THE CLINICAL AND GENETIC EPIDEMIOLOGY OF ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY IN NEWFOUNDLAND

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By

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ABSTRACT

Background

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a cause of sudden cardiac death (SCD) in young people due to ventricular tachyarrhythmias. One autosomal dominant genetic subtype of ARVC in Newfoundland, linked to a founder haplotype at 3p25 (ARVD5) provided a genetically homogenous population in which to define the epidemiology of ARVD5 and find the causative gene using a retrospective and prospective familial cohort.

Methods

The final sample comprised 496 well ascertained subjects from 15 families (270 men, 226 women) born at an a priori 50% risk of ARVD5. Subjects were 'affected' if they i) had the founder haplotype OR the causative mutation, ii) were an obligate carrier, iii) had SCD or cardioversion for ventricular arrhythmia under age 50 years. 'Unaffected' subjects' did not have the founder haplotype/ mutation and formed a comparison group. Genetic information was used to determine ARVD5 penetrance. Incident and prevalent clinical events, symptoms, hospitalization and death were compared between affected and unaffected subjects. Affected subjects with an implantable cardioverter defibrillator (ICD) were matched to affected controls to assess treatment efficacy.

Results

The causative gene for ARVD5 was *TMEM43*, a transmembrane protein of unknown function. ARVD5 was 100% penetrant for signs and symptoms over the lifespan. Novel clinical findings were poor R wave progression, and dilated cardiomyopathy. All abnormal test results occurred significantly earlier in affected men and women compared with their unaffected same-sex relatives. Survival was decreased in affected subjects where 50% of men and 5% of women were dead by age 40 years: a relative risk of death between affected men and women of 5.1 (95% CI 3-8.5). The time course of disease was prolonged in affected women by 1-2 decades, who were four times less likely to be hospitalized than affected men. Heart failure eventually occurred in those who did not experience SCD. Holter monitoring for ectopy was diagnostically useful (likelihood ratio > 10). In men, the five year mortality rate post ICD was zero compared with 28% in the comparison group (p=0.009). The issues of overlap between genetic research and clinical genetics, privacy and duty to warn are addressed.

Conclusions

ARVD5 is caused by a 100% penetrant novel gene for ARVC. It is lethal in men, who are significantly more affected at all stages of the disease than women. ICD provides effective treatment. Clinical genetic and research practice should not be differentiated for severe conditions.

PROLOGUE

"I am the family face;
Flesh perishes, I live on,
Projecting trait and trace
Through time to times anon,
And leaping from place to place
Over oblivion

The years-heired feature that can
In curve and voice and eye
Despise the human span
Of durance—that is I;
The eternal thing in man,
That heeds no call to die."

Heredity

Thomas Hardy

This poem captures the juxtaposition of history and biology, where inherited traits link individuals across time. This is an awesome concept: that genes, which make each individual unique, are conserved across generations: that a single gene which gives ones family a 'distinctive nose' has been inherited by individuals lost in the centuries prior to one's birth, and will do so by unknown descendants. This biological link is true immortality for genes: an immortality that sometimes comes at the expense of human mortality. This truth is the impetus behind the story of this piece of work: the story of one gene, passed across multiple generations on the island of Newfoundland in Canada, "*the family face...that heeds no call to die*". This gene has caused the deaths of many young men, some named and remembered, some forgotten; the gravestones of whom can be found in isolated communities across this temperamental island. This is the burden that has been faced by Newfoundland families with this "*eternal*" gene. These people and

their stoicism in the face of tragedy have provided the material that allowed this thesis to be written.

This piece of work tells a story in which my role began in 1996. Several families had arrhythmogenic right ventricular cardiomyopathy, with striking histories of sudden death in young individuals. The following five years were spent meeting with these families, extending their genealogy, listening to their stories and collecting all available data on both alive and deceased family members with and without disease. In 2002, I embarked on a Ph.D. and with the collection of more data I began the journey to determine the clinical and genetic epidemiology of this lethal disorder. All work of this nature requires the concerted effort of a team of people: this was a journey therefore taken alongside my clinical and academic colleagues and teachers.

This piece of work was far more than just a thesis. Genetic research of this type becomes almost a crusade, revealing as it does the inequalities inherent in a health care system struggling to provide care in rural de-populated areas, and defines a desire to help individuals reluctant sometimes to face the inevitability of inheritance. Health care can be proactive, and preventative, but only if the full spectrum of a disease is known. Comparing those with disease (including those who are asymptomatic and those who die before seeing a health worker), and those without disease, (including those who may look as if they have the disease but in actuality do not), one can determine disease related features; how to diagnose appropriately, and how to treat. This should be the aim of this type of health research: to effect change in the lives of the people affected with and at risk of disease, by the acquisition of knowledge.

"No man is an Island, entire of itself; every man is a piece of the Continent, a part of the main; if a clod be washed away by the sea, Europe is the less, as well as if a promontory were, as well as if a manor of thy friends or of thine own were; any man's death diminishes me, because I am involved in Mankind; And therefore never send to know for whom the bell tolls; It tolls for thee."

John Donne

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"Grown-ups never understand anything for themselves, and it is tiresome for children to be always and forever explaining things to them."
Antoine de Saint-Exupery
"Children are the only form of immortality that we can be sure of."
Peter Ustinov

Emily and Alice Macgregor: for explaining things to me. You have taught me the real miracle of genetics.

"My husband gave me a necklace. It's fake. I requested fake. Maybe I'm paranoid but in this day and age I don't want something around my neck that's worth more than my head."
Rita Rudner

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"Adoption carries the dimension of connection not only to your own tribe but beyond widening the scope of what constitutes love ties and family. It is a larger embrace."
Isabella Rossellini

Betty and Stanley Hodgkinson and Jane Quambro: For years of love and support

Friends

"Friendship is born at that moment when one person says to another: What! You too? I thought I was the only one"
C. S. Lewis
"A true friend is someone who thinks that you are a good egg even though he knows that you are slightly cracked."
Bernard Meltzer
"The middle of the road is where the white line is and that's the worst place to drive."
Robert Frost

To my friends: thank you for never driving on the white line! Particular thanks to Peter Sheppard for help with formatting.

"*Father Time is not always a hard parent, and, though he tarries for none of his children, often lays his hand lightly upon those who have used him well; making them old men and women inexorably enough, but leaving their hearts and spirits young. . .*"*
Charles Dickens

Breda Jones: (1908-1995) my first High School science teacher. You were 64 years old, ready to retire, yet you befriended an 11 year old girl. I came to realise that friendship is unrelated to age and I bless the day I was fortunate enough to attend your science class. Everything I do relates to the lessons you taught.

Supervisory Committee

"Education: that which reveals to the wise, and conceals from the stupid the vast limits of their knowledge."
Mark Twain

Sean Connors, Terry Young, for highlighting my limits.

Supervisors

"Human beings, who are almost unique in having the ability to learn from the experience of others, are also remarkable for their apparent disinclination to do so."
Douglas Adams
"We gain comfort from those who agree with us...and growth from those who don't."
Frank A Clark

Patrick Parfrey: for sharing your knowledge of clinical epidemiology, and for the opportunity to grow. Despite great efforts to the contrary, we both managed to overcome our inherent (probably genetic) disinclination.

"The difference between good teachers and great teachers is that great teachers have mastered the art of teaching people things they didn't know they needed to learn."
Jeff Wahl
"A gifted teacher is as rare as a gifted doctor, and makes far less money."
Anonymous

Anne Bassett: for being a gifted and tolerant teacher; for being a supreme physician; for putting up with me; for being my mentor; and my friend and for the continued supply of my favourite sherry! I am indebted to the quirk of fate that effected our original introduction and I am grateful that you agreed to be my co-supervisor.

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DEDICATION

"I have seen flowers come in stony places
And kind things done by men with ugly faces
And the Gold Cup won by the worst horse at the races
So I trust too"

John Masefield

This thesis is presented as a testament to all the families with ARVC in Newfoundland.

I trust that as new generations arise, the blight that has affected previous generations will be lessened.

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LIST OF ABBREVIATIONS AND SYMBOLS

AD	Autosomal dominant
AMGGI	Atlantic medical genetics and genomics initiative
AR	Autosomal recessive
ARVC	Arrhythmogenic right ventricular cardiomyopathy
ARVD	Arrhythmogenic right ventricular dysplasia
bp	Base pairs
CF	Cystic Fibrosis
CIHR	Canadian Institute of Health Research
cM	Centimorgan
CM	Cardiomyopathy
DCM	Dilated cardiomyopathy
DSC	Desmocollin
DSG	Desmoglein
DSP	Desmoplakin
ECG	Electrocardiogram
HCM	Hypertrophic cardiomyopathy
ICD	Implantable cardioverter defibrillator
LBBB	Left bundle branch block
LOD score	Log of the odds ratio
LQT	Long QT syndrome
LV	Left ventricular
LVEDD	Left ventricular end diastolic dimension
LVESD	Left ventricular end systolic dimension
MI	Myocardial infarct
MOI	Mode of inheritance
MRI	Magnetic resonance imaging
NOS	Not otherwise specified
nsVT	Non sustained VT
PKGB	Plakoglobin
PKP	Plakophylin
PRWP	Poor R wave progression
RBBB	Right bundle branch block
RCT	Randomised controlled trial
RR	Relative risk
RV	Right ventricular
RVFW	Right ventricular free wall
RYR	Ryanodine receptor
SAECG	Signal Averaged ECG
SCD	Sudden cardiac death
TMEM43	Transmembrane protein 43
TGF ß3	Transforming growth factor ß3
UTR	Untranslated region
VF	Ventricular fibrillation
VT	Ventricular tachycardia
ZASP	z-band alternatively spliced PDZ motif-containing protein

LIST OF APPENDICES

1 INTRODUCTION

"You see only what you look for; you recognize only what you know" (1)

M.C. Sosman

"We sometimes forget that diseases have no intrinsic being; that their names represent only convenient rubrics, classes that have logistic uses but no precise conceptual content particularly as to cause" (2)

Childs and Motulsky

1.1.1 Thesis history and organization

This research project, and by definition this thesis, has a long history. It may be helpful to understand this history, and my role in the process. The introduction therefore starts with an explanation of how and why events unfolded as they did, and provides a temporal sequence. One can, however, begin reading from the literature review page 25 section 1.2 and skip this 'introduction to the introduction' if desired.

This is a body of work that focused on an eventual sample of 15 Newfoundland and Labrador families with arrhythmogenic right ventricular cardiomyopathy (ARVC) linked to a genetic locus at 3p25 (ARVD5). The sample comprised a familial cohort defined in a retrospective and prospective manner. Subjects were defined as those born at an a priori 50% risk of inheriting the ARVD5 gene, alive or dead. This included all affected subjects (as they, by default, had to be born at an a priori 50% risk) and all those born to affected subjects. Data from all generations in the family pedigree were included (in some cases up to nine generations). Current generations were enrolled into the study and followed

clinically in a prospective manner resulting in a final sample size of 496 subjects (270 males, 226 females).

This is a cohort (from the Latin word for one of the 10 divisions of an ancient Roman legion) study: the non-experimental observational analytical analogue of the randomised control trial (RCT). A classic cohort study assigns two groups free of disease who differ according to an exposure and followed over time for an outcome (usually disease). A historical cohort study accesses past records to classify individuals and then follows the cohort forward through time, a longitudinal study from past to present rather than present to future. This study uses both approaches. A retrospective and prospective analysis of a cohort, divided by their 'exposure' (gene mutation positive or gene mutation negative), assessed for signs and symptoms of disease.

In 1996 I became a Memorial University research associate funded by the Canadian Genetic Diseases Network. I recruited families with sudden cardiac death (SCD) and cardiomyopathy (CM) starting with those originally ascertained through the Newfoundland and Labrador Medical Genetics Program. Extended pedigrees were drawn and computerized, a unique identifier for all subjects was created, all current and past cardiac medical records were obtained and genetic counselling was provided for all subjects. This included summary letters to participants, general practitioners and other health care providers.

One large family (Figure 8 page 79) was shown to have ARVC in the 1980's (3) and family members were recruited to a research study based out of Baylor University, Texas in the early 1990's. In 1998, linkage to the short arm of chromosome three (3p23:

ARVD5) was published by these researchers (4). Concurrently, Dr. Ludwig Thierfelder, a German cardiologist and molecular geneticist, requested collaboration with Newfoundland and Labrador aimed at finding the underlying ARVD5 gene. Thus, in 1998, a Canadian/European research group was convened which included Dr. William McKenna, a senior cardiologist from London, England. Subjects were assessed and consented to the research project. In the absence of a formal clinic, their cardiology follow up was handled by the research team, which now included Dr. Sean Connors, an electrophysiological cardiologist. With Dr. Connors' recruitment, Newfoundland and Labrador could provide implantable cardioverter defibrillator (ICD) treatment. Prior to this, patients were referred to mainland Canada.

Five additional unrelated ARVC families were ascertained, and linked by Dr. Thierfelder's group to ARVD5 (4). At this time, the locus was repositioned by Dr. Thierfelders group to 3p25. All affected subjects in all six families were shown to have an identical DNA haplotype on 3p25 (a series of concurrent DNA markers inherited as a linear block across generations). This indicated that they all shared the same mutation-carrying ancestor (founder). Thus presymptomatic diagnosis of ARVD5 in at-risk relatives in these six families could occur: if the haplotype associated with disease was present, it was likely that the ARVC gene mutation was also present. This was important for a clinically difficult to diagnose condition. I coordinated the research and clinical work relating to this endeavour, and discussed all research results with participating subjects.

In 2002 my part-time PhD formally began. Its remit was to determine the genetic epidemiology of ARVD5 in Newfoundland and Labrador, the natural history, phenotype and efficacy of treatment. A secondary outcome was to find the causative gene. Initially the six already ascertained families were used. Abstraction forms were designed for all genetic and cardiac data. This data was input to a statistical package for social sciences (SPSS, Versions 11 and 15) dataset comprising over 1000 variables (Appendix A page 255). This dataset was the basis for all statistical analyses. Informed consent in compliance with the requirements of Memorial University and Eastern Health was obtained from all subjects or appropriate surrogates. This included permission for access to medical records and for a blood sample for DNA extraction. Progress reports were compiled, and a research website was created (http://www.med.mun.ca/arvc/). Information was provided for at–risk families living in other parts of Canada and the USA, including the referral of family members to local genetic and cardiology practitioners. An information sheet about ARVD5 was produced for the use of health care professionals, and one for the use of patients.

Information from this project provided the data central to the creation of a genetics-cardiomyopathy clinic within Eastern Health. This clinic began in February 2004 and took direct referrals of patients with familial cardiomyopathies and arrhythmias. The main clinical diagnoses referred to the clinic were ARVC, SCD not otherwise specified (NOS), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), Cardiomyopathy NOS, arrhythmias NOS and Long QT syndrome (LQTS). In September 2004, the Canadian Institutes for Health Research (CIHR) funded a larger epidemiological study to define the molecular genetic etiology and epidemiology of

inherited cardiomyopathy in Newfoundland and Labrador. All family members referred to the genetics-cardiomyopathy clinic were asked if they wished to participate in this study. In this manner, nine subsequent families with ARVD5 were found. Thus the 15 families which form the final sample for this thesis are a subset of an extensive epidemiological sample of cardiomyopathy families in Newfoundland and Labrador.

In 2004, a new molecular genetic collaborator joined the ARVC team. Dr. Terry-Lynn Young returned to Newfoundland and Labrador after her post doctoral training in Seattle. With CIHR funding Dr. Young's molecular genetics laboratory recapitulated Dr. Thierfelder's linkage analysis and worked towards finding the causative gene. In 2006, Dr. Young headed the Atlantic Medical Genetics and Genomics Initiative; (AMGGI) receiving a nine million dollar award from Genome Canada via Genome Atlantic. The primary aim of AMGGI was to find novel genes within the Atlantic Provinces: the ARVD5 study formed a major part of this initiative.

This thesis details the genetic epidemiology of ARVD5 in Newfoundland Labrador and is organised in manuscript format. The manuscripts form a cohesive body of work produced since 2002. The number of families and the number of subjects vary across chapters as the ascertainment of new families (and family members within already ascertained families) continued throughout the study period (Table 1 page 24).

Table 1: Number of subjects and families represented in each chapter

Chapter	Number of families with ARVD5	Affected subjects (N)		Unaffected subjects (N)	
		Male	Female	Male	Female
2	15	148	109	77	74
3	15	148	109	77	77
4	11	123	74	46	46

I describe the discovery of the causative gene, provide a description of the natural history, the phenotype, the efficacy of treatment, methods of managing ARVD5 and several ethical issues. Appendix B details the academic and lay sequelae to this project. The chapters are presented in a pathophysiologically logical manner, not in the order in which they were written. Thus Chapter 2 defines the finding of the ARVD5 causative gene and mutation and its penetrance. Chapter 3 describes the phenotype and natural history. Chapter 4 analyses the efficacy of ICD treatment. The authorship of chapters 2-4 represent the clinical and laboratory personnel involved in the multidisciplinary nature of the extended research project, including my supervisors. My position as first author represents the lead role in writing the manuscripts, designing each clinical epidemiological sub-study, and ascertaining and analyzing the clinical epidemiology data. Ethical issues that arose from my work in chapters 2-4 culminated in research collaboration with Daryl Pullman, a bioethicist. All ethics related issues are contained in chapters 5-6. The discussion (chapter 7) provides broad conclusions and suggests future research directions.

1.2 LITERATURE REVIEW

ARVC is a cause of SCD in young people, due to ventricular tachyarrhythmias, caused by fat and fibrous tissue within the myocardium. ARVC is genetically heterogeneous, with many different genes, mutations and loci implicated in causation. One autosomal dominant (a 50% risk to first degree relatives of inheriting the mutated gene) form of ARVC is prevalent in Newfoundland and Labrador (ARVD5). This genetic subtype is the focus of this thesis. This introduction serves as a literature survey of ARVC in general and is organized in parallel structure to the order of the thesis chapters.

A short history of ARVC

It is likely that ARVC has been present throughout human history, but death at young ages in previous eras would be common and less noteworthy. ARVC may share a history with adipositas cordis (5) a myocardial condition characterized by fatty infiltration of the myocardium. Whether this was the disease to which Dr. Lydgate referred in George Eliot's Middlemarch (1872) (6, 7) when he said: "...you are suffering from what is called fatty degeneration of the heart...it is my duty to tell you that death from this disease is often sudden..." or whether it was ARVC is unclear. However, 19th century pathology text books clearly describe adipositas cordis, considered within the differential diagnosis for ARVC (8), and thus may be mistaken for ARVC. Conversely, the fictional Dr. Lydgate may have been talking about deposition of cholesterol in coronary arteries. For the purposes of this thesis, I would like to think that it was ARVC.

Convention credits the recognition of ARVC as an arrhythmic disorder to a French team (9) . Earlier case reports do exist describing probable ARVC (10), including one of four patients, three with SCD, all with patchy fatty infiltration of the right ventricle (RV), and T wave inversion on ECG (11). However, Frank Marcus is credited as being the first to systematically describe the disease in 1982 (12). ARVC has undergone several name changes. It was initially known as a pre-excitation syndrome (9), it then became a dysplastic (where cells, tissues or organs abnormally develop or grow) disorder (13), it expanded to include an arrhythmic portion to become arrythmogenic right ventricular dysplasia (ARVD) (14), and is currently considered to be a cardiomyopathy (ARVC) (15).

1.3 THE GENETICS OF ARVC

Table 2 Known loci and genes for ARVC

Locus	Gene	Protein	Chromosomal location	MOI *	Disease(s)
ARVD1	TGFß3	Transforming growth factor ß3	14q23-q24	AD	ARVC
ARVD2	RYR2	Ryanodine receptor-2	1q41.2-q43	AD	1) ARVC 2) Catecholiminergic polymorphic VT
ARVD3	?	?	14q12-q22	AD	ARVC
ARVD4	?	?	2q32.1-q32.3	AD	ARVC
ARVD5	See thesis	See thesis	3p25	AD	ARVC + left ventricular involvement (LVI)
ARVD6	?	?	10p14-p12	AD	ARVC
ARVD7	?	?	10q22.3	AD	ARVC
ARVD8	DSP	Desmoplakin	6p24	AD	1) ARVC 2) DCM with woolly hair syndrome. 3) Epidermolysis bullosa (lethal acantholytic)
ARVD9	PKP2	Plakophylin2	12p11	AD	ARVC
ARVD10	DSG2	Desmoglein2	18q12.1-12.2	? AD	ARVC + LVI
ARVD11	DSC2	Desmocollin	18q12.1	? AD	ARVC + LVI
ARVD12	PKGB	Plakoglobin	17q21	AD	ARVC
NAXOS	PKGB	Plakoglobin	17q21	AR	ARVC with woolly hair and keratoderma

* **MOI**: Mode of Inheritance, **AD**: autosomal dominant, **AR**: autosomal recessive

Early reports supported the case that ARVC was usually inherited as an autosomal dominant disorder, before the search for the underlying genes began in the early 1990's (16-23). Familial cases were present in the 1982 systematic description by Marcus (12) and many have suggested that about 30% of ARVC cases are familial (24, 25). As the

definition of 'familial' requires a comprehensive assessment of extended family trees to define variability of expression, it is likely to be an underestimate, as such family histories are rarely documented (12). A more realistic estimate may be that >90% is familial (26). Despite the preponderance of dominant families, the first gene to be cloned was, somewhat surprisingly, a recessive syndromic form of the disease, found at high prevalence on the Greek island of Naxos.

1.3.1 Autosomal recessive ARVC

Naxos Disease

Naxos disease, described clinically in 1986 (27) presents at birth with 'woolly hair' (described as being like 'steel wire'), erythema of the palms of the hands (redness of the skin caused by capillary congestion), leading to keratoderma ('horny' skin). Cardiac arrhythmias follow, causing SCD in late adolescence or early adulthood due to ventricular tachyarryhthmias (28). Four families contributing nine affected subjects were originally described with ARVC-like features, three of the cases with left ventricular (LV) involvement. The disease occurs on many Hellenic islands, in addition to Naxos. The extracardiac congenital manifestations can be considered 'markers' for Naxos disease, and are thus a form of presymptomatic diagnosis (the diagnosis of a serious disorder before major clinical symptoms become apparent).

Naxos disease was mapped to the long arm of chromosome 17 (17q21) in the late 1990s (29) and because of the defect in epidermal cells, investigators focused on the region of 17q where keratin genes had been mapped. The gene for plakoglobin (PKGB), a desmosomal protein was located within this region. PKBG occurs in two isoforms, the

first with 15 exons (NM_021991), the second with 14 exons (NM_002230), both coding for 745 amino acids. Screening of PKGB in the subjects with Naxos disease showed a two nucleotide deletion (2157 del 2TG) in affected individuals resulting in a frame shift, predicting a stop codon in the protein (30).

Autosomal dominant ARVC

In less than a decade, twelve autosomal dominant forms of ARVC were linked to chromosomal regions, numbered sequentially in order of discovery: ARVD1-ARVD12.

ARVD1 (MIM # 107970)

The first locus assignment for ARVC was described by an Italian group. Linkage studies in two large families (the largest with 19 affected subjects over four generations), determined a maximum LOD (log. of the odds ratio) score of 6.04 ($\theta = 0.0$) with marker D14S42, at 14q23-q24 (31). Another four generation family was linked to this locus in 2003 (32) with maximum LOD scores of 4.41 and 4.06 with markers D14S254 and D14S983 respectively. A family from Southern Germany in this latter publication was also said to be linked despite a) being small, b) several recombination events c) a maximum LOD score of 1.15 at marker D14S59 and d) a different background haplotype from the other two families (32). The authors screened the exonic sequences of four positional candidate genes expressed in cardiac tissue (*POMT2, TGFβ3, KIAA1036*, and *KIAA0759*[1]). However, no mutations were found (32).

The gene, transforming growth factor beta 3 (*TGFβ3)* comprises seven exons (NM_003239) and codes for 412 amino acids. In 2005, a mutation in the 5-prime

[1] OMIM: http://www.ncbi.nlm.nih.gov/omim/

untranslated region (UTR) of the *TGFβ3* gene was found in nine affected and three unaffected members of the four-generation Italian family initially described in 2003 (32, 33). Thirty unrelated probands were screened, and an additional mutation in the 3-prime UTR of the *TGFβ3* gene was found in one patient. Of interest (and unexplained) was the lack of mutations detected in the other large family originally described in 1994 which was said to link to the same locus (31) and in the family from Southern Germany (32).

ARVD2 (MIM # 600996)

Three further families were reported from the Italian group, one from Switzerland and two from northern Italy. One northern Italian family, previously described in 1988, presented with effort-induced polymorphic ventricular tachycardia (VT), despite a normal 12 lead ECG and functional studies (22). Four juvenile SCDs had occurred in the family and autopsy showed grossly normal hearts with large areas of fatty-fibrous replacement (34). Linkage to 1q42-q43 was demonstrated with a lod score of 4.02 at $\theta = 0.0$, using 95% penetrance. The two other families (from northern Italy and Switzerland) showed no linkage to either chromosome 14 or chromosome 1. In 2000, another Italian family with polymorphic VT was described, and the original family from 1988 (22) was updated (35). In 2001, two further Italian families with a similar type of ARVC (manifesting polymorphic VT) were reported. These were used along with the families previously described in 2000 (35) to refine the 1q42 physical map (36). Four missense mutations were identified in the ryanadine receptor-2 gene (RYR2: a calcium channel) in two highly conserved regions of the protein. One mutation was found in two separate families, and in one family, two *RYR2* mutations were found to co-segregate with disease (as a compound heterozygote) (36). The mechanism of different *RYR2* mutations is thus

unknown (37). It may be that RYR2 mutations are dominant with reduced penetrance, or that some mutations require an additional RYR2 mutation in the opposite allele to become penetant, as with recessive mutations. RYR2 is the cardiac counterpart to the skeletal muscle protein RYR1. Mutations in analogous regions in the *RYR1* skeletal muscle gene cause malignant hyperthermia susceptibility (a disorder of calcium regulation resulting in uncontrolled skeletal muscle hyper-metabolism), or central core disease (variable skeletal muscle weakness associated with a high risk of malignant hyperthermia). *RYR2* is one of the largest human genes with 105 exons (NM_0010352) encoding 4967 amino acids.

ARVD3 (MIM # 602086)

A locus for ARVD was described (14q12-q22) proximal to the chromosome 14 locus of ARVD1 (14q23–q24) in three small families from Italy, Slovenia and Belgium (38). Following a multipoint linkage analysis, a maximal cumulative LOD of 4.7 between D14S252 and D14S257 was found. This locus, 30cM nearer the centromere than ARVD1, was called ARVD2, later changed to ARVD3 (as ARVD2 on chromosome 1 had been published between acceptance of the Severini et al manuscript and the proofs).

ARVD4 (MIM # 602087)

With two Italian families, and a family from the USA (with European ancestry) (39), a locus at 2q32.1–q32 was reported (40). Two of the families showed LV involvement, yet family sizes were small and there were no details on the unaffected or unknown family members. The two families previously reported by this research group and found not to

be linked to chromosomes 1 and 14 (34), were also found not to be linked to this chromosome 2 locus.

ARVD5 (MIM # 604400)

This genetic subtype, prevalent in Newfoundland, is the focus of this thesis. It was recognised in the 1970's that a disorder caused SCD in young Newfoundland males, many of whom were distantly related (41). This family (now known as family AR1) was the focus of a research project in the early 1980s headed by Drs. Patrick Ko (cardiologist) and William Marshall (immunologist). At this time the diagnosis of ARVD was made, and at least one patient from this family was treated for ARVD in Ontario (41). The remit of this research was to determine if ARVD was associated with specific HLA types, following several reports in the literature implicating linkage between HLA and other cardiomyopathies (42, 43). Many subjects from AR1 were ascertained, and blood samples taken. A letter describing the family was published in the late 1980's (3) following a comprehensive ARVD clinical publication by the Italian group (44). Unfortunately, prior to 1995, the biological samples from this study were destroyed due to a lack of storage space (45). As several of the early subjects were now deceased, this loss was irreplaceable. This is not a criticism of an event that occurs regularly when research grants end and storage space is at a premium. It does however emphasise the serious issue of amalgamating genetic research on diseases with a high morbidity and mortality with clinical care (Chapter 6 page 173). Linkage to a region on the short arm of chromosome 3 was published in 1998 (4), and the underlying gene mutation described in 2008 (46) (Chapter 2)

ARVD6 (MIM # 604401)

In a large North American Caucasian family, with 40 extant individuals over five generations, the first five ARVD loci were excluded by microsatellite marker analysis, with a LOD score of < -2 for each locus (47). This ARVC phenotype was highly penetrant and malignant, with diagnoses in young children. A genome wide scan identified a novel locus at 10p14–p12, with a peak 2-point LOD score of 3.92 with marker D10S1664 on a shared haplotype of 10.6 cM between markers D10S547 and D10S1653. This region has been reduced to 2.9 cM in a South African family with 13 affected individuals (48).

ARVD7 (MIM # 609160)

The locus for ARVD7 was mapped in a Swedish family with autosomal dominant myofibrillar myopathy (MFM) and cardiomyopathy (49). Myopathic changes (rimmed vacuoles, and the accumulation of desmin, dystrophin, and other proteins) on muscle biopsy and disorganization of myofibrils on electron microscopy were seen. Severity was variable, with an onset between the third and sixth decades. One deceased patient had RV dilatation on autopsy, with fibrofatty replacement of the myocardium, and mild changes in the LV. Three males were defined as having ARVC with non-sustained (ns) VT, atrial flutter, and RV dilatation. A multipoint peak lod score of 3.06 between markers D10S605 and D10S215 suggested linkage to 10q22.3 (49). Mutations in the *ZASP* (z-band alternatively spliced PDZ motif-containing protein) gene (10q22.3) have been identified in myofibrillar myopathy patients (50), some of whom had cardiac involvement. ZASP is a lim domain binding protein (LDB3), comprising 16 exons and spanning 70 kb. LIM

domain binding proteins bind other LIM domain proteins. This brings together various transcription factors to form activation complexes or to block formation of such complexes (thus they act as either an enhancer or a repressor of transcription). PDZ proteins (fundamental building blocks in protein organization) interact in cytoskeletal assembly. The PDZ domain of ZASP therefore interacts with the C terminus of alpha-actinin-2 (51), whose isoform, localized to the Z-disc, anchors myofibrillar actin filaments in cardiac muscle. Although this seems a reasonable candidate for ARVD7 in these patients, to date, there have been no reports of mutations in *ZASP* causing ARVC.

ARVD8 (MIM # 607450)

The Rampazzo group described an Italian family comprising 26 individuals over four generations, ascertained via an 18 year old proband with ventricular fibrillation (VF) (52). After excluding the known loci, a 5cM 700 microsatelite genome wide scan produced a maximum LOD score of 4.32 for marker D6S309 at 6p24 and revealed a disease associated haplotype. This paper presented both the linked locus and the causative mutation in *desmoplakin* (DSP) (52). The *DSP* gene was considered an excellent functional candidate within this critical region because the authors recognised that Carvajal syndrome (manifesting DCM as part of the phenotype) (53) was caused by homozygosity for a mutation in *DSP* (54) at 6p24. The mutation caused a premature stop codon (7901 del G), resulting in a truncated protein with absence of the C domain. Carvajal syndrome is an autosomal recessive Ecuadorian disorder initially described in 1998 (53). Eighteen patients were originally described with epidermolytic (of the skin) palmoplantar (on the palms of the hands or soles of the feet) keratoderma (as found in Naxos disease), woolly hair, and DCM. Additionally they had altered cardiac

contractility, leading occasionally to heart failure and death in the second and third decade. In ARVD8, the resulting heterozygous mutation in *DSP* was a missense mutation (S299R). The *DSP* gene spans 45 kb and comes in 2 isoforms, *DSP I* (NM_004415) and *DSP II* (NM_001008844), each with 24 exons (2871 and 2272 amino acids respectively).

