Sex-Influenced Mortality in a large Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) cohort caused by *RYR2* p.R420W

by

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Abstract:

Background:

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is an arrhythmia syndrome causing sudden cardiac death (SCD). We have ascertained three multiplex, multigenerational families with CPVT due to *RYR2* (p.R420W) disrupting calcium channel regulation. The effect of *RYR2* p.R420W on survival is described.

Methods:

Cases of sudden death were ascertained from clinical and genetics chart reviews following informed research consent from individuals or their next of kin (Study ID 00-176). Individuals were considered well-ascertained if disease status of ≥ 50% of their sibship was known (n=60). Affected individuals included mutation positive, obligate carriers (OC), and/or documented SCD <50 years (n=32). Unaffected status was defined as mutation negative (n=23). Remaining individuals were designated unknown (n=5). Unaffected and Unknown groups were combined. Time to death was compared using Kaplan-Meier time-to-event analysis and multivariate Cox regression. Survival was compared to families with Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) due to *TMEM43* p.S358L.

Results:

Affected status with *RYR2* p.R420W was significantly associated with mortality (RR=4, 95% CI: 1.1-14.5), with 24% mortality in the affected group by age 30. Affected males died earlier than females (RR=6, 95% CI: 1.7-20.4). Median survival in males with *RYR2* p.R420W was 50 years (95% CI: 5.5-94.5), compared to *TMEM43* 44 years (95% CI: 42.0-46.8). Median survival

in females with *RYR2* was 76 years (95% CI: 37.7-115.2), compared to *TMEM43* 73 years (95% CI: 69.6-76.1).

Conclusions:

The *RYR2* p.R420W mutation significantly affects mortality. There is a clear sex-influence. Median survival is comparable to another highly lethal mutation *TMEM43* p.S358L causing ARVC.

General Summary:

Catecholaminergic polymorphic ventricular tachycardia is a rare inherited syndrome that causes early sudden cardiac death in affected individuals. A mutation (*RYR2* p.R420w*)* was identified in three families in Newfoundland and Labrador with early sudden cardiac death. Here we show that individuals with this mutation suffer early mortality. Further, we found males with *RYR2* p.R420w die earlier than females with the same mutation suggesting a sex influence. Finally, mortality with this mutation is similar to mortality with a well-known sudden death syndrome (arrhythmogenic right ventricular cardiomyopathy), which affects the same population.

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Finally, I would like to thank the patients who agreed to be enrolled in our study. The opportunity to report on this condition is a valuable opportunity to move the needle on our understanding of this disease and a step closer to early identification of this tragic disease. I cannot thank those who shared their information so willingly enough, despite the obvious emotional grief attached to this disease and its devastating outcomes.

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List of Abbreviations:

ARVC	Arrhythmogenic Right Ventricular Cardiomyopathy
ASCVD	Atherosclerotic Cardiovascular Disease
ATP	Adenosine Triphosphate
CPVT	Catecholaminergic Polymorphic Ventricular Tachycardia
CT	Computed Tomography
ECG	Electrocardiogram
HCM	Hypertrophic Cardiomyopathy
ICD	Implantable Cardioverter Defibrillator
LBBB	Left Bundle Branch Block
LCSD	Left Cardiac Sympathetic Denervation
LVNC	Left Ventricular Non-Compaction
LQTS	Long QT Syndrome
MI	Myocardial Infarction
MRI	Magnetic Resonance Imaging
NL	Newfoundland and Labrador
NSVT	Non-Sustained Ventricular Tachycardia
PET	Positron Emission Tomography
PMVT	Polymorphic Ventricular Tachycardia
PVC	Premature Ventricular Contraction
QTc	Correct QT interval
RVOT VT	Right Ventricular Outflow Tract Ventricular Tachycardia
RBBB	Right Bundle Branch Block
RyR2	Ryanodine Receptor 2
SIDS	Sudden Infant Death Syndrome
SQTS	Short QT Syndrome
SR	Sarcoplasmic Reticulum

SUD	Sudden Unexplained Death
SVT	Supraventricular Tachycardia
TDP	Torsades De Pointes
VF	Ventricular Fibrillation

Chapter 1: Introduction

1.1 Inherited arrhythmia syndromes:

Inherited arrhythmia syndrome is a diagnostic term describing inherited conditions related to primary electrical disorders in the setting of a structurally normal heart. All disorders which can create disruption in the normal electrical activity of the heart i.e., arrhythmias, are classified under this term. These inherited arrhythmia syndromes are caused by gene mutations encoding ion channels.

Inherited arrhythmia syndromes are of specific interest due to their capacity to produce potentially malignant arrhythmias in otherwise healthy individuals. These malignant arrhythmias can ultimately lead to sudden cardiac death (SCD). It is recognized that nearly one third of autopsy-negative sudden unexplained death (SUD) in young persons and approximately 10% of sudden infant death syndrome (SIDS) are caused by inherited arrhythmia syndromes [1].

Long QT Syndrome (LQTS), Short QT Syndrome (SQTS), Brugada syndrome, and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) are the most commonly described inherited arrhythmia syndromes in the literature. Despite this, most data on these syndromes are limited to case studies of probands and small family studies. The focus of this thesis is on CPVT and the natural history of a single mutation.

1.2 Genetics of Inherited Arrhythmia Syndromes:

Most inherited arrhythmia syndromes are monogenic disorders, in that they are caused by a single gene mutation or variation. Monogenic disorders are passed through families following typical Mendelian inheritance patterns. Five patterns of inheritance of physical traits over multiple generation; autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, or

Y-linked. [2]. In Mendel's description each parent contributes an element, an allele, to each offspring. In autosomal dominant inheritance, the presence of only one allele is required for expression of a trait, whereas in autosomal recessive, two alleles are required. For example, diseases such as Huntington's require the presence of only a single mutated allele to produce disease phenotype. Whereas diseases such as cystic fibrosis require two mutated alleles (one from each parent), to produce disease. X-linked disorders are transmitted from mothers to their offspring. X-linked recessive disorders are exclusively transmitted through the X chromosome and predominantly affect males. X-linked dominant disorders affect males and females equally. Y-linked transmission is theoretical only.

Penetrance is defined as the proportion of individuals with a genotype that is causative of a disease who manifest disease. Using Huntington's disease as an example; all individuals who carry a pathogenic variant will manifest disease. This differs from genetic variants with variable penetrance such as pathogenic BRCA1 variants which are implicated in breast cancer. The lifetime prevalence of developing breast cancer with a pathogenic BRCA1 variant is estimated at 60-80%. Penetrance is influenced by age, allelic heterogeneity, modifier genes and environmental exposures.

Here we describe the autosomal dominant transmission of a single gene mutation which is associated with CPVT, *RYR2* p.R420W.

1.3: Clinical Description of CPVT

CPVT is an inherited arrhythmia syndrome or channelopathy. CPVT was first characterized in the 1970's and was set apart from other inherited arrhythmias with similar phenotypes including right ventricular outflow tract ventricular tachycardia (RVOT VT) and

Torsades de pointes (TDP) (1). CPVT has been defined by the production and progression of potentially lethal cardiac arrhythmias, including polymorphic premature ventricular contractions (PVCs), non-sustained ventricular tachycardia (NSVT), bidirectional and polymorphic ventricular tachycardia (PMVT), and ventricular fibrillation (VF) (Figure 1) [3]. Phenotypically, CPVT occurs in the absence of overt changes on surface electrocardiograms (ECG), and in the absence of structural heart disease.

Clinically patients normally present with syncopal episodes or SCD induced by exertional or emotional stress, attributed to the onset of PMVT. CPVT has also been implicated in several cases of unexplained or near drowning in young healthy swimmers [4]. Several descriptions in the literature report up to one-third of CPVT patients experience arrhythmic events prior to initiation of therapy, with many patients suffering repeated syncopal events prior to diagnosis. In many cases convulsions as well as fecal and urinary incontinence related to cerebral hypoperfusion may accompany syncopal episodes and are inappropriately attributed to neurologic disorders leading to delay in diagnosis [5]. In these patients CPVT is often only diagnosed in retrospect when antiepileptic therapies are found to be ineffective.

Up to 30% of patients report a family history of exercise-related syncope, seizure, or SCD with family history highlighting the importance of through ascertainment of family history in clinical interviews. This also underlines the genetic predisposition to adverse events among patients in families with CPVT-causing mutations.

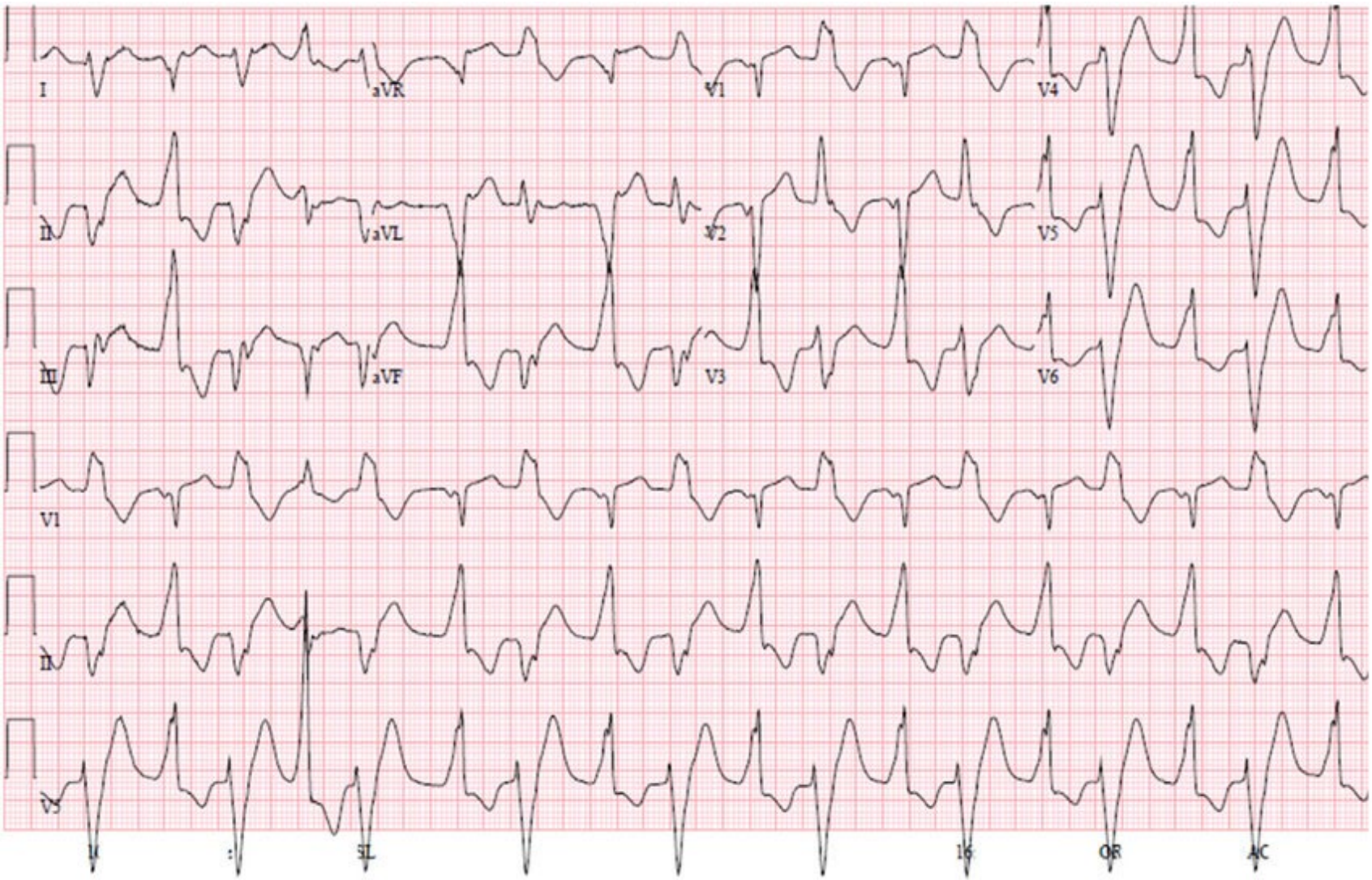

Figure 1: Bidirectional Ventricular Tachycardia in a patient with CPVT (adapted from Koene et al, licensed by CC BY-NC-ND).

Rapid uncoordinated cardiac activity is displayed in this ECG. This arrhythmia can lead to loss of consciousness or even sudden death

1.4 Prevalence and Onset of CPVT

The prevalence of CPVT has not been established in the literature however estimates by expert groups suggests a prevalence of approximately 1:10,000 in the general population [6]. This number likely underestimates the true prevalence of CPVT as it is likely a cause in a significant portion of SCD labelled 'idiopathic VF' or 'autopsy negative' sudden death. In a single Spanish series, CPVT-related mutations were implicated in 15% of cases of 'idiopathic VF' [7].

CPVT normally first presents in the first or second decade of life; however, it can occur as late as the fourth decade of life [4]. Furthermore, genetic mutations specific to CPVT have been attributed to at least two cases of SIDS [8,9]. CPVT does hold measurable clinical significance given the mortality rate in untreated patients under the age of twenty is estimated at as high 50% representing one of the most severe inherited arrhythmogenic disorders [4]. Furthermore, family studies have revealed a family history of SCD before the age of 40 years old for more than one third of individuals with CPVT and as many as 60% of families with ryanodine receptor 2 (RyR2*)* mutations [10].

1.5 Diagnostic Testing in CPVT:

Several diagnostic tools are imperative in the clinical diagnosis of CPVT. 12-lead ECG, the cornerstone of diagnosis in nearly all cardiac disease, is unremarkable in CPVT. The absence of ECG features suggestive of alternative diagnoses, however, is helpful in leading to correct diagnosis of CPVT. Specifically, the absence of a prolonged corrected QT interval suggestive of LQTS, ST segment elevation suggestive of Brugada syndrome, precordial T wave inversions and epsilon waves suggestive of arrhythmogenic right ventricular cardiomyopathy (ARVC), and

evidence of ventricular hypertrophy suggestive of hypertrophic cardiomyopathy (HCM). Patients with CPVT may be bradycardic, however this is neither sensitive nor specific in the diagnosis [4,9].

Ambulatory ECGs (Holter monitors) are useful in patients who are unable to perform exercise testing, specifically infants and patients with physical limitations who are suspected of having CPVT. Furthermore, among patients suspected to have emotion induced arrhythmias by clinical history, ambulatory monitoring is useful in capturing arrhythmias that would otherwise go undetected. Ambulatory monitoring can also detect other arrhythmias, specifically atrial fibrillation or supraventricular arrhythmias, which is helpful in raising or lowering clinical suspicion for CPVT [11]. Though not robustly studied, the sensitivity of Holter monitoring is likely lower than that of exercise testing [12].

