predictive genetic testing, early detection and family counselling. Here, we argue that the emerging role of genetics in AF necessitates that lone AF be defined more strictly, to inform who may be most likely to benefit from gene testing, family screening, and precision therapy.

Limitations:

This is a population level study of retrospective administrative data. The burden and frequency of AF cannot be ascertained using this study design. We were also unable to adjust for environmental contributors to AF like alcohol intake, exercise, diet and smoking as they are not reliably coded in the medical record. Similarly, the codes for comorbidities and procedures were not universally validated, however, ICD codes of AF have strong positive and negative predictive

power in previous validation cohorts.[88-90] To increase the probability that lone AF was unexplained/idiopathic, we classified all cases with an identifiable AF predisposing condition as being secondary AF. However, it remains possible that some of these conditions did not contribute to the pathogenesis of AF. Additionally, although we could link prescriptions to individual patients, we were unable to verify if they were filled or if medications were taken properly. Finally, the literature and evidence that defines the maximal age at which lone AF can be diagnosed remains ambiguous and controversial. Here, we used a cut-off of 65 years to define young onset of AF because this was similar to that of recent studies,[14, 85] and reflects the age at which OAC should be started on all AF patients in Canada.[7]

2.6 Conclusions

While lone AF manifests early in life among males, women consistently suffer from poorer outcomes despite receiving similar therapies compared to their male counterparts. The true incidence of lone AF is likely much lower than previously reported. The present study suggests that lone AF is a unique clinical entity with trends and outcomes that differ substantially from that of secondary AF. A greater emphasis needs to be placed on managing and studying lone AF as a distinct cardiovascular condition.

Chapter 3: A Population-Based Study of Syncope in the Young

3.1 Abstract

Background: The prevalence, hospitalization patterns, and outcomes of pediatric and adolescent syncope have not been rigorously characterized.

Methods: Patients <20 years-old presenting to an emergency department (ED) with a primary diagnosis of syncope (ICD-10 code: R55) between fiscal-year (FY) 2006/07 and FY 2013/14 in the province of Alberta, Canada, were grouped according to discharge status from the ED: admitted to hospital and discharged without admission. Temporal trends and differences in baseline characteristics, medication use, and outcomes between admitted and discharged patients were examined.

Results: The prevalence of syncope increased from 143/100,000 population in FY 2006/07 to 166/100,000 population in FY 2013/14 ($p<0.01$). The majority of the 11,488 patients that presented to the ED with syncope were discharged home (n=11,214, 98%). Cardiac disease was present in 12.7% and thoracic conditions were present in 8% of the study population. A majority of patients (66.2% admitted and 56.4% discharged, $p=0.018$) had a prescription drug in the year prior to presentation. By 30-days, 26.1% of admitted patients had a repeat ED presentation and 8.1% had a re-hospitalization. Among discharged patients 30-day repeat ED presentation rate was 11.7% and hospitalization rate was 1.1%. By 1-year, repeat ED rates increased to 64.1% and 47.5%, and (re)hospitalization rates increased to 21.4% and 6.8% among admitted and discharged patients, respectively.

Conclusions: Our data suggest that pediatric and adolescent syncope is increasing in prevalence and represents a growing public health problem. This population has a high burden of comorbidities which likely contribute to increased healthcare resource utilization and polypharmacy.

3.2 Introduction

Syncope is defined as a brief loss of consciousness due to sudden onset cerebral hypoperfusion followed by spontaneous and complete recovery.[24] The lifetime risk of syncope has been conservatively estimated at 35%,[106] and the condition accounts for a substantial burden of healthcare expenditure,[107] with leading experts advising against the use of low yield testing in adults.[26, 108] Recent population data suggest that presentation and hospitalization for syncope is declining in adults.[109] This progress may be in part related to the development of risk stratification scores and evidence-based care pathways for adults with syncope.[110, 111]

Pediatric syncope is also common. The Rochester Epidemiology Project reported a syncope incidence of 125.8 per 100,000 in children and adolescents between 1987 and 1991,[112] and another study showed that a third of children will faint prior to adulthood.[106] The etiology of pediatric and adolescent syncope is usually vasovagal, a generally benign dysregulation of the autonomic nervous system. However, the dramatic appearance of syncope coupled with the misconception that serious cardiac pathology is a common precipitant,[25] may lead to unnecessary hospitalization and costly testing in kids. While pediatric guidelines also advise a minimalistic approach to assessment of the syncopal child,[25, 108, 113] the data supporting these recommendations are mainly limited to case series and extrapolations from adult population studies. This is problematic as many pediatric providers may be unfamiliar with the adult data supporting the syncope guidelines, and the etiology of syncope in the young is more likely to be neurocardiogenic in nature.[25] As such, robust tools for risk assessment and safe discharge planning are lacking for children. To better characterize the incidence, demographic characteristics, prescription drug use, and outcomes of syncope in the young, we undertook a population study of children and adolescents presenting to the ED with a primary diagnosis of syncope in Alberta, Canada.

3.3 Methods

Study population: Individuals aged <20 years at the time of the first (index) visit to the ED from April 2006 to March 2014 (fiscal year [FY] 2006-2013) with a primary diagnosis of syncope [International Classification of Diseases, 10th revision (ICD-10): R55][114] were included in this study. Patients were categorized according to their ED discharge status as admitted (within 24 hours) to hospital discharged from the ED without admission to hospital. All patients were followed for one year from the index ED visit.

Data source: Data were obtained by linking 6 databases maintained by the Alberta Ministry of Health: (1) the Ambulatory Care database tracks all visits to the 101 EDs in the province and was used to identify the study cohort; (2) Discharge Abstract database records all admissions to acute care facilities; (3) Physician Claims database tracks all fee-for-service claims for insured health services; (4) Alberta Population Registry and Vital Statistics track vital statistics for all inhabitants of the province; and (5) Pharmacy Information Network provides access to all prescriptions of medications that were filled.[115]

Patient characteristics and medication use: We used previously validated international classification of diseases codes to identify patient comorbidities.[116] Several additional comorbidities more relevant to pediatrics were added[117-119] (see Appendix C - Supplemental data for complete list of codes). Comorbidities were considered present if they were recorded for the index ED visit or hospitalization within 24 hours of ED discharge (if it occurred) and all other contacts with the healthcare system in the four years preceding the index visit using the ambulatory care and hospitalization databases. Children were defined as age <10 years and adolescents were ≥10 years of age (to a maximal age of 19 years).[120] Residence was categorized as urban or rural based on the second digit of the home address postal code. Prescription medication claims for the cohort were available as of January 1, 2009. We examined prescription claims one year before, and one year after, the index syncope event among patients presenting to the ED between fiscal years 2009 and 2014.

Outcomes: The main outcomes of interest were 30-day and 1-year re-presentation to the ED or (re) admission to hospital. We documented the number of deaths at one-year, overall, and among admitted and discharged patients.

Statistical analysis: We summarized patient characteristics using means (±SD), medians (interquartile ranges), counts and percentages, as appropriate. Values were compared across cohorts using t-tests for means and Mann-Whitney for medians. All analyses were performed using Stata version 14 (Stata Corporation, College Station, Texas); Two-sided P values < 0.05 were considered statistically significant. The study was reviewed and approved by the ethics board at the University of Alberta.

3.4 Results

Between April 1, 2006 and March 31, 2014, the annual prevalence of ED presentations with a primary diagnosis of syncope among children and adolescents increased from 143 per 100,000 population to 166 per 100,000 population (p<0.01, Figure 3.1). Of the 11,488 patients in the study, 234 patients (2%) were admitted to hospital and 11,214 (98%) were discharged from the ED (Table 3.1). Among admitted patients, 96 (41%) were subsequently discharged with a primary diagnosis of syncope and 138 (59%) with a primary diagnosis other than syncope. The most common discharge diagnoses in the latter group were convulsions, not elsewhere classified (6%), other symptoms and signs involving cognitive functions and awareness (3%) and epilepsy and recurrent seizure (2.6%). Amongst all EDs, the admission rate for index syncope ranged from 0 (37 EDs) to 21.1% (small, rural hospital). The average ED admission rate at the two pediatric hospitals in the province of Alberta was similar (2.1%) to that at non-pediatric hospitals (2.0%, p=0.752).

Table 3.1

Variables	All patients	Admitted (N=234)	Discharged (N=11,214)	p-value
Demographic characteristics				
Age, y mean (SD)	14 (4.3)	12.3 (5.9)	14.1 (4.3)	<0.001
Age, y median (IQR)	15 (12-17)	14 (9-17)	15 (12-17)	<0.001
Age group, n (%)				
Child (<10 years)	1,763 (15.4)	62 (26.5)	1,701 (15.2)	<0.001
Adolescent (>=10 years)	9,685 (84.6)	172 (73.5)	9,513 (84.8)	
Male, n (%)	4,222 (36.9)	91 (38.9)	4,131 (36.8)	0.520
Urban residence, n (%)	8,648 (75.5)	160 (68.4)	8,488 (75.7)	0.004
Selected comorbidity, n (%)				
Heart condition	1,455 (12.7)	49 (20.9)	1,406 (12.5)	0.001
Malignancy	38 (0.3)	3 (1.3)	35 (0.3)	0.011
Thoracic condition	920 (8.0)	31 (13.3)	889 (7.9)	0.003
Abdominal condition	16 (0.1)	1 (0.4)	15 (0.1)	0.234
Renal condition	34 (0.3)	4 (1.7)	30 (0.3)	<0.001
Neurological condition	1,348 (11.8)	38 (16.2)	1,310 (11.7)	0.032
Depression and other mood disorders	343 (3.0)	12 (5.1)	331 (3.0)	0.053
Diabetes	67 (0.6)	3 (1.3)	64 (0.6)	0.158
Hemiplegia or paraplegia	53 (0.5)	4 (1.7)	49 (0.4)	0.005

Sepsis	26 (0.2)	3 (1.3)	23 (0.2)	0.001

Table 3.1**:** Patient characteristics. Co-morbid conditions identified in the 4 years preceding index syncope.

Figure 3.1

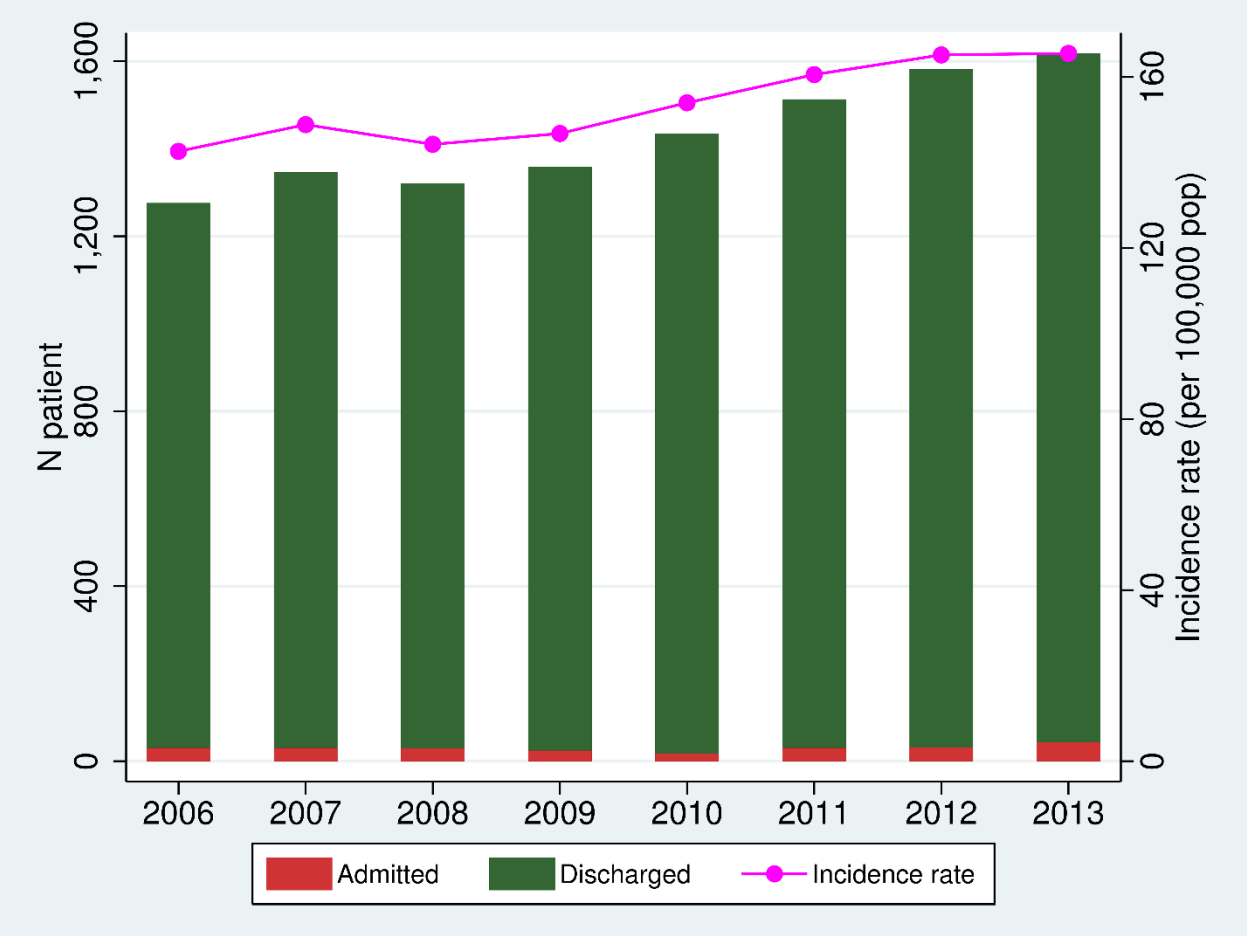


Figure 3.1: Prevalence and discharge status among children admitted ED with a primary diagnosis of syncope in Alberta, 2006- 2013.

The median age of admitted patients was significantly lower 14 (IQR 9-17) years than among discharged patients (15 (IQR 12-17) years, $p<0.001$). Female sex predominated in the cohort, but did not differ among admitted and discharged patients. Most subjects resided in an urban setting (75.5%), and discharged patients were more likely to reside in an urban setting (75.7%) than admitted patients (68.4%, $p<0.001$). Cardiac conditions were identified in 12.7% and were less common in discharged patients (12.5%) than among admitted patients (20.9%, $p<0.001$).

The three most common cardiac diagnoses were: other & complications of heart disease (88.5%), arrhythmias & cardiac arrest (9%) and conductive heart disease (3%). Similarly, thoracic conditions were more prevalent in admitted patients (13.3%) compared to patients who were discharged (7.9%, p<0.01). The three most common thoracic diagnoses were: chronic pulmonary disease (99%), pulmonary embolism (0.1%) and other secondary pulmonary hypertension (0.1%). The rates of neurological conditions were 16.2% and 11.7% among admitted and discharged patients, respectively. Head injuries accounted for most neurological diagnoses (97%). Co-morbid epilepsy was identified in 0.7% of patients with neurological conditions.

Medications: Medication data were available for all patients presenting to the ED in FY 2009 and after (145 admitted patients and 7361 discharged patients). Overall, 66.2% of admitted patients and 56.4% of discharged patients (p=0.018) had a prescription drug filled in the one year before the index syncope ED presentation (Table 3.2). In the year following the index syncope presentation, 73.8% admitted patients and 63.6% of discharged patients had a prescription medication claim (p=0.012). Regardless of the time period (either before or after the index syncope event), the most common class of drugs, in both admitted and discharged patients were anti-infectives for systemic use and drugs related to the nervous system. A full list of prescription medications is provided in Table 3.2.

Table 3.2

	Before			**After**		
Medication class (n, %*)	**Admitted (N=145)**	**Discharged (N=7,361)**	**p**	**Admitted (N=145)**	**Discharged (N=7,361)**	**p-value**
Any medication	96 (66.2)	4,149 (56.4)	0.018	107 (73.8)	4,684 (63.6)	0.012
Alimentary tract and metabolism	28 (19.3)	657 (8.9)	<0.001	30 (20.7)	859 (11.7)	0.001
Blood and blood forming organs	6 (4.1)	119 (1.6)	0.019	10 (6.9)	226 (3.1)	0.009
Cardiovascular system	7 (4.8)	177 (2.4)	0.062	12 (8.3)	263 (3.6)	0.003

Dermatologics	28 (19.3)	986 (13.4)	0.039	31 (21.4)	1,126 (15.3)	0.045
Genito-urinary system and sex hormones	18 (12.4)	1,036 (14.1)	0.569	21 (14.5)	1,300 (17.7)	0.320
Systemic hormonal preparations, excluding sex hormones and insulins	16 (11)	250 (3.4)	<0.001	15 (10.3)	343 (4.7)	0.001
Antiinfectives for systemic use	64 (44.1)	2,567 (34.9)	0.021	71 (49)	2,914 (39.6)	0.022
Antineoplastic and immune-modulating agents	2 (1.4)	21 (0.3)	0.018	2 (1.4)	37 (0.5)	0.146
Musculo-skeletal system	21 (14.5)	645 (8.8)	0.016	21 (14.5)	800 (10.9)	0.167
Nervous system	33 (22.8)	1,183 (16.1)	0.030	47 (32.4)	1,636 (22.2)	0.004
Antiparasitic products, insecticides and repellents	5 (3.5)	141 (1.9)	0.186	8 (5.5)	183 (2.5)	0.022
Respiratory system	21 (14.5)	1,196 (16.3)	0.568	27 (18.6)	1,296 (17.6)	0.751
Sensory organs	16 (11)	360 (4.9)	0.001	12 (8.3)	397 (5.4)	0.130

Table 3.2: Medications according to main anatomical therapeutic chemical (ATC) group during one year before and after ED index syncope, 2009-2013

The 30-day re-presentation rate to the ED was 26.1% in admitted patients and 11.7% in discharged patients ($p<0.01$, Figure 3.2). The most common reason for ED re-presentation was for syncope (13.1% in admitted and 13.3% in discharged patients). Similarly, 8.1% of admitted patients were re-admitted and 1.1% of patients discharged were admitted to hospital by 30-days. Syncope was the most responsible reason for re-hospitalization in 21.1% of admitted and 10.3% of discharged patients. At one year, the rate of repeat presentation to the ED was 64.1% among admitted patients and 47.5% among patients who were discharged from the index ED presentation. Similarly, 1-year (re)hospitalization rates were 21.4% among admitted patients and 6.8% among patients discharged from the index ED presentation. Overall, there were a total of 5 deaths at one-year (0.04%), 3 of which were among admitted patients and 2 among discharged patients. The

causes of death in admitted patients were (1) malignant neoplasm, without specification of site, (2) pulmonary hypertension, (3) disorder of male genital organs, unspecified. Both deaths in discharged patients were attributed to motor vehicle accidents/collisions.

Figure 3.2

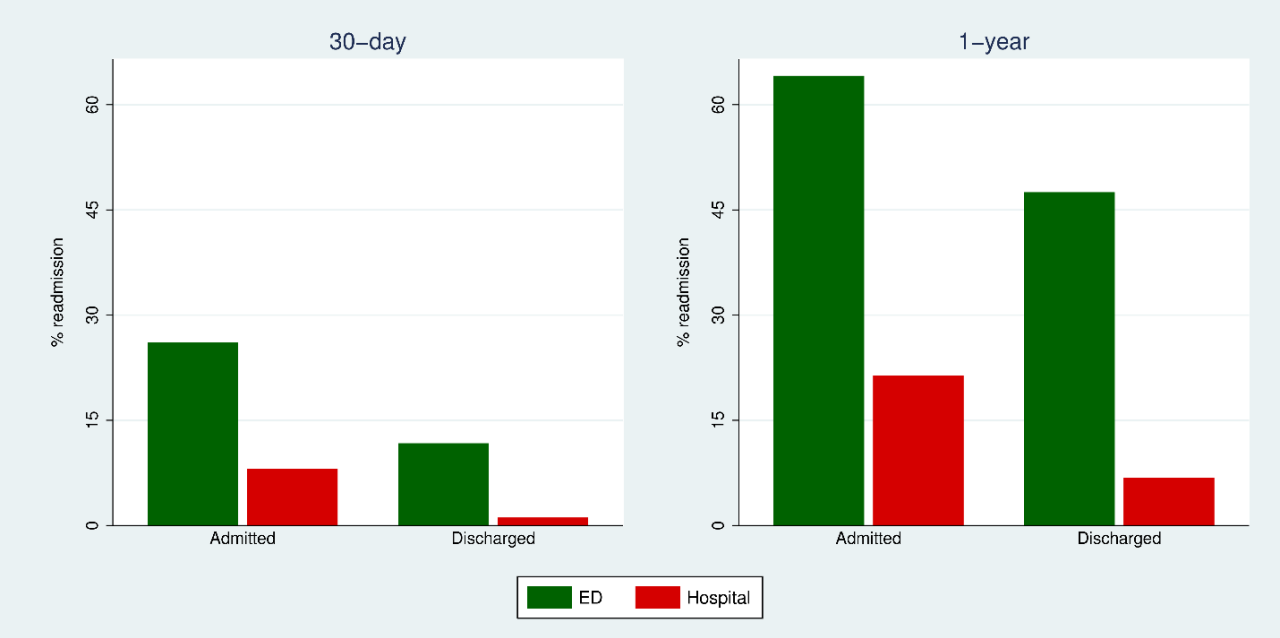


Figure 3.2: Readmission to ED and hospital at 30-days and 1-year in pediatric syncope patients according to discharge status.

3.5 Discussion

In a population-based study of 11,488 children and adolescents presenting to the ED with a primary diagnosis of syncope, we found that the population incidence rate of syncope is increasing over time. Although most patients were discharged home from the ED, 30-day and 1-year rates of repeat visits to the ED and of (re) hospitalization were substantial in both patients who were admitted as well as in those who were discharged. The rates of prescription medication claims, especially for anti-infectives, were high in admitted and discharged patients, both before and after the syncope event. Collectively, our data suggest that pediatric and adolescent syncope is an under-recognized public health problem. Since most patients either do not seek medical care, or seek outpatient assessment,[121] the incidence of syncope in the young may be higher than reported in our study.

We identified a substantial burden of comorbidities and evidence of polypharmacy in our cohort. The widespread prevalence of cardiac and thoracic disease in patients admitted to hospital

was unanticipated, as were the neurological diagnoses found in more than 10% of discharged patients. Further studies should be undertaken to better characterize our findings and explore whether these comorbidities contribute to syncope. The use of prescription medications further supports a potential link between concurrent medical illness and syncope in children. A recent pediatric study identified a similarly alarming use of prescription drugs, especially psychiatric medications, in a population with generally benign forms of syncope.[122] Despite such a high rate of re-presentation, comorbidities, prescription medication usage, the overall mortality was very low in our cohort (5 of 11,488 subjects).

Hospitalization trends for pediatric syncope differ between Canada and the United States. In a single Michigan-based hospital, Goble and colleagues report a 10% admission rate in children with syncope. In their study, almost all admissions were deemed to be unnecessary when examined retrospectively.[123] While these trends are different from our cohort, the incidence of life-threatening cardiac disease appears to be comparably low in both countries.[124] The medico-legal landscape and private model of healthcare delivery in the United States may account for a greater risk of hospitalization. Accordingly, in contrast to American and European guidelines,[108, 113] recent expert Canadian recommendations advise against routine ECGs in syncopal children.[25] The ECG probably offers little added benefit to a thorough history, and can instead lead to unnecessary admission.[123] The low rate of mortality observed in our cohort, and in previous studies[123, 125] support this minimalistic diagnostic approach. Future work directed at adapting validated tools for adult syncope[110, 125, 126] in this population may help to minimize unnecessary hospital admissions.

Disposition from the ED in children with syncope may be uniquely challenging. Of the several possible causes, life-threatening ones are rare[24] but are probably most feared. The event itself can be sudden and transient,[24] and diagnostic clues, such as a change in heart rate or rhythm, are likely to have resolved by the time of presentation. Thus, the clinician must predominantly rely on a clinical history.[127] However, syncope commonly occurs at young ages,[106, 128] which can affect the reliability of the history. Frequent head injuries may further hinder assessment. Pediatric providers may also not be as aware of guidelines on syncope, which are usually written by adult cardiologists and pertain most to an older population. In our study, ED physicians appeared to rarely admit syncopal patients, and tended to reserve hospitalization to those patients residing rurally or living with comorbidities. This approach is supported by recent Canadian guidelines which advise against hospitalization for the majority of syncopal children.[25] The higher incidence

of cardiac co-morbidities in admitted children may reflect care pathways which exclude syncopal patients with established cardiovascular abnormalities.

Several other factors observed in the present study merit discussion. Three quarters of patients meeting entry criteria resided in urban locations. By comparison, Statistics Canada reports that 83% of Albertans resided in urban environments during the 2011 fiscal year.[129] While our study did not include a control group for comparison, it appears that young patients from rural settings were managed differently compared to the general population as per Statistics Canada. While the present study cannot characterize this finding further, we hypothesize that patients living rurally may be more frequently admitted for observation and further testing, given their greater distance from medical care. The rising incidence of ED presentation for syncope in the young may also be multifactorial. It may be as a result of better access to ED facilities in the province. It could also reflect changing parental attitudes. For example, with increasing media attention on sudden death among young athletes, the recognition that syncope could be a manifestation of underlying disease in children may be more appreciated in the contemporary era. As such, parents may be more inclined to seek emergency care for their children, as opposed to visiting outpatient care on an elective basis. Another reason may be a tendency to over-investigate and/or over-treat young patients for fear of missing a potentially dangerous diagnosis. This is reflected in the literature examining anti-microbial usage in children, who appear to be over-prescribed these agents.[130] In part, this is driven by physicians' perceptions of caregiver expectations,[131] which could similarly play a role in the decision to admit and/or investigate pediatric syncope cases.

Limitations: The administrative code for syncope has not been validated for the pediatric population or the combined database used in this study. Validation of the syncope code in a Danish cohort showed that approximately one third of syncope cases are not coded in discharge diagnoses.[114] Therefore, the true incidence of syncope may be higher than reported. Our study only includes patients with a primary diagnosis of syncope which may result in fewer failures in coding. These descriptive data also lack clinical information, so we cannot report on clinical findings in the ED or on follow-up testing. The extent to which proximity to an ED influenced the likelihood of presentation and subsequent admission was not examined and requires further study. Pharmacy data was only available from the period of 2009-2012. Lastly, a validated comorbidity index does not exist for children. We did our utmost to assure that the comorbidities generated were based on

clinically relevant ICD codes. Developing and validating a pediatric comorbidity index should be a topic of future study.

3.6 Conclusions

Our data suggest that pediatric and adolescent syncope is increasing in prevalence and represents a growing public health problem. This population has a high burden of comorbidities which likely contribute to increased health care resource utilization and polypharmacy. These findings highlight the growing need for an international strategy on syncope in the young. The recent Canadian guidelines on pediatric syncope may enhance decision-making and prevent re-admissions.

PART III:

CLINICAL CHARACTERIZATIONS OF AN INHERITED ARRHYTHMIA SYNDROME IN THE YOUNG

Chapter 4: Implantable Cardioverter Defibrillator Use in Catecholaminergic Polymorphic Ventricular Tachycardia: A Systematic Review & Meta-analysis of Observational Data

4.1 Abstract

Background: The implantable cardioverter defibrillator (ICD) may be associated with a high risk of complications in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT). However, ICDs in this population have not been systematically evaluated.

Objective: To characterize the use and outcomes of ICDs for CPVT.

Methods: We conducted a systematic review using EMBASE, MEDLINE, Pubmed, and Google Scholar to identify studies that included CPVT patients with ICDs.

Results: Fifty-three studies describing 1,429 CPVT patients were included. In total, 503 (35.2%) patients had an ICD (median age was 15.0 years (IQR 11.0-21.0)). Among ICD recipients with a reported medication status, 96.7% were prescribed beta-blockers, and 13.2% flecainide. Sympathetic denervation was performed in 23.2%. Nearly half of patients received an ICD for primary prevention (47.3%), and 12.8% were prescribed optimal anti-arrhythmic therapy. During follow-up, 40.1% had ≥1 appropriate shock(s), 20.8% experienced ≥1 inappropriate shock(s), 19.6% had electrical storm, and 7 (1.4%) patients died. An ICD-associated electrical storm was implicated in 4 deaths. Additional complications, such as lead failure, endocarditis, or surgical revisions were observed in 96 of 296 patients (32.4%). Sub-analysis of the 10 studies encompassing 330 patients with the most detailed ICD-related data showed similar trends.

Conclusions: In this CPVT population, ICDs were common, and associated with a high burden of shocks and complications. The reliance on primary prevention ICDs, and poor uptake of adjuvant anti-arrhythmic therapies, suggests that improved adherence to guideline-directed management could reduce ICD utilization and harm.

4.2 Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a channelopathy defined by stress-induced arrhythmias.[53] Beta-blocker administration is a universal recommendation (Class I),[53] and flecainide (Class IIa) and left cardiac sympathetic denervation (LCSD) (Class IIb) are often needed for this life-threatening condition.[53, 132] Implantable cardioverter defibrillators (ICD) are reserved for cardiac arrest survivors and those who fail to respond to maximal medical therapy.[53] Life-threatening events remain common in the contemporary era, which may account for the high utilization of ICDs for CPVT.[58]

Although the ICD seems like a logical step in the management of a potentially-lethal cardiac condition, nuances that are unique to CPVT make their utilization controversial, which include: (1) painful, adrenaline-provoking shocks are potentially pro-arrhythmic in CPVT,[58, 59] (2) complex atrial and ventricular arrhythmias (characteristic of CPVT) trigger inappropriate shocks,[59] (3) excessive ICD-related harms occur in the setting of inherited cardiac conditions,[133] and (4) the typically young age of CPVT onset[58] accentuates lifetime risk of device complications. These detractors need to be weighed against the potentially life-saving benefits of ICDs in young, otherwise healthy patients and the lack of alternative options for rescue therapy. In CPVT, the frequent use of primary prevention ICDs,[58] and geographic trends in implantation,[58, 134, 135] suggest that data around ICD decision-making are lacking. We undertook a systematic review to better understand the use and outcomes of ICDs for CPVT.

4.3 Methods

Search Strategy: We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Appendix D - supplemental data).[136] We searched EMBASE, MEDLINE, Pubmed, Google Scholar, and bibliographies of major reports in the field published up until May 2017. Retrospective or prospective cohort, case control, and cross-sectional human studies in the English language were included. A primary literature review was first performed by two authors (T.M.R. and F.P.) through Pubmed and MEDLINE scientific databases using the following terms: catecholaminergic polymorphic ventricular tachycardia, CPVT, catecholaminergic polymorphic ventricular tachycardia + ICD, catecholaminergic polymorphic ventricular tachycardia + defibrillator, catecholaminergic polymorphic ventricular tachycardia + implantable cardioverter defibrillator, CPVT + ICD, CPVT + defibrillator, and CPVT +

implantable cardioverter defibrillator. To expand the number of eligible studies, we performed a secondary search (author K.J.) using EMBASE, MEDLINE, and Google Scholar, using the following combination of terms: CPVT + ICD, catecholaminergic + ICD, CPVT + defibrillator, catecholaminergic + defibrillator. Search terms were determined by literature review, database query and consensus among the authors. Two authors (T.M.R. and K.J.) verified that each publication met inclusion criteria, with reconciliation through discussion. Studies included in the main analysis needed to report the following: (1) basic demographic data, (2) at least two CPVT patients, and (3) at least one patient who had received an ICD. Data presented solely in abstract form were not considered, nor were case reports describing a single patient. During this process, we identified several academic centers that published overlapping cohorts.[134, 135, 137-142] To minimize this source of bias, we made every effort to avoid analyzing one cohort multiple times by using the following protocol: (1) The authors/affiliations of manuscripts were reviewed to identify papers with potentially overlapping cohorts; (2) If overlap between cohorts was suspected based on this screening, we then contacted the senior author on each manuscript in question to ask if patient overlap existed across more than one paper; (3) If the senior author confirmed that overlap among cohorts existed, we only included the paper with the largest number of patients. If the cohort sizes were similar, we preferentially selected the paper with more detailed ICD data; (4) In the event that a senior author could assist in excluding individual subjects who appeared in more than one paper, we still included both papers, removing any duplicated patients. When there was uncertainty at any of these steps, or the number of potentially overlapping patients was felt to be a small minority within a larger cohort, we erred on being inclusive, given this is a known limitation of systematic review and meta-analysis.[143] At the conclusion of this process, 53 of 80 unique papers identified in the systematic search were analyzed (Figure 4.1). The supplemental data (Appendix D) lists all studies from the primary analysis. We also undertook a sub-analysis of studies reporting detailed ICD-related data, and of those published after the seminal reports of contemporary CPVT therapy (LCSD and flecainide).

Figure 4.1

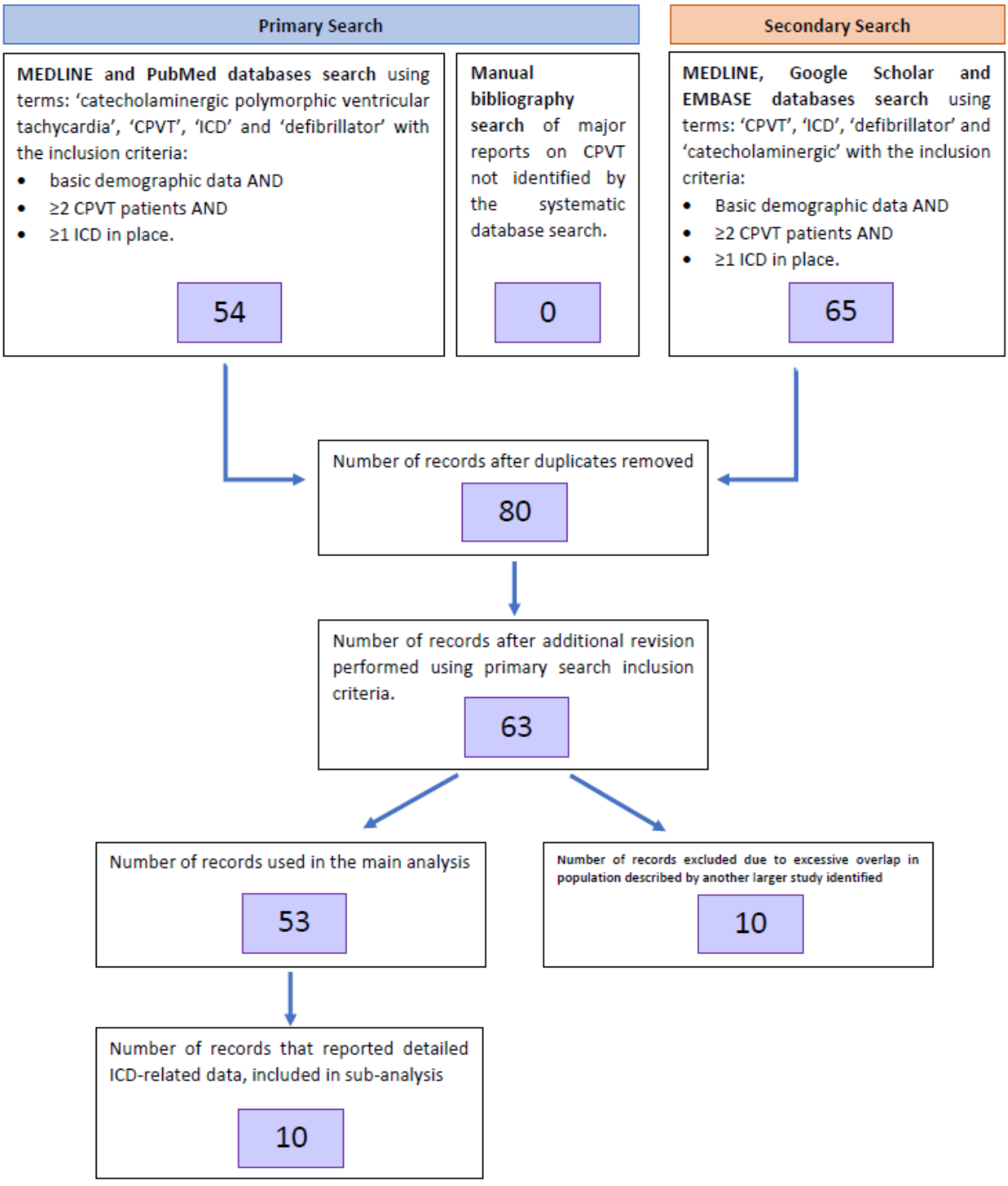


Figure 4.1. Summary of systematic search strategy and yield.

Data Collection, Analysis, and Definitions: Collected variables were determined *a priori*, expanded after pilot review of initial studies, and recorded and analyzed using Microsoft Excel (Microsoft Corporation, 2013). Data on ICDs were extracted from eligible studies, which included the number of ICD recipients, frequency of life-threatening events (ventricular tachycardia (VT), syncope, sudden cardiac arrest (SCA), and sudden death), number of shocks, appropriateness of shock(s), administration of anti-arrhythmic therapies, and outcomes. If the ICD was inserted in the absence of a prior SCA, the indication was classified as primary prevention. Based on extrapolation from the HRS/EHRA/APHRS expert guideline document for inherited arrhythmias and recent randomized trial data,[53, 144] optimal anti-arrhythmic therapy (OAT) was defined as a beta-blocker and flecainide as minimum therapy. To adjust for contemporary advancements in OAT, a sub-analysis of studies published after the seminal descriptions of LCSD[138] in 2008 and flecainide[132] in 2011 were undertaken. For both therapies, we conducted the sub-analysis on studies published in the calendar year following the release of the seminal report (calendar year 2009 inclusive and onwards for LCSD and calendar year 2012 inclusive and onwards for flecainide). We also undertook a sub-analysis of studies reporting detailed ICD-related outcomes, defined as publications describing: (1) ≥10 patients with ICDs; (2) device indication(s); (3) number of appropriate shocks; (4) number of inappropriate shocks and (5) mortality for each patient. One large study on LCSD included a substantial number of patients with ICDs, but data on pharmacologic therapies specific to each ICD recipient could not be extracted.[140] Therefore, this population was excluded from the pharmacologic outcomes data. Ages are reported as medians (interquartile range). Age at the time of ICD insertion was recorded; if unavailable, the listed age of each subject was used (typically age at diagnosis or symptom-onset).

4.4 Results

Population: The search identified 53 publications describing 1,429 CPVT patients. In this population, 503 (35.2%) underwent ICD implantation and 101 of 181 ICD recipients were probands (55.8%) when family history was reported. The median age of the ICD population was 15.0 years (IQR 11.0-21.0). Females predominated (56.1%), and nearly half of patients received primary prevention implants (47.3%). Fifteen studies (28.3%) included ≥10 patients, only 10 of which reported detailed ICD outcomes (Table 4.1). For comparison, the clinical characteristics and outcomes of treated patients without ICDs in this population are reported in Table 4.2.