ARVD9 (MIM # 609040)

At the Max Delbruck centre in Germany, a plakophillin 2 (PKP2) null mouse was created which exhibited changes in cardiac morphogenesis (55). The absence of PKP2 disrupted the desmosome by dissociating DSP from the adherens junctions. Human *PKP2* is found in 2 isoforms, *PKP2*a (NM_001005242) and *PKP2*b (NM_004572). *PKP2*a has 13 exons coding for 837 AAs and *PKP2*b has 14 exons coding for 881 amino acids. Dr's. Gerull and Thierfelder shared a research floor with the PKP2 null mouse research team and recognised that this might have relevance to human ARVC (56) so they assessed 120 unrelated ARVC probands of Western European descent (101 males and 19 females). All 14 *PKP*2 exons, including flanking intronic splice sequences, were sequenced and 25 different heterozygous mutations were found in 32 (27 male, 5 female) /120 probands (27%). In a cohort from Johns Hopkins University (57), nine families with eight *PKP2* mutations between them were assessed. Wide intra-familial variability was noted with male *PKP2* carriers exhibiting more phenotypic features. The families were small and the ages at which subjects were tested varied across the lifespan thus firm conclusions about the penetrance or presentation of each *PKP2* mutation remains to be defined.

ARVD10 (MIM # 610193)

The Johns Hopkins team found no mutations in *PKP2* (ARVD 9) and *DSP* (ARVD 8) in 33 probands. They then chose to look at other desmosomal proteins (as functional candidates). They screened desmoglein2 (DSG2), a desmosomal cadherin protein, mapped in 1992 (58), and found four ARVC probands with *DSG2* mutations that were not present in 120 control individuals (59). *DSG2* is a 15 exon gene (NM_001943) coding for 1118 amino acids. Three of the mutations (in exons 3, 11 and 15) were heterozygous missense, highly conserved amino acid changes in functional domains of the protein; the fourth was a nonsense mutation in exon 8. The segregation of these mutations in the families, and whether they were all pathogenic was unclear. The families presented were small and it was not clear whether there were other relatives potentially available to screen.

The Italian group (60) looked at 80 unrelated probands for known ARVC mutations, 26 of whom had mutations in either *DSP* (ARVD8), *PKP2* (ARVD9), or *TGFβ3* (ARVD1). Nine *DSG2* (ARVD10) mutations (five missense, two insertion-deletions, one nonsense, and one splice site mutation) were found in 8 probands (five males and three females): none were found in 560 unrelated chromosomes from 280 controls. As with the Johns Hopkins team, the segregation involved combinations of mutations in affected and unaffected family members. The families were small, and it was again unclear whether there were more relatives available to determine whether two mutations were required for phenotypic expression. The authors argued that all the missense mutations were pathogenic. A high penetrance for DSG mutations (in 9 probands) has been described (61).

ARVD11 (MIM # 610476)

The McKenna London team screened 77 unrelated ARVC probands for mutations within *desmocollin-2 (*DSC2); the one major desmosomal protein remaining as a possible candidate for ARVC. They found two heterozygous mutations in affected members of four unrelated families in the *DSC2* gene, resulting in frame shifts and premature truncation of the DSC2 protein (62). In one family a 1-bp deletion in exon 10 in two female first degree relatives resulted in a frame shift and premature truncation of the protein at codon 480. In three unrelated families a 2-bp insertion (2687insGA) in exon 17 of the *DSC2b* gene caused a frame shift and premature truncation of the protein at codon 900. No disease-associated haplotype was found to link the three families. Two isoforms of *DSC2* exist due to alternate splicing in exon 16. *DSC2a* contains 16 exons (NM_024422a) and codes for 901 amino acids, *DSC2b* has 17 exons (NM_004949b) and codes for 847 amino acids.

The German team at the Max Delbruck centre investigated 88 unrelated patients with ARVC for mutations in *DSC2* (63) and identified a heterozygous splice acceptor site mutation (c.631-2A-->G) in a 58-year-old male patient with ARVC in intron 5 which caused the use of a cryptic splice acceptor site and the creation of a downstream premature stop codon. This mutation was not present in 500 control chromosomes. The family history was limited, and other than the affected proband, was clinically negative. The team looked at cardiac *DSC2* expression in lymphocytes and cardiac tissue obtained from biopsy and observed a marked reduction in the abundance of the mutant transcript. The Italian team screened 54 ARVC probands for DSC-2 mutations and found two heterozygous mutations (c.304G>A (p.E102K)) and (c.1034T>C (p.I345T)) in two

probands. Both mutations mapped to the N-terminal region, affecting the normal localisation of DSC2 (64).

ARVD12 (MIM # 611528)

The London group found a *PKGB* mutation (S39_K40insS; predicted to insert an extra serine residue in the N terminus of the protein) in a German family, where a father and three sons were affected with ARVC (65). Unlike Naxos disease (where a homozygous *PKGB* mutation causes syndromic ARVC with extracardiac hair and skin manifestations), these patients had a heterozygous mutation and presented with only cardiac findings.

1.3.2 ARVC: a disease of the desmosome or too early to tell?

Of the 12 known ARVC loci, there are now eight known genes for ARVC, including our discovery (46) (Chapter 2). Five of these eight involve genes coding for proteins present in the desmosomal complex (the last six listed in Table 2 page 27). This has been a classic example of the 'light under the lamppost' effect. Following the first desmosomal gene assignment, other desmosomal genes were assessed. They were 'lit' by that initial discovery. However, as the previous pages have hopefully highlighted, the evidence for causation for some of the desmosomal mutations described is not always robust. Some of these mutations may be modifiers in a complicated pathway, and other genes, currently lying in shadow remain to be found.

Cell adhesion

Connections between cells are controlled by cell-to-cell junctions, separated into 'tight', 'gap', 'adherans', and 'desmosomes' (the latter may be considered an adherans junction).

Briefly, tight junctions seal adjacent epithelial cells. Gap junctions permit the passage of small molecules between cells and are made from transmembrane connexin proteins. Adherens junctions provide strong attachments between adjacent cells, particularly important in areas of high mechanical stress. Adherens junctions comprise cadherin transmembrane proteins whose extracellular segments bind to each other and whose intracellular segments bind to catenins, which connect to actin filaments.

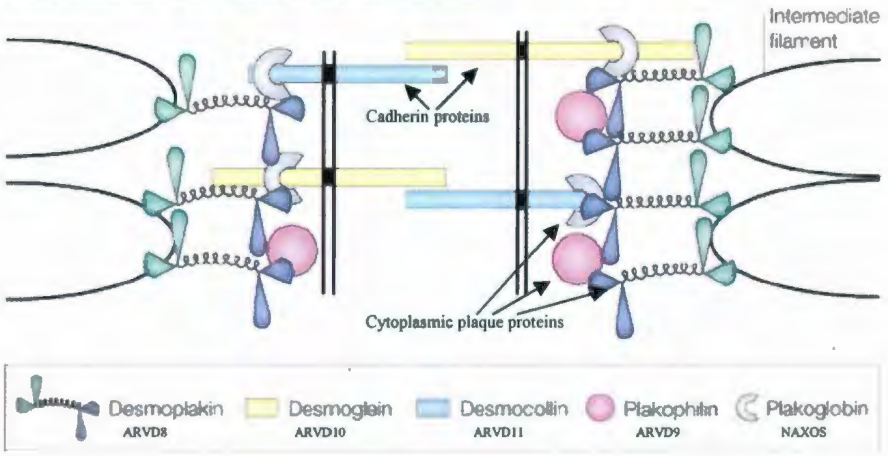
Desmosomes

"The Victorians would have approved of the desmosome–intermediate filament complex — a structure that buttons cells together and forms a web of filamentous constraints — a system as staid and solid as the queen's corsets. The recent bodice-ripping reports of mutant desmosomal proteins that curl the hair, break hearts, and leave us blistered and thick-skinned would have reinforced their moral perspective that lapses that undo buttons and let down suspenders lead to loss of integrity."(66)

Desmosomes are cell-cell adhesion multiprotein complexes (67), critical for providing rigidity in epidermal and cardiac tissue (Figure 1 page 40). They function as cell junctions, as signalling centres in embryogenesis, and they anchor cytoskeletal elements by linking to cytokeratins (intermediate filaments) at the plasma membrane. The desmosomal structure comprises several transmembrane adhesive glycoproteins, members of the cadherin superfamily of proteins (desmocollins and desmogleins) and cytoplasmic plaque proteins (DSP2, PKGB and PKP2). The physical relationship between desmosomes and intermediate filaments provides cellular structural integrity and adhesion. This allows for the assembly of cells into three dimensional tissues and organs.

Desmosomal protein defects in areas of high mechanical stress, such as the heart and the skin thus have a significant impact on tissue integrity (68).

Figure 1 Desmosome's role in cell adhesion[2]



Plakoglobin (Naxos disease and ARVD12) (MIM *173325)

PKGB is a cytoplasmic protein found in adherens junctions and desmosomes, thought to act as a 'linker molecule' between the inner and outer portions of the desmosomal plaque by binding tightly to the cytoplasmic domain of cadherins. The central domain of PKGB is formed by highly conserved armadillo repeats. The armadillo gene, involved in segment polarity, was initially discovered in Drosophila and many related proteins have been identified based on sequence homology. These proteins all have a central domain comprising several amino acid repeats forming a highly conserved 3D structure. They provide both structural (as cell-cell contact and with cytoskeleton-associated proteins) and signalling (by generating and sending signals affecting gene expression) functions (69). These functions are seen in PKGB where the central domain adheres to other

[2] http://www.nature.com/nrm/journal/v1/n3/images/nrm1200_208a_f4.gif

desmosomal and cytosolic proteins (69) (70), and where changes in adherens junctions are signalled directly to the nucleus (71, 72).

Desmoplakin (ARVD 8) (MIM +125647)

DSP2 is part of the plakin family of cytoskeletal proteins and is exclusive to desmosomes. It is the most abundant multi-functional desmosomal protein. It binds intermediate filaments at its carboxy-terminal site, it dimerizes through a central coiled rod; and with its pair of amino-terminal binding domains, it plugs into desmosomes and adherens junctions. DSP2 binds to PKP2, which in turn connects to desmosomal cadherins. Additionally, it binds to the juxtamembrane domain of DSC2-1a and associates indirectly through PKGB, with cadherins in adherens junctions (Figure 1 page 40) (66). Mouse models over-expressing a DSP C terminal mutation (R2834H) showed cardiac bi-ventricular apoptosis (cell death), fibrosis, enlargement and dysfunction. Over expression of wild type DSP had no adverse effects on the myocardium (73).

Plakophilin-2 (ARVD 9) (MIM*602861)

PKP2 is also an armadillo repeat protein of the desmosome. Plakophilins are located in the outer dense plaque of desmosomes, linking desmosomal cadherins with DSP2 and the intermediate filament system. Like other armadillo-repeat proteins, plakophilins probably have a role in transcriptional regulation in the nucleus. PKP2 (in its two alternatively spliced isoforms, a, and b) is the primary cardiac plakophilin. It interacts with several cell adhesion proteins, has an important role in the assembly of junctional proteins in embryogenesis and is an essential morphological component of the heart. Mouse hearts in the absence of PKP2 fall apart (74).

Desmoglein (ARVD 10) (MIM *125671)

Desmogleins are desmosomal cadherins and with the desmocollins, form the essential transmembrane components of the desmosome (75) (Figure 1 page 40). All desmogleins (DSG1–4) have four extracellular cadherin domains and a transmembrane domain. Calcium binding to the extracellular domains stabilizes their structure and function. Seven of the nine mutations causing ARVD10 (60), were located in the extracellular amino terminal domain which directly participates in adhesive interactions. The authors postulated that a single amino acid change could result in changes in molecular affinity, altering the adhesive capacity of the cadherins (DSG2 andDSC2). In-vitro antisense experiments against DSC2 resulted in cell detachment and knockout mice for *DSG2* died as early embryos (76), supporting the role of impaired cell-to-cell adhesion in some forms of cardiomyopathy (77).

Desmocollin 2 (ARVD 11) (MIM *125645)

DSC2 is like desmoglein, a desmosomal cadherin: a glycoprotein of between 115 and 130 kD. The potential pathology of *DSC2* mutations in ARVC subjects was investigated by cloning full-length wild-type and mutated cDNAs in eukaryotic vectors to obtain a fusion protein. Constructs were transfected into neonatal rat cardiomyocytes and in HL-1 cells. N terminus mutations altered the location of DSC2 to a primarily cytoplasmic position compared with wild type (64). Knockout zebrafish embryos had a reduced desmosomal plaque area in cardiomyocytes, and had associated contractility defects. A physiologic level of DSC2, like other desmosomes, appears to be crucial for normal cardiac desmosome formation, cardiac morphogenesis, and cardiac function (63).

Non-desmosomal genes in ARVC

Of the three non-desmosomal genes now known to be involved in causing ARVC, two were described before this thesis; the third is described in Chapter 2 (page 74).

Transforming growth factor β3 (TGFβ3) (ARVD1)

TGFβ3 is a member of the transforming growth factor family, comprising proteins that regulate several physiological processes, including embryonic development, homeostasis, chemotaxis and control of the cell cycle. TGFβ1, β2 and β3 inhibit proliferation in many cells and induce apoptosis of epithelial cells. They also stimulate mesenchymal cells to proliferate, and can induce a fibrotic response in several tissues in vivo (78). The presence of *TGFβ3* mutations in ARVC families may thus relate to the production of myocardial fibrosis, disrupting the electrical behaviour of the myocardium, predisposing to re-entrant ventricular arrhythmias (79).

Ryanadine Receptor 2 (ARVD2)

Functional studies have provided good evidence for the role of RYR2 in SCD (37). RYR2 channels couple the excitation of myocardial cells to their actin/myosin contractile apparatus by a calcium induced-calcium release mechanism. This works by the stimulation of voltage sensitive calcium channels in the outer myocardial membrane which allows the entry of some calcium ions. This then activates the release of calcium into the cytoplasm from the sarcoplasmic reticulum lumen via RYR2 which thus initiates myocardial contraction (80, 81).

RYR2 mutations account for different clinical phenotypes: ARVD2 (36, 82) and catecholiminergic polymorphic VT (stress induced polymorphic VT in the absence of

structural heart disease and a prolonged QT interval) (83-85). Characteristics shared by these diseases are effort-induced ventricular arrhythmias and the risk of SCD. Shared pathogenesis would comprise the effect of the mutations on the ability of the calcium channel to remain closed. Thus intense adrenergic stimulation due to stress (emotional or physical) could lead to calcium overload. These seemingly different clinical phenotypes may thus represent variable expressivity or allelic heterogeneity (multiple alleles within the same gene causing the same phenotype).

Genetic subtypes with a common pathophysiological mechanism for ARVC.

The involvement of multiple desmosomal proteins, and TGFβ3 and RYR2 leads to several hypotheses regarding a unifying pathophysiology of ARVC (52) (86). Thus defective desmosomal proteins would impair cell to cell contact and affect the ability of the myocardium to stretch. This would occur preferentially in areas of high strain in the RV (which can stretch more than the left (52): the so called 'triangle of dysplasia' (Figure 6 page 52). Mechanical forces applied to adherens junctions activate 'stretch sensitive' calcium channels via cadherin mechanical intracellular signalling (87). These stretch activated channels transform mechanical forces into an electrochemical signal due to an increase in intracellular calcium concentration (88-90). Those with defective desomosomal proteins would produce 'over stretch'. Additionally, stretching of cardiomyocytes modulates calcium release from RYR2 release channels (91). A genetically impaired stretch response thus might affect intracellular calcium concentration (resulting in excessive calcium load) and the excitation/contraction coupling. The result may be the production of arrhythmias (92). The existence of the *RYR2* mutations supports the concept of impaired intracellular calcium playing a key

pathogenic role in the pathogenesis in at least some forms of ARVC. TGFβ's are known to modulate the expression of genes encoding desmosomal proteins (93), suggesting that the effect of un-translated region mutations causing over expression of TGFβ3 might affect cell to cell stability. Links may be made between all the putative genes and at least part of the ARVC phenotype.

Cardiac remodelling and the interdependence of the protein 'players' in the myocyte 'orchestra'.

Although the term 'remodelling' may at first glance imply improvement, it is in fact the opposite. Remodelling defines the concept that the size, shape and function of the heart change in response to various adverse stressors. The term 'reverse remodelling' is used to define an improvement in ventricular mechanics and function after stress. Remodelling occurs as a response when strain is applied to the heart, and describes a series of histopathological and structural changes in the ventricular myocardium that leads to a progressive decline in cardiac performance, and possibly reduced contractile function.

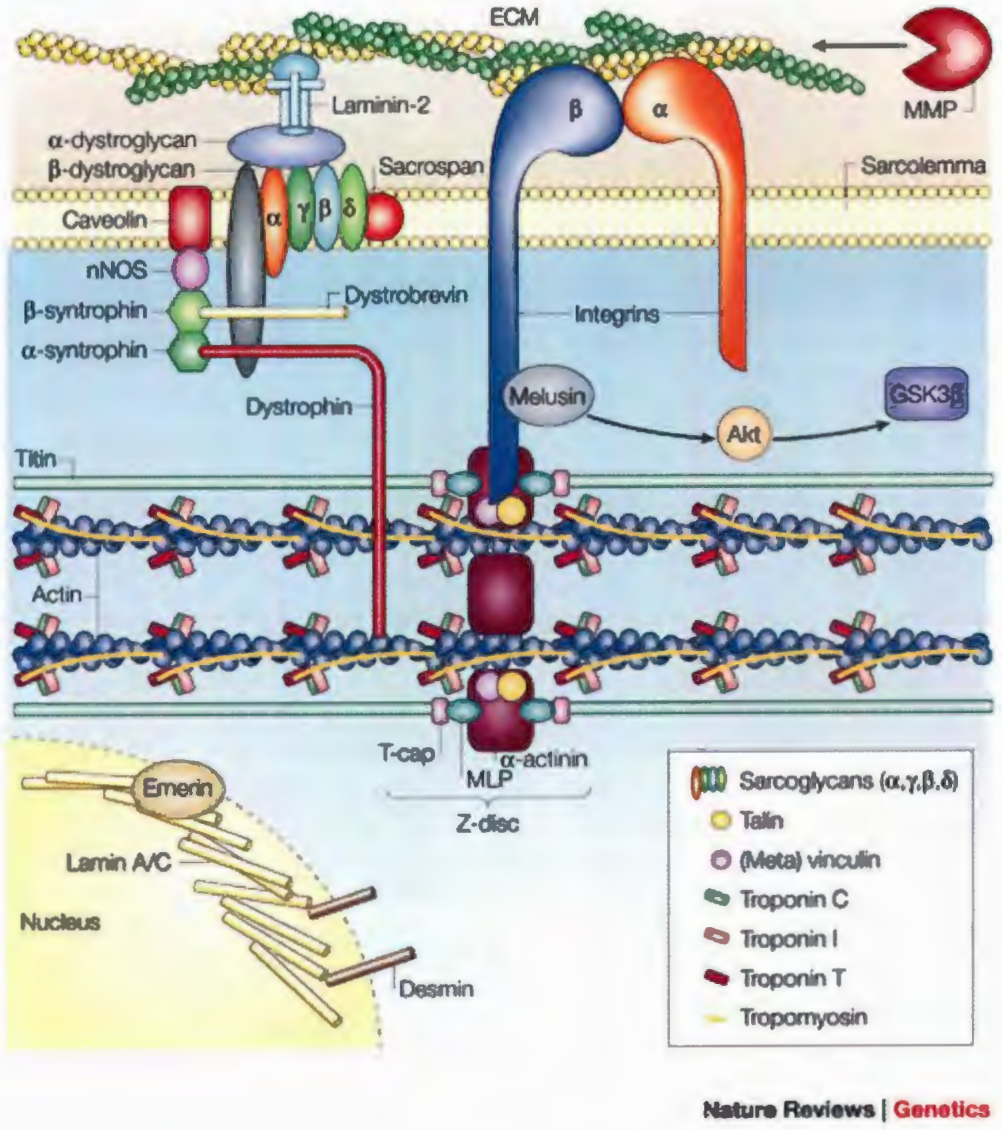
Heart failure is characterized by impaired systolic and diastolic dysfunction and occurs in the final stages of disease caused by the *TMEM43* mutation. Both dilatation and hypertrophy occur as a remodelling response of the heart to an underlying insult. Dilatation is classically thought to be related to volume overload with hypertrophy a response to increased pressure (e.g. hypertension). (94). In the case of the primary cardiomyopathies however, these occur in the absence of an obvious cause for either volume overload or increased pressure. Thus, prior to the definition of some of the causative genes, primary cardiomyopathies were known as 'idiopathic'. DCM is presumed to be caused by mutations in contractile or calcium cycling proteins, nuclear

membrane proteins, or cytoskeletal proteins (95), and HCM by sarcomere protein mutations (96), although overlap (where sarcomere protein mutations can cause DCM for example) exists.

1.3.3 How force is generated within the heart

The sarcomere comprises a group of proteins organised into thick and thin filaments. When calcium and ATP are present, they slide past one another creating force. Calcium is the most important molecule in this scenario. It enters myocytes by L type calcium channels via a sodium (Na+) and calcium ion (Ca2+) exchanger, which causes calcium to be released from the sarcoplasmic reticulum via ryanadine receptors. During relaxation, calcium is removed from the cytosol via the exchanger. Intracellular calcium rises in response to electrical depolarisation which causes calcium to bind to troponin C, I and T, and also alpha tropomyosin (Figure 2 page 47 and Figure 3 page 48).

Figure 2: Schematic illustrating the relationship between the desmosomal, cytoskeletal, sarcomeric and nuclear membrane proteins in the cardiac myocyte. (97)[3]

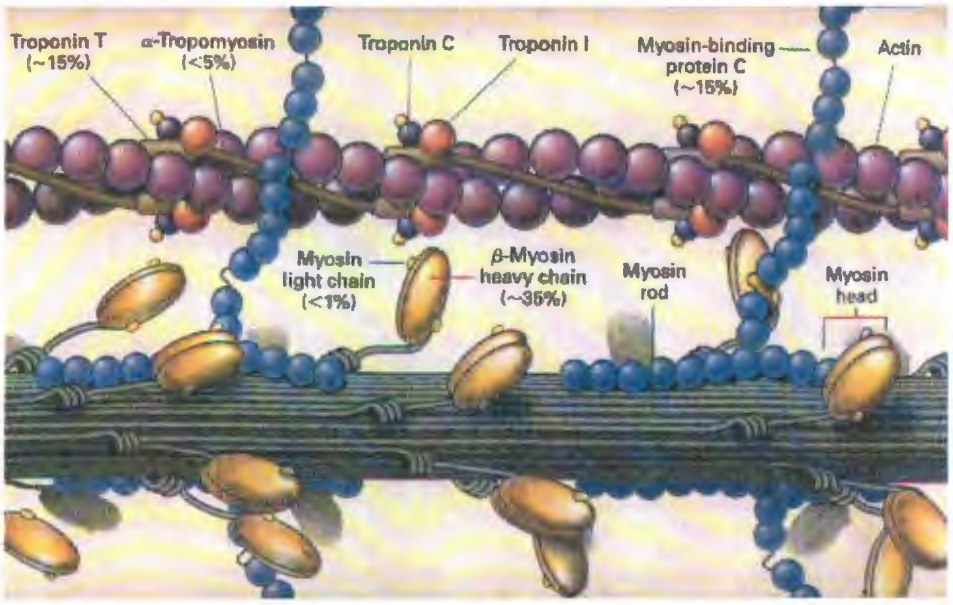


This releases troponin I inhibition of the actin myosin connections (Figure 3 page 48) which allows myosin to bind to actin then ATP to myosin, thus allowing for the myosin

[3] http://www.nature.com/nrg/journal/v5/n11/thumbs/nrg1470-f2.jpg

head to move along the thin filament generating force following ATP hydrolysis (Figure 3 page 48). This force is transmitted to the cytoskeleton (intermediate filaments, desmin, microfilaments, microtubules and lamin proteins which comprise the intermediate filaments of the inner nuclear membrane (Figure 2 page 47)) via the proteins linking the sarcomere to the sarcolemma and the outside of the cell. There are multiple costomeres that interconnect the cytoskeleton to the sarcolemma, including the complex comprising dystrophin, alpha and beta dystroglycans, alpha beta gamma and delta sarcoglycans, dystrobrevin and α and β syntrophin (Figure 2 page 47).

Figure 3: The relationship between the different sarcomere proteins in the cardiac myocyte. (98)[4]



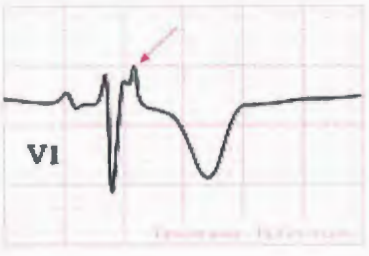
[4] gilead.org.il/hcm/sarcomere.jpg

Phenotype/Diagnosis/Natural History

Clinical presentation

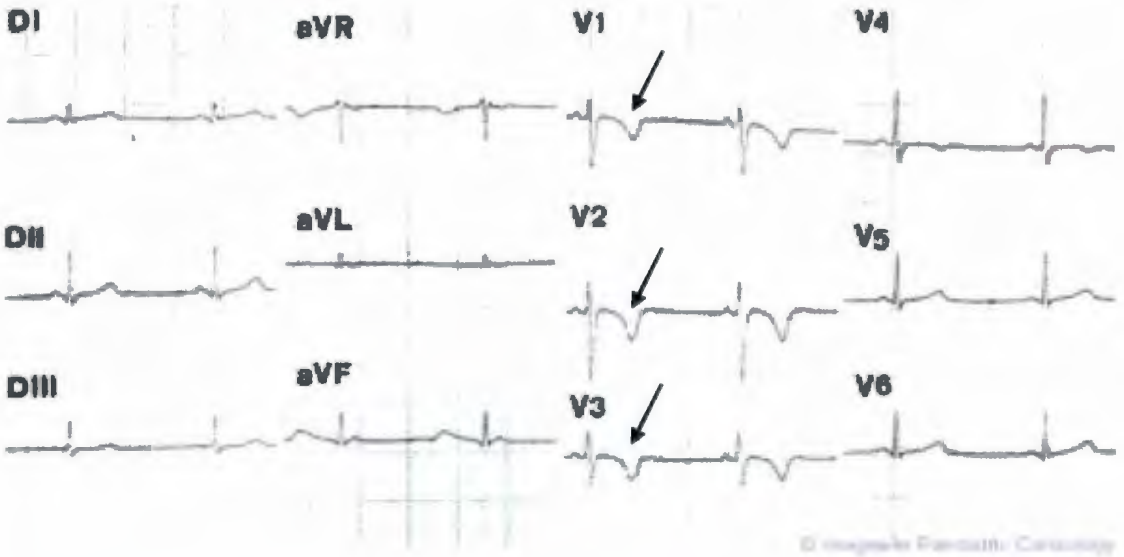
ARVC is an arrhythmic disorder, thus it alters the normal heartbeat (Appendix C page 284). 'Classic' ARVC presents with palpitations, presyncope (light-headedness), syncope (temporary loss of consciousness), and dizziness: symptoms related to VT (Appendix C). A common mechanism for VT in ARVC is re-entry (99, 100). Re-entry occurs when a conducting pathway is blocked in one direction, and re-stimulation of the pathway occurs. Thus in ARVC, the fat and fibre lead to unidirectional block and slow conduction, creating re-entry circuits and initiating VT (100), a similar mechanism to re-entry (and VT) after a myocardial infarct (MI), as a result of scarring following the death of a portion of the myocardium (101). After presenting with symptoms, classic ARVC features on 12 lead ECG include epsilon waves (small notched deflections just after the QRS in lead V1) and inverted T waves in leads v1 to v5 in the absence of right bundle branch block (RBBB).

Figure 4 Epsilon wave in 12 lead ECG[5] and normal V1 lead



[5] http://en.ecgpedia.org/wiki/Arrythmogenic_Right_Ventricular_Cardiomyopathy

Figure 5 T wave inversion in ARVC[6]



The etiology of T wave inversion in ARVC in general is not understood (102). Epsilon waves however are thought to relate to the fractionation of the electrical activity due to myocardial scarring and the position of the epsilon wave in V1 and V2 indicates RV involvement (102).

The early, prodromal stages of ARVC have been called the 'concealed phase' of the disease (34), where patients present with mild symptoms (or have inadvertent findings on clinical tests). Subsequent testing of such patients over time reveals fat and fibre on cardiac biopsy, ventricular arrhythmias and 12 lead ECG findings (103).

Heart failure is an ARVC presenting feature in some circumstances, with an enlarged RV and oedema (12) (104). Occasionally the first presentation is SCD, which occurs when

[6] http://www.health.gov.mt/impaedcard/issue/issue6/1837/1837.htm

VT progresses to VF (Appendix C). SCD due to ARVC may be more common in athletes (related to exertion) (105). In general, SCD accounts for about 50% of all deaths from cardiovascular causes (106), with about 20% of these deaths due to a genetic cardiomyopathy, or congenital heart defect (107). SCD occurs with many myocardial diseases, including, heart failure, coronary artery disease, other arrhythmias and various cardiomyopathies. In all situations, the VT may terminate spontaneously.

Diagnosis of ARVC

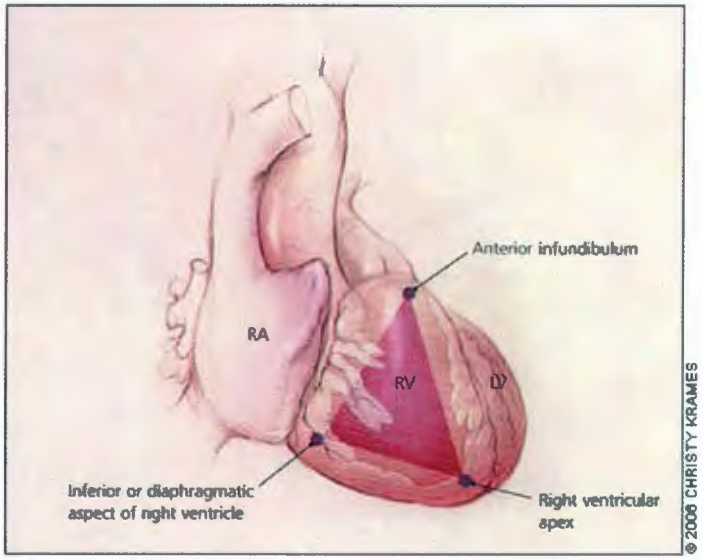
The quotation by Sosman which opens this introduction was used by Frank Marcus in the only book so far written on ARVC (7). Both Sosman and Childs (page 19) eloquently underline the difficulties associated with disease definition in general. ARVC is like the tip of the iceberg (a fitting analogy for Newfoundland and Labrador) where the disease that presents to clinical services probably represents about 1/10 of the true prevalence in the population.

A definition of phenotype is problematic for any disease. In most diseases it is that which presents clinically because the disease has caused problems significant enough to require assistance. For a true understanding of the disease process however, longitudinal data is required: to recognise the prodromal stages, the presenting stages, and the terminal stages. For a disease which causes SCD, the terminal event may be the only stage recognized. For ARVC, the definition of phenotype has proved difficult from the earliest recorded cases. Initially, the disease was described as manifesting VT, with massive RV involvement, and a myocardium infiltrated by fat and fibre. The pathological signs and symptoms thus became a gold standard, defined following a RV biopsy or at autopsy.

Pathology and proposed pathophysiological mechanisms for ARVC

In the 24 patients originally described by Marcus (12) the pathophysiological findings were outlined. Marcus described the process as affecting the posterior and inferior areas of the RV inflow tract adjacent to the tricuspid valve, the anterior infundibulum and the apex, forming the 'triangle of dysplasia' (Figure 6 page 52).

Figure 6 Triangle of dysplasia[7]



The restriction of ARVC to the RV may be pathognomonic to many. Sole involvement has historically been assigned to the right free wall, sparing the septum (and the LV) (108, 109), with a loss of myocardium due to fibrofatty replacement leading to aneurysms (bulges) (24). In Marcus's original description, aneurysms were described in the triangle of dysplasia. Other pathology findings included a decrease in the number of myocardial fibres and their replacement by fat and fibre; white thick sclerotic endocardium in the areas of dysplasia, and hypertrophy of the few remaining myocytes.