Cardiac imaging studies are useful only in excluding the presence of structural heart disease, a requirement of contemporary diagnostic criteria [13]. Transthoracic and transesophageal echocardiography, cardiac magnetic resonance imaging (MRI) and computed tomography (CT) are normal in CPVT patients. Coronary angiography documenting normal coronary arteries is also a requirement for the diagnosis of CPVT. Cardiac imaging is useful in ruling out causes of arrhythmia with overt structural changes including coronary artery anomalies, ARVC, HCM, and left ventricular non-compaction (LVNC). A single small study of 20 patients revealed 3 cases with structural abnormalities on cardiac MRI suggestive of LVNC, generating the hypothesis that more sensitive imaging modalities may be able to detect structural abnormalities in patients with CPVT. The prevalence of structural heart disease on MRI and prognostic value of dedicated testing is yet to be fully elucidated, and is the subject of ongoing studies [14].

The gold standard for the diagnosis of CPVT is exercise ECGs. In a large survey of 44 European centers, exercise testing was valuable in the diagnosis of asymptomatic patients with

CPVT [15]. Exercise testing mimics the physiologic substrate to produce ventricular arrhythmia. Studies have shown an exercise threshold of 110-130 beats/minute is required to produce PVCs. Predominant PVC morphologies that have been reported are left bundle branch block (LBBB) with superior axis and right bundle branch block (RBBB) with inferior axis [12]. As exercise duration and intensity increases ventricular arrhythmias also progress in severity. Isolated PVCs progress to ventricular couplets or NSVT. Occasionally, PMVT or bidirectional ventricular tachycardia can be elicited with exercise (Figure 1). A small study on the impact of sudden high workload exercise revealed that burst exercise stress testing could induce new and more complex arrhythmias than standard exercise testing. Further studies on this exercise protocol are needed, however it is hypothesized to improve diagnostic sensitivity and impact clinical decision making [16].

Some groups have advocated for the use of the sympathomimetic agent epinephrine, for the provocation of ventricular arrhythmias among patients without arrhythmia on ambulatory ECG or exercise testing, in whom clinical suspicion remains high [17]. The sensitivity of such testing has been shown to be lower than that of exercise testing [18].

The role of electrophysiology testing in patients with CPVT remains limited. Given the requirement of emotional or exertional stress, and the associated adrenergic stimulation, there is limited ability to trigger ventricular arrhythmia. Current guidelines support the lack of diagnostic or prognostic value among patient with CPVT [13]. There is a role however to assess for the presence of coexisting arrhythmias, specifically supraventricular tachycardia (SVT) and sinus node dysfunction [19].

Genetic testing for mutations causative of CPVT are useful even when a clinical diagnosis is made to confirm the diagnosis. Further, the identification of a causative mutation is of particular importance in identifying asymptomatic relatives during cascade screening. Identification of

asymptomatic mutation carriers allows them to be surveilled and treated appropriately, halting the possibility of SCD as the first presentation. Current guideline documents suggest genetic screening for all first-degree relatives of a individuals who meet diagnostic criteria for CPVT or have a causative mutation.

In 2013, an expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes was published by the Heart Rhythm Society/European Heart Rhythm Association/Asia Pacific Heart Rhythm Society. This document specifically presents diagnostic criteria for CPVT. The diagnosis of CPVT is usually made in the presence of a structurally normal heart in patient with unexplained exercise or catecholamine induced arrhythmia before, as well as in the presence of a pathogenic mutation [13]. Table 1 demonstrates the specific requirements for a diagnosis of CPVT.

Table 1: Diagnostic Criteria for CPVT (HRS/EHRA/APHRS Expert Consensus Statement on the Diagnosis and Management of Patients with Inherited Primary Arrhythmia Syndromes) [13].

CPVT is diagnosed in the presence of a structurally normal heart, normal ECG, and unexplained exercise or catecholamine-induced bidirectional VT or polymorphic ventricular premature beats or VT in an individual <40 years of age.
CPVT is diagnosed in patients (index case or family member) who have a pathogenic mutation.
CPVT is diagnosed in family members of a CPVT index case with a normal heart who manifest exercise-induced premature ventricular contractions (PVCs) or bidirectional/polymorphic VT.
CPVT can be diagnosed in the presence of a structurally normal heart and coronary arteries, normal ECG, and unexplained exercise or catecholamine-induced bidirectional VT or polymorphic ventricular premature beats or VT in an individual >40 years of age.

1.6 Differential Diagnosis for CPVT:

The clinical phenotype of CPVT closely resembles the phenotype of several other inherited arrhythmias. Long QT Syndrome 1 (LQTS1) resembles the phenotype of patients with CPVT closely. Both syndromes present with exercise-induced syncope or sudden death often with swimming as a potentially lethal arrhythmia-precipitating trigger. Both syndromes have been shown to underlie cases of sudden unexplained drownings in young, healthy swimmers. CPVT, however, appears to be more lethal than LQTS1 [10,20]. Though the clinical presentation is similar in both diseases, 12-lead ECG is often helpful as it is normal in patients with CPVT with patients with LQTS1 having a prolonged corrected QT interval (QTc). In the event of a normal baseline

ECG, exercise testing is of further utility. Patients with LQTS will often demonstrate QTc interval prolongation in the recovery phase of the exercise test [21], whereas the presence of exercise induced arrhythmia is in keeping with a diagnosis of CPVT [22].

Andersen-Tawil is a rare inherited arrhythmia syndrome which in some cases can phenotypically mimic the presentation of CPVT. Though Andersen-Tawil is commonly associated with periodic paralysis, facial and limb dysmorphia, along with ventricular arrhythmia, penetrance of these features is variable. In the absence of overt non-cardiac features, the diagnosis of Andersen-Tawil can easily be mistaken for CPVT [23].

Arrhythmia syndromes related to diseases with structural heart disease can often be mistaken for CPVT, especially in the absence of obvious findings on first line diagnostic imaging (i.e transthoracic echocardiography). Diseases such as HCM, mitral annular disjunction, and ARVC may initially mimic CPVT. Advanced imaging such as cardiac MRI, positron emission tomography (PET), along with genetic testing are vital in differentiating these diagnoses.

The overlap between CPVT and ARVC is of specific interest in the Newfoundland and Labrador (NL) population due to the presence of mutations for both in the NL population [24]. Both diseases present with ventricular arrhythmias and SCD among young patients in the same population. Furthermore, many of the individuals described herein were initially considered to have ARVC given the prevalence in the NL population and the earlier description of ARVC's natural history in NL (many ARVC at risk family members would initially have no features on clinical testing) [25].

Beyond the above-mentioned inherited arrhythmia syndromes, it is likely that a significant portion of presentations labelled 'idiopathic VT' and other unexplained SCD are likely attributable to CPVT causing mutations based on autopsy studies [7]. Finally, the hallmark arrhythmia of CPVT,

bidirectional VT, has also been reported in other disorders, specifically digoxin toxicity, myocarditis, and sarcoidosis [26,27]. Figure 2 shows common differential diagnoses to be considered among patients with SCD below the age of 40 [28].

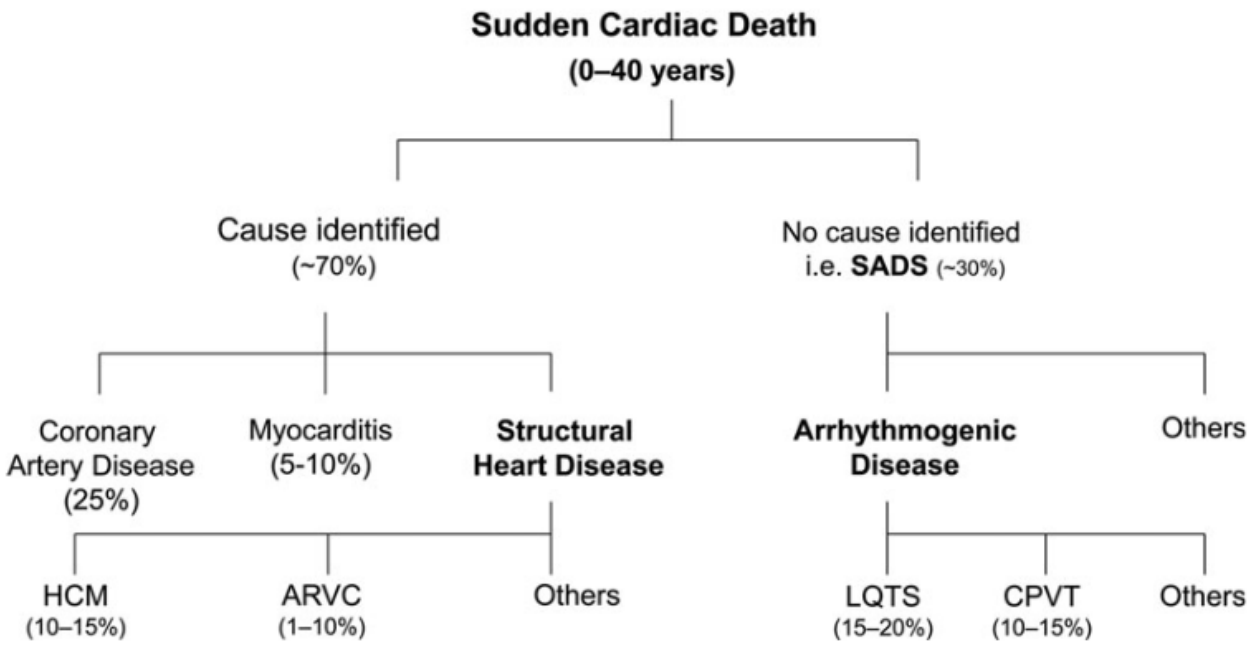

Figure 2: Differential diagnosis of CPVT (adapted from Semsarian et al 10.1093/eurheartj/ehv063, reproduced with permission).

1.7 Sudden Cardiac Death in the Newfoundland and Labrador population:

CPVT is of specific clinical interest in the Canadian province of Newfoundland and Labrador (NL) due to a higher incidence of SCD in this population and the relative under diagnosis of CPVT worldwide [29]. The high prevalence of SCD syndromes in NL is likely attributable to the founder population derived from 20-30,000 settlers of predominantly English and Irish extraction [30]. A significant driver of the high prevalence of SCD in NL is the high rates of Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) with a higher prevalence than in the general population [25]. Though the prevalence of CPVT is estimated at 1:10,000 by expert consensus groups [13], with the cohort of 32 affected patients described in this study the prevalence of CPVT in NL is likely much higher. Furthermore, 980 families have been referred to the Cardiac Genetics Clinic at Eastern Health in St. John's, NL with SCD as the most common reason for referral to the clinic highlighting the burden of arrhythmic syndromes in this population.

Though genetic causes are likely the predominant driver of early SCD in the NL population, it is also important to acknowledge the increased prevalence of risk factors for atherosclerotic cardiovascular disease (ASCVD) among the population. NL has the highest mortality rate for cardiovascular disease and ischemic heart disease among Canadian provinces [31]. Furthermore, with regards to traditional risk factors for ASCVD, NL has among the highest prevalence of diabetes, hypertension, obesity, and cigarettes smoking. The province also has some of the highest rates of individuals with 2 or more risk factors for ASCVD [32]. A clear link between these ASCVD risk factors and myocardial infarction (MI) has been described in the literature [33]. MI can produce SCD by the production of ventricular arrhythmias, either by PMVT during acute ischemia, or by scar-mediated reentry ventricular arrhythmias after MI. The increased mortality rate among those with cardiovascular disease and ischemic heart disease in the NL population is

likely at least partially attributable to the production of these arrhythmias. Finally, though the above mentioned ASCVD risk factors are classically classified as 'modifiable' risk factors, the increasing prevalence among the founder NL population suggests the possibility of a genetic role in their development.

1.8 CPVT Pathophysiology:

In cardiac muscle an action potential initiates excitation-contraction coupling, leading to the opening of voltage gated calcium influx channels (L-type calcium channels). Intracellular calcium triggers calcium release from the sarcoplasmic reticulum (SR) through ryanodine receptor channels (RyR2), in a process known as calcium induced calcium release. Released calcium binds to troponin and initiates a cycle of crossbridge formation and movement. Myocyte relaxation begins with SR calcium release being halted. Calcium unbinds from troponin and is transported back into the SR using the calcium-ATPase or removed from the cell using the sodium/calcium exchanger [34] (Figure 3).

RyR2 is a tetrameric intracellular calcium release channel required for cardiac excitation contraction coupling (Figure 4). Located in the junctional sarcoplasmic reticulum, it is responsible for regulating calcium induced calcium release [35]. Gain of function mutations in RyR2 result in 'leaky' calcium release channels and increased calcium-induced calcium release from the sarcoplasmic reticulum. This is particularly an issue during sympathetic stimulation which can precipitate calcium overload, delayed after depolarizations and ventricular arrhythmias [36]. Mutations in RyR2 represent the most common genetic subtype of CPVT (CPVT1) and account for 60% of CPVT cases.