Table 4.1

Study	Design	Recruitment period (yrs)	Inclusion criteria	Total N	Number with ICD n (%)	Primary Prevention (%)	≥1 appropriate shock (%)	≥1 in-appropriate shock (%)	Electrical storm (%)	Other complications (%)	OAT (%)	Death despite ICD (%)
Hayashi 2009[135]	Retro	NR	Clinical and/or genetic CPVT	101±	16 (16)	12 (75)	4 (25)	6 (38)	NR	2 (13)	0	0
Van der Werf 2011[132]	Retro	2009-2010	Clinical and genetic CPVT and received flecainide	33	12 (36)	11 (92)	1 (8)	1 (8)	1 (8)	NR	10 (83)	0
Sy 2011[145]	Retro	NR	Clinical and/or genetic CPVT	27	15 (56)	6 (40)	3 (20)	6 (40)	1 (1)	NR	4 (27)	2 (13)
Roses-Noguer 2012[146]	Retro	NR	Clinical and/or genetic CPVT with ICD	13	13 (100)	6 (46)	8 (62)	5 (38)	NR	12 (92)	7 (54)	0
Miyake 2013[59]	Retro	1999-2001	Clinical and/or genetic CPVT with ICD and symptom onset <21 yrs	24	24 (100)	8 (33)	10 (42)	11 (46)	4 (17)	8 (33)	0*	0
Kozlovski 2014[147]	Retro	NR	Clinical and/or genetic CPVT	35	27 (77)	10 (37)	11 (41)	4 (15)	NR	NR	NR	0
Roston 2015[58]	Retro	2012-2014	Clinical and/or genetic CPVT and symptom onset <19 yrs	226¥	118 (54)	51 (43)	55 (47)	21 (17)	21 (18)	28 (23)	NR	3 (2)
De Ferrari 2015[140]	Retro	1988-2014	Clinical and/or genetic CPVT	63Ω	37 (60)	23 (62)	23 (62)	7 (19)	12 (32)	17 (46)	NR	1 (3)
Wanguemert 2015[148]	Retro	1994-2015	Carriers of *RYR2* p.G357S mutation	182	40 (22)	24 (60)	3 (10)	1 (3)	NR	NR	0	0
Broendberg 2017[149]	Retro	Up to June 2016	*RYR2* genotype positive CPVT	51	28 (55)	7 (14)	8 (16)	4 (8)	NR	9 (18)	NR	0
TOTAL±				**750**	**330 (44)**	**158 (48)**	**126 (38)**	**66 (20)**	**39 (19)**	**76 (32)**	**21 (20)**	**6 (2)**

Table 4.1: Summary of patients who received an ICD in the 10 studies with detailed outcomes

±Two subjects excluded who were in another cohort (analysis undertaken on 99 patients)
¥Eight subjects excluded who were in another cohort (analysis undertaken on 218 patients)
ΩOne subject excluded who was in another cohort (analysis undertaken on 62 patients)
*pre-ICD
NR=not reported/able to extrapolate data on ICD patients; OAT=optimal anti-arrhythmic therapy; ICD=implantable cardioverter defibrillator

Table 4.2

Study	Design	Recruitment period (yrs)	Inclusion criteria	Total N	Number without ICD (%)	VT, syncope, seizure or cardiac arrest before treatment (%)	VT, syncope, seizure or cardiac arrest after treatment (%)	Death on Treatment without ICD (%)
Hayashi 2009[135]	Retro	NR	Clinical and/or genetic CPVT	101±	83	N/A	18	1 (1)
Van der Werf 2011[132]	Retro	2009-2010	Clinical and genetic CPVT and received flecainide	33	21 (64)	10 (48)	10 (48)	0
Sy 2011[145]	Retro	NR	Clinical and/or genetic CPVT	27	12 (44)	8 (67)	0	0
Roses-Noguer 2012[146]	Retro	NR	Clinical and/or genetic CPVT with ICD	13	0	0	0	0
Miyake 2013[59]	Retro	1999-2001	Clinical and/or genetic CPVT with ICD and symptom onset <21 yrs	24	0	0	0	0
Kozlovski 2014[147]	Retro	NR	Clinical and/or genetic CPVT	35	8 (33)	NR	NR	0
Roston 2015[58]	Retro	2012-2014	Clinical and/or genetic CPVT and symptom onset <19 yrs	226¥	98 (46)	NR	NR	2 (2)
De Ferrari 2015[140]	Retro	1988-2014	Clinical and/or genetic CPVT	63Ω	25 (40)	21# (84)	11 (44)	0
Wanguemert 2015[148]	Retro	1994-2015	Carriers of *RYR2* p.G357S mutation	182	142 (78)	NR	NR	3α (2)
Broendberg 2017[149]	Retro	Up to June 2016	RyR2 genotype positive CPVT	51	23 (45)	NR	NR	3α (13)
TOTAL±				**750**	**412 (56)**	**39 (68)**	**39 (68)**	**9 (2)**

Table 4.2: Summary of patients who did not receive an ICD in the 10 studies with detailed outcomes

±Two subjects excluded who were in another cohort (analysis undertaken on 99 patients)
¥Eight subjects excluded who were in another cohort (analysis undertaken on 218 patients)
ΩOne subject excluded who was in another cohort (analysis undertaken on 62 patients)
*pre-ICD
#prior to LCSD treatment
α Includes only patients with follow-up after diagnosis
NR=not reported/able to extrapolate data on ICD patients; OAT=optimal anti-arrhythmic therapy; ICD=implantable cardioverter defibrillator

Anti-arrhythmic Therapies: Treatment status could be determined for 271 of 503 ICD recipients (53.9%). Beta-blockers were prescribed in 235 (96.7%), flecainide in 31 (13.2%) and LCSD in 63 (23.2%) patients. Amongst studies reporting medication usage, 13.6% of ICD patients were prescribed OAT. In studies from 2012 onwards, 15 of 200 ICD recipients (7.5%) were taking flecainide. After the seminal description of LCSD for CPVT in 2008, 53 of 260 subjects (20.4%) had LCSD, although this was largely driven by a single study with 37 ICD recipients.[140] These 37 patients were excluded from OAT analysis, as concurrent drug usage could not be determined. Figure 4.2 summarizes the studies with detailed ICD-outcomes.

Figure 4.2

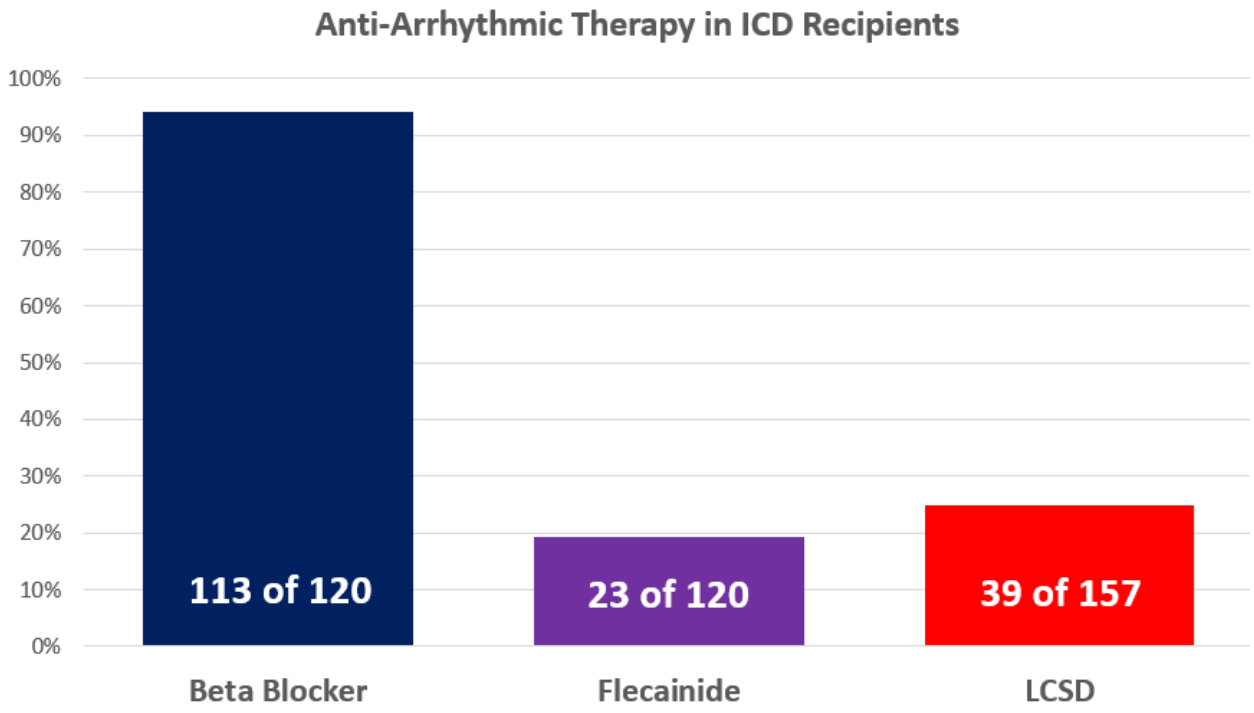


Figure 4.2: Types of anti-arrhythmic drug therapy used in patients who received an ICD in 6 of 10 studies with detailed ICD data. In the remaining 4 studies, data on anti-arrhythmic drug therapy could not be extracted. An additional study was only included in the LCSD category (7 studies) as no anti-arrhythmic drug therapy could be ascertained.

Outcomes: During follow-up, 178 of 444 patients (40.1%) received ≥1 appropriate shock(s), and 84 of 402 (20.8%) suffered ≥1 inappropriate shock(s). Electrical storm occurred in 49 of 250 patients (19.6%). ICD-related complications, such as lead failure, endocarditis, or surgical revisions were observed in 96 of 396 patients (32.4%). Eighty-nine percent of patients with appropriate shock(s) were not prescribed OAT. In the setting of appropriate shock(s), 35.6% of patients reported after the year 2008 underwent LCSD, although this was largely driven by a single study on LCSD,[140] and 28.0% reported after the year 2011 received flecainide. Among 434 patients with mortality data, there were 7 deaths (1.4%) despite ICD placement, 4 of which were associated with electrical storm. The mortality of treated patients without ICDs was similar (Table 4.2). Statistical comparisons between anti-arrhythmic therapies with respect to shock appropriateness were not undertaken given the large amount of missing data in most reports and the heterogenous study designs. Supplemental tables 4.1, 4.2, and 4.3 provide raw data on shock appropriateness for each guideline-based therapy.

Inciting Arrhythmia and Shock Efficacy: Two series included details regarding the appropriateness of a shock by inciting arrhythmia and shock outcome (Table 4.3).[59, 146] Shocks for VT almost universally failed (71 of 72; 98.6%) despite being appropriate. Conversely, shocks that occurred for ventricular fibrillation (VF) were almost always successful (66 of 70; 94.3%). Among patients with shocks, 89 of the 227 discharges were deemed inappropriate (39.0%). These frequently occurred in the setting of atrial arrhythmias (45%), noise/T wave oversensing (27%) and self-aborted VT/VF (26%). Only one of the two studies outlined the device programming used in each patient.[146] The median combined follow-up from ICD implantation in these two studies was 4.0 years (IQR 2.4-7.1).

Table 4.3

Study	Number of patients	Median duration of follow-up (years)	Number of patients with shocks (%)	Number of appropriate shocks (% of total shocks)	Number of appropriate shocks for VT (% successful)	Number of appropriate shocks for VF (% successful)	Number of Inappropriate Shocks (% of total shocks)	Number of Inappropriate shocks for atrial arrhythmias (% of inappropriate shocks)	Number of Inappropriate shocks for self-resolved ventricular arrhythmia (% of inappropriate shocks)	Number of patients with both inappropriate and appropriate shocks	Number of Inappropriate shocks for noise/T wave oversensing (% of inappropriate shocks)
Roses-Noguer 2013[146]	13	4.0 (range 1.7-19.9)	10 (77)	63 (72)	40 (3)	23 (79)	24 (28)	16 (67)	1 (4)	NR	7 (29)
Miyake 2013[59]	24	3.3 (IQR 1.1-5.8)	14 (58)	75 (54)	32 (0)	43 (100)	65 (46)	24 (36)	22 (34)	7 (29)	17 (26)
TOTAL	**37**	**4.0 (IQR 2.4-7.1)**	**24 (65)**	**138 (61)**	**72 (1)**	**66 (94)**	**89 (39)**	**40 (45)**	**23 (26)**		**24 (27)**

Table 4.3: Summary of the two studies reporting shock appropriateness and efficacy by inciting arrhythmia. NR=not reported, VT=ventricular tachycardia

Subcutaneous ICD: Three patients had fully subcutaneous ICDs (S-ICD).[146] One was needed after infective endocarditis of a transvenous system. In two S-ICDs, inappropriate shocks related to T-wave oversensing occurred, and one required revision after wound dehiscence. Six of the 7 inappropriate shocks (85.7%) due to T-wave oversensing were in S-ICDs.

4.5 Discussion

CPVT is a life-threatening syndrome characterized by an early-onset of exercise or emotional stress-induced VT and VF.[53] Side effects, non-adherence, under-dosing, and breakthrough events on beta-blockers complicate management in many patients.[58] Additionally, in CPVT, persistent ventricular bigeminy, couplets or tachycardia on exercise testing is a risk factor for events.[142] These factors, coupled with medico-legal concerns and the possibility of a tragic outcome, likely

motivates liberal ICD utilization. Although ICD implantation may seem like a logical intervention for a life-threatening and incurable arrhythmic condition, ICDs have never undergone rigorous study in the setting of CPVT.

In over five-hundred ICD recipients ascertained from the primary literature, we show that ICDs result in frequent shocks, electrical storm and device complications, especially in the setting of beta-blocker monotherapy, which was almost universally prescribed. In 4 patients, electrical storm precipitated incessant VT, and lethal VF. In at least one case, the initiating shock was inappropriate, indicating that the ICD was directly implicated in the death.[145] Another publication showed that appropriate shocks for VT could precipitate more malignant, and potentially fatal arrhythmias in many patients.[59] Several observations should be considered when weighing the risks and benefits of an ICD: 1 in 5 patients will have electrical storm, 1 in 5 patients will have inappropriate shocks, 1 in 3 patients will have device-related complications (which usually required surgical revisions) and death can occur despite having an ICD in situ. Additionally, while duration of follow-up could not be reliably determined for the population, the study with the longest known ICD follow-up was 6 years,[145] and the median age of the entire population was only 15 years. As such, the life-time risk of at least one adverse ICD-related event is undoubtedly higher than estimated in the present study. These factors must be counter-balanced with the finding that nearly half of ICD patients have at ≥1 appropriate discharge over the same period. However, not all appropriate shocks indicate that a life-threatening event has been prevented, since arrhythmias may be terminated spontaneously or by anti-tachycardia pacing.[150, 151] Also, while the mortality was similar between ICD and non-ICD recipients (Table 4.2), this observation is likely biased by the fact that ICDs are more likely to be recommended to severely affected patients. In the existing literature, it was not possible to determine whether the high inappropriate shock burden was mainly confined to the very symptomatic group who also had appropriate shocks. If this were the case, then some inappropriate shocks may be acceptable in patients who receive frequent appropriate shocks, since they may derive a net benefit.

In 2008, Wilde et al. reported LCSD for CPVT,[138] and in 2011, van der Werf and colleagues showed that flecainide reduced arrhythmias in beta-blocker refractory CPVT patients.[132] Theoretically, these advances should have reduced ICD utilization and improved outcomes.[140, 144] However, a sub-analysis of studies published after the initial report of flecainide suggests that OAT remained scarcely prescribed in the ICD population. While some delay in knowledge translation

is understandable, it is alarming that less than 1 in 3 appropriately shocked patients were on flecainide. Now that flecainide is supported by randomized data,[144] it should be used routinely before ICD implantation. The uptake of LCSD was slightly better in the contemporary era, however this was largely driven by one study which exclusively enrolled LCSD patients.[140] For those with a bona fide ICD indication, concurrent use of LCSD and flecainide may improve ICD-specific outcomes. We emphasize that both therapies appear to be safer, less invasive, and supported by more robust evidence compared to the ICD.

Device programming has an important role in CPVT.[59, 146] Miyake et al. and Roses-Noguer et al. showed that defibrillation is ineffective for VT (99% shock failure), but usually successful for VF (94% shock effectiveness). In CPVT, programming deferred shocks and setting a single VF zone to a shorter cycle length should allow for episodes to self-terminate, and may decrease reconfirmation intervals, leading to fewer unsuccessful shocks for VT.[59] Given the frequent complications from transvenous leads,[152] the S-ICD may be an alternative. However, a potential limitation of the S-ICD may be inappropriate shocks triggered by T-wave oversensing.[153] The programming template used in S-ICDs may also not be optimal for CPVT. A randomized trial on the S-ICD is ongoing, which may clarify their role in inherited arrhythmias (ClinicalTrials.gov/NCT02881255).

The main limitation of this study is the lack of ICD data and follow-up periods reported in most publications. No prospective studies on the ICD in CPVT exist, and only a few studies provided robust ICD-related data on a larger number of patients.[59, 146] Data were aggregated from retrospective, observational studies, as no randomized trials on CPVT existed during the inclusion period. Formal meta-analysis would have limited validity, noting the absence of controls, heterogeneity in study inclusion criteria, and uncertain follow-up durations. We attempted to summarize the studies with the most complete datasets separately (Tables 4.1, 4.2 and 4.3). Missing data may not have occurred at random. For example, the absence of complications may be under-reported, which over-emphasizes ICD risk. This is an established limitation of a systematic review. Nearly half of CPVT patients receive primary prevention ICDs, which is typically not recommended. Thus, the risk-benefit ratio may be more favorable in those receiving secondary prevention devices. For the sub-analyses of studies published in the contemporary treatment era, some of the patients may have been last followed prior to these time periods. Therefore, uptake of OAT may be higher in patients presently diagnosed and treated. Based on

existing data, we could not reach conclusions about the outcomes of ICDs in the setting of OAT, nor was it known whether target doses were achieved. A prospective, preferably randomized study of OAT versus OAT plus ICD for SCA survivors would help to address this question.

4.6 Conclusions

This systematic review reports concerning trends in the indications, outcomes and uptake of OAT for ICD recipients with CPVT. This population has a high burden of shocks, and a 20% chance of electrical storm. Knowledge translation appears to be delayed for CPVT therapies, and ICD outcomes in patients receiving OAT remains unknown. These findings suggest that better adherence to guideline-directed management may theoretically improve outcomes. The role of the ICD for CPVT in the contemporary era remains unclear.

Chapter 5: Chronotropic Incompetence as a Risk Predictor in Children and Young Adults with Catecholaminergic Polymorphic Ventricular Tachycardia

5.1 Abstract

Introduction: Risk stratification tools for catecholaminergic polymorphic ventricular tachycardia (CPVT) are limited. The exercise stress test (EST) is the most important diagnostic and prognostic test. We aimed to determine whether heart rate (HR) and blood pressure (BP) response during EST were associated with risk of arrhythmias.

Methods: We studied the association between HR and BP response and ventricular arrhythmia burden on EST in 20 CPVT patients. HR reserve values < 80% and ≤62% were used to define chronotropic incompetence (CI) off and on therapy respectively. Symptoms and ventricular arrhythmia score (VAS) in all patients with respect to chronotropic incompetence (CI) and BP during index EST off therapy and on maximal therapy were compared.

*Results***:** CI in CPVT patients off therapy was associated with a worse VAS during EST (p =0.046). Patients with CI also more frequently presented with syncope and/or cardiac arrest compared to patients with a normal chronotropic response (p=0.008). Once on therapy, patients with CI had similar VAS compared to patients without CI (p=0.50), suggesting that treatment attenuates risk related to CI. Patients with CI also had a lower peak systolic BP (p=0.041) which persisted on maximal therapy (p=0.033).

*Conclusion***:** Untreated CPVT patients with CI have more ventricular arrhythmias than those without CI. This may serve as a simple disease prognosticator that can be modified by anti-arrhythmic therapy. A mechanistic link between CI and arrhythmia susceptibility remains unknown. Larger studies are needed to confirm and establish the mechanism of these findings.

5.2 Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a potentially lethal heritable channelopathy defined by increasing ventricular ectopy (VE) during exercise stress testing (EST) in the absence of structural heart disease.[54] Early data suggested that CPVT had an unfavorable natural history with nearly 80% of affected patients suffering a life-threatening cardiac event by 40 years of age, and treatment failures and device complications remain common.[58, 154, 155] In the current era of cascade genetic screening, there is a growing population of phenotypically silent CPVT patients.[134, 145, 156] This observation creates additional dilemmas as optimal management of these patients is unknown, and risk stratification tools are dangerously lacking. The EST provides data on VE burden which is a predictor of subsequent cardiac events.[135] Chronotropic incompetence (CI), or an attenuated heart rate (HR) response during exercise, is an established prognosticator of arrhythmias[157, 158] and cardiac death[159-161] in other conditions. Sinus node dysfunction, which is frequently present in CPVT,[162] is a common cause of CI. We therefore hypothesized that CI during exercise may predict a greater arrhythmic burden in CPVT patients.

5.3 Methods

Study population: This is a retrospective study of young patients (≤ 21 years old at first EST) with a diagnosis of CPVT made by expert consensus criteria.[53] The proband was defined as the first identified case of CPVT in a family. Eligible patients were those entered in the Pediatric and Congenital Electrophysiology Society (PACES) CPVT Registry from either British Columbia Children's Hospital or the Monroe Carell Jr. Children's Hospital at Vanderbilt. Only patients from one of these two tertiary centers were eligible because an additional, more in-depth collection of EST-specific data were needed for this study, as these details were not fully captured in the general Pediatric CPVT Registry population. All centers obtained ethical approval from their respective institutional review boards.

Exercise Stress Testing: CPVT patients underwent ESTs using standard exercise protocols with continuous electrocardiographic monitoring. HR and BP were obtained at rest and at the end of each stage of exercise. Two ESTs were analyzed for each patient whenever possible (total n=14 ESTs off therapy and n=17 ESTs on maximal therapy included in analyses); the index EST which was the first EST performed in a subject off therapy and the EST at maximal therapy (defined as the maximum tolerated dose of therapy (beta-blocker and/or adjunctive therapy) that was

administered to suppress arrhythmia). HR and BP were recorded at regular intervals throughout the EST. We recorded maximal workload achieved (in metabolic equivalents (METS) and total exercise time (in seconds)). Ventricular arrhythmia score (VAS) was determined from ESTs using an established grading scale in CPVT as previously described (no VE=0, isolated ventricular premature beats (VPBs)=1, bigeminy=2, couplets=3 and non-sustained ventricular tachycardia (NSVT)=4).[132]

Calculation of Heart Rate Reserve: CI was defined as a %HR reserve of <80%.[161] HR reserve is the difference between maximal age-predicted HR (MA-PHR) which is calculated as 220- age (in years) and HR at rest. The following equation was used: %HR reserve = ([peak HR – rest HR]/[MA-PHR – rest HR]) x 100. For patients on beta-blockers, CI was defined as %HR reserve of ≤62% as previously reported in a large cohort of patients taking beta-blockers with normal ECGs at rest referred for symptom-limited exercise testing.[163]

Statistical Analysis: For descriptive purposes, patients were initially divided into two groups according to the presence or absence of CI. Results of quantitative variables are presented as mean ± standard error of the mean (SEM). Qualitative variables are presented as absolute values and percentages. Comparison of qualitative characteristics was performed using the Chi square or Fisher's exact test. Quantitative variables were compared using the student's t-test for independent samples and one-way ANOVA with Tukey post hoc comparisons. A two-tailed $p<0.05$ was considered significant. Statistical analysis was performed using Graph Pad 7.0 (Graph Pad Software, San Diego, CA, USA).

5.4 Results

The baseline demographic and EST characteristics of the 20 eligible CPVT patients are summarized in Table 5.1. The median age at first EST was 12 years (range 6-21). Nine of 20 subjects (45%) were male and 14 (70%) were probands. The most severe symptoms reported were syncope in 6 patients (30%) and sudden cardiac arrest (SCA) in 8 patients (40%), while the remaining 6 (30%) were asymptomatic and ascertained through cascade family screening. Genetic testing was performed in 19 of 20 patients. A mutation in *RYR2* was found in 18 of 19 subjects (95%), including one treatment-refractory patient who was also found to be homozygous for a *CASQ2* mutation (Subject 1). All but three asymptomatic patients (Subject 5, 8 and 12) were prescribed beta blockers. There were 12 patients (60%) who required ancillary treatments which

included flecainide in 8 (40%), implantable cardioverter defibrillator in 9 (45%) and left cardiac sympathectomy in 3 patients (15%; mean 6.7 ± 4 years after initial cardiac evaluation). Demographic and treatment characteristics of the study population are summarized in Table 5.1.

Table 5.1

Subject	Sex	Case Status	Age at first EST	Presenting symptoms	Worst VE on EST	Worst symptom	Genetic Mutation	Therapies
1	M	Proband	7	Syncope	PMVT	SCA	RYR2 M1107T (heterozygous) & CASQ2 IVS5+1G>C (homozygous)	Nadolol, flecainide, ganglionectomy, defibrillator
2	F	Proband	12	Syncope	PMVT	Syncope	RYR2 E4183Q	Nadolol
3	F	Proband	8	Syncope	PMVT	Syncope	RYR2 L2200F	Nadolol, verapamil, flecainide, defibrillator
4	M	Relative	12	Asymptomatic, referred for family screening	Ventricular bigeminy	Asymptomatic	RYR2 R122H	Nadolol
5	F	Relative	12	Asymptomatic, referred for family screening	None	Asymptomatic	RYR2 R420W	None
6	M	Relative	10	Asymptomatic, referred for family screening	Frequent VPBs	Asymptomatic	RYR2 R420W	Nadolol switched to Bisoprolol (intolerance)
7	F	Relative	8	Asymptomatic, referred for family screening	Frequent VPBs	Asymptomatic	RYR2 R420W	Nadolol
8	M	Relative	6	Asymptomatic, referred for family screening	Frequent VPBs	Asymptomatic	RYR2 R420W	None
9	F	Proband	11	Asymptomatic, screening prior to psychiatric medications	Frequent VPBs	SCA	RYR2 I4855M	Carvedilol, defibrillator
10	M	Proband	11	Syncope	PMVT	Syncope	RYR2 V4771I	Nadolol switched to bisoprolol (intolerance), flecainide
11	M	Proband	17	Syncope	PMVT	Syncope	RYR2 R15P	Nadolol
12	M	Relative	21	Asymptomatic, referred for	Frequent VPBs	Asymptomatic	RYR2 R15P	None

				family screening				
13	M	Proband	11	SCA	Ventricular couplets	SCA	No genetic testing performed	Nadolol, flecainide, defibrillator
14	F	Proband	15	SCA	Ventricular bigeminy	SCA	RYR2 V4125F	Nadolol, flecainide, sympathectomy, defibrillator
15	F	Proband	15	SCA	Ventricular couplets	SCA	RYR2 L1894F	Nadolol, defibrillator
16	F	Proband	14	Syncope	PMVT	Syncope	RYR2 K4751Q	Nadolol, flecainide
17	F	Proband	6	Syncope	PMVT	SCA	RYR2 A4091T	Nadolol, flecainide, sympathectomy, defibrillator
18	F	Proband	6	Syncope	Ventricular bigeminy	Syncope	RYR2 R4201H	Nadolol, flecainide
19	M	Proband	14	SCA	PMVT	SCA	No RYR2 variants identified	Nadolol, defibrillator
20	F	Proband	15	SCA	PMVT	SCA	RYR2 E2314K	Nadolol, flecainide, defibrillator

Table 5.1: Characteristics of CPVT patients included in the pilot study

CASQ2 = calsequestrin-2, EST = Exercise stress test, PMVT = polymorphic ventricular tachycardia, RYR2 = Ryanodine receptor-2, SCA = sudden cardiac arrest, VE = ventricular ectopy, VPB = ventricular premature beat.

Baseline and exercise characteristics of patients off therapy (Subjects 2-12, 17, 19-20) according to the presence or absence of CI are listed in Table 5.2. Off therapy, the most common reasons for stopping the EST in the CI group was fatigue (40%), cardiac (arrhythmia, bidirectional VT) in 40% and target heart rate met (20%). Those with normal chronotropy stopped the EST due to fatigue (67%), cardiac (ventricular tachycardia, dizziness) in 22% and target heart rate met (11%). Five out of 14 patients (36%) had CI (HR Reserve <80%) off therapy (Subjects 3, 9, 10, 17 and 19). Patients with CI had a worse VAS as compared to those with normal chronotropy (3.4 ± 0.6 vs 1.4 ± 0.6; p = 0.046). Those with CI had lower resting diastolic BP (65 ± 3 vs 74 ± 2; p =0.045, lower peak systolic BP (133 ± 10 vs 168 ± 10; p =0.041) and lower delta systolic BP (peak systolic BP minus resting systolic BP; 24 ± 8 vs 59 ± 11; p =0.047). There was no association between CI and age, HR at rest and systolic BP at rest off therapy (p > 0.05).

Table 5.2

Variable		HR Reserve <80% (n=5)	HR Reserve ≥ 80% (n=9)	p-value
age		9.8 ± 1.4	13.2 ± 1.2	0.10
Exercise duration (sec)		361.8 ± 98.3	754.9 ± 62.6	0.0041
METS achieved		4.9 ± 1.3	13.4 ± 1.4	0.0018
HR at rest (beats/min)		69.4 ± 3.9	73.6 ± 5.1	0.59
HR at peak exercise (beats/min)		161.2 ± 5.4	197.8 ± 3.3	<0.0001
Blood Pressure (mm Hg)				
	Systolic Blood Pressure at rest	109.0 ± 3.3	110.2 ± 4.3	0.85
	Diastolic Blood Pressure at rest	65.0 ± 2.9	73.2 ± 2.2	0.045
	Peak Systolic Blood Pressure	133.4 ± 9.7	169.4 ± 10.3	0.041
	Delta Systolic Blood Pressure (peak-rest)	24.4 ± 7.9	59.2 ± 10.7	0.047
Ventricular Arrhythmia Score		3.4 ± 0.6	1.6 ± 0.5	0.046

Table 5.2: Baseline and exercise characteristics of subjects off medication based on percent heart rate reserve (index EST). HR = heart rate, METS = metabolic equivalents

Baseline and exercise characteristics of patients on maximal therapy according to the presence or absence of CI are listed in Table 5.3. Ten out of 17 patients (59%) had CI (HR Reserve ≤62%) on maximal therapy (Subjects 1, 2, 3, 10, 14-19). On maximal therapy, the most common reasons for stopping the EST in the CI group was due to fatigue (80%), shortness of breath (10%) and anxiety (10%). Similarly, those with normal chronotropy generally stopped the EST due to fatigue (86%) followed by chest pain and shortness of breath (14%). Importantly, once CPVT was recognized and treated, the follow-up EST was always symptom/arrhythmia limited, and not stopped due to target HR being met, which was a rare cause for discontinuation on initial diagnostic EST off-therapy. There was no difference in ventricular arrhythmia score between those with CI and those with normal chronotropy on maximal therapy (p=0.50). After maximal therapy, the chronotropic status of 64% remained unchanged, 18% developed CI and 18% of patients developed normal chronotropy. CPVT patients with CI on maximal therapy continued to have lower peak systolic BP (126 ± 6.2 vs 151.4 ± 9.5; p=0.033) compared to patients without CI. There was no association between age, exercise duration, METS achieved, resting HR, resting systolic BP resting diastolic BP and delta systolic BP, and CI in CPVT patients on maximal therapy. All 10 patients on maximal therapy with CI (100%) were probands and symptomatic at presentation (syncope or sudden cardiac arrest) as compared to 43% of CPVT patients with a normal

chronotropic response (p=0.008). This difference is less likely a medication effect since no difference in dosage of beta blocker therapy was observed between those with CI versus normal chronotropy (CI: 69.8 ± 13.3 mg nadolol vs normal chronotropy: 40 ± 8.2 mg nadolol; p = 0.2) nor was beta blocker dosage different between those with normal chronotropy who were symptomatic versus asymptomatic at presentation (symptomatic: 73.3 ± 43.7 mg nadolol vs asymptomatic: 50 ± 10 mg nadolol; p=0.71). Beta-blocker therapy had a general depressive effect on heart rate at peak exercise in both the CI (off therapy: 161.2 ± 5.4 vs maximal therapy: 128.5 ± 6.4; p = 0.0058) and normal chronotropy group (off therapy: 197.8 ± 3.3 vs maximal therapy: 157.1 ± 3.4; p<0.0001) as expected. Nine out of 10 CPVT patients (90%) with CI on maximal therapy required an adjunctive therapy in addition to beta blockers compared to 43% with a normal chronotropic response (p=0.042). No significant difference was found in number of CPVT patients undergoing defibrillator implantation between those with CI versus patients with a normal chronotropic response on maximal therapy (60% vs 43%; p=0.49).

Table 5.3

Variable		HR Reserve ≤62% (n=10)	HR Reserve >62% (n=7)	p-value
age		14.7 ± 0.5	15.6 ± 1.1	0.41
Exercise duration (sec)		557.6 ± 44.1	685.1 ± 63.1	0.11
METS achieved		8.0 ± 1.1	9.8 ± 1.5	0.33
HR at rest (beats/min)		59.3 ± 2.7	54.9 ± 6.1	0.47
HR at peak exercise (beats/min)		126.7 ± 5.6	159.7 ± 2.3	0.0003
Blood Pressure (mm Hg)				
	Systolic Blood Pressure at rest	101.4 ± 3.3	107.9 ± 4.7	0.26
	Diastolic Blood Pressure at rest	61.9 ± 2.5	67.7 ± 1.9	0.11
	Peak Systolic Blood Pressure	126.0 ± 6.2	151.4 ± 9.5	0.033
	Delta Systolic Blood Pressure (peak-rest)	24.6 ± 6.0	43.6 ± 10.2	0.11
Ventricular Arrhythmia Score		1.8 ± 0.5	2.3 ± 0.5	0.50

Table 5.3: Baseline and exercise characteristics of subjects on medication based on percent heart rate reserve (EST on maximal therapy)

HR = heart rate, METS = metabolic equivalents

As shown in Table 5.4, we compared both %HR reserve and VAS on EST off therapy and on maximal therapy in patients with worst symptom of syncope, SCA and asymptomatic patients.

Off therapy, %HR reserve was significantly different in patients with a worst symptom of SCA (%HR reserve <80% = CI) as compared to asymptomatic patients ($p<0.01$). VAS distinguished asymptomatic patients from patients with syncope ($p<0.001$) and patients with SCA as worst symptom ($p>0.05$). On maximal therapy, %HR reserve significantly distinguished asymptomatic patients from patients with syncope (%HR reserve ≤62% = CI; $p<0.01$) and patients with SCA (%HR reserve ≤62% = CI) as worst presenting symptom ($p <0.05$) whereas VAS did not distinguish between groups ($p>0.05$). Results would indicate that CI is able to distinguish patients with SCA as worst symptom both off and on maximal therapy whereas VAS is able to distinguish patients with SCA as worst symptom only off therapy.

Table 5.4

	Worst Symptom			
	Asymptomatic (n=6)	Syncope (n=4)	SCA (n=4)	p-value
EST Off Therapy				
%HR Reserve	93.2 ± 1.5	85.9 ± 8.5	64.7 ± 3.5	0.0032
Ventricular Arrhythmia Score	0.7 ± 0.3	4 ± 0	2.75 ± 1.2	<0.0001
	Asymptomatic (n=3)	Syncope (n=6)	SCA (n=8)	p Value
EST On Maximal Therapy				
%HR Reserve	73.6 ± 1.5	51.7 ± 3.8	52.9 ± 4.1	0.0078
Ventricular Arrhythmia Score	1.3 ± 0.3	2.5 ± 0.6	1.9 ± 1.3	0.69

Table 5.4: CI and VAS on and off therapy according to worst symptom
EST = exercise stress test, HR = heart rate, SCA = sudden cardiac arrest

5.5 Discussion

In this retrospective study of young CPVT patients, we found that CI off therapy is associated with ventricular arrhythmia and symptom burden. Untreated patients with CI had a worse VAS on EST (Table 5.2) and were more likely to have presented with life-threatening symptoms compared to those without CI. Maximal anti-arrhythmic therapy reduced the VAS in those with CI to a score which was not significantly different from that in patients with normal chronotropy (Table 5.3). Collectively, these data suggest that the EST off therapy (i.e. the initial diagnostic EST) can risk stratify CPVT patients based on CI, which may inform the initial therapy, closeness of follow-up and effectiveness of therapy for CPVT.

Although the EST is the most important test for suspected CPVT, it lacks sensitivity,[142] and sudden death can occur despite a normal, or near normal result.[135, 164] Thus, we sought other simple metrics that can be acquired on a standard EST to predict risk. CI has previously been associated with prognosis in other cardiac disorders, such as atrial fibrillation,[158] hypertrophic cardiomyopathy[165] and coronary artery disease.[159] CI might be particularly useful beyond the VAS since it detected worst symptom (SCA) in the maximally treated patient. CI may be used as a screening tool for those patients who present for family screening, those who are asymptomatic or who have a low VAS on EST. Further work is needed to compare the sensitivity and specificity of these predictors in a larger cohort. Several previous studies support the findings related to CPVT presented here. Firstly, atrial manifestations, like sinus node dysfunction, are common in CPVT, and may occur in the setting of more damaging mutations.[166, 167] Secondly, pharmacologically increasing sinus rate may protect against CPVT in mice.[168] And thirdly, ventricular arrhythmias actually subsided late in exercise in a subset of CPVT patients who reached >85% of their maximum-predicted HR.[168] While the mechanism of exercise-induced CI and arrhythmic risk in CPVT is unknown, one possible explanation is that some CPVT patients have an inappropriate autonomic response that favors parasympathetic dominance. Faggioni et al hypothesized that a higher HR shortens the diastolic interval, which exceeds the frequency of spontaneous Ca^{2+} release and delayed after depolarizations, ultimately leading to VE suppression.[168] Our study provides clinical data to support these concepts.

Risk related to CI appears to be attenuated by CPVT therapy. Once treated, the occurrence of ventricular arrhythmias was lower in all patients, regardless of CI on initial testing. While it may seem counter-intuitive that beta-blockers, which induce CI, would be protective, several factors may account for this apparent paradox. CPVT arrhythmias are most likely to manifest during an "arrhythmic window" of risk defined by HR.[169] Thus, the benefit of beta-blockers may be derived from narrowing the range of HR during which CPVT is most likely to manifest, rather than by simply decreasing maximal HR. Additionally, the molecular mechanism of CI may be different than the pharmacologic mechanism of beta-blocker protectiveness.

BP response to exercise may also be a CPVT risk predictor. In the present study, lower resting diastolic BP, peak systolic BP and delta systolic BP in those with CI off therapy were associated with a higher VAS. This finding persisted despite maximal therapy in patients with CI. The significance of this remains speculative at present. Since BP is influenced by HR, it may be

that CI itself leads to relative hypotension, which would mean that BP response provides no incremental prognostic utility over CI.

Some limitations warrant discussion. The population was small, and we limited our analyses to the index EST and EST on maximal therapy. All patients were on beta-blockers at a minimum, but we did not adjust for beta-blocker type or dosage equivalency on maximal therapy. While it is possible that CI was associated with a higher VAS due to early discontinuation of the EST due to severe CPVT, the practice at both participating centers is to continue the test unless hemodynamically unstable arrhythmias develop, prohibitive symptoms occur, like pre-syncope or exhaustion or no further diagnostic information would be obtained. Further prospective studies of CI comparing maximal HR with METS achieved, exercise duration and symptoms are warranted. Also, in the CI group, ancillary anti-arrhythmic treatments were more often prescribed ($p=0.042$), suggesting that these patients were indeed more severely affected.

5.6 Conclusion

The lack of prognostic tools in CPVT is a major clinical problem. These data suggest that the EST provides valuable clinical information beyond making a CPVT diagnosis. Namely, CI is a marker of increased CPVT risk, which appears to be attenuated by anti-arrhythmic therapy. A relative hypotensive response to exercise may be a novel prognosticator, although this observation may be confounded by the concurrent presence of CI. Our findings imply that the autonomic nervous system plays a role in disease modulation.

PART IV: MOLECULAR CHARACTERIZATIONS OF AN INHERITED ARRHYTHMIA SYNDROME IN THE YOUNG

Chapter 6: The Clinical and Genetic Spectrum of Catecholaminergic Polymorphic Ventricular Tachycardia: Findings from an International, Multicenter Registry

6.1 Abstract

Aims: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an ion channelopathy characterized by ventricular arrhythmia during exertion or stress. Mutations in *RYR2-coded* Ryanodine Receptor-2 (RyR2) and *CASQ2-coded* Calsequestrin-2 (CASQ2) genes underlie CPVT1 and CPVT2, respectively. However, prognostic markers are scarce. We sought to better characterize the phenotypic and genotypic spectrum of CPVT, and utilize molecular modeling to help account for clinical phenotypes.