[7] www.aafp.org/afp/20060415/1391.html

Much of our knowledge of the pathology of ARVC has come from Padua in Italy, due to the prevalence of ARVC in the Veneto region. The Italian group describe two types of ARVC based on pathology: the first a lipomatous pattern (infiltrative group) comprising mainly fatty tissue, restricted to the RV sparing the septum and the LV, and the second, a fibrolipomatous pattern (cardiomyopathic group), comprising fat and fibre, with parchment thin walls and translucent and sac like aneurysms at the apex, including the LV (108, 110, 111). It is likely that these descriptions will be modified following the determination of the genetic etiologies for ARVC.

Several mechanisms have been postulated for the pathological findings in ARVC. These include incremental apoptosis, leading to loss of normal cardiac myocardium; transdifferentiation (myocardial cells changing from muscle to fat and fibre), following an infectious or inflammatory pathological injury repair process. The obvious genetic heterogeneity in ARVC may mean that the pathology reflects different genetic subtypes of ARVC. Alternately the pathology may reflect different stages of a progressive disease process.

ARVC may be an injury repair process, where fat and fibre replace myocardium following myocyte degeneration and death (112). Two thirds of Italian cases, following autopsy or biopsy, exhibited inflammatory cell infiltrates (CD43 positive T lymphocytes) associated with a focal myoctye necrosis (112). The authors suggested that a recurrent myocarditis occurs, triggering electrical instability leading to SCD, or a progression to heart failure to mimic DCM (112). Similar features of repair and injury and an infectious inflammatory process have been described in a cat model of ARVC (113).

Whether viruses play a role in some forms of ARVC has been explored with both negative (114) and positive (115) results. In the latter, Bowles et al 2002 publication (115), where the role of viruses was implicated, all cases were considered to be sporadic based on the lack of a family history of cardiac disease or SCD, and following negative clinical screening of family members. The authors recognized that viruses may cause a diseased myocardium, or be a secondary effect in a diseased heart rendered susceptible to infection. That the latter is a possibility is supported by the observation of viruses in the myocardium of cardiac pathology specimens exhibiting signs of rejection from routine heart transplant biopsies (116). Dystrophin deficient mice have also developed enteroviral cardiomyopathy. This may be due to the disruption to the sarcolemma resulting in an increased susceptibility to the virus (117): a case where an underlying mutation predisposes to an infectious process.

Apoptosis has been documented many times in both ARVC post mortem specimens (118) (119) and biopsies (120) (121) (122) as well as *DSP2* mutation positive mice (73). However whether apoptosis is a primary cause, or a secondary response, to the disease process is unknown.

Fat in the heart: what is the relevance?

The question of whether fat in the myocardium is pathological or a natural secondary phenomenon has been raised (123) (124). Burke suggested that the site of the fat, rather than the gross amount, was the relevant issue, (125). Thus fat in the RV outflow tract was abnormal but the same amount in the anterior wall near the apex was not. Fat had also to be associated with 'significant' fibrosis for a diagnosis of ARVC to be made (125). The

Padua group have tried to quantify 'significant amount' and their pathological diagnosis was based on the relative quantities of fat and fibre (8), where fat and fibre at > 3% and > 45% respectively were considered pathognomonic for ARVC (8). Pathology is extremely important in the absence of a presymptomatic genetic test, so in many families a biopsy documenting pathological change may be the principal method of diagnosis of ARVC.

Diagnostic Criteria

In an attempt to create order from the diagnostic maelstrom of ARVC, in 1994 a consensus committee of leading cardiologists produced diagnostic criteria (25). Six major classes of anomalies were defined: dysfunction and structural abnormalities, histological changes, repolarisation and depolarisation abnormalities, arrhythmias and family history. Muliple features were defined for each class, separated into major and minor criteria, where 2 major or 1 major plus 2 minor, or 4 minor criteria were required for diagnosis (Table 3 page 57).

These criteria were based upon the experience of those on the committee and are thus the direct result of a selection bias, determined from patients with a disease severe enough (yet who were still alive) to bring them to medical attention. Those with mild disease may never present, and more severe cases may die before presenting, and the cause of death misclassified or misunderstood. How we diagnose and understand a disease thus takes on aspects which become almost philosophical in nature. This is exemplified by the example of cystic fibrosis (CF), which for years was diagnosed exclusively by clinical signs and symptoms (126). However, the determination of the causative gene (127) and the recognition that there are thousands of pathological mutations within the CF gene (allelic

heterogeneity) has resulted in an expansion of the clinical picture. In some cases the 'disease' expression is so mild as to be considered a normal variant rather than pathology (128) (129) (130). It is likely that this scenario will recur for many single gene disorders.

Table 3; Task Force of the Working Group Myocardial and Pericardial disease of the European Society of Cardiology and the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology diagnostic criteria (25)

	Major criteria	Minor criteria
Global and/or regional dysfunction and structural alterations	Severe dilatation and reduction of right ventricular ejection fraction with no (or only mild) LV impairment Localised right ventricular aneurysms (akinetic or dyskinetic areas with diastolic bulging) Severe segmental dilatation of the right ventricle	Mild global right ventricular dilatation and/or ejection fraction reduction with normal left ventricle Mild segmental dilatation of the right ventricle Regional right ventricular hypokinesia
Tissue characterization of walls	Fibrofatty replacement of myocardium on endomyocardial biopsy	
Repolarisation abnormalities		Inverted T waves in right precordial leads (V2 and V3) (people aged older than 12 years in the absence of right bundle branch block)
Depolarisation /conduction abnormalities	Epsilon waves or localized prolongation (>110ms) of the QRS complex in right precordial leads (V1-V3)	Late potentials (signal averaged ECG)
Arrhythmias		Left bundle branch block type ventricular tachycardia (sustained and non-sustained) (ECG, Holter, exercise testing) Frequent ventricular extrasystoles (more than 1000/24 hours on Holter)
Family History	Familial disease confirmed at necropsy or surgery	Family history of premature sudden death (< 35 years) due to suspected ARVC Family history (clinical diagnosis based on present criteria)

2 major or 1 major plus 2 minor, or 4 minor criteria required for diagnosis

The early report on 24 patients with ARVC suffered an ascertainment bias (a complication present in virtually all reports), focusing on cases with a severe phenotype (12). All patients had a dilated RV, sustained VT and LBBB. The conclusion was that this was a rare, severe, disorder. Over time, the spectrum of ARVC expanded, where less severe cases were recognised (19) and where the prevalence of SCD, and the relevant histopathology was noted (44). With time, it became clearer that many cases exhibited biventricular disease, with some exhibiting mainly LV disease(131-141), and the spectrum became wider.

The clinical diagnostic criteria therefore suffer the same 'severe case' bias. This becomes a concern when relatives are assessed, who despite manifesting minor clinical features, do not fulfill the defined criteria. This issue was addressed in 2002 by Hamid and Dr. McKenna's London group of researchers, who found that the accepted criteria were insufficient to make a diagnosis of ARVC in a subject with a definitely affected relative (142). This team modified the criteria to require fewer clinical features for diagnosis within a known family. These families were not defined by an underlying ARVC mutation; rather they were defined by their clinical presentation. The relatives thus had cardiac testing in order to look for cardiac clinical signs which might be considered part of the diagnostic spectrum. Despite the fact that 21% of affected relatives had LV involvement, and 3% fulfilled a DCM diagnosis, the modified criteria did not add LV findings to the modified listing (Table 4 page 59).

Table 4 Modification of original task force diagnostic criteria (142).

ARVC in first degree relative plus one of the following	
ECG	T wave inversion in right precordial leads (V2 and V3)
Signal averaged ECG (SAECG)	Late potentials seen on SAECG
Arrhythmia	LBBB type VT on ECG, Holter monitor or during exercise testing > 200 PVCs over a 24 hour period
Structural or functional abnormality of the RV	Mild global RV dilatation and/or reduced ejection fraction with normal LV. Mild segmental dilatation of the RV Regional RV hypokinesis

The London group has recently assessed the utility of the original and the modified criteria (61). They looked at family members with *DSG2* mutations and showed that the penetrance of the disease was 58% if the original Task Force criteria were used (25), and 75% using the modified criteria (142). Of interest in this paper was the absence of RV anomalies in 34% of gene carriers: 25% of whom had LV involvement, and in whom 74% did not show the classic T wave inversion. The phenotype of ARVD5 presented in this thesis supports the need to widen the diagnostic criteria even further.

Diagnostic Criteria developed for ARVD5

The diagnostic criteria as defined and discussed above cannot be used for a large epidemiological study as presented in this thesis. The pedigree structures of the families have SCD as a presenting feature, with an autosomal dominant mode of inheritance. Whilst autopsy may have defined ARVC in several deceased individuals, an autopsy diagnosis alone could not diagnose ARVC based on the original criteria (25).

Additionally, the tertiary nature of the clinical testing would minimise the ability to define affected individuals. We therefore created our own straightforward diagnostic criteria, which utilised even minimal information.

Table 5 The ARVD5 epidemiological diagnostic criteria

All subjects at a priori 50% birth risk ascertained Disease Status	**Definition**
Affected	1. Obligate carrier. 2. SCD under 50 years. 3. Cardioversion for VT or VF under 50 years. 4. Genetic diagnosis: high-risk DNA haplotype or gene mutation positive.
Unaffected	1. Low risk DNA haplotype or gene mutation negative.
Unknown	1. Did not fulfil criteria as set out above.

1.4 VARIABLE EXPRESSION AND REDUCED PENETRANCE

All autosomal dominant disorders are variably expressive; some have reduced penetrance. Variable expressivity describes the tendency for a disease to affect related individuals in different ways: thus the disease does not necessarily run 'true' within a family, or between families with the same mutation. Reduced penetrance is the extreme form of variable expression: where the presence of the gene does not always result in manifestation of the full disease. A non penetrant disease is one where no sign of the disease is present (143). Diagnosis relies upon finding clinical features that are associated with disease. The move towards modified criteria for ARVC (142), and the recognition that gene carriers manifest a varied phenotype (61) speak to a growing recognition within

cardiology of the variability in disease presentation. In genetic disorders, variable expression is ubiquitous: ARVD5 is no exception.

1.5 DIFFERENTIAL DIAGNOSES FOR ARVC

Adipositas cordis This rare disorder is characterized by fatty infiltration of myofibers, and often causes restrictive cardiomyopathy (144). The fatty infiltration may be confused with ARVC.

Right ventricular outflow tract (RVOT) disorder. This is a primary disorder of the outflow tract of the RV, presenting usually with VT. It is considered to be benign and non-familial. It usually responds to beta blockers and calcium channel blockers and is considered distinct from ARVC (145).

Uhl's anomaly (146) This presents with a paper thin RV free wall that is almost transparent. It is a rare lethal condition that presents during infancy and childhood leading to congestive heart failure and death. Although named after Uhl, it was originally recognised by Osler who described a 'parchment' heart in 1905 (147). ARVC was (and sometimes still is) considered part of Uhl's anomaly although it is usually now considered a separate entity (148-150). Supporting this conclusion is the lack of evidence that Uhl's anomaly is familial (145, 151).

Chagas disease (152) Chagas' disease is a human tropical parasitic disease caused by the flagellate protozoan *trypanosoma cruzi* transmitted primarily by insect bite. The chronic stage may occur decades after initial infection, and can cause cardiomyopathy which can be mistaken for ARVC. Apoptosis has been recognised in this disease (153).

Viral cardiomyopathies: Multiple viral infections that have been considered part of the differential diagnosis of ARVC including Enterovirus infection (154) and Bartonella infection (155).

1.5.1 Prevalence and incidence

Prevalence and incidence are measures of the frequency of disease events in a population. Prevalence is derived by counting 'cases' at a single point in time. Incidence is derived by counting cases over a specified period of time: both require a definitive diagnosis. As ARVC is difficult to clinically diagnose, incidence and prevalence figures are unreliable (156). The prevalence in Italy is quoted as 1/5000 accounting for 20% of deaths in young adults (110). A higher frequency is noted in Padua, Northern Italy where an incidence of 6/10,000 is quoted (31). Rampazzo recognised that this figure had limitations as they had actively searched for cases with ARVC as part of a research project, yet they may still have missed prodromal forms of the disease, yet in one small geographic area the incidence was believed as high as 4.4/1000 (31). The incidence remains essentially unknown in the USA. One study reported a frequency at autopsy of 0.55% among young adults following SCD in Maryland (157), and 17% of sudden death victims between 20 and 40 years of age were diagnosed with ARVC in a subsequent study (158). In France, 18/50 cases of sudden perioperative deaths were considered to have ARVC (159). There are those who consider that this disease is both under diagnosed (because of reduced penetrance, biased ascertainment, and because family history is not present) (160) and those who think that it is often over diagnosed, particularly with newer imaging techniques (161, 162). Issues of diagnosis make incidence and prevalence difficult to quantify: a situation which will hopefully be rectified with the more widespread use of

genetic testing. Whatever the true incidence and prevalence, ARVC has been reported worldwide (163) (164) (165) (166).

1.6 NATURAL HISTORY

The natural history of disease is the clinical presentation from birth to death. In ARVC this has historically been subdivided into four discrete stages (156). The first is a 'concealed' phase, with subtle RV changes, where SCD may present catastrophically and unexpectedly. The second is an 'overt electrical disorder' phase, with RV arrhythmias and clear RV manifestations. The third is a RV failure stage, with preserved LV function. The final stage is biventricular pump failure, with significant LV involvement that mimics other causes of DCM. These stages are necessarily descriptive, and suffer major biases. Studies are scarce. Most have involved small genetically heterogeneous groups (multiple genetic causes) and/or groups of subjects with variations in presentation, family history, causes of death and rates of death (167-169). Most natural history data comes from Italy (103, 108) (170), is descriptive, and uses etiologically heterogenous ARVC subjects. Comparing heterogeneous groups with ARVC will miss differences due to the differing genetic (and possibly environmental) causes. Using the homogeneous group with ARVD5 in Newfoundland has allowed us to define in a robust manner the natural history of this genetic subtype.

Cardiac Tests

Many cardiac clinical tests are used to diagnose ARVC (Appendix C page 284). Their utility in the ARVD5 population is presented in chapter 3 page 105.

Electrocardiography

ECG abnormalities considered part of the ARVC spectrum (25) include T wave (Figure 5 page 50), and epsilon waves (Figure 4 page 49) indicating right ventricular late potentials (171) detected by running the ECG at double speed and amplitude using a 40 hz filter (172). Extended QRS duration on 12 lead ECG in V1, V2 or V3 is also part of the ARVC diagnostic criteria (25, 173) (Table 3 page 57).

The benefit of the ECG is that it is easy to apply, inexpensive and ubiquitous compared with many other cardiac tests that are only available at tertiary health care centres. It has been recognised as a valuable first test for the clinical detection of ARVC, with lengthened QRS duration in the right precordial leads (V1-V3) present in 98% of clinically diagnosed ARVC patients (174).

The signal-averaged ECG (SAECG)

The first use of SAECG in ARVC was in the late 1980's (175), when diagnostic criteria were assessed and the test considered diagnostically useful (176). However, the problem of assessing utility in individuals already diagnosed by another method (in this paper by angiography (176)) remains methodologically problematic.

The Holter monitor

The Holter monitor has been used to document ectopy in those with ARVC from the 1980s. It has also been used to assess heart rate variability and diurnal variations in arrhythmia activity (177). It is considered the most sensitive test for determining ARVC in Boxer dogs (178).

Echocardiography

Echocardiography provides important diagnostic information with which to diagnose ARVC (179) (180) (181) (182). Its value is constantly being reassessed. For example, a recent study compared ARVC probands with matched controls (by age, sex, body size and year of echo) (183). The enlarged right sided heart structures were 'obvious' on echocardiography, but as the subjects were already diagnosed by standard criteria (25), a major ascertainment bias existed. Echocardiography has been used by some as a gold standard for diagnosing ARVC (184) correlating well with MRI findings in patients for RV measurements, volumes and ejection fractions (185). As newer techniques evolve (e.g. real-time 3 dimensional echocardiography (186) and Doppler tissue imaging) and evaluated in ARVC (140) (187, 188) echocardiography may become even more useful. Like the ECG, echocardiography is available in many non-tertiary centres. Unlike the ECG, it is operator and analyser dependent. As the phenotype of ARVC expands and as patients are followed prospectively, heart failure is one outcome that is readily diagnosed by echocardiography (104).

Cardiac Biopsy

Cardiac biopsies remove cardiac tissue in vivo, and the tissue is assessed for the presence of fat and fibre. The procedure carries a risk of serious sequalea[8]. It is dependent upon the location of the tissue sample, as a negative biopsy (an absence of fat and fibre) may rule out ARVC, or, more problematically, may indicate that the sample was taken from an unaffected area of the myocardium. Of serious concern is ventricular perforation, which

[8] http://www.health.qld.gov.au/informedconsent/ConsentForms/cardiac/cardiac_08.pdf.

may occur in thin and friable ventricular free walls (111). Genetic testing would overcome these limitations.

Magnetic Resonance Imaging (MRI)

The first use of nuclear MRI in humans was as recent as 1977 (189), and it has overtaken cardiac biopsy as the diagnostic tool of choice for diagnosing ARVC by assessing the presence of fat and fibre in the myocardium. This is despite that when MRI diagnosis was compared with histopathological biopsy diagnosis, the latter was considered more sensitive (190). However, MRI is a much less invasive clinical test. MRI differentiates fat from fibre through signal density compared with normal myocardium, as the former increases signal density (the image is brighter) and the latter decreases signal density. A more recent MRI technique uses contrast to better visualise fibre (191) (192). The eventual reliability of MRI in terms of diagnostic accuracy remains to be determined (193) (161). MRI as a technique is restricted to tertiary centres. The use of MRI in Newfoundland for ARVC diagnosis was terminated for ARVD5 following the use of the 3p25 DNA haplotype.

1.6.1 Left ventricular enlargement in ARVC and overlap with DCM

Early descriptions of ARVC recognised that the LV could be affected in the same way as the RV (131, 133, 134, 194, 195), and decades of evidence suggests that ARVC is a biventricular disease (131-141, 196-198) that may mimic DCM (44) (199) with the involvement of the LV reflecting disease progression. Forty-two hearts from autopsy and transplant ARVC patients showed histological involvement of the LV 76% of the time (199), and five of the seven individuals affected in the four families with ARVD10

(desmoglein mutations) (61) had evidence of significant LV involvement that was more obvious than the RV disease in two individuals.

DCM is a cardiomyopathy where the cardiac wall is the correct size, but the internal dimensions are enlarged. Mitral regurgitation occurs and the heart muscle becomes thin and floppy, with systolic dysfunction and eventual heart failure. There are many possible causes of DCM including hypertension, coronary artery disease, valvular disease, viral infections (e.g. cocsackie B virus), alcohol abuse, drugs (particularly some chemotherapies), hemochromatosis, sarcoidosis, amyloidosis, mitochondrial disorders, some inborn errors of metabolism and pregnancy (which may unmask a genetic type or be pregnancy related). Familial DCM requires the presence of other family members with DCM or SCD less than 35 years (200).

Like ARVC, there are descriptive criteria to diagnose DCM (15, 201) (200). The presence of an ejection fraction (the fraction of blood pumped out of a ventricle with each heart beat) less than 45%, or fractional shortening (FS) less than 25% where FS is defined as left ventricular end diastolic diameter (LVEDD) minus left ventricular end systolic diameter (LVESD) divided by LVEDD, and LVEDD > 117% indexed to height and weight (202, 203) makes the diagnosis. Like the alternate criteria determined for ARVC (142), it is recognised that relatives of probands with DCM may variably express disease, so major and minor criteria for assessment of familial DCM have been proposed (204, 205).

There are an ever increasing number of genetic causes of DCM. They include sarcomere genes, known to cause hypertrophic cardiomyopathy (HCM), and genes coding for

cytoskeletal proteins (206). To date, the only overlapping gene with mutations shown to cause both a DCM and ARVC phenotype is desmoplakin which is implicated in both autosomal recessive DCM (Carvajal syndrome) and ARVD9.

1.6.2 Treatment of ARVC

Disconnection of the right ventricular free wall (RVFW)

Early treatment for ARVC involved surgical disconnection of the RV free wall (RVFW) from the LV, separating the electrical activity of the two lower heart chambers. Theoretically VT arising from the RV would then be restricted to the RV. The RV flow of blood was apparently maintained by the contraction of the right atrium and the movement of the septum towards the RVFW during LV contraction. This operation in humans was first done in the early 1980's (41), and a patient in this early publication (case 1) was a member of family AR1 (Figure 8 page 79). This early paper reported positive postoperative recovery and hemodynamic function after 4 months of follow up. Multiple animal studies followed which showed that although the procedure worked, hemodynamic compromise often occurred because of a lack of contractility of the RV resulting in RV failure. Although a rare form of treatment now, it is occasionally still used, alongside adjunct therapy of RV pacing to maintain RV contractility (207, 208).

Antiarrhythmic drug therapy

Antiarrhythmic drugs are placed into one of five classes based on the primary mechanism of their antiarrhythmic effect (209). This is a limited classification, as many agents have more than one primary mechanism and the metabolites of several drugs themselves have an antiarrhythmic effect which would place them in another class. The most common

antiarrhythmic drugs for ARVC are amiodorone and sotolol, (210-212) both class III agents acting primarily as potassium channel blockers prolonging the cardiac action potential. Amiodarone is the drug of choice for reducing the frequency of fatal arrhythmias. It has significant side effects, including hypothyroidism, liver problems (including jaundice and hepatitis), a bluish tinge to the skin (the 'smurf syndrome') and interstitial lung disease. Sotalol has additional beta blocking properties (slowing down the heart rhythm by acting upon the β adrenergic receptors in the heart). One of the most serious side effects to Sotolol is the proarrythmic response to the drug in some individuals. Procinamide (class 1a) which acts on sodium channels is occasionally used.

Catheter ablation

ARVC may be suitable for an ablation procedure: a decision most often made during an electrophysiological study (where catheters are introduced into the heart to map abnormal electrical circuits) (213). Ablation therapy destroys areas of re-entry in the myocardium that lead to VT by burning the foci of re-entry away. This is done via a catheter which burns away the foci, creating a myocardial scar disrupting the abnormal circuit. Typically this is reserved for either VT in a structurally normal heart or for a patient with frequent episodes refractory to drug therapy. It was treatment with a catheter ablation, and mapping of the myocardium in the 1970's that first brought ARVC to the attention of French cardiologists (214). Ablation continues to be used in ARVC (215), and may be considered as a possible ICD alternative (216).

Implantable Cardioverter Defibrillator (ICD)

Pharmacological treatment for serious arrhythmias has, since the 1990's, been supplanted by ICD therapy. The ICD is a battery-operated system that recognizes VT and VF and terminates them by synchronized counter shocks, thus preventing SCD. The ICD can also pace abnormal heart rhythms back to sinus rhythm.

The first cardiac defibrillator was implanted in Seattle in 1980 (217), and until 1985 (when the US Food and Drug Administration approved commercial devices), ICDs were limited to patients following cardiac arrest due to VF. The early implants were cumbersome, abdominally placed and had wires running through the diaphragm leading to external patches on the heart (218). In the 1990's ICDs evolved to incorporate pacing and Holter functions, and were placed pectorally (219). ICD sizes have decreased until now they are smaller than a pager. The recognition of major side effects following anti-arrhythmic therapy (220) (221), and the randomized studies comparing anti-arrhythmic medication with the ICD has meant that the ICD is the standard of care for high-risk arrhythmia patients (222). The randomised trials included both primary (assessment of those at high risk of MI or death from cardiac compromise before the first MI) (223, 224) and secondary (assessment of those given drug or ICD therapy following a previous MI) (225-228) studies. ICD was also found to be effective in those at risk for SCD due to HCM (229).

Rare problems occur with ICDs, including device failure issues, infections (230), surgical complications (231) and intractable numbers of shocks (232). Although usually discounted, the possibility that the ICD itself is pro-arrhythmic has been raised (233). The

mortality rate in the initial patients implanted (a very high-risk group) was 2% per annum (234). In the series studying HCM, the mortality rate post ICD was 1% per annum (229).

Few studies have assessed the efficacy of ICD therapy in ARVC (235) (236-238). In an Italian series (238), a heterogeneous group of 132 patients was described, but the absence of a control group restricted the conclusions. The same problem occurred with a more recent report on ICDs in ARVC, where frequency of shocks and survival was assessed (239). The most recent paper was descriptive and detailed 15 ARVC patients matched to patients with coronary artery disease, and again lacked a disease based control group (240). The efficacy of ICD for ARVD5, utilising a control group is presented in chapter 4 page 139.

Genetics and ethics

Genetic information burgeoned following the re-discovery of Mendel's laws of segregation in the early 20th century. Progress was fast, with the first human gene to be assigned to a locus occurring in 1911 (colour blindness on the X chromosome) (241). The determination of gene loci accelerated following the advent of recombinant DNA technology in the 1970's and over the last 40 years, thousands of human genes have been mapped and cloned[9]. Alongside the genetic information have been parallel ethical issues, exemplified in the early 20thcentury in the USA by the Cold Spring Harbour Eugenics Office mirrored by eugenics issues in Canada and Europe, culminating in the Nazi atrocities of WWII. Following WWII, the Nuremberg laws were enacted to protect research subjects, in all biomedical research (242). Our current ethical disquiet over

[9] www.ncbi.nlm.nih.gov/sites/entrez?db=omim

genetics has its roots in the old eugenics horrors, and in the perceived tension between the needs of researchers and the clinical needs of individuals.

As the scientific part of this thesis was being conducted, several ethical issues arose which reflect some of these historic situations. I present those in chapters 5 and 6, but do not provide in this introduction an exhaustive literature review of the subject matter. These chapters are added as additional information, tangential to the thrust of the thesis, but hopefully interesting and thought provoking.

Thus the thesis is separated into seven chapters. Chapters one and seven are the broad introduction and conclusion for the body of work contained in the five middle chapters. It should be noted that the manuscript nature of this thesis means that each chapter contains its own introduction and discussion.

1.7 CO-AUTHORSHIP STATEMENT

Design and identification of research proposal

The design of the clinical and genetic epidemiology sections of the research proposal was by Kathy Hodgkinson, except for the gene discovery portion of chapter 2.

Practical aspects of the research

Kathy Hodgkinson collected all the clinical and genetic epidemiology data, and the majority of the blood samples for DNA analysis. The DNA analysis in chapter 2 was done by members of Terry Young's laboratory.

Data analysis

The author analysed all the clinical epidemiology data. The molecular data in chapter 2 was analysed by members of Terry Young's laboratory.

Manuscript preparation

The clinical and genetic epidemiology sections of chapter 2 were authored by Kathy Hodgkinson. The molecular genetic sections were authored by Terry Young.

Chapters 5 and 6 were co-authored with Daryl Pullman.

Chapters 1, 3, 4, and 7 were authored by Kathy Hodgkinson

2 ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY TYPE 5 (*ARVD5*) IS A FULLY PENETRANT, LETHAL ARRHYTHMIC DISORDER CAUSED BY AN AMINO ACID SUBSTITUTION IN THE NOVEL *TMEM43* GENE

"Each success only buys an admission ticket to a more difficult problem."

Henry Kissinger

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2.1 ABSTRACT

Autosomal dominant arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) causes sudden cardiac death and is characterized by clinical and genetic heterogeneity. Fifteen unrelated ARVC families with a disease associated haplotype on chromosome 3p (*ARVD5*) were ascertained from a genetically isolated population. Identification of key recombination events reduced the disease region to a 2.36 Mb interval containing 20 annotated genes. Bi-directional resequencing showed one rare variant in *transmembrane protein 43* (*TMEM43* 1073 C>T, S358L), was carried on all recombinant *ARVD5* ancestral haplotypes from affected subjects and not found in population controls. The mutation occurs in a highly conserved transmembrane domain of TMEM43 and is predicted to be deleterious. Clinical outcomes in 257 affected and 151 unaffected subjects were compared, and penetrance determined. We concluded that ARVC at locus *ARVD5* is a lethal, fully penetrant, sex-influenced, morbid disorder. Median life expectancy was 41 years in affected males compared to 71 years in affected females (relative risk 6.8, 95% CI 1.3-10.9). Heart failure was a late manifestation in survivors. Although little is known about the function of the *TMEM43* gene, it contains a response element for PPARγ (an adipogenic transcription factor), which may explain the fibrofatty replacement of the myocardium, a characteristic pathological finding in ARVC.

2.2 INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D [MIM 107970]) is an inherited disorder, often involving both ventricles, and is characterized by ventricular tachycardia (VT), heart failure, sudden cardiac death (SCD) and fibrofatty replacement of cardiomyocytes (151). It is usually inherited as an autosomal dominant disorder, although recessive forms exist (28). To date, eleven genetic loci have been mapped, and seven genes identified (60, 63, 74, 243, 244). Five genes code for desmosomal proteins (desmoplakin [MIM 125647], plakophilin-2 [MIM 602861], desmoglein-2 [MIM 125671], desmocollin-2 [MIM 125645] and plakoglobin [MIM 173325]) that are predicted to succumb to mechanical stress (74, 243). The remaining two genes are *cardiac ryanadine receptor 2* (*RYR2* [MIM 180902]) (36) and *transforming growth factor beta-3* (*TGFβ3* [MIM 190230]) (33).

Clinical diagnosis of ARVC is difficult as it relies on physiological and pathological testing for the presence of right ventricular structural and functional anomalies, fibrofatty replacement of the ventricular myocardium, premature ventricular contractions (PVC's) on Holter monitor, extended QRS, epsilon waves and T wave inversion on 12 lead ECG and late potentials on signal averaged ECG (SAECG) (25). Phenotypic variation of ARVC, in both presentation and clinical course, has led to a modification of the McKenna diagnostic criteria (25) to facilitate the identification of relatives at risk in ARVC families (142). ARVC can have both adverse (sudden death and heart failure) (245), and favourable outcomes (246), which may be due to genetic heterogeneity, although clearly mutations at different loci do not explain intrafamilial variation (247) or

the sex-influence towards greater clinical severity in males (167). There are no known studies to date that assess clinical features in mutation-negative subjects born at *a-priori* 50% pedigree risk to determine mutation specific cardiac features: most studies assess small families to determine cardiac features, and gene specific (not mutation specific) penetrance (61). Ultimately, the determination of the penetrance of mutation specific clinical manifestations of ARVC will provide accurate, mutation specific, clinical information for appropriate genetic counselling and treatment options.

The *ARVD5* locus (MIM 604400) on 3p was mapped in an extended 8 generation family from the genetically isolated population of the island of Newfoundland, Canada (4). This family was first identified in the 1980's (3) and at least one family relative participated in right ventricular disconnection studies, as a possible treatment for ARVC (41). We have identified 14 additional families with Newfoundland ancestry that share the *ARVD5* haplotype and have previously shown that the use of prophylactic treatment with implantable cardioverter defibrillator (ICD) therapy in relatives at risk of ARVC greatly improved survival (248). We report here that we have identified the *ARVD5* disease gene and determined the mutation specific penetrance of its major clinical manifestations.

2.3 MATERIALS AND METHODS

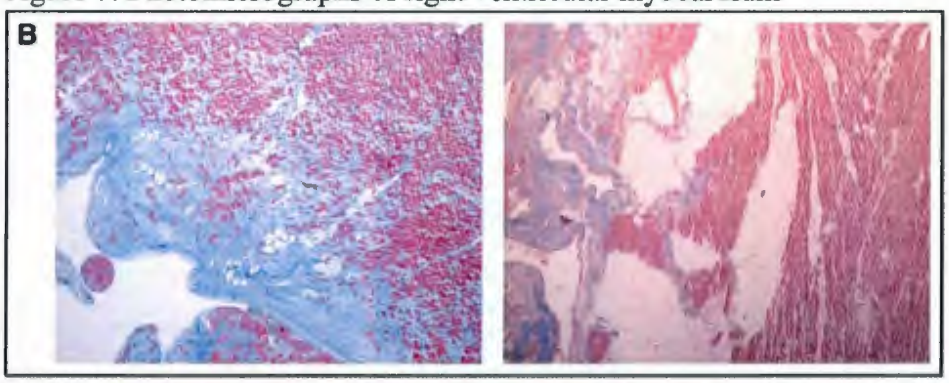
2.3.1 Study population

Fifteen of 150 apparently unrelated families referred either to the Newfoundland Provincial Medical Genetics Program, or the Newfoundland Labrador genetics cardiomyopathy clinic because of a family history of cardiomyopathy and sudden death were included int his study. These families were determined to have ARVC based on

clinical testing using established criteria (25), post mortem pathology (Figure 7 page 78) and an autosomal dominant pattern of inheritance (Figure 8 page 79). These families are characterized by deep genealogies, a Newfoundland ancestry and a disease associated haplotype originally identified in family AR1 used to map *ARVD5* (Figure 8 page 79).