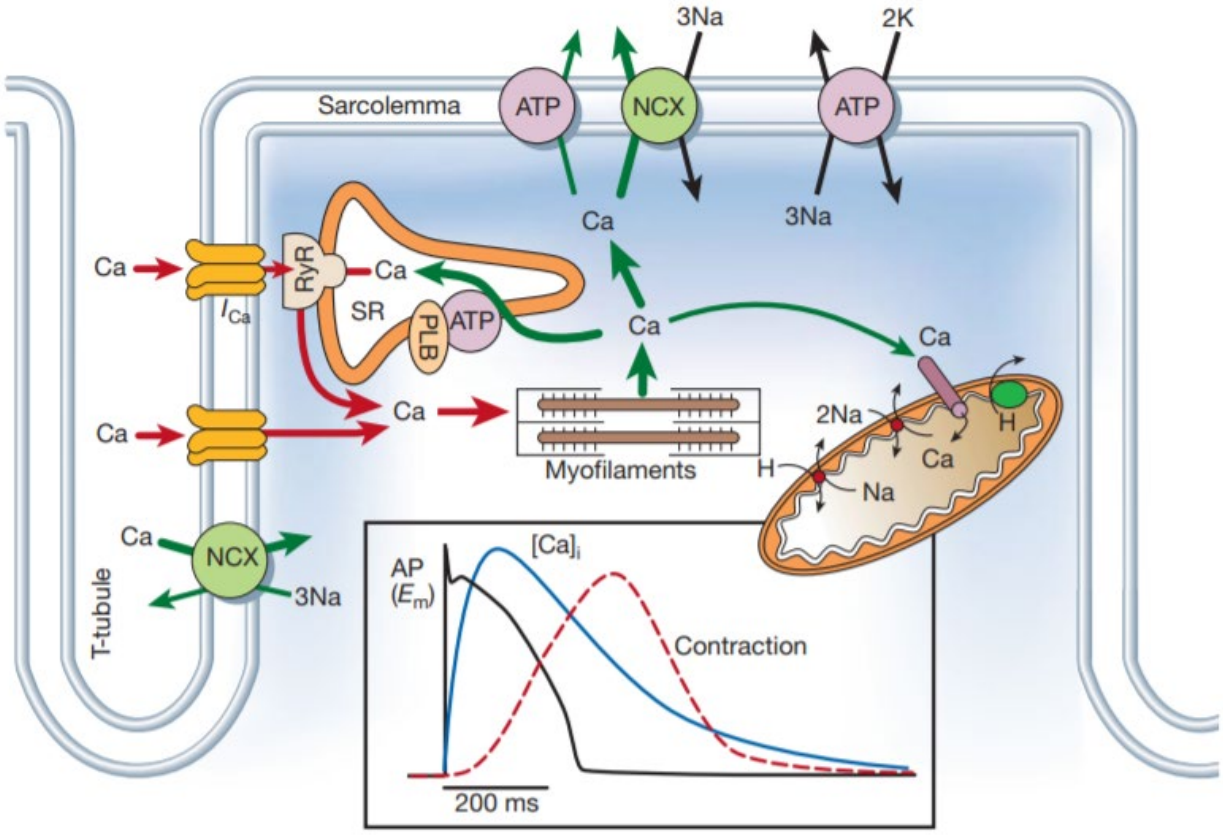

Figure 3: Cardiac excitation-contraction coupling. I_{Ca}=Calcium influx channels/L-Type Calcium channel, NCX=Sodium/Calcium exchange, ATP=ATPase, RyR= ryanodine receptor channels. (Adapted from Bers 2002, DOI: 10.1038/415198a, reproduced with permission).

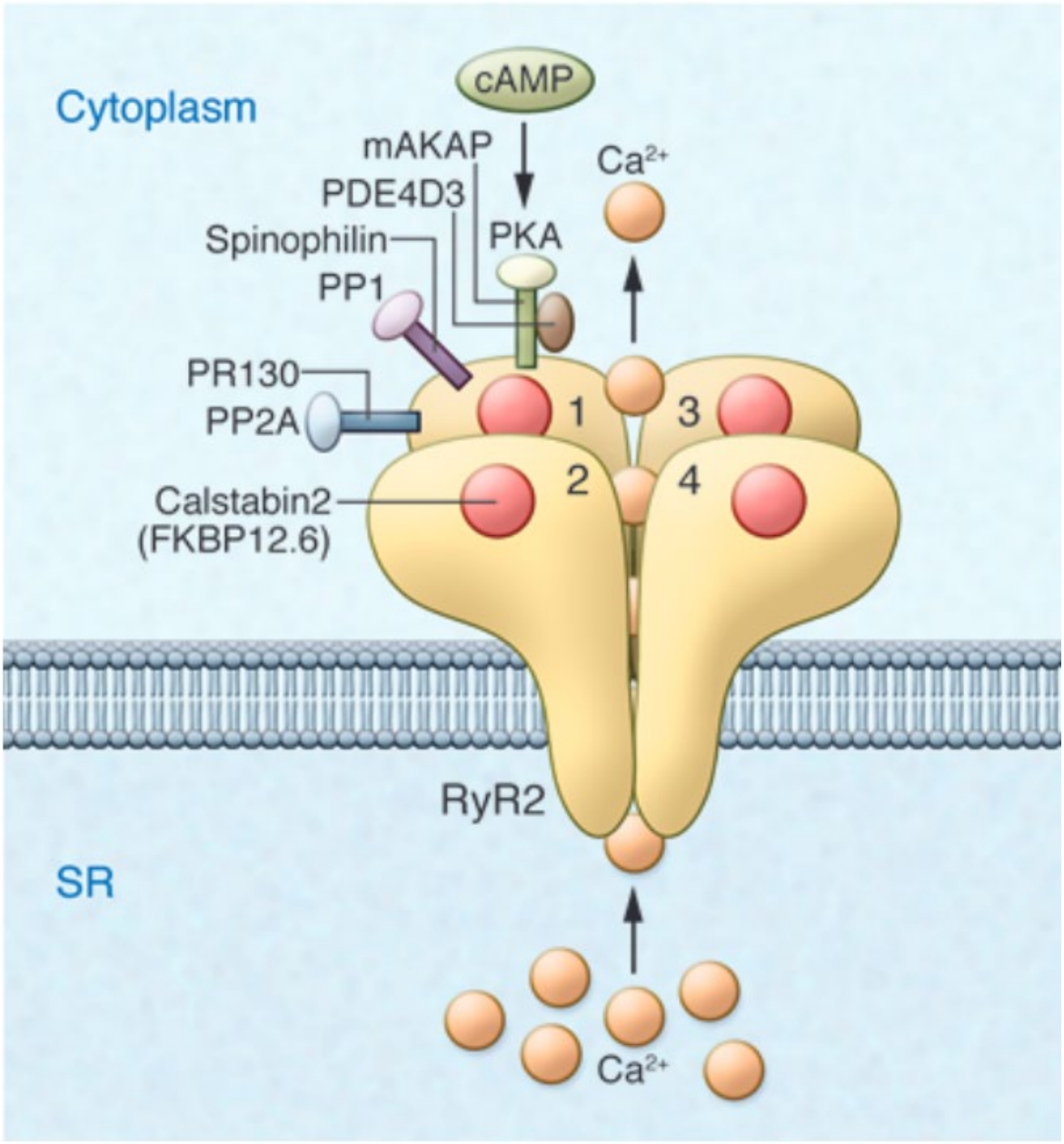

Figure 4: Illustration of the tetrameric structure of RyR2 (adapted from Marks, 2013, DOI: 10.1172/JCI62834, reproduced with permission).

Several mechanisms for the inappropriate release of calcium have been proposed. Firstly, it is suggested that RyR2 mutations impair the relationship between RyR2 and the regulatory protein Calstabin2. Calstabin2 is responsible for stabilizing the RyR2 channel in its closed state during diastole. Disruption in this relationship leads to 'leakiness' of the RyR2 channel [37]. Secondly, RyR2 mutations have been implicated in disruption of the interaction between the N-terminal and central domain of RyR2 which plays a role in stabilizing the closed conformation of the channel. This allows for diastolic calcium leakage [38]. Finally, CPVT mutations have been postulated to lower the threshold amount of luminal calcium required for RyR2 activation. It is hypothesized that at lower levels of SR calcium RyR2 channels open in the presence of mutations. This is further accentuated during adrenergic stimulation [5].

Excessive SR calcium release by the above mechanisms is one of two factors in a proposed double-hit mechanism required for the propagation of ventricular arrhythmias. Though calcium 'leak' due to RyR2 is necessary for the initiation of arrhythmia, myocytes can readjust their calcium homeostasis at rest by decreasing the amount of calcium in the SR. This autoregulatory cycle however is broken during adrenergic stimulation, due to the increased likelihood of RyR2 complexes opening. Further, enough cells need to display delayed afterdepolarizations and triggered activity, i.e., enough cells need to exhibit the ability to propagate arrhythmia, to overcome protective mechanisms preventing arrhythmia [5]. Genes encoding calsequestrin, *CASQ2*, calmodulin, *CALM*, and triadin, *TRDN*, play an important role in the release of calcium from the SR. Mutations in the genes encoding these proteins can also lead to dysfunctional calcium release, delayed after depolarizations and are pathogenic for CPVT [39,40].

1.9 Genetics of CPVT:

Mutations in several genes have been implicated in the pathogenesis of CPVT. RyR2, CASQ2, and KCNJ2 have all been implicated in CPVT [41,42]. RyR2 mutations have been implicated in 60-70% of cases of CPVT [5]. Most affected families have a private mutation (found in their family only) [43]. Mutations in RyR2 have been demonstrated to be inherited in an autosomal dominant pattern.

RyR2 represents one of the largest genes in the human genome with 105 exons that transcribe one of the largest cardiac ion channel proteins. The vast majority of documented RyR2 mutations are missense. However, up to 5% of CPVT patients may host large gene rearrangements suggestive of whole-exon deletions [43].

The RyR2 channel consists of three distinct regions, a C-terminal domain, a central domain, and an N-terminal domain. The p.R420w mutation is found in the N-terminal domain of the RyR2 *channel* (Figure 5) [44]. A comparison of mutations in these respective domains among 116 mutation carriers reveals higher rates of NSVT among patients with mutations in the C-terminal domain. There is no obvious difference in disease penetrance based on the domain where a mutation is found [45]. The p.R420W mutation has previously mainly only been described in studies examining probands. The largest available family study to date describes 61 relatives of a proband with the mutation [45].

The specific missense mutation (c.1258C>T coding p.R420W, Figure 6) responsible for producing p.R420W is rarely reported in clinical databases, with only three reported cases in gnomAD [46,47] (V2.1.1: Allele Frequency: 0.00001205 (3/249,018 alleles). This mutation has also been evaluated and flagged as pathogenic by multiple prediction programs including FATHMM-MKL [48], MutationTastor [49] and SIFT [50]. Varsome has classified *RyR2* p.R420W mutation as

pathogenic using American College of Medical Genetics (ACMG) criteria [51]. In addition to DNA being taken from individuals in families with clinical disease for confirmation of the presence of mutation, concurrent research into the molecular genetics of RyR2 is being performed at our center. Figure 6 shows a sequence of the missense mutation responsible for *RYR2* p.R420W identified through said dedicated genotype investigation.

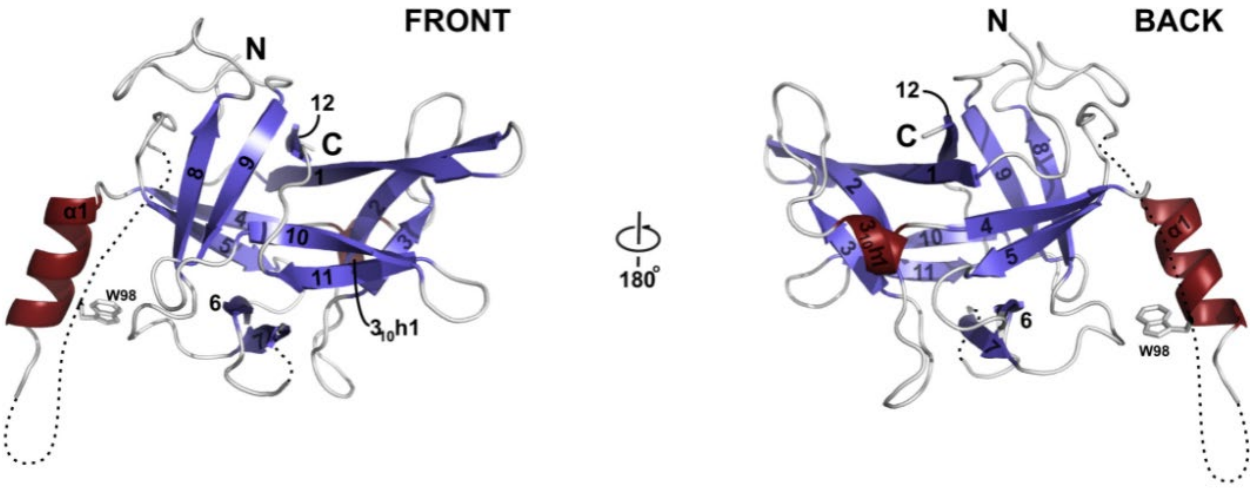

Figure 5: Crystal structure of the RyR2 N-Terminal domain (Adapted from Lobo et. al., 2009, DOI: 10.1016/j.str.2009.08.016, reproduced with permission)

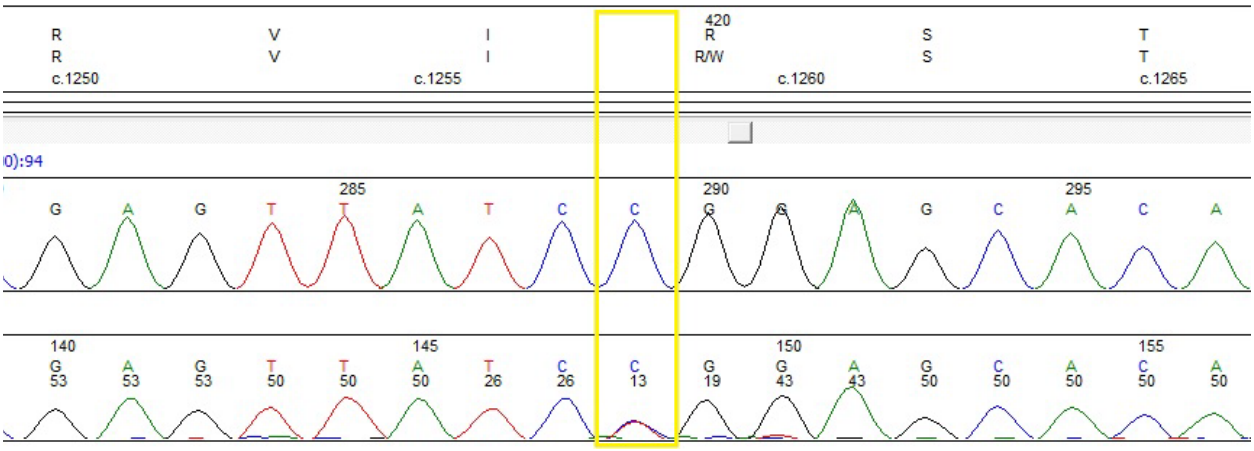

Figure 6: Sequence tracing of c.1258C>T coding p.R420W missense mutation responsible for *RYR2* p.R420W mutations. Included with permission.

1.10 Risk stratification:

Accurate risk stratification for patients with CPVT remains elusive and is incompletely described in the literature. Several other inherited arrhythmia syndromes have well defined risk stratification scores which guide clinical follow-up, diagnostic testing, and therapy. LQTS, for example, is guided by a validated and easy to use risk score which incorporates ECG findings, clinical features, as well as family history [52]. Further, Brugada syndrome can be risk stratified based on the presence of typical ECG findings along with symptoms suggestive of ventricular arrhythmia [13].

Among patients with CPVT there are no obvious patient characteristics which put patients at low risk for arrhythmic events. Based on this; current guidelines suggest that all patients with a diagnosis of CPVT be actively treated [13]. Available patient series suggest decreased events among those presenting at older age, likely owing to a milder phenotype being present. Further, patients who have had an aborted cardiac arrest are more likely to have future malignant arrhythmias [53].