Methods: This is a Pediatric and Congenital Electrophysiology Society multicenter, retrospective cohort study of CPVT patients diagnosed at <19 years of age and their first-degree relatives.

Results: Genetic testing was undertaken in 194 of 236 subjects (82%) during 3.5 (1.4-5.3) years of follow-up. The majority (60%) had RyR2-associated CPVT1.Variant locations were predicted based on a 3D structural model of RyR2. Specific residues appear to have key structural importance, supported by an association between cardiac arrest and mutations in the intersubunit interface of the N-terminus, and the S4-S5 linker and helices S5 and S6 of the RyR2 C-terminus. In approximately one quarter of symptomatic patients, cardiac events were precipitated by only normal wakeful activities.

Conclusion: This large, multicenter study identifies contemporary challenges related to the diagnosis and prognostication of CPVT patients. Structural modeling of RyR2 can improve our understanding severe CPVT phenotypes. Wakeful rest, rather than exertion, often precipitated life-threatening cardiac events.

6.2 Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an uncommon but often lethal ion channelopathy[170-172] characterized by bidirectional or polymorphic ventricular tachycardia (VT) during stress.[170] In 2001, Priori and colleagues reported that mutations in *RYR2*-coded Ryanodine Receptor-2 (RyR2) underlie CPVT1. [171] RyR2 is the largest known ion channel in the human genome and is responsible for calcium regulation in the cardiomyocyte.[173] CPVT1 mutations usually cluster in one of 4 areas known as "hotspots."[173] Arrhythmias are generally related to a gain of function in RyR2, which are likely a consequence of excessive or untimely calcium release in the sarcoplasmic reticulum, leading to delayed after-depolarizations.[173] Calsequestrin-2 (CASQ2) mutations account for autosomal recessive CPVT2,[174] probably through abnormal attenuation of RyR2.[173]

Data on prognosis in CPVT are limited.[171, 172, 175] Initially, males with CPVT1 were thought to have more severe phenotypes.[171] However, more recent data has not reported this association.[175] Furthermore, expressivity is variable in CPVT, making clinical diagnosis challenging.[175] A higher incidence of non-sustained VT has been observed in patients with variants in the C-terminal channel-forming region of RyR2,[175] however this has not changed treatment guidelines.[2] We sought to better characterize the genotypic and phenotypic spectrum of CPVT in a large, multicenter registry cohort.

6.3 Methods

This is a retrospective, observational cohort study of CPVT that enrolled [1] children (<19 years of age) with CPVT and [2] their affected first-degree relatives (≥19 years of age). Adults (≥19 years) were only included if a pediatric proband (<19 years) from their family was also enrolled. Centers were solicited through the Pediatric and Congenital Electrophysiology Society and all obtained ethical approval locally. The study adheres to the Declaration of Helsinki. Treatment outcomes from this population have been reported previously.[176]

Data Collection: Data were obtained from existing medical records, entered by each site into a data collection form and verified by the coordinating center. Genetic testing was undertaken prior to the study period by the enrolling center as part of routine care. REDCap[177] electronic data capture tools hosted by the Child and Family Research Institute at British Columbia Children's Hospital were used for data acquisition and storage.

Modeling: A 3D model of human RyR2 was created based on available crystal structures and cryo-electron microscopy (EM) structures. The program MODELLER (https://salilab.org/modeller/) was used to produce the homology-based fragments, using available structures of human RyR2, mouse RyR2 (97.4% sequence identity), and rabbit Ryanodine Receptor-1 (RyR1) (65.4% sequence identity). The high sequence identity implies basically unaltered domain folds, thus allowing for the construction of reliable models. The utilized crystal structure templates were as follows: human RyR2 N-terminal region (PDB: 4JKQ),[178] with missing loops modeled based on the mouse RyR2 crystal structure (PDB: 3IM6); mouse RyR2 SPRY1 (PDB 5C33)[179]; rabbit RyR1 repeat12 (PDB 5C30)[179]; mouse RyR2 SPRY2 domain (PDB 4P9I)[180]; mouse RyR2 Repeat34 domain (PDB 4ETV).[181] All of the remaining regions were built based on the cryo-EM model of rabbit RyR1 (PDB: 3J8H).[182] The best model for each was selected based on the MolPDF score. The final full-length human RyR2 model was created by superposing the structural models of individual domains onto the cryo-EM structural model of rabbit RyR1 using UCSF Chimera (https://www.cgl.ucsf.edu/chimera/).

Definitions: A proband was defined as the index case of confirmed CPVT in a family. Pathogenicity was recorded from the original genetic report whenever possible, usually in the setting of commercial genetic testing. When no rare genetic variant (pathogenic mutation, probable pathogenic mutation or variant of unknown significance (VUS)) was identified despite molecular analysis, the case was classified as gene-elusive. Known benign variants were also classified into the gene-elusive group. Subjects who did not undergo genetic testing as part of routine care were not included in genetic comparisons. Definitions of RyR2 hotspots[173] and regions[183] are published elsewhere. RyR2 variants that did not localize to a known region and/or hotspot are termed "non-hotspot" and/or "non-region." Ventricular arrhythmias (VA) were classified as mild (ventricular couplets, ventricular bigeminy and/or frequent premature ventricular complexes) or severe (non-sustained VT, bidirectional sustained VT, and/or cardiac arrest) based on the highest grade of ectopy documented on any recording irrespective of drug therapy. Variable expressivity was defined by ≥1 asymptomatic subject and ≥1 severely affected subject (history of syncope and/or cardiac arrest) within a family. An arrhythmic syncope or cardiac arrest while on a beta-blocker was defined as beta-blocker failure.

Statistical Analysis: Contingency tables were generated for all categorical data with the frequency (percentage) reported. Data are presented as the median (95% distribution-free confidence intervals). Dates of birth were collected as year and month only as per ethical considerations. Durations listed vary by ± 15 days. All statistical analyses were completed using SAS Statistical Software Version 9.4 (SAS Institute, Cary, NC).

6.4 Results

This study describes 236 CPVT subjects (52% female) including 171 probands (72%) and 65 relatives (28%) followed for 3.5 (95% CI 2.9-3.9) years, equivalent to 795 patient-years. Diagnosis occurred at a median age of 12.6 (11.9-13.2) years with a delay to diagnosis of 0.5 (0.3-0.9) years in those presenting symptomatically. Further detailed demographic data from this cohort are described in a previous publication.[176]

Clinical and Genetic Assessment: Cardiac symptoms were reported in 179 of 236 patients (76%) at presentation, including 24 patients (13%) with more than one presenting symptom. Presenting symptoms included 112 with syncope (54%), 58 with cardiac arrest (28%), 12 with palpitations (6%), 8 with seizures (4%), 6 with chest pain (3%), 3 with pre-syncope (1.5%), 3 with dyspnea (1.5%) and 5 with other miscellaneous symptoms/incidental findings leading to diagnosis (2%). The remaining 57 patients (24%) presented with a family history of CPVT or of possible CPVT, such as unexplained sudden death in a young relative. Genetic testing was undertaken in 194 of 236 patients (82%) prior to the study period by the enrolling center as part of routine care. Some subjects had more than one gene sequenced, resulting in a greater total number of genetic test results than study subjects. Variants were as follows: RyR2 in 117 of 194 (60%), CASQ2 in 9 (5%), KCNJ2 in 1 (1%), 17 patients (9%) who tested positive for more than one potential mutation, and 27 (14%) had a variant but the participating center could not provide further details. There were 23 (12%) gene-elusive patients. Genetic testing occurred in private/commercial labs for 162 of 256 tests (63%), research labs for 9 tests (4%) and unknown/unreported for 85 (33%) tests. Ninety-six of 194 (49%) subjects had a known or probable disease-causing variant, 33 (17%) had a VUS, and 65 (34%) had no available prediction at the time of testing. A comparison between CPVT1 and gene-elusive CPVT subjects is summarized in Table 6.1a. Demographic data by pathogenicity of *RYR2* variant appears in Table 6.1b. Data on RyR2 variants organized by hotspot and region are summarized in Table 6.2 and Figures 1 and 2.

Table 6.1a

	CPVT1* **n = 117**	**Gene Elusive** **n = 23**
Male Sex	59/117 (50%)	10/23 (43%)
Probands	77/117 (66%)	22/23 (96%)
Median age at diagnosis (years)	11.7 (95% CI: 10.6-12.8)	14.8 (95% CI: 12.3-17.2)
Median delay to diagnosis (years)	0.6 (95% CI: 0.3-1.2)	0.4 (95% CI: 0-2.2)
Syncope	36/117 (31%)	6/23 (26%)
Cardiac Arrest	43/117 (37%)	9/23 (39%)
Atypical Trigger for Syncope	8/33 (13%)	3/9 (33%)
Atypical Trigger for Cardiac Arrest	10/37 (26%)	4/10 (40%)
Ventricular Arrhythmia	89/117 (76%)	23/23 (100%)
Atrial Arrhythmia	26/117 (22%)	5/23 (22%)
Beta-blocker Failure	15/117 (13%)	5/23 (22%)
ICD use	56/115 (49%)	15/23 (65%)
Deaths	4/117 (3%)	0/23 (0%)

Table 6.1a: Clinical comparisons between patients with CPVT1 and gene-elusive CPVT.

*Defined as carrying a rare variant in *RYR2* classified as a pathogenic mutation, probable pathogenic mutation or VUS at the time of genetic testing.

Table 6.1b

	Pathogenic/Probable Pathogenic *RYR2* Variant n=90	*RYR2* Variant of Undetermined/ Unknown Significance n=27
Male Sex	42 (47%)	17 (63%)
Probands	60 (67%)	17 (63%)
Median Age at Diagnosis (years)	11.7 (8.0-14.7)	11.8 (9.8-14.9)
Median Delay to Diagnosis (years)	0.5 (0.1-4.1)	0.7 (0.1-4.1)
Syncope	30 (33%)	6 (22%)
Cardiac Arrest	33 (37%)	10 (37%)
Atypical Trigger for Syncope	10 (11%)	3 (11%)
Atypical Trigger for Cardiac Arrest	6 (7%)	2 (7%)
Ventricular Arrhythmia	69 (77%)	20 (74%)
Atrial Arrhythmia	20 (22%)	6 (22%)
Beta-Blocker Failure	12 (13%)	3 (11%)
ICD Use	46 (51%)	10 (37%)
Deaths	4 (4%)	0 (0%)

Table 6.1b: Clinical comparisons between patients with CPVT1 by pathogenicity of *RYR2* variant

Table 6.2

	Mild VA (n=13)	Severe VA (n=76)	Syncopal Events (n=42)	Cardiac Arrest Events (n=52)[€]
Hotspot I	5 (38%)	12 (16%)	6 (14%)	8 (16%)
Hotspot II	4 (30%)	16 (21%)	10 (24%)	11 (21%)
Hotspot III	1 (8%)	15 (20%)	8 (19%)	12 (23%)
Hotspot IV	1 (8%)	19 (25%)	12 (29%)	9 (17%)
Non-hotspot†	1 (8%)	11 (14%)	5 (12%)	8 (15%)
Unknown*	1 (8%)	3 (4%)	1 (2%)	4 (8%)
C-terminal region	2 (15%)	35 (46%)	19 (45%)	21 (42%)
Central region	4 (31%)	18 (24%)	10 (24%)	13 (26%)
N-terminal region	6 (46%)	12 (16%)	6 (14%)	8 (16%)
Non-region†	0	5 (6%)	2 (5%)	3 (6%)
Unknown*	1 (8%)	6 (8%)	5 (12%)	5 (10%)

Table 6.2: Phenotypes of CPVT1 patients by RyR2 hotspot and region.

[€]The number of cardiac arrest events reported for Regions is n=50; †Variant does not localize to a known hotspot and/or region in RyR2; *Insufficient data provided by participating center to determine localization of RyR2 variant; VA = ventricular arrhythmias

Figure 6.1

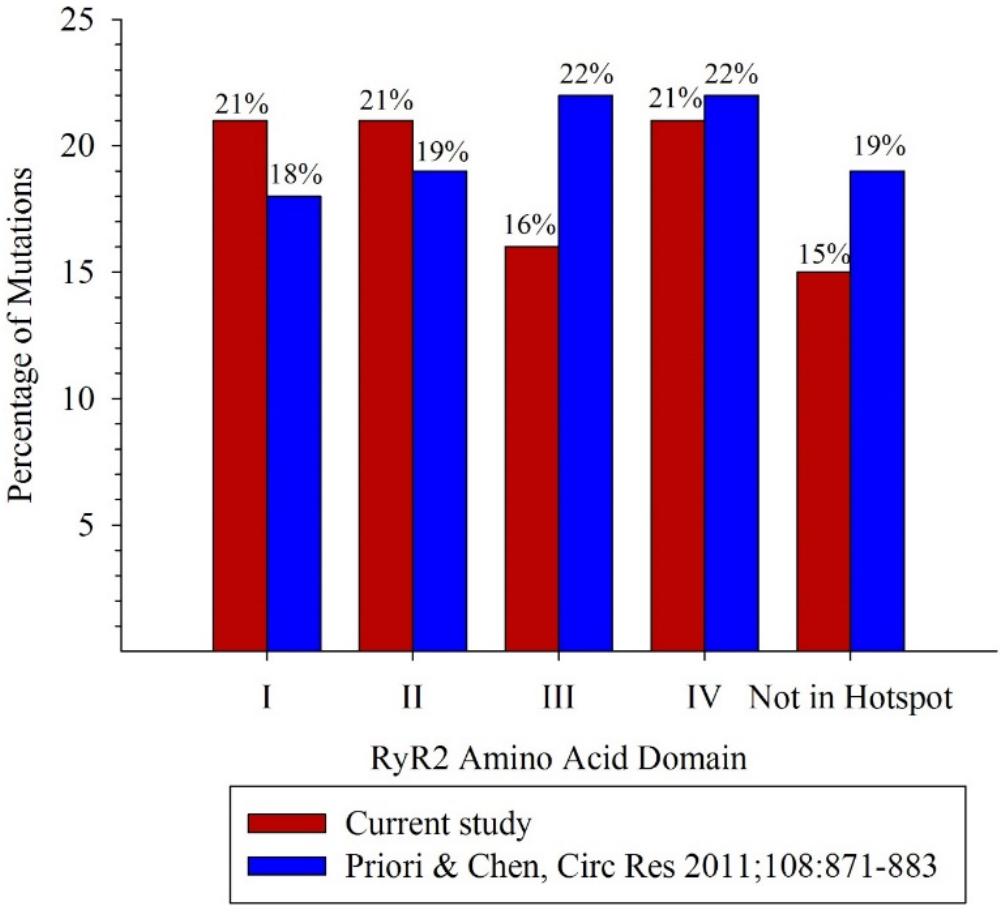


Figure 6.1: Plot of the percentage of variants found in hotspot areas on RyR2 thought to underlie most cases of CPVT (Priori & Chen, 2011)[173] in comparison to hotspot localization of RyR2 variants identified in the present study.

Figure 6.2

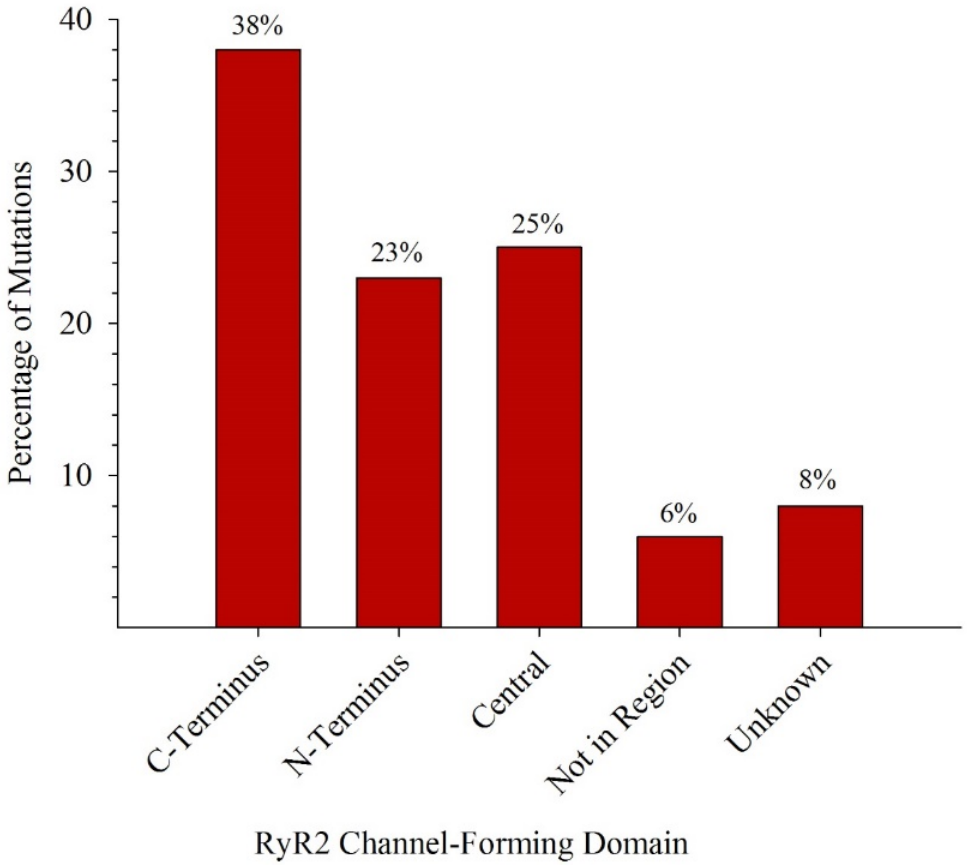


Figure 6.2: Plot of the percentage of variants by RyR2 region.

Triggers for Life-Threatening Events: An atypical triggering event for syncope, defined by normal wakeful activity (eg. playing an instrument, resting), occurred in 15 of 66 patients (23%) in whom the preceding circumstance was known. Cardiac arrest was atypically triggered in 12 of 45 (27%) patients. Six patients (3%) died as a result of confirmed/suspected CPVT-related arrhythmias including 4 with CPVT1. In 3 of the 6 deceased patients, an atypical trigger preceded death. An additional 2 decedents were excluded from mortality analysis as they were ascertained from a molecular autopsy. Both had variants in RyR2 and pre-mortem symptoms consistent with CPVT. Figure 6.3 summarizes the circumstances preceding life-threatening events.

Figure 6.3

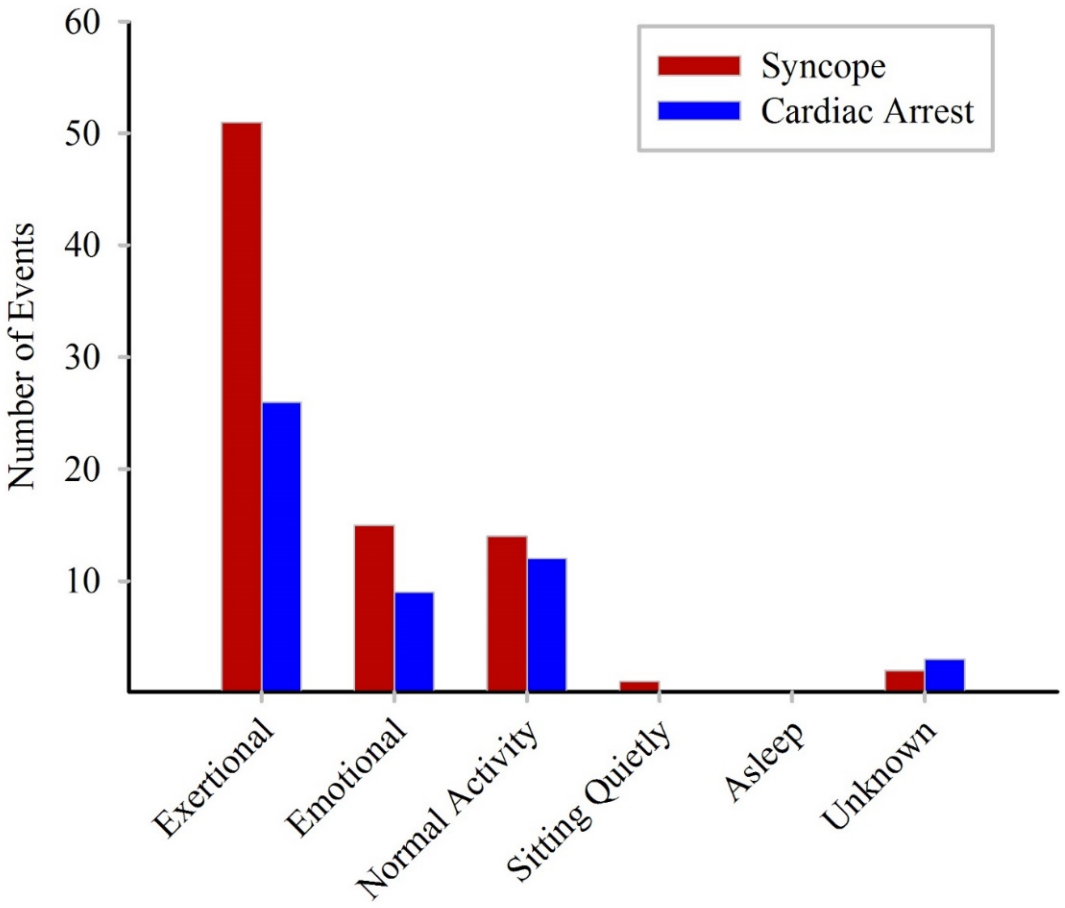


Figure 6.3: Circumstances immediately preceding all life-threatening events defined as syncope and/or cardiac arrest.

CPVT Families: This cohort included 32 families comprising 79 patients (33%). Fifty-four patients from 23 families had CPVT1, while 5 patients from 2 families had CPVT2. In 13 of 32 families (41%), expressivity was variable among relatives. In 16 families (50%), there was ≥1 asymptomatic, genotype-positive case. A *RYR2* variant at R420 was found in 14 of 117 CPVT1 patients (12%) (R420Q in 2 and R420W in 12). Nine of the *RYR2*-R420W subjects made up 4 families, all with variable expressivity.

CPVT2: There were 4 of 194 patients (2%) affected by homozygous *CASQ2*-associated CPVT2, and all had a history of life-threatening symptoms. The *CASQ2* variants identified were as follows (by amino acid change): R251H, I270T and Q245X. In addition, there was one family potentially affected by heterozygous CPVT2, which included 3 subjects with a heterozygous, probably pathogenic *CASQ2* variant (D340stop). The proband experienced life-threatening symptoms, while her relatives had exercise-induced ventricular bigeminy.

Molecular Modeling: A direct analysis of RyR2 variants on the overall 3D structure of our homology model shows that: (1) most variants cluster close to the 4-fold symmetry axis and virtually none are found towards the cytosolic corner regions, which are located more at the periphery (Figure 6.4a), (2) all but one variant (I4867V) in the S5 and S6 helices, as well as the S4-S5 linker of the C-terminus, were implicated in cardiac arrest (Figure 6.4b), (3) in hotspot 1, all 7 variants found at the interface between neighbouring subunits are associated with cardiac arrest (Figure 6.5),

Figure 6.4a

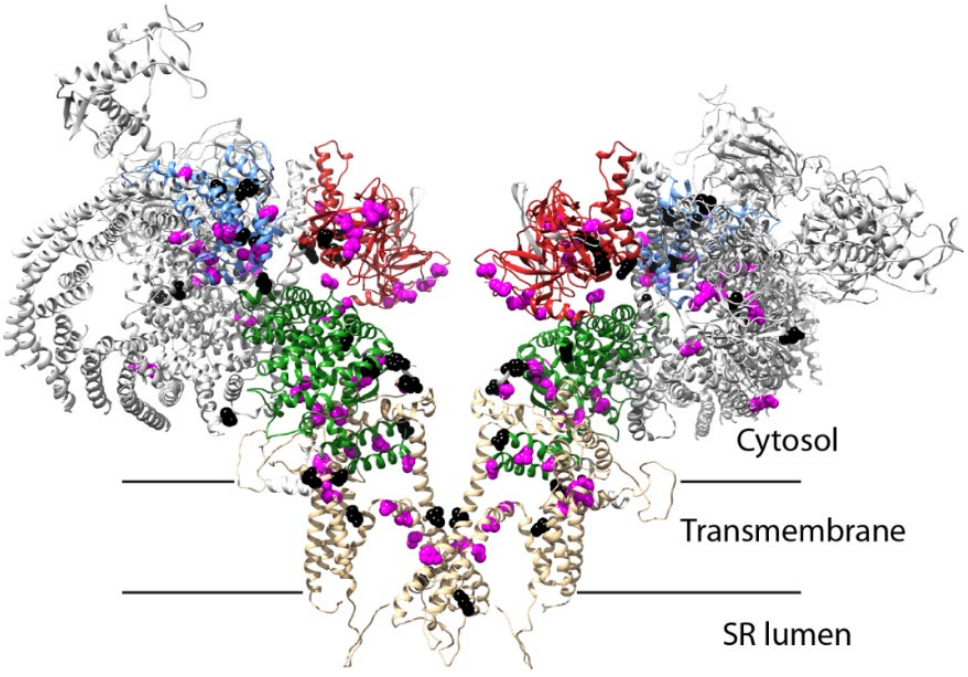


Figure 6.4a: Homology model of human RyR2. The protein is shown in cartoon form, with the disease hotspots highlighted in colors (hotspot 1: blue, hotspot 2: red; hotspot 3: green; hotspot 4: beige). View is from the 'side', parallel to the membrane. Only 2 out of four subunits are shown for clarity. Positions for CPVT-associated variants are highlighted, with atoms shown in Van der Waals representation (purple: associated with cardiac arrest, black: all others).

Figure 6.4b

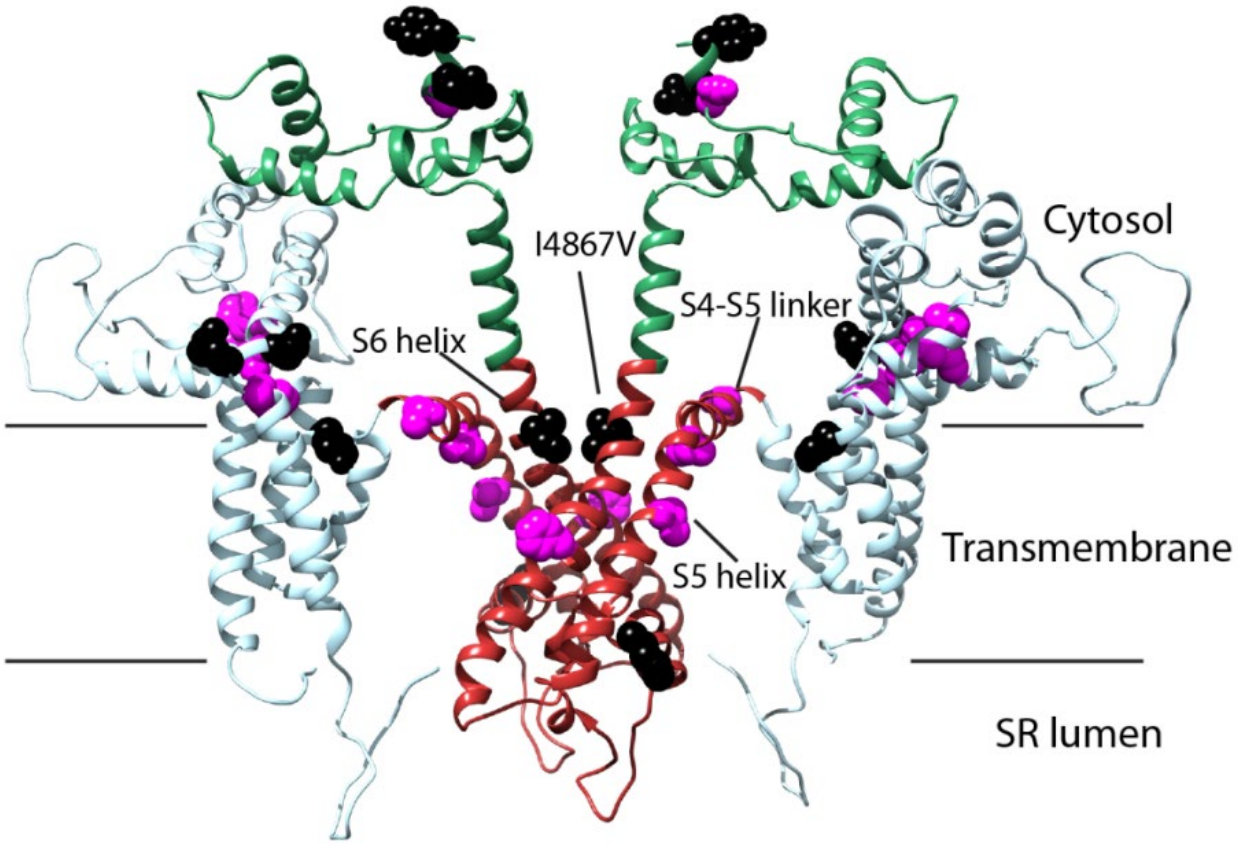


Figure 6.4b*:* Close-up of the transmembrane region of the RyR2. This region forms the bulk of disease hotspot 4. The following subregions are highlighted: pore-forming region (red), additional transmembrane (cyan), C-terminal cytosolic extension to the pore (green). Positions of CPVT-associated variants associated with cardiac arrest are highlighted in purple, and all others in black. The S5 and S6 helices and the S4-S5 linker are labeled.

Figure 6.5

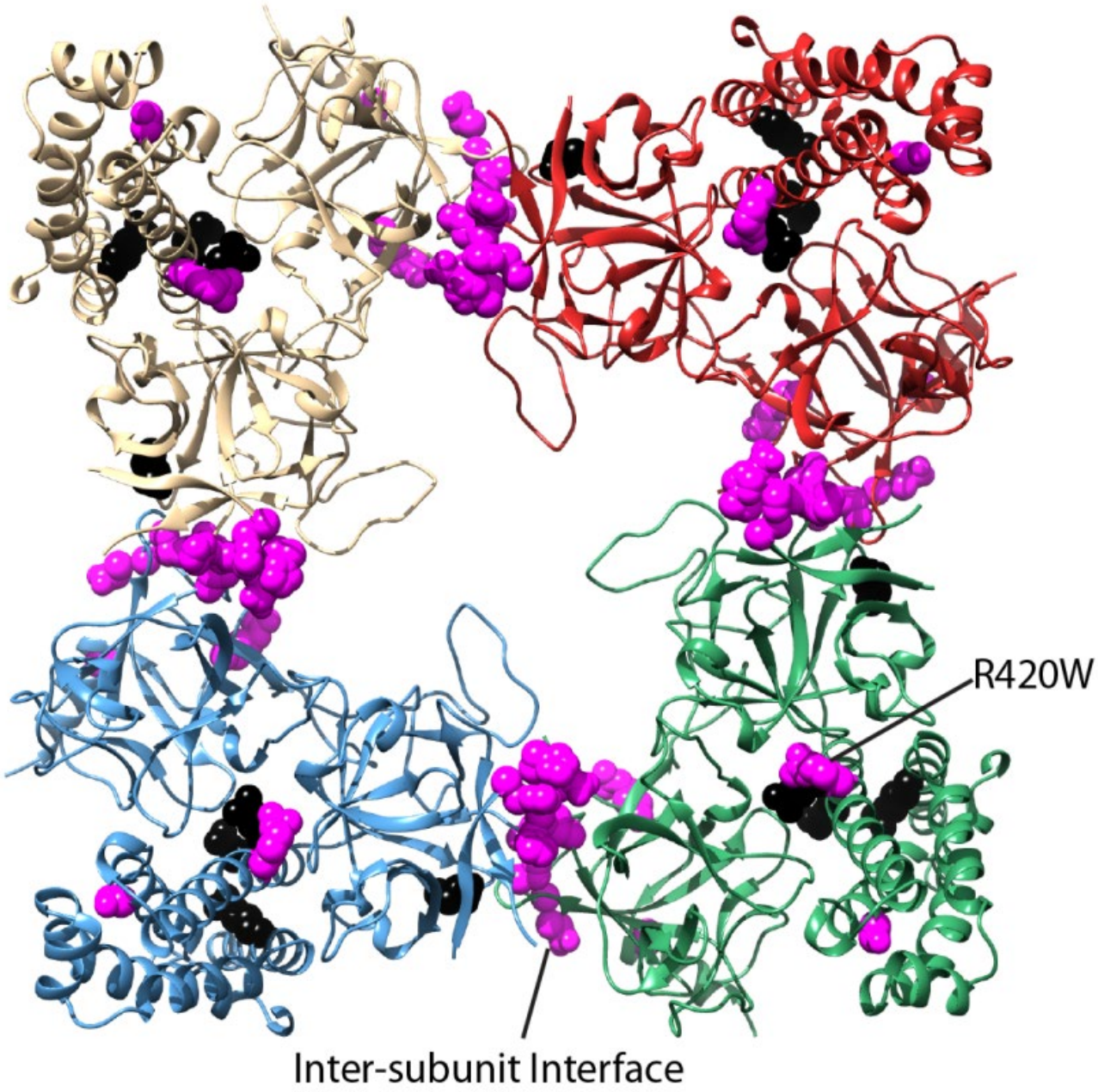


Figure 6.5: Close-up of the N-terminal disease hotspot (hotspot1) within RyR2. Different subunits are indicated in different colors. Variants identified in our cohort are indicated in Van der Waals spheres, with purple indicating those variants associated with cardiac arrest, and all others in black. The interface between neighboring subunits forms a cluster of variants linked with cardiac arrest, highlighting its functional importance. The position of the founder R420W variant is labeled.

6.5 Discussion

CPVT is a potentially lethal syndrome that almost entirely lacks prognostic data. Since the seminal description of *RYR2*-related CPVT more than 15 years ago, genotypic factors still do not inform prognosis or management. In some cohorts, CPVT1 patients appeared to be at higher arrhythmic risk,[184] however this phenomenon has not been consistently observed. [176] In 2012, van der Werf et al showed that variant location in RyR2 could predict arrhythmia in several Dutch CPVT families, [175] including a correlation between non-sustained VT on initial exercise stress test and C-terminus variants in RyR2. However, this potential prognosticator has not changed treatment standards.[2] Instead, clinicians may make life-altering decisions based on anecdotal experience, patient sex, age and/or family history despite a lack of reproducible evidence supporting any of these perceived risk factors. Herein, we describe the outcomes of multiple probands and families with CPVT1, and use molecular modeling to characterize the structural changes in RyR2 that underlie the most malignant CPVT phenotypes, in a large, heterogeneous registry population.

To better characterize the structural channel alterations in cases of CPVT1 complicated by life-threatening cardiac events, we modeled *RYR2* variants from our cohort using a RyR2 homology model. The only available high-resolution 3D structure for human RyR2 is for the N-terminal region.[178] However, reliable homology-based models can be built when templates are available with >50% sequence identity.[185] The availability of multiple structures for mouse RyR2 domains, which displays >97% sequence identity with human RyR2, as well multiple crystal and cryo-EM structures of rabbit RyR1 (>65% identity)[186] allowed for the building of a near complete homology-based model of RyR2 on which all variants could be mapped. Although portions of the cryo-EM study of RyR1 are at comparatively lower resolution, most variants mapped to the better-ordered regions and those for which high-resolution crystal structures are available. Figure 6.4b shows the location of the variants on our homology model of the RyR2 transmembrane region, which contains the C-terminus. This area forms the minimal 'channel' through which calcium ions permeate the sarcoplasmic reticulum membrane, whereas the cytosolic assembly mostly serves to allosterically modulate the gating properties of the channel region. Variants in the latter would thus be expected to be more permissive, whereas variants in the channel region would have a much higher impact on function. Within the transmembrane region, key structural elements are formed by the helices S5 and S6 of the pore-forming domain, and the linker between S4 and S5 has been suggested as an important allosteric coupling element between triggers and gating of the pore in

several other ion channels [187]. Accordingly, 4 of the 5 variants that map to these elements led to cardiac arrest in our cohort.

Our 3D model also confirms a previous study on the importance of the N-terminus in channel gating (Figure 7.2) during channel opening, this area undergoes large conformational changes, whereby an interface between neighboring subunits is thought to be disrupted.[188] Variants that weaken this interface are thus predicted to facilitate channel opening. All 7 variants that mapped to this interface were associated with cardiac arrest, confirming a recent report that this interface is a crucial determinant of channel gating.[188] These correlations within the C- and N-termini suggest that amino acid residues with key importance in RyR2 gating can give rise to the most severe disease phenotypes. In 2011, Priori and Chen also proposed that CPVT variants cluster in 1 of 4 "hotspots" on RyR2,[173] Hotspot 1 forms a gating ring at the cytosolic face,[188] whereas hotspot 4 forms the transmembrane assembly.[182] Hotspots 2 and 3 form a physical link with hotspots 1 and 4. Variant localizations by hotspot in our cohort mirror the data of Priori and Chen (Figure 6.1a), supporting the theory that alterations to these areas are most likely to underlie an arrhythmia phenotype.

A large number of CPVT1 families are reported in this cohort, probably due to utilization of cascade genetic screening. Half of families included ≥1 relative(s) with genetically confirmed, asymptomatic CPVT1. These cases of concealed CPVT1 are poorly understood. For example, in both the present study and existing data,[175, 189] the *RYR2*-R420W variant demonstrated incomplete penetrance and expressivity. R420 forms part of an extensive network of interactions between the domains in hotspot 1 (Figure 7.2), coordinating a central chloride ion.[190] Mutation of this residue leads to a marked disruption of the chloride binding site and allosteric changes within the hotspot in the setting of *RYR2*-R420W and R420Q.[190, 191] The growing number of concealed CPVT1 cases should be a topic of further study, including efforts to elucidate genetic modifiers that can account for phenotypic variability and to identify genotype-specific management strategies.

Traditionally, CPVT arrhythmias are described during periods of intense emotion or physical exertion, prompting experts to advise strict exercise restriction.[170] However, a substantial proportion of events are triggered by wakeful rest in our cohort. Non-exercise induced ventricular fibrillation has been described in loss-of-function *RYR2* mutation carriers, suggesting that other mechanisms could underlie arrhythmias in these patients.[192] This cohort also includes a

disproprortionately large number of children, who have elevated baseline heart rates compared to adult patients. This elevated heart rate at rest could potentially account for the higher number of arrhythmias precipitated by rest in this pediatric cohort.

Autosomal recessive CPVT2 is highly malignant.[174] The present study includes 4 individuals with homozygous CPVT2, all of whom suffered from severe arrhythmias. We also report one small family with CPVT possibly related to a heterozygous variant in *CASQ2*, including one proband with life-threatening symptoms. Recently, the existence of heterozygous CPVT2 was documented using a whole exome sequencing approach,[193] suggesting that the heterozygous *CASQ2* variant observed in our family may also behave in an autosomal dominant fashion. Further molecular studies of *CASQ2*-related CPVT variants are needed.

Our research was limited by selection bias, as those patients presenting with sudden death are rarely diagnosed or seen by a cardiologist, and minimally symptomatic or gene-elusive patients may have been excluded from enrollment. Adult subjects were under-represented as only pediatric centers participated, which may over-estimate disease severity. A diagnosis of CPVT was required for enrollment; however, we were unable to determine strength of diagnosis in all cases. A validated severity model for CPVT does not currently exist. The definition of mild versus severe VA is based on clinical experience rather than robust data. Genetic reporting spans more than a decade of molecular advances in CPVT. Some centers did not have robust genotypic data beyond the amino acid sequence and pathogenicity as testing spanned a nearly 15 year period in this retrospective cohort. We could not determine disease-causation without linkage analysis. In some cases, it was not known whether one gene or multiple genes were analyzed during the sequencing process. We attempted to verify genetic information with centers whenever possible. Future prospective studies are needed to assure that all patients undergo broad sequencing of all genes now known to underlie CPVT. For the RyR2 model, the portions for which crystal structures are available are the best defined, followed by the C-terminal region, for which the resolution of recent cryo-EM studies is the highest. As such, a direct analysis of variants in the N-terminal and C-terminal hotspots is the most reliable. For most other sections, a direct analysis of hydrogen bonds and ionic interactions of the variants is not yet possible, but there is no uncertainty regarding their general location in the 3D structure, since the overall fold is easily observed throughout current cryo-EM structures. Homology modeling is predictive in nature and cannot be independently used to determine pathogenicity.