A total of 496 subjects born at *a priori* 50% risk (clinically affected and their first-degree relatives) were available for study across the 15 families (Figure 8 page 79). Subjects were determined to be clinically affected if they fulfilled the ARVC modified criteria (142). Blood samples from 295 subjects born at *a priori* 50% risk were collected and informed consent obtained in compliance with the Human Investigation Committee requirements of the Eastern Health Corporation of St. John's, Newfoundland, Canada (study number 00-176).

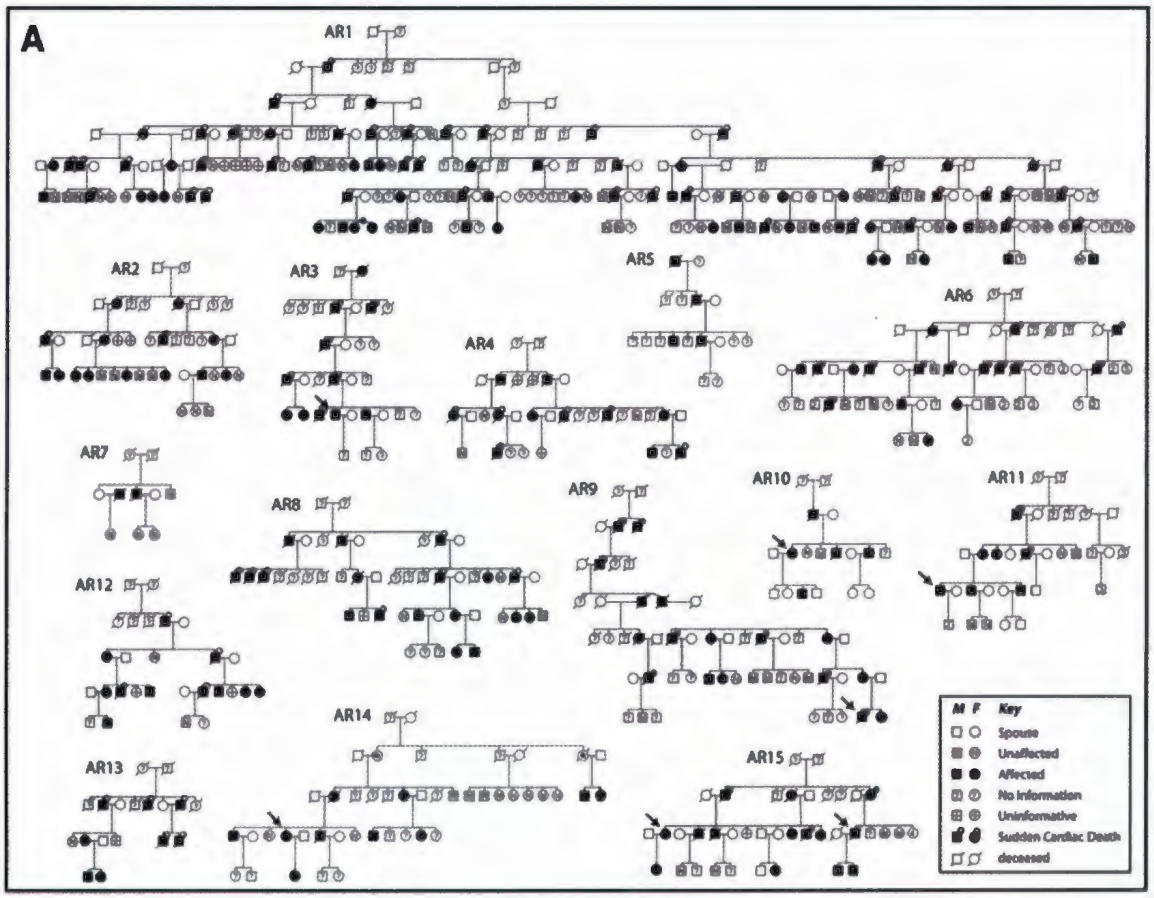
Figure 7: Photomicrographs of right ventricular myocardium



Paraffin-embedded post mortem right ventricular myocardium stained with masson trichrome showing fibrofatty replacement of myocytes from a male teenager following sudden cardiac death (left: 40x magnification) and a second degree relative with sudden cardiac death in his 8th decade (right: 20x magnification). Pink = normal myocardium, blue = fibre, white = fat.

Figure 8: ARVC families linked to chromosome 3p

Pedigrees of 15 autosomal dominant ARVC families linked to *ARVD5*. Affected subjects are shown as blackened squares (male) and circles (female). Subjects deceased due to SCD are noted by a circle above the symbol.



Fine mapping of the *ARVD5* locus

Mapping the *ARVD5* locus in family AR1 (Figure 7 page 78) previously defined a 9.3 cM disease region between markers *D3S3610-D3S3659* (4). Eighteen polymorphic microsatellite markers were genotyped on clinically affected subjects across all families to identify a disease associated haplotype at 3p25 that was shared amongst families from this genetic isolate and presumed to be ancestral. We identified key recombination events on the *ARVD5* ancestral haplotype and recombinations seen in two or more families were used to narrow the region.

Screening candidate *ARVD5* genes

A mutation-screening panel was established that comprised seven genomic DNA samples from four clinically affected subjects from three families (AR1, AR8, AR15; Figure 8 page 79) and three spouses (controls). All coding and non-coding exons and intron-exon boundaries of positional candidate genes for *ARVD5* were sequenced. All sequences were amplified by polymerase-chain reaction (PCR) assay from genomic DNA in 25 µl reaction volume. Primer sequences are available from the authors on request. The PCR products were purified using 50% sephacryl (Amersham Biosciences) and MultiScreen HTS filter plates (Millipore Corporation). Purified PCR products were cycle sequenced in both forward and reverse directions with the use of BigDye Terminator V3.1 cycle sequencing kit on an automated ABI 3700 DNA analyzer (Applied Biosystems). Sequencing electropherograms were inspected manually and analyzed with Mutation Surveyor software (Transition Technologies). Sequencing variants found exclusively in clinically affected subjects on the mutation-screening panel were experimentally determined to reside on the *ARVD5* haplotype by segregation analysis in family AR14 (Figure 8 page 79). The allele frequencies of the *ARVD5* sequencing variants were determined using Newfoundland population-based controls obtained through random phone dialling, as part of a large colorectal cancer study (249). Newfoundland is a known genetic isolate where 98% of its residents are of English and Irish descent (250, 251). Key recombinant families (AR2 and AR10) were analyzed to determine which rare variants (<1% of the alleles screened) were retained on recombinant ancestral haplotypes in clinically affected subjects.

Expression of *TMEM43* in myocardium and lymphocytes of clinically affected subjects

Total RNA was extracted from Epstein Barr virus (EBV) transformed B lymphocytes from two affected subjects and one unaffected control and cardiac tissue from one affected subject and an unrelated control using Trizol (Invitrogen) followed by DNase1 treatment (Ambion). Complementary synthesis was performed and analyzed by both size fractionation and direct sequencing with over-lapping primers designed to cover the complete coding sequence of *TMEM43*.

Bioinformatic Analysis

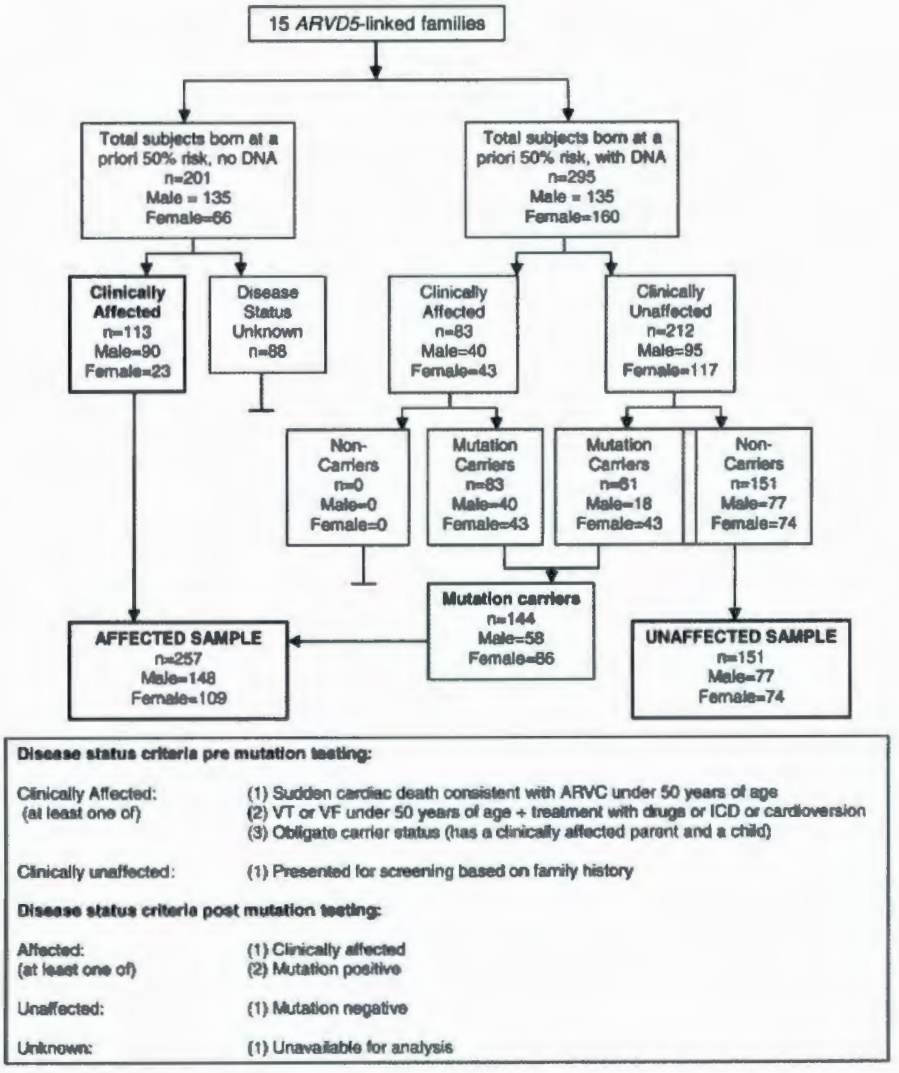
Conservation of the TMEM43 protein across species was determined using ClustalW and Weblogo (252-254). Potential protein localization, function, structure and post-translational modification sites were predicted using the online tools via the ExPASy web site (254). The effects of amino acid substitutions on protein function were predicted (255-259).

Clinical assessment of affected vs. unaffected subjects

Clinical data were prospectively collected over eleven years. This involved annual visits by subjects to the genetics cardiomyopathy clinic where 12 lead ECGs, Holter monitors, MRI's, signal averaged ECG's and echocardiograms were done. All cardiac anomalies were noted following clinical testing. Clinical data were also obtained retrospectively from medical records including 'at risk' relatives not seen in clinic and autopsy results. Subjects were categorized as affected, unaffected or unknown (Figure 9 page 83). Only subjects from well ascertained sibships (where disease status was known in ≥50% of siblings) were included in this study.

Anomalies on 12 lead ECGs were determined by two physicians blind to disease status. Left ventricular enlargement (LVE) was defined as ≥2 standard deviations (SD) above a predicted mean: left ventricular end diastolic diameter (LVEDD)>112% (>2SD) (202). Late potentials on SAECG were classified based on recognized criteria: QRS (filtered QRS duration)>114 ms, LAS (low amplitude signals)>38 ms, and RMS (root mean square voltage of the terminal 40ms of filtered QRS)<20 ms (260). The presence of arrhythmias in the form of PVCs was determined from Holter monitor analysis. Heart failure was classified according to the New York Heart Association (NYHA) Functional Classification (261). Subjects were identified with heart failure (NYHA categories 1-4) if they presented prospectively in the study with heart failure, or it was documented in a medical record. Date of death was confirmed via autopsy records and archival records.

Figure 9: Workflow and mutation status of subjects born at a priori 50% risk of ARVC.



Penetrance is defined as the proportion of subjects with a specified genotype known to cause a disease who have any signs or symptoms of the disease (262). To determine the disease penetrance of ARVC at ARVD5, a subset of disease features based on the ARVC modified diagnostic criteria (142) were assessed from the first available clinical test result to show an anomaly to determine which clinical features truly segregated with affection status in this population. A Kaplan Meier analysis was then done using a subset of

subjects alive at the start of the prospective study (1996), who had an available medical record, and who were either mutation positive or an obligate carrier (60 males and 77 females). Based on the analysis of prevalent disease features, a male subject was considered 'penetrant' at the age when any one of the following clinical events occurred: (i) SCD (ii) VT on clinical testing (iii) heart failure (iv) >200 PVC's in a 24 hour period (v) LVE>2SD (vi) QRS>110 ms on 12 lead ECG or (vii) any late potential on SAECG (142). Female subjects were considered penetrant for all these features apart from QRS>110 ms on 12 lead ECG, and any late potential on SAECG. We also determined the penetrance of two major morbid outcomes of the disease, death and heart failure, using both the prospective and retrospective data set (n=257, 148 males, 109 females).

Cardiac tests were not available in all subjects at *a priori* 50% risk, mainly due to SCD as a presenting feature for many family members. For example, of 114 affected subjects who did not have an available echocardiogram, 86 were deceased (75%) whereas only 2 were dead in 50 unaffected subjects with no echocardiogram. Testing is in progress in this latter group.

Statistical Analysis

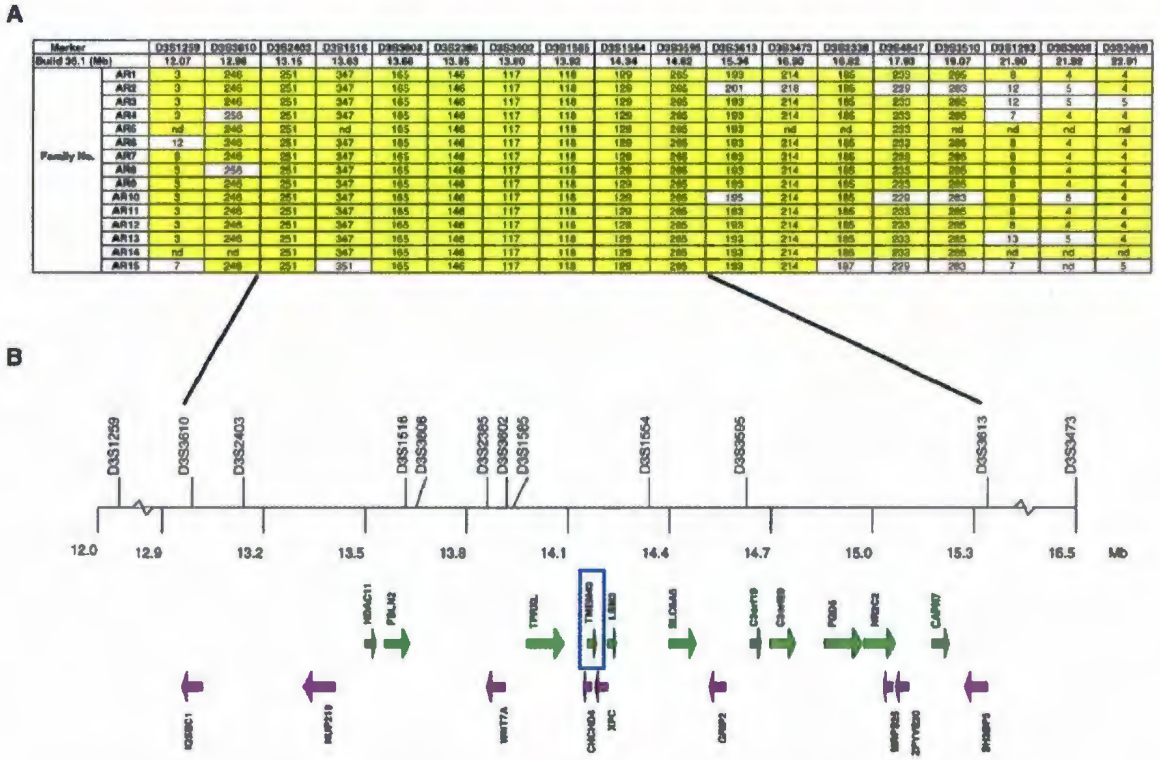
Comparisons between affected versus unaffected subjects were calculated by the Kaplan Meier product limit method with censoring occurring at the time of ICD therapy, heart transplantation or last follow-up (defined as the age at the last clinic visit). Relative risk was calculated using Cox's Regression model. A p-value of <0.05 was considered significant (SPSS software, version 14, Chicago USA). Documentation of the statistical methods utilised throughout this thesis are described in detail in Appendix C page 284.

2.4 RESULTS

Fine mapping and mutation screening/gene sequencing

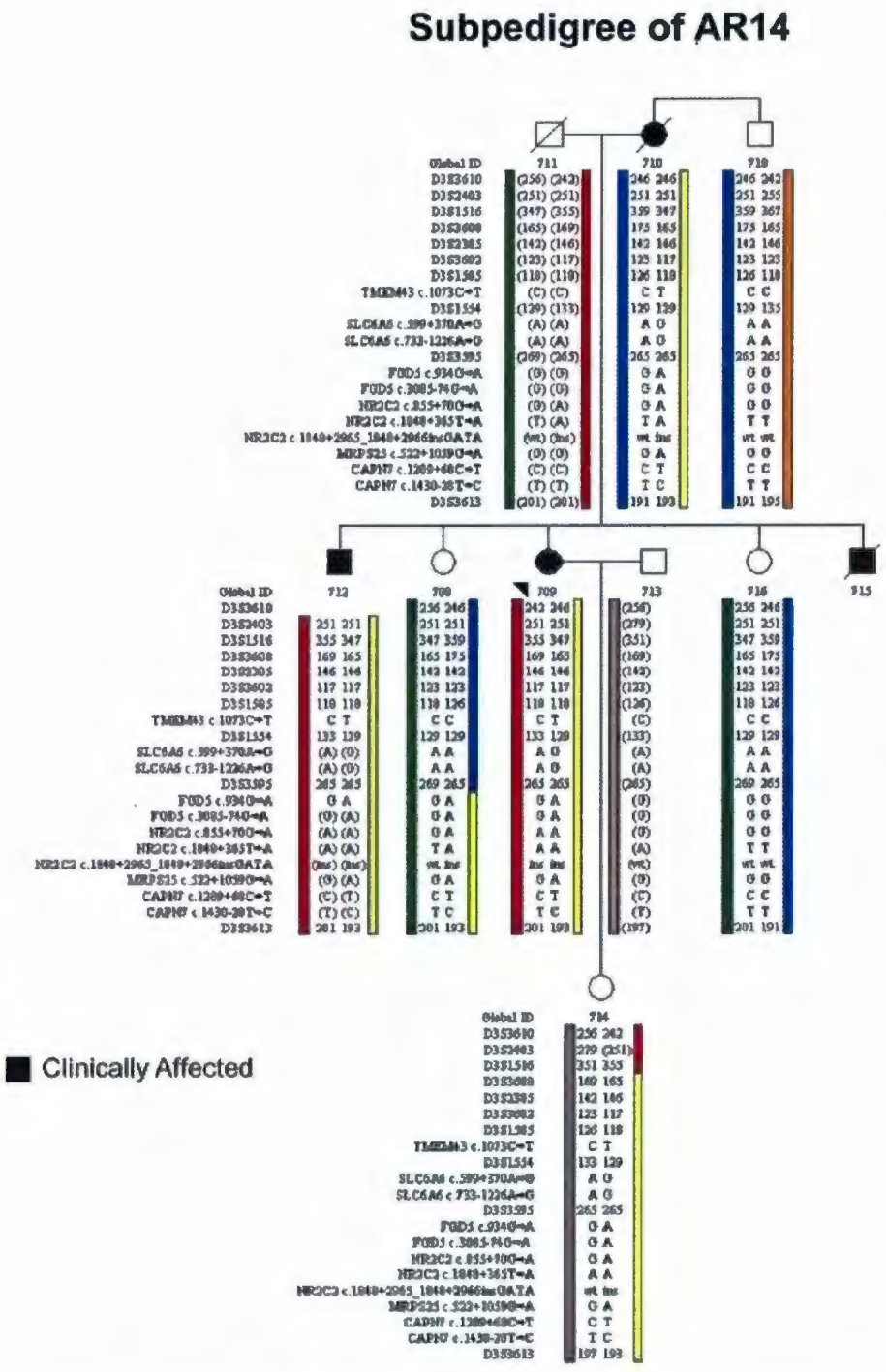
Comparison of the *ARVD5* ancestral haplotypes at 3p25 across clinically affected subjects identified key recombinations and reduced the disease region to a 2.36 Mb interval containing 20 annotated genes (Figure 10 page 86, Table 6 page 89). Bi-directional resequencing of the 20 physical candidate *ARVD5* genes revealed 240 variants (Table 6 page 89). Nineteen variants were found exclusively in clinically affected subjects on the mutation screening panel and 11 were determined to reside on the *ARVD5* ancestral haplotype through segregation analysis in AR14 (Table 7 page 90, Figure 11 page 87). Screening of population controls showed that five of these variants were rare (<1% of the alleles screened; Table 7 page 90). Only one of the five, *TMEM43* 1073 C>T (S358L) (Figure 13 page 91 D), was shared by all clinically affected subjects across the 15 families, and was retained on key recombinant *ARVD5* haplotypes identified in clinically affected subjects from families AR10 (Figure 12 page 88) and AR2 (data not shown). This suggested that *TMEM43* is *ARVD5*. Additional supportive evidence included clinically unaffected adult subjects who shared distal sections of the *ARVD5* haplotype and lacked the *TMEM43* mutation (e.g. Figure 11 page 87). All available spouses (n= 47) and population controls (n= 161) were negative for the *TMEM43* mutation (416 mutation negative chromosomes).

Figure 10: Physical map of the ARVD5 critical region.



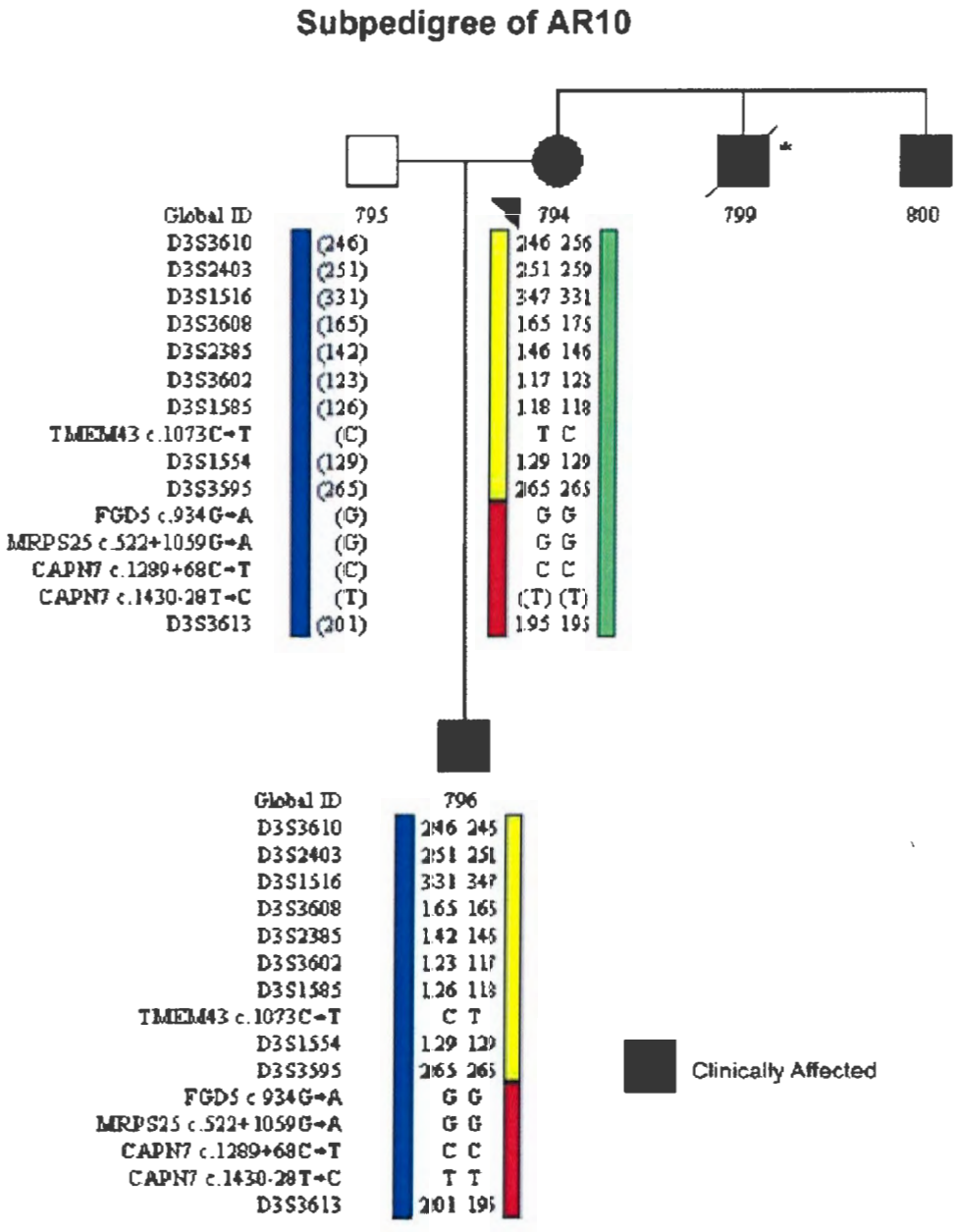
Panel A: Summary recombinant ARVD5 haplotypes identified across the 15 ARVC families using microsatellite markers (alleles are either numbered (1-9) or given in base pairs).
Panel B: The physical map of the *ARVD5* critical region. Physical distances were captured from the March 2006 freeze of the UCSC Genome Browser. Arrows show the direction of transcription of each annotated gene.

Figure 11: Segregation analysis in sub pedigree AR14



Of the 19 variants found exclusively in clinically affected subjects on the mutation screening panel, only 11 were found to reside on the *ARVD5* ancestral haplotype (yellow) through segregation analysis of clinically affected subjects (Global ID's 709, 710, and 712). Note that a clinically unaffected subject (Global ID 708) inherited a recombinant *ARVD5* haplotype from her clinically affected mother that lacks *TMEM43*. Alleles in brackets have been inferred.

Figure 12: Segregation analysis in sub pedigree AR10



Clinically affected subjects (Global ID 794 and 796) only have one of the five rare variants due to a historical recombination event on the *ARVD5* haplotype (yellow). Alleles in brackets have been inferred.

Table 6: The 20 physical candidate genes for ARVD5

Genes	Accession Number	MIM number	Strand	Genomic Position		Exons
				Start	End	
IQSEC1	NM_014869	610166	−	13003536	12917079	13
NUP210	NM_024923	607703	−	13436809	13332737	40
HDAC11	NM_024827	607226	+	13496824	13521834	10
FBLN2	NM_001004019	135821	+	13565625	13654922	18
WNT7A	NM_004625	601570	−	13896619	13835083	4
TPRXL	AK092426	611167	+	13953902	14082480	3
CHCHD4	NM_144636	611077	−	14141323	14128584	4
TMEM43	NM_024334	na	+	14141546	14160180	12
XPC	NM_004628	278720	−	14195143	14161651	16
LSM3	NM_014463	607283	+	14195341	14214840	4
SLC6A6	NM_003043	186854	+	14419110	14503973	15
GRIP2	NM_001080423	na	−	14558592	14510177	25
C3orf19	NM_016474	na	+	14668278	14689167	11
C3orf20	NM_032137	na	+	14691658	14789544	17
FGD5	NM_152536	na	+	14835810	14950899	20
NR2C2	NM_003298	601426	+	14964240	15065782	15
MRPS25	NM_022497	na	−	15081820	15065024	4
ZFYVE20	NM_022340	609511	−	15115659	15086584	14
CAPN7	NM_014296	606400	+	15222737	15269426	21
SH3BP5	NM_004844	605612	−	15349108	15271250	9
					Total	275

Table 7 Sequencing variants identified exclusively in clinically affected subjects

Gene Name	Accession number	Variant Nomenclature	Classification	Allele Frequency (# of chromosomes)	
HDAC11	NM_024827	c.369+18_369+19insG	Non-coding	nd	nd
TMEM43	**NM_024334**	**c.1073C>T**	**Missense (S>L)**	**0 / 322**	**0.00%***
TMEM43	NM_024334	c.1203+115T>C	Non-coding	nd	nd
XPC	NM_004628	c.2823+684G>C	Non-coding	nd	nd
SLC6A6	NM_003043	c.1-27420G>A	Non-coding	nd	nd
SLC6A6	**NM_003043**	**c.599+370A>G**	**Non-coding**	**dbSNP**	**46.00%**
SLC6A6	**NM_003043**	**c.733-1226A>G**	**Non-coding**	**50 / 90**	**55.60%**
FGD5	**NM_152536**	**c.934G>A**	**Missense (V>M)**	**2 / 318**	**0.60%***
FGD5	NM_152536	c.2186+22G>A	Non-coding	nd	nd
FGD5	NM_152536	c.2187-82G>A	Non-coding	nd	nd
FGD5	NM_152536	c.2220G>T	Synonymous (L>L)	nd	nd
FGD5	NM_152536	c.2613+50C>T	Non-coding	nd	nd
FGD5	**NM_152536**	**c.3085-74G>A**	**Non-coding**	**15 / 166**	**9.00%**
NR2C2	**NM_003298**	**c.855+70G>A**	**Non-coding**	**8 / 88**	**9.10%**
NR2C2	**NM_003298**	**c.1848+365T>A**	**Non-coding**	**16 / 90**	**17.80%**
NR2C2	**NM_003298**	**c.1848+2965_1848+2966insGATA**	**Non-coding**	**23 / 126**	**18.30%**
MRPS25	**NM_022497**	**c.522+1059G>A**	**Non-coding**	**0 / 138**	**0.00%***
CAPN7	**NM_014296**	**c.1289+68C>T**	**Non-coding**	**1 / 162**	**0.01%***
CAPN7	**NM_014296**	**c.1430-28T>C**	**Non-coding**	**0 / 160**	**0.00%***

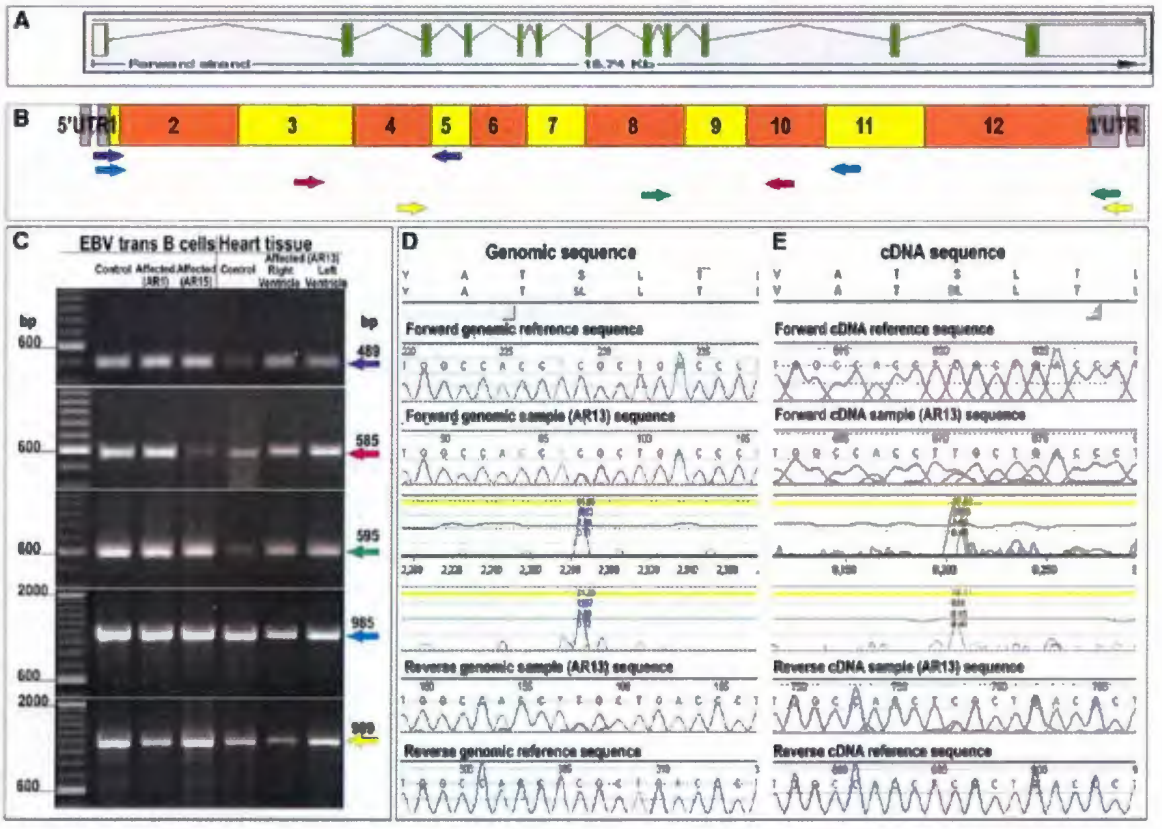
Eleven of the 19 variants were determined to be in phase on the *ARVD5* ancestral haplotype and were subsequently sequenced on population controls (bold). The allele frequency of variants previously reported in the NCBI dbSNP was used. Rare variants (<1% of the alleles screened) are marked with an *.