1.11 Therapeutic options:

Therapy for CPVT is multipronged, with the first tenet of therapy being lifestyle modification. Though robust data supporting the clinical benefit of activity restrictions, governing bodies recommend limiting/avoiding competitive sports, strenuous exercise, and limiting exposure to stressful environments among all patients with diagnosed CPVT [13,54].

First-line medical therapy CPVT are beta-blockers with observational data showing decreased risk of arrhythmic events. Nadolol is the preferred drug for therapy of CPVT over other beta-blockers with decreased arrhythmic events in both adults and children [53]. Propranolol is suggested as an alternative as it is also a non-selective beta-blocker. Carvedilol has also been

suggested as an alternative as it directly acts on RyR2 [55]. Eight-year rates of arrhythmic events among patients treated with beta-blockers vary in the literature from 11% to 27% [53,56]. The majority of events are attributed to medication non-adherence in these studies, highlighting the importance of reiterating strict medication compliance to patients with CPVT.

Flecainide is a class IC antiarrhythmic, which exerts anti-arrhythmic effects through sodium channel blockade. In patients with refractory arrhythmia despite adequate therapy with beta-blockers, flecainide is recommended for reducing arrhythmia burden. Flecainide is thought to exert direct RyR2 blocking properties [57]. Limited data suggests the efficacy of flecainide in reducing ventricular arrhythmia [58].

Small series have shown a short-term benefit of left cardiac sympathetic denervation (LCSD) on arrhythmic events [59]. This procedure involves video assisted thoracoscopic surgery for the removal of the lower half of left stellate and thoracic ganglia from T2-T4. Though long-term follow-up on the effectiveness of LCSD is lacking, physiologic plausibility of this therapy exists as therapy would prevent norepinephrine release in the heart [60].

Implantable cardioverter defibrillator (ICD) therapy should be considered in patients with CPVT who are unresponsive to medical and invasive therapy, along with patients with aborted cardiac arrest [13]. Though ICD therapy is generally preferred for treating inherited arrhythmia syndromes, there are several case reports that have shown proarrhythmic effects among patients with CPVT. ICD shocks lead to catecholaminergic surges which may lead to incessant ventricular arrhythmias, and death [61]. The concern for catecholaminergic surges due to ICD shocks guide the recommendation to limit ICDs to patient's refractory to therapy.

Further medical therapies with potential for use in CPVT include propafenone, verapamil, and ivabradine. None have been rigorously tested at the time of this thesis.

1.12 Study Aims:

This thesis aims to describe the natural history of a *RYR2* p.R420W mutation through three well-ascertained, multiplex, autosomal dominant, pedigrees. The strength of this study is the near complete ascertainment of clinical events in each generation. Further, given the rare nature of mutations causing CPVT, here we can avoid the inherent issues with describing probands alone. Probands, predominantly, since they must present clinically, often present with more severe disease. By describing all members of families with presumed mutations a more complete picture of the pathogenicity of the mutation can be drawn. To our knowledge this is the largest well-ascertained familial study conducted on the *RYR2* p.R420W mutation.

Furthermore, given the similar clinical phenotype (a propensity to SCD) between CPVT and ARVC and their increased prevalence in the NL population, we took this opportunity to compare the effect of the underlying mutations (*RYR2* p.R420W in CPVT; *TMEM43* p.S358L in ARVC*)* on mortality. *TMEM43* p.S358L was previously studied in NL and the data collected using identical methodology. Furthermore, the shared exposures, genetic backgrounds, and access to care in both studies allows for a more direct comparison. Herein we compare the pathogenicity of the two mutations to give context to the pathogenicity of *RYR2* p.R420W.

Methods:

2.1 Study Design:

This study is a retrospective cohort study of families with CPVT due to *RYR2* p.R420W. The aim is to describe the natural history of patients with *RYR2* p.R420W and analyze their clinical outcomes. The goal is to contribute to our understanding of the natural history of disease-causing RyR2 mutations in patients with CPVT.

2.2 Study Families:

2.2.1 Selection Criteria:

All patients referred to the only tertiary cardiac genetic clinic for heritable cardiac disease in Newfoundland and Labrador, Canada are screened for SCD syndromes. Since 1996, 980 families have been referred. Families are identified as potentially having a heritable SCD syndrome through the presence of a history suggestive of an inherited condition. Often, families are identified following unexplained SCD in a young family member. However, families are also identified through a history of aborted SCD or symptoms highly suggestive of malignant arrhythmia, either in a proband or a family member. Blood was collected and genetic screening outsourced to accredited clinical laboratories for diagnosis. Of the 980, three families were identified as having the *RYR2* p.R420W variant (Family 0034, Family 0979, Family 1879). Robust clinical and historical phenotypic information was obtained going back several generations on each of the three families using thorough family histories. Each of the three families were characterized by an autosomal dominant inheritance pattern of SCD. Using extended family history, multigenerational, multiplex pedigrees were constructed and analyzed (Figure 7). Newfoundland and Labrador is a small province, with just over half a million population. Families in which early SCD occurs are noteworthy and likely identifiable. As this thesis however deals with sex

differences in mortality, pedigrees not showing this feature are not helpful. We thus decided to amalgamate the families by artificially inserting a common set of ancestors. So, two individuals were added in the earliest generations to allow for easier amalgamation. In this manner we created one obfuscated family tree. Though the additional individuals would be at *a priori* 50% risk of having the mutation by this pedigree structure, they were not included in the analysis as they do not exist. Based on the clinical and genetic information available at the time of this thesis there is no evidence that these families are related.

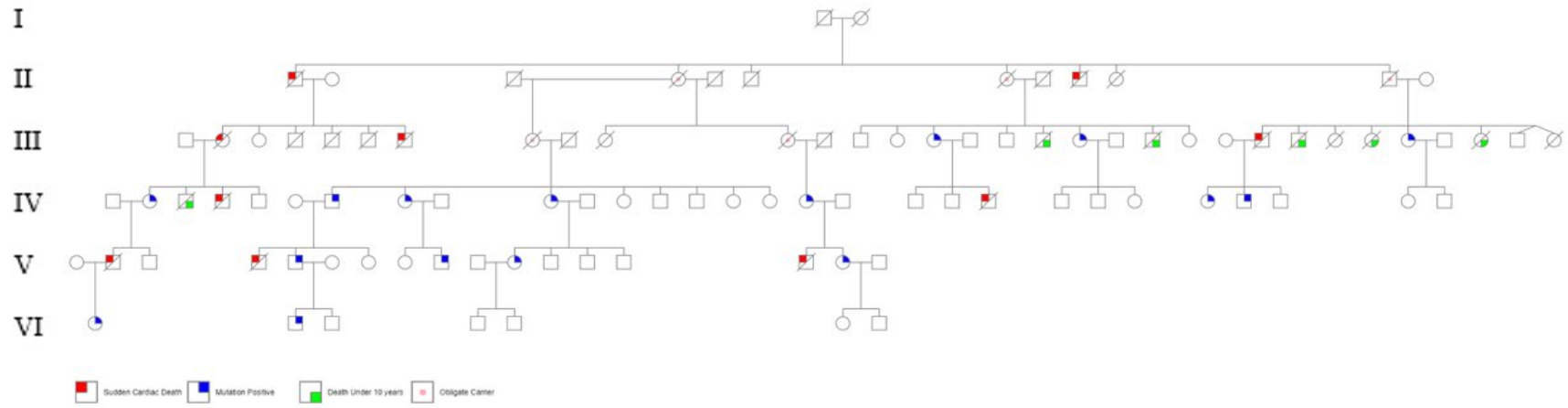

Figure 7: A well-ascertained multigeneration pedigree (comprising three separate families, amalgamated as one pedigree to preserve confidentiality) with autosomal dominant inheritance of *RYR2* p.R420W mutation.

2.2.2 Study Population:

From the aforementioned three families all subjects born at a 50% *a priori* risk of disease were identified using pedigree analysis (n=79). Given that RyR2 mutations are inherited in an autosomal dominant manner; any subject with a parent or sibling with a known mutation, early SCD, or was an obligate carrier by pedigree analysis was at an *a priori* 50% risk of having the *RYR2* p.R420W mutation. Obligate carriers were identified if by pedigree analysis the only plausible method of transmission to an affected offspring was by inheriting from the individual in question.

To avoid recall bias associated with remembering only individuals with severe clinical consequences, and conversely missing cases with clinically insignificant events, only 'well ascertained sibships' were included in this study. We defined a well ascertained sibship as a sibship where ≥ 50% of disease status was known. Disease status was deemed to be adequately ascertained for an individual if disease status, including age of death was available (n=60).

Six individuals in this analysis were treated with beta-blockers, an established therapy for CPVT. This potentially masks the pathogenicity of the mutation among these individuals.

We further subdivided our study population by disease status i.e., affected, unaffected, or unknown. Subjects were considered affected if they had one of the following: (i) mutation positive, (ii) obligate carriers (by pedigree analysis), (iii) SCD or ventricular arrhythmia under the age of 50 (n=32). Of these 32 patients, 16 were mutation positive, six were obligate carriers and 10 had SCD under the age of 50. Unaffected status was defined as all patients who were mutation negative (n=23). The remaining subjects were deemed unknown (n=5) (Figure 8).

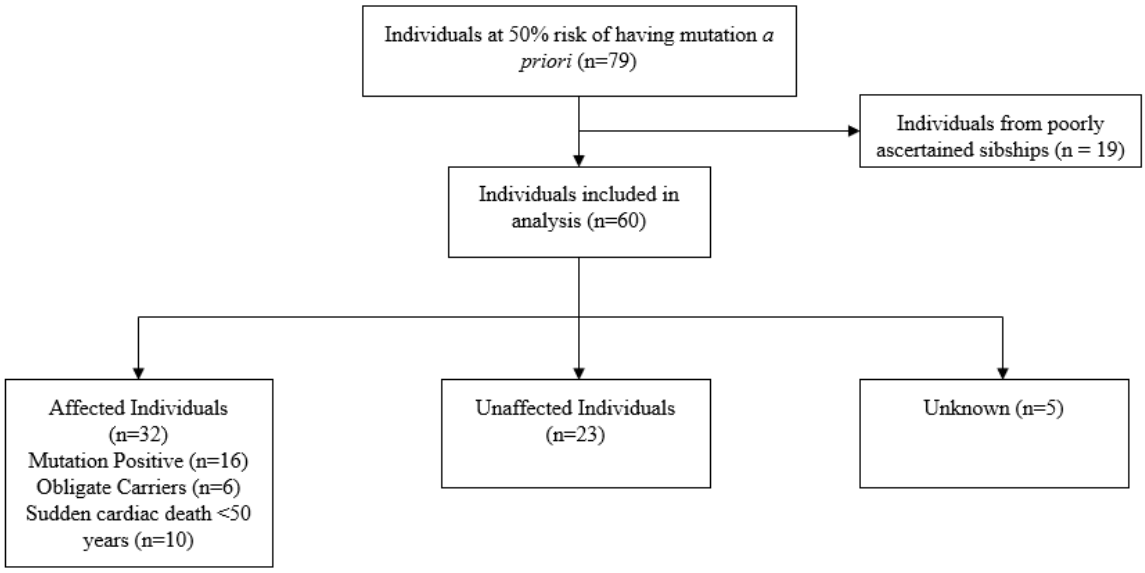

Figure 8: Flowchart showing how patients were grouped for the final analysis

2.3 Ethical Considerations:

Given the use of clinical information for our analysis, particularly given that the data is on a rare disease in a relatively small population, an application was submitted to the Health Research Ethics Board (HREB) of Newfoundland and Labrador, which governs all research involving human subjects or their data in the province. The sudden cardiac death study is longstanding and has ethics approval (Study ID 00-176). This study was an amendment following the submission of a *Secondary Use of Data/Chart audit application form*. This study also received approval from the research Proposal Authority Committee (RPAC) through the Eastern Health Care Corporation of St. John's. Given the sensitive and confidential nature of the clinical data being collected, data was housed in the Unit of Clinical Epidemiology at the Faculty of Medicine at Memorial University. Data was stored in a locked cabinet and remained in the department during the entire research period with access limited to members of the research team. Each patient received appropriate genetic counseling as per normal clinical practice. All participants enrolled in the study

provided informed research consent. Informed research consent for deceased individuals was obtained through next of kin.

2.4 Data Collection:

2.4.1. Data Abstraction form:

To obtain robust clinical and genetic data to fulfil the aims of this study, a standardized data abstraction form was created to collect all clinically relevant information. Furthermore, all patient identifying information was removed to maintain confidentiality. The abstraction form was created by Omar Abdel-Razek and the clinical data collected in the abstraction form is shown in Appendix 1. Disease status positivity was defined as mutation positive, obligate carrier, or severe clinical event with no DNA available. CPVT criteria was defined per the HRS/EHRA/APHRS Expert Consensus Statement on the Diagnosis and Management of Patients with Inherited Primary Arrhythmia Syndromes (see introduction). Method of diagnosis was defined as, presence of a clinical event, presence of DNA sample (positive or negative for the mutation), pedigree information, or any combination. Method of presentation was defined as clinical event, clinical screen, DNA screen, or inadvertent. Sudden death was defined as SCD with no obvious alternative explanation.

2.4.2 Charts:

Patient charts used in this retrospective analysis were a combination of general clinical and specific genetics clinic charts. Clinical charts were accessed using the electronic medical system employed by the Eastern Health Care Corporation of the St. John's (Meditech). Clinical charts included all electronic medical records available from all hospital visits within the Eastern Health Care Corporation. These visits include clinic visits, emergency room visits, along with hospital admissions and all diagnostic testing performed. In addition to electronic documentation, scanned

handwritten notes from hospital admissions, as well as from visits outside of this health jurisdiction were available.

Genetics charts were compiled by our genetics team as part of genetic investigation and management into SCD in the Newfoundland and Labrador population. Charts were compiled by contacting patients directly, either by phone or in person, to obtain research consent to access medical and genetics information. In addition to available clinical information, thorough genetic histories were recorded in written notes added to the genetics charts. In cases where information regarding the clinical status of individuals in a sibship were not readily available, or incompletely remembered, community resources including obituaries and church records were utilized. DNA testing as performed through our genetics department was also available in the genetics charts. All clinical genetics charts were stored securely in the Provincial Medical Genetics Program and any additional research information was stored in the Unit of Clinical Epidemiology at the Faculty of Medicine at Memorial University, in a locked cabinet with access available only to members of the research team.