6.6 Conclusions

This multicenter registry-based study of a large, heterogeneous, and extensively genotyped CPVT population describes contemporary challenges related to the diagnosis and prognostication of CPVT patients. Utilizing predictive homology modeling, areas of key structural importance in the RyR2 channel are elucidated and supported by clinical outcome data. Modeling *RYR2* variants may be especially helpful for patients and families facing genetic uncertainty. Life-threatening events often occur during resting wakeful activities, highlighting the unpredictable nature of CPVT arrhythmias. Genetic expressivity of CPVT1 is also variable among relatives, which suggests that a family history of sudden death is an unreliable prognosticator. The present study moves us toward a better understanding of the interplay between RyR2 structure and clinical outcomes, and also identifies the challenges that still exist in determining arrhythmic risk, interpreting positive family histories and equivocal genetic testing. Further functional and linkage analysis is needed to establish genetic causation in most cases of CPVT.

Chapter 7: Clinical and Molecular Evidence of a Gene Dosage Effect in Patients with Catecholaminergic Polymorphic Ventricular Tachycardia related to Multiple Genetic Mutations: A Report from the PACES CPVT Registry

7.1 Abstract

Background: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is often a life-threatening arrhythmia disorder with variable penetrance and expressivity. Little is known about the incidence or outcomes of CPVT patients with ≥2 variants.

Methods: The phenotypes, genotypes and outcomes of patients in the Pediatric and Congenital Electrophysiology Society CPVT Registry with ≥2 variants in genes linked to CPVT were ascertained. The American College of Medical Genetics & Genomics (ACMG) criteria and structural mapping were used to predict the pathogenicity of variants (3D model of pig RyR2 in open-state).

*Results***:** Among 237 CPVT subjects, 193 (81%) had genetic testing. Fifteen patients (8%) with a median age of 9 years (IQR 5-12) had ≥2 variants. Sudden cardiac arrest occurred in 11 children (73%), although none died during a median follow-up of 4.3 years (IQR 2.5-6.1). Thirteen patients (80%) had at least two *RYR2* variants, while the remaining two patients had *RYR2* variants plus variants in other CPVT-linked genes. Among all variants identified, re-classification of the commercial laboratory interpretation using ACMG criteria led to the upgrade from variant of unknown significance (VUS) to pathogenic/likely pathogenic (P/LP) for 5 variants, and downgrade from P/LP to VUS for 6 variants. For *RYR2* variants, 3D mapping using the RyR2 model suggested that 2 VUS by ACMG criteria were P/LP, while 2 variants were downgraded to likely benign.

Conclusions: This severely affected cohort demonstrates that a minority of CPVT cases are related to ≥2 variants, which may have implications on family-based genetic counselling and prognosis. While multi-variant CPVT patients were usually severely affected, further research is needed to determine the significance and generalizability of this observation. This study also shows that a rigorous approach to variant re-classification using the ACMG criteria and 3D mapping is important in reaching an accurate diagnosis, especially in the multi-variant population.

7.2 Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare inherited arrhythmia syndrome characterized by ventricular tachycardia (VT) provoked by adrenergic stress.[53] The condition is caused by excessive calcium leak from the sarcoplasmic reticulum, leading to delayed after-depolarizations and arrhythmias.[53] Most cases are attributed to mutations in *RYR2*-coded ryanodine receptor (RyR2) or *CASQ2*-coded calsequestrin-2.[53] Although less recognized, *SCN5A, TRDN,* and *CALM1-3* have also been implicated in catecholamine sensitive polymorphic VT.[194-199]

To date, genotype-based risk predictors have not been clinically useful. In other inherited arrhythmic conditions, like long QT syndrome (LQTS) and hypertrophic and arrhythmogenic cardiomyopathies, patients with double and compound mutations fare especially poorly.[200-204] We used the Pediatric and Congenital Electrophysiology Society (PACES) Registry[58, 205] to characterize CPVT patients with ≥2 variants. To systematically assess the likelihood of pathogenicity, variants were mapped on to the 3D structure of RyR2, which provides mechanistic insights into their function and enhances the analysis compared to sequence-based scoring algorithms alone.

7.3 Methods

This is a retrospective study derived from the PACES CPVT Registry, which is an international multicenter registry of children (≤19 years) and their first-degree relatives with a diagnosis of CPVT made by consensus criteria. [53] Clinical, genotypic and outcome data were previously reported.[58, 205] Participating sites received ethical approval locally and the protocol adhered to the 1975 Declaration of Helsinki. A stepwise bioinformatics approach was implemented to classify variant pathogenicity, including structural mapping using 3D model of pig RyR2 in open-state (Fig. 7.1). Continuous data are presented as the median (interquartile range). Detailed methods are available in the supplemental data (Appendix E).

Figure 7.1

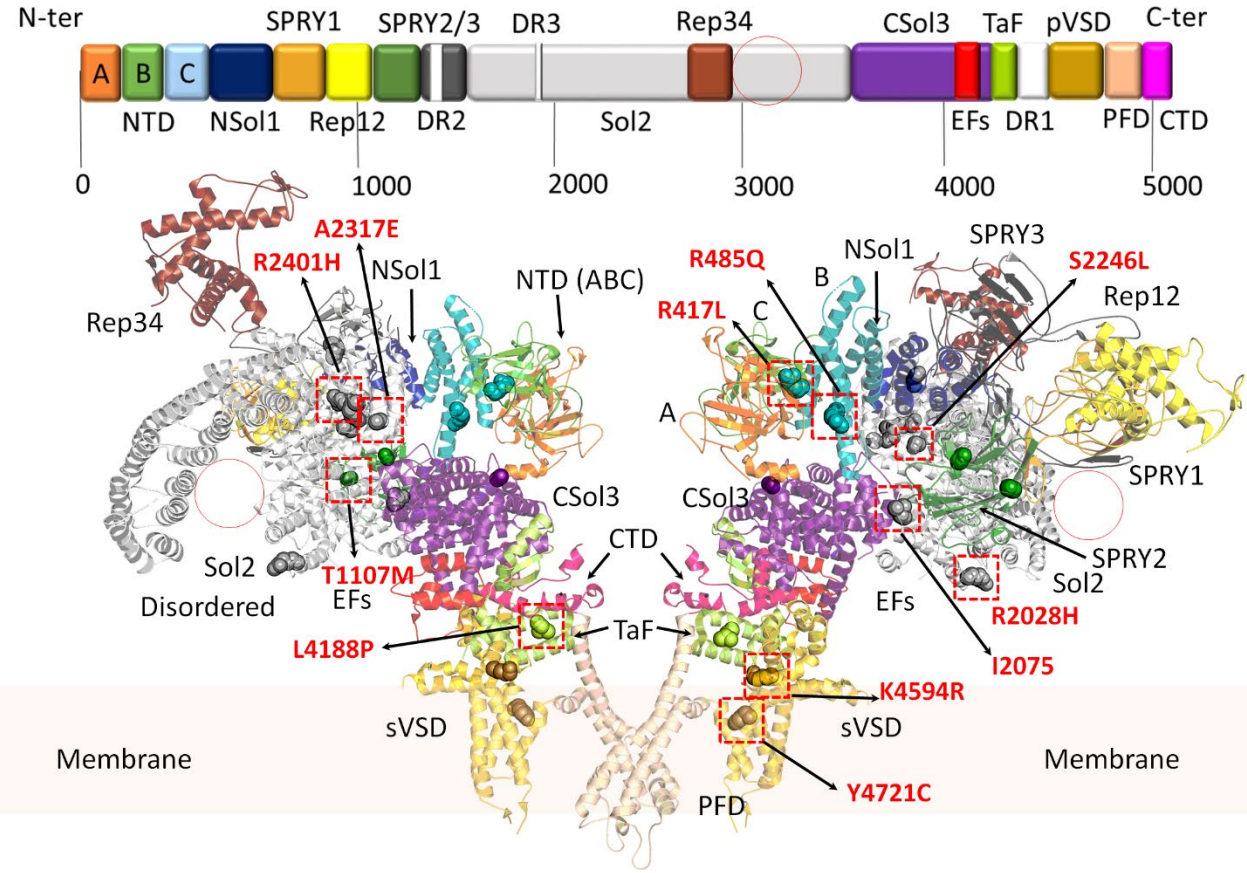


Figure 7.1: Domain architecture of the cardiac RyR2.

Domains are coloured according to the linear sequence scheme shown above the ribbon diagram. Structure obtained from PDB: 5GOA.[206] The ribbon diagram shows a RyR2 dimer from the side. Red circles represent large alpha solenoid regions which are unstructured in the cryo-electron micrograph (CryoEM) structure. All variants discussed in the manuscript are shown in sphere representations. Red squares highlight location of the most likely damaging variants (expanded in Fig. 7.2). Domains are named according to nomenclature used by des Georges, et al., 2016. A (*dark orange*), B (*green*), and C (*light blue*) domains: form part of the N-terminal domain (NTD); NSol (*dark blue*): alpha-solenoid region near the NTD; SPRY1 (*light orange*), SPRY2 (*dark green*), and SPRY3 (*dark grey*): three domains named after splA kinase and RyRs where they were first identified; Sol2 (*light grey*): second alpha-solenoid region centrally located on RyR2; Rep12 (*yellow*) and Rep23 (*brown*): four repeats (~100 aa each) in two tandem arrangements, Repeats 1 and 2 located between SPRY 1 and SPRY2, and Repeats 3 and 4 located within Sol2; CSol3 (*magenta*): third alpha-solenoid region located near the C-terminal; EFs (*red*): pair of EF hand-like motifs located within CSol3 region; TaF (*light green*): thumb and forefingers domain; DR1/2/3 (*white*): evolutionary divergent regions of RyR isoforms (not shown in the ribbon diagrams); pVSD (*gold*): pseudo voltage-sensing domain; PFD (*wheat*): pore-forming domain; CTD (*pink*): C-terminal domain.

7.4 Results

Population: Of 237 patients entered in the PACES CPVT Registry, 193 (81%) underwent genetic testing. There were 15 patients (8%) from 12 families with ≥2 variants. Table 7.1 summarizes the genotypes and phenotypes of these multi-variant carriers. The median age at presentation was 9 years (IQR 5-12) and 9 (60%) were female. Thirteen children (87%) had ≥2 *RYR2* variants, one had *CASQ2* and *RYR2* variants, and one had *RYR2* and *SCN5A* variants (Table 7.1, Table 7.2 and Supplemental Table 7.1. There were 12 children (80%) who were probands. Inheritance could not be established in 6 children (40%) owing to a lack of parental genetic data. A family history of suspected/confirmed CPVT was reported in 10 patients (67%). VT and/or sudden cardiac arrest (SCA) occurred in 13 of 15 cases (87%). The exceptions were subject #6 who was asymptomatic and subject #12 who had exertional syncope and seizures. Pedigrees for patients from select families are appear in Figure 7.2.

Table 7.1

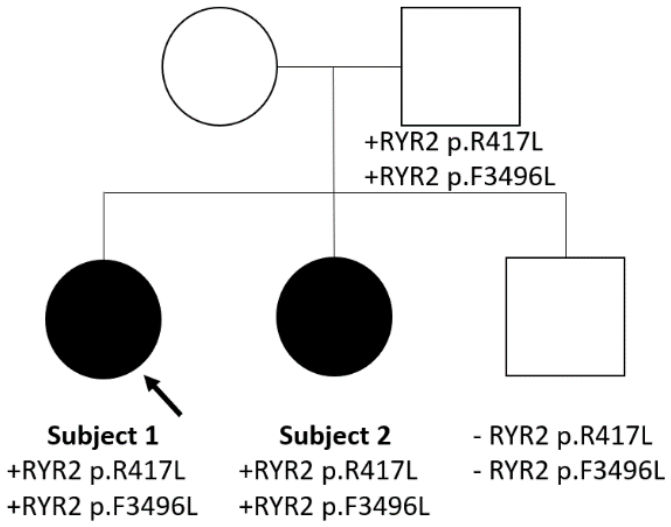
Subject	Sex	Ethnicity	Age (years)	Proband	Variant #1	Variant #2	Inheritance	Phase (cis vs trans)	Family History	Symptoms	Treatments	Treatment Failure
1	F	Hispanic	16	Yes	*RYR2*-p.R417L	*RYR2*-p.F3496L	Paternal	Cis	Father is gene carrier, asymptomatic	Exertional syncope, exertional VT	Nadolol	No
2	F	Hispanic	9	No	*RYR2*-p.R417L	*RYR2*-p.F3496L	Paternal	Cis	Sibling of subject 1	Family screening, exertional VT	Untreated	N/A
3	M	Caucasian	12	Yes	*RYR2*-p.S3938R	*RYR2*-p.R485Q	Unknown	Unknown	No suspected/known CPVT	Exertional SCA	Atenolol & ICD	No
4	F	Caucasian	12	Yes	*RYR2*-p.I2075T	*RYR2*-p.K4594R	Obligate paternal inheritance	Cis	Positive for SCA in sister	SCA	Metoprolol & ICD	No
5	F	Caucasian	10	No	*RYR2*-p.I2075T	*RYR2*-p.K4594R	Obligate paternal inheritance	Cis	Sister of subject 5	Exertional SCA	Metoprolol & ICD	No
6	F	Caucasian	5	No	*RYR2*-p.I2075T	*RYR2*-p.K4594R	Paternal inheritance	Cis	Paternal cousin of subject 5	Asymptomatic	ICD	N/A
7	F	Caucasian	12	Yes	*RYR2*-p.R2028H	*RYR2*-p.Y4721C	Variant #1 from mother, variant #2 from father	Trans	Parents are phenotypically silent heterozygous carriers	Exertional SCA	Atenolol & ICD	Yes
8	M	Arab	7	Yes	*RYR2*-p.T1107M	*CASQ2*-c.IVS5+1G>C (homozygous)	*CASQ2* inherited from consanguineous parents	Trans	Parents are first cousins No suspected/known CPVT	Exertional syncope, SCA, VT on EST	Nadolol, flecainide, ICD, sympathectomy	Yes
9	M	Caucasian	newborn	Yes	*RYR2*-p.R2474K	*RYR2*-p.A1136V	De novo	Unknown	No suspected/known CPVT	Exertional SCA	Atenolol, later changed to nadolol & ICD	Yes

10	F	Hispanic	11	Yes	*RYR2*-p.L4188P	*RYR2*-p.G1886S	Unknown	Unknown	No suspected/known CPVT	Seizures, emotional SCA	Nadolol	No
11	F	Caucasian	4	Yes	*RYR2*-p.S2246L	*RYR2*-p.G1886S	Unknown	Unknown	No suspected/known CPVT	SCA	Nadolol & ICD	No
12	M	Caucasian	9	Yes	*RYR2*-p.H2464D	*RYR2*-p.G1885E	Mother gene negative, father unknown	Unknown	No suspected/known CPVT	Exertional syncope, epilepsy	Atenolol, later changed to nadolol, flecainide & valproate	No
13	M	White	7	Yes	*RYR2*-p.R2401H	*DSG*-p.V288I	Unknown	Unknown	No suspected/known CPVT	Exertional SCA	Nadolol	No
14	M	White	5	Yes	*RYR2*-p.G4772S	Multiple*	Unknown	Unknown	SCA in multiple relatives (symptomatic cousin carries *RYR2*-G4772S)	Exertional syncope, SCA, & VT storm	Nadolol & ICD	Yes
15	F	Arab	8	Yes	*RYR2*-p.A2317E	*SCN5A*-p.Q692K	Unknown	Unknown	Sudden death in maternal grandfather (swimming at 39 years old)	Exertional SCA	Nadolol, ICD & LCSD	No

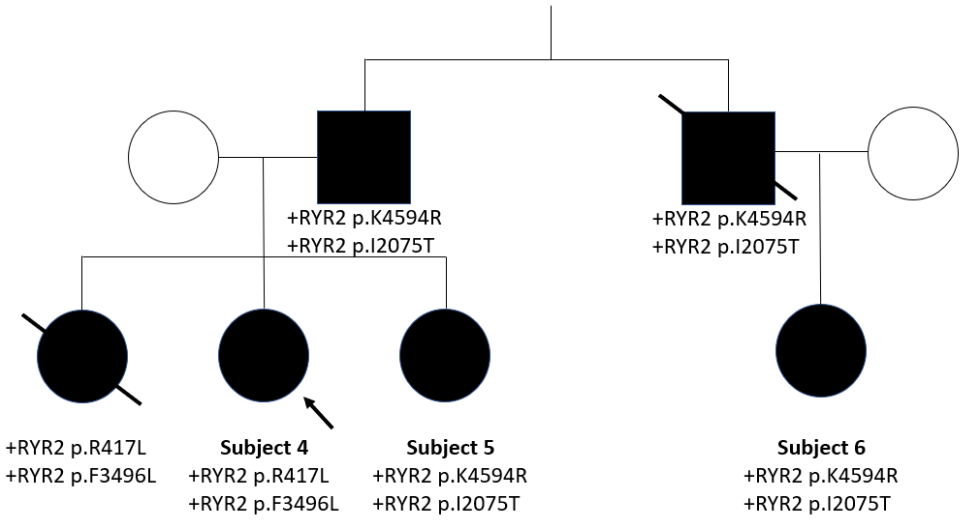
Table 7.1: Clinical characteristics and outcomes of multi-variant carriers

*Subject 14 had additional variants as follows: *RYR2*-c.3599-9delT, *RYR2*-c.14091-11dupT, *CACNA1c*-p.T1870M, *CACNA1C*-c.5680+11C>T, *TMEM43*-c.512+19G>T, *PKP2*-c.2300-4G>C, *DSP*-p.R1458G. N/A = not applicable

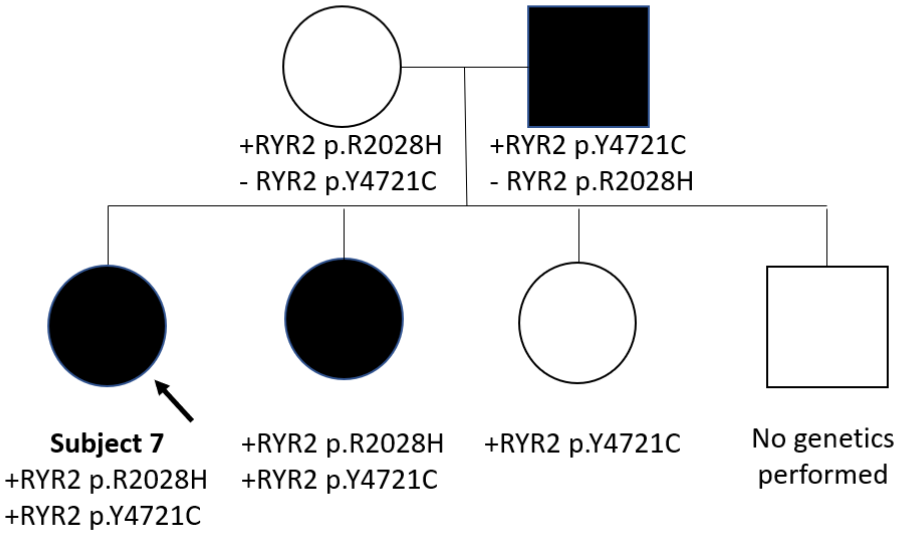
Figure 7.2



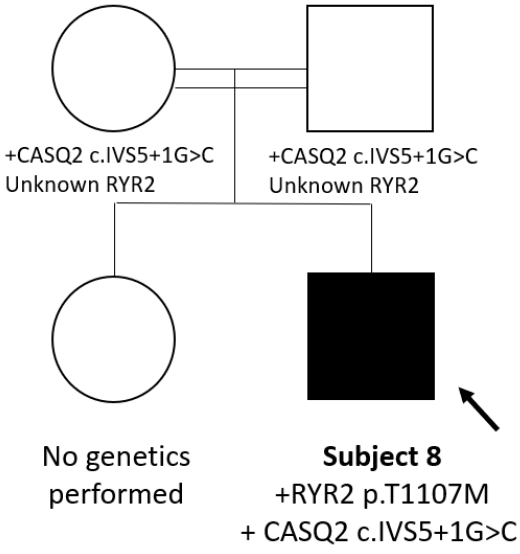
Pedigree 1: Family of Subjects 1 & 2



Pedigree 2: Family of Subjects 4, 5 & 6



Pedigree 3: Family of Subject 7



Pedigree 4: Family of Subject 8 (note parents are 1st degree cousins)

Figure 7.2: Pedigrees of select families

Therapies & Outcomes: Anti-arrhythmic therapy was instituted in 13 of 15 patients (87%). One patient received no treatment (subject #2), and in one patient, only an implantable cardioverter-defibrillator (ICD) was used (subject #6). A beta-blocker was prescribed in all treated patients. Therapeutic escalation with flecainide and left cardiac sympathectomy was necessary in subject #8 due to arrhythmias on beta-blockers. He continued to have events despite these ancillary treatments. Subject #12 also received flecainide for refractory arrhythmias and valproate for seizures, while subject #15 eventually had a left cardiac sympathectomy. An ICD was implanted in 10 of 15 patients (67%), 9 of which were for secondary prevention after SCA. Treatment failure occurred in 4 of 13 subjects (31%) on medication. No patients died during a median follow-up of 4.3 years (IQR 2.5-6.1).

Genetic Analysis: Based on American College of Medical Genetics and Genomics (ACMG) criteria, 13 of 29 variants (45%) were defined as pathogenic/likely pathogenic (P/LP) after a review of the literature and population allele frequencies in the Exome Aggregation Consortium (ExAC) browser.[32] A remaining 16 (65%) were variants of unknown significance (VUS) by ACMG criteria. Table 7.2 and the supplemental material (Appendix E) summarize the variants in this population and the clinical and molecular data to support variant pathogenicity using our stepwise

approach to classification. We then undertook a detailed analysis of *RYR2* variants using the 3D structure of pig and mouse RyR2[206, 207] (Fig. 7.1 & 7.2) based on several rationales. Firstly, predicting the pathogenicity of variants on sequence alone does not consider the chemical environment of the affected residues. Substitutions of amino acid residues involved in protein folding, domain-domain interactions, and interactions with auxiliary ligands are much more likely to affect function than residues simply pointing to solvent. This type of information is not available from sequence-based algorithms like the Polyphen score. Secondly, knowledge of the 3D environment can give possible clues on the disease mechanism.[208] Of 21 *RYR2* variants identified in this study, 12 could be mapped on the open-state structure of RyR2 (Fig. 7.2 and 7.3), and 11 would likely have a damaging effect on channel function (p.R417L, p.R485Q, p.S3938R, p.K4594R, p.Y4721C, p.S2246L, p.H2464D, p.R2401H, p.L4188P, p.A2317E and p.T1107M). Of note, 2 of these were initially classified as VUS by ACMG criteria (p.L4188P and p.A2317E). In contrast, one *RYR2* pathogenic variant (p.A1136V) and one VUS (p.R2028H) by ACMG standards appeared benign based on structural mapping and sequence conservation. The model for each of these variants appear in Figures 7.2 & 7.3.

Table 7.2

Subject(s)	Variants	Reported pathogenicity from commercial testing lab	ExAC browser allele frequency	Pathogenicity re-classification based on ACMG Criteria	Predicted structural impact based on RyR2 model
1, 2	*RYR2*-p.R417L (Figure 7.3A)	P/LP	Absent	Likely Pathogenic	R417 is located near the anion-binding site in domain C, at domains A-C and B-C interfaces. The inter-domain area is dominated by hydrophilic and charged residues. The R417L variant would introduce a shorter, hydrophobic side chain in place of a bulky, positively charged side chain, which may alter the anion binding and cause domain-domain rearrangements.
	RYR2-p.F3496L	VUS	Absent	Likely Pathogenic	F3496 is located in an intrinsically disordered alpha-solenoidal region of RyR2 (Sol2).
3	*RYR2*-p.R485Q (Figure 7.3A)	VUS	0.00008645	Likely Pathogenic	R485 is located inside an alpha helix of domain C, buried within the helical bundle. The R485 side chain forms a salt bridge with the E411, located in another helix facing domains A and B. The R485Q variant would break this interaction, destabilizing domain C, and affect the anion binding site.
	RYR2-p.S3938R	P/LP	Absent	Likely Pathogenic	S3938 is located in the CSol3 region of RyR2. S3938 is near the pore, within the cytosolic side of the channel. Mutation to bulkier, positively charged side chain may alter hydrogen bonding pattern at this site and/or disrupt surrounding alpha helices structure.
4, 5, 6	*RYR2*-p.I2075T (Figure 7.3B)	P/LP	Absent	VUS	I2075 is located within the Sol2 region, where it is buried between two helices. The Ile residue is surrounded by hydrophobic residues. The variant is close to an interface with Csol3 region, and thus the variant may impact this inter-domain interaction.
	RYR2-p.K4594R (Figure 7.3C)	VUS	Absent	Likely Pathogenic	K4594 is located at the cytosolic edge of the pseudo voltage-sensing domain (pVSD), next to the thumb and forefingers (TaF) domain. These domains are implicated in the binding of activating ligands and channel opening. Although the

					K4594R substitution is conservative, the guanidinium group of Arg allows for a larger number of interactions or may facilitate a stronger interaction with nearby E4200. The ATP/Caffeine binding sites located nearby, thus any small perturbation in this area is likely to alter channel gating.
7	*RYR2*-p.R2028H	P/LP	Absent	VUS	R2028 is found in Sol2 region of RyR2, pointing toward the solvent. The variant is unlikely to have a major impact on the function, but may influence binding to an unknown auxiliary protein.
	RYR2-p.Y4721C (Figure 7.3D)	P/LP	Absent	Likely Pathogenic	This residue is located within the transmembrane region of pVSD. This region plays an important role in allosteric gating of the channel and the Tyr is surrounded by other hydrophobic residues. Mutation to cysteine is likely to perturb channel gating and domain packing.
8	*RYR2*-p.T1107M (Figure 7.4A)	VUS	Absent	Pathogenic	T1107 is located within the SPRY2 domain, where it is buried and surrounded by hydrophobic residues. The variant would form steric clashes with W1156 and cause destabilization of the domain, as shown in a crystallographic study of this mutant[209]. Functional experiments have shown it affects Ca^{2+} release properties (see suppl. Appendix E).
	CASQ2-c.IVS5+1G>C	P/LP	Absent	VUS	Not performed
9	*RYR2*-p.R2474K (Figure 7.4B)	P/LP	Absent	Likely pathogenic	R2474 is located in the Sol2 region of RyR2, near two other mutations. Region is poorly resolved in CryoEM structures. The variant is subtle and structural predicted suggests a minimal impact. It is currently unknown whether any auxiliary protein binds to this region.
	RYR2-p.A1136V	VUS	0.007063	Likely pathogenic	A1136 is located within the SPRY2 domain. The equivalent residue in both RyR1 and RyR3 is a valine, therefore the mutation is unlikely to have significant negative impact on the overall structure of RyR.
10	*RYR2*-p.L4188P (Figure 7.4C)	VUS	Absent	VUS	L4188 is located within a helix as part of the TaF domain that clamps the C-terminal extension of the RyR. This interaction is critical for channel gating. The substitution to Pro may promotes helix breaking, and potentially perturb channel gating.
	RYR2-p.G1886S	VUS	0.04385	VUS	G1886 is located in a flexible unstructured loop as part of Sol2 region. Though the substitution alone is unlikely to have an impact on channel gating, it may have indirect effects such as creation of a new phosphorylation site.
11	*RYR2*-p.S2246L (Figure 7.4D)	P/LP	Absent	Pathogenic	S2246 is located within the Sol2 region, where the side chain is tightly packed next to an alpha helix. Mutation to a longer side chain likely results in steric clashes, and will impact helix packing in this region.
	RYR2-p.G1886S	VUS	0.01540	VUS	G1886 is located in a flexible unstructured loop as part of Sol2 region. Though the substitution alone is unlikely to have an impact on channel gating, it may have indirect effects such as creation of a new phosphorylation site or alter bidding to auxiliary protein(s).
12	*RYR2*-p.H2464D	P/LP	Absent	Pathogenic	H2464 is located within a poorly resolved Sol2 region of RyR2 structure. The variant may impact binding of an unknown auxiliary protein to this region.
	RYR2-p.G1885E	VUS	0.04385	VUS	G1885 is located in a flexible unstructured loop of RyR2, and thus cannot be mapped onto the existing structure.
13	*RYR2*-p.R2401H (Figure 7.4B)	P/LP	Absent	Likely Pathogenic	R2401 is located within the Sol2 region, near two other CPVT associated mutations. Substitution to His may have an impact on helix stability.
	DSG-p.V288I	VUS	Absent	VUS	Not performed
14	*RYR2*-p.G4772S	P/LP	Absent	VUS	G4772 is located in the pore forming domain (PFD), as part of the outer helix. Substitution to less flexible Ser may affect helical packing within the membrane and cause subtle domain rearrangements.
	CACNA1c-p.T1870M	VUS	Absent	VUS	Not performed
	RYR2-c.3599-9delT	VUS	Absent	VUS	Not performed
	RYR2-c.14091-11dupT	VUS	Absent	VUS	Not performed

	CACNA1C-c.5680+11C>T	VUS	Absent	VUS	Not performed
	TMEM43-c.512+19G>T	VUS	Absent	VUS	Not performed
	PKP2-c.2300-4G>C	VUS	0.00008079	VUS	Not performed
	DSP-p.R1458G	P/LP	0.001737	VUS	Not performed
15	*RYR2*-p.A2317E (Figure 7.B)	P/LP	Absent	VUS	A2317 is in an alpha solenoid region, near two other CPVT associated mutations. Mutation to the larger Glu residue likely forms steric clashes with nearby residues, and this is likely to affect packing and stability of the region.
	SCN5A-p.Q692K	P/LP	0.0002822	VUS	Mutation site is on the 1-2 linker (DI-II loop) near the start the VSD of the cardiac voltage-gated sodium channel (NaV1.5). The region is important to gating function of the channel and is in close proximity to the CaMKII binding site. The mutation may also influence binding to other auxiliary proteins.

Table 7.2: Clinical and molecular data supporting variant classification

Figure 7.3

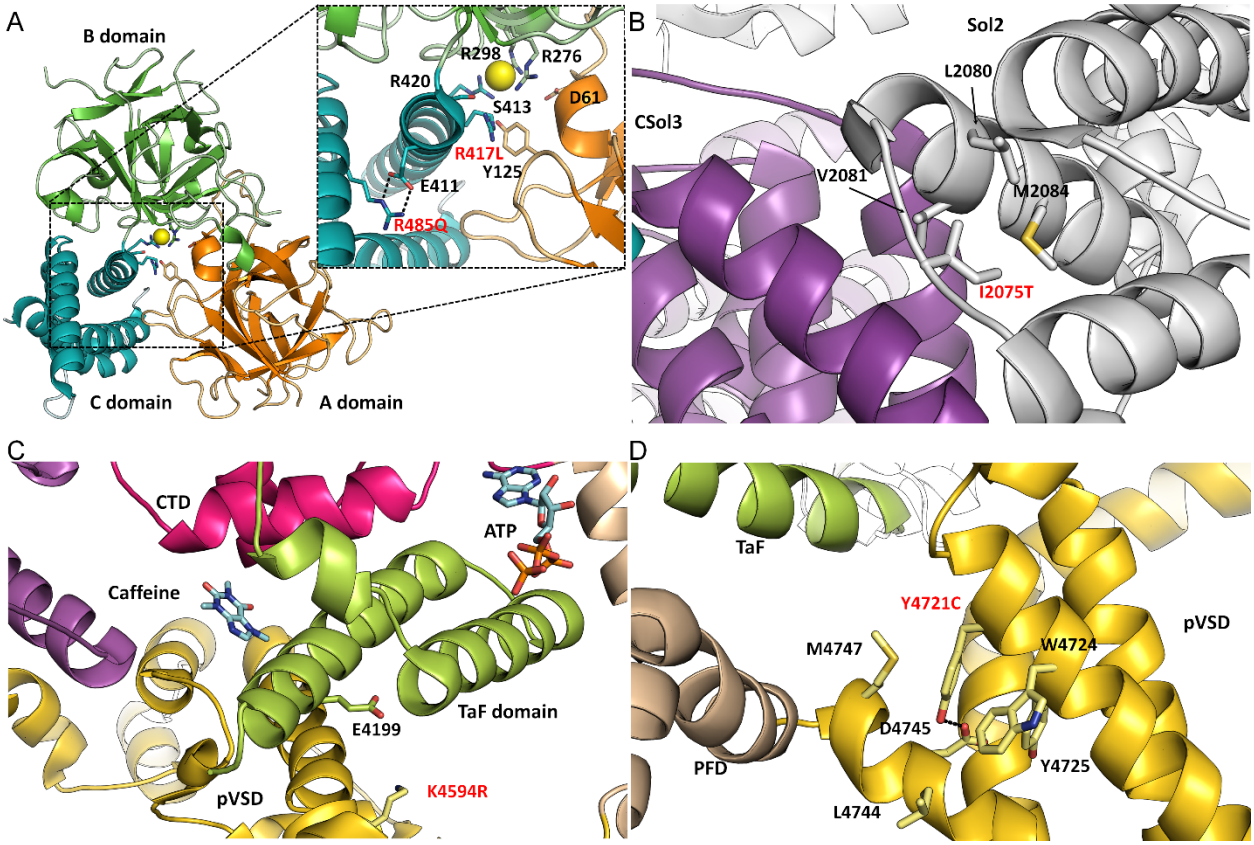


Figure 7.3: Location of CPVT-associated variants on RyR2 structure.

Enlarged view of the insets are presented to highlight location of the variants and nearby residues/secondary structures. Domains are coloured according to the linear sequence scheme shown in Figure 7.1. All variants occur in highly conserved regions of all three RyR isoforms.

Unless specified, the structures are based on the 4.2 Å resolution CryoEM open-state structure of RyR2 from porcine heart[206], PDB: 5GOA. (**A**) *RYR2*-p.R417L and *RYR2*-p.R485Q mutants are located centrally within RyR2 ABC domains, near the anion binding site. Residues involved in the formation of the Cl^- pocket are highlighted. R485 forms a salt bridge interaction with nearby E411 residue. (**B**) The *RYR2*-p.I2075T is located within a flexible linker, joining two alpha-helices together in the Sol2 region. The Ile is surrounded by nearby hydrophobic residues. (**C**) The *RYR2*-p.K4594R variant is located in the pVSD domain, near the TaF domain. Mutation to Arg, may potentially form a salt bridge with a nearby E4199 located on the TaF domain. In RyR1 open-state structure[210] the K4594 is located near activating ligands such as caffeine and ATP, PDB: 5TAQ. (**D**) The *RYR2*-p.Y4721C variant is located within the transmembrane region of pVSD, and unsurprisingly the Y4721 is surrounded by other hydrophobic residues. Y4721 is potentially forming a polar contact with carboxyl group D4745.

Figure 7.4

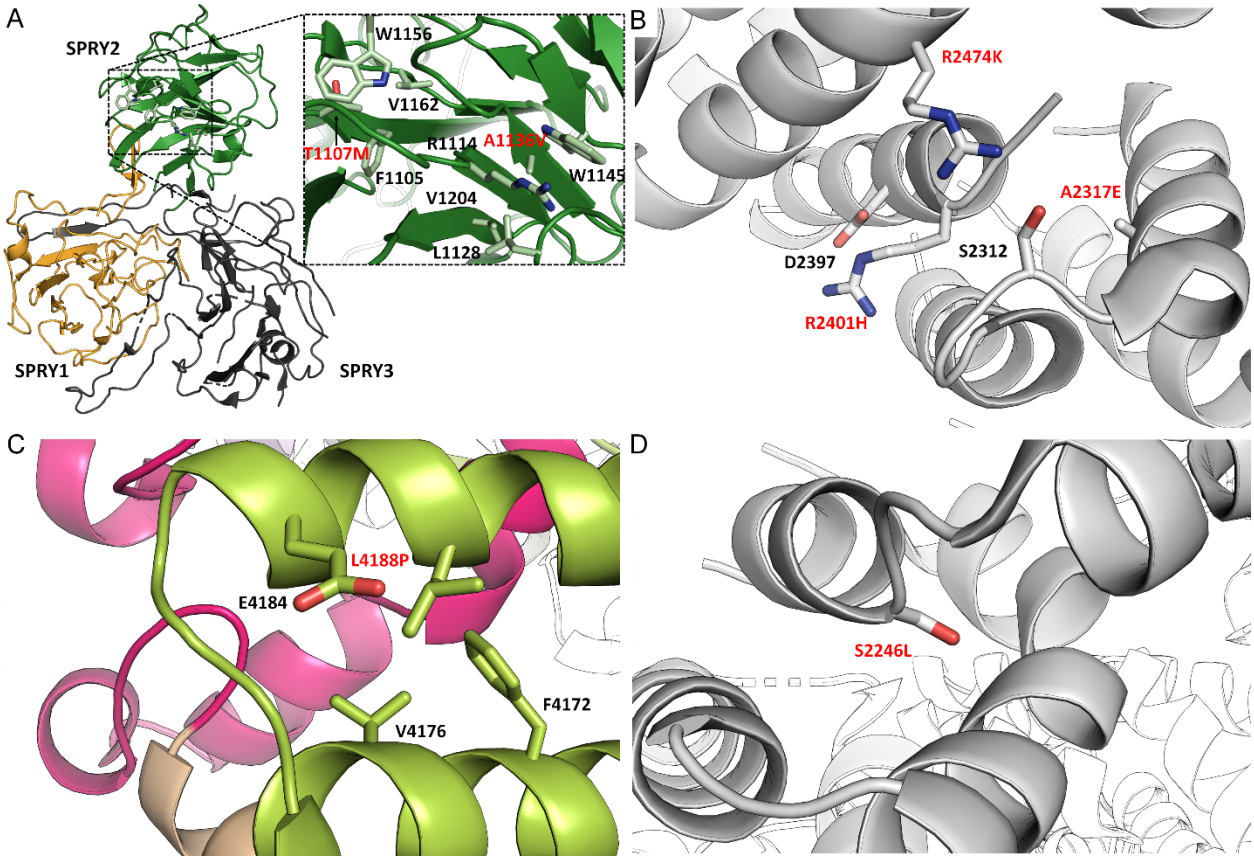


Figure 7.4: Location of CPVT-associated variants on RyR2 structure (continued).

(**A**) *RYR2*-p.T1107M and p.A1136V mutations are located within the SPRY2 domain, where they are buried and surrounded by hydrophobic residues. (**B**) The *RYR2*-p.R2474K, R2401H, and RYR2-A2317E mutants are clustered within the central alpha-solenoid region of RyR2 (Sol2). The R2401 and R2474 potentially interacts with nearby D2397 and S2312 respectively. These mutations are in close proximity to the CSol3 region and the unstructured region of Sol2. This region is intrinsically flexible and generally poorly resolved in the CryoEM map. (**C**) *RYR2*-

p.L4188P mutation is located in the TaF domain where it is surrounded by hydrophobic residues. (**D**) *RYR2*-p.S2246L mutation is located in the better resolved region of Sol2, where the side chain is tightly packed against neighbouring alpha helix.

7.5 Discussion

In this study, multi-variant CPVT occurred in 8% of the PACES CPVT Registry. Nearly three-quarters of these children were SCA survivors and many had variants that were likely pathogenic. Collectively, our data contribute to the hypothesis that inherited arrhythmia patients with multiple variants have poor outcomes, termed a cumulative dosage effect. Even in the absence of prognostic utility, which cannot be clearly established in the present study, multi-variant CPVT is relevant to decision-making around cascade family screening. In total, there were 3 possible situations observed: (1) double variants in cis, (2) compound heterozygous variants in trans, and (3) digenic heterozygous variants (different genes). As will be discussed, each scenario creates a unique set of diagnostics, cascade screening and prognostic implications.