ARVC at locus *ARVD5* is caused by a missense mutation in *TMEM43*

The longest isoform of *transmembrane protein 43* (*TMEM43,* Genbank Accession number NM_024334) has 12 exons (Figure 13 A page 91) predicting a 400 amino acid protein that is 98% similar to the mouse protein (Figure 14 page 92). This conserved gene is found across all eukaryotic and prokaryotic species (Figure 14 page 92). We were able to extract full-length *TMEM43* cDNA from white blood cells and cardiac tissue from both patients and controls, demonstrating that *TMEM43* is expressed in both blood and

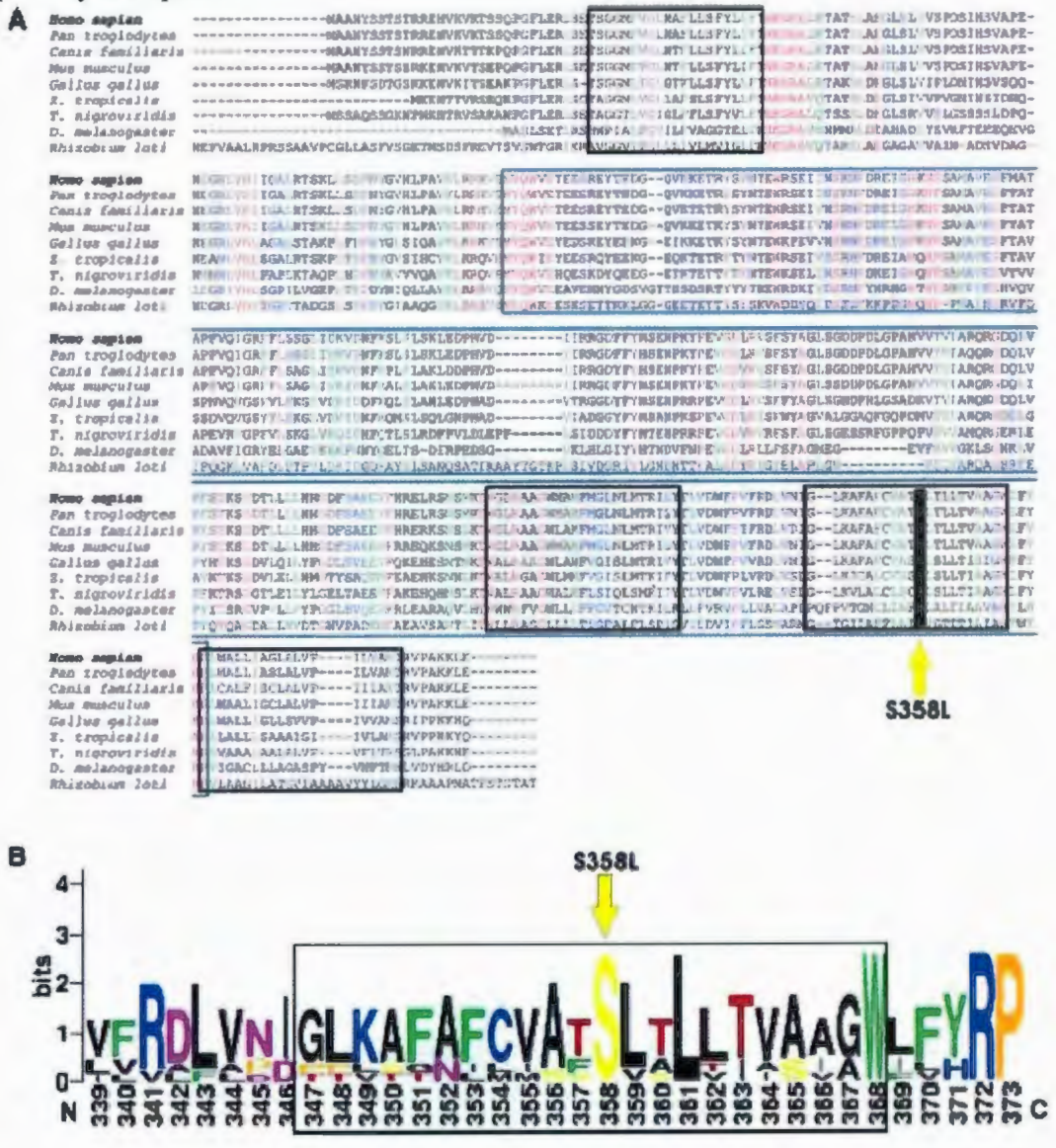
cardiac tissue and that the *TMEM43* 1073 C>T (S358L) mutation does not appear to affect splicing (Figure 13 B, C and E page 91).

Figure 13: Gene structure and mutation analysis of TMEM43



Panel A: Gene structure of *TMEM43*. Exons are represented by boxes. Translated exons are solid green and un-translated exons are clear. Introns are represented by green lines.
Panel B: Coverage of primers designed to amplify cDNA showing position of PCR primer pairs: Exons 1-4 (purple), Exons 4-9 (red), Exons 9-12 (green); Exons 1-10 (blue) 5-12 (yellow).
Panel C: PCR products amplified from cDNA of EBV transfected B cells of affected subjects from the mutation screening panel (affected AR1 and affected AR15) and unaffected (control) subjects. cDNA of heart tissue from both the left and right ventricle of an affected subject (AR13) and a heart biopsy from a control subject were size fractionated by electrophoresis.
Panel D and **E**: Forward and reverse sequencing traces showing the *TMEM43* 1073 C>T mutation of an affected subject's (AR13) genomic and cDNA. The amino acid translations (top) shows the S358L amino acid substitution.

Figure 14: Multiple alignment of the TMEM43 gene across eight eukaryotic and prokaryotic species



Panel A: Clustal W align was used to align orthologues from *Homo sapiens* (NP_077310), *Pan troglodytes* (XP_516299), *Canis familiaris* (XP_541751), *Mus musculus* (NP_083042), *Gallus gallus* (XP_414378), *Zenopus tropicalis* (UP10004D5297), *Tetraodon nigroviridis* (Q4RXL8), *Drosophila melanogaster* (NP_64162) and *Rhizobium loti* (Q98HF3) (253). The blue box outlines the DUF1625 domain and the black boxes outline predicted transmembrane domains. Completely conserved residues are red, strongly similar residues are green and weakly similar residues are blue. The S358L mutation is marked by a yellow arrow.
Panel B: The web logo format was used to align eukaryotic species. The third transmembrane domain is outlined (black box). The S358L mutation is marked by a yellow arrow.

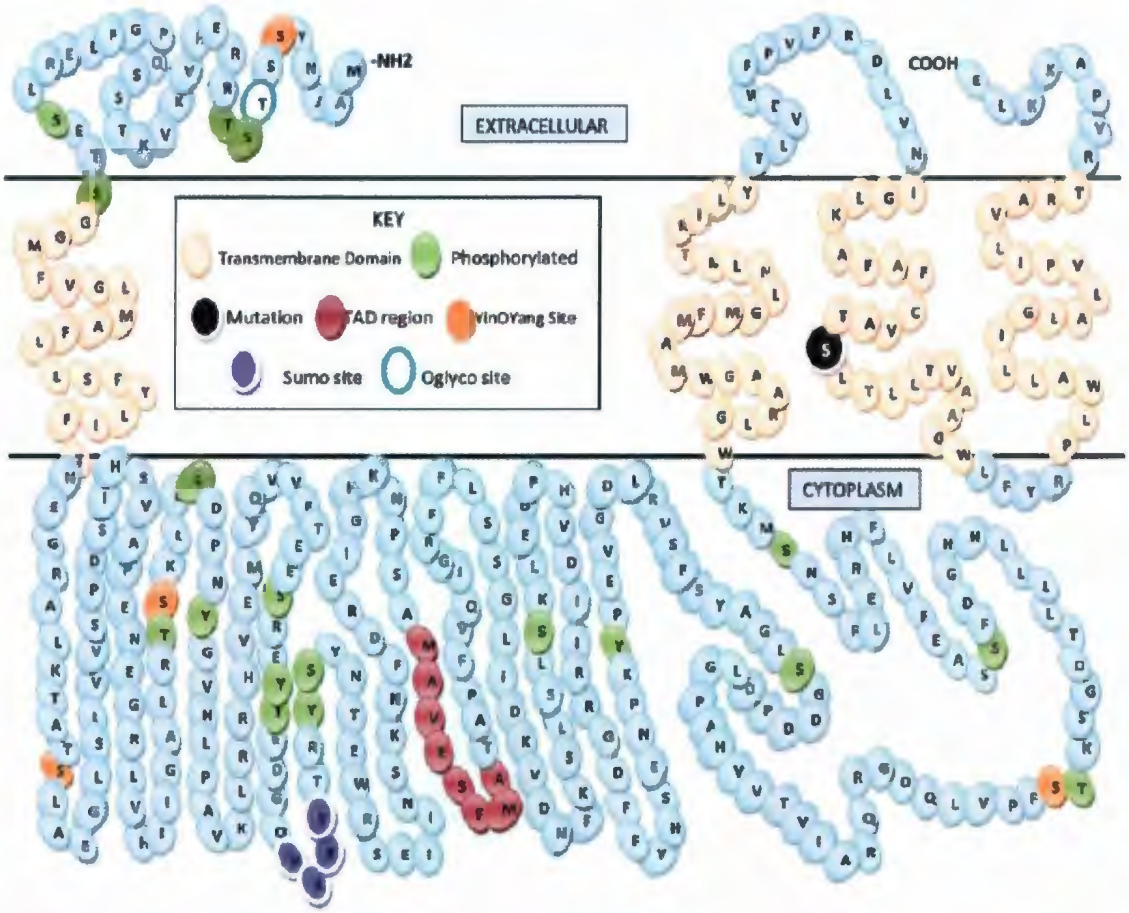
Bioinformatic analysis of TMEM43 predicts it to be a cytoplasmic membrane protein with several potential post-translation modification sites (Figure 15 page 94). Unlike the transmembrane proteins of the desmosome (desmocollin and desmoglein), TMEM43 does not have a cadherin domain. Furthermore protein sequence alignments with desmocollin and desmoglein show less than 10% identity and less than 12% similarity (254). The mutation, S358L, occurs within the third predicted transmembrane domain and is highly conserved in mammalian, avian, amphibian, and insect orthologs (Figure 14 page 92, Figure 15 page 94). Interestingly, a leucine at this position is found in the bacterium *Rhizobium loti* but it is not found in any multicellular organisms (Figure 14 page 92). The S358L mutation is predicted to be deleterious (Table 8 page 93) but little is known about the function of TMEM43 protein.

Table 8: Prediction of the TMEM43 1073 C>T (S358L) mutation effect.

Program	SIFT	Panther	PolyPhen	SNPs3D	PMut
S358L mutation	deleterious	deleterious	benign	deleterious	deleterious

Five different bioinformatic programs were used to predict the effect of the S358L mutation. Note that sequence homology used by the PolyPhen analyses was calculated with alignments of orthologs from Eukaryota and bacteria.

Figure 15: Predicted topography of the TMEM43 protein



Indicated are transmembrane domains (beige), phosphorylation sites (green), a transactivation domain (red), YingOYang sites (orange), a SUMO attachment site (purple), and an O-glycosylation site (blue open). The extracellular and cytoplasmic regions may be reversed: there is evidence supporting either orientation.

TMEM43 mutation screening in *ARVD5* linked families

We sequenced genomic DNA from all available subjects born at *a priori* 50% risk (n=295) across the 15 ARVC families for the presence of the 1073 C>T *TMEM43* mutation. All clinically affected subjects (n=83/83, 40 males, 43 females) and 28.8% of clinically unaffected subjects (n=61/212, 18 males, 43 females) were mutation carriers (Figure 9 page 83). Interestingly, 57% (35/61) of unaffected mutation carriers were found on subsequent testing to have clinical signs of ARVC. These included ectopy on Holter

monitor (≥ 200 PVC's over 24 hours (142)), extended QRS on 12 lead ECG or SAECG (25), or an enlarged left ventricle (>2SD above the mean). The remaining 26 clinically unaffected mutation carriers (3 males, 23 females) were at a median age of 22 years, and 33 years respectively. The 151 subjects with no clinical signs who did not have the *TMEM43* variant were considered unaffected (Figure 9 page 83).

2.4.1 Clinical assessment of penetrance in *ARVD5*

12 lead ECG

Data on a total of 297 subjects (167 affected, 130 unaffected) that had at least one 12 lead surface ECG was available. Extended QRS>110 ms was significantly different between affected vs. unaffected males (Table 9 page 97). Epsilon waves and T wave inversion commonly seen in ARVC (25) were seen in less than 3% of affected subjects in this cohort.

Holter Monitor

Of the 239 subjects (146 affected, 93 unaffected) with at least one Holter Monitor report, the most prevalent feature was PVC's ≥ 200 and the presence of at least one run of non sustained VT (Table 9 page 97), both significantly more common in affected than unaffected subjects.

Echocardiography

Of the 244 subjects (143 affected, 101 unaffected) that had at least one 2D echocardiogram available for analysis, the most prevalent feature was LVE based on LVEDD (Table 9 page 97) (202, 203), and was significantly different between affected and unaffected subjects.

SAECG

Of the 198 subjects (97 affected, 101unaffected) who had at least one SAECG available for analysis, all late potentials were significantly more common in affected versus unaffected males, although not in females (Table 9 page 97).

Table 9: Prevalent cardiac features on first clinical test

12 lead ECG, Holter, Echocardiographic and SAECG manifestations used in penetrance analysis in subjects from 15 families with ARVC due to a mutation in *TMEM43*.

12 lead ECG												
	Affected					**Unaffected**						
	Male		**Female**		**Tot.**	**Male**		**Female**		**Tot.**	χ^2	χ^2
N	**78**		**89**		**167**	**64**		**66**		**130**	**Affected Males vs Unaffected Males**	**Affected Females vs Unaffected Females**
Mean Age	**30**		**37.7**			**33.1**		**38.2**				
SD	**12.1**		**15.8**			**16**		**13.2**				
	n	**%**	**n**	**%**	**N (%)**	**n**	**%**	**n**	**%**	**N (%)**		
QRS> 110 ms	25	32	8	9	**33 (19.8)**	4	6	2	3	**6 (4.6)**	**p≤0.001**	p≤0.2ns
Holter Monitor												
	Affected					**Unaffected**						
Sex	**Male**		**Female**		**Total**	**Male**		**Female**		**Tot.**	χ^2	χ^2
N	**67**		**79**		**146**	**49**		**44**		**93**	**Affected Males vs Unaffected Males**	**Affected Females vs Unaffected Females**
Mean Age	**31.1**		**37.7**			**33.1**		**38.2**				
SD	**13.3**		**15.5**			**15.9**		**12.8**				
	n	**%**	**n**	**%**	**N (%)**	**n**	**%**	**n**	**%**	**N (%)**		
PVCs ≥200/24 h	47	70	47	59	**94 (64.4)**	0	0	1	2.3	**1.0**	**p<0.001**	**p<0.001**
≥ 1 run ns VT	13	19	18	23	**31 (21.2)**	0	0	0	0.0	**0.0**	**p<0.01**	**p<0.001**
Echocardiograph												
	Affected					**Unaffected**						
Sex	**Male**		**Female**		**Tot.**	**Male**		**Female**		**Tot.**	χ^2	χ^2
N	**67**		**76**		**143**	**50**		**51**		**101**	**Affected Males vs Unaffected Males**	**Affected Females vs Unaffected Females**
Mean Age	**31.58**		**39.61**			**32.46**		**38.27**				
SD	**13.15**		**15.14**			**14.91**		**13.28**				
	n	**%**	**n**	**%**	**N (%)**	**n**	**%**	**n**	**%**	**N (%)**		
LVE>2SD	35	52	27	20	**62 (43.3)**	10	20	4	8	**14 (13.9)**	**p<0.001**	**p<0.001**
SAECG manifestations												
	Affected					**Unaffected**						
Sex	**Male**		**Female**		**Tot.**	**Male**		**Female**		**Tot.**	χ^2	χ^2
N	**32**		**65**		**97**	**50**		**51**		**101**	**Affected Males vs Unaffected Males**	**Affected Females vs Unaffected Females**
Mean Age	**30.91**		**39.83**			**36.74**		**38.86**				
SD	**14.54**		**14.49**			**16.25**		**10.99**				
	n	**%**	**n**	**%**	**N (%)**	**n**	**%**	**n**	**%**	**N (%)**		
QRS>114 ms	19	59	19	29	**38 (39.2)**	10	20	2	4	**12 (11.9)**	**p≤ 0.001**	p<0.1 ns
RMS<20 ms	15	47	18	28	**33 (34)**	12	24	9	18	**21 (20.8)**	**p≤0.05**	p<1.0 ns
LAS>38 ms	17	53	21	32	**38 (39.2)**	14	28	13	25	**27 (26.7)**	**p≤0.025**	p<1.0 ns

PVC: premature ventricular complex, **VT:** Ventricular tachycardia, **LVE**: Left ventricular enlargement indexed to height and weight, **RMS**: root mean square voltage of the terminal 40millisecond of filtered QRS, **LAS**: low amplitude signals.

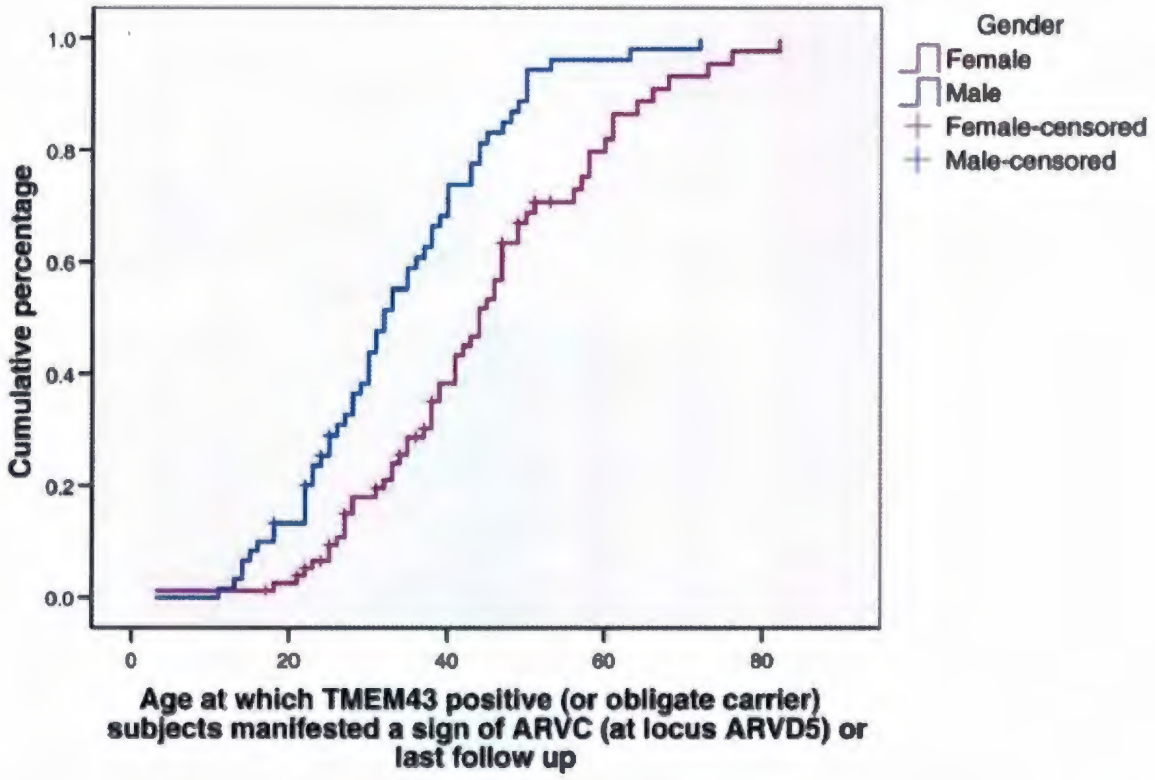
ARVC penetrance

One hundred and thirty seven subjects (60 affected males, 77 affected females) were used to determine penetrance. Median age to develop an ARVD5 associated phenotype was 32 years (95% CI 28-35) for males and 44 years (95% CI 39-48) for females, with 100% of males and females penetrant by 63 and 76 years respectively (Figure 16 page 99). Males were twice as likely to reveal the disease phenotype than females (RR 2, 99% CI 1.2-3.3) ($p \leq 0.0001$). The commonest clinical features for which subjects were initially penetrant were ectopy (44%), and LVE (27%), then VT (9%), QRS>110 ms (9%), late potentials on SAECG (7%), SCD (2%) and heart failure (2%).

Heart Failure

Fourteen of 89 affected males developed heart failure at a median age of 63 years (95% CI 41-84 years) compared with no unaffected males ($p \leq 0.0001$, log rank) (Figure 17 A1 page 100). One unaffected female at age 79 developed heart failure compared to seven affected females (median age 73 years, 95 % CI 69-77 years) ($p \leq 0.001$, log rank) (Figure 17 A2 page 100). Affected males were three times more likely to develop heart failure than females (RR 3.4, 95% CI 1.36-8.57, $p \leq 0.009$) (Figure 17 A3 page 100).

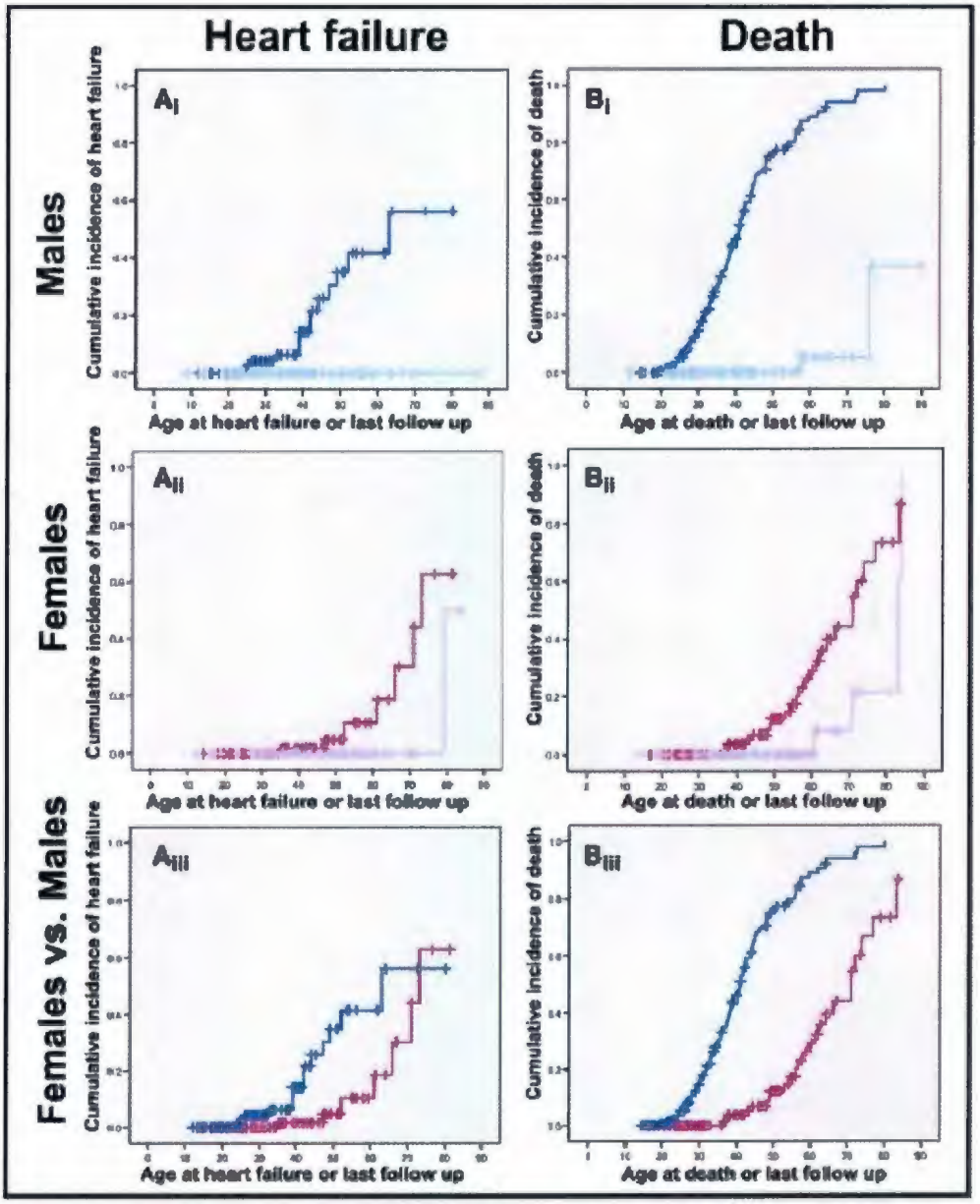
Figure 16: ARVC penetrance caused by the TMEM43 1073 C>T mutation



Panel A: Cumulative proportion by age at which male (n=60) and female (n=77) mutation positive affected subjects manifested a first clinical sign of ARVC. Subjects were censored at last follow-up.
Panel B: Cumulative percentage of subjects penetrant by decade.

Age (years)	11-20	21-30	31-40	41-50	51-60	61-70	71-80
Males	13	39	68	89	96	100	100
Females	3	18	38	67	80	97	100

Figure 17: Time to event analysis of heart failure and death in affected subjects



Panels A1-3: Cumulative incidence of heart failure: **A1)** Affected males (n=89, dark blue) compared to unaffected males (n=71, light blue) ($p \leq 0.0001$: log rank), **A2)** Affected females (n=87, dark pink) compared to unaffected females (n=68, light pink) ($p \leq 0.001$, log rank), **A3)** Affected males (dark blue) vs. affected females (dark pink).
Panels B1-3: Life expectancy: **B1)** Time to death in affected males (n=148, dark blue) and unaffected males (n=77, light blue), **B2)** Time to death in affected females (n=109, dark pink) and unaffected (n=74, light pink), **B3)** Affected males (dark blue) vs. affected females (dark pink).

Life expectancy

In affected subjects (n=257) there were 123 (48%) deaths (99 males and 24 females). Sudden cardiac death occurred in 86% (86/99) of the males and 42% (10/24) of the females. Survival was significantly reduced in affected versus unaffected subjects. Median survival was 41 compared to 83 years ($p \leq 0.0001$, log rank) in affected vs. unaffected males (Figure 17 B1 page 100) and 71 compared to 83 years in affected vs. unaffected females ($p \leq 0.002$, log rank) (Figure 17 B2 page 100). The relative risk of dying was 6.8 times higher in affected males versus affected females (95% C.I: 4.3–10.9, $p \leq 0.0001$) (Figure 17 B3 page 100).

2.5 DISCUSSION

Mutations in desmosomal genes underlie several genetic subtypes of ARVC, suggesting that ARVC is primarily a disease of the desmosome (74, 243). We used a positional mapping approach and 15 ARVC families that shared a disease associated haplotype on 3p from a genetic isolate to identify ARVD5. All clinically affected subjects that were screened for the *TMEM43* 1073 C>T mutation (n=83) were mutation carriers. This mutation was not detected in spouses or population controls. The *TMEM43* gene is expressed in cardiac tissue, is evolutionarily conserved and the amino acid substitution is predicted to be deleterious.

Recently, signalling pathways have been implicated in ARVC pathogenesis (74, 263). For example, plakoglobin, when freed from desmosomal complexes, translocates to the nucleus where it competes and opposes the action of β-catenin and down regulates the

canonical Wnt/β-catenin signaling pathway (263). Suppression of the canonical Wnt/β-catenin signaling up regulates two adipogenic transcription factors, C/EBP-α (MIM 116897) and PPARγ (MIM 601487) (263). A genome wide scan for peroxisome proliferator response elements (PPREs) identified 1085 potential target genes of PPARγ, including *TMEM43* (264). If TMEM43 is a part of an adipogenic pathway regulated by PPARγ, then perhaps dysregulation of this pathway may explain the fibrofatty replacement of the myocardium in ARVC patients.

Studies that assess the ARVC phenotype in patients with mutations in *plakophilin-2* (265), *desmoglein-2* (61) and *plakoglobin* (28) typically evaluate clinical features in mutation carriers only. The assumption is that all cardiac signs present in affected subjects are due to the underlying genetic defect. However, it may be that some signs are due to the genetic background of either the family or the source population. The size of the ARVC cohort in this study provides an opportunity to compare and define ARVC-specific clinical features in affected and unaffected subjects.

Using the benefit of a control group, we evaluated modified clinical criteria (142) in affected versus unaffected subjects. We included LVE, a clinical feature noted in other ARVC studies, (132, 135, 140, 266, 267) and prevalent in this cohort. For all clinical features examined, a significant difference was found between affected versus unaffected males. Females were significantly different for all features with the exception of late potentials on SAECG and a QRS>110 ms on 12 lead ECG.

For the penetrance study, we followed subjects who were alive at the start of the study (1996), who had a medical record and had a genetic diagnosis (mutation carriers or

obligate carriers). The results showed that ARVC was fully penetrant in males by the age of 63 and in females by the age of 76; ectopy and LVE were often the first presenting features, and few presented with death or heart failure. Therefore, in order to assess the penetrance of heart failure and death, we used all affected subjects. Heart failure and death were morbid outcomes at early ages in both males and females, with far more serious early events in males. In both sexes heart failure occurs as a later manifestation in subjects who did not succumb to SCD. These major manifestations define ARVC, due to the *TMEM43* mutation, as a lethal, fully penetrant, sex-influenced, autosomal dominant disorder.

Biases exist in studies that use both retrospective and prospective data; the use of well ascertained sibships may mitigate some of these. Although formal assessment of right ventricular structure is not available for the majority of subjects in this cohort, the cardiomyopathy fulfills diagnostic criteria for ARVC (142), it has been historically diagnosed as ARVC (4, 41) and fibrofatty replacement of the myocardium is present. The involvement of the left ventricle in this cohort supports the recent call to place ARVC within a group called 'arrhythmic cardiomyopathies,' which would take into account various presentations of disease (268). These findings may not be applicable to other forms of inherited ARVC. However, it is likely that mutations in *TMEM43* are present in other populations. Issues of classification in the cardiomyopathies in general will be solved by their underlying genetic etiology, thus disease specific genotype/phenotype information will become the most useful and relevant.