I manually searched each clinical and genetics chart on the 60 individuals meeting study inclusion criteria and completed the extraction form for each individual. This was done under the direct supervision of our research team.

2.4.3 Chart Reviews:

Qualitative data on 60 individuals was collected by reviewing clinical and genetics charts. Chart reviews took place from January 2017-June 2017. I collected all data on each of the 60 included individuals. Clinical charts were reviewed for all clinical encounters, including but not limited to clinic visits in inherited arrhythmia clinic, general cardiology visits, device clinic visits, emergency room visits, inpatient hospital admissions and clinical notes, pediatric visits, along with

autopsy reports if applicable. Clinical encounters were specifically screened for information on family history suggestive of SCD, clinical visits for syncope or loss of consciousness suggestive of ventricular arrhythmia, documented ventricular arrhythmias by ECG or device interrogation, along with presentations suggestive of SCD. All diagnostic testing performed was also reviewed. Primary data included 12 lead ECGs, signal averaged ECGs, Holter monitoring, transthoracic/transesophageal echocardiograms, advanced cardiac imaging including cardiac MRIs, electrophysiology testing, implanted device interrogation records, cardiac CTs, invasive coronary angiography, as well as cardiac pathology if available. If at any point during data abstraction from clinical charts there was ambiguity or need for clinical clarification, I consulted with Dr. Sean Connors, a member of the SCD research team and a clinical cardiologist with specific clinical interest in inherited arrhythmia syndromes.

Genetics charts were reviewed for all information regarding family history, specifically pedigree analysis if performed. Genetic counselors performed interviews with probands, along with all family members who were living and reachable for interviewing. In cases where family members were either deceased or not available for interviews, data was obtained through information available in the community regarding dates, ages, and mechanisms of death. A particular advantage of this study is the completeness with which family information was obtained by our genetics team. Given the relatively small population, important phenotypic data was able to be obtained from church records (specifically dates and ages at death, as well as limited information on mode of death), available obituaries, and patient interviews. Genetics charts were further reviewed for information on DNA screening and presence of the *RYR2* p.R420W mutation. If during data extraction there was need for clinical clarification, I consulted with my thesis supervisors.

Once each chart was reviewed and data abstraction form filled, all data was organized and entered in a password protected Microsoft Excel (v16.0) spreadsheet. The organized data was then analyzed with IBM Software Package for the Social Sciences (SPSS), version 18 for further statistical analysis.

2.5 Groups Analyzed:

To assess the effect of mortality on individuals with affected status, data was analyzed comparing affected patients (n=26) with a combined cohort of unaffected and unknowns (n=28). This was done to ensure that only patients that met the strictest clinical and genetic definitions (mutation positive, obligate carriers, SCD/ventricular arrhythmia under the age of 50). This definition was used to reduce confirmation bias associated with including patients with clinical events not clearly demonstrative of CPVT, for example syncopal events suspicious for ventricular arrhythmia. Though these patients may meet clinical criteria for possible CPVT, they were not included in the affected group as they did not meet our rigorous combined clinical/genetic definition. Our strict clinical/genetic definition allows greater certainty that any differences noted in clinical outcomes are truly related to the presence of a mutation and not to chance inclusion of a clinical mimicker with adverse short- and long-term outcomes.

Through pedigree analysis the sex-influence of the disease was immediately apparent, with serious clinical events occurring earlier among affected males versus affected females. As a result, data was also analyzed comparing affected males (n=11) and affected females (n=15) to quantitatively illustrate the pathogenicity of disease among males.

An analytic dilemma presented to the study group was the inclusion of SCD as both an outcome measure as well as an inclusion criterion for the affected groups. The use of sudden death

as an outcome measure and as an inclusion criterion was justified by the obvious clinical importance of sudden death as an outcome measure. Further, the use of sudden death as an inclusion criterion meets both clinical definitions for the diagnosis of CPVT, among other inherited arrhythmia syndromes[13], as well as genetic definitions of affected status used in the literature for other inherited arrhythmia syndromes [24]. To establish the pathogenicity of the *RYR2* p.R420W mutation and ascertain the effect of the mutation without confounding by subjects who passed away under the age of 50 without DNA, the data was further analyzed using a genetic definition alone. This genetic definition compared a combined group of those who were mutation positive or obligate carriers (n=22) with those who were mutation negative (n=23).

A further dilemma in the analysis of the study group was the higher-than-expected incidence of sudden death among patients under the age of ten years. In our initial analysis deaths under the age of 10 were excluded as clinical information was relatively sparse compared to adults and teenagers analyzed in this study. Previous literature has described sudden infant death among individuals with pathogenic mutations implicated in CPVT [8,9]. Based on this literature, and the biologic plausibility of sudden infant death among this disease causing CPVT mutation, we further analyzed our data to include individuals under the age of 10. An affected group including juveniles (defined as age under 10 years) (n=32), was compared to an unaffected/unknown group including juveniles (n=28). Affected males including juveniles (n=15) with affected females including juveniles (n=17). Table 2 below shows each analysis performed for this study.

To further illustrate the pathogenicity of *RYR2* p.R420W, survival data was compared directly to a previously described 26 family cohort with the *TMEM43* p.S358L mutation associated with ARVC [24]. This analysis was done to compare survival to a known highly lethal mutation. Furthermore, given that the *TMEM43* and *RYR2* data come from the same Newfoundland and

Labrador population, a direct comparison of survival can be made with minimization confounding factors such as environmental and lifestyle factors. Clinical data for both familial studies were collected in an identical fashion. Affected males with *RYR2* p.R420W mutation (n=11) were compared with affected males with *TMEM43* p.S358L (n=212) mutation and affected females *RYR2* p.R420W (n=15) were compared with affected females *TMEM43* p.S358L (n=152).

2.6 Statistical Analysis:

Groups were analyzed using the Kaplan-Meier time-to-event analysis. Subjects were censored (removed prior to reaching end point of the analysis) at last clinic visit, and death. Relative risk (RR) was calculated using Cox's proportional regression. The SPSS statistics package (v18) was used for analyses (SPSS, Chicago, IL). Data was stratified by sex given the influence of male sex on mortality. I performed the statistical analysis with the help of Dr. Susan Stuckless (Statistician, Unit of Clinical Epidemiology, Memorial University).

Table 2: List of Between Group Analyses Performed

Affected vs Unaffected/Unknown group
Affected Males vs Affected Females
Affected vs Unaffected/Unknown group (Juveniles Included)
Affected Males vs Affected Females (Juveniles Included)
Affected vs Unaffected (Genetic Definition)
Affected males with *RYR2* p.R420W vs affected males with *TMEM43* p.S358L
Affected females with *RYR2* p.R420W vs affected females with *TMEM43* p.S358L

Chapter 3: Results

These results are based upon clinical and genetic data from 6o individuals from three NL families from with CPVT due to *RYR2* p.R420W.

3.1 Affected vs Unaffected/Unknown group (Death < 10 years excluded):

From the 60 individuals from well-ascertained sibships, six had unexplained death prior to the age of 10. These individuals were excluded from this analysis as any putative association with the *RYR2* p.R420W mutation is speculative leaving a total of 54 individuals. Those who were affected (n=26), were compared to those who were unaffected/unknown (n=28).

Affected status in families with *RYR2* p.R420W was significantly associated with mortality (RR=4, CI=95% CI:1.1-14.5, p=0.034). There were 11 total events in the affected group, with 4 events in the unaffected/unknown group. Mortality at age 30 years was 24% in the affected group and 0% in the unaffected/unknown group. This trend is shown in Table 3 which demonstrates greater proportions of mortality in the affected group at ages >10 years old with the percentages of mortality only equalizing among octogenarians as the mortality curves approach zero. The value of the log rank statistic comparing the survival of the two groups was 5.223 (p-value = 0.022), indicating a significant difference in the survival distributions between the two groups. This difference between the two groups is illustrated in Figure 9, with affected individuals having earlier mortality than unaffected/unknown individuals. Median age of death/loss to follow up was similar between groups (Affected: 76.48 years 95% CI: 51.9-101.0, 85.00 years unaffected/unknown).

Table 3: Time to death outcomes for affected individuals compared to unaffected/unknown individuals (percent survival by decade of life).

	Cumulative Survival (decade of life)							
Affected Status	<10	<20	<30	<40	<50	<60	<70	<80
Affected	100%	85%	76%	76%	76%	67%	67%	50%
Unaffected/Unknown	100%	100%	100%	100%	95%	82%	82%	82%

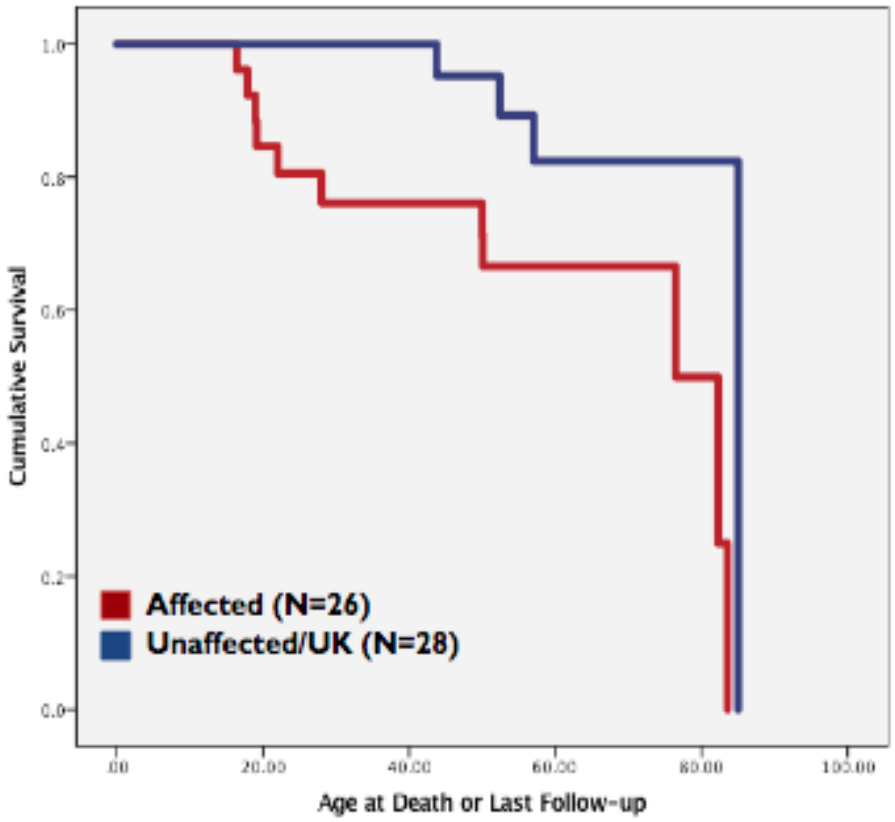

Figure 9: Cumulative Survival in Affected individuals in families with *RYR2* p.R420W compared to a combined unaffected/unknown group.

Affected group has earlier mortality than unaffected/unknown.

3.2 Affected Males vs Affected Females (Death < 10 years excluded):

Qualitative analysis of the constructed pedigrees reveals a clear sex-influence with death occurring earlier in affected males. Of the 60 individuals from well-ascertained sibships a total of 26 were affected (11 males and 15 females). Affected males were compared with affected females with regards to survival. This analysis again excluded juveniles given the speculative association of *RYR2* p.R420W with those deaths.

Male affected status was significantly associated with early mortality (RR=7, 95% CI: 1.5-34.5, p=0.013). There were 8 events among affected males, with 3 events in affected females. Mortality at 30 years was 47% among affected males and 8% among affected females. This trend is demonstrated in Table 4 which shows a greater proportion of affected males dying in the affected groups at ages > 10 years old with the percentages of mortality only equalizing over the age of 70. The value of the log rank test was 8.224 (p-value = 0.004), indicating a significant difference in the survival distributions between affected males and affected females. Figure 10 illustrates the effect of male sex on mortality among affected individuals with affected males having earlier mortality than affected females. The median age of death/loss to follow-up was markedly decreased among affected males (Male 50 years (95% CI: 5.5-94.5), Female 76 years (95% CI: 37.7-115.2).

Table 4: Time to death outcomes for affected males compared to affected females (percent survival by decade of life).

	Cumulative Survival (decade of life)							
Sex	<10	<20	<30	<40	<50	<60	<70	<80
Male	100 %	64%	53%	53%	53%	32%	32%	32%
Female	100 %	100%	92%	92%	92%	92%	92%	46%

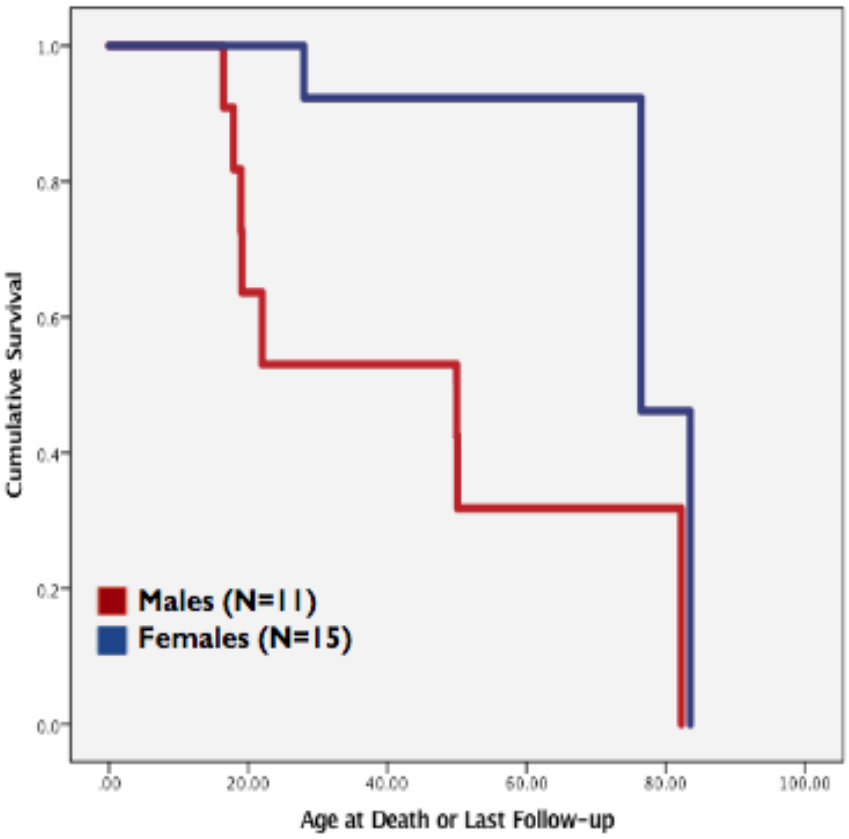

Figure 10: Cumulative Survival Affected Males vs Affected Females in families with *RYR2* p.R420W.