The cumulative gene dosage phenomenon is similarly rare for hypertrophic cardiomyopathy,[201, 202] arrhythmogenic right ventricular cardiomyopathy,[203] and LQTS,[200, 204] compared to CPVT in the PACES Registry. Multi-variant CPVT children were usually severely affected (73% with SCA). In comparison to the rest of the Registry population (38% with SCA), the severity of multi-variant CPVT appeared greater. However, statistical comparisons between single vs. multiple variant phenotypes were not undertaken due to the relatively small population described here, and the historical uncertainty around the genetic testing protocols/techniques which pre-dated enrollment in the registry. For example, early on, commercial genetic testing companies were only testing select *RYR2* exons and older reports in the Registry were sometimes incomplete. Additionally, the second variant was sometimes a VUS, and/or potentially benign based on our mapping and/or found in cis phase. In such settings, CPVT may be driven by a single variant or the second variant could be a risk modifier. An example is *RYR2*-p.G1886S, which occurred in 2 of our patients, and was also seen in the general population.[32] A recent study has shown that this variant is a significant risk factor for ventricular arrhythmias in heart failure patients,[211] suggesting that it could be a candidate risk modifier in CPVT. A parallel effect exists in the LQTS genes where common variants underlie a susceptibility to drug-induced QT prolongation [212, 213] but do not cause overt LQTS. For example, *KCNE1*-p.D85N is too common

in the population to independently cause LQTS, but significantly increases risk if a second LQTS mutation occurs.[214] In our cohort, subject #14 carried a *CACNA1C* VUS and a pathogenic *RYR2* mutation (plus several other *RYR2* VUS), and had a classic CPVT phenotype (catecholamine triggered bidirectional VT) with some QT prolongation. We hypothesize that a possible LQTS variant, plus multiple variants linked to CPVT, may have collectively contributed to his severe overlap phenotype (ie. digenic heterozygosity). Quite remarkably, another boy had two forms of CPVT (type-1 due to *RYR2*-p.T1107M and type-2 due to *CASQ2*-c.IVS5+1G>C). While CPVT type-2 alone can be especially dangerous, the *RYR2*-p.T1107M variant also likely has a damaging role based on the present data, and previous studies showing both a clinical and *in vitro* phenotype.[209, 215] The growing international CPVT registry and prospective data are needed to clarify the risk and incidence of multi-variant CPVT.

The presence ≥2 variants creates other logistical problems. We could not differentiate between cis and trans variants in some cases owing to the inconsistent screening of the parents. Incomplete parental screening may be due to the clinicians' uncertainty regarding the disease-causing variant in the family, thus demonstrating another challenge in this circumstance. Variants in cis phase similarly confounds screening in hypertrophic cardiomyopathy.[216] In cis phase, the CPVT phenotype would not necessarily be worse than any given single variant. However, the presence of ≥2 cis variants is relevant, as it demonstrates the complexities around family screening in the setting of CPVT. *RYR2* variants in trans phase have a theoretical mechanism for increased severity. RyR2 is a large, homotetrameric protein made up of 4 subunits. Two variants in trans phase would mean that all 4 subunits making up the channel would be mutated. In contrast, in the typical case of autosomal dominant CPVT, half of the four subunits would be wildtype. Theoretically, this could account for a more severe phenotype in compound multi-variant CPVT. Based on the present study, we propose that targeted sequencing for both variants be performed in the clinical setting, and that relatives, especially parents, need to be evaluated by an expert to clarify the role of each variant.

The unclear pathogenicity of *RYR2* variants is a growing concern in CPVT.[217] We used a standardized bioinformatics approach to variant interpretation to avoid overcalling pathogenicity. After applying the ACMG criteria, we mapped variants on the open-state structure of RyR2 to see if any other insights could be obtained. This technique relies on the 3D structure derived from high resolution studies of the ryanodine receptor. A good example of this is the N-terminal region of

RyR2, which consists of three domains: domain A (residues 1–217) and domain B (residues 218–409), and domain C (residues 410–543) (Fig. 7.1).[191, 218, 219] A chloride ion is coordinated by residues of all three domains and disruption of this binding site *via* disease-causing variants results in domain reorientations.[190, 191, 207] These observations suggest that CPVT variants may destabilize domain interfaces or disrupt the folding of individual domains, which would impair domain-domain interactions and cause adverse effects on channel gating.[190, 191, 207, 210, 219-221] Structural analyses supported downgrading p.A1136V and p.R2028H to likely benign. *RYR2*-p.A1136 is located in a relatively non-conserved region within the SPRY2 domain, where its equivalent residue in both RyR1 and RyR3 is a valine, thus substitution to valine is unlikely to alter the function of RyR2 significantly. Further, structural mapping showed that valine substitution can be easily accommodated without the formation of steric clashes. The p.R2028H mutation is located in a flexible region of RyR2, where the side chain is pointing towards the solvent, and thus the mutation is unlikely to have a major impact on channel function. These are not functional assays, so the conclusions are predictive in nature.

This study is limited by its retrospective design. Genetic testing spanned nearly 15 years, and not all commercial testing companies provided technical details as would be required in the contemporary era. Results for family members were sometimes not available (often if followed by a non-participating center). Early commercial sequencing methods could not differentiate between two variants in the cis vs. trans position of *RYR2* (GeneDx™, unpublished communication). Limitations also exist in the structural analysis of variants, whereby some portions of RyR2 structure are poorly defined in the CryoEM structure. The best-defined regions are domains whose structures have been determined *via* X-ray crystallography (N-terminal domains, SPRY1/2, Rep12, and Rep34 domains), followed by the C-terminal and transmembrane regions, for which the resolution of CryoEM studies is the highest.[208] As such, direct analysis of variants in the N-terminal and C-terminal hotspots is the most reliable. For most other sections, direct analysis of hydrogen bonds and ionic interactions of the variants is not yet possible, however their general location in the 3D structure can be determined at the current resolution for RyR2. A detailed supplemental file (Appendix E) disclosing all the supporting data is provided to facilitate re-classification by future researchers as the field advances.

7.6 Conclusions

More than one variant may underlie a minority of CPVT cases. This poses challenges with respect to diagnosis and family counselling. While multi-variant CPVT patients were usually severely affected, further research is needed to determine the significance and generalizability of this observation. We demonstrate that a rigorous approach to variant re-classification using the ACMG criteria and 3D mapping is important in reaching an accurate diagnosis, especially in the multiple variant population.

Chapter 8: A Novel *RYR2* Loss-of-Function Mutation (I4855M) is Associated with Left Ventricular Non-compaction and Atypical Catecholaminergic Polymorphic Ventricular Tachycardia

8.1 Abstract

Background: CPVT is a channelopathy usually caused by gain-of-function mutations ryanodine receptor type-2 (RyR2). Left ventricular non-compaction (LVNC) is an often genetic cardiomyopathy. A rare LVNC-CPVT overlap syndrome may be caused by exon 3 deletion in RyR2. We sought to characterize the phenotypic spectrum and molecular basis of a novel RyR2 mutation identified in a family with both conditions.

Methods: Several members of an affected family underwent clinical and genetic assessment. A homology model of the RyR2 pore-region was generated to predict the location and potential impact of their RyR2 mutation. Ca^{2+}-release assays were performed to characterize the functional impact of the RyR2 mutant expressed in HEK293 cells.

Results: A multigenerational family presented with a history of sudden death and a phenotype of atypical CPVT and LVNC. Genetic testing revealed a *RYR2* mutation (p.I4855M) in two affected individuals. A homology model of the RyR2 pore-region showed that the p.I4855M mutant residue is located in the highly conserved 'inner vestibule', a water-filled cavity. p.I4855M may interfere with Ca^{2+} permeation and affect interactions between RyR2 pore subunits, and is thus predicted *in silico* to be damaging. Expression and functional studies in HEK293 cells revealed that p.I4855M inhibited caffeine-induced Ca^{2+} release and exerted a dominant-negative impact on wildtype RyR2.

Conclusions: This study identifies a potentially lethal overlapping syndrome of LVNC and atypical CPVT related to a novel *RYR2* variant. Structural and functional studies suggest this is a loss-of-function mutation, which exerts a dominant-negative effect on wildtype RyR2.

8.2 Introduction

Encoded by *RYR2,* the cardiac ryanodine receptor type 2 (RyR2) is a large, highly conserved ion channel that releases Ca^{2+} from the sarcoplasmic reticulum (SR), allowing the rapid efflux of Ca^{2+} needed for muscle contraction into the cytoplasm.[56, 57] In 2001, Priori et al. reported that autosomal dominant, gain-of-function (GOF) *RYR2* mutations cause CPVT,[56] a syndrome typified by exertional polymorphic VT in the setting of normal cardiac structure.[54] More recently, loss-of-function (LOF) *RYR2* variants have been implicated in idiopathic ventricular fibrillation (VF).[192, 222]

LVNC is an uncommon cardiomyopathy characterized by deep intertrabecular sinusoids communicating with the ventricular cavity.[223, 224] Causes include sarcomere gene mutations among other rare factors.[223] Recent reports of LVNC associated with exon 3 deletion in *RYR2* provided evidence of a link between LVNC and this channel.[225-227] However, these cases are rare and the role of *RYR2* in structural disease is poorly understood. We studied a multigenerational family with a phenotype of atypical CPVT and LVNC so as to better understand the molecular basis for this presentation.

8.3 Methods

Clinical Assessment: Clinical phenotyping of the proband (Figure 8.1 patient IV:4) and her living relatives was undertaken, including resting ECG, EST and echocardiography in at-risk living family members. Historical data were gathered during clinic visits and through coroner reports for deceased relatives. Commercial testing of 72 genes related to inherited arrhythmia and/or cardiomyopathy, including sarcomere-encoding genes, was undertaken in the proband. The family was consented for enrollment in our International CPVT Registry, which is approved by our institutional ethical review board.

Figure 8.1

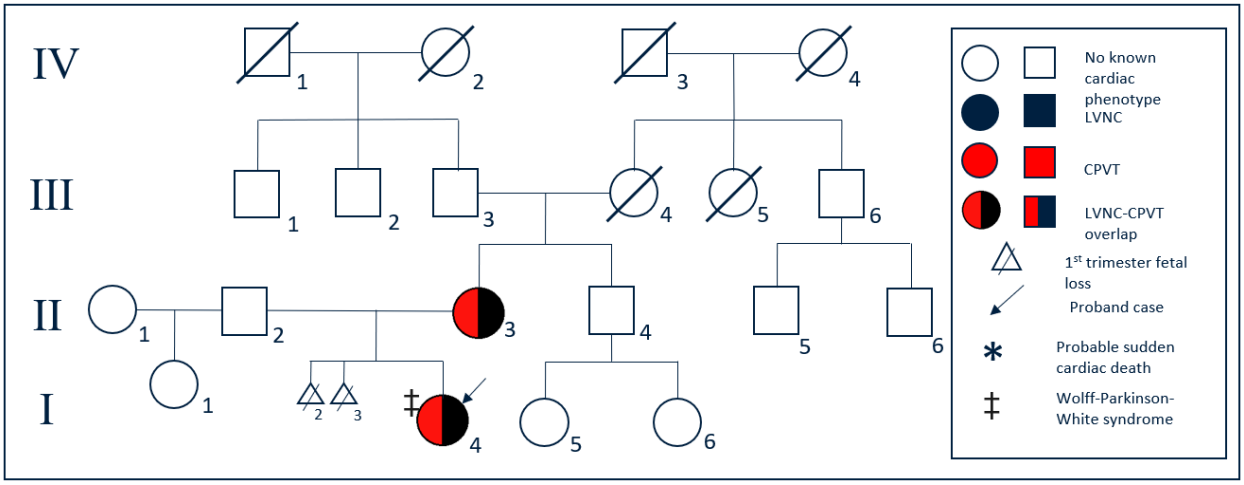


Figure 8.1: Pedigree of the family. Patient II:4 is presumed to have a CPVT-like phenotype on the basis of SUD, family history and autopsy not suggestive of significant structural/coronary heart disease. Patient I:4 had a presumed cardiac death at 40 years of age. Patient II:5 died of a brain aneurysm. Patient II:6 is asymptomatic. Patient III:6 had a reportedly normal echocardiogram. CPVT=catecholaminergic polymorphic ventricular tachycardia; LVNC=left ventricular non-compaction

Homology Modeling: We created a homology model of the RyR2 pore-forming region to determine variant location based on existing molecular data.[181, 182, 209, 228, 229] The program MODELLER (https://salilab.org/modeller/) was used, based on the cryo-EM structure of rabbit RyR1 at 3.8Å resolution.[182] This structure has an overall 65.4% sequence identity with human RyR2, and thus formed a reliable template for homology modeling.

Caffeine-induced Ca^{2+} Release in HEK293 Cells: The p.I4855M mutant was functionally characterized by using a caffeine-induced Ca^{2+} release assay in HEK293 cells as described previously[230]. HEK293 cells were transfected with 12 μg of wildtype (WT) or p.I4855M mutant RyR2 cDNAs. Cells grown for 18–20 h after transfection were harvested and loaded with 10 μM Fluo-3 AM (Molecular Probes) in high glucose Dulbecco's Modified Eagle Medium at room temperature for 60 min. The fluorescence intensity of Fluo-3 AM at 530 nm was measured before and after repeated additions of various concentrations of caffeine (0.025-5 mM) in an SLM-Aminco series 2 luminescence spectrometer (SLM Instruments) with 480 nm excitation at 25 °C. The peak levels of each caffeine-induced Ca^{2+} release were determined and normalized to the highest level (100%) of caffeine-induced Ca^{2+} release for each experiment.

8.4 Results

Clinical Phenotypes and Genetic Testing: The female proband (Figure 8.1, patient IV:4) initially presented as an asymptomatic 10 year–old for cardiac screening prior to beginning psychostimulant medication for attention deficit hyperactivity disorder due to a family history of SUD in her maternal grandmother. Her resting ECG showed pre-excitation (Figure 8.2A), which resolved at a heart rate of 150 beats/minute (Figure 8.2B) during exercise, although she did have several VPBs (Figure 8.2C). Her ECG during rest and exercise showed no evidence of QT interval prolongation. An echocardiogram revealed LVNC with normal function (Figure 8.2D, E). She was advised to undergo serial monitoring, but did not begin any treatment. Shortly after diagnosis, the girl suffered an exertionally-triggered sudden cardiac arrest (SCA). Immediate resuscitation, including two shocks from an automated external defibrillator, led to a full neurological recovery. Her left ventricular function was mildly depressed afterwards. She received an ICD and started taking carvedilol. A follow-up EST showed ventricular bigeminy, so her carvedilol dose was increased, resulting in an improved EST with only VPBs. Genetic testing revealed a novel variant in *RYR2* (p.I4855M). An electrophysiologic study was not undertaken, and magnetic resonance imaging could not be performed after an ICD was placed.

Figure 8.2

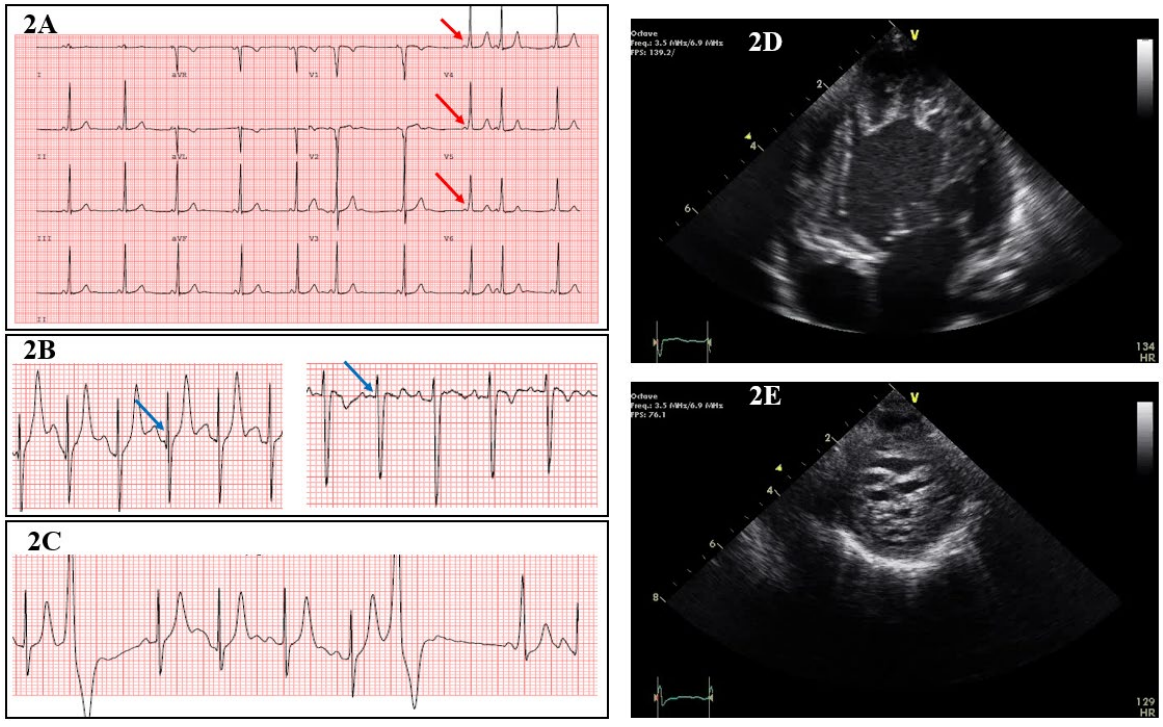


Figure 8.2: Resting ECG demonstrating pre-excitation, highlighted by red arrows (A); Abrupt resolution of pre-excitation during exercise as seen in leads II (left) and V1 (right), and highlighted by blue arrows (B); Exercise-induced VPBs (C); LVNC on resting echocardiogram in apical four chamber (D) and apical (E) views.

Cascade family screening for inherited cardiac disease was undertaken. The mother (Figure 8.1, patient III:3) of the proband was already undergoing evaluation for dyspnea by a cardiologist. She was found to have repolarization abnormalities with ST segment flattening, diffuse T-wave inversion and QT interval prolongation (500 ms) on resting ECG (Figure 8.3A). She developed polymorphic ventricular bigeminy early in exercise (Figure 8.3B) and echocardiogram showed LVNC with normal ejection fraction (Figure 8.3C, D). A signal average ECG was done but could not be interpreted due to intermittent paced beats. Genetic testing was positive for the p.I4855M variant in *RYR2*. She received an ICD shortly after diagnosis. The father and maternal uncle to the proband (Figure 8.1, patients III:2 and III:4, respectively) are asymptomatic with normal ESTs and cardiac structure, and both tested negative for the familial *RYR2* variant. The maternal grandfather to the proband (Figure 8.1, patient II:3) was also negative for *RYR2*-p.I4855M.

Figure 8.3

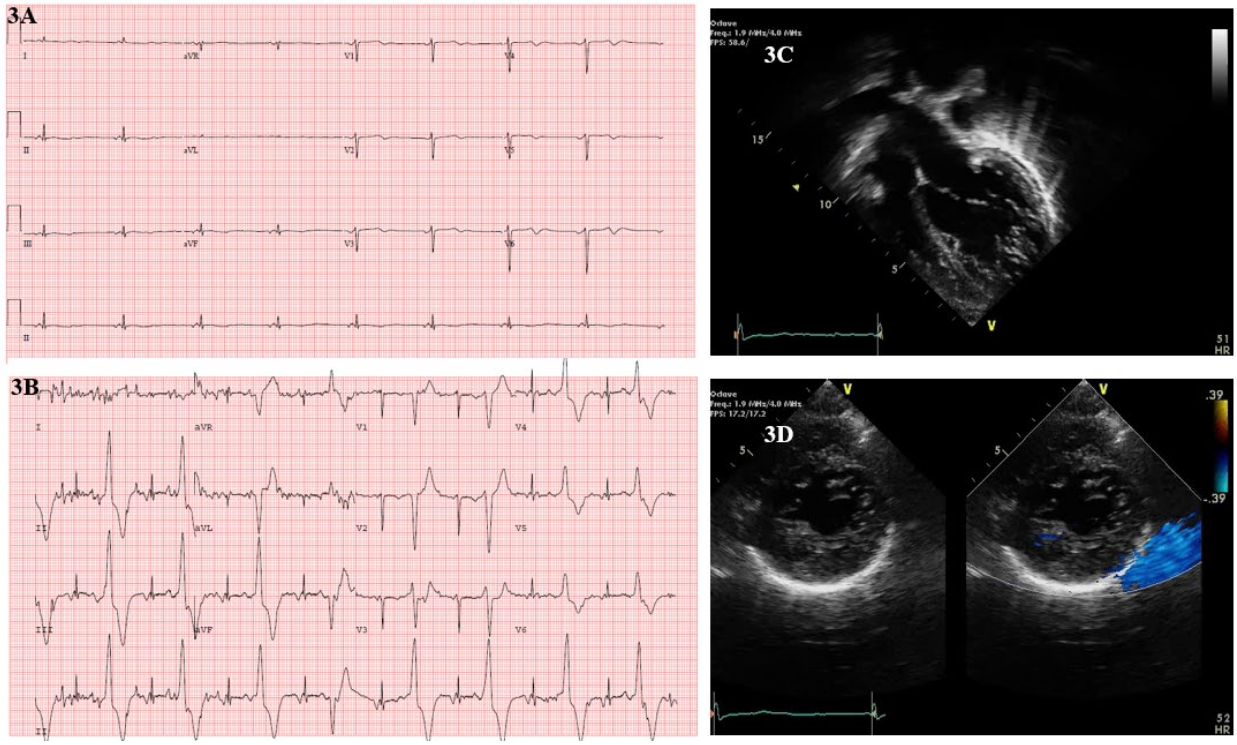


Figure 8.3: Resting ECG in the mother of the proband (patient II:4) (A); ventricular bigeminy during exercise (B), and echocardiogram showing LVNC in parasternal long (C) and parasternal short (D) axis views.

More than 15 years prior to this presentation, the proband's maternal grandmother (Figure 8.1, patient II:4) experienced an unclear progression of cardiac symptoms in the setting of a right bundle branch block, sinus bradycardia and first degree atrioventricular block. She was scheduled to have an ICD placed for unknown reasons. However, just days prior to implantation, she had a non-exercise induced SUD at 51 years of age. A standard autopsy was significant for mild right ventricular dilation, a small inter-atrial septal defect, and normal left ventricular wall thickness. No significant coronary artery disease was found. Molecular testing was not performed, however she is an obligate carrier of p.I4855M presuming the mutation was not *de novo* in patient III:3. The proband's maternal great-aunt (Figure 8.1, patient I:4) also died suddenly at 40 years of age, which was believed to be cardiac in nature. No further details are available.

Structural and Functional Analysis: Our homology model of the RyR2 pore-region showed that p.I4855M is in the 'inner vestibule', a water-filled cavity where ions can remain in a hydrated fashion (Figure 8.4). *RYR2*-p.I4855M may therefore interfere with Ca^{2+} permeation by affecting its hydration, but could also affect the position of the pore helices, thus affecting the conformation of the selectivity filter. Either of those two scenarios would likely result in a LOF by decreasing the permeability. p.I4855M affects a highly conserved amino acid residue, predicted in silico to be damaging, and is absent from the ExAc database of 60,000+ exomes.

Figure 8.4

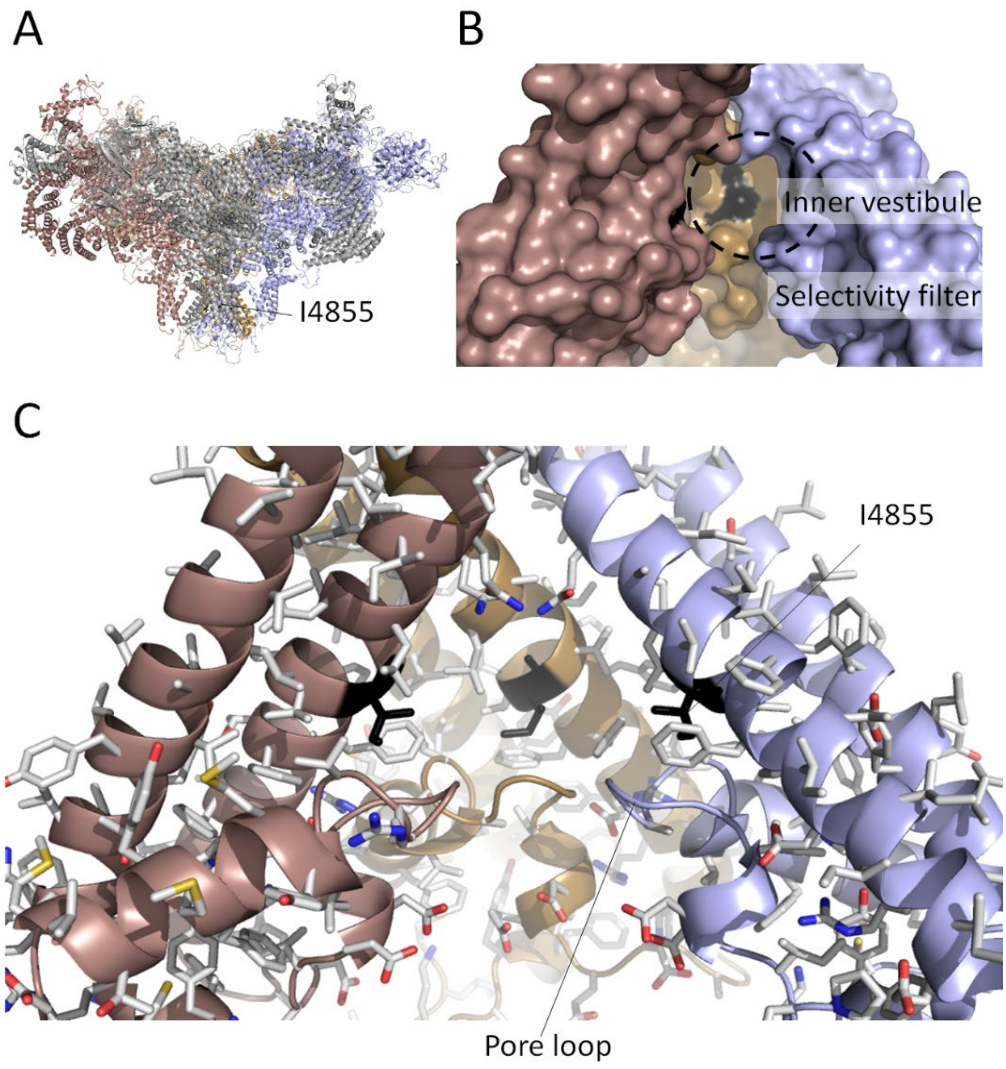


Figure 8.4A: Overall structure of human RyR2 protein, with a homology model based on the cryo-EM structure of rabbit RyR1. The four subunits are indicated in different colors. The overall location of I4855 is in the pore-forming domain.

Figure 8.4B: Surface representation of the pore forming domain, with the I4855 residue highlighted in black. One subunit is omitted for clarity. I4855 is lining the wall of the inner vestibule, an area where permeating ions can become fully hydrated.

Figure 8.4C: Detailed view of the model of the RyR2 pore domain with side chains shown in stick representations, and I4855 highlighted in black. The I4855 side chain points towards the inner vestibule. Substitution by a longer Met side chain is likely to interfere with the hydration of permeating ions. Alternatively, the Met side chain may also affect the conformation of the pore loop and thus affect permeation indirectly.

To gain insights into the effect of p.I4855M on RyR2 function, we determined the response of RyR2-WT and the p.I4855M mutant to activation by increasing concentrations of caffeine. As shown in Figure 8.5, the level of Ca^{2+} release in HEK293 cells transfected with RyR2-WT increased progressively with each consecutive addition of caffeine (from 0.05 mM to 0.5 mM), and then decreased with further additions of caffeine (1.0 mM to 5 mM), likely due to the depletion of the intracellular Ca^{2+} stores by the prior additions of caffeine (0.025 to 0.5 mM) (Fig. 8.5A, D). The p.I4855M mutation markedly inhibited caffeine activation of RyR2, leading to a dramatic rightward shift in caffeine response (Fig. 8.5B, D). HEK293 cells co-transfected with the p.I4855M mutant and RyR2-WT displayed a significant rightward shift in caffeine response (Fig. 8.5C, D), indicating a dominant negative effect of p.I4855M on the WT channel.

Figure 8.5

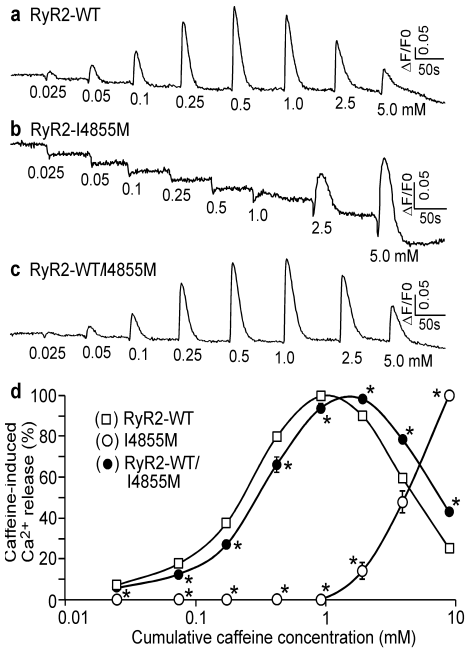


Figure 8.5: Effect of the P.I4855M mutation on the sensitivity of caffeine activation of RyR2. HEK293 cells were transfected with RyR2-WT (a) or P.I4855M (b), or co-transfected with RyR2-WT and P.I4855M (c). Fluorescence intensity of the fluo-3 loaded transfected cells before and after repeated additions of increasing concentrations of caffeine (0.025-5mM) was monitored continuously. (d) Ca^{2+} release–cumulative caffeine concentration relationships in transfected HEK293 cells. The amplitude of each caffeine peak was normalized to that of the maximum peak (100%) for each experiment. Data shown are mean ± SEM (n = 8) (*$P < 0.05$ vs WT).

WT = wildtype

8.5 Discussion

While *RYR2* mutations are traditionally implicated in young CPVT patients with life-threatening ventricular arrhythmias,[56] *RYR2* may also influence myocardial development and structure.[226, 231, 232] Data from this multigenerational family support the pathogenic role of a novel *RYR2* variant in LVNC, atypical CPVT and SUD. In contrast to most known CPVT mutations, we report that a LOF *RYR2* variant can underlie this rare and variable phenotype.

Several theories exist to account for the overlapping phenotypes seen in *RYR2* mutation carriers. GOF variants likely reduce the threshold for RyR2 channel activation through increased sensitivity to luminal or cytosolic Ca^{2+}.[56] Conversely, pathogenic LOF variants attenuate channel activation by Ca^{2+}.[192] In an early report implicating *RYR2* in arrhythmogenic right ventricular cardiomyopathy (ARVC), Tiso et al. suggested that excessive Ca^{2+} leak from the SR induces myocardial necrosis.[232] Knowledge has since evolved, including recent evidence implicating *RYR2* variants in up to 9% of genetically-elusive ARVC cases,[231] which may be explained by altered signaling pathways dependent on intracellular Ca^{2+} homeostasis, leading to structural heart disease.[231] However, neither of these publications are supported by functional studies, making it difficult to hypothesize a potential mechanism, or comment on the probability of causation. It is important to note that *RYR2* variants related to cardiomyopathy may alternatively exert a modifier, rather than a causative effect in this phenotype. Accordingly, in our study and in a previous report,[227] the CPVT phenotype was more penetrant than the structural phenotype, suggesting that an added genetic "hit" may be needed in cardiomyopathy. It remains possible, although unlikely, that this family also has an ARVC phenotype related to the *RYR2* mutation. However, in the setting of a SUD, LVNC and a *RYR2* variant, it seems more likely that either LVNC and/or CPVT is the major contributing cause of the proband's arrest. Unfortunately, a magnetic resonance image could not be obtained after placement of ICDs in both living patients.

A link between *RYR2* and other structural phenotypes already exists, including an in-frame deletion in the N-terminal region in *RYR2*, which includes exon 3, leading to conduction disease, atrial arrhythmia and dilated cardiomyopathy.[226] This observation is supported by similar overlapping LVNC-CPVT phenotypes in the setting of exon 3 deletion in *RYR2*.[225, 227] These large deletions appear to reduce the threshold for termination of Ca^{2+} release and increase fractional SR Ca^{2+} release, causing a further excess of Ca^{2+}, which may induce LVNC.[215, 227] This mechanism is

still unproven, and the missense *RYR2* variant found in our family is not in exon 3 or the N-terminus, and does not appear to result in excess Ca^{2+} release. However, our data do support a relationship between LVNC and *RYR2* through a loss of channel function, and a missense mutation, rather than total deletion of exon 3. This adds to a growing body of literature implicating abnormal Ca^{2+} homeostasis in structural heart disease, which includes a highly penetrant arrhythmogenic cardiomyopathy related to phospholamban,[233] a protein that also regulates Ca^{2+}. Although the mechanism of *RYR2*-related LVNC is presently elusive, several studies now implicate *RYR2,* a highly conserved gene, in families with various forms of structural and arrhythmic heart disease,[226, 227, 231, 232] supporting the hypothesis that the *RYR2* phenotype extends beyond typical CPVT. As such, it is reasonable to consider sequencing *RYR2* in particularly arrhythmogenic cases of cardiomyopathy, or in the setting of genetically-elusive LVNC.

Polymorphic VT is a hallmark of CPVT. In our proband, we were unable to obtain a recording of the arrhythmia leading to SCA (i.e. monomorphic VT vs. polymorphic VT/VF), which makes it challenging to distinguish between a structural and non-structural trigger. In the ARVC-CPVT overlap population, arrhythmias tend to be exertional,[226, 227, 232] and the phenotype can more closely resemble CPVT rather than a primary structural cardiomyopathy. This was also observed in our proband case, suggesting a shared predilection to the CPVT phenotype.[231, 232] p.I4855M is an unusual *RYR2* variant as it suppressed caffeine-induced Ca^{2+} release, suggesting a LOF effect. The few verified LOF *RYR2* mutations are more closely associated with idiopathic VF[192, 222] than with CPVT arrhythmias, and none appear related to structural heart disease. While the circumstances preceding SUD in patients II:4 and I:4 were not clearly exercise-related, non-exertional triggers of CPVT arrhythmias may be more common than previously thought.[58] This family has some features suggestive of CPVT, but also findings consistent with idiopathic VF. For example, the identification of a LOF *RYR2* mutation and relatively mild exercise-induced ventricular ectopy are not typical for CPVT. However, idiopathic VF cannot be diagnosed in the setting of structural heart disease,[53] or presumably in the setting of pre-excitation, which is likely a complication of non-compaction *in utero.*[234] As such, there is no single unifying channelopathy diagnosis for this family, although the presence of low grade exertional ventricular ectopy suggests an atypical form of CPVT. Accordingly, our understanding of the arrhythmic phenotype of LOF mutations is still evolving.

CPVT treatment focuses on modulating the sympathetic nervous system by using β-blockers and advising exercise restriction.[53] At present, no optimal treatment strategy exists for patients with overlapping arrhythmic and structural phenotypes, or for those with LOF *RYR2* mutations. LVNC can be associated with a malignant course in children, and risk stratification is lacking.[223] We treated the proband with an ICD only after cardiac arrest, and carvedilol, given its superior efficacy in systolic dysfunction,[235] although non-selective β-blockers are most protective in CPVT.[53, 169] The decision to proceed to ICD placement in the mother of the proband was challenging, as prophylactic ICDs may be harmful in CPVT.[58] However, given the highly malignant family history and uncertainty regarding β-blocker efficacy in the setting of RyR2 LOF, an ICD was placed. Further work is needed to better understand the optimal treatment and natural history of LVNC.

Limitations: We have limited clinical data for deceased relatives. Despite evidence that the phenotype co-segregates with genotype and *in vitro* testing suggested that the *RYR2* (p.I4855M) variant is pathogenic, it remains possible that p.I4855M alone does not account for the entire phenotype. Although p.I4855M affects the intrinsic properties of the RyR2 channel as revealed by our heterologous HEK293 cell studies, its impact on Ca^{2+} handling in cardiac cells has yet to be studied.

8.6 Conclusion

A novel variant in *RYR2* is associated with an overlapping phenotype of atypical CPVT and LVNC that appears to be highly penetrant with evidence of multigenerational sudden cardiac arrest. Expression and functional studies in HEK293 cells suggest that p.I4855M is a *RYR2* LOF mutation, adding to a small but growing body of evidence implicating these variants in a distinct arrhythmia syndrome. Further work is needed to identify the mechanistic basis of *RYR2*-related structural heart disease.

PART V: DISCUSSION AND FUTURE IMPLICATIONS

Chapter 9: Summary, Conclusions and Future Work

9.1 Discussion & summary of findings

The genetic basis of the first identified genetic arrhythmia syndrome, LQTS type-1, has been known for over 25 years,[67] and the last major monogenic IAS gene remaining, *RYR2,* was described in 2001.[56] Gene discovery efforts are now mainly focused on variant re-classification, arrhythmia mechanisms, and the advent of precision therapy in this field. However, knowledge translation continues to be hindered by the siloed nature of basic, clinical and population-level research, which generally have no formally aligned goals. This dissertation explored potentially genetic causes of arrhythmia in young patients using methodologies and techniques that span the spectrum of translational science. The works have identified areas in which future collaborative, translational studies are feasible, as well as those that are not likely to be informative. These 2 population-based studies, 4 registry-based retrospective studies, and one systematic review and meta-analysis of observation data, have also brought us closer to understanding atrial and ventricular arrhythmias of genetic origin.

In Chapter 2, we chose to address AF in the young because: (1) administrative diagnostic coding is well-studied for AF,[88, 89, 236, 237] (2) pre-existing studies used antiquated definitions of truly lone/idiopathic AF, which could be improved upon and (3) recent data suggest that it has a strong, but previously underappreciated genetic origin.[61, 62, 104] This latter hypothesis is supported by numerous population-level and molecular data. In the Framingham cohort, parental AF was one of the strongest predictors of AF occurring in offspring.[10] GWAS have shown the existence of some mildly predictive SNPs for AF.[103, 238] Additionally, three major studies in the past two years have replicated the role of truncating variants in the gene encoding Titin in AF.[61, 62, 104] Limitations of this finding are that the prevalence of genetically mediated AF is unknown and most gene discovery studies have focused on the general AF population. Instead, it is more likely that genetic variants are enriched in younger onset, idiopathic disease. This is demonstrated in the traditional ventricular IAS, like LQTS and CPVT, which often present in childhood and are far less likely to be found in adults with VT/VF.[53] In IAS, an underappreciated but consistent observation has been that these patients also suffer from atrial arrhythmias, including AF.[58, 63] All these considerations suggest that lone AF has a strong genetic basis and should be considered an atrial form of IAS. Thus, by creating a more robust definition of idiopathic/lone AF (young age, lack of comorbidities, etc.), the yield of gene discovery studies could be improved in this population.

In the study outlined in Chapter 2, which included nearly 1,000 patients with idiopathic AF based on the medical record represented to our knowledge, the largest lone AF cohort examined to-date. Strengths of the study were the ability to compare truly lone AF in the young to other forms of AF and to establish incidence. While the primary composite endpoint of death and thromboembolic events was lowest in young lone AF, it was non-zero (1.4%), and there was a frequent burden of AF recurrence (16.0%) and dependence on electrical cardioversion (27.1%) by one-year. Furthermore, the preponderance towards AF in males was even more dramatic in lone AF in the young compared to older onset AF, and yet females had worse outcomes. The explanation may implicate sex-based hormonal and/or environmental risk factors, like extreme physical activity and alcohol intake. Unfortunately, we were not able to capture family history through administrative coding, which would have been helpful in predicting the potential contribution of genetics. Instead, the cohort provided an estimate of the prevalence of truly lone AF and moved us closer to predicting who is most likely to benefit from genetic testing and future gene discovery efforts. Given that ~2-3% of all AF diagnosed constituted lone AF based on our definition, it is likely that amassing a large enough cohort to conduct widescale genetic studies will take time, though some efforts are currently in progress. Beyond this, the outcome data related to AF recurrence, event-free survival and sex differences suggested that lone AF warrants dedicated study as a unique cardiovascular entity.