This study has the advantage of having access to past medical records and extensive genealogical data, and a single cardiac care team followed all subjects. Clinical signs due to the genetic background of either the family or the source population were controlled for by the use of unaffected subjects from well ascertained sibships. This genetic subtype does not show the typical ARVC ECG findings of T wave inversion or epsilon waves (25). A direct benefit of this process therefore has been the provision of accurate mutation specific clinical data for precise genetic counselling and follow-up of those at-risk (248).

We have identified the gene that causes ARVC at locus *ARVD5*. A missense mutation in *TMEM43* causes a fully penetrant, sex-influenced lethal form of ARVC. Although little is known about the function of this gene, it encodes a transmembrane protein that could be a target of PPARγ, which may explain the fibrofatty replacement of the myocardium in ARVC patients. Future directions include functional studies of the TMEM43 protein.

Web Resources

UCSC Genome Browser (http://genome.ucsc.edu/)

Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/Omim)

NCBI dbSNP (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp&cmd=search&term=)

3 THE NATURAL HISTORY AND PHENOTYPIC EXPRESSION OF ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVD5) CAUSED BY A MUTATION IN *TMEM43*

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3.1 ABSTRACT

Objective

To determine the phenotype and natural history of arrhythmogenic right ventricular cardiomyopathy (ARVC) caused by a 1073 C>T mutation in TMEM43 (ARVD5).

Background

The phenotype and natural history of ARVC caused by a mutation in TMEM43 have not been described.

Methods

We studied the age of onset of cardiac symptoms, clinical events and test abnormalities in 412 subjects from 15 families. Subjects were considered positive (n=258) if they had the TMEM3 mutation, were an obligate carrier, or major clinical sequalea occurred before age 50 years. We compared their outcomes to mutation negative relatives (n=154).

Results

All cardiac symptoms and outcomes occurred in positive males significantly earlier, and more often than negative males. Positive males were hospitalised four times more often than positive females and died at a younger age ($p \leq 0.001$). The temporal sequence from symptoms to death was prolonged in females by 1-2 decades. The most prevalent ECG manifestation was poor R wave progression (PRWP) with positive males twice as likely to develop PRWP as positive females ($p \leq 0.05$). Forty three percent of positive subjects had left ventricular enlargement, with 11% fulfilling criteria for dilated cardiomyopathy. The most sensitive test for diagnosis of ARVC was Holter monitoring.

Conclusions

Cardiac symptoms occurred at a young age in ARVC males with a *TMEM43* mutation, in whom onset of symptoms was often closely followed by death. Heart failure was a later manifestation of ARVC in survivors. The ARVD5 genetic subtype of ARVC is both an arrhythmogenic and a cardiomyopathic disease.

3.2 INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a recognised cause of sudden cardiac death (SCD) in young people (105), making early diagnosis for prophylactic treatment critical. Clinical diagnosis of ARVC is based on a combination of electrophysiological, structural and functional abnormalities (25). Multiple genetic causes of ARVC have been described (269). Variable expressivity (gene carriers manifest different phenotypes), reduced penetrance (some gene carriers show no signs of disease) and difficulty imaging the right ventricle make diagnosis problematic. It is likely a disease of both ventricles, as left ventricular involvement is common in ARVC (61, 132, 134, 135, 138-140, 194, 266, 267, 270, 271)and clinical overlap is increasingly recognised (141, 142, 272-275).

The latest gene to be found as a cause of autosomal dominant ARVC is TMEM43, a transmembrane protein of unknown function (46). A missense *TMEM43* 1073 C>T mutation (S358L) is causative in Newfoundland families with ARVD5. In subjects born at *a priori* 50% risk of inheriting ARVC, the identification of the mutation, comprehensive analysis of the pedigree and the documentation of the early onset of severe cardiac events has allowed us to determine clinically affected and/or mutation positive subjects with ARVC and mutation negative subjects who do not have the *TMEM43* mutation. To ease this accurate (cumbersome) terminology, we will refer to these groups in this manuscript as 'positive' and 'negative' respectively. The ability to define mutation carriers has allowed for presymptomatic diagnosis of ARVC, and the

recognition of the negative subjects has allowed us to determine the natural history and the true phenotype by utilising an in-built control group.

Most studies of the natural history of ARVC have involved small heterogeneous samples where variable clinical presentation, family history, causes of death and rates of death are described (167-169). Corrado et al defined the natural history as having four discrete stages (156). An Italian study of 37 families, ten of whom had an assigned genetic locus, found similar variable expression but they did not report clinical symptoms, nor differentiate subjects according to locus (170).

We have previously reported that ARVD5 is a lethal disease, particularly in males who often die suddenly at a young age, that survival is improved with implantable cardioverter defibrillator (ICD) therapy (248), and that the disease appears to be 100% penetrant for known disease clinical manifestations by age 70 years in males and 80 years in females (46). In the current study, we obtained comprehensive clinical data from medical charts and interviews, assessed the prevalence and incidence of symptoms and clinical events and analysed serial electrocardiograms (ECG), echocardiograms, Holter monitors and signal averaged ECGS (SAECGs). We compared the prevalence and incidence of clinical manifestations in positive and negative subjects from 15 families segregating ARVD5, and assessed the diagnostic utility of symptoms and clinical tests.

3.3 METHODS

3.3.1 Study population

Of 150 families with a history of SCD and/or cardiomyopathy referred to the provincial medical genetics program in Newfoundland and Labrador, 15 were found to have a disease associated haplotype at the ARVD5 locus at 3p25 (248) (OMIM: http://www.ncbi.nlm.nih.gov/omim/) and subsequently shown to have a missense 1073 C>T mutation (S358L) in *TMEM43* (46). Cardiac disease in these large families was characterized by autosomal dominant (AD) inheritance, a single causative mutation and cardiac pathology consistent with ARVC. We first identified all individuals born at a 50% *a priori* risk of ARVC as potential subjects for the study. Subjects in these families were identified as positive if they met one or more of the following criteria: 1) they had the *TMEM43* 1073 C>T mutation 2) they were an obligate carrier (that is, they had an affected first degree ancestor and descendant in the pedigree), 3) SCD occurred under age 50 years or 4) they were resuscitated for ventricular tachycardia (VT) or fibrillation (VF) under age 50 years. Subjects were defined as negative if they were proven not to have the *TMEM43* 1073 C>T mutation. All remaining subjects were considered to have unknown disease status.

In the 15 families we ascertained 638 subjects who were born at *a priori* 50% risk for ARVC. We limited our analysis however to subjects from 'well ascertained' sibships, that is, we knew the disease status of $\geq$ 50% of their siblings (n=496). This strategy was designed to minimise the bias present in recognising clinical disease in serious cases, and

missing it in less serious cases. Subjects in the unknown group (n=84) were not studied further. In this way, 412 (226m, 186f) subjects met the criteria for inclusion in the study.

3.3.2 Data collection

All subjects in the family pedigree in the line of descent from a positive subject, born at *a priori* 50% risk of inheriting ARVC, were enrolled in the study. This included long deceased family members whose only information was dates of birth and death, from census records or family bibles on family trees. Following genetic counselling and informed consent (obtained from each subject (or surrogate) in compliance with the Human Investigation Committee requirements of the Health Care Corporation and Memorial University of St. John's, Newfoundland) all available past and present medical records were obtained and reviewed from all subjects, alive or dead. Formal clinical assessments were conducted as part of a prospective screening regime and for subjects presenting with a clinical problem. As occurs in most family studies of inherited disease, subjects were ascertained at different stages of their disease process.

Beginning in 1998, all extant subjects at 50% risk of ARVC were screened at a genetics-cardiomyopathy clinic at between one and three yearly intervals starting at 10 years of age. Subjects ascertained prior to 1998 were seen because of a clinical problem. Subjects were offered DNA testing. This was initially a DNA haplotype at locus ARVD5 at 3p25 which segregated with ARVC, done in the research laboratory of Dr. Thierfelder (248). Direct mutation analysis was feasible following the definition of the causative gene (46)). A detailed symptomology and medical history was obtained from all subjects by one of two highly trained cardiac nurses, blind to any previous test results (clinical or DNA).

Data were included on four male (one positive, three mutation negative) and 10 female (eight positive and two negative) subjects who were from other provinces in Canada, or the USA. In these cases, with subject consent, we obtained past cardiac records, and offered DNA analysis. Follow-up was organized through local genetics and cardiology clinical services (276).

Sudden death at an early age, particularly in males, influenced the non-availability of medical records. Among 149 positive males, medical records were available for 87 (58%), and not available for 62 (42%), of whom 61 (98%) were dead. Notably, however the median age to death or last follow-up for positive males with no medical records (n=62) was 39 years similar to the 46 years in the group with medical records, (n=87) (data not shown). Among 109 positive females, medical records were available for 85 (78%) and not for 24 (22%), of whom 15 (62%) were dead. Medical records were available in 65 of 77 (84%) negative males and 68 of 77 (88%) negative females. The negative subjects with no medical records comprised those who had provided DNA, but were lost to follow-up.

We documented age at the first time a subject had palpitations (an awareness of a fast or irregular heartbeat), syncope (a sudden brief loss of consciousness from which recovery was spontaneous), pre-syncope (a transitory feeling of impending loss of consciousness which did not proceed to syncope), chest pain, heart failure, hospitalization secondary to a cardiac problem (Table 10 page 113). In addition, age at death or last follow-up was obtained.

Table 10: Median age to clinical cardiac manifestations in ARVD5 subjects with medical records (n=305).

			Cardiac manifestation						
Subject group			Palp	PS	CP	S	HF	Hosp	Death
Positive males N=87		n	45	44	29	23	15	39	31
		%	52	50	33	26	17	45	36
		Median age (y) to event (95% CI)	39 (35-44)	39 (33-45)	45 (38-52)	61 (35-86)	63 (46-80)	43 (38-47)	45 (38-52)
Negative males N=65		n	14	20	15	9	0	6	1
		%	21	31	23	14	0	9	1.5
		Median age (y) to event (95%CI)3	67 (50-84)	64 (48-79)	66 (47-85)	-	-	-	-
+ve males vs. –ve males	C R	p	0.0001	0.006	0.031	0.04	0.0001[2]	0.0001	0.001
		RR (95% CI)[3]	3.4 (1.8-6.3)	2.2 (1.2-3.8)	2.0 (1.1-3.9)	2.3 (1.1-4.9)	-	10.2 (3.9-26.4)	31.5 (4.3 – 231.4)
Positive females N=85		n	49	37	26	24	6	21	9
		%	66	43	30	28	7	24	10
		Median age (y) to event (95%CI)3	41 (36-46)	49[1] (41-57)	59 (44-74)	66 (52-79)	71 (71-71)	66 (55-76)	-
Negative females N=68		n	25	18	14	5	0	6	2
		%	40	39	20	7	0	9	3
		Median age (y) to event (95% CI)3	62 (42-82)	-	66 (37-95)	-	-	-	-
+ve females vs. –ve females	C R	p	0.02	0.06	0.4	0.006	0.06[2]	0.08	0.4
		RR (95% CI)[3]	1.8 (1.1-2.9)	ns	ns	3.8 (1.4-10.3)	ns	ns	ns
+ve males vs. +ve females	C R	p	0.8	0.007	0.04	0.45	0.002	0.0001	0.001
		RR (95% CI)[3]	ns	1.9 (1.2-2.9)	1.8 (1.1-3.1)	ns	5.2 (1.8-14.5)	4.4 (2.3-8.0)	7.4 (3.3-16.2)

Palp: palpitations, PS: presyncope, CP: chest pain, S: syncope, HF: heart failure, Hosp: hospitalization for a clinical cardiac event. CR: Cox's proportional regression analysis.

[1]: This analysis was done for females with a formal clinical assessment (see text). The denominators for this analysis therefore were 74 positive vs. 63 negative females

[2]. This p value is derived from the log rank assessment of the Kaplan Meier analysis

[3]. Where median ages are not provided, 50% of the subjects did not reach the end point in the Kaplan Meier analysis.

We determined whether subjects seen through clinic, who were asked directly about symptoms, differed statistically from those who had only a medical record available. There were no significant differences in time to various cardiac manifestations comparing male subjects with medical records to those with both a medical record and a formal clinical assessment. The data for pre-syncope, syncope, chest pain, heart failure and palpitations in males is therefore presented here without differentiation by data source. Palpitations were three times more likely to occur in clinically affected and/or mutation positive females who had a formal clinical assessment (n=74) compared with those with a medical record only (n=11) (p=0.01, RR 3.1 (95% CI 1.2-7.6)). Thus data on palpitations in females is presented for the group that had clinical assessment only.

3.3.3 Direct clinical assessment

In addition to taking a medical history and a physical examination, subjects attending the cardiomyopathy /genetics clinic had a 12 lead electrocardiogram (ECG), a signal averaged ECG (SAECG), an echocardiogram and a 24 hour Holter monitor test. Serial assessments were done at one to three year intervals. The presence of abnormalities on each clinical test was assessed. The ECGs were read by two physicians blind to clinical status (SC and PP). Poor R wave progression (PRWP) was defined as R wave in V3 of less than 3mm (277). QRS was defined as being greater than 110ms. Prevalence of abnormalities on first ECG was calculated (Table 11 page 115). In positive subjects with serial ECGs, the age to onset of *de novo* PRWP and PVCs was calculated by time to event analysis, after excluding those with PRWP and PVCs present at baseline (Table 12 page 116).

Table 11: Anomalies on initial 12 lead ECG in ARVD5 (n=300).

	Positive group					Negative group					Analyses	
	Male		Female		Tot.	Male		Female		Tot.	χ^2	χ^2
N	78		89		167	64		69		133	Positive Males vs. negative Males	Positive Females vs negative Females
Mean age at test (SD)	30.0 (12.0)		37.8 (15.8)			33.3 (16.1)		38.2 (12.9)				
12 lead ECG	n	%	n	%	N (%)	n	%	n	%	N (%)		
PRWP	23	29	25	28	48 (29)	0	0	2	3	2 (1)	p≤0.0001	p≤0.0001
PVC's	25	32	22	25	47 (28)	0	0	1	1	1 (<1)	p≤0.0001	p≤0.0001
QRS> 110 ms	25	32	8	9	33 (19.8)	4	6	2	3	6 (4.6)	p≤0.0001	p=0.119 (ns)
Septal Q waves	10	13	14	16	24 (14)	0	0	0	0	0	p≤0.003	p≤0.001

Features analysed, but with data not shown due to low prevalence (with most < 5%) included ventricular tachycardia, atrial fibrillation, L axis, T wave inv. in V2 V3, R axis, 1° AV block, Long QT, Short QT, Short PR, Epsilon wave, Tall R in V1, dilated L Atrium, dilated R atrium, L ventricular hypertrophy, R ventricular hypertrophy, L bundle branch block, R bundle branch block, L anterior hemiblock, L. posterior hemiblock, Inferior Q waves, Lateral Q waves, Anterior Q waves, Inferior ST depression, Anterior ST depression, Lateral ST depression, Inferior ST elevation, Anterior ST elevation, Lateral ST elevation, Flat Inferior T wave, Flat lateral T wave, Inverted lateral T wave.

Table 12: Incident events on 12 lead ECG, Holter monitoring and echocardiogram in ARVD5.

Test	Feature	Sex	Disease group status	# without anomaly on first test who had one or more follow-up tests.	IE	Median age to onset of anomaly	Log rank p value *	RR	95% CI	Cox p value	Positive males and positive females compared
12 lead ECG	PRWP	M	Positive	38	11	55	0.001	No events	No events	No events	p≤0.05 RR=2.3 95% CI (1.01-5.2)
			Negative	41	0	-					
		F	Positive	64	14	58	0.001	8.4	1.9-37.4	0.005	
			Negative	43	2	-					
	PVCs	M	Positive	37	11	48	0.001	28.2	3.6-221	0.002	p≤0.004 RR=3.7 95% CI (1.5-9.3)
			Negative	40	1	-					
		F	Positive	65	11	77	0.023	4.9	1.1-22.6	0.041	
			Negative	40	2	70					
Holter Monitor	> 200 PVCs	M	Positive	10	6	25	0.001	48.1	5.3-431	0.01	p≤0.02 RR=3.6 95% CI (1.3-10.9)
			Negative	31	2	72					
		F	Positive	21	9	48	0.002	5.6	16-18.8	0.005	
			Negative	25	4	-					
	> 1000 PVC's	M	Positive	12	6	26	0.001	No events	No events	No events	p≤0.003 RR=5.4 95% CI (1.8-16.9)
			Negative	35	0	-					
		F	Positive	23	8	52	0.009	6.2	1.2-29.8	0.02	
			Negative	30	2	-					
Echocardiogram	LVE > 2SD	M	Positive	19	10	45	0.006	6.5	1.4-30.2	0.02	p≤0.3
			Negative	19	2	-					
		F	Positive	35	14	51	0.01	4.5	1.3-15.7	0.02	
			Negative	23	3	60					
	LVE > 3SD	M	Positive	22	7	-	0.009	No events	No events	No events	≤0.5
			Negative	23	0	-					
		F	Positive	38	9	59	0.05	4.0	0.8-1.9	0.07	
			Negative	25	2	-					

IE: incident events, PRWP: poor R wave progression, PVC: premature ventricular contraction, LVE: left ventricular enlargement. * p value from the Kaplan Meier analysis comparing positive and negative subjects of the same sex.

Left ventricular enlargement (LVE) was defined as left ventricular end diastolic diameter (LVEDD)>112% corresponding to 2 standard deviations (SD) above the predicted mean value (Henry's formula) (202). We also assessed LVE > 117% (> 3 SD above the predicted mean). Prevalence of abnormalities on first echocardiogram was calculated (Table 13 page 118). In positive subjects with serial echocardiograms, the age to onset of *de novo* LVE was calculated by time to event analysis, after excluding those with LVE present at baseline (Table 12 page 116).

Ectopy was determined on Holter monitor testing over 24 hours, and the prevalence of number of PVCs, VT, and other forms of ectopy was determined (Table 14 page 118). In positive subjects with serial Holter monitors, the age of onset of *de novo* ≥ 200 PVCs or ≥ 1000 PVCs was calculated by time to event analysis after excluding those with ≥ 200 or ≥ 1000 PVCs at baseline (Table 12 page 116). This number of PVCs was chosen because of their use within the original and the modified diagnostic criteria for ARVC (25, 142)

Prevalent late potentials on first SAECG (Table 15 page 119) were classified based on recognized criteria: QRS (filtered QRS duration)>114 ms, LAS (low amplitude signals)>38 ms, and RMS (root mean square voltage of the terminal 40ms of filtered QRS)<20 ms(260). The SAECG test is considered abnormal when 2/3 of these criteria are abnormal.

Table 13: Anomalies on initial echocardiogram in ARVD5 (n=248).

	Positive group					Negative group					Analyses	
Sex	**Male**		**Female**		**Tot.**	**Male**		**Female**		**Tot.**	χ^2	χ^2
N	**68**		**76**		**144**	**50**		**54**		**104**	**Positive males vs negative males**	**Positive females vs negative females**
Mean Age at test (SD)	**31.6 (13.0)**		**39.6 (15.1)**			**32.6 (15)**		**38.4 (12.1)**				
Echo-cardiogram	**n**	**%**	**n**	**%**	**N (%)**	**n**	**%**	**n**	**%**	**N (%)**		
LVE > 2SD	35	52	27	20	**62 (43)**	10	20	4	8	**14 (14)**	**p≤0.001**	**p≤0.001**
LVE > 3SD	24	35	20	26	**44 (30)**	4	8	2	4	**6 (6)**	**p≤0.001**	**p≤0.001**
Dyskinesia global/focal	19	28	15	20	**34 (24)**	0	0	0	0	**0**	**p≤0.001**	**p≤0.001**
LAE	16	23	8	10	**24 (17)**	5	10	4	7	**9 (9)**	**ns**	**ns**
RVD	12	18	6	8	**18 (12)**	2	4	4	7	**6 (6)**	**p≤0.05**	**ns**
FS<25%	11	16	8	10	**18 (12)**	2	4	1	2	**3(3)**	**p≤0.02**	**ns**
LVH	7	10	7	9	**14 (10)**	3	6	3	5	**6 (6)**	**ns**	**ns**

PVC: premature ventricular complex, **VT:** Ventricular tachycardia, **LVE**: Left ventricular enlargement indexed to height and weight, **LAE:** left atrial enlargement, **RVD,** right ventricular dilatation, **LVH:** left ventricular hypertrophy, **FS:** Fractional shortening

Table 14: Anomalies on initial Holter monitor in ARVD5 (n=239).

	Positive group					Negative group					Analyses	
Sex	**Male**		**Female**		**Total**	**Male**		**Female**		**Tot.**	χ^2	χ^2
N	**67**		**79**		**146**	**49**		**44**		**93**	**Positive males vs negative males**	**Positive females vs negative Females**
Mean Age at test (SD)	**31.1 (13.3)**		**37.7 (15.5)**			**33.1 (15.9)**		**38.2 (12.8)**				
Holter Monitor anomalies	**n**	**%**	**n**	**%**	**N (%)**	**n**	**%**	**n**	**%**	**N (%)**		
PVCs ≥200/24 h	47	70	47	59	**94 (64)**	0	0	1	2	**1 (1)**	**p≤0.0001**	**p≤0.0001**
PVC's ≥ 1000/24h	45	67	37	47	**82 (56)**	0	0	1	2	**1 (1)**	**p≤0.0001**	**p≤0.0001**
≥ 1 couplet	39	58	43	56	**82 (56)**	0	0	2	4	**2 (2)**	**p≤0.0001**	**p≤0.0001**
≥ 1 run bigeminy	24	36	33	42	**57 (39)**	0	0	1	2	**1 (1)**	**p≤0.0001**	**p≤0.0001**
≥ 1 run ns VT	30	45	24	30	**54 (37)**	0	0	0	0	**0**	**p≤0.0001**	**p≤0.0001**
≥ 1 triplet	20	30	14	18	**34 (23)**	0	0	0	0	**0**	**p≤0.0001**	**p≤0.01**

nsVT: non-sustained ventricular tachycardia

Table 15: Anomalies on initial SAECG in ARVD5 (n=202).

	Positive group					Negative group					Analyses	
Sex	**Male**		**Female**		**Tot.**	**Male**		**Female**		**Tot.**	χ^2	χ^2
N	**33**		**65**		**98**	**50**		**54**		**104**	**Positive males vs negative males**	**Positive females vs negative females**
Mean Age at test (SD)	**31.2 (14.3)**		**39.8 (14.5)**			**36.8 (16.3)**		**39 (10.8)**				
SAECG anomalies	**n**	**%**	**n**	**%**	**N (%)**	**n**	**%**	**n**	**%**	**N (%)**		
Total QRS >114ms	19	58	19	29	**38 (38)**	10	20	2	4	**12 (12)**	**p≤ 0.0001**	**p≤ 0.0001**
RMS<20 ms	15	46	18	28	**33 (34)**	12	24	9	18	**21 (21)**	**p≤0.041**	p=0.2(ns)
LAS>38 ms	17	52	21	32	**38 (38)**	15	30	13	25	**27 (27)**	**p≤0.049**	p=0.42 (ns)
2/3 Abnormal	16	49	17	26	**33 (34)**	10	14	8	10	**18 (18)**	**P≤0.041**	**p≤0.041**

3.3.4 Genetic analysis

Screening for the *TMEM43* 1073 C>T mutation:

Venous blood was drawn from 295 subjects and DNA was extracted from peripheral lymphocytes (278). Presence or absence of the *TMEM43* 1073 C>T mutation was determined for each subject in each family as described previously (46).

Statistical analysis

The known sex-differences in survival in ARVC linked to 3p25, (248) determined that data for males and females were examined separately. For time to event analysis subjects were censored at last follow-up (defined as the age at last clinic visit), death, date of ICD surgery or heart transplant. Survival was calculated according to the Kaplan Meier product limit method. Relative risk (hazard ratio) was calculated using Cox's Proportional Regression model. Version 15 of the SPSS statistics package was used for analyses (SPSS, Chicago USA).

The diagnostic utility (sensitivity, specificity, positive predictive value, negative predictive value) of symptoms and test results present at the first clinic assessment was assessed for the identification of those clinically affected and/or mutation positive subjects. The likelihood ratio of having, and not having inherited ARVC was calculated by sensitivity /1-specificity, and 1-sensitivity/specificity respectively. In general, likelihood ratios over 10 (for diagnosis), or under 0.1 (for ruling out diagnosis) are considered to indicate good diagnostic utility.

3.4 RESULTS

Clinical manifestations of cardiac disease in males

Table 10 page 113 and Figure 18 page 122 summarize the incidence of cardiac symptoms and events in 87 positive and 65 negative males with medical records. Positive males were significantly more likely than negative males to develop each of the cardiac symptoms and outcomes. Fifty three percent of positive males had presyncope and palpitations before age 40 years (Figure 18 page 122) compared with 27% and 17% of negative males respectively (data not shown). Heart failure developed in 16% positive males before age 40 years, and in 46% before age 60 years, with no events in negative males (p=0.0001). Median age at first hospitalisation for a cardiac indication was 43 years (95% CI 38 years-47 years), with 43% of positive males hospitalised before age 40 years, compared with 11% of negative males (RR 10.2 (95% CI 3.9-26.4) p=0.0001). Of the six negative males hospitalised, three were for investigation of chest pain, one following an MI, one with ectopy and one at age 15 years following syncope.

Median survival in positive males was 45 years (95% CI 38-52), with one death in the mutation negative group (p=0.001). Using a strictly conservative ascertainment strategy, incorporating those with a medical record, where positive status was defined by genetic means only (having the disease mutation or being an obligate carrier), and where all subjects in a sibship had a known disease status (100% ascertainment), mortality was similar (median survival 42 years, 95% C I 38-46) (Figure 18 page 122).

Figure 18: Cumulative incidence of cardiac symptoms or outcomes in positive males: temporal sequence.

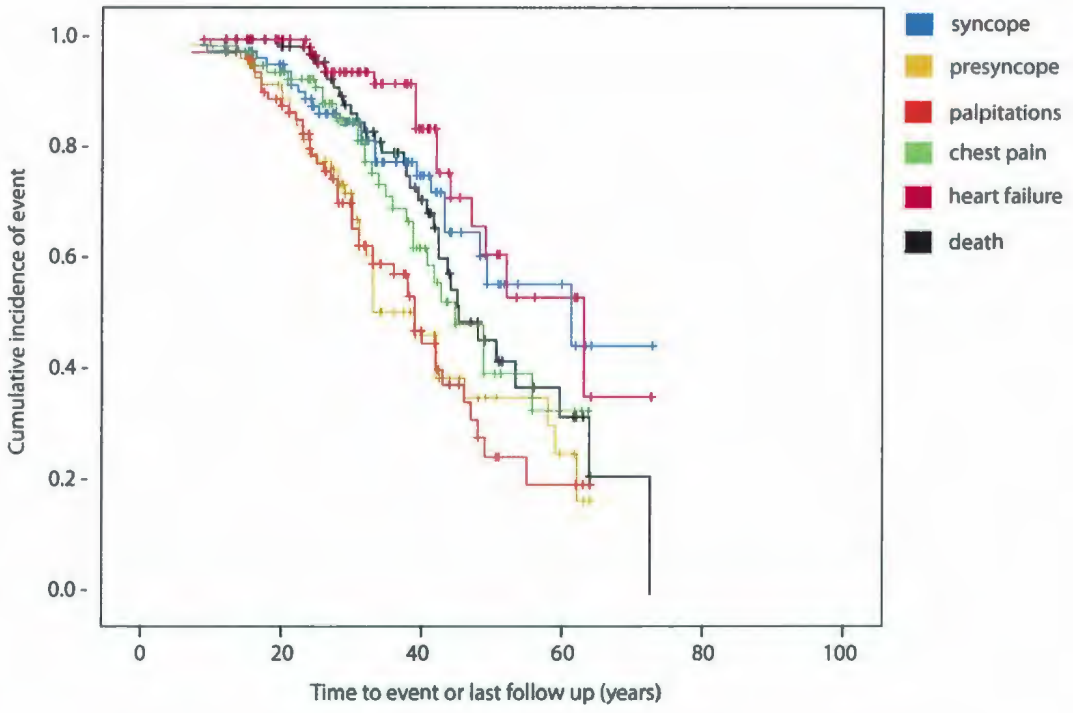


Table 16: Cumulative percentage with symptom or outcome defined by age group.

	Age groups: affected males (percentage)							
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	70+
Presyncope	2	10	28	53	65	75	81	
Syncope	1	5	15	25	44	44	56	
Palpitations	3	12	29	53	85	80		
Chest Pain	0	6	15	38	61	67		
Heart Failure	0	0	6	16	39	46	46	65
Hospitalisation following a clinical event *	0	0	18	43	79	95		
Death	0	1	13	29	54	68	79	100

* Not shown on graph

Clinical manifestations of cardiac disease in females

Table 10 page 113 and Figure 19 page 124 summarise the incidence of cardiac symptoms and events in 85 positive and 68 negative females with medical records. Rates were higher for all events in the positive group. Presyncope and heart failure showed a trend towards significance but only syncope and palpitations reached statistical significance. Positive females were 4 times more likely to have syncope (p=0.006) and 2 times more likely to have palpitations (p=0.02) than negative females. A greater proportion of positive females (n=21, 24%) were admitted to hospital for cardiac problems than negative females (n=6, 9%) (ns). Of the latter, three were for chest pain, one for Wolf-Parkinson-White syndrome, and one at age 12 years with palpitations. There were more deaths in positive females than negative females, but this did not reach significance.

Comparison of positive males and positive females

Males were significantly more likely to have had presyncope p=0.007; RR 1.9 (95% CI 1.2-9), chest pain p=0.04, RR 1.8 (1.03-3.1) and heart failure (p=0.002); RR 5.2 (95% CI 1.8-14.5) than females (Table 10 page 113). The temporal sequence from cardiac symptoms to death was compressed in males but more prolonged in females by 1-2 decades (Fig. 1 and Fig. 3). Males were 4 times more likely to be hospitalised, p=0.0001, RR = 4.4 (95% CI 2.3-8), and died significantly earlier than females (p=0.001) (RR=7.4, 95% CI 3.3-16.2). These mortality results were similar using the conservative ascertainment strategy, where those analyzed included only those in whom the diagnosis was made using genetic data and where all siblings had known disease status (p=0.001) (RR=9, 95% CI 2.5-32) (Figure 20 page 125).

Figure 19: Cumulative incidence of cardiac symptoms or outcomes in positive females: temporal sequence.