Affected males have earlier mortality than affected females.

3.3 Affected vs Unaffected/Unknown group (Juveniles included):

Given the uncertainty regarding the pathogenicity of *RYR2* p.R420W among juveniles the original analysis excluded juveniles. However, upon reviewing the available literature as well as the relatively higher proportion of juvenile deaths among the three *RYR2* p.R420W families, I repeated the analysis to include juveniles. With juvenile deaths included, the total number of individuals in the affected and unaffected/unknown groups were 32 and 28 respectively.

With juveniles included the affected status was significantly associated with mortality (RR=6, 95% CI: 1.7-20.4, p=0.004). There were 17 events among affected individuals, with 4 events among the unaffected/unknown group. Mortality at 30 years was 38% in the affected group and 0% in the unaffected/unknown group. This trend is demonstrated in Table 5 which confirms a greater proportion of events in the affected group with a difference between the two groups occurring before the age of 10 and persisting into the 8th decade of life and only equalizing as the survival curves approach zero. The value of the log rank test was 10.399 (p-value = 0.001), indicating a significant difference in the survival distributions between the affected group and the unaffected/unknown group, when juveniles are included. This difference between the two groups is illustrated in Figure 11, with affected individuals having earlier mortality than unaffected/unknown individuals with juvenile deaths included. The median of age of death/loss to follow-up was 76.47 (95% CI: 35.9-117.0) years in the affected group and 85.00 (Std Error=0.00) in the unaffected/unknown group.

Table 5: Time to death outcomes for affected individuals compared to unaffected/unknown individuals with juveniles included (percent survival by decade of life).

	Cumulative Survival (decade of life)							
Affected Status	<10	<20	<30	<40	<50	<60	<70	<80
Affected	81%	69%	62%	62%	62%	54%	54%	40%
Unaffected/ Unknown	100%	100%	100%	100%	95%	82%	82%	82%

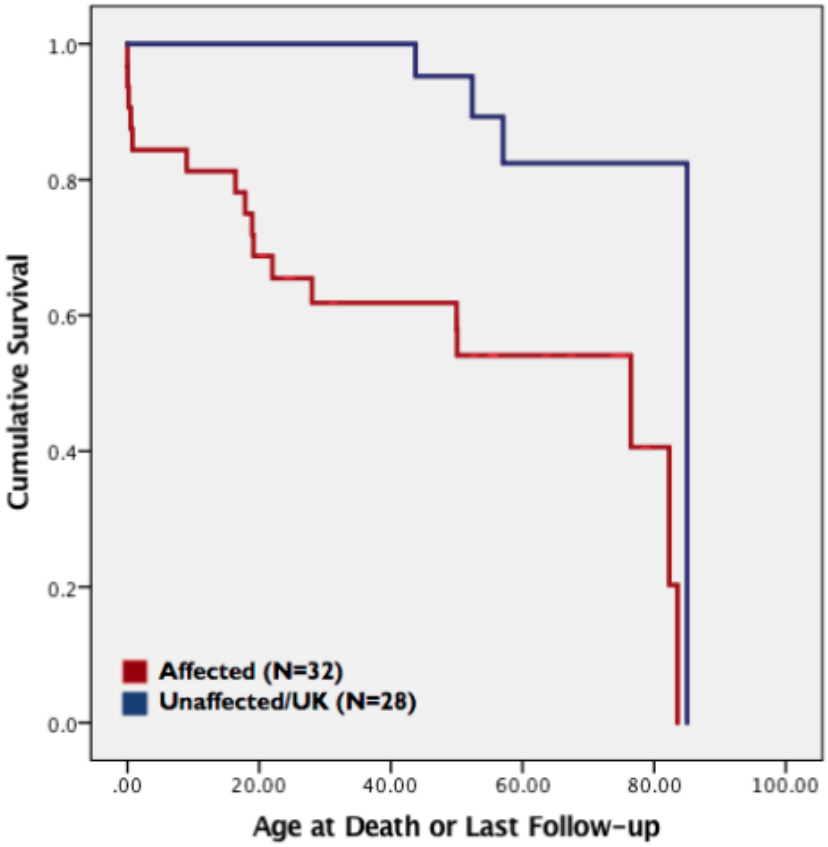

Figure 11: Cumulative Survival in Affected individuals in families with *RYR2* p.R420W compared to a combined unaffected/unknown group (juveniles included).

Affected individuals have earlier mortality when compared to unaffected/unknown individuals when juveniles are included.

3.4 Affected Males vs Affected Females (Juveniles Included):

As with the above results, initial analysis on the sex-influence of the *RYR2* p.R420W mutation excluded individuals under the age of 10. For the same rationale as the affected vs unaffected/unknown analysis the analysis of affected males vs affected females was repeated to include juveniles; when the total number of individuals in the affected males and affected females was 15, and 17 respectively.

With juveniles included male affected status was significantly associated with early mortality (RR=4.7, 95% CI: 1.5-14.6, p=0.008). There were 12 events among affected males, with 5 events in affected females. Mortality at 30 years was 61% among affected males and 19% among affected females. This trend is demonstrated in Table 6 which shows a greater proportion of affected males dying in the affected groups beginning under the age of 10 and persisting into the 8th decade of life and only equalizing as the survival curves approach zero. The value of the log rank test was 8.282 (p-value = 0.004), indicating a significant difference in the survival distributions between affected males and affected females with juveniles included. Figure 12 illustrates the effect of male sex on mortality among affected individuals with affected males having earlier mortality than affected females. The median age of death/loss to follow-up was markedly decreased among affected males (Male 19.146 years (95% CI: 14.1-24.1), Female 76 years (95% CI: 8.4-144.6)).

Table 6: Time to death outcomes for affected males compared to affected females with juveniles included (percent survival by decade of life).

	Cumulative Survival (decade of life)							
Affected Status	<10	<20	<30	<40	<50	<60	<70	<80
Male affected	73%	47%	39%	39%	39%	23%	23%	23%
Female affected	88%	88%	81%	81%	81%	81%	81%	40%

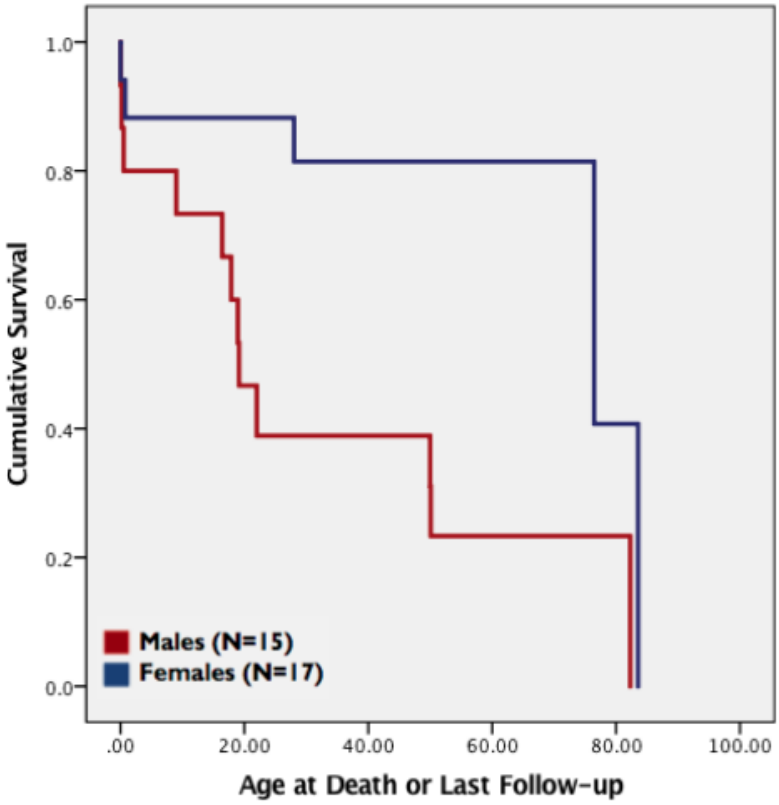

Figure 12: Cumulative survival in Affected Males compared to Affected Females (juveniles included).

Affected males have earlier mortality than affected females when juveniles are included.

3.5 Affected vs Unaffected (Genetic Definition):

Given the potential confounding by including SCD as both an outcome measure as well as an inclusion criterion the primary data was further analyzed using a stricter genetic definition of affected status. In this analysis affected status was defined as individuals who were mutation positive or obligate carriers (n=22). The unaffected group was defined as those who were mutation negative (n=23).

In this analysis there were 0 events in patients who were unaffected with 5 events in the affected group. The trend in mortality is shown in Table 7. There was a trend towards statistical significance p-value for log rank test = 0.062, this trend is illustrated in Figure 13.

Table 7: Time to death outcomes for affected individuals compared to unaffected individuals (Genetic Definition) (percent survival by decade of life).

	Cumulative Survival (decade of life)						
Affected Status	<10	<20	<30	<40	<50	<60	<70
Affected	100%	95%	90%	90%	90%	84%	84%
Unaffected	100%	100%	100%	100%	100%	100%	100%

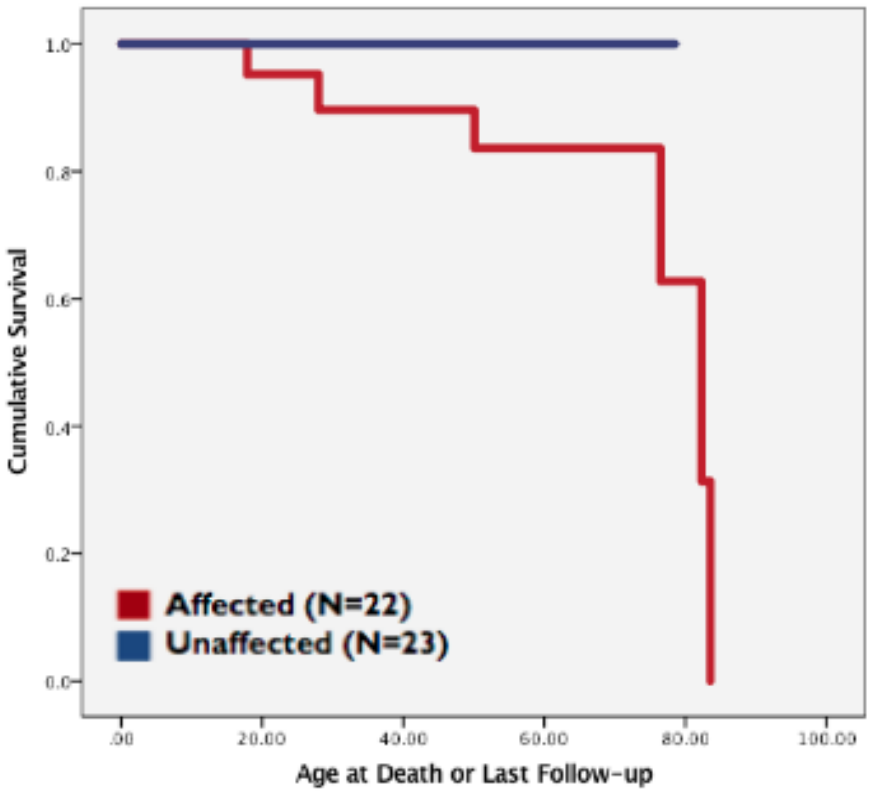

Figure 13: Cumulative Survival Affected versus Unaffected using a genetic definition. Affected in this analysis include only those who are *RYR2* p.R420W positive or obligate carriers, Unaffected in this analysis includes only those who are *RYR2* p.R420W negative. A trend towards increased mortality when among affected individuals using a genetic definition.

3.6 Affected males with *RYR2* p.R420W vs affected males with *TMEM43* p.S358L (juveniles excluded):

Given the similar worst-case outcome (SCD) of those affected by the CPVT causing mutation *RYR2* p.R420W and the ARVC causing mutation *TMEM43* p.S358L our study group compared the pathogenicity of the two mutations. Identical methodologies were used to abstract and analyze data making a direct comparison suitable. Fifteen affected males with *RYR2* p.R420W were compared with 212 affected males with *TMEM43* p.S358L.

When affected males in families with *RYR2* p.R420W were compared to affected males in families with *TMEM43* p.S358L median survival was 50 years (95% CI: 5.5-94.5) compared with 44 years (95% CI: 42.0-46.8). There were 8 events among the *RYR2* p.R420W group and 133 among the *TMEM43* p.S358L group. There was no statistically significant difference between the two groups (RR=1.8, 95% CI=0.95-3.28, p=0.07), however the earlier deaths among males with *RYR2* p.R420W is shown in Table 8 and further illustrated in Figure 14.

Table 8: Time to death outcomes for Affected males with *RYR2* p.R420W compared to affected males with *TMEM43* p.S358L (percent survival by decade of life).

	Cumulative Survival (decade of life)							
Gene/Mutation	<10	<20	<30	<40	<50	<60	<70	<80
RYR2 p.R420W	100%	85%	76%	76%	76%	67%	67%	50%
TMEM43 p.S358L	100%	99%	89%	64%	32%	22%	8%	3%

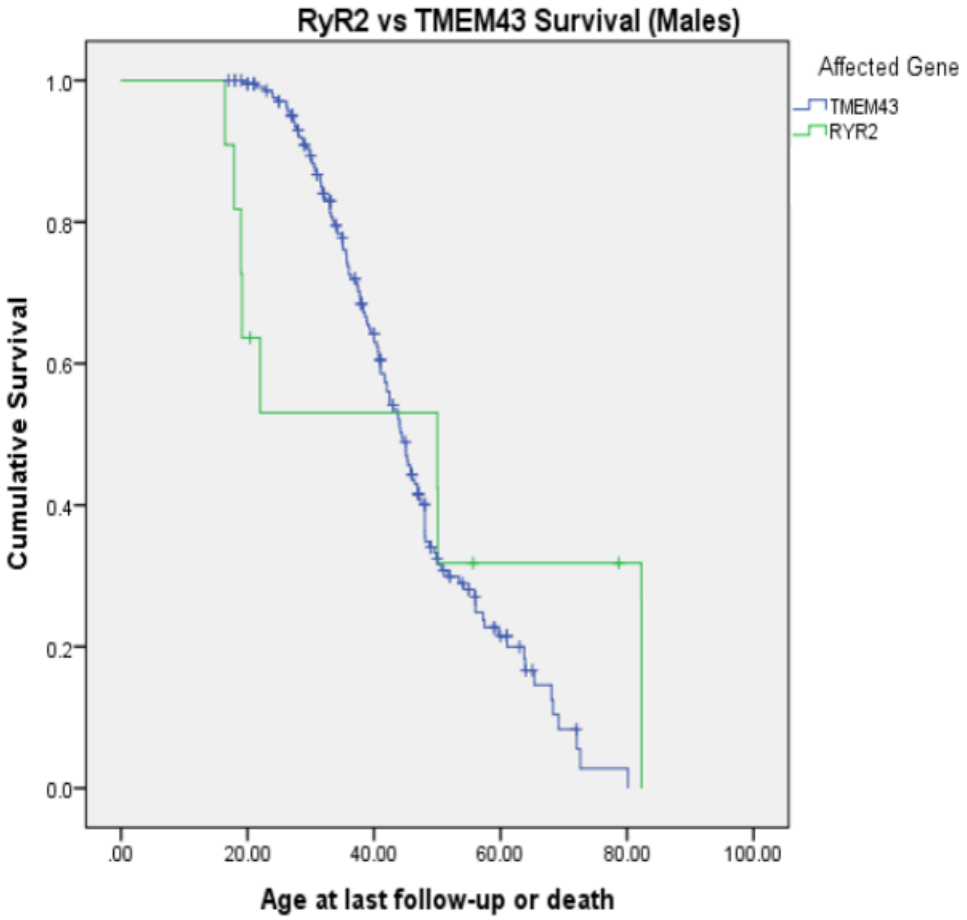

Figure 14: Cumulative survival among affected males in in families with *RYR2* p.R420W were compared to affected males in families with *TMEM43* p.S358L.