In Chapter 3, we examined trends in syncope incidence and outcomes amongst children and adolescents because it is a common, albeit non-specific, presentation that potentially points towards a genetic arrhythmia syndrome. The very high risk of re-admission and re-presentation to the ED for syncope within 30-days and 1-year of the sentinel syncope diagnosis was the most dramatic and unanticipated finding. Although syncope was associated with a cardiovascular diagnosis in 20.9% of admitted children, the risk of a cardiac death at 1-year was exceedingly low, with just one of 11,488 syncopal children (0.000087%) having an underlying cardiovascular diagnosis (pulmonary hypertension, which is not a genetic IAS, nor a typical cause of SCD). Rather, the rising incidence of syncope in the young may largely stem from non-cardiac causes, like psychiatric illness and infections. Thus, population-based pediatric syncope cohorts are not an optimal resource to identify patients with potentially genetic arrhythmia syndromes. However, while syncope was a non-specific finding in children, it is a growing public health problem that was linked to substantial healthcare resource utilization and polypharmacy. A

national/international strategy for syncope in the young is needed, which need not focus on potentially genetic arrhythmias, but rather on pediatric evidence-based tools to guide admission and appropriate testing, and prevent re-hospitalization.

Although the great majority of syncope in the young is benign in etiology, syncope associated with IAS is an ominous symptom. In Part III (Chapters 4-5), we turned our focus to CPVT, one of the most lethal, yet poorly understood genetic causes of syncope and SCD in the young. In work predating this dissertation,[58] we and others had anecdotally observed that ICD outcomes were often poor in CPVT. Miyake et al.[59] and Roses-Noguer et al.[146] both independently proposed that this may be due to the pro-arrhythmic potential of defibrillating bidirectional/polymorphic VT which would have otherwise been self-limiting and benign. Instead, the painful, catecholamine-induced ICD shock could precipitate a dangerous incessant ventricular storm. In Chapter 4, we showed that when the major studies on CPVT are collated from the existing literature (nearly 1,500 patients) that ICDs appeared most effective for VF, yet almost entirely ineffective for bidirectional/polymorphic VT. Implantation of an ICD also came with substantial lifelong complication risk that may have exceeded their overall benefit. Since the publication of Chapter 4 in *Heart Rhythm*, our group, in collaboration with another larger CPVT registry based in Amsterdam have more robustly defined this problem by comparing SCA survivors who received a secondary prevention ICD to those who were only treated with medical therapy.[239] The ICD group did not experience improved survival during follow-up, but did have a high shock burden (appropriate and inappropriate). Collectively, these findings should result in the international guideline committee downgrading the indication for ICDs in the next iteration (currently Class I for those who survive SCA, or experience recurrent ventricular arrhythmias despite medical therapy).[53] The greatest focus should be on providing OMT, especially flecainide, which is effective and well-tolerated,[144] yet was under-prescribed based on our data in Chapter 4.

Understanding who may benefit from more advanced therapies, like flecainide, ICD, and LCSD, was traditionally a major challenge. All CPVT studies suffer from ascertainment bias, and the mechanisms and triggers of CPVT, especially in atypical cases, hinder decision-making. In Chapter 5, we focused on a simple clinical marker of risk – chronotropic incompetence, which is a failure to reach 80% of predicted heart rate on exercise treadmill testing. CPVT registry patients with CI had worse outcomes during follow-up, and thus, CI may be an effective marker to decide who receives flecainide early in the course of disease. Since the publication of this manuscript in

the *Journal of Cardiovascular Electrophysiology,* the importance of heart rate on treadmill testing was further described in nearly 200 CPVT patients, including a contribution from the PACES Registry, amongst other groups, which showed a greater change in heart rate from peak exercise to one-minute in recovery (heart rate recovery) was associated with a higher risk of cardiac events (accepted in *Circulation: Arrhythmia & Electrophysiology*, December 2019). However, predicting and avoiding the specific triggers of CPVT arrhythmias remains a challenge, as shown in Chapter 6, where we found that approximately one-quarter experience syncope and SCA during normal activities of daily living. It is plausible that CI and heart rate recovery in exercise are not helpful markers in these individuals. These phenotypic differences may be due to the variety of RyR2 mechanisms underlying CPVT, which can be predicted using homology mapping. This technique may be especially useful in the rare cases of two or more competing RyR2 variants in a single patient or family (as described in Chapter 7), where family screening can become complex. This technique can lead to the discovery of new disease mechanisms as well. For example, Chapter 8 investigated the *RyR2*-p.I4855M variant, which appeared to have a novel mechanism whereby a dominant-negative LoF variant likely resulted in decreased Ca^{2+} permeability. Supporting this finding was the unique phenotype of highly arrhythmogenic LVNC observed in the family, which was distinct from classic CPVT, and the absence of this variant in over 60,000 ExAC controls. Further international efforts are underway to better characterize this novel mechanism of RyR2-related LoF disease, and at the time of writing this dissertation, >20 confirmed cases are known to exist, several of which have been ascertained through the PACES collaborative CPVT network (unpublished data from Prof. Wayne Chen et al). The condition will be termed "cardiac ryanodine receptor release deficiency syndrome," or CRDS. Risk stratification for CRDS is not presently established, and therefore all members of the affected p.I4855M family have ICDs. Our success in identifying a new syndrome within a CPVT database supports the need for further deep phenotyping of existing registry cohorts to identify subtle dissimilarities among cases, and to create a pipeline for mechanistic discovery that can inform precision therapies for these patients.

9.2 Limitations

The potential limitations of the study designs were comprehensively described within in each chapter. In brief, for the population-based studies in Chapters 2-3, administrative diagnostic coding was unlikely to properly capture all cases of syncope and AF. For the systematic review in Chapter 4, the validity of the findings was dependent on the accuracy of the originally published studies,

and not all necessary data could be collected despite our best efforts. For the PACES CPVT Registry studies, there were a few inherent limitations related to population size with incomplete follow-up, the theoretical nature of homology mapping, and the use of HEK293 cellular models, which may have behaved differently from cardiac cells.

9.3 Future directions: Towards population-wide deep phenotyping and genotyping

A standardized, translational approach spanning multiple centres, provinces and countries is needed to address ongoing unanswered questions in genetic arrhythmia in the young. However, like in most rare diseases, there are a few fundamental obstacles that presently prevent more rapid advancements, including:

1. Small patient numbers at individual centres seen by a variety of providers with variable expertise
2. A lack of collectively agreed upon, universally available, and gold-standard tests to make a consensus diagnosis
3. Multiple researchers working independently to try to achieve similar objectives, without coordination, collaboration or a translational element that can link basic science to clinical to population-level data

The studies presented in this dissertation have a few of these limitations, but represent a step forward in trying to bring together translational research expertise to answer simple questions about genetic arrhythmias in the young. Other larger scale initiatives not focused on genetic IAS have already overcome some of these barriers, like the UK Biobank and Geisinger MyCode initiatives. In time, hopefully efforts stemming from UK Biobank will lead to the development of a polygenic risk score for ventricular arrhythmia in the general population, which could conceivably be applied to risk stratify genetic arrhythmia syndromes in the young.

National single payer healthcare systems represent the ideal opportunity to also advance the field. In Canada, there are robust population-level health databases (described in Chapters 2-3, in addition to the Canadian Institute for Health Information reports), that can be used to link individual registry patients to vital statistics, pharmaceutical drug prescription, and clinical outcomes. Additionally, countries like Canada typically only rely on a few centres of excellence in genetic arrhythmias, which minimizes practice heterogeneity and maximizes recruitment and engagement. The Hearts in Rhythm Organization (https://hiro.heartsinrhythm.ca) has a mandate to bring these programs together for the purposes of advocacy, education and research. However,

we have previously shown that funding and indications for clinical genetic testing[240] and the availability of advanced diagnostic imaging are non-uniform. Furthermore, only a few pediatric centres participate in this research. These factors introduce bias and confounders. Research into the genetic arrhythmia syndromes could be advanced by universally pursuing deep phenotyping and deep genotyping in all Canadian patients referred for expert evaluation. Although costly, this effort would address the first two of the three key limitations listed previously, and would provide an ideal population to discover and validate polygenic risk markers. Finally, strong coordination amongst basic scientists working in this field in Canada would advance knowledge translation to the clinic related to new disease mechanisms and potential precision therapies. At present, coordination among basic scientists is limited and there is no organized framework to connect them with relevant clinical data.

In conclusion, a Canadian translational initiative, involving basic, clinical and population scientists, focused on universal deep genotypic and phenotypic evaluation for suspected genetic arrhythmia would have several unique strengths:

1. Availability of geographically broad, highly inclusive data set from an ethnically and socioeconomically diverse national population
2. Opportunity to minimize redundancy and re-direct resources to unaddressed questions
3. Ability to link individual genetic arrhythmia patient follow-up and outcomes to national population health and pharmacy databases
4. Development of an unselected cohort to discover and validate current and future polygenic risk scores, like those for AF and VT, and to apply them as incremental risk modifiers in genetic arrhythmia syndromes
5. More rapid and relevant knowledge translation from the bench to the genetic IAS population, which may identify precision therapies, risk markers and new diseases

Bibliography:

1. Bhatnagar P, Wickramasinghe K, Wilkins E and Townsend N. Trends in the epidemiology of cardiovascular disease in the UK. *Heart*. 2016;102:1945-1952.

2. Priori SG, et al. Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. *Europace*. 2013;15:1389-406.

3. van der Werf C, van Langen IM and Wilde AA. Sudden death in the young: what do we know about it and how to prevent? *Circ Arrhythm Electrophysiol*. 2010;3:96-104.

4. Antzelevitch C and Burashnikov A. Overview of Basic Mechanisms of Cardiac Arrhythmia. *Cardiac electrophysiology clinics*. 2011;3:23-45.

5. Ackerman MJ, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm*. 2011;8:1308-39.

6. Khera AV, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet*. 2018;50:1219-1224.

7. Andrade JG, et al. 2018 Focused Update of the Canadian Cardiovascular Society Guidelines for the Management of Atrial Fibrillation. *Can J Cardiol*. 2018;34:1371-1392.

8. Al-Khatib SM, et al. 2017 AHA/ACC/HRS Guideline for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. *Heart Rhythm*. 2017.

9. Schnabel RB, et al. 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. *Lancet*. 2015;386:154-62.

10. Fox CS, et al. Parental atrial fibrillation as a risk factor for atrial fibrillation in offspring. *JAMA*. 2004;291:2851-5.

11. Lane DA, Skjoth F, Lip GYH, Larsen TB and Kotecha D. Temporal Trends in Incidence, Prevalence, and Mortality of Atrial Fibrillation in Primary Care. *J Am Heart Assoc*. 2017;6.

12. Brand FN, Abbott RD, Kannel WB and Wolf PA. Characteristics and Prognosis of Lone Atrial Fibrillation: 30-Year Follow-up in the Framingham Study. *JAMA*. 1985;254:3449-3453.

13. Kopecky SL, et al. The natural history of lone atrial fibrillation. A population-based study over three decades. *N Engl J Med*. 1987;317:669-74.

14. Wyse DG, et al. Lone atrial fibrillation: does it exist? *J Am Coll Cardiol*. 2014;63:1715-23.

15. Skov MW, et al. Electrocardiographic Preexcitation and Risk of Cardiovascular Morbidity and Mortality: Results From the Copenhagen ECG Study. *Circ Arrhythm Electrophysiol*. 2017;10.

16. Go AS, et al. Contemporary Burden and Correlates of Symptomatic Paroxysmal Supraventricular Tachycardia. *J Am Heart Assoc*. 2018;7.

17. Migliore F, et al. Prevalence of Cardiomyopathy in Italian Asymptomatic Children With Electrocardiographic T-Wave Inversion at Preparticipation Screening. *Circulation*. 2012;125:529-538.

18. Papadakis M, et al. The magnitude of sudden cardiac death in the young: a death certificate-based review in England and Wales. *Europace*. 2009;11:1353-8.

19. Meyer L, et al. Incidence, causes, and survival trends from cardiovascular-related sudden cardiac arrest in children and young adults 0 to 35 years of age: a 30-year review. *Circulation*. 2012;126:1363-72.

20. Pilmer CM, Kirsh JA, Hildebrandt D, Krahn AD and Gow RM. Sudden cardiac death in children and adolescents between 1 and 19 years of age. *Heart Rhythm*. 2014;11:239-45.

21. Lim Z, Gibbs K, Potts JE and Sanatani S. A review of sudden unexpected death in the young in British Columbia. *Can J Cardiol*. 2010;26:22-6.

22. Pilmer CM, et al. Scope and nature of sudden cardiac death before age 40 in Ontario: a report from the cardiac death advisory committee of the office of the chief coroner. *Heart Rhythm*. 2013;10:517-23.

23. Werf Cvd, Langen IMv and Wilde AAM. Sudden Death in the Young. *Circulation: Arrhythmia and Electrophysiology*. 2010;3:96-104.

24. Saklani P, Krahn A and Klein G. Syncope. *Circulation*. 2013;127:1330-9.

25. Sanatani S, et al. Canadian Cardiovascular Society and Canadian Pediatric Cardiology Association Position Statement on the Approach to Syncope in the Pediatric Patient. *Can J Cardiol*. 2016.

26. Sheldon RS, et al. Standardized approaches to the investigation of syncope: Canadian Cardiovascular Society position paper. *Can J Cardiol*. 2011;27:246-53.

27. Ruwald MH, et al. The relation between age, sex, comorbidity, and pharmacotherapy and the risk of syncope: a Danish nationwide study. *Europace*. 2012;14:1506-14.

28. Soteriades ES, et al. Incidence and prognosis of syncope. *N Engl J Med*. 2002;347:878-85.

29. Magnusson P, Palm A, Branden E and Morner S. Misclassification of hypertrophic cardiomyopathy: validation of diagnostic codes. *Clin Epidemiol*. 2017;9:403-410.

30. Caulfield T, et al. Reflections on the cost of "low-cost" whole genome sequencing: framing the health policy debate. *PLoS Biol*. 2013;11:e1001699.

31. Karczewski KJ, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. *bioRxiv*. 2019:531210.

32. Lek M, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536:285-291.

33. Sudlow C, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12:e1001779.

34. Bycroft C, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562:203-209.

35. Schwartz MLB, et al. A Model for Genome-First Care: Returning Secondary Genomic Findings to Participants and Their Healthcare Providers in a Large Research Cohort. *Am J Hum Genet*. 2018;103:328-337.

36. Haggerty CM, et al. Genomics-First Evaluation of Heart Disease Associated With Titin-Truncating Variants. *Circulation*. 2019;140:42-54.

37. Nannenberg EA, et al. Effect of Ascertainment Bias on Estimates of Patient Mortality in Inherited Cardiac Diseases. *Circulation: Genomic and Precision Medicine*. 2018;11:e001797.

38. Carruth ED, et al. Prevalence and Electronic Health Record-Based Phenotype of Loss-of-Function Genetic Variants in Arrhythmogenic Right Ventricular Cardiomyopathy-Associated Genes. *Circ Genom Precis Med*. 2019;12:e002579.

39. Haggerty CM, et al. Electronic health record phenotype in subjects with genetic variants associated with arrhythmogenic right ventricular cardiomyopathy: a study of 30,716 subjects with exome sequencing. *Genet Med*. 2017;19:1245-1252.

40. Cerrone M, Remme CA, Tadros R, Bezzina CR and Delmar M. Beyond the One Gene-One Disease Paradigm. *Circulation*. 2019;140:595-610.

41. Ikawa Y, et al. Gene therapy of hemoglobinopathies: progress and future challenges. *Human Molecular Genetics*. 2019;28:R24-R30.

42. Roberts JD and Gollob MH. Impact of genetic discoveries on the classification of lone atrial fibrillation. *J Am Coll Cardiol*. 2010;55:705-12.

43. Roberts JD and Gollob MH. A contemporary review on the genetic basis of atrial fibrillation. *Methodist DeBakey cardiovascular journal*. 2014;10:18-24.

44. Arking DE, et al. Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. *Nat Genet*. 2014;46:826-36.

45. Bezzina CR, et al. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. *Nat Genet*. 2013;45:1044-9.

46. Krahn AD, et al. Systematic assessment of patients with unexplained cardiac arrest: Cardiac Arrest Survivors With Preserved Ejection Fraction Registry (CASPER). *Circulation*. 2009;120:278-85.

47. Chen YH, et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science*. 2003;299:251-4.

48. Li N, Dobrev D and Wehrens XH. PITX2: a master regulator of cardiac channelopathy in atrial fibrillation? *Cardiovasc Res*. 2016;109:345-7.

49. Jervell A and Lange-Nielsen F. Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval, and sudden death. *American Heart Journal*. 1957;54:59-68.

50. Brugada J, Campuzano O, Arbelo E, Sarquella-Brugada G and Brugada R. Present Status of Brugada Syndrome: JACC State-of-the-Art Review. *J Am Coll Cardiol*. 2018;72:1046-1059.

51. Rodriguez-Manero M, et al. Prevalence, clinical characteristics and management of atrial fibrillation in patients with Brugada syndrome. *Am J Cardiol*. 2013;111:362-7.

52. Hosseini SM, et al. Reappraisal of Reported Genes for Sudden Arrhythmic Death. *Circulation*. 2018;138:1195-1205.

53. Priori SG. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm*. 2013;10:1932-63.

54. Leenhardt A, et al. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. *Circulation*. 1995;91:1512-9.

55. Berg KJ. Multifocal ventricular extrasytoles with Adams-Stokes syndrome in siblings. *American Heart Journal*. 1960;60:965-970.

56. Priori SG, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2001;103:196-200.

57. Priori SG and Chen SR. Inherited dysfunction of sarcoplasmic reticulum Ca2+ handling and arrhythmogenesis. *Circ Res*. 2011;108:871-83.

58. Roston TM, et al. Catecholaminergic polymorphic ventricular tachycardia in children: analysis of therapeutic strategies and outcomes from an international multicenter registry. *Circ Arrhythm Electrophysiol*. 2015;8:633-42.

59. Miyake CY, et al. Efficacy of implantable cardioverter defibrillators in young patients with catecholaminergic polymorphic ventricular tachycardia: success depends on substrate. *Circ Arrhythm Electrophysiol*. 2013;6:579-87.

60. Qin D, et al. Comparative effectiveness of antiarrhythmic drugs for rhythm control of atrial fibrillation. *Journal of Cardiology*. 2016;67:471-476.

61. Ahlberg G, et al. Rare truncating variants in the sarcomeric protein titin associate with familial and early-onset atrial fibrillation. *Nat Commun*. 2018;9:4316.

62. Choi SH, et al. Association Between Titin Loss-of-Function Variants and Early-Onset Atrial Fibrillation. *JAMA*. 2018;320:2354-2364.

63. Johnson JN, et al. Prevalence of early-onset atrial fibrillation in congenital long QT syndrome. *Heart Rhythm*. 2008;5:704-9.

64. Tabor HK, Risch NJ and Myers RM. Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nature Reviews Genetics*. 2002;3:391-397.

65. Wang Q, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell*. 1995;80:805-11.

66. Keating M, et al. Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey ras-1 gene. *Science*. 1991;252:704-6.

67. Wang Q, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet*. 1996;12:17-23.

68. Veerman CC, et al. hiPSC-derived cardiomyocytes from Brugada Syndrome patients without identified mutations do not exhibit clear cellular electrophysiological abnormalities. *Sci Rep*. 2016;6:30967.

69. Ng PC and Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res*. 2003;31:3812-4.

70. Adzhubei I, Jordan DM and Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet*. 2013;Chapter 7:Unit7.20.

71. Roston TM, Van Petegem F and Sanatani S. Catecholaminergic polymorphic ventricular tachycardia: a model for genotype-specific therapy. *Curr Opin Cardiol*. 2017;32:78-85.

72. Kottkamp H. Human atrial fibrillation substrate: towards a specific fibrotic atrial cardiomyopathy. *Eur Heart J*. 2013;34:2731-8.

73. Lubbers ER, Price MV and Mohler PJ. Arrhythmogenic Substrates for Atrial Fibrillation in Obesity. *Frontiers in Physiology*. 2018;9.

74. Prabhu S, et al. Catheter Ablation Versus Medical Rate Control in Atrial Fibrillation and Systolic Dysfunction: The CAMERA-MRI Study. *J Am Coll Cardiol*. 2017;70:1949-1961.

75. Cong T, et al. Quantitative analysis of mitral valve morphology in atrial functional mitral regurgitation using real-time 3-dimensional echocardiography atrial functional mitral regurgitation. *Cardiovascular Ultrasound*. 2018;16:13.

76. Soulat-Dufour L, et al. Biatrial remodelling in atrial fibrillation: A three-dimensional and strain echocardiography insight. *Archives of Cardiovascular Diseases*. 2019;112:585-593.

77. Grace A, et al. High-resolution noncontact charge-density mapping of endocardial activation. *JCI insight*. 2019;4.

78. Havranek S, et al. Multivariate Analysis of Correspondence between Left Atrial Volumes Assessed by Echocardiography and 3-Dimensional Electroanatomic Mapping in Patients with Atrial Fibrillation. *PLOS ONE*. 2016;11:e0152553.

79. Robinson PN. Deep phenotyping for precision medicine. *Hum Mutat*. 2012;33:777-80.

80. Dainis AM and Ashley EA. Cardiovascular Precision Medicine in the Genomics Era. *JACC Basic Transl Sci*. 2018;3:313-326.

81. Robinson PN, Mungall CJ and Haendel M. Capturing phenotypes for precision medicine. *Cold Spring Harb Mol Case Stud*. 2015;1:a000372.

82. Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405-24.

83. Zoni-Berisso M, Lercari F, Carazza T and Domenicucci S. Epidemiology of atrial fibrillation: European perspective. *Clin Epidemiol*. 2014;6:213-20.

84. Fumagalli S, et al. Age-Related Differences in Presentation, Treatment, and Outcome of Patients With Atrial Fibrillation in Europe: The EORP-AF General Pilot Registry (EURObservational Research Programme-Atrial Fibrillation). *JACC Clin Electrophysiol*. 2015;1:326-334.

85. Jahangir A, et al. Long-term progression and outcomes with aging in patients with lone atrial fibrillation: a 30-year follow-up study. *Circulation*. 2007;115:3050-6.

86. Scardi S, et al. Lone atrial fibrillation: prognostic differences between paroxysmal and chronic forms after 10 years of follow-up. *Am Heart J*. 1999;137:686-91.

87. Go AS, et al. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. *JAMA*. 2001;285:2370-5.

88. Ehlert AN, Heckbert SR, Wiggins KL and Thacker EL. Administrative billing codes accurately identified occurrence of electrical cardioversion and ablation/maze procedures in a prospective cohort study of atrial fibrillation patients. *Clin Cardiol*. 2017;40:1227-1230.

89. Cozzolino F, et al. A diagnostic accuracy study validating cardiovascular ICD-9-CM codes in healthcare administrative databases. The Umbria Data-Value Project. *PloS one*. 2019;14:e0218919-e0218919.

90. Rix TA, et al. Validity of the diagnoses atrial fibrillation and atrial flutter in a Danish patient registry. *Scand Cardiovasc J*. 2012;46:149-53.

91. Roston TM, et al. A Population-Based Study of Syncope in the Young. *Can J Cardiol*. 2018;34:195-201.

92. WHO. Guidelines for ATC classification and DD assignment., 9th Edition. *World Health Organization Collaborating Centre for Drug Statistics Methodology*. 2016.

93. Inteventions. CCoH. Volume 3. *Canadian Institute for Health Infomration*. 2012.

94. Benjamin EJ, et al. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. *JAMA*. 1994;271:840-4.

95. Lloyd-Jones DM, et al. Lifetime risk for development of atrial fibrillation: the Framingham Heart Study. *Circulation*. 2004;110:1042-6.

96. O'Neal WT, et al. Sex hormones and the risk of atrial fibrillation: The Multi-Ethnic Study of Atherosclerosis (MESA). *Endocrine*. 2017;58:91-96.

97. Chen LY, Herron KJ, Tai BC and Olson TM. Lone atrial fibrillation: influence of familial disease on gender predilection. *J Cardiovasc Electrophysiol*. 2008;19:802-6.

98. Slade T, et al. Birth cohort trends in the global epidemiology of alcohol use and alcohol-related harms in men and women: systematic review and metaregression. *BMJ Open*. 2016;6:e011827.

99. Kirkland S, Greaves L and Devichand P. Gender Differences in Smoking and Self Reported Indicators of Health. *BMC women's health*. 2004;4 Suppl 1:S7-S7.

100. Schnabel RB, et al. Gender differences in clinical presentation and 1-year outcomes in atrial fibrillation. *Heart*. 2017;103:1024-1030.

101. Arnson Y, et al. Comparing Management and Outcomes in Men and Women With Nonvalvular Atrial Fibrillation: Data From a Population-Based Cohort. *JACC: Clinical Electrophysiology*. 2018;4:604-614.

102. Feghaly J, Zakka P, London B, MacRae CA and Refaat MM. Genetics of Atrial Fibrillation. *J Am Heart Assoc*. 2018;7:e009884.

103. Nielsen JB, et al. Genome-wide Study of Atrial Fibrillation Identifies Seven Risk Loci and Highlights Biological Pathways and Regulatory Elements Involved in Cardiac Development. *Am J Hum Genet*. 2018;102:103-115.

104. Goodyer WR, et al. Broad Genetic Testing in a Clinical Setting Uncovers a High Prevalence of Titin Loss-of-Function Variants in Very Early Onset Atrial Fibrillation. *Circ Genom Precis Med*. 2019;12:e002713.

105. Piccini JP, et al. Bucindolol for the Maintenance of Sinus Rhythm in a Genotype-Defined HF Population: The GENETIC-AF Trial. *JACC Heart Fail*. 2019;7:586-598.

106. Ganzeboom KS, Colman N, Reitsma JB, Shen WK and Wieling W. Prevalence and triggers of syncope in medical students. *Am J Cardiol*. 2003;91:1006-8, a8.

107. Sun BC, Emond JA and Camargo CA, Jr. Inconsistent electrocardiographic testing for syncope in United States emergency departments. *Am J Cardiol*. 2004;93:1306-8.

108. Shen W-K, et al. 2017 ACC/AHA/HRS Guideline for the Evaluation and Management of Patients With Syncope: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines, and the Heart Rhythm Society. *Circulation*. 2017.

109. Sandhu RK, Sheldon RS, Savu A and Kaul P. Nationwide Trends in Syncope Hospitalizations and Outcomes from 2004-2014. *Canadian Journal of Cardiology*.

110. Thiruganasambandamoorthy V, et al. Development of the Canadian Syncope Risk Score to predict serious adverse events after emergency department assessment of syncope. *Cmaj*. 2016;188:E289-98.

111. Saccilotto RT, et al. San Francisco Syncope Rule to predict short-term serious outcomes: a systematic review. *CMAJ*. 2011;183:e1116-e1126.

112. Driscoll DJ, Jacobsen SJ, Porter CJ and Wollan PC. Syncope in children and adolescents. *J Am Coll Cardiol*. 1997;29:1039-45.

113. Moya A, et al. Guidelines for the diagnosis and management of syncope (version 2009). *Eur Heart J*. 2009;30:2631-71.

114. Ruwald MH, et al. Accuracy of the ICD-10 discharge diagnosis for syncope. *Europace*. 2013;15:595-600.

115. Alberta Health. Overview of Administrative Health Datasets. 2016.

116. Quan H, et al. Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. *Med Care*. 2005;43:1130-9.

117. Cramer JA, et al. Healthcare utilization and costs in children with stable and uncontrolled epilepsy. *Epilepsy Behav*. 2014;32:135-41.

118. Quan H, et al. Validity of AHRQ patient safety indicators derived from ICD-10 hospital discharge abstract data (chart review study). *BMJ Open*. 2013;3:e003716.

119. Ramanathan R, et al. Validity of International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) screening for sepsis in surgical mortalities. *Surg Infect (Larchmt)*. 2014;15:513-6.

120. Age limits and adolescents. *Paediatrics & Child Health*. 2003;8:577-577.

121. Ganzeboom KS, et al. Lifetime cumulative incidence of syncope in the general population: a study of 549 Dutch subjects aged 35-60 years. *J Cardiovasc Electrophysiol*. 2006;17:1172-6.

122. Paris Y, et al. Regional Implementation of a Pediatric Cardiology Syncope Algorithm Using Standardized Clinical Assessment and Management Plans (SCAMPS) Methodology. *J Am Heart Assoc*. 2016;5.

123. Goble MM, Benitez C, Baumgardner M and Fenske K. ED management of pediatric syncope: searching for a rationale. *Am J Emerg Med*. 2008;26:66-70.

124. Hurst D, et al. Syncope in the Pediatric Emergency Department - Can We Predict Cardiac Disease Based on History Alone? *J Emerg Med*. 2015;49:1-7.

125. Guse SE, et al. Implementing a Guideline to Improve Management of Syncope in the Emergency Department. *Pediatrics*. 2014;134:e1413-e1421.

126. Quinn J, McDermott D, Stiell I, Kohn M and Wells G. Prospective validation of the San Francisco Syncope Rule to predict patients with serious outcomes. *Ann Emerg Med*. 2006;47:448-54.

127. Massin MM, et al. Syncope in pediatric patients presenting to an emergency department. *J Pediatr*. 2004;145:223-8.

128. Sheldon RS, et al. Age of first faint in patients with vasovagal syncope. *J Cardiovasc Electrophysiol*. 2006;17:49-54.

129. Statistics Canada: Population, urban and rural, by province and territory (Alberta). *https://wwwstatcangcca/tables-tableaux/sum-som/l01/cst01/demo62j-enghtm*. 2011.

130. Finkelstein JA, et al. Antimicrobial use in defined populations of infants and young children. *Arch Pediatr Adolesc Med*. 2000;154:395-400.

131. Mangione-Smith R, McGlynn EA, Elliott MN, Krogstad P and Brook RH. The relationship between perceived parental expectations and pediatrician antimicrobial prescribing behavior. *Pediatrics*. 1999;103:711-8.

132. van der Werf C, et al. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. *J Am Coll Cardiol*. 2011;57:2244-54.

133. Olde Nordkamp LR, et al. Implantable cardioverter-defibrillator harm in young patients with inherited arrhythmia syndromes: A systematic review and meta-analysis of inappropriate shocks and complications. *Heart Rhythm*. 2015.

134. van der Werf C, et al. Familial evaluation in catecholaminergic polymorphic ventricular tachycardia: disease penetrance and expression in cardiac ryanodine receptor mutation-carrying relatives. *Circ Arrhythm Electrophysiol*. 2012;5:748-56.

135. Hayashi M, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2009;119:2426-34.

136. Moher D, Liberati A, Tetzlaff J and Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ*. 2009;339.

137. Postma AV, et al. Catecholaminergic polymorphic ventricular tachycardia: RYR2 mutations, bradycardia, and follow up of the patients. *J Med Genet*. 2005;42:863-70.

138. Wilde AAM, et al. Left Cardiac Sympathetic Denervation for Catecholaminergic Polymorphic Ventricular Tachycardia. *New England Journal of Medicine*. 2008;358:2024-2029.

139. Tester DJ, et al. Genotypic heterogeneity and phenotypic mimicry among unrelated patients referred for catecholaminergic polymorphic ventricular tachycardia genetic testing. *Heart Rhythm*. 2006;3:800-5.

140. De Ferrari GM, et al. Clinical Management of Catecholaminergic Polymorphic Ventricular Tachycardia: The Role of Left Cardiac Sympathetic Denervation. *Circulation*. 2015;131:2185-93.

141. Ostby SA BM, Owen H, Wackel PL, Cannon BC, Ackerman MJ. Competitive Sports Participation in Patients With Catecholaminergic Polymorphic Ventricular Tachycardia: A Single Center's Early Experience. *JACC: Cardiac Electrophysiology*. 2016;2.

142. Hayashi M, et al. The role of stress test for predicting genetic mutations and future cardiac events in asymptomatic relatives of catecholaminergic polymorphic ventricular tachycardia probands. *Europace*. 2012;14:1344-51.

143. Choi W-S, et al. Duplicate publication of articles used in meta-analysis in Korea. *SpringerPlus*. 2014;3:182.

144. Kannankeril PJ, et al. Efficacy of Flecainide in the Treatment of Catecholaminergic Polymorphic Ventricular Tachycardia: A Randomized Clinical Trial. *JAMA Cardiol*. 2017;2:759-766.

145. Sy RW, et al. Arrhythmia characterization and long-term outcomes in catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm*. 2011;8:864-71.

146. Roses-Noguer F, Jarman JW, Clague JR and Till J. Outcomes of defibrillator therapy in catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm*. 2014;11:58-66.

147. Kozlovski J, et al. Delay to diagnosis amongst patients with catecholaminergic polymorphic ventricular tachycardia. *Int J Cardiol*. 2014;176:1402-4.

148. Wanguemert F, et al. Clinical and molecular characterization of a cardiac ryanodine receptor founder mutation causing catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm*. 2015;12:1636-43.

149. Broendberg AK, et al. Nationwide experience of catecholaminergic polymorphic ventricular tachycardia caused by RyR2 mutations. *Heart*. 2017;103:901-909.

150. Wathen MS, et al. Prospective Randomized Multicenter Trial of Empirical Antitachycardia Pacing Versus Shocks for Spontaneous Rapid Ventricular Tachycardia in Patients With Implantable Cardioverter-Defibrillators. *Pacing Fast Ventricular Tachycardia Reduces Shock Therapies (PainFREE Rx II) Trial Results*. 2004;110:2591-2596.

151. Ellenbogen KA, et al. Are Implantable Cardioverter Defibrillator Shocks a Surrogate for Sudden Cardiac Death in Patients With Nonischemic Cardiomyopathy? *Circulation*. 2006;113:776-782.

152. Atallah J, et al. A Multi-Institutional Study of Implantable Defibrillator Lead Performance in Children and Young Adults: Results of the Pediatric Lead Extractability and Survival Evaluation (PLEASE) Study. *Circulation*. 2013.

153. El-Chami MF, Harbieh B, Levy M, Leon AR and Merchant FM. Clinical and electrocardiographic predictors of T wave oversensing in patients with subcutaneous ICD. *Journal of Arrhythmia*. 2016;32:181-185.

154. Celiker A, Erdogan I, Karagoz T and Ozer S. Clinical experiences of patients with catecholaminergic polymorphic ventricular tachycardia. *Cardiol Young*. 2009;19:45-52.

155. Mohamed U, Napolitano C and Priori SG. Molecular and electrophysiological bases of catecholaminergic polymorphic ventricular tachycardia. *J Cardiovasc Electrophysiol*. 2007;18:791-7.

156. Roston TM, et al. The clinical and genetic spectrum of catecholaminergic polymorphic ventricular tachycardia: findings from an international multicentre registry. *Europace*. 2018;20:541-547.

157. Rib Y, Zhussupova G, Igimbayeva G, Abdrakhmanov A and Jalali SF. Can chronotropic incompetence predict life-threatening ventricular arrhythmias in patients with stable ischemic heart disease? *Caspian J Intern Med*. 2018;9:164-170.

158. O'Neal WT, et al. Chronotropic Incompetence and Risk of Atrial Fibrillation: The Henry Ford ExercIse Testing (FIT) Project. *JACC Clin Electrophysiol*. 2016;2:645-652.

159. Lauer MS, Okin PM, Larson MG, Evans JC and Levy D. Impaired heart rate response to graded exercise. Prognostic implications of chronotropic incompetence in the Framingham Heart Study. *Circulation*. 1996;93:1520-6.

160. Lauer MS, et al. Impaired chronotropic response to exercise stress testing as a predictor of mortality. *JAMA*. 1999;281:524-9.

161. Azarbal B, et al. The incremental prognostic value of percentage of heart rate reserve achieved over myocardial perfusion single-photon emission computed tomography in the prediction of cardiac death and all-cause mortality: superiority over 85% of maximal age-predicted heart rate. *J Am Coll Cardiol*. 2004;44:423-30.

162. Faggioni M, van der Werf C and Knollmann BC. Sinus node dysfunction in catecholaminergic polymorphic ventricular tachycardia: risk factor and potential therapeutic target? *Trends Cardiovasc Med*. 2014;24:273-8.

163. Khan MN, Pothier CE and Lauer MS. Chronotropic incompetence as a predictor of death among patients with normal electrograms taking beta blockers (metoprolol or atenolol). *Am J Cardiol*. 2005;96:1328-33.

164. Nof E, et al. Postpacing abnormal repolarization in catecholaminergic polymorphic ventricular tachycardia associated with a mutation in the cardiac ryanodine receptor gene. *Heart Rhythm*. 2011;8:1546-52.

165. Magri D, et al. Clinical and prognostic impact of chronotropic incompetence in patients with hypertrophic cardiomyopathy. *International Journal of Cardiology*. 2018;271:125-131.

166. Faggioni M, van der Werf C and Knollmann B. Sinus node dysfunction in catecholaminergic polymorphic ventricular tachycardia – risk factor and potential therapeutic target? *Trends in cardiovascular medicine*. 2014;24:273-278.

167. Sumitomo N, et al. Association of atrial arrhythmia and sinus node dysfunction in patients with catecholaminergic polymorphic ventricular tachycardia. *Circ J*. 2007;71:1606-9.

168. Faggioni M, et al. Accelerated sinus rhythm prevents catecholaminergic polymorphic ventricular tachycardia in mice and in patients. *Circ Res*. 2013;112:689-97.

169. Leren IS, et al. Nadolol decreases the incidence and severity of ventricular arrhythmias during exercise stress testing compared with beta1-selective beta-blockers in patients with catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm*. 2016;13:433-40.

170. Leenhardt A, et al. Catecholaminergic polymorphic ventricular tachycardia in children: A 7-year follow-up of 21 patients. *Circulation*. 1995;91:1512-1519.

171. Priori S, et al. Mutations in the cardiac ryanodine receptor gene (*hRyR2)* underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2001;103:196-200.

172. Hayashi M, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2009;119:2426-2434.

173. Priori S and Chen S. Inherited dysfunction of sarcoplasmic reticulum Ca^{2+} handling and arrhythmogenesis. *Circ Res*. 2011;108:871-883.

174. Lahat H, et al. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Am J Hum Genet*. 2001;69:1378-84.

175. Van der Werf C, et al. Familial evaluation in catecholaminergic polymorphic ventricular tachycardia. *Circ Arrhythm Electrophysiol*. 2012;5:748-756.

176. Roston T, et al. Catecholaminergic polymorphic ventricular tachycardia in children: An analysis of therapeutic strategies and outcomes from an international multicenter registry. *Circ Arrhythm Electrophysiol*. 2015;8:633-642.

177. Harris PA, et al. Research Electronic Data Capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of biomedical informatics*. 2009;42:377-381.

178. Borko L, et al. Structural insights into the human RyR2 N-terminal region involved in cardiac arrhythmias. *Acta crystallographica Section D, Biological crystallography*. 2014;70:2897-912.

179. Yuchi Z, et al. Crystal structures of ryanodine receptor SPRY1 and tandem-repeat domains reveal a critical FKBP12 binding determinant. *Nature communications*. 2015;6:7947.

180. Lau K and Van Petegem F. Crystal structures of wild type and disease mutant forms of the ryanodine receptor SPRY2 domain. *Nature communications*. 2014;5:5397.

181. Yuchi Z, Lau K and Van Petegem F. Disease mutations in the ryanodine receptor central region: crystal structures of a phosphorylation hot spot domain. *Structure*. 2012;20:1201-11.