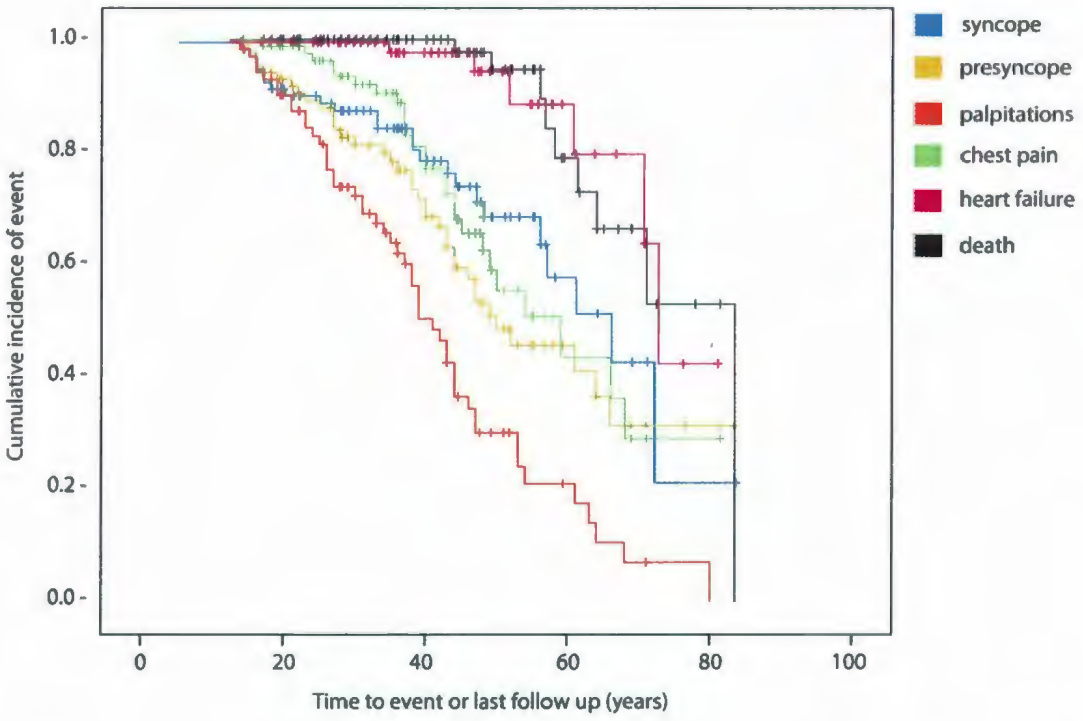
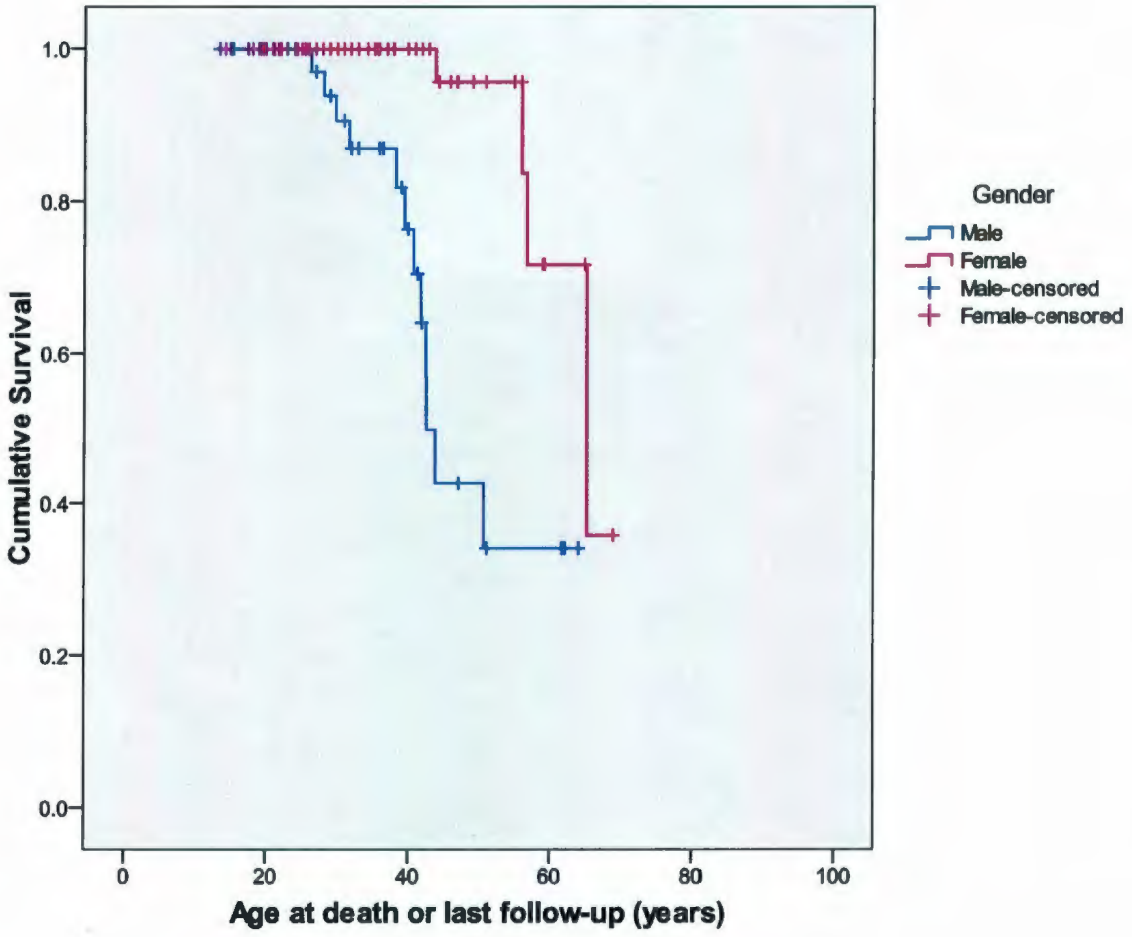


Table 17: cumulative percentage with symptom or outcome defined by age group.

	Age groups: affected females (percentage)							
	0-10	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**70+**
Presyncope	0	7	19	38	52	54	69	69
Syncope	0	8	12	21	31	42	57	88
Palpitations	0	10	26	49	70	79	93	100
Chest Pain	0	1	7	19	41	57	71	71
Heart Failure	0	0	0	2	6	11	20	58
Hospitalisation secondary to clinical event*	0	0	6	14	25	40	66	82
Death	0	0	0	0	6	21	34	100

* Not shown on graph

Figure 20: Cumulative survival in positive males and females, censored at ICD, heart transplant or last follow-up, in subjects defined by genetic means (mutation analysis or obligate carrier status), and where all siblings in a sibship had a known disease status.



12-lead ECG

Three hundred subjects (167 positive, 133 negative) had at least one 12-lead surface ECG available. The most prevalent features were poor R wave progression (PRWP: also septal Q waves; a subset of PRWP), the presence of PVC's, and extended QRS (Table 11 page 115). T wave inversion in leads V2-V3, and epsilon waves were noted in $\leq 4\%$ of positive subjects. Of the positive subjects with no ECG available for analysis, 86% were dead.

Serial ECGs were available in 84 (50%) positive and 84 (63%) negative subjects and in these we assessed the incidence of PRWP and PVCs (Table 12 page 116). PRWP in the anterior chest leads was identified at baseline in 48 (29%) positive subjects (Table 11 page 115). Twenty-five positive subjects without PRWP at baseline subsequently developed *de novo* PRWP. Two negative females had PRWP at baseline: two negative females developed PRWP on a subsequent ECG. The median age to onset of *de novo* PRWP for positive males was 55 years ; 16% had PRWP by age 30 years, 30% by age 40 yrs and 69% by age 60 years (Fig. 4). The median age to onset of *de novo* PRWP for positive females was 58 years; none had PRWP by age 25 years, 9% by age 40 yrs and 69% by age 60 years (Fig. 5). Positive males were twice as likely to develop PRWP as clinically affected and/or mutation positive females (RR 2.3 (95% CI 1.1-5.2) (Table 12 page 116).

PVCs in any of the 12 leads on the ECG were identified at baseline in 47 (28%) positive subjects. Twenty two positive subjects without PVCs at baseline subsequently developed PVCs. One negative female had one PVC at baseline: two negative females and one negative male developed PVCs on a subsequent ECG. The median age to onset of *de novo* PVCs for positive males was 48 years; 6% had PVCs by age 30 years, 27% by age 40 yrs and 100% by age 60 years (Fig. 4). The median age to onset of *de novo* PVCs for positive females was 77 years; none had PVCs by age 25 years, 12% by age 40 yrs and 25% by age 60 years (Fig. 5). Positive males were four times as likely to develop PVCs on 12 lead ECG as positive females (RR 3.7 (95% CI 1.5-9.3) (Table 12 page 116).

Echocardiography

Two hundred and forty eight subjects (144 positive and 104 negative) had at least one 2D echocardiogram available for analysis. Five reports without available measurements were excluded, four of which (3 males and 1 female, all deceased) were reported to have a 'massively dilated heart'. Of the positive subjects with no echocardiogram, 85% were dead. The most prevalent feature was LVE 112% (2SD above the mean) based on LVEDD (Table 13 page 118) (203).

Fifty-four positive and 42 negative subjects who showed no LVE at baseline had serial echocardiograms. Sixty-two of the 144 positive subjects had LVEDD >2SD at baseline (LVE). Twenty-four (44%) positive subjects without LVE at baseline subsequently developed LVE. The median age to onset of *de novo* LVE for positive males was 45 years ; 14% had LVE by age 30 years, 42% by age 40 yrs and 52% by age 60 years (Figure 21 page 129). The median age to onset of *de novo* LVE for positive females was 51 years; none had LVE by age 30 years, 4% by age 40 yrs and 69% by age 60 years (Figure 22 page 130). There was no significant difference between positive males and positive females for time to development of LVE (Table 12 page 116).

Seven males (10% of all positive males who had an echocardiogram) and nine females (12% of all positive females who had an echocardiogram) fulfilled criteria for DCM (279). Six positive male subjects with marked LVE and systolic dysfunction received a heart transplant and one died while waiting for a transplant.

Although RV echocardiography was not formally assessed, RV thinning/bulges or dilatation was reported in 12 of 68 (18%) of positive males, and 6 of 76 (8%) females, as well as in six (2 m,4f) of 104 mutation negative subjects (Table 13 page 118).

Holter Monitor

Two hundred and forty three subjects (146 positive, 97 negative) had at least one Holter monitor available for analysis. Of the positive subjects with no Holter Monitor, 82% were dead. The most prevalent feature was ≥ 200 PVCs over 24 hours (Table 14 page 118).

Thirty one positive and 40 negative subjects who did not have ≥ 200 PVCs at baseline had serial Holter monitors. Ninety four (47 male and 47 female) had ≥ 200 PVCs on first Holter monitor (Table 14 page 118). Fifteen (48%) positive subjects without ≥ 200 PVC's at baseline subsequently developed ≥ 200 PVCs. The median age to onset of *de novo* ≥ 200 PVCs for positive males was 25 years ; 64% had ≥ 200 PVCs by age 30 years (Figure 21 page 129). The median age to onset of *de novo* ≥ 200 PVCs for positive females was 48 years ; 9% had ≥ 200 PVCs by age 30 years, 10% by age 40 yrs and 62% by age 60 years (Figure 22 page 130).

Eighty two positive subjects (45 m, 37 f) had ≥ 1000 PVCs on first Holter monitor (Table 14 page 118). There were 14 incident cases in the positive group. In the negative group, 2 females developed ≥ 1000 PVCs. There were significant differences between positive and negative males (p=0.0001), positive and negative females (p=0.02) and between positive males and positive females (p= 0.003, RR 5.4 95% CI 1.8-9.2) (Table 12 page 116).

Figure 21: Cumulative incidence of clinical signs on 12 lead ECG, Holter monitor and echocardiogram in positive males: temporal sequence.

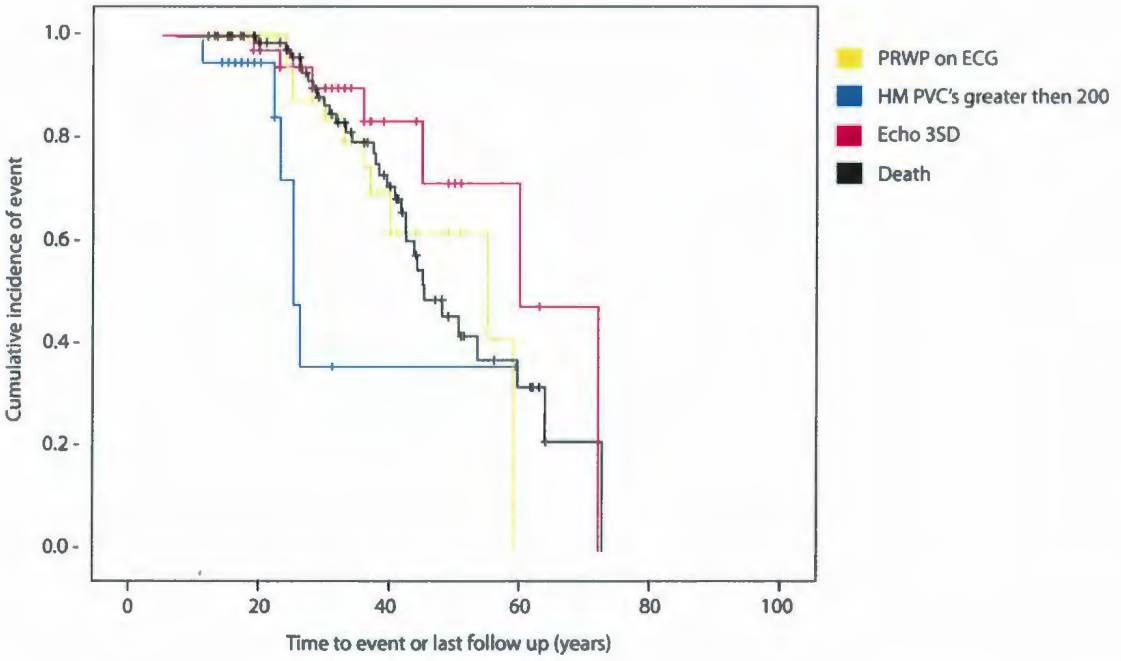


Table 18: cumulative percentage with clinical sign defined by age group.

		Cumulative percentage by age in positive males							
Test	**Feature**	**<20**	**<30**	**<40**	**<50**	**<60**	**<70**	**<80**	**<90**
12 lead ECG	**PRWP**	0	16	30	38	69	100		
	PVC *	2	6	27	100				
Holter Monitor	**>200 PVCs**	5	64						
	> 1000 PVCs *	5	60						
Echo	**>2SD LVE***	4	14	42	52	52	68	100	
	> 3SD LVE	3	11	17	39	39	53	100	

* Not shown on graph

Figure 22: Cumulative incidence of clinical signs on 12 lead ECG, Holter monitor and echocardiogram in positive females: temporal sequence.

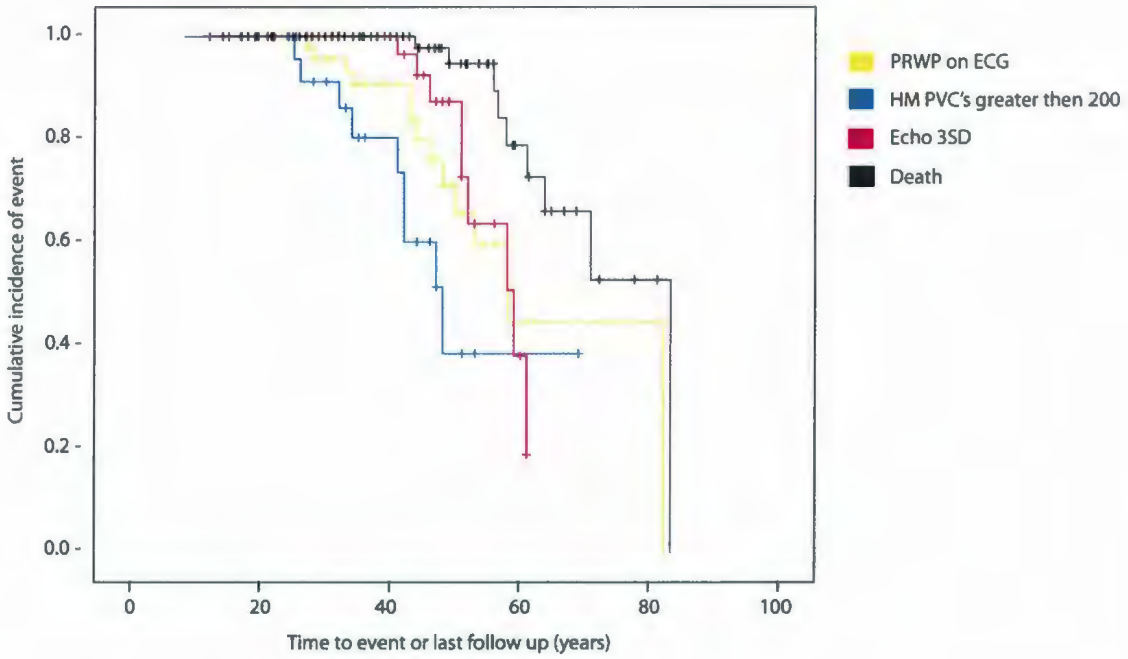


Table 19: Cumulative percentage with clinical sign defined by age group.

		Cumulative percentage by age in positive females							
Test	**Feature**	**<20**	**<30**	**<40**	**<50**	**<60**	**<70**	**<80**	**<90**
12 lead ECG	**PRWP**	0	4	9	29	66	66	100	
	PVC*	0	6	12	15	25	25	66	100
Holter Monitor	**>200 PVCs**	0	9	10	62				
	> 1000 PVCs*	0	3	12	35	51			
Echo	**>2SD LVE***	0	0	3	33	68	84		
	> 3SD LVE	0	0	0	13	62	81		

*Not shown on graph

SAECG

Two hundred and two subjects (98 positive, 104 negative) had at least one SAECG available for analysis. Extended QRS, and the presence of 2/3 of the SAECG parameters abnormal were both significantly different between positive and negative subjects. The development of SAECG abnormalities was more common however in negative than positive males (Table 12 page 116), and there was no significant difference between the positive and negative groups in the Cox regression analysis.

Diagnostic Utility

A likelihood ratio of above 10 is considered the level at which a test is diagnostically robust. The LR's of having ARVC are all less than four for all cardiac manifestations with the exception of males with heart failure where the LR is 17. For clinical testing results, PRWP and PVCs on ECG (in males) and PVC's (>200 and >1000) on Holter monitor for males and females are all well above 10. The likelihood ratios of not having inherited ARVC are greater than 0.2 for each manifestation and for each clinical test result, suggesting that the absence of clinical symptoms or the absence of the test outcomes as presented are inadequate to rule out the diagnosis of ARVC (Data not shown).

3.5 DISCUSSION

This paper reports the natural history, and the phenotype of ARVC caused by a founder mutation in TMEM43, where clinical manifestations of disease in positive and negative family members were compared.

3.5.1 Natural history

Results for negative family members helped to determine disease associated cardiac symptoms. In positive males, the phenotype was malignant, with early age of onset of cardiac symptoms followed soon after by death. A more benign phenotype was observed in positive females with a later age of onset of symptoms, less frequent hospitalisations, and longer survival. Other than syncope and palpitations, there was no difference in symptoms between negative and positive females. The development of cardiac symptoms in either sex had no utility in determining those who did, and did not, inherit ARVC.

Heart failure and chest pain were late manifestations of disease in positive males and females, occurring in subjects who did not succumb to malignant arrhythmia. The data suggest that heart failure will occur in a majority of male subjects with this *TMEM43* missense mutation whose lives have been prolonged following ICD therapy.

Despite methodological and sampling differences, our results are similar in magnitude to those reported in other studies of ARVC, including 26 subjects with mutations in desmoplakin (ARVD8) (280), and within a heterogeneous group of 130 affected individuals (281). The lack of a comparison group however in these studies make comparisons problematic.

3.5.2 Clinical features on cardiac testing

The phenotype of this form of ARVC includes (i) ECG manifestations of arrhythmogenic disease (PVCs), ECG features of presumptive conduction disease (PRWP and septal Q waves), as well as prolonged QRS, also delineated on SAECG; (ii) echocardiographic manifestations of LVE, (iii) frequent early ventricular ectopy on Holter monitor, at an early age in males, and (iv) a high risk of SCD at a young age in males.

This genetic subtype of ARVC has both similarities and differences to more typical ARVC. Absent are T wave inversions in the anterior precordial leads, and epsilon waves (25), but PRWP in the precordial leads was present in almost half of all positive subjects on first ECG. Anterior myocardial infarction can cause PRWP (277), although angiography in several subjects was negative. The mechanism by which PRWP occurs is unknown but we speculate that fibrous tissue in the septum may produce a pattern similar to that following an anteroseptal infarct a suggestion supported by the presence of septal Q waves. The presence of PRWP in 2% of negative subjects may reflect the polymorphic nature of this finding in the general population, with reports of its presence in up to 10% of hospitalised adult patients having a routine ECG (282).

This genetic subtype of ARVC showed substantial LV involvement. While there is clear evidence for LVE in ARVC (266, 267), DCM with LVE and systolic dysfunction meeting strict DCM criteria (15) is not often reported. However, a study of a heterogeneous group of ARVC patients did note the occurrence of congestive heart failure (281). In the current study, the LVE was not restricted to a single family or set of siblings, reducing the likelihood of familial clustering. Although LVEDD > 2 or 3 SD

above the mean is not diagnostic of DCM, LVE may be an early phase of a dilating heart as it progresses to depressed ejection fraction in relatives (283, 284).

Substantial ectopy occurred and was the earliest clinical feature noted on testing on both Holter monitoring and 12 lead ECG. As these tachyarrhythmias are considered responsible for SCD in young people, it provides clinical evidence supporting ventricular arrhythmia as a cause of the early deaths.

The reduction in survival, particularly in males is a major concern. Previous studies of genetically heterogeneous families with ARVC have reported better survival than observed in our families (170), although the consensus has been from the earliest reports that males have a worse survival than females (12). In this study of ARVC linked to 3p25, the sex influence on mortality was mirrored in the earlier time to development of PRWP, LVE, and ectopy in positive men compared to positive women.

3.5.3 Limitations of the study

Describing the natural history of an uncommon inherited disease, which is difficult to diagnose and associated with early death, is challenging. Limitations inherent in these studies relate to (i) study design, (ii) definition of affected status,(iii) ascertainment bias, (iv) survivor bias and (v) intervention bias.

(i) Subjects in this cohort study were assessed in both a retrospective and prospective manner. The same consistency of assessment therefore may not have occurred in the group studied retrospectively, where data was abstracted from clinical records, and where there is almost certainly missing data. Despite this, there were few differences in results

between those subjects ascertained in a retrospective manner and those who were also assessed in clinic and followed prospectively. The results therefore, for all but one symptom (palpitations in females) were analysed together.

(ii) Defining affected status using the occurrence of a major clinical event (SCD or resuscitation for VT before 50 years) could introduce biases particularly through identification of those at the more severe end of the disease spectrum. However, exclusion of those defined by the clinical event criteria and including only those defined by genetic criteria did not alter the conclusions: this form of ARVC is associated with early death in males.

(iii) Ascertainment bias is unavoidable in family studies of inherited disease but we tried to limit this by analyzing only subjects in well ascertained sibships, where the disease status of at least 50% of siblings was known. The fact that there were more male than female subjects in the study may indicate some residual bias in ascertainment where the severity of the disease in males may have increased the likelihood of identifying positive males.

(iv) Survivor bias is relevant to this study as a substantial number of positive males died prior to presentation to the health care system and had no clinical record available. However, median survival of positive males with no medical records was similar to those with medical records supporting the validity of the overall results. It is possible that the clinical features observed may relate more to the male survivors in families who may be expressing a milder form of the disease. Prospective studies with younger subjects will help to address this important issue.

(v) We have demonstrated that ICD treatment, as a primary or secondary preventative intervention significantly improves survival in males (248).Intervention bias was controlled by censoring at the time of ICD implant. We were unable to assess in any meaningful way the possible impact of drug treatment on clinical outcomes due to the multiplicity of drugs used and the fact that positive subjects were treated over the course of several decades, with changing regimes.

PRWP can occur due to lead misplacement, patient obesity, LV hypertrophy and Wolff Parkinson White syndrome (WPW) amongst others, and can also be a normal variant (285). In our study, most of the 12-lead ECGs showing PRWP were repeated several times, the observed pattern was not restricted to the obese, LV hypertrophy was not present on echocardiography and WPW was excluded on ECG. We used the clinical criterion of less than 3mm in V3 from ECGs read in a blinded manner as a measure of PRWP, which does not take amplitude into account. However, this potential bias would be the same across both positive and negative groups.

Despite ARVC associated pathology and the fulfilment of diagnostic criteria in the probands in each family, formal echocardiographic assessment of RV structure was not done. Consequently, only limited conclusions can be made on the presence of abnormalities in the right ventricle.

3.5.4 Advantages of the study

For most subjects we were able to determine the presence of ARVC using genetic data, (the *TMEM43* 1073 C>T mutation, or being an obligate carrier), a diagnostic strategy independent of clinical manifestations. This large genetically homogeneous population

was a robust resource in which to investigate this form of ARVC, made stronger by the use of a negative group of family members, and the presence of medical records from extant and deceased subjects from which disease manifestations were carefully and consistently classified. We have serial assessments of both positive and negative subjects from regular visits to a genetics/cardiomyopathy clinic, where subjects were screened prior to a genetic diagnosis. These data provide necessary information for genetic counselling and management of the disease in at-risk subjects. The advantage of studying large families with a founder haplotype and deep genealogies is offset by the fact that these findings may not be generalisable to other forms of inherited ARVC. However, it is likely that in the future, ARVC and other cardiomyopathies will be classified by their molecular genetic etiology, thus disease specific genotype/phenotype information will become the most useful and relevant. It is also likely that mutations in TMEM43 will be identified in other populations as a cause of ARVC.

3.6 CONCLUSIONS

Disease related symptoms of this genetic subtype ARVD5 caused by a mutation in TMEM43 include palpitations, presyncope and syncope which tend to occur at a young age, particularly in positive males in whom onset of symptoms is often closely followed by death. Hospitalization for cardiac disease, heart failure and SCD were significantly more common in positive males than positive females, and occurred at a younger age. Heart failure in positive males presented as a late manifestation in survivors. This phenotype includes manifestations of both arrhythmogenic and cardiomyopathic disease, is more malignant in males than females, frequently exhibits PRWP in the precordial leads and ventricular ectopics on ECG, has features of left ventricular disease with LVE

which can lead to DCM, and ectopy on Holter monitor at an early age is a reasonably sensitive diagnostic test for both sexes.

4 THE IMPACT OF IMPLANTABLE CARDIOVERTER DEFIBRILLATOR THERAPY ON SURVIVAL IN AUTOSOMAL DOMINANT ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVD5)

"Medical science has proven time and again that when the resources are provided, great progress in the treatment, cure, and prevention of disease can occur."

Michael J. Fox

"Extreme remedies are very appropriate for extreme diseases."

Hippocrates of Iphicrates

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4.1 SUMMARY

Objective

To determine the impact of Implantable Cardioverter Defibrillator (ICD) therapy in an autosomal dominant form of arrhythmogenic right ventricular cardiomyopathy (ARVC).

Background

ARVC is a cause of ventricular arrhythmias causing sudden cardiac death (SCD), which may be prevented by ICD.

Methods

We studied 11 ARVC families, where a 3p25 DNA haplotype (ARVD5) segregates with disease and compared mortality in subjects with an ICD to that in a control group matched for age, sex, ARVC risk status, and family. 367 subjects at 50% a-priori risk of inheriting ARVC were classified as high risk (HR=197), low risk (LR=92) or unknown (UK=78) based on clinical events, DNA haplotyping, and/or pedigree position. Forty-eight HR subjects (30 males, [median age 32 years] and 18 females [median age 41 years]) were followed after ICD implantation (secondary to documented ventricular tachycardia (VT) in 27%). Survival was compared to 58 HR historical control subjects who were alive at the same age to-the-day at which the ICD subject received the device.

Results

In the HR group (n=197), 50% of males were dead by 39 years, and of females by 71 years, a relative risk of death of 5.1 (95% CI 3-8.5) for males. SCD's occurred in 62 (76%) males and 7 (33%) females. The 5-year mortality rate for males with an ICD was

zero compared to 28% in controls (p=0.009). Within 5 years, the ICD fired for VT in 70%, and for VT >240 beats per minute in 30%. There was no difference in rate of discharge when analysed by indication for ICD.

Conclusions

The unknown mutation at the ARVD5 locus on chromosome 3 causing ARVC in this population results in high mortality. Risk stratification, including genetic haplotyping, led to ICD therapy that resulted in substantially improved survival for males.

4.2 INTRODUCTION

Treatment of ventricular tachyarrhythmias, which often cause sudden cardiac death (SCD), includes antiarrhythmic drugs and implantable cardioverter defibrillator (ICD) therapy. Several randomized control trials have compared these treatment modalities (223, 224, 226, 228) and shown that anti-arrhythmic medications have limited effectiveness (286). ICD therapy is therefore often now considered the treatment of choice for ventricular tachyarrhythmias (222). ICD therapy is safe; rare complications include infections (230), surgical complications (231) electrode problems (239) and inappropriate shocks (232).

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a cause of SCD, usually due to tachyarrhythmias (287), for which ICD therapy may be effective. ARVC may be a significant cause of SCD in young adults in the USA (158),and Italy (105) although its true prevalence is unknown (156). Diagnostic criteria are based on clinical, structural and functional features with the gold standard for diagnosis being the presence of fat and fibrous tissue on cardiac biopsy or autopsy (19, 25, 173). However, antemortem diagnosis is difficult despite investigations including 12-lead electrocardiogram (ECG), signal averaged ECG (SAECG), echocardiography, magnetic resonance imaging (MRI), and Holter monitoring (HM). These tests are non-specific, particularly as the true penetrance of the disease is not clear and variable expression is present both between and within families (19).

ARVC is recognized to be familial (16-19, 21, 23, 288) in an estimated 30% of cases (24, 25). However, comprehensive assessments of extended family trees are rarely documented (12), and familial cases are likely under recognized (142). Known causes of autosomal dominant ARVC include mutations in the cardiac ryanodine receptor (*RYR2*) (36, 83) and desmoplakin (52) genes. Other AD ARVC loci include regions on chromosomes 2, (40), 3 (4), 10 (47, 49) and 14 (31, 38). It is clear that ARVC is a genetically heterogeneous group of diseases with phenotypic overlap (151). The number of ARVC subjects in previous studies defined by genotype is small (22, 31, 32, 34-36, 38, 40, 47, 49, 52, 170).

A Newfoundland family with autosomal dominant ARVC was reported in 1988 (3). The diagnosis of ARVC was made following post mortem documentation of fat and fibre in the myocardium, and clinical features fulfilling published criteria (25). ARVC in this family was subsequently linked to the short arm of chromosome 3 encompassing a critical region of 9.3cM (4) at 3p25 (ARVD5). Subsequently, ten additional ARVC families were studied and the critical region was reduced to 2cM, at which locus the identical set of DNA markers (haplotype) co-segregates with ARVC. This segregation with disease across generations suggests a common founder in these 11 Newfoundland families (251) and provides by far the largest group of subjects with ARVC defined by genotype. Risk stratification of subjects by ARVC status is therefore feasible in these families, based upon family history, clinical history and genetic haplotype (linkage analysis) data: a methodology used clinically in several genetic disorders prior to determining an underlying causative mutation (289, 290).

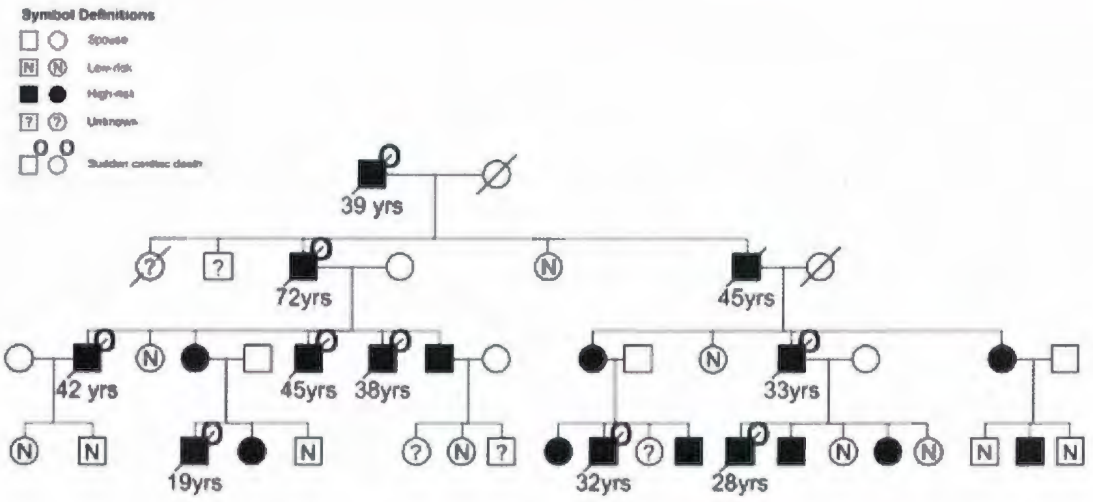
Previous studies of ICD therapy in ARVC retrospectively assessed efficacy in individual patients in the absence of appropriate controls (236-239, 291). We investigated mortality after implantation of ICD's in 48 high risk (HR) subjects from 11 families with an autosomal dominant homogeneous form of ARVC linked to 3p25, and compared mortality to that in HR control subjects, matched for age and sex from the same families.

4.3 METHODS

4.3.1 Sample population

Thirty-five families were referred to the provincial genetics program because of SCD and a family history of cardiomyopathy. Eleven were determined to have autosomal dominant ARVC (Figure 23 page 146) based on established histopathological, clinical and genetic criteria (25). One of these was the original family in which linkage to 3p was defined (4)(OMIM: http://www.ncbi.nlm.nih.gov/omim/), and 10 other families were found to be linked to the same region. Five hundred and fifty one individuals from extant and ancestral generations from these 11 families who were born at ≥ 50% pedigree risk (ARVC affected individuals and their first degree relatives) were identified. To minimize the effects of ascertainment and referral bias 367 subjects (212 male, 155 female) from 'well-ascertained' sibships were studied. Well ascertained sibships were defined as those where ≥ 50% of siblings were known to be at high or low risk of ARVC based on clinical, pedigree and/or haplotype data. High-risk (HR) was defined as documented sustained ventricular tachycardia (VT) or otherwise unexplained SCD < 50-years, the presence of a HR DNA haplotype and/or obligate carrier status (affected offspring and parent) by pedigree analysis. Subjects could be defined based on any or all of these criteria for HR status. Low-risk (LR) was defined as LR DNA haplotype. Otherwise subjects were considered unknown risk status (UK). The terms HR and LR are used because the common disease haplotype is assumed to co-segregate with the as yet unknown disease-causing mutation.

Figure 23 One of 11 autosomal dominant ARVC families linked to 3p25 showing family members at high-risk, low-risk, and unknown status



Consent for analysis of past and current cardiology records and for DNA analysis was obtained from each subject (or surrogate) in compliance with the Human Investigation Committee requirements of the Health Care Corporation of St. John's. All available clinical records and autopsy reports were obtained.

A cardiomyopathy genetics research clinic was initiated in 1998. Serial data (retrospective and prospective) on subjects at 50% risk were collected (including clinical events, 12-lead ECG, signal averaged ECG (SAECG), Holter monitor, echocardiography and MRI) and subjects were offered genetic haplotype analysis. ICD therapy was offered to these subjects following a HR DNA haplotype and an abnormal clinical test compatible with ARVC (n=15 males and 18 females). None of these subjects had documented VT. Two more males had an ICD following a HR DNA result alone. Seven males prior to 1998, and 6 males implanted in other North American centres had ICD's

because of sustained VT requiring cardioversion. No individual to date has declined an ICD, although one HR subject refused follow-up and died suddenly aged 31 years. One subject died 6 weeks after first attending clinic, during clinical work-up, and prior to receipt of a HR haplotype result. Thus we report on the outcome of 30 male and 18 female subjects with ICD's, 73% for primary prevention of VT.

All ICD recipients were followed bi-annually at which time the ICD was interrogated. After confirmation of sustained ventricular tachyarrhythmias confirmed by the stored electrocardiograms following ICD discharge, time to first appropriate discharge for documented VT was recorded, as was time to first VT > 240 bpm. A rate of 240 bpm was chosen to delineate potentially fatal arrhythmias (in the absence of the ICD) from probable non-fatal arrhythmias (292, 293). ICD discharge was not included if it occurred within one week of the implant date. Inappropriate ICD discharge was defined as atrial fibrillation, sinus tachycardia or non-sustained VT.

4.3.2 Haplotype analysis

Of 367 subjects from well ascertained sibships, 201 (55%) had a venous blood sample collected. DNA was extracted from peripheral lymphocytes as previously described (294). For each polymorphic short tandem repeat (STR) DNA marker, fluorescent labelled primers were used to amplify di-, tri-, or tetra nucleotide repeats by the polymerase chain reaction, and fragments were analyzed by capillary gel electrophoresis, using an ABI 310 genetic analyzer (Applied Biosystems). Presence or absence of the 2cM HR haplotype was determined for each family member assessed.

4.3.3 Study design

Age to death was determined in the initial cohort of 197 HR, 92 LR and 78 UK subjects from the 11 ARVC families, with censoring at last follow-up or at time of ICD implantation. Survival following ICD was determined, and compared with that of control subjects following entry to the study. For each ICD subject, control HR subjects were selected who did not receive an ICD, were the same sex, were alive at the age to-the-day at which the ICD subject received the ICD, and were 1st or 2nd degree relatives of the ICD subject. All available 1st and 2nd degree relatives meeting these criteria were used as controls (n=40; 69%). If there were no age/sex matched 1st or 2nd degree relatives, one 3rd or 4th degree relative was used (n=11; 19%). In the absence of any appropriate relatives, one age and sex matched control subject from a family in the same geographical region was used (n=7; 12%).

The primary outcome was age at death defined to the day. Secondary outcomes were time to initial appropriate ICD device discharge for sustained VT, and time to first VT >240 bpm. Time to first appropriate discharge was compared in those who had ICD for primary versus secondary prophylaxis, and in those with, versus those without left ventricular enlargement (LVE) based on the Henry calculations defined as 2 SD above the mean for LVE (112%) (202, 203).

Statistical analysis

We used version 11.5 of the SPSS statistics package for statistical analyses of clinical tests (SPSS, Chicago USA). Categorical data were compared by chi-squared analyses. A p-value of <0.05 was considered significant. Survival was calculated according to the

Kaplan Meier product limit method. Relative risk was calculated using Cox's Regression model. Follow-up continued until death or June 3 2003.

4.4 RESULTS

An affected only linkage analysis was done in the first large Newfoundland family described (3). Of 17 affected individuals who had DNA samples available for molecular analysis, 11 manifested symptomatic sustained VT before 50 years of age, one demonstrated ARVC pathology at autopsy, and 5 were obligate ARVC disease gene carriers. Twenty-one deceased family members who transmitted ARVC through five generations were also considered obligate carriers. Linkage of disease to 3p25 was confirmed with a maximum multi-point LOD score of 9.3 at D3S1585. Analysis of 8 markers allowed the construction of a chromosome 3p25 HR haplotype with D3S3610-Fibulin2-D3S2385-D3S1585-D3S1554-708d1CA-316A10CA-D3S3613 ordered from telomere to centromere shared by all affected family members. The HR haplotype was also identified in all affected subjects from ten other families with autosomal dominant ARVC.

As shown in Table 20 page 150, 197 (54 %) of the well ascertained sample was designated HR. The method of determining risk-status in men was approximately evenly split between pedigree position (33 %), DNA haplotype (31 %) and subjects with SCD or VT < 50 years (36 %). Few women were diagnosed as HR because they had SCD or VT. Two hundred and seven (56%) of the 367 subjects were born after 1950. Most HR male subjects, but only about one quarter of HR female subjects had died. 76% of male and 39% of female deaths were due to SCD. Only 4 LR subjects died, compatible with

survival being necessary to obtain a blood sample for DNA tests (Table 20 page 150). It is likely that the majority of those of UK status are in fact LR.

Table 20 Demographic data, method of risk determination and cause of death in 367 subjects at an a-priori 50% risk of inheriting ARVC, from 11 families linked to 3p25: defined by ARVC risk status

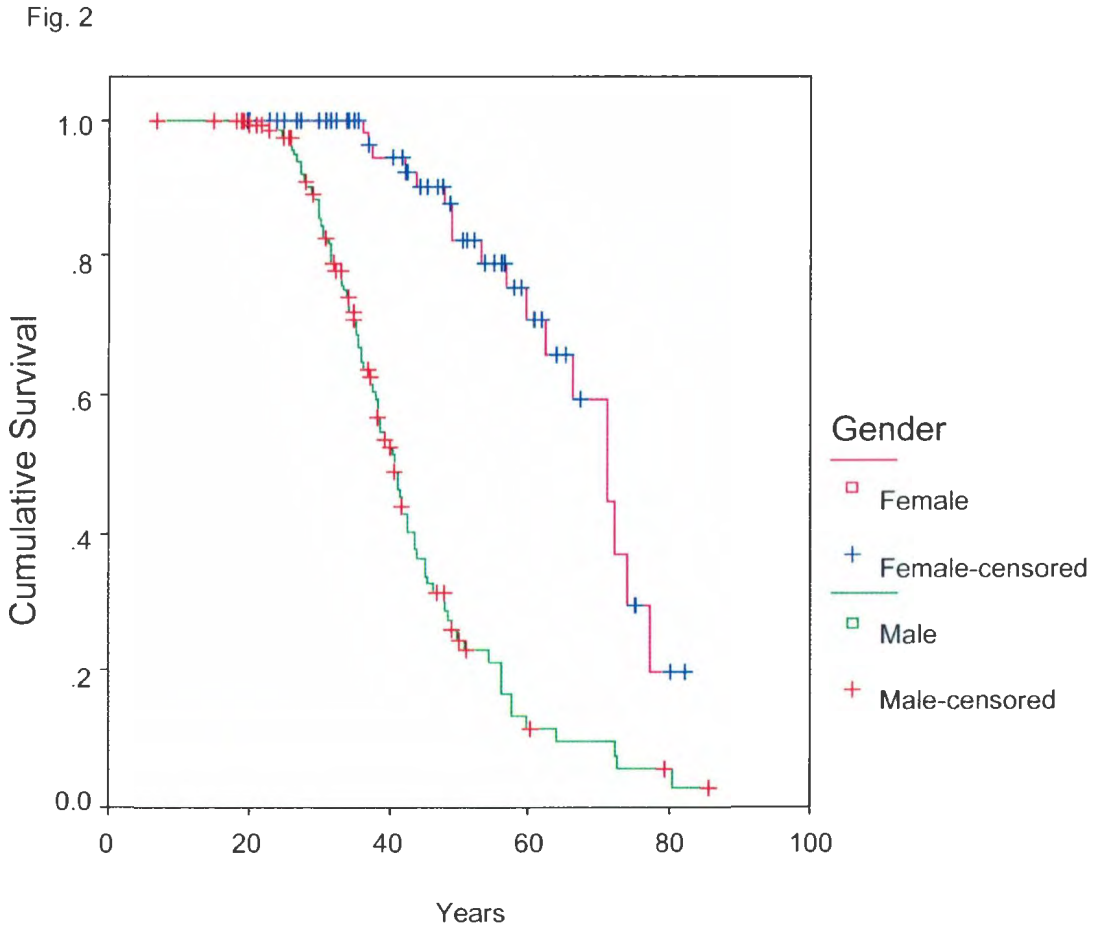
	High Risk N = 197				**Low Risk N = 92**				**Unknown N = 78**			
	M N=123		F N=74		M N=46		F N=46		M N=43		F N=35	
	N	%	N	%	N	%	N	%	N	%	N	%
Born												
< 1950 (n = 160)	61	50	32	43	10	22	13	28	27	63	17	49
≥ 1950 (n = 207)	62	50	42	57	36	78	33	72	16	37	18	51
Methods of diagnosis												
Obligate carrier (OC)	41	33	30	41	-	-	-	-	-	-	-	-
DNA haplotype	38	31	41	55	46	100	46	100	-	-	-	-
Clinical event (SCD or VT)	44	36	3	4	-	-	-	-	-	-	-	-
Dead	82	67	18	24	1	2	3	6	19	44	11	31
SCD	62	50	7	9	-	-	-	-	1	2	-	-

Overall mortality in the ARVC population.

Median survival in HR males (n=123) was 39 years (95% CI 36-42), in LR males (n=46) 76 years (95% CI 76), and in unknown males (n=43) 67 years (95% CI 63-71). Median survival for HR females (n=74) was 71 years (95% CI 64-78), for LR females (n= 46), median age could not be computed because of the lack of events, and UK females (n=35) 83 years (95% CI 51-115 years). The mortality rate was significantly greater in HR than LR males (p=0.00001), and HR and LR females (p=0.01). The youngest age at death in HR males was 19 years, and the oldest 72 years. In HR females the respective ages were 37 and 88 years. Female HR subjects survived significantly longer than male HR subjects (p=0.00001) (Figure 24 page 151). The RR of death for males compared to females in the HR group was 5.1 (95% CI 3-8.5). We also assessed mortality in subjects born before and

after 1950 to take into account possible effects of differences in risk status determination: results remained the same (data not shown).

Figure 24 Time to death or last follow-up in high-risk males and females with ARVC linked to 3p25



4.4.1 Subjects studied

There were 30 males who received ICD's, at median age of 33 years, [range 15-51years] and 18 females at median age of 42 years, [range 22-56 years]. ICD's were implanted in 17 males and 18 females in the absence of documented VT (primary prophylaxis) and in the remaining 13 males following VT (secondary prophylaxis). Fifty eight control subjects comprised 36 males whose median age at study entry was 33 years, [15-51

years], and 22 female subjects whose median age at study entry was 39 years, [22-56 years] (Table 21 page below).

Table 21 Demographic data and method of risk determination of ARVC in 48 subjects at high risk who received an ICD and 58 matched controls from 11 families with ARVC linked to 3p25

Demographic Parameters	**ICD Cohort**				**Control Cohort**				**ICD M+F**	**Control M+F**	**p value ***
	Males N=30		Females N=18		Males N=36		Females N=22		48	58	
	N	%	N	%	N	%	N	%			P*
Born < 1950	2	6	1	5	25	69	19	86	3	44	<0.0001
Born ≥ 1950	28	93	17	95	11	31	3	14	45	14	
Diagnosis											
OC	4	13	4	50	18	51	17	49	8	35	<0.0001
Haplotype	22	74	14	39	5	50	5	50	36	10	<0.0001
SCD or VT	4	13	0	0	13	10	0	0	4	13	<0.05
In years											
Mean AAE	33.3		40.3		32.2		40				ns
Median AAE	32.9		42		32.7		39.2				ns
SD	9.8		9.0		9.6		9.5				ns

* P=Chi square with 1 df for ICD M+F vs. Control M+F

* OC: obligate carrier, SCD: sudden cardiac death, VT: Ventricular tachycardia, AAE: age at entry

The majority of the ICD subjects had risk-status defined by DNA haplotype, and were born after 1950. In contrast, 36 of 58 of the controls were born before 1950 and had risk status defined by pedigree position. The age at entering the study was the same in the control group as the age of receipt of ICD, reflecting the matching strategy. There were no statistically significant differences between ICD cases and controls in medication profile, presence of syncope or chest pain, or abnormalities on SA ECG, echocardiogram, Holter monitoring or MRI. However a significantly higher proportion of cases had documented palpitation and presyncope than the controls (Table 22 page 153). The

abnormalities noted on clinical testing are defined in Table 22 (page 153). (25, 125, 131, 136, 161, 202, 295-299).

Table 22 Clinical manifestations of ARVC in 48 subjects at high risk who received an ICD and 58 matched controls from 11 families with ARVC linked to 3p25.

	ICD Cohort n = 48			Control Cohort n = 58			Chi^2 1df
	N with manifestation	N with documentation	%	N with manifestation	N with documentation	%	p
Antiarrhythmics							
Class 1	10	44	23	11	42	26	ns
Sotalol	13	44	33	6	31	13	ns
Amiodarone	11	44	27	5	30	17	ns
Symptoms							
Palpitations	35	44	79	15	32	47	0.003
Pre-syncope	29	44	66	12	32	37	0.01
Syncope	15	44	34	5	33	15	ns
Chest Pain	14	44	32	18	35	51	ns
Heart Failure	6	47	13	10	31	32	0.04
Abn Tests**							
12-lead ECG[1]	33	48	69	32	33	97	0.002
SA ECG[2]	17	31	55	5	12	42	ns
Echocardiogram[3]	24	42	57	20	25	80	ns
Holter monitor[4]	33	42	79	20	24	83	ns
MRI[5]	4	23	23	0	4	0	ns
Catheterization[6]	0	18	0	3	15	20	0.05
Autopsy[7]	0	0		16	17	94	

Table 3: Definitions

***Class 1 medications:** Mexilitine, Propafenone, Procainamide, Quinidine and Phenytoin The larger denominator for the class 1 medication takes into account those with no records because they never visited a doctor.

** Abnormalities on clinical tests

1. ECG: T wave inversion, Septal Q waves, poor R wave progression in V_1 V_2 and V_3, or presence of PVC's(25, 295)
2. Signal averaged ECG (SAECG): > 115 ms QRS duration (25)
3. Echocardiogram: > 112% Left ventricular dilatation(202), any right ventricular dilatation, focal or global hypokinesis or akinesis of any cardiac wall (25, 131, 136, 296)
4. Holter Monitor, > 1000 PVC's in 24 hours, documented runs of VT of >4 beats (25, 297)
5. Magnetic Resonance Imaging (MRI): evidence of fatty / fibrous infiltrate or ventricular thinning ((161, 298, 299)
6. Cardiac catheterization: any critical coronary artery disease
7. Autopsy: evidence of fat and fibre in the myocardium (25, 125)

Mortality following ICD

The male ICD group was followed for a median of 2.6 years (from 3 weeks to 12.8 years) and the control group for a median of 9.5 years (from 4.5 months to 31 years). There were no deaths in the ICD group. Three subjects went on to successfully receive a heart transplant after several (2-10) years of ICD therapy. Indications for transplant included increasing heart failure as a secondary consequence of cardiomyopathy and intractable VT. One subject in the control group received a heart transplant; the remaining 35 subjects had died. The 5-year mortality rate for males following ICD therapy was zero compared to 28% in controls (p=0.009) (Figure 25 page 155).

The female ICD group (n=18) was followed for a median of 0.7 years, from 2 weeks to 3.9 years and the control group (n=22) for a median of 28.8 years, from 1.9 years to 37.8 years. There were no deaths in the ICD group, whereas in the control group, 10 of 22 (45%) subjects had died. Five year mortality in the control group was 9% not significantly different from that in the females who received an ICD (Figure 26 page 155).

Figure 25 Time to death or last follow-up in 30 male subjects at high-risk for ARVC who received ICD and 36 matched controls

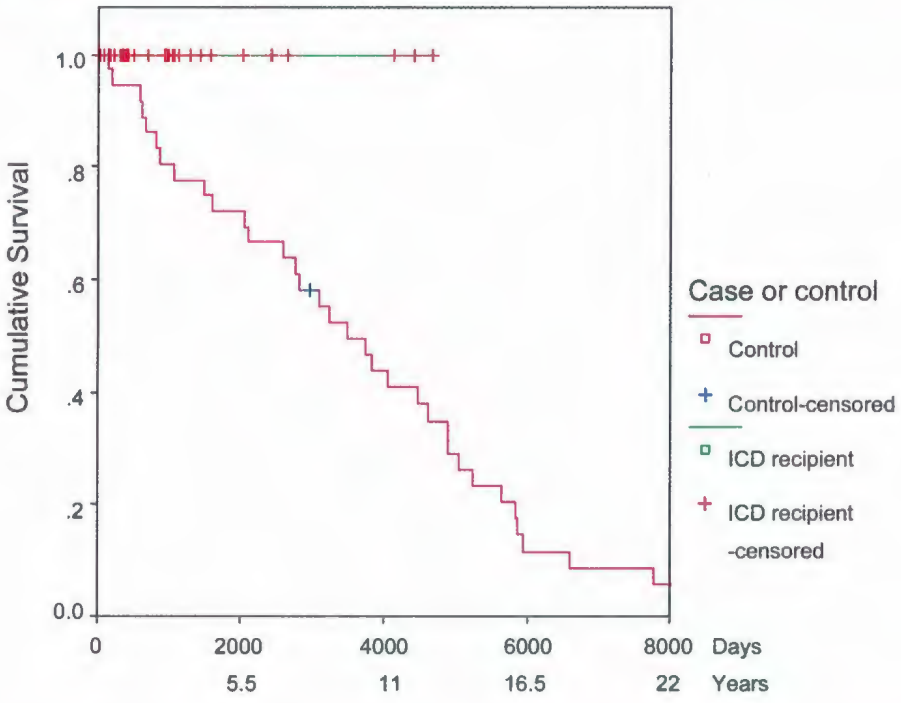
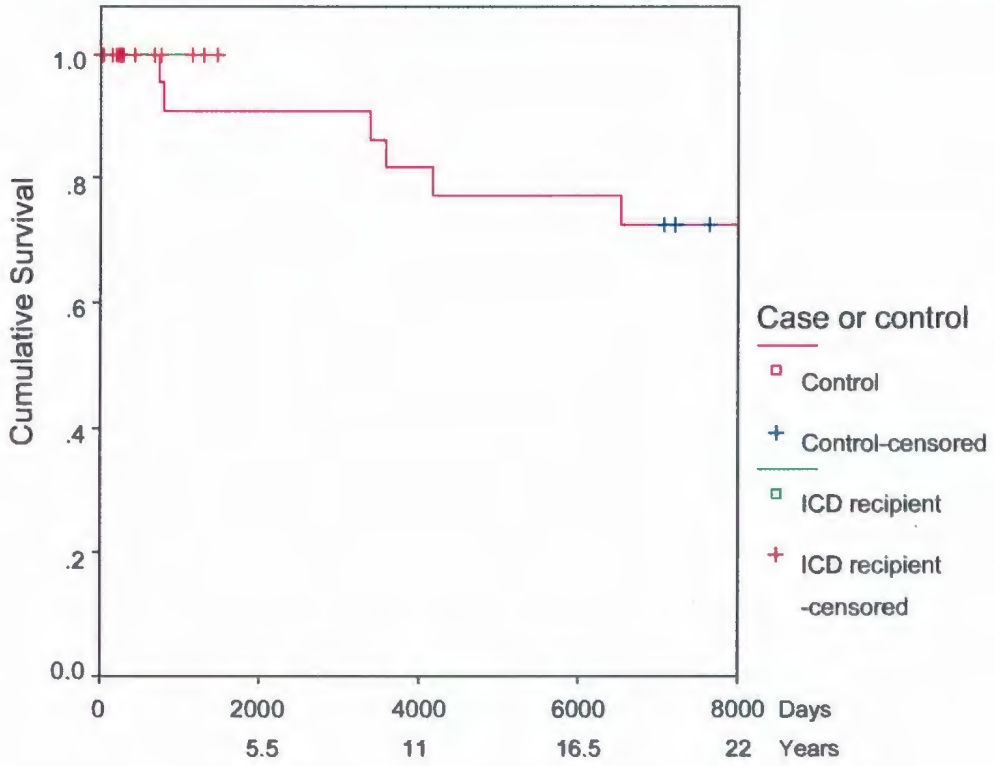


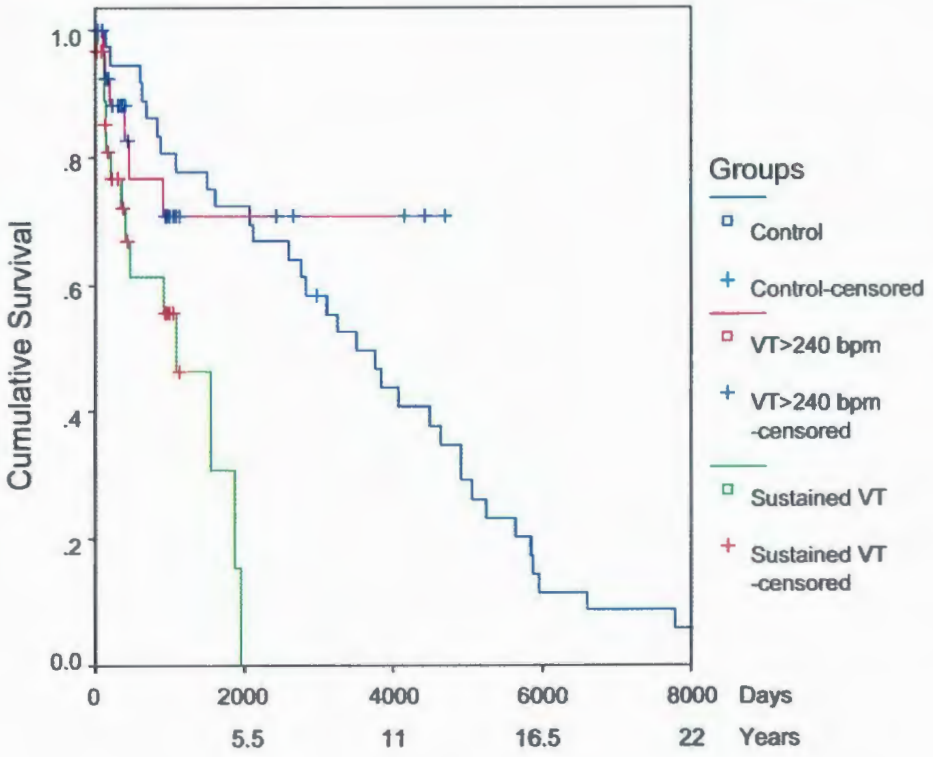
Figure 26 Time to death or last follow-up in 18 female subjects at high risk for ARVC who received ICD and 22 matched controls



4.4.2 Time to appropriate first discharge of the ICD

In the male ICD group (n=30), 14 subjects (47%) had at least one appropriate discharge for VT. The median time to first discharge was 2.9 years (95% CI 1.17-4.6years) (range 2 weeks to 5.1 years). The 5-yr. cumulative frequency for first appropriate ICD discharge was 70%, significantly greater than the 5-year mortality rate for the control group (p=0.0005). However, the 5 year mortality in the control group was similar to the 5 year cumulative rate of 28% for VT >240 bpm in the ICD group (Figure 27 page 156). Two (11%) of 18 female ICD subjects had an appropriate first discharge, both for VT >240 bpm.

Figure 27 Time to first appropriate firing or last follow-up for VT (ICD1), and for VT >220bpm (ICD2), in males who received an ICD and time to death or last follow up in matched controls with ARVC.



We compared discharge for VT in males based on whether the ICD was for primary or secondary indications. There was no significant difference between the groups (median time 2.5 years, 95% CI 0-5.2 years vs. 2.9 years, 95% CI 0-6 years respectively).A similar observation was made when time to first discharge for VT>240bpm was assessed (results not shown). No significant difference in discharge in those with or without LVE was observed. In 16 subjects with LVE, 45% had fired within 5 year, and in 10 subjects without LVE, all had fired.

With respect to side effects observed, 5 (10%) of the 48 ICD subjects have had single inappropriate shocks and 3 have had lead fracture requiring replacement.

4.5 DISCUSSION

4.5.1 Mortality

The results of this study, from a large genetically homogenous population, demonstrate that ARVC, caused by an unknown mutation at the ARVD5 locus on chromosome 3, is a life-shortening disorder. There is a significantly increased relative risk of earlier death in men compared to women, in line with previous studies (12, 167), although the mortality rates in the current study are higher than those for other familial forms of ARVC (170).

Guidelines for the prophylactic use of ICD in ARVC are not established (300). The majority of subjects in this study were essentially asymptomatic, presenting a dilemma with respect to therapy application. The use of ICD was thus influenced by the fact that the presenting symptom was often SCD and the extensive family histories defined a malignant natural history. Clinical testing in subjects from ARVC families in this study

who were at an a-priori 50% pedigree risk, and the availability of genetic haplotyping, allowed accurate determination of those at greatest risk, permitting prophylactic implantation of an ICD.

Efficacy of ICD

Few studies have assessed the efficacy of ICD therapy in ARVC (235-239, 291). The largest series to date (238), studied a multicentre group of 132 patients: a smaller study assessed mainly primary prophylaxis (291), and a single centre study (239) reported on 60 subjects with advanced disease. These studies of heterogenous ARVC subjects estimated ICD mortality benefit based on discharge free survival. In contrast, the 11 large ARVC families linked by a founder haplotype in this current study provided the etiologic homogeneity that facilitated the identification of a robust control group matched for high risk status, age, sex and family. As a result, a closer estimate of the true magnitude of ICD therapy could be derived. The results clearly demonstrated that ICD therapy significantly reduced mortality in high risk men from the ARVC families studied. Interestingly, these ICD mortality benefit results (28% 5-year total mortality reduction in males compared with familial controls) appear similar to those in previous studies where discharge free survival was estimated: 36% 5-year (239) and 28% 3 year (238). Few side effects of ICD therapy occurred and all were resolved. It is notable that decreased mortality was observed, despite the fact that the majority of men studied received an ICD prior to the documented onset of ventricular arrhythmias. The results for women were not statistically significant. This was likely attributable to the reduced statistical power due to the less malignant course of ARVC in women, the smaller sample size and shorter duration of follow-up.

Theoretically, first ICD discharge would be analogous to death given certain criteria: that the ICD is programmed to fire at only those times when a potentially lethal arrhythmia occurs and that the insertion of the ICD is not pro-arrhythmic (301), the latter being very unlikely given previous primary prevention studies (302). By the end of this study about half of the male group and 10% of the female group had had an appropriate ICD discharge. It was demonstrated however that time to first discharge in the ICD group occurred much earlier than death occurred in the control group. There are several potential explanations for this finding. Firstly, subjects with ICD's may have progressed further in the disease process than the controls, thus were at greater risk of having an arrhythmic event. If this were correct then the benefit of ICD may be greater than it would appear in this study. Against this possibility however is that the degree of ectopy observed on Holter monitoring was similar in the ICD and control groups, and there were fewer ICD subjects with an abnormal echocardiogram. The conclusion that ICD and control groups were similar should be tempered however by the fact that the course of disease in the control group was determined retrospectively, and data on clinical manifestations of disease were missing in a substantial minority of controls. Finally, individuals in the control group may have survived runs of VT that triggered discharge in the ICD group. This explanation seems the most likely, given that we found that time to first ICD discharge for sustained VT > 240bpm was similar to time to death in the control group. Unlike one recent study of ICD efficacy in ARVC (238), we did not find a relationship between LVE and rate of discharge of the ICD.

There are several limitations to this study. Firstly, it is a cohort study with historic matched controls. Secondly, there was selection bias in the choice of cases for the ICD

group and to a lesser extent the control group. Thirdly, clinical data from the control group and from a minority of the ICD group were collected retrospectively. Finally, only families linked to 3p25 were studied and the results may not be generalizable to all families with ARVC. However, a randomized control trial is not possible in ARVC and all previous studies have been descriptive, without an appropriate control group. The control group for this study was matched for multiple factors, which may influence outcome: ARVC risk status, family, sex, age and survival. Selection bias was limited by including all available controls, and by studying only well ascertained sibships in which ≥ 50% of siblings at 50% risk had a known ARVC status. Furthermore, selection bias in the cases was likely to limit the effect size of the ICD as some cases had ICD because they had already developed VT and may therefore have been more severely affected. However, these individuals did not seem to have ICD discharge more often than other subjects. The retrospective nature of the study did not influence the determination of the primary outcome (mortality) but did limit the conclusions that could be drawn on the comparability of the clinical manifestations of disease in the ICD and control groups. Although these results may not be generalizable to other causes of ARVC, this ARVD5 linked population is by far the largest genetically homogeneous group of ARVC subjects studied and ARVC caused by this mutation may be prevalent in other populations. Careful family history will detect an autosomal dominant mode of inheritance consistent with this form of ARVC but positive family histories can be missed if the mutation is transmitted through several generations of females. Further studies will be necessary to determine the long term clinical course of ARVC following ICD therapy and to assess the utility of ICD in females with ARVC.

We conclude that this form of ARVC is a malignant disease, particularly in males, who frequently die suddenly in early adulthood. The study is unique in that DNA haplotyping and extensive family history data are used for risk stratification and subsequent prophylactic ICD therapy in a large genetically homogeneous sample. Results demonstrate that this genetic risk stratification model for ICD therapy has a beneficial impact on survival in males in this population regardless of indication (primary or secondary therapy) implying that SCD may be the first symptom in those at high-risk. These results support the use of ICD's as a primary prevention therapy in familial ARVC linked to 3p25 for individuals at high genetic risk.

5 GENETIC KNOWLEDGE AND MORAL RESPONSIBILITY: AMBIGUITY AT THE INTERFACE OF GENETIC RESEARCH AND CLINICAL PRACTICE

"Conventionality is not morality."

Charlotte Bronte

Daryl Pullman and Kathy Hodgkinson

This paper was published in Clinical Genetics 2006 Mar;69(3):199-203.

5.1 ABSTRACT

Despite a rapidly expanding