No statistically significant difference in deaths among males with *RYR2* p.R420W is seen when compared to males with *TMEM43* p.S358L.

3.7 Affected females with *RYR2* p.R420W vs affected females with *TMEM43* p.S358L:

Using the same rationale as above, we compared 17 affected females with *RYR2* p.R420W and 152 affected females with *TMEM43* p.S358L. When affected females in families with *RYR2* p.R420W were compared to affected males in families with *TMEM43* p.S358L median survival was 76 years (95% CI:37.7-115.2) compared with 73 years (95% CI: 69.6-76.1). There were 3 events among the *RYR2* p.R420W group and 37 among the *TMEM43* p.S358L group. There was no statistically significant difference between the two groups (RR=1.4, 95% CI=0.64-3.08, p=0.41). This is demonstrated in Table 9 and Figure 15.

Table 9: Time to death outcomes for affected females with *RYR2* p.R420W compared to affected females with *TMEM43* p.S358L (percent survival by decade of life).

	Cumulative Survival (decade of life)							
Gene/Mutation	<10	<20	<30	<40	<50	<60	<70	<80
RYR2 p.R420W	100%	64%	53%	53%	53%	32% %	32%	32%
TMEM43 p.S358L	100%	100%	100%	98%	89%	78%	61%	38%

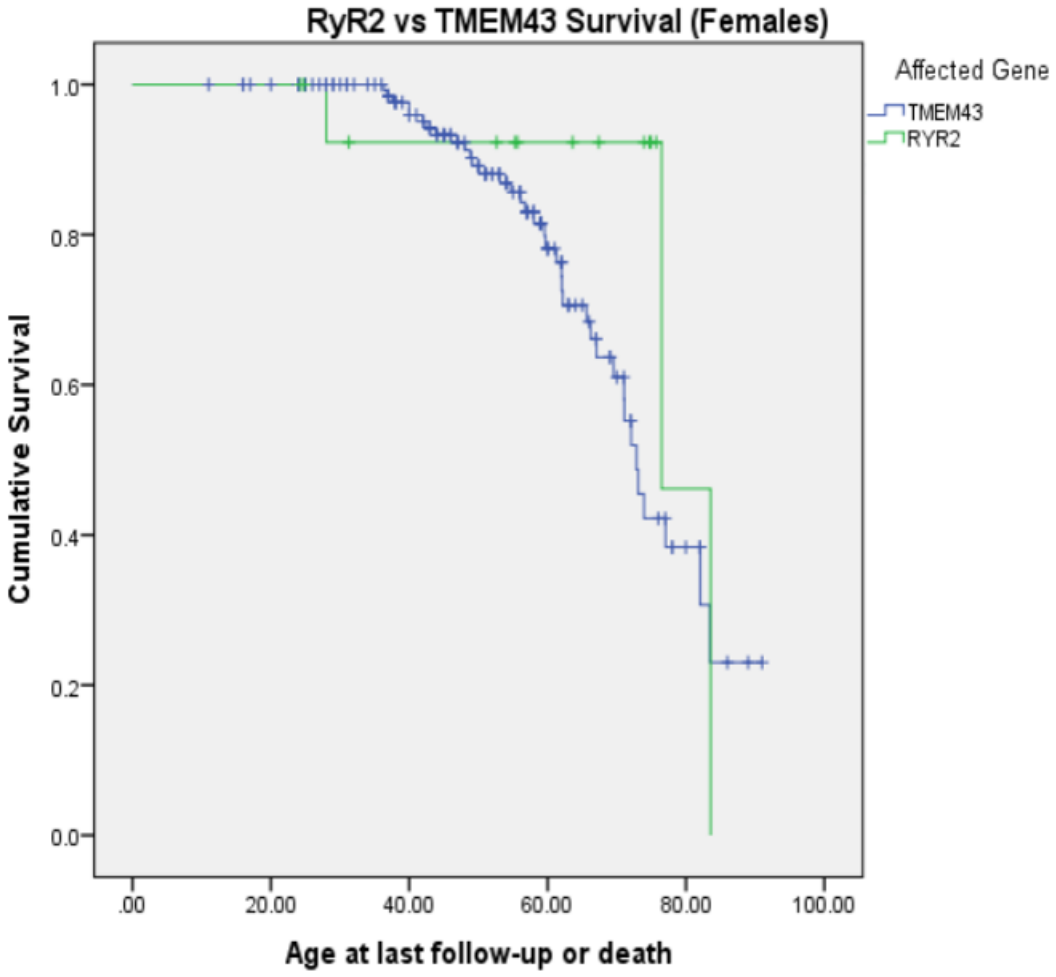

Figure 15: Cumulative survival among affected females in in families with *RYR2* p.R420W were compared to affected males in families with *TMEM43* p.S358L.

Survival among females with *RYR2* p.R420W is like females with *TMEM43* p.S358L.

Chapter 4: Discussion

CPVT is a relatively incompletely described rare inherited arrhythmia syndrome. In this study we describe the natural history of CPVT due to *RYR2* p.R420W mutation. Through three well-ascertained families we report the pathogenicity of *RYR2* p.R420W and compare mortality to that experienced in ARVC patients due to *TMEM43* p.S358L in the same population. Further, we describe the sex-influence of this mutation as well as speculate on potential effects in juveniles.

4.1 Study population:

Newfoundland and Labrador is a genetic isolate and founder population which represents a unique opportunity to assess genotype-phenotype relationships for highly variable rare diseases, such as CPVT. We describe the effect on mortality of the *RYR2* p.R420W mutation, with minimal ascertainment bias. Ascertainment bias is minimized by the ability to assess all individuals born at a-priori 50% risk, as opposed to only cases presenting for medical care due to clinical symptoms. In this study, we have assessed all those who are unaffected (mutation negative), as well as those who meet affected criteria (early clinical manifestations, obligate carriers, mutation positive). This allows the ability to accurately attribute clinical signs and symptoms across an individual's lifespan to the *RYR2* p.R420W mutation. This also helps exclude issues related to their common genetic background or shared environment.

4.2 Survival among affected individuals:

Mortality in individuals with untreated CPVT is estimated at 30-50% by age of 40 years old [62]. A review of 403 individuals with CPVT revealed an 8-year mortality of 6.4% despite beta-blocker therapy [56]. Furthermore, a study of 34 individuals with CPVT revealed 20.6% of patients developed fatal cardiac events however 85.7% of those with events were medication nonadherent

[63]. The effect on survival of *RYR2* p.R420W represents a pathogenic mutation with similar mortality as that quoted in current literature. There is limited data in the current literature regarding the pathogenicity of the *RYR2* p.R420W mutation. Case studies describing SCD and arrhythmic events with this mutation have been described [64]. However, to our knowledge this is the largest study on the natural history of the mutation. Furthermore, given that 19% (n=6) of those included in the affected group were treated with beta-blockers the effect on survival on mortality is likely blunted in this study.

To date, 795 variants in the *RyR2* gene have been noted in the literature [65] and it is likely that not all mutations cause exactly the same phenotypic expression. Notably, other mutations in *RYR2* (*RYR2* p.R176L) have been found to display lower penetrance when exploring beyond first degree relatives[66]. A lack of family studies on CPVT due to *RYR2* p.R420W leaves a gap in our understanding of the natural history of this lethal mutation. A previous study from the Netherlands reported 61 individuals from three families [45], as well as several case reports of probands with this mutation [64,67]. This study has contributed clinical data on 27 *RYR2* p.R420W patients, suggesting this variant is clinically important in patients with CPVT, especially in those of Northern European ancestry.

4.3 Phenotypic differences by sex:

Previous studies have shown a difference in phenotypic patterns between males and females. Several studies have established the frequency of asymptomatic presentations of females with CPVT with as many as 80% of late presenters (age >21) being female in one study [12]. In addition, a 2002 study showed a male predominance for syncopal episodes in patients with CPVT, with a relative risk of 4.2 [68]. Though these previous studies speak to the pathogenicity of CPVT and associated mutations in male sex, to our knowledge this study represents the first study to

describe such a dramatic sex-influence. The mechanism of phenotypic difference between sexes is not entirely elucidated, although likely is due to a combination of hormonal, environmental, and catecholaminergic factors. One possible hypothesis includes the presence of sex differences in autonomic cardiovascular regulation [69]. In addition to hormonal and autonomic differences between males and females, environmental factors including diverging experiences of exertional and emotional stressors in youth may also play a role in the witnessed differences. Further study into the etiology of the sex differences is required.

4.4 Comparison with ARVC and TMEM43 p.S358L mutations:

Although pathogenesis of CPVT and ARVC differ greatly, a comparison has been made between the two diseases in this study. This comparison has been made on the basis of the prevalence of both *TMEM43* p.S358L and *RYR2* p.R420W in the Newfoundland and Labrador population [24]. Both mutations and their natural history have been described in an identical population with similar access to health care, socioeconomic status, and similar genetic baselines. Data collection for both *TMEM43* p.S358L and *RYR2* p.R420W families was done in an identical fashion making for an apt comparison. In addition, both mutations were studied in well-ascertained large pedigree cohorts. In each analysis, owing to the population studied, accurate clinical data is ascertained in many sibships through multiple generations in multiple families.

The comparison between these two mutation-based conditions highlights the pathogenicity of the *RYR2* p.R420W mutation. Both conditions occur because of a single nucleotide missense mutation, and both have phenotypically similar yet catastrophic outcomes. The availability of a potentially effective therapy in CPVT and its significant effect on mortality underlines the need for an early and effective genetic screening strategy in individuals of families with *RYR2* p.R420W mutations, as effective therapy can be initiated [53].

4.5 Juvenile Deaths:

Six deaths under the age of 10 were ascertained in the analysis of this population. The cause of these deaths is not known. A decision was made not to include these individuals in the initial analysis. This was done to ensure the survival affect reported represents those with clearest evidence of being affected by the mutation (mutation positive, obligate carrier, sudden death <50). However, a lack of juvenile deaths in the large family histories spanning several generations in the *TMEM43* p.S358L group when compared to those in the *RYR2* p.R420W group raises the question of this mutation being a potential cause of these deaths in children. The relative paucity of juvenile deaths in the *TMEM43* p.S358L group is despite both family studies being conducted during the same time frame, with similar comorbid conditions and access to healthcare issues affecting both groups. *RYR2* p.R420W mutations have previously been described in families with juvenile deaths, however without DNA confirmation [67]. Though this study does not include any juvenile deaths with DNA confirmation of mutation status, the results at least raise the possibility of *RYR2* p.R420W mutations as a cause of very young death. It is our current practice to genetically test all juveniles born at an a-priori 50% risk of carrying the *RYR2* p.R420W mutation. Furthermore, therapy with beta-blockers would be offered to all those who test positive regardless of age.

When juveniles are included in the analysis, affected status is more strongly associated with mortality (RR=6, 95% CI: 1.7-20.4), with 38% mortality in the affected group and 0% mortality in the unaffected group by age 30. Male affected status remains significantly associated with mortality (RR=4.7, 95% CI: 1.5-14.6), Mortality at 30 years was 61% among affected males and 19% among affected females. Median age of death among affected males decreases to 19.146 years when juveniles are included, compared with 50 years when they are not, and remains at 76 years among affected females.

Firm conclusions on the impact of this mutation on infant and juvenile deaths cannot be made. Given the timeframe (mid-20th century), and geographic isolation of the juveniles described the distinct possibility that these deaths are attributable to other causes of juvenile death endemic to this population exists. This does however raise the possibility of *RYR2* p.R420W as a potential cause of juvenile death and warrants further dedicated studies on its potential effect on juveniles.

4.6 Ethical Implications of Pedigree Studies

Our study describes three families from sparsely populated rural Newfoundland, Canada. Publication of pedigrees with clinical information represented a difficult challenge in balancing the risk of inferential identification from a pedigree against emphasizing the malignant nature of the mutation described. The risk of inferential identification is exacerbated by the small population studied and the relative familiarity of individuals and families in rural communities. Further, the seriousness of the clinical events experienced, (e.g. SCD in young individuals), increases the risk of inferential identification. Though informed consent was obtained for this study several risks remain if a family is identifiable. Family members may be able to learn specific clinical and personal information about family members that some members would prefer not to disclose. Specific examples include clinical events, number of pregnancies, consanguinity of marriages, nonpaternity, and the existence of family members not previously known to a family member examining a pedigree. Beyond this there is a risk of drawing unfounded conclusions based on the information presented leading to unwise decisions regarding reproduction, or employment [70].

To reduce the risk of this inferential identification we combined three families into one and added family members to disguise the identities of each family. Family members were added to the earlier generations of the pedigrees to ensure that attempts to obfuscate the identities of families

were not a misrepresentation of the data. Further, to avoid diluting the importance/magnitude of the mutation no family members were removed and sex was not omitted or changed. Though no strategy for minimizing risk of identification aside from pedigree omission is perfect, the strategies used represent an earnest and effective attempt to obfuscate identity without diluting the scientific relevance of our findings.

4.7 Study Limitations:

Several biases are present when describing the natural history of a rare inherited disease. Firstly, given that it is a rare disease with few who are affected, and even fewer who are properly diagnosed, our sample size is smaller than what would be seen in studies of more frequently encountered arrhythmia syndromes (although these are often clinical diagnosis specific, not mutation specific). Despite this limitation, this study still represents the largest well-ascertained family study of patients with a homogeneous mutation causing CPVT.

A further limitation of this study is the uncertain effect of therapy on the outcomes. In the three families studied, six individuals denoted as affected had been treated with beta-blocker therapy at the time of the study. Of the six treated individuals, five were females. Beta-blockers have been proven efficacious in control of malignant arrhythmias in individuals with CPVT [53]. The effect on survival likely dampens the overall effect of affected status on survival, protecting those affected from events. The effect of beta-blocker on sex-influenced survival however is uncertain. Though the use of beta-blocker possibly exaggerates the sex-influence of *RYR2* p.R420W, it is more likely that females survive long enough to be diagnosed and receive therapy, whereas affected males present with sudden death before being diagnosed.

A further limitation is the use of SCD as both an inclusion criterion as (SCD below age 50 has been denoted as affected) as well as an outcome. Though it is felt that sudden death is both an important outcome as well as an important definition of disease, this represents a possible source of bias. To assess this possibility, analyses were repeated using only a genetic definition of disease. An affected group of mutation positive and obligate carriers was compared to an unaffected group. This analysis trended to a statistically significant difference with statistical significance likely not reached due to extremely small sample size and subsequent lack of power.

Finally, these results are generalizable only to individuals with the *RYR2* p.R420W mutation. Though these results are hypothesis generating for those with other RyR2 mutations, along with other CPVT causing mutations, the generalizability of these results to all RyR2 mutations is limited. These results underline the importance of early testing and early therapy in individuals with the *RYR2* p.R420W mutation. They further underline the need for future studies describing the natural history of specific mutations causing CPVT.

4.8 Future Work:

CPVT, as with other rare inherited diseases, remains incompletely described in the literature. The available literature is mainly comprised of studies describing probands and small familial studies. Larger, well-ascertained familial studies, such as this one, are required to describe the natural history of CPVT as a disease-causing entity. Furthermore, larger well-ascertained familial studies will provide further information on individuals who are genotype-positive, but phenotype-negative or show mild disease. Larger studies on this population will also shed light on protective genetic, environmental, and therapeutic factors leading to milder disease. In addition to larger family studies, the compilation of large international registries to combine most known cases

of CPVT will be incredibly useful. Such large registries and randomized studies can also shed light on the presence of a sex difference and create firmer hypotheses on the etiology of our observed difference. Population-based genomic databases also represent an area of potential further research. These databases include members of the general population who may have lethal genetic variants without an obvious phenotype. Describing asymptomatic individuals with this, among other mutations, will be vital in clarifying the natural history of CPVT.

Another area of important future study will be the impact of this CPVT causing mutation, along with other CPVT causing mutations, on juvenile deaths. We have described six juvenile deaths which may be attributed to the *RYR2* p.R420W mutation. Further exploration of the frequency and nature of juvenile deaths among families with CPVT will help elucidate the risk to children. In addition, as post-mortem molecular autopsies of unexpected death are legislated, the discovery of more disease-causing mutations for CPVT (and other causes of SCD) is likely inevitable [71]. A greater understanding of this and other mutations as potential causes of juvenile death will inevitably lead to earlier monitoring and treatment of those found to have mutations. These interventions have the potential to save lives.

A final area of further work in this area is specifically regarding therapy. Though observational data suggests a benefit of non-selective beta-blockers over cardio-selective beta-blockers, randomized data does not exist and would be beneficial for therapeutic decisions [53]. Though studies exist suggesting flecainide as an option as a second antiarrhythmic in patients with recurrent arrhythmias [58], high quality studies supporting this practice are limited and should be the focus of future research. Finally, whether a subset of the CPVT population can be treated with medical therapy alone without the obligatory use of ICD's as in other inherited arrhythmias remains to be studied. Given the risks associated with invasive devices, as well as the

psychological burden among young patients with devices, this represents an important area of further research.

Chapter 5: Conclusions

5.1 Conclusions:

In summary, we have described the natural history of three families with a known *RYR2* p.R420W mutation. Among patients *RYR2* p.R420W carriers there is a statistically significant association with death, further validating the pathogenicity of *RYR2* p.R420W. Furthermore, we have observed that mortality occurs earlier in males than in females. Of seven deaths occurring under the age of 50, six occurred in males, highlighting the sex-specific effects attributable to *RYR2* p.R420W. Also relevant is that we have confirmed six juvenile deaths in families where *RYR2* p. R420W is segregating, raising the possibility that this mutation may be the cause.

Finally, when compared to an established SCD causing mutation in a genetic subtype of ARVC (*TMEM43* p.S358L), survival with the *RYR2* p.R420W mutation is similar. This comparison further highlights the pathogenicity of this mutation and CPVT as an important cause of early SCD.

The use of the Newfoundland and Labrador founder population and the availability of multiplex, multigenerational families allows for robust ascertainment strategies and thus a relatively unbiased assessment of natural history can be obtained. Ascertainment bias is minimized by the ability to assess all persons at a-priori 50% risk, as opposed to only those who present due to clinical symptoms. In this study, we could assess all those who are unaffected, as well as all who meet affected criteria. This allows the ability to accurately ascribe clinical signs and symptoms across the lifespan to the *RyR2* p.R420W mutation. This also helps to exclude issues related to their common genetic background or shared environment. This study represents the

largest single mutation multigenerational cohort with robust ascertainment in CPVT, highlighting the power of well ascertained family histories.

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Appendix 1: Data Extraction Form

CPVT

Family # _____ Generation #_____ First Name:_____ Last Name:_____

Gender trans. Parent 1=Mother___ 2=Father___ 3=UNK___ Gender 1=M____ 2= F ___ DOB _____

Sibship #_____ MCP_____ Ascert 1 Well_____ 2 Poor _____ Ascert 100 1 Well____ Poor____

DNA Obtained: 1 Yes___ 2 No ___ 3 Unknown___ 4 Blocks ___ Date of last follow up _____

Disease status positive: 1 Mutation positive___ 2 Obligate Carrier ___ 2 Severe clinical event without DNA*_____

Disease status negative: 4 Mutation negative___

Disease status unknown: 5 Unknown clinical status or unknown DNA status___

CPVT criteria positive Y _____ N_____

Which criteria? 1 exercise induced polymorphic ventricular arrhythmias___ 2 syncope with activity ___

3 V fib in stress ____ 4 exercise/emotion induced palpitations/dizziness ____

5 absence of structural abn___ 6 SUD/SCD triggered by stress/ exercise____

* SCD under 50 years
Clinical information: PP = pedigree information, CE: clinical event, NYD: not yet diagnosed, MOD: method of diagnosis, MOP: Mode of presentation

MOD: 1 CE no DNA__ 2 DNA__ 3 CE +DNA__ 4 PP __ 5 NYD__ 6 PP+DNA__ 7 PP+DNA+CE __ 8 PP+CE__

MOD hierarchy PP_____ DNA_____ CE_____ NYD_____

MOP: 1 CE_____ 2 Clinical screen _____ 3 DNA screen _____4 Inadvertent_____

MOP2 1 Chart review _____ 2 Chart rev +interview (incl screening clinic)_____3 No chart avail _____

Attended the genetic screening clinic? 1 Y___ 2 N____

Clin. Prob: 1 Y___ 2 N___ 3N/A___ Type 1 Syncope___ 2Palpitations ___ 3 Death___ 4Other___ 5 HF___

6 Chest pain ___ 7 presyncope___ 8 anxiety___

Age at 1st Clin prob ____ Longstanding 1 Yes ____ 2 No_____ 3 unknown _____

Visited a doctor 1 yes____ 2 no _____ 3 unknown_____ Age at presentation___

Presyncope 1 yes____ 2 no _____ 3 unknown_____ Age at presentation___

Syncope 1 yes____ 2 no _____ 3 unknown_____ Age at presentation___

Palpitations 1 yes____ 2 no _____ 3 unknown_____ Age at presentation___

Chest pain 1 yes____ 2 no _____ 3 unknown_____ Age at presentation___

Heart failure 1 yes____ 2 no _____ 3 unknown_____ Age at presentation___

hospitalizations 1 yes____ 2 no _____ 3 unknown_____ Age at presentation___

# of hospitalizations_____ days in hospital _____

Dead: 1 yes _____ 2 no _____ age at death _____

Cause of death: 1 Sudden death ____ 2 HF_____ 3 Coronary disease_____ 4 Other cardiac_____

5 Accident_____ 6 Other_____ 7 Not available _____

Describe_____

_____

Family #_____ Generation #_____

Treatment:

Class I drugs 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Name_____

Sotolol 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Name_____

Amiodorone 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Name_____

Betablockers 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Name_____

Cardiac meds 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Name_____

Defibrillator 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Comments_____

Pacemaker 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Comments_____

cardioversion 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Comments_____

Heart Tx. 1 yes ____ 2 no _____ 3 n/a____ Age at Rx____

Comments_____

Investigations:

ECG 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

Signal averaged ECG 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

Echo 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

Holter 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

MRI 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

Stress test 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

Heart cath 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

EP studies 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

Gated scan 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

Autopsy 1 yes____ 2 no ____ 3 N/A___ Age _____

Other heart tissue 1 yes____ 2 no ____ 3 N/A___ Age at 1st _____

Signal Averaged ECG

SAEKG Reports

Age (1st) _____ Age_____ Age_____ Age_____

Total QRS____ Total QRS____ Total QRS____ Total QRS____

RMS_____ RMS_____ RMS_____ RMS_____

LAS_____ LAS_____ LAS_____ LAS_____

EP study reports

EP study: yes ____ no ____

Age at EP study_____ Age at EP study_____ Age at EP study_____

Ventricular Arrhythmia:

Yes____ No_____ Yes____ No_____ Yes_____ No_____

MRI

Age at 1st MRI_____

# of MRI reports_____

Age_____

RV Dysplasia_____

LV dysplasia_____

Comment:

Cardiac catheterization

Age at cath:_____

CAD 1: 1 vessel____ 2: 2vessel____ 3: 3 vessel____ 4: non critical disease ____ 5: Normal____

LVEDP_____ Pulmonary Artery Pressure (PAP)_____ Wedge Pressure (WP)_____

Right atrial pressure(RAP)_____

Dyskinesia 1 focal_____ 2 global____ 3normal _____

Stress test report

Age at 1st stress test_____ age at stress test_____ age at stress test_____

Normal ___ Abnormal___ normal___ abnormal___ normal___

abnormal___

Autopsy abstract

Age at autopsy: _____

Heart weight_____

RV dilation yes_____ no _____ LV dilation yes _____ no _____

RV hypertrophy yes____ no_____ RV hypertrophy yes___ no ____

CAD: 1 critical _____ 2. Non-critical (minor)_____ 3 negative _____

Light microscopy

LV hypertrophy yes____no _____ LV fibrosis yes_____ no _____

RV fibrosis yes____no _____

RV fatty infiltration yes_____no____

LV fatty infiltration yes____no____

Holter

Age____ Age____ Age____ Age____

PVCs (24 hrs)____ PVCs (24 hrs)____ PVCs (24 hrs)____ PVCs (24 hrs)____

Right sided____ Right sided____ Right sided____ Right sided____

Left sided_____ Left sided_____ Left sided_____ Left sided_____

Couplets____ Couplets____ Couplets____ Couplets____

Triplets____ Triplets____ Triplets____ Triplets____

Runs of VT_____ Runs of VT_____ Runs of VT_____ Runs of VT_____

Idiovent rhythm____ Idiovent rhythm____ Idiovent rhythm____ Idiovent rhythm____

Sustained VT Sustained VT Sustained VT Sustained VT

Yes_____ no ____ Yes_____ no ____ Yes_____ no ____

Yes_____ no ____

Supravent tot_____ Supravent tot_____ Supravent tot_____ Supravent tot_____

Couplets_____ Couplets_____ Couplets_____ Couplets_____

Runs____ Runs____ Runs____ Runs____

Longest run_____ Longest run_____ Longest run_____ Longest run_____

Max rate_____ Max rate_____ Max rate_____ Max rate_____

Brady runs____ Brady runs____ Brady runs____ Brady runs____

Longest BR____ Longest BR____ Longest BR____ Longest BR____

Min HR_____ Min HR_____ Min HR_____ Min HR_____

Max HR_____ Max HR_____ Max HR_____ Max HR_____

Avg HR_____ Avg HR_____ Avg HR_____ Avg HR_____

Pauses_____ Pauses_____ Pauses_____ Pauses_____

Longest pause_____ Longest pause_____ Longest pause_____ Longest pause_____

Right Heart Biopsy

Age at biopsy:_____

Light microscopy

Hypertrophy yes__ no ___ fibrosis yes___ no____ fatty infiltration yes___ no ____

Electron microscopy

Hypertrophy yes___no___ fibrosis yes___no_____

Gated scan report

Age at gated scan ____ Age at gated scan ____

Ventricular wall abn yes _____ no _____ Ventricular wall abn yes _____ no _____

Ejection fraction yes_____ no _____ Ejection fraction yes_____ no _____

Appendix 2: Ethics Approval Letter

From: administrator@hrea.ca [mailto:administrator@hrea.ca]
Sent: April 28, 2022 10:36 AM
To: Connors, Sean <sconnors@mun.ca>
Cc: Hodgkinson, Kathleen <khodgkin@mun.ca>; Hreaadministrator <administrator@hrea.ca>
Subject: HREB - Approval of Ethics Renewal 571769

Researcher Portal File #: 40001315

Dear Dr. Sean Patrick Connors:

This e-mail serves as notification that your ethics renewal for study HREB # 2000.176 – The clinical and genetic epidemiology of Cardiomyopathy, Channelopathy, and Sudden Cardiac Death in Newfoundland (NL) – has been **approved**. Please log in to the Researcher Portal to view the approved event.

Ethics approval for this project has been granted for a period of twelve months effective from May 11, 2022 to May 11, 2023.

Please note, it is the responsibility of the Principal Investigator (PI) to ensure that the Ethics Renewal form is submitted prior to the renewal date each year. Though the Research Ethics Office makes every effort to remind the PI of this responsibility, the PI may not receive a reminder. The Ethics Renewal form can be found on the Researcher Portal as an "Event".

The ethics renewal [will be reported] to the Health Research Ethics Board at their meeting dated May 5, 2022.

Thank you,

Research Ethics Office

(e) info@hrea.ca
(t) 709-777-6974
(f) 709-777-8776
(w) www.hrea.ca
Office Hours: 8:30 a.m. – 4:30 p.m. (NL TIME) Monday-Friday