182. Yan Z, et al. Structure of the rabbit ryanodine receptor RyR1 at near-atomic resolution. *Nature*. 2015;517:50-5.

183. Medeiros-Domingo A, et al. The RYR2-encoded ryanodine receptor/calcium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long QT syndrome: a comprehensive open reading frame mutational analysis. *J Am Coll Cardiol*. 2009;54:2065-74.

184. Priori SG, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2002;106:69-74.

185. Sali A and Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. *Journal of Molecular Biology*. 1993;234:779-815.

186. Van Petegem F. Ryanodine Receptors: Allosteric Ion Channel Giants. *Journal of Molecular Biology*. 2015;427:31-53.

187. Blunck R and Batulan Z. Mechanism of Electromechanical Coupling in Voltage-Gated Potassium Channels. *Frontiers in Pharmacology*. 2012;3:166.

188. Kimlicka L, Lau K, Tung CC and Van Petegem F. Disease mutations in the ryanodine receptor N-terminal region couple to a mobile intersubunit interface. *Nature communications*. 2013;4:1506.

189. Bauce B, et al. Screening for ryanodine receptor type 2 mutations in families with effort-induced polymorphic ventricular arrhythmias and sudden death: early diagnosis of asymptomatic carriers. *J Am Coll Cardiol*. 2002;40:341-9.

190. Kimlicka L, et al. The cardiac ryanodine receptor N-terminal region contains an anion binding site that is targeted by disease mutations. *Structure*. 2013;21:1440-9.

191. Tung CC, Lobo PA, Kimlicka L and Van Petegem F. The amino-terminal disease hotspot of ryanodine receptors forms a cytoplasmic vestibule. *Nature*. 2010;468:585-8.

192. Jiang D, Chen W, Wang R, Zhang L and Chen SR. Loss of luminal Ca2+ activation in the cardiac ryanodine receptor is associated with ventricular fibrillation and sudden death. *Proc Natl Acad Sci U S A*. 2007;104:18309-14.

193. Gray B, et al. A novel heterozygous mutation in cardiac calsequestrin causes autosomal dominant catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm*. 2016.

194. Swan H, et al. Gain-of-function mutation of the SCN5A gene causes exercise-induced polymorphic ventricular arrhythmias. *Circ Cardiovasc Genet*. 2014;7:771-81.

195. Lieve KV, et al. Gain-of-function mutation in SCN5A causes ventricular arrhythmias and early onset atrial fibrillation. *Int J Cardiol*. 2017;236:187-193.

196. Nyegaard M, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. *Am J Hum Genet*. 2012;91:703-12.

197. Gomez-Hurtado N, et al. Novel CPVT-Associated Calmodulin Mutation in CALM3 (CALM3-A103V) Activates Arrhythmogenic Ca Waves and Sparks. *Circ Arrhythm Electrophysiol*. 2016;9.

198. Makita N, et al. Novel calmodulin mutations associated with congenital arrhythmia susceptibility. *Circ Cardiovasc Genet*. 2014;7:466-74.

199. Roux-Buisson N, et al. Absence of triadin, a protein of the calcium release complex, is responsible for cardiac arrhythmia with sudden death in human. *Hum Mol Genet*. 2012;21:2759-67.

200. Mullally J, et al. Risk of Life Threatening Cardiac Events among Patients with Long QT Syndrome and Multiple Mutations. *Heart Rhythm*. 2013;10:378-382.

201. Ingles J, et al. Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. *Journal of Medical Genetics*. 2005;42:e59-e59.

202. Burns C, Bagnall RD, Lam L, Semsarian C and Ingles J. Multiple Gene Variants in Hypertrophic Cardiomyopathy in the Era of Next-Generation Sequencing. *Circulation: Cardiovascular Genetics*. 2017;10.

203. Rigato I, et al. Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet*. 2013;6:533-42.

204. Rohatgi RK, et al. Contemporary Outcomes in Patients With Long QT Syndrome. *J Am Coll Cardiol*. 2017;70:453-462.

205. Roston TM, et al. The clinical and genetic spectrum of catecholaminergic polymorphic ventricular tachycardia: findings from an international multicentre registry. *Europace*. 2017.

206. Peng W, et al. Structural basis for the gating mechanism of the type 2 ryanodine receptor RyR2. *Science*. 2016;354.

207. Kimlicka L, Lau K, Tung CC and Van Petegem F. Disease mutations in the ryanodine receptor N-terminal region couple to a mobile intersubunit interface. *Nat Commun*. 2013;4:1506.

208. Yuchi Z and Van Petegem F. Ryanodine receptors under the magnifying lens: Insights and limitations of cryo-electron microscopy and X-ray crystallography studies. *Cell Calcium*. 2016;59:209-27.

209. Lau K and Van Petegem F. Crystal structures of wild type and disease mutant forms of the ryanodine receptor SPRY2 domain. *Nat Commun*. 2014;5:5397.

210. des Georges A, et al. Structural Basis for Gating and Activation of RyR1. *Cell*. 2016;167:145-157.e17.

211. Francia P, et al. RyR2 Common Gene Variant G1886S and the Risk of Ventricular Arrhythmias in ICD Patients with Heart Failure. *J Cardiovasc Electrophysiol*. 2015;26:656-61.

212. Kääb S, et al. A Large Candidate Gene Survey Identifies the KCNE1 D85N Polymorphism as a Possible Modulator of Drug-Induced Torsades de Pointes. *Circulation Cardiovascular Genetics*. 2012;5:91-99.

213. Schwartz PJ and Woosley RL. Predicting the Unpredictable: Drug-Induced QT Prolongation and Torsades de Pointes. *J Am Coll Cardiol*. 2016;67:1639-1650.

214. Lane CM, et al. Long QT syndrome type 5-Lite: Defining the clinical phenotype associated with the potentially proarrhythmic p.Asp85Asn-KCNE1 common genetic variant. *Heart Rhythm*. 2018;15:1223-1230.

215. Tang Y, Tian X, Wang R, Fill M and Chen SR. Abnormal termination of Ca2+ release is a common defect of RyR2 mutations associated with cardiomyopathies. *Circ Res*. 2012;110:968-77.

216. Blair E, Price SJ, Baty CJ, Ostman-Smith I and Watkins H. Mutations in cis can confound genotype-phenotype correlations in hypertrophic cardiomyopathy. *J Med Genet*. 2001;38:385-8.

217. Kapplinger JD, et al. Yield of the Genetic Test in Suspected Catecholaminergic Polymorphic Ventricular Tachycardia and Implications for Test Interpretation. *Circulation: Genomic and Precision Medicine*. 2018;11.

218. Zalk R, et al. Structure of a mammalian ryanodine receptor. *Nature*. 2015;517:44-49.

219. Yan Z, et al. Structure of the rabbit ryanodine receptor RyR1 at near-atomic resolution. *Nature*. 2015;517:50-55.

220. Lobo Paolo A, Kimlicka L, Tung C-C and Van Petegem F. The Deletion of Exon 3 in the Cardiac Ryanodine Receptor Is Rescued by β Strand Switching. *Structure*. 2011;19:790-798.

221. Amador FJ, et al. Type 2 ryanodine receptor domain A contains a unique and dynamic alpha-helix that transitions to a beta-strand in a mutant linked with a heritable cardiomyopathy. *J Mol Biol*. 2013;425:4034-46.

222. Paech C, et al. Ryanodine receptor mutations presenting as idiopathic ventricular fibrillation: a report on two novel familial compound mutations, c.6224T>C and c.13781A>G, with the clinical presentation of idiopathic ventricular fibrillation. *Pediatr Cardiol*. 2014;35:1437-41.

223. Hoedemaekers YM, et al. The importance of genetic counseling, DNA diagnostics, and cardiologic family screening in left ventricular noncompaction cardiomyopathy. *Circ Cardiovasc Genet*. 2010;3:232-9.

224. Maron BJ, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation*. 2006;113:1807-16.

225. Campbell MJ, Czosek RJ, Hinton RB and Miller EM. Exon 3 deletion of ryanodine receptor causes left ventricular noncompaction, worsening catecholaminergic polymorphic ventricular tachycardia, and sudden cardiac arrest. *Am J Med Genet A*. 2015;167a:2197-200.

226. Bhuiyan ZA, et al. Expanding spectrum of human RYR2-related disease: new electrocardiographic, structural, and genetic features. *Circulation*. 2007;116:1569-76.

227. Ohno S, et al. Exon 3 deletion of RYR2 encoding cardiac ryanodine receptor is associated with left ventricular non-compaction. *Europace*. 2014;16:1646-54.

228. Borko L, et al. Structural insights into the human RyR2 N-terminal region involved in cardiac arrhythmias. *Acta Crystallogr D Biol Crystallogr*. 2014;70:2897-912.

229. Yuchi Z, et al. Crystal structures of ryanodine receptor SPRY1 and tandem-repeat domains reveal a critical FKBP12 binding determinant. *Nat Commun*. 2015;6:7947.

230. Chen W, et al. The ryanodine receptor store-sensing gate controls Ca2+ waves and Ca2+-triggered arrhythmias. *Nat Med*. 2014;20:184-92.

231. Roux-Buisson N, et al. Prevalence and significance of rare RYR2 variants in arrhythmogenic right ventricular cardiomyopathy/dysplasia: results of a systematic screening. *Heart Rhythm*. 2014;11:1999-2009.

232. Tiso N, et al. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet*. 2001;10:189-94.

233. van der Zwaag PA, et al. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. *Eur J Heart Fail*. 2012;14:1199-207.

234. Ichida F, et al. Clinical features of isolated noncompaction of the ventricular myocardium: long-term clinical course, hemodynamic properties, and genetic background. *J Am Coll Cardiol*. 1999;34:233-40.

235. Poole-Wilson PA, et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. *Lancet*. 2003;362:7-13.

236. Jensen PN, et al. A systematic review of validated methods for identifying atrial fibrillation using administrative data. *Pharmacoepidemiol Drug Saf*. 2012;21 Suppl 1:141-7.

237. Norberg J, Backstrom S, Jansson JH and Johansson L. Estimating the prevalence of atrial fibrillation in a general population using validated electronic health data. *Clin Epidemiol*. 2013;5:475-81.

238. Lin H, et al. Targeted sequencing in candidate genes for atrial fibrillation: the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Targeted Sequencing Study. *Heart Rhythm*. 2014;11:452-7.

239. van der Werf C, et al. Implantable cardioverter-defibrillators in previously undiagnosed patients with catecholaminergic polymorphic ventricular tachycardia resuscitated from sudden cardiac arrest. *Eur Heart J*. 2019.

240. Roston TM, et al. The accessibility and utilization of genetic testing for inherited heart rhythm disorders: a Canadian cross-sectional survey study. *J Community Genet*. 2018;9:257-262.

241. Liberati A, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*. 2009;339.

242. Bottillo I, et al. Prediction and visualization data for the interpretation of sarcomeric and non-sarcomeric DNA variants found in patients with hypertrophic cardiomyopathy. *Data in Brief*. 2016;7:607-613.

243. Kaartinen M, et al. Characterization of familial and sporadic arrhythmogenic right ventricular cardiomyopathy in Finland. *Ann Med*. 2007;39:312-8.

244. LaPage MJ, Russell MW, Bradley DJ and Dick M, 2nd. Novel ryanodine receptor 2 mutation associated with a severe phenotype of catecholaminergic polymorphic ventricular tachycardia. *J Pediatr*. 2012;161:362-4.

245. Jabbari J, et al. New exome data question the pathogenicity of genetic variants previously associated with catecholaminergic polymorphic ventricular tachycardia. *Circ Cardiovasc Genet*. 2013;6:481-9.

246. Hernández JJ, et al. Abstract 17750: A CPVT Mutation Confers Gain of Function to the Cardiac Ryanodine Receptor Channel. Characterization Using Cardiomyocytes Derived From Patient-Specific Ips Cells. *Circulation*. 2016;128:A17750.

247. George CH, Higgs GV and Lai FA. Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. *Circ Res*. 2003;93:531-40.

248. Suetomi T, et al. Mutation-linked defective interdomain interactions within ryanodine receptor cause aberrant Ca(2)(+)release leading to catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2011;124:682-94.

249. Milting H, et al. Composite polymorphisms in the ryanodine receptor 2 gene associated with arrhythmogenic right ventricular cardiomyopathy. *Cardiovascular Research*. 2006;71:496-505.

250. Koop A, Goldmann P, Chen SR, Thieleczek R and Varsanyi M. ARVC-related mutations in divergent region 3 alter functional properties of the cardiac ryanodine receptor. *Biophys J*. 2008;94:4668-77.

251. Fujino N, et al. Abstract 915: A Novel Missense Mutation in Cardiac Ryanodine Receptor Gene as a Possible Cause of Hypertrophic Cardiomyopathy: Evidence From Familial Analysis. *Circulation*. 2015;114:II_165.

252. Liu X LJ, Shen Y, Wan R, Xiong QM, Zhou QQ, Xie JY, Jin JJ, Yan X, Yu JH, Hong K. Recurrent Syncope Related to Catecholaminergic Polymorphic Ventricular Tachycardia Due to De Novo RyR2-R2401H Mutation. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2017;45:39-43.

253. Creighton W, Virmani R, Kutys R and Burke A. Identification of Novel Missense Mutations of Cardiac Ryanodine Receptor Gene in Exercise-Induced Sudden Death at Autopsy. *The Journal of molecular diagnostics : JMD*. 2006;8:62-67.

254. Aizawa Y, et al. A novel mutation in FKBP12.6 binding region of the human cardiac ryanodine receptor gene (R2401H) in a Japanese patient with catecholaminergic polymorphic ventricular tachycardia. *Int J Cardiol*. 2005;99:343-5.

255. Millat G, et al. Contribution of long-QT syndrome genetic variants in sudden infant death syndrome. *Pediatr Cardiol*. 2009;30:502-9.

256. Kapplinger JD, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm*. 2010;7:33-46.

257. Haywood AF, et al. Recurrent missense mutations in TMEM43 (ARVD5) due to founder effects cause arrhythmogenic cardiomyopathies in the UK and Canada. *Eur Heart J*. 2013;34:1002-11.

258. Cox MG, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: pathogenic desmosome mutations in index-patients predict outcome of family screening: Dutch arrhythmogenic right ventricular dysplasia/cardiomyopathy genotype-phenotype follow-up study. *Circulation*. 2011;123:2690-700.

259. Pugh TJ, et al. The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. *Genet Med*. 2014;16:601-8.

Appendix A: Permissions to reproduce published materials

Figures appearing in the introductory comments of Chapter 1 are previously published, and permissions to reproduce were approved by the respective publishers.

Versions of Chapters 3, 4 & 8 are published by Elsevier. Permissions to reproduce each article for the purposes of an academic dissertation are provided as follows:

Chapter 3

Chapter 4

Chapter 8


s100.copyright.com/AppDispatchServlet
Copyright Clearance Center
RightsLink
Home
Account Info
Help
LIVE CHAT
Title: A novel RYR2 loss-of-function mutation (I4855M) is associated with left ventricular non-compaction and atypical catecholaminergic polymorphic ventricular tachycardia
Author: Thomas M. Roston,Wenting Guo,Andrew D. Krahn,Ruiwu Wang,Filip Van Petegem,Shubhayan Sanatani,S.R. Wayne Chen,Anna Lehman
Publication: Journal of Electrocardiology
Publisher: Elsevier
Date: March–April 2017
© 2016 Elsevier Inc. All rights reserved.
Logged in as:
Thomas Roston
Account #:
3001078053
LOGOUT
Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: https://www.elsevier.com/about/our-business/policies/copyright#Author-rights
BACK
CLOSE WINDOW
Copyright © 2019 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

A version of Chapter 5 is published by Wiley-Blackwell. Permissions to reproduce each article for the purposes of an academic dissertation are provided as follows:

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Aug 20, 2019

This Agreement between Thomas Roston ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4653340525443
License date	Aug 20, 2019
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Journal of Cardiovascular Electrophysiology
Licensed Content Title	Chronotropic incompetence as a risk predictor in children and young adults with catecholaminergic polymorphic ventricular tachycardia
Licensed Content Author	Shubhayan Sanatani, Prince J. Kannankeril, Bjorn C. Knollmann, et al
Licensed Content Date	Jul 11, 2019
Licensed Content Volume	0
Licensed Content Issue	0
Licensed Content Pages	7
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title of your thesis / dissertation	Genetic Cardiac Arrhythmias in the Young: From Population Trends to Cellular Mechanisms
Expected completion date	Feb 2020
Expected size (number of pages)	300
Requestor Location	Thomas Roston 2775 Laurel St Vancouver, BC V5Z 1M9 Canada Attn: Thomas Roston
Publisher Tax ID	EU826007151
Total	0.00 USD
Terms and Conditions	

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

A version of Chapter 6 is published by Oxford University Press, which freely allows the reproduction of materials for a dissertation. An excerpt from the policy is provided, and available in full here: https://global.oup.com/academic/rights/permissions/autperm/?cc=us&lang=en&

After publication

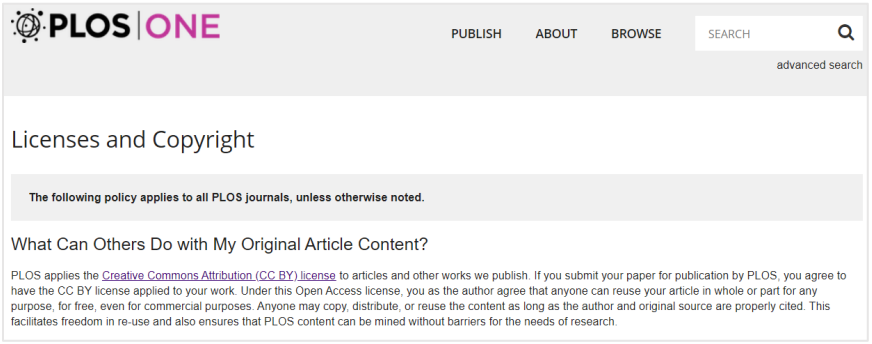
After publication you may reuse the following portions of your content without obtaining formal permission for the activities expressly listed below:

- one chapter or up to 10% of the total of your single author or co-authored book,
- a maximum of one chapter/article from your contribution to an edited book or collection (e.g. Oxford Handbooks),
- a maximum of one chapter/article of your contribution to an online only, or digital original publication, or
- three figures/illustrations/tables of your own original work

OUP is pleased to grant this permission for the following uses:

- posting on your own personal website or in an institutional or subject based repository after a **12 month** period for **Science and Medical** titles and a **24 month** period for **Academic, Trade and Reference** titles;
- inclusion in scholarly, not-for-profit derivative reuses, (these can include the extension of your contribution to a book-length work, or inclusion in an edited collection of your own work, or any work of which you are an author or editor);
- reproduction within coursepacks or e-coursepacks for your own teaching purposes, (with the proviso that the coursepacks are not sold for more than the cost of reproduction);
- inclusion within your thesis or dissertation.

A version of Chapter 7 is published by PLOS ONE, which is an open-access publisher that allows reproduction of materials if acitation is listed. An excerpt from the policy is provided, and available in full here: https://journals.plos.org/plosone/s/licenses-and-copyright


PLOS ONE

PUBLISH ABOUT BROWSE SEARCH

advanced search

Licenses and Copyright

The following policy applies to all PLOS journals, unless otherwise noted.

What Can Others Do with My Original Article Content?

PLOS applies the Creative Commons Attribution (CC BY) license to articles and other works we publish. If you submit your paper for publication by PLOS, you agree to have the CC BY license applied to your work. Under this Open Access license, you as the author agree that anyone can reuse your article in whole or part for any purpose, for free, even for commercial purposes. Anyone may copy, distribute, or reuse the content as long as the author and original source are properly cited. This facilitates freedom in re-use and also ensures that PLOS content can be mined without barriers for the needs of research.

Chapter 1 includes excerpts from an article published by Wolters Kluwer Health Inc. Permission to reproduce excerpts from this manuscript for an academic dissertation are provided as follows:


s100.copyright.com/AppDispatchServlet#formTop

Copyright Clearance Center RightsLink®

Home Account Info Help LIVE CHAT

Wolters Kluwer

Title: Catecholaminergic polymorphic ventricular tachycardia: a model for genotype-specific therapy

Author: Thomas Roston, Filip Van Petegem, and Shubhayan Sanatani

Publication: Current Opinion in Cardiology

Publisher: Wolters Kluwer Health, Inc.

Date: Jan 1, 2017

Copyright © 2017, Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

Logged in as:
Thomas Roston
Account #:
3001078053
LOGOUT

License Not Required

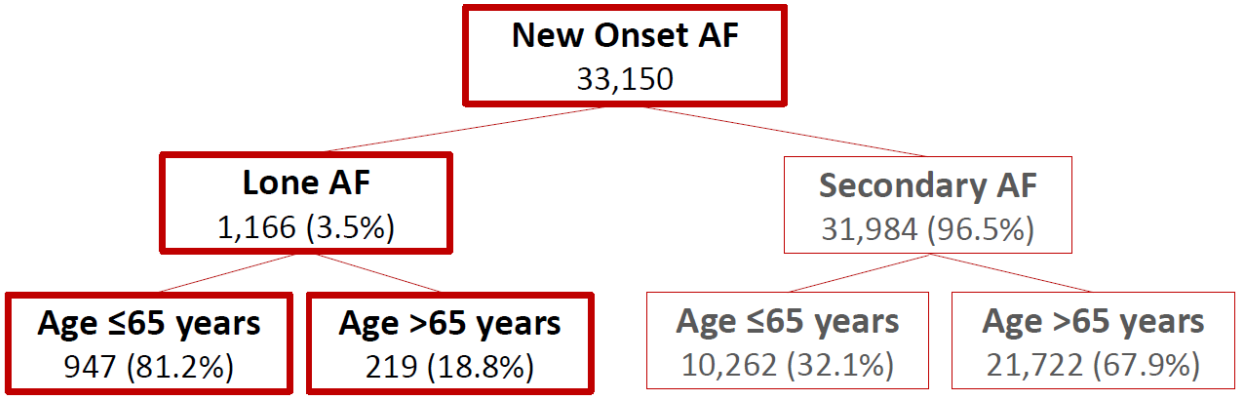
This request is granted gratis and no formal license is required from Wolters Kluwer. Please note that modifications are not permitted. Please use the following citation format: author(s), title of article, title of journal, volume number, issue number, inclusive pages and website URL to the journal page.

BACK CLOSE WINDOW

Copyright © 2019 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

Appendix B: Supplemental data from Chapter 2

Supplemental Figure 2.1: Flow chart for cohort selection



Supplemental Table 2.1

Procedural Intervention	CCI Codes	Notes
Cardiac Ablation	1.HH.59.^^	Destruction, cardiac conduction system, including: • Using cryoprobe • Using radiofrequency • Using device not elsewhere classified • Using ultrasound device
Cardiac Ablation	1.HZ.59	Destruction, heart not elsewhere classified, including: • Using cryoprobe • Using device not elsewhere classified • Using laser
Electrical Cardioversion	1.HZ.09.^^	Stimulation, heart not elsewhere classified, including: • Using electrode cardioverter/defibrillator • Using electrode with synchronized direct current shock • Using open manual massage

Supplemental Table 2.1: Procedural interventions used to identify catheter ablation and electrical cardioversion that occurred during follow-up in incident AF patients

Supplemental Table 2.2

Chronic Comorbidity	ICD-9 Codes	ICD-10 Codes
Malignancy	140-239.9	C00-C97
Thyroid disease	240-246.9	E00-E07
Cardiovascular and cerebrovascular disease	390-429 439-459.9	I00-I59 I70-I99
Chronic lung disease	490-496 500-508.9	
Diabetes	250-250.9	E10-E14
Obesity and metabolic disorders		E65-E68

		E70-E90

Supplemental Table 2.2: Chronic comorbidities used to define secondary AF in patients who received one or more of these diagnoses in any clinical setting 3 years prior to or 1 year after incident AF diagnosis.

Supplemental Table 2.3

Acute Event	ICD-9 Codes	ICD-10 Notes
Selected injuries and poisoning	800-809.1 850-854.1 950-957.1 960-979.9	S00-S19 T36-T50 T51-T65
Burns	940-949.9	
Acute respiratory infection	460-466.1	J00-J06 J40-47 J20-J22 J30-J39 J85-J86
Pneumonia and influenza	480-487.8	J09-J18
Appendicitis	540-543	K35-38
Other infections	001-139.8 680-686.9	A00-B-99 L00-L08

Supplemental Table 2.3: Acute medical events used to define secondary AF in patients who received one or more of these diagnoses in any clinical setting 1 month prior to or 1 month after incident AF diagnosis.

Supplemental Table 2.4a

Variable Name	Lone	Secondary	Total	P-value
Total N	701	20274	20975	
Death	20 (2.9)	3415 (16.8)	3435 (16.4)	<0.001
Stroke/TIA/Embolism/Death	27 (3.9)	4192 (20.7)	4219 (20.1)	<0.001
Bleeding	21 (3.0)	1967 (9.7)	1988 (9.5)	<0.001
AF	171 (24.4)	5834 (28.8)	6005 (28.6)	0.012
Non-CV hospitalization/ED visit	390 (55.6)	15043 (74.2)	15443 (73.6)	<0.001

Supplemental Table 2.4a: 3-year outcome among all ages incident AF patients (lone vs secondary)

Supplemental Table 2.4b

Variable Name	Lone	Secondary	Total	P-value
Total N	572	6414	6986	
Death	8 (1.4)	378 (5.9)	386 (5.5)	<0.001
Stroke/TIA/Embolism/Death	10 (1.7)	516 (8.0)	526 (7.5)	<0.001
Bleeding	16 (2.8)	345 (5.4)	361 (5.2)	0.008
AF	136 (23.8)	1949 (30.4)	2085 (29.9)	0.001
Non-CV hospitalization/ED visit	324 (56.6)	4402 (68.6)	4726 (67.7)	<0.001

Supplemental Table 2.4b: 3-year outcome among incident AF patients aged ≤65 years (lone vs secondary)

Supplemental Table 2.5a

Variable Name	Lone	Secondary	Total	P-value
Total N	422	4579	5001	
Warfarin	50 (11.8)	1659 (36.2)	1709 (34.2)	<0.001
NOAC	21 (5.0)	617 (13.5)	638 (12.8)	<0.001
OAC	64 (15.2)	1968 (43.0)	2032 (40.6)	<0.001
Antiarrhythmic drug including Sotalol	45 (10.7)	815 (17.8)	860 (17.2)	0.002
Antiplatelet	32 (7.6)	1054 (23.0)	1086 (21.7)	<0.001
Beta blocker	133 (31.5)	2716 (59.3)	2849 (57.0)	<0.001
Calcium channel blocker	34 (8.1)	1274 (27.8)	1308 (26.2)	<0.001
Digoxin	6 (1.4)	455 (9.9)	461 (9.2)	<0.001

Supplemental Table 2.5a: 3-year medication uptake among young (aged ≤65 years) incident AF patients (lone vs secondary)

Supplemental Table 2.5b

Variable Name	Age ≤65y	Age >65y	P-value	Females	Males	P-value
Total N	422	93		119	396	
Warfarin	50 (11.8)	37 (39.8)	<0.001	22 (18.5)	65 (16.4)	0.60
NOAC	21 (5.0)	13 (14.0)	0.002	6 (5.0)	28 (7.1)	0.43
OAC	64 (15.2)	44 (47.3)	<0.001	24 (20.2)	84 (21.2)	0.81
Antiarrhythmic drug including Sotalol	45 (10.7)	6 (6.5)	0.22	11 (9.2)	40 (10.1)	0.78
Antiplatelet	32 (7.6)	9 (9.7)	0.49	11 (9.2)	30 (7.6)	0.56
Beta blocker	133 (31.5)	46 (49.5)	0.001	43 (36.1)	136 (34.3)	0.72
Calcium channel blocker	34 (8.1)	15 (16.1)	0.016	17 (14.3)	32 (8.1)	0.043
Digoxin	6 (1.4)	7 (7.5)	0.001	6 (5.0)	7 (1.8)	0.046

Supplemental Table 2.5b: 3-year medication uptake among all ages incident lone AF patients-stratified by age (young vs old) and sex (females vs males)

Appendix C: Supplemental data from Chapter 3

Supplemental Table 3.1

Major group	Malformation	ICD-9	ICD-10
Heart condition	Heart failure[116]	398.91, 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.13, 404.91, 404.93, 425.4–425.9, 428.x	I09.9, I11.0, I13.0, I13.2, I25.5, I42.0, I42.5–I42.9, I43.x, I50.x, P29.0
	Transposition of great vessels	745.1X	Q20.1, Q20.3, Q20.5, Q20.8
	Tetralogy of Fallot	745.2	Q21.3
	Common ventricle	745.3	Q20.4
	Endocardial cushion defects	745.6X	Q21.2
	Anomalies of pulmonary valve congenital	746.0X	Q22.0-3
	Other specified congenital anomalies of heart	746.8X	Q24.0-6, Q24.8
	Patent ductus arteriosus	747.0	Q25.0
	Coarctation of aorta (preductal) (postductal)	747.10	Q25.1
	Anomalies of pulmonary artery	747.3	Q25.5, Q25.6, Q25.71, Q25.72, Q25.79
	Hypoplastic left heart syndrome	746.7	Q23.04
	Acute rheumatic fever	391.0, 391.1, 391.2, 391.8, 391.9	I01.0-I01.9
	Chronic rheumatic heart disease	394.0, 394.1, 394.2, 394.9	I05.0-I05.9
	Ischemic heart diseases	410, 411, 412, 413, 414	I20-I25
	Pericarditis & other pericardial disease	420.0, 420.91, 420.90, 420.99, 423.0, 423.1, 423.2, 423.8, 423.9	I30.0, I30.1, I30.8, I30.9, I31.0, I31.1, I31.2, I31.3, I31.8, I31.9, I32
	Valvular heart disease	424.0, 424.1, 424.2, 424.3, 421.1	I34.0, I34.1, I34.2, I34.8, I34.9, I36.0, I36.1, I36.2, I36.8, I36.9, I35.0, I35.1, I35.2, I35.8, I37.0, I37.1, I37.2, I37.8, I37.9, I39.0, I39.1, I39.2, I39.3
	Myocarditis & cardiomyopathy	422.0, 422.90, 422.91, 422.92, 422.93, 422.99, 425.0, 425.11, 425.18, 425.3	I40.0, I40.1, I40.8, I40.9, I41.0, I41.1, I41.2, I41.8, I42.1, I42.2, I42.3, I42.4
	Conductive heart disease	426.0, 426.11, 426.12, 426.13, 426.2, 426.3, 426.50, 426.51, 426.52, 426.53, 426.54, 426.7, 426.81, 426.9	I44.0, I44.1, I44.2, I44.3, I44.4, I44.5, I44.6, I44.7, I45.2, I45.3, I45.4, I45.6, I45.8, I45.9

	Arrhythmias & cardiac arrest	427.X	I46.0, I46.1, I46.9, I47.0, I47.2, I47.9, I47.1, I48, I49.0, I49.1, I49.2, I49.3, I49.4, I49.5, I49.8, I49.9
	Other & complications of heart disease	429.X	I51
Malignancy	Cancer[116]	140.x–172.x, 174.x–195.8, 200.x–208.x, 238.6	C00.x–C26.x, C30.x–C34.x, C37.x–C41.x, C43.x, C45.x–C58.x, C60.x–C76.x, C81.x–C85.x, C88.x, C90.x–C97.x
	Metastatic solid tumor[116]	196.x–199.x	C77.x–C80.x
Thoracic condition	Chronic pulmonary disease[116]	416.8, 416.9, 490.x–505.x, 506.4, 508.1, 508.8	I27.8, I27.9, J40.x–J47.x, J60.x–J67.x, J68.4, J70.1, J70.3
	Agenesis, hypoplasia, and dysplasia of lung	748.5	Q33.2, Q33.3, Q33.6
	Tracheosophageal fistula, esophageal atresia and stenosis	750.3	Q39.0-4
	Pulmonary embolism	415.0, 415.12, 415.13, 415.19	I26.0, I26.9
	Other secondary pulmonary hypertension	416.8	I27.2
Abdominal condition	Liver disease	070.22, 070.23, 070.32, 070.33, 070.44, 070.54, 070.6, 070.9, 570.x, 571.x, 573.3, 573.4, 573.8, 573.9, V42.7, 456.0–456.2, 572.2–572.8	B18.x, K70.0–K70.3, K70.9, K71.3–K71.5, K71.7, K73.x, K74.x, K76.0, K76.2–K76.4, K76.8, K76.9, Z94.4, I85.0, I85.9, I86.4, I98.2, K70.4, K71.1, K72.1, K72.9, K76.5, K76.6, K76.7
	Atresia and stenosis of small intestine	751.1	Q41.9
	Atresia and stenosis of large intestine, rectum, and anal canal	751.2	Q42.9
	Anomalies of abdominal wall	756.7	Q79.2-4, Q79.51, Q79.59
Renal condition	Renal disease[116]	403.01, 403.11, 403.91, 404.02, 404.03, 404.12, 404.13, 404.92, 404.93, 582.x,	I12.0, I13.1, N03.2–N03.7, N05.2–N05.7, N18.x, N19.x, N25.0, Z49.0–Z49.2, Z94.0, Z99.2

		583.0–583.7, 585.x, 586.x, 588.0, V42.0, V45.1, V56.x	
	Polycystic kidney	753.12-14	Q61.2, Q61.3, Q61.19
	Renal dysplasia	753.15	Q61.4
	Congenital single renal cyst	753.11	Q61.01
	Other specified cystic kidney disease	753.19	Q61.8
	Renal agenesis and dysgenesis	753.0	Q60.2, Q60.5
Neurological condition[117]	Head injury	854.X	S01, S06
	Brain tumor	191.X, 198.3, 225.X, 237.5, 239.6	C71, C79.31, D33.2-4, D33.7, D33.9, D32.0, D32.1, D32.9, D43.2, D43.4, D49.6
	Cerebrovascular disease[116]	362.34, 430.x–438.x	G45.x, G46.x, H34.0, I60.x–I69.x
	Epilepsy	245.XX, 780.39	E06.1, E06.3-E06.5, E06.9, R56.9
Other condition	Depression and other mood disorders	296.XX, 298.0, 300.4, 309.1, 311	F30-F33, F34.8, F39
	Diabetes[116]	250.0–250.3, 250.8, 250.9, 250.4–250.7	E10.0, E10.1, E10.6, E10.8, E10.9, E11.0, E11.1, E11.6, E11.8, E11.9, E12.0, E12.1, E12.6, E12.8, E12.9, E13.0, E13.1, E13.6, E13.8, E13.9, E14.0, E14.1, E14.6, E14.8, E14.9, E10.2–E10.5, E10.7, E11.2–E11.5, E11.7, E12.2–E12.5, E12.7, E13.2–E13.5, E13.7, E14.2–E14.5, E14.7
	Hemiplegia or paraplegia[116]	334.1, 342.x, 343.x, 344.0–344.6, 344.9	G04.1, G11.4, G80.1, G80.2, G81.x, G82.x, G83.0–G83.4, G83.9
	Other conditions due to autosomal anomalies: Accessory autosomes NEC, Triploidy	758.5	Q92.7
	Sepsis[118, 119]	995.91, 995.92, 785.52	A400-A403, A408, A409, A410-A415, A418, A419, R578, T811.

Supplemental Table 3.1: List of comorbidities

Appendix D: Supplemental data from Chapter 4

Supplemental Material 4.1: PRISMA Checklist (directly quoted)[136, 241]

Section/topic	#	Checklist item	Report page # in published manuscript
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	N/A
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
Methods			
Protocol	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	N/A
Selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Collection	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5, 6
Bias in studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used	N/A

		in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A
Synthesis	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5, 6
Bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	N/A
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5, 6
Results			
Selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	16
Characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	14, 16, suppl
Bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	N/A
Results	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	N/A
Synthesis	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression).	N/A
Discussion			
Summary	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8-11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusion	26	Provide a general interpretation of the results in the context of other evidence, implications future research.	11
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

Supplemental Material 4.2: Studies included in primary analysis

- Adler, A., Sadek, M. M., Chan, A. Y., Dell, E., Rutberg, J., Davis, D., . . . Gollob, M. H. (2016). Patient Outcomes From a Specialized Inherited Arrhythmia Clinic. Circ Arrhythm Electrophysiol, 9(1), e003440. doi:10.1161/circep.115.003440
- Aizawa, Y., Mitsuma, W., Ikrar, T., Komura, S., Hanawa, H., Miyajima, S., . . . Aizawa, Y. (2007). Human cardiac ryanodine receptor mutations in ion channel disorders in Japan. Int J Cardiol, 116(2), 263-265. doi:10.1016/j.ijcard.2006.02.024
- Al-Hassnan, Z. N., Tulbah, S., Al-Manea, W., & Al-Fayyadh, M. (2013). The Phenotype of a CASQ2 Mutation in a Saudi Family with Catecholaminergic Polymorphic Ventricular Tachycardia. Pacing and Clinical Electrophysiology, 36(5), e140-e142. doi:10.1111/j.1540-8159.2012.03434.x
- Allouis, M., Probst, V., Jaafar, P., Schott, J.-J., & Le Marec, H. Unusual clinical presentation in a family with catecholaminergic polymorphic ventricular tachycardia due to a G14876A ryanodine receptor gene mutation. American Journal of Cardiology, 95(5), 700-702. doi:10.1016/j.amjcard.2004.10.057
- Andrsova, I., Valaskova, I., Kubus, P., Vit, P., Gaillyova, R., Kadlecova, J., . . . Novotny, T. (2012). Clinical characteristics and mutational analysis of the RyR2 gene in seven Czech families with catecholaminergic polymorphic ventricular tachycardia. Pacing Clin Electrophysiol, 35(7), 798-803. doi:10.1111/j.1540-8159.2012.03399.x
- Arad, M., Glikson, M., El-Ani, D., & Monserrat-Inglesias, L. (2012). A family with recurrent sudden death and no clinical clue. Ann Noninvasive Electrocardiol, 17(4), 387-393. doi:10.1111/anec.12024
- Arakawa, J., Hamabe, A., Aiba, T., Nagai, T., Yoshida, M., Touya, T., . . . Shimizu, W. (2015). A novel cardiac ryanodine receptor gene (RyR2) mutation in an athlete with aborted sudden cardiac death: a case of adult-onset catecholaminergic polymorphic ventricular tachycardia. Heart Vessels, 30(6), 835-840. doi:10.1007/s00380-014-0555-y
- Atallah, J., Fynn-Thompson, F., Cecchin, F., DiBardino, D. J., Walsh, E. P., & Berul, C. I. (2008). Video-assisted thoracoscopic cardiac denervation: a potential novel therapeutic option for children with intractable ventricular arrhythmias. Ann Thorac Surg, 86(5), 1620-1625. doi:10.1016/j.athoracsur.2008.07.006
- Bailey, C., Blair, E., Garratt, C., & Newman, W. G. (2016). Effective cascade screening through identification of a mutation in RYR2 in a large family with a history of sudden death. Journal of Cardiology Cases, 13(1), 9-13. doi:https://doi.org/10.1016/j.jccase.2015.08.015
- Beery, T. A., Shah, M. J., & Benson, D. W. (2009). Genetic characterization of familial CPVT after 30 years. Biol Res Nurs, 11(1), 66-72. doi:10.1177/1099800409333369
- Broendberg, A. K., Nielsen, J. C., Bjerre, J., Pedersen, L. N., Kristensen, J., Henriksen, F. L., . . . Jensen, H. K. (2017). Nationwide experience of catecholaminergic polymorphic ventricular tachycardia caused by RyR2 mutations. Heart, 103(12), 901-909. doi:10.1136/heartjnl-2016-310509
- Celiker, A., Erdogan, I., Karagoz, T., & Ozer, S. (2009). Clinical experiences of patients with catecholaminergic polymorphic ventricular tachycardia. Cardiol Young, 19(1), 45-52. doi:10.1017/s1047951108003338
- Clausen, H., Pflaumer, A., Kamberi, S., & Davis, A. (2013). Electrical Storm in Children. Pacing and Clinical Electrophysiology, 36(3), 391-401. doi:10.1111/pace.12050
- Costa, R., Silva, K. R., Mendonca, R. C., Nishioka, S. A., Siqueira Sde, F., Tamaki, W. T., . . . Filho, M. M. (2007). Incidence of shock and quality of life in young patients with implantable cardioverter-defibrillator. Arq Bras Cardiol, 88(3), 258-264.
- Costello, J. P., Wilson, J. K., Louis, C., Peer, S. M., Zurakowski, D., Nadler, E. P., . . . Nath, D. S. (2014). Surgical Cardiac Denervation Therapy for Treatment of Congenital Ion Channelopathies in

Pediatric Patients: A Contemporary, Single Institutional Experience. World Journal for Pediatric and Congenital Heart Surgery, 6(1), 33-38. doi:10.1177/2150135114555203

- De Ferrari, G. M., Dusi, V., Spazzolini, C., Bos, J. M., Abrams, D. J., Berul, C. I., . . . Schwartz, P. J. (2015). Clinical Management of Catecholaminergic Polymorphic Ventricular Tachycardia: The Role of Left Cardiac Sympathetic Denervation. Circulation, 131(25), 2185-2193. doi:10.1161/circulationaha.115.015731
- de la Fuente, S., Van Langen, I. M., Postma, A. V., Bikker, H., & Meijer, A. (2008). A case of catecholaminergic polymorphic ventricular tachycardia caused by two calsequestrin 2 mutations. Pacing Clin Electrophysiol, 31(7), 916-919. doi:10.1111/j.1540-8159.2008.01111.x
- di Barletta, M. R., Viatchenko-Karpinski, S., Nori, A., Memmi, M., Terentyev, D., Turcato, F., . . . Priori, S. G. (2006). Clinical phenotype and functional characterization of CASQ2 mutations associated with catecholaminergic polymorphic ventricular tachycardia. Circulation, 114(10), 1012-1019. doi:10.1161/circulationaha.106.623793
- Else, S. D., Potts, J. E., & Sanatani, S. (2012). Postexertional supraventricular tachycardia in children with catecholaminergic polymorphic ventricular tachycardia. Case Rep Cardiol, 2012, 329097. doi:10.1155/2012/329097
- Gray, B., Bagnall, R. D., Lam, L., Ingles, J., Turner, C., Haan, E., . . . Semsarian, C. (2016). A novel heterozygous mutation in cardiac calsequestrin causes autosomal dominant catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm, 13(8), 1652-1660. doi:10.1016/j.hrthm.2016.05.004
- Hayashi, M., Denjoy, I., Extramiana, F., Maltret, A., Buisson, N. R., Lupoglazoff, J. M., . . . Leenhardt, A. (2009). Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. Circulation, 119(18), 2426-2434. doi:10.1161/circulationaha.108.829267
- Haydin, S., Saygi, M., Ergul, Y., Ozyilmaz, I., Ozturk, E., Akdeniz, C., & Tuzcu, V. (2013). Subxiphoid approach to epicardial implantation of implantable cardioverter defibrillators in children. Pacing Clin Electrophysiol, 36(8), 926-930. doi:10.1111/pace.12158
- Heiner, J. D., Bullard-Berent, J. H., & Inbar, S. (2011). Deadly proposal: a case of catecholaminergic polymorphic ventricular tachycardia. Pediatr Emerg Care, 27(11), 1065-1068. doi:10.1097/PEC.0b013e3182360606
- Hofferberth, S. C., Cecchin, F., Loberman, D., & Fynn-Thompson, F. (2014). Left thoracoscopic sympathectomy for cardiac denervation in patients with life-threatening ventricular arrhythmias. J Thorac Cardiovasc Surg, 147(1), 404-409. doi:10.1016/j.jtcvs.2013.07.064
- Illikova, V., Hlivak, P., & Hatala, R. (2015). Cardiac channelopathies in pediatric patients - 7-years single center experience. J Electrocardiol, 48(2), 150-156. doi:10.1016/j.jelectrocard.2014.11.010
- Jang, S. Y., Cho, Y., Kim, N. K., Kim, C.-Y., Sohn, J., Roh, J.-H., . . . Kim, G. J. (2017). Video-Assisted Thoracoscopic Left Cardiac Sympathetic Denervation in Patients with Hereditary Ventricular Arrhythmias. Pacing and Clinical Electrophysiology, 40(3), 232-241. doi:10.1111/pace.13008
- Kawamura, M., Ohno, S., Naiki, N., Nagaoka, I., Dochi, K., Wang, Q., . . . Horie, M. (2013). Genetic background of catecholaminergic polymorphic ventricular tachycardia in Japan. Circ J, 77(7), 1705-1713.
- Kawata, H., Ohno, S., Aiba, T., Sakaguchi, H., Miyazaki, A., Sumitomo, N., . . . Shimizu, W. (2016). Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) Associated With Ryanodine Receptor (RyR2) Gene Mutations- Long-Term Prognosis After Initiation of Medical Treatment. Circ J. doi:10.1253/circj.CJ-16-0250
- Khoury, A., Marai, I., Suleiman, M., Blich, M., Lorber, A., Gepstein, L., & Boulos, M. (2013). Flecainide therapy suppresses exercise-induced ventricular arrhythmias in patients with CASQ2-

associated catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm, 10(11), 1671-1675. doi:10.1016/j.hrthm.2013.08.011

- Kozlovski, J., Ingles, J., Connell, V., Hunt, L., McGaughran, J., Turner, C., . . . Semsarian, C. (2014). Delay to diagnosis amongst patients with catecholaminergic polymorphic ventricular tachycardia. Int J Cardiol, 176(3), 1402-1404. doi:10.1016/j.ijcard.2014.08.020
- Leren, I. S., Saberniak, J., Majid, E., Haland, T. F., Edvardsen, T., & Haugaa, K. H. (2016). Nadolol decreases the incidence and severity of ventricular arrhythmias during exercise stress testing compared with beta1-selective beta-blockers in patients with catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm, 13(2), 433-440. doi:10.1016/j.hrthm.2015.09.029
- Marai, I., Khoury, A., Suleiman, M., Gepstein, L., Blich, M., Lorber, A., & Boulos, M. (2012). Importance of ventricular tachycardia storms not terminated by implantable cardioverter defibrillators shocks in patients with CASQ2 associated catecholaminergic polymorphic ventricular tachycardia. Am J Cardiol, 110(1), 72-76. doi:10.1016/j.amjcard.2012.02.049
- McNamara, C., Cullen, P., Rackauskas, M., Kelly, R., O'Sullivan, K. E., Galvin, J., & Eaton, D. (2017). Left cardiac sympathetic denervation: case series and technical report. Ir J Med Sci. doi:10.1007/s11845-017-1577-0
- Miyake, C. Y., Webster, G., Czosek, R. J., Kantoch, M. J., Dubin, A. M., Avasarala, K., & Atallah, J. (2013). Efficacy of implantable cardioverter defibrillators in young patients with catecholaminergic polymorphic ventricular tachycardia: success depends on substrate. Circ Arrhythm Electrophysiol, 6(3), 579-587. doi:10.1161/circep.113.000170
- Nof, E., Belhassen, B., Arad, M., Bhuiyan, Z. A., Antzelevitch, C., Rosso, R., . . . Glikson, M. (2011). Postpacing abnormal repolarization in catecholaminergic polymorphic ventricular tachycardia associated with a mutation in the cardiac ryanodine receptor gene. Heart Rhythm, 8(10), 1546-1552. doi:10.1016/j.hrthm.2011.05.016
- Nyegaard, M., Overgaard, M. T., Sondergaard, M. T., Vranas, M., Behr, E. R., Hildebrandt, L. L., . . . Borglum, A. D. (2012). Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. Am J Hum Genet, 91(4), 703-712. doi:10.1016/j.ajhg.2012.08.015
- Ostby SA, B. M., Owen H, Wackel PL, Cannon BC, Ackerman MJ. (2016). Competitive Sports Participation in Patients With Catecholaminergic Polymorphic Ventricular Tachycardia: A Single Center's Early Experience. JACC: Cardiac Electrophysiology, 2(3).
- Priori, S. G., Napolitano, C., Memmi, M., Colombi, B., Drago, F., Gasparini, M., . . . DeLogu, A. (2002). Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation, 106(1), 69-74.
- Roses-Noguer, F., Jarman, J. W., Clague, J. R., & Till, J. (2014). Outcomes of defibrillator therapy in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm, 11(1), 58-66. doi:10.1016/j.hrthm.2013.10.027
- Rosso, R., Kalman, J. M., Rogowski, O., Diamant, S., Birger, A., Biner, S., . . . Viskin, S. (2007). Calcium channel blockers and beta-blockers versus beta-blockers alone for preventing exercise-induced arrhythmias in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm, 4(9), 1149-1154. doi:10.1016/j.hrthm.2007.05.017
- Roston, T. M., Vinocur, J. M., Maginot, K. R., Mohammed, S., Salerno, J. C., Etheridge, S. P., . . . Sanatani, S. (2015). Catecholaminergic polymorphic ventricular tachycardia in children: analysis of therapeutic strategies and outcomes from an international multicenter registry. Circ Arrhythm Electrophysiol, 8(3), 633-642. doi:10.1161/circep.114.002217
- Schneider, H. E., Steinmetz, M., Krause, U., Kriebel, T., Ruschewski, W., & Paul, T. (2013). Left cardiac sympathetic denervation for the management of life-threatening ventricular tachyarrhythmias in young patients with catecholaminergic polymorphic ventricular tachycardia and long QT syndrome. Clin Res Cardiol, 102(1), 33-42. doi:10.1007/s00392-012-0492-7

- Song, M. K., Baek, J. S., Kwon, B. S., Kim, G. B., Bae, E. J., Noh, C. I., & Choi, J. Y. (2010). Clinical spectrum and prognostic factors of pediatric ventricular tachycardia. Circ J, 74(9), 1951-1958.
- Sy, R. W., Gollob, M. H., Klein, G. J., Yee, R., Skanes, A. C., Gula, L. J., . . . Krahn, A. D. (2011). Arrhythmia characterization and long-term outcomes in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm, 8(6), 864-871. doi:10.1016/j.hrthm.2011.01.048
- Tulumen, E., Schulze-Bahr, E., Zumhagen, S., Stallmeyer, B., Seebohm, G., Beckmann, B. M., . . . Borggrefe, M. (2015). Early repolarization pattern: a marker of increased risk in patients with catecholaminergic polymorphic ventricular tachycardia. Europace. doi:10.1093/europace/euv357
- van der Werf, C., Kannankeril, P. J., Sacher, F., Krahn, A. D., Viskin, S., Leenhardt, A., . . . Wilde, A. A. (2011). Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. J Am Coll Cardiol, 57(22), 2244-2254. doi:10.1016/j.jacc.2011.01.026
- van der Werf, C., Nederend, I., Hofman, N., van Geloven, N., Ebink, C., Frohn-Mulder, I. M., . . . Wilde, A. A. (2012). Familial evaluation in catecholaminergic polymorphic ventricular tachycardia: disease penetrance and expression in cardiac ryanodine receptor mutation-carrying relatives. Circ Arrhythm Electrophysiol, 5(4), 748-756. doi:10.1161/circep.112.970517
- Von Bergen, N. H., Atkins, D. L., Dick, M., 2nd, Bradley, D. J., Etheridge, S. P., Saarel, E. V., . . . Law, I. H. (2011). Multicenter study of the effectiveness of implantable cardioverter defibrillators in children and young adults with heart disease. Pediatr Cardiol, 32(4), 399-405. doi:10.1007/s00246-010-9866-7
- Walsh, M. A., Stuart, A. G., Schlecht, H. B., James, A. F., Hancox, J. C., & Newbury-Ecob, R. A. (2016). Compound Heterozygous Triadin Mutation Causing Cardiac Arrest in Two Siblings. Pacing Clin Electrophysiol, 39(5), 497-501. doi:10.1111/pace.12813
- Wang, S., Zhu, W., Hamilton, R. M., Kirsh, J. A., Stephenson, E. A., & Gross, G. J. (2010). Diagnosis-specific characteristics of ventricular tachycardia in children with structurally normal hearts. Heart Rhythm, 7(12), 1725-1731. doi:10.1016/j.hrthm.2010.07.037
- Wanguemert, F., Bosch Calero, C., Perez, C., Campuzano, O., Beltran-Alvarez, P., Scornik, F. S., . . . Brugada, R. (2015). Clinical and molecular characterization of a cardiac ryanodine receptor founder mutation causing catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm, 12(7), 1636-1643. doi:10.1016/j.hrthm.2015.03.033
- Watanabe, H., Chopra, N., Laver, D., Hwang, H. S., Davies, S. S., Roach, D. E., . . . Knollmann, B. C. (2009). Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. Nat Med, 15(4), 380-383. doi:10.1038/nm.1942
- Yu, T. C., Liu, A. P., Lun, K. S., Chung, B. H., & Yung, T. C. (2016). Clinical and genetic profile of catecholaminergic polymorphic ventricular tachycardia in Hong Kong Chinese children. Hong Kong Med J, 22(4), 314-319. doi:10.12809/hkmj154653

Supplemental Material 4.3: Studies excluded from primary analysis due to excessive overlap in another study:

- Postma, A. V., Denjoy, I., Kamblock, J., Alders, M., Lupoglazoff, J. M., Vaksmann, G., . . . Wilde, A. A. (2005). Catecholaminergic polymorphic ventricular tachycardia: RYR2 mutations, bradycardia, and follow up of the patients. J Med Genet, 42(11), 863-870. doi:10.1136/jmg.2004.028993
- Krahn, A. D., Gollob, M., Yee, R., Gula, L. J., Skanes, A. C., Walker, B. D., & Klein, G. J. (2005). Diagnosis of unexplained cardiac arrest: role of adrenaline and procainamide infusion. Circulation, 112(15), 2228-2234. doi:10.1161/circulationaha.105.552166

- Wilde, A. A. M., Bhuiyan, Z. A., Crotti, L., Facchini, M., De Ferrari, G. M., Paul, T., . . . Schwartz, P. J. (2008). Left Cardiac Sympathetic Denervation for Catecholaminergic Polymorphic Ventricular Tachycardia. New England Journal of Medicine, 358(19), 2024-2029. doi:10.1056/NEJMoa0708006
- Hayashi, M., Denjoy, I., Hayashi, M., Extramiana, F., Maltret, A., Roux-Buisson, N., . . . Leenhardt, A. (2012). The role of stress test for predicting genetic mutations and future cardiac events in asymptomatic relatives of catecholaminergic polymorphic ventricular tachycardia probands. Europace, 14(9), 1344-1351. doi:10.1093/europace/eus031
- Marai, I., Boulos, M., & Khoury, A. (2015). Left Cardiac Sympathetic Denervation in Patients with CASQ2-Associated Catecholaminergic Polymorphic Ventricular Tachycardia. Isr Med Assoc J, 17(9), 538-540.
- Haugaa, K. H., Leren, I. S., Berge, K. E., Bathen, J., Loennechen, J. P., Anfinsen, O. G., . . . Amlie, J. P. (2010). High prevalence of exercise-induced arrhythmias in catecholaminergic polymorphic ventricular tachycardia mutation-positive family members diagnosed by cascade genetic screening. Europace, 12(3), 417-423. doi:10.1093/europace/eup448
- Olde Nordkamp LRA, Driessen AHG, Odero A, Blom NA, Koolbergen DR, Schwartz PJ and Wilde AAM. Left cardiac sympathetic denervation in the Netherlands for the treatment of inherited arrhythmia syndromes. *Netherlands Heart Journal*. 2014;22:160-166.
- Ohno, S., Omura, M., Kawamura, M., Kimura, H., Itoh, H., Makiyama, T., . . . Horie, M. (2014). Exon 3 deletion of RYR2 encoding cardiac ryanodine receptor is associated with left ventricular non-compaction. Europace, 16(11), 1646-1654. doi:10.1093/europace/eut382
- Loar, R. W., Bos, J. M., Cannon, B. C., & Ackerman, M. J. (2015). Sudden cardiac arrest during sex in patients with either catecholaminergic polymorphic ventricular tachycardia or long-QT syndrome: a rare but shocking experience. J Cardiovasc Electrophysiol, 26(3), 300-304. doi:10.1111/jce.12600
- Coleman, M. A., Bos, J. M., Johnson, J. N., Owen, H. J., Deschamps, C., Moir, C., & Ackerman, M. J. (2012). Videoscopic left cardiac sympathetic denervation for patients with recurrent ventricular fibrillation/malignant ventricular arrhythmia syndromes besides congenital long-QT syndrome. Circ Arrhythm Electrophysiol, 5(4), 782-788. doi:10.1161/circep.112.971754

Supplemental Table 4.1

Study	Type of Study	Recruitment period (yrs)	Inclusion/Exclusion criteria	Total number of patients	Number of patients with ICD	Number of ICD patients on BB	Number of ICD patients with appropriate shock(s) on BB	Number of ICD patients with inappropriate shock(s) on BB	Number of ICD patients with electrical storm on BB	Median (range) follow-up in ICD patients on BB (yrs)	Death(s) despite BB and ICD
Hayashi et al., 2009	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	101‡	16 (16%)	NR	3	NR	0	NR	0
Van der Werf et al., 2011	Observational, retrospective	2009-2010	Clinical and genetic CPVT diagnosis and received flecainide	33	12 (36%)	12	3	NR	1	NR	0
Sy et al., 2011	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	27	15 (56%)	15	4	5	2	42 (16-162)	2
Roses-Noguer et al., 2012	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis and received an ICD	13	13 (100%)	13	10	5	0	4.0 (1.7-19.9)	0

Miyake et al., 2013	Observational, retrospective	1999-2001	Clinical and/or genetic CPVT diagnosis in patients with ICDs and symptom onset <21 yrs	24	24 (100%)	24	10	11	4	3.3 (1.1-5.8)	0
Kozlovski et al, 2014	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	35	27 (77%)	NR	NR	NR	NR	NR	0
Roston et al., 2015	Observational, retrospective	2012-2014	Clinical and/or genetic CPVT diagnosis and symptom onset <19 yrs	226¥	118 (54%)	NR	NR	NR	NR	NR	2
De Ferrari et al., 2015	Observational, retrospective	1988-2014	Clinical and/or genetic CPVT diagnosis	63Ω	37 (60%)	NR	NR	NR	NR	NR	1
Wanguemert et al., 2015	Observational, retrospective	1994-2015	Carriers of *RYR2* p.G357S mutation	182	40 (22%)	40	0	0	0	NR	0
Broendberg et al, 2017	Observational, retrospective	Up to June 2016	RyR2 genotype positive CPVT	51	28	28	8	4	2	7.3 (IQR 4.3-14) in probands; 4.0 (IQR 3.5-6.8) in relatives	0
TOTAL±				**750**	**330 of 750(44%)**	**132 of 132 (100%)**	**38 of 135 (28%)**	**25 of 129 (19%)**	**9 of 132 (7%)**		**5 of 132 (4%)**

±Two subjects excluded who were in another cohort (analysis undertaken on 99 patients)
¥Eight subjects excluded who were in another cohort (analysis undertaken on 218 patients)
ΩOne subject excluded who was in another cohort (analysis undertaken on 62 patients)
BB = Beta blocker; ICD = implantable cardioverter defibrillator; NR = not reported; NA = not applicable; IQR = interquartile range

Supplemental Table 4.1: Beta blocker usage amongst CPVT patients who received ICDs.

Supplemental Table 4.2

Study	Type of Study	Recruitment period (yrs)	Inclusion/Exclusion criteria	Total number of patients	Number of patients with ICD	Number of ICD patients on flecainide	Number of ICD patients with appropriate shock(s) on flecainide	Number of ICD patients with inappropriate shock(s) on flecainide	Number of ICD patients with electrical storm on flecainide	Median (range) follow-up in ICD patients on flecainide (yrs)	Death(s) despite flecainide and ICD
Hayashi et al., 2009	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	101±	16 (16%)	0	NA	NA	NA	NA	NA
Van der Werf et al., 2011	Observational,	2009-2010	Clinical and genetic CPVT diagnosis and	33	12 (36%)	12	1	NR	0	20 (12-40)	0

	retrospective		received flecainide								
Sy et al., 2011	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	27	15 (56%)	4	1	1	0	75 (17-143)	0
Roses-Noguer et al., 2012	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis and received an ICD	13	13 (100%)	7	2	NR	NR	40.8 (28.8-88.8)	0
Miyake et al., 2013	Observational, retrospective	1999-2001	Clinical and/or genetic CPVT diagnosis in patients with ICDs and symptom onset <21 yrs	24	24 (100%)	3	0	1	NR	29 (7-82)	0
Kozlovski et al, 2014	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	35	27 (77%)	0	NA	NA	NA	NA	NA
Roston et al., 2015	Observational, retrospective	2012-2014	Clinical and/or genetic CPVT diagnosis and symptom onset <19 yrs	226¥	118 (54%)	NR	NR	NR	NR	NA	0
De Ferrari et al., 2015	Observational, retrospective	1988-2014	Clinical and/or genetic CPVT diagnosis	63 Ω	37 (60%)	NR	NR	NR	NR	NR	0
Wanguemert et al., 2015	Observational, retrospective	1994-2015	Carriers of *RYR2* p.G357S mutation	182	40 (22%)	NR	NR	NR	NR	NR	NR
Broendberg et al, 2017	Observational, retrospective	Up to June 2016	RyR2 genotype positive CPVT	51	28	NR	1	NR	NR	NR	0
TOTAL±				**750**	**330 of 750 (44%)**	**26 of 115 (23%)**	**5 of 26 (19%)**	**2 of 7 (29%)**	**0 of 16**		**0**

±Two subjects excluded who were in another cohort (analysis undertaken on 99 patients)[132]
¥Eight subjects excluded who were in another cohort (analysis undertaken on 218 patients)[59]
ΩOne subject excluded who was in another cohort (analysis undertaken on 62 patients)
BB = Beta blocker; ICD = implantable cardioverter defibrillator; NR = not reported; NA = not applicable; IQR = interquartile range

Supplemental Table 4.2: Flecainide usage amongst CPVT patients who had ICDs

Supplemental Table 4.3

Study	Type of Study	Recruitment period (yrs)	Inclusion/Exclusion criteria	Total number of patients	Number of patients with ICD	Number of ICD patients with LCSD	Number of ICD patients with appropriat	Number of ICD patients with inappropriat	Number of ICD patients with electric	Median (range) follow-up in	Death(s) despite LCSD and ICD

							e shock(s) with LCSD	e shock(s) with LCSD	al storm with LCSD	ICD patients with LCSD (yrs)	
Hayashi et al., 2009	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	101±	16 (16%)	0	NA	NA	NA	NA	NA
Van der Werf et al., 2011	Observational, retrospective	2009-2010	Clinical and genetic CPVT diagnosis and received flecainide	33	12 (36%)	NR	NR	NR	NR	NR	NR
Sy et al., 2011	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	27	15 (56%)	1	1	0	0	144	0
Roses-Noguer et al., 2012	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis and received an ICD	13	13 (100%)	2	1	0	0	2.1 and 1.7	0
Miyake et al., 2013	Observational, retrospective	1999-2001	Clinical and/or genetic CPVT diagnosis in patients with ICDs and symptom onset <21 yrs	24	24 (100%)	0	NA	NA	NA	NA	NA
Kozlovski et al, 2014	Observational, retrospective	NR	Clinical and/or genetic CPVT diagnosis	35	27 (77%)	NR	NR	NR	NR	NR	NR
Roston et al., 2015	Observational, retrospective	2012-2014	Clinical and/or genetic CPVT diagnosis and symptom onset <19 yrs	226¥	118 (54%)	14	1	NR	0	NR	0
De Ferrari et al., 2015	Observational, retrospective	1988-2014	Clinical and/or genetic CPVT diagnosis	63Ω	37 (60%)	29*	10	NR	4	NR	1
Wangemert et al., 2015	Observational, retrospective	1994-2015	Carriers of *RYR2* p.G357S mutation	182	40 (22%)	0	NA	NA	NA	NA	NA
Broendberg et al, 2017	Observational, retrospective	Up to June 2016	RyR2 genotype positive CPVT	51	28	0	NA	NA	NA	NA	NA
TOTAL±				**750**	**330 of 750 (44%)**	**56 of 270 (21%)**	**13 of 46 (28%)**	**0 of 3**	**4 of 46 (9%)**		**1 of 46 (2%)**

±Two subjects excluded who were in another cohort (analysis undertaken on 99 patients)
¥Eight subjects excluded who were in another cohort (analysis undertaken on 223 patients)
ΩOne subject excluded who was in another cohort (analysis undertaken on 62 patients)
*the authors excluded 8 ICD patients from analysis with insufficient follow up post-ICD, did not have an ICD prior to LLCSD, or had it removed.
BB = Beta blocker; LCSD = cardiac sympathetic denervation; ICD = implantable cardioverter defibrillator; NR = not reported; NA = not applicable; IQR = interquartile range

Supplemental Table 4.3: LCSD amongst CPVT patients who had ICDs

Appendix E: Supplemental data for Chapter 7

Supplemental Material 7.1: **Detailed Material and Methods**

Clinical Analysis & Definitions: Genetic testing was performed for routine patient care and was therefore not standardized. Patients who had ≥2 variants in CPVT-associated gene(s) reported to be pathogenic, likely pathogenic (P/LP) and/or variant(s) of uncertain pathogenicity (VUS) were included. Data from medical records were inputted by individual sites in a secure online database (REDCap – Research Electronic Data Capture), [177] which was hosted and overseen by the coordinating center (British Columbia Children's Hospital). A proband was defined as the index case of confirmed catecholaminergic polymorphic ventricular tachycardia (CPVT) in a family, and treatment failure was defined as sudden cardiac arrest, syncope and/or appropriate implantable cardioverter defibrillator shock while on pharmacologic therapy. Duration of follow-up was calculated as the time from initial diagnosis/sentinel CPVT symptom to most recent known cardiology assessment.

Genetic Analysis and Pathogenicity Classification: A systematic, stepwise approach to variant classification was undertaken. The commercial lab interpretation of pathogenicity was initially recorded in the database. While the terminology used by laboratories has become more standardized in recent years, for earlier reports, commercial companies used variable terminology to define variant pathogenicity. We often needed to update their terminology using the following modern classification scheme: pathogenic, likely pathogenic (grouped together as P/LP) or VUS. The most common historical term used by laboratories was the variant of "possible" pathogenicity. We re-classified this as a P/LP, given that the commercial testing company using this term also had a separate category for VUS. These commercial interpretations were then re-classified using the American College of Medical Genetics and Genomics (ACMG)[82] criteria, followed by 3D mapping using an RyR2 model. To apply the ACMG criteria, we first accessed the Exome Aggregation Consortium (ExAC) browser determine the population frequency of each variant. ExAC is an online repository of genetic information from 60,706 unrelated individuals sequenced as part of various disease-specific and population studies.[32] Next, we performed a detailed literature review using PubMED, Google Scholar, and the bibliographies of major papers in the field to identify any clinical and functional data on candidate mutations. After this process, variants were then re-classified according to contemporary criteria for pathogenicity defined by the ACMG[82], which require the latter two

steps to undertake a complete assessment of a variant. We then used the 3D model of pig (*Sus scrofa*) RyR2 in the open-state (PDB ID: 5GOA) to predict structural impact of variants whenever possible, which can inform the underlying mechanism behind CPVT.[205] Analysis of the 3D structural environment of all variants was performed using Pymol (v2.1Schrödinger, LLC.). The supplemental tables below summarize the criteria applied for determination of pathogenicity, including the results of the literature review, ExAC allele frequencies and 3D structural mapping.

Supplemental Table 7.1

Variant	Consequence	Reported pathogenicity*	Published evidence	First author of previous reports	ExAC Allele Frequency[32]	ACMG Criteria[82]	ACMG Conclusion	Structural modeling in present study	Updated pathogenicity based on modeling vs. ACMG criteria
RYR2-p.R417L	Missense	P/LP	No reports	N.A.	Absent	Moderate (PM1, PM2, PM6); Supporting (PP2, PP4)	Likely pathogenic	R417 is located inside an alpha helix in domain C at domains A-C and B-C interfaces, near the anion-binding site. The inter-domain area is dominated by hydrophilic and charged residues. The R417L mutation would introduce a shorter, hydrophobic side chain in place of a bulky, positively charged side chain, which may	Unchanged

								alter the anion binding and cause domain-domain rearrangements.	
RYR2-p.F3496L	Missense	VUS	No reports	N.A.	Absent	Moderate (PM2, PM6); Supporting (PP1, PP2, PP4)	Likely pathogenic	F3496 is located in an intrinsically disordered alpha-solenoidal region of RyR2 (Sol2), and can thus not be visualized. It is currently unknown whether any auxiliary protein binds to this region.	Unchanged
RYR2-p.S3938R	Missense	P/LP	2 cases; no co-segregation or in vitro testing reported	Medeiros-Domingo et al.[183] Tester et al.[139]	Absent	Moderate (PM1, PM2, PM6); Supporting (PP2, PP4)	Likely pathogenic	S3938 is located in the CSol3 region of RyR2. S3938 is near the pore, within the cytosolic side of the channel. Mutation to bulkier, positively charged side chain may alter hydrogen bonding pattern at this site and/or disrupt surrounding alpha helices	Unchanged

								structure. Due to the large open space surrounding this residue, the site may also influence binding to an unknown auxiliary protein.	
RYR2-p.R485Q	Missense	VUS	Structural modeling showing loss of π-cation interaction	Bottillo et al.[242]	0.00008645	Moderate (PM1, PM6); Supporting (PP2, PP4)	Likely pathogenic	R485 is located inside an alpha helix of domain C, buried within the helical bundle. The R485 side chain forms a salt bridge with the E411, located in another helix facing domains A and B. The R485Q mutation would break this interaction, destabilizing domain C, and affect the anion binding site.	Unchanged
RYR2-p.R2474K	Missense	P/LP	1 case; no co-segregation or in vitro testing reported	Kozlovski et al.[147]	Absent	Moderate (PM1, PM2, PM6); Supporting (PP2)	Likely pathogenic	R2474 is located in the Sol2 region of RyR2. Region is poorly resolved in CryoEM structures. The variant is subtle and	Unchanged

								structural predicted suggests a minimal impact. It is currently unknown whether any auxiliary protein binds to this region.	
RYR2-p.A1136V	Missense	VUS	1 case reported[183], present in healthy controls[243]; no co-segregation or in vitro testing reported	Medeiros-Domingo et al.[183] Kaartinen et al.[243]	0.007063	Strong (PS2); Supporting (PP2, PP4)	Likely pathogenic	A1136 is located within the SPRY2 domain. It is buried inside a hydrophobic core, and a substitution to a larger side chain may perturb the folding and form clashes with nearby residues such as R1114 and L1128. The equivalent residue in both RyR1 and RyR3 is a valine, therefore the mutation is unlikely to have significant negative impact on the overall structure of RyR.	Downgraded
RYR2-p.I2075T	Missense	P/LP	Same family previously published; no co-segregation	Paech et al.[222]	0.000009395	Supporting (PP1, PP2, PP4)	VUS	I2075 is located within an alpha-solenoid	Unchanged

			or in vitro testing reported					region, where it is buried between two helices. Substitution by Thr is likely to affect the helical packing. Importantly, it is very close to an interface with another alpha solenoid region, and the variant may thus impact this interdomain interaction.	
RYR2-p.K4594R	Missense	VUS	Same family previously published; no co-segregation or in vitro testing reported	Paech et al.[222]	Absent	Moderate (PM1, PM2); Supporting (PP1; PP2; PP4)	Likely pathogenic	K4594 is located at the cytosolic edge of the pseudo voltage-sensing domain (pVSD), with potential interactions with the thumb and forefingers (TaF) domain. These domains are implicated in the binding of activating ligands and channel opening. Although the K4594R substitutio	Unchanged

								n is conservative, the guanidinium group of Arg allows for a larger number of electrostatic interactions. Further, the higher pKa of the guanidinium group may facilitate a stronger interaction with nearby E4200, which could impact on the ATP/Caffeine binding sites located nearby (Fig. 2H). Thus any small perturbation in this area is likely to alter channel gating.	
RYR2-p.R2028H	Missense	P/LP	No reports	N.A.	Absent	Moderate (PM2); Supporting (PP2, PP4)	VUS	R2028 is found in Sol2 region of RyR2, where the side chain is pointing toward the solvent. The variant is unlikely to have a major impact on the function, but may	Downgraded

								influence binding to an unknown auxiliary protein.	
RYR2-p.Y4721C	Missense	P/LP	No reports	N.A.	Absent	Moderate (PM1, PM2); Supporting (PP2, PP4)	Likely pathogenic	This residue is located within the transmembrane region of pVSD. This region plays an important role in allosteric gating of the channel and the Tyr is surrounded by other hydrophobic residues. Mutation to cysteine is likely to perturb channel gating and domain packing.	Unchanged
RYR2-p.L4188P	Missense	VUS	Study subject also reported previously[244]; Polyphen-2 score consistent with probable damaging effect but present in population[245]	Jabbari et al.[245] LaPage et al.[244]	Absent	Strong (BS2); Moderate (PM1, PM2, PM6); Supporting (PP2; PP4)	VUS	L4188 is located within the TaF domain that clamps the C-terminal extension of the RyR. This interaction is critical for channel gating. The substitution by Pro, which promotes helix breaking, and may perturb channel gating.	Upgraded

RYR2-p.H2464D	Missense	P/LP	1 case; human derived pluripotent stem cells showing gain of function[246]	Hernandez et al.[246]	Absent	Strong (PS3); Moderate (PM1, PM2, PM6); Supporting (PP2, PP4)	Pathogenic	H2464 is located within a poorly resolved Sol2 region of RyR2 structure. The variant may impact binding of an unknown auxiliary protein to this region.	Unchanged
RYR2-p.S2246L	Missense	P/LP	Reported in multiple human CPVT patients[137, 183]; augmented calcium release to beta-adrenergic agents in HL-1 derived cardiomyocytes;[247] Knock-in mouse model showing increase calcium spark frequency and lethal arrhythmia[248]	George et al.[247] Sueto mi et al.[248] Medeiros-Domingo et al.[183] Postma et al.[137]	Absent	Strong (PS3); Moderate (PM1, PM2, PM6); Supporting (PP2; PP4)	Pathogenic	S2246 is located within the Sol2 region, where the side chain is tightly packed next to an alpha helix. Mutation to a longer side chain likely results in steric clashes, and will impact helix packing in this region..	Unchanged
RYR2-p.G1885E	Missense	VUS	3 cases of compound heterozygous (trans) ARVC with *RYR2*-G1886S[249] and two ARVC families with double mutations (cis) with *RYR2*-*Q*2958R[232]; HEK293 mutants demonstrate	Milting et al.[249] Tiso et al.[232] Koop et al.[250]	0.01540	Strong (PS3, BS1; BS2); Supporting (PP2, PP4)	VUS	G1885 is located in a flexible unstructured loop. Though the substitution is unlikely to have an impact on channel gating, because this region is highly conserved among in RyRs, the	Unchanged

			gain of function[250]					region may play an important allosteric role or is a part of a binding site for an auxiliary protein.	
RYR2-p.G1886S	Missense	VUS	3 cases of compound heterozygous (trans) ARVC with *RYR2*-G1885E[249] and associated with increased susceptibility in population of ICD patients with heart failure[211]	Milting et al.[249] Francia et al.[211]	0.04385	Strong (BS1, BS1); Supporting (PP2, PP4)	VUS	G1886 is located in a flexible unstructured loop as part of Sol2 region. Though the substitution alone is unlikely to have an impact on channel gating, it may have indirect effects such as creation of a new phosphorylation site.	Unchanged
RYR2-p.T1107M	Missense	VUS	1 family with hypertrophic cardiomyopathy[251]; HEK293 mutants demonstrate loss of function[215] and structural modeling shows steric clash with neighboring hydrophobic residues[209]	Noboru et al.[251] Tang et al.[215] Lau et al.[209]	0.0003230	Strong (PS2, PS3); Supporting (PP2, PP3, PP4)	Pathogenic	T1107 is located within the SPRY2 domain, where it is buried and surrounded by hydrophobic residues. The mutation would form steric clashes with W1156 and cause destabilization of the domain. Functional experiments have	Unchanged

								shown it affects Ca^{2+} release properties.	
RYR2-p.G4772S	Missense	P/LP	No reports	N.A.	Absent	Moderate (PM2); Supporting (PP1, PP2, PP4)	VUS	G4772 is located in the pore forming domain (PFD), as part of the outer helix. Substitution to less flexible Ser may affect helical packing within the membrane and cause subtle domain rearrangements.	Unchanged
RYR2-p.R2401H	Missense	P/LP	Several isolated cases[252-254]	Aizawa et al.[254] Liu et al.[252] Creighton et al.[253]	Absent	Moderate (PM1, PM2); Supporting (PP2, PP4)	Likely pathogenic	R2401 is located within the Sol2 region. It forms a salt bridge with a neighboring D2397 residue. Substitution to His may have a minor impact on helix stability.	Unchanged
RYR2-c.3599-9delT	Splice site	VUS	No reports	N.A.	Absent	Moderate (PM2); Supporting (PP4, BP3)	VUS	Not performed	N.A.
RYR2-c.14091-11dupT	Splice site	VUS	No reports	N.A.	Absent	Moderate (PM2); Supporting (PP4, BP3)	VUS	Not performed	N.A.

RYR2-p.A2317E	Missense	P/LP	1 case[134]; Polyphen score consistent with probable damaging effect but present in general population[245]	Van der Werf et al.[134] Jabbari et al.[245]	Absent	Strong (BS3); Moderate (PM1); Supporting (PP2, PP4)	VUS	A2317 is in an alpha solenoid region, where it is involved in packing with neighbouring residues. Mutation to the larger Glu residue likely forms steric clashes with nearby residues, and this is likely to affect packing and stability of the region.	Upgraded
CASQ2-IVS5+1G>C	Splice site	P/LP	No reports	N.A.	Absent	Very Strong (PVS1); Moderate (PM2); Supporting (PP4, PP5)	Pathogenic	Not performed	N.A.
SCN5A-p.Q692K	Missense	P/LP	1 sudden infant death case[255] but reclassified as polymorphism based on population data[256]	Millat et al.[255] Kapplinger et al.[256]	0.0002822	Strong (BS2); Supporting (PP4)	VUS	Not performed	N.A.
DSG-p.V288I	Missense	VUS	No reports	N.A.	Absent	Moderate (PM2); Supporting (PP4)	VUS	Not performed	N.A.
CACNA1C-p.T1870M	Missense	VUS	No reports	N.A.	Absent	Moderate (PM2); Supporting	VUS	Not performed	N.A.

						(PP2, PP4)			
CACNA1C - c.5680+11C>T	Intronic	VUS	No reports	N.A.	Absent	Moderate (PM2); Supporting (PP4, BP3)	VUS	Not performed	N.A.
TMEM43- c.512+19G>T	Intronic	VUS	1 case of ARVC[257]	Haywood et al.[257]	Absent	Moderate (PM2); Supporting (PP4, BP3)	VUS	Not performed	N.A.
PKP2- c.2300-4G>C	Splice site	VUS	No reports	N.A.	0.00008079	Supporting (PP4, BP3)	VUS	Not performed	N.A.
DSP- p.R1458G	Missense	P/LP	1 ARVC[258] and 1 dilated cardiomyopathy[259] patient, both with additional VUS	Cox et al.[258] Pugh et al.[259]	0.001737	Supporting (PP4)	VUS	Not performed	N.A.

* P/LP=pathogenic/likely pathogenic; ACMG=American College Medical Genetics; N.A.=not applicable; VUS=variant of uncertain significance

Supplemental Table 7.1: Detailed classification scheme for all variants in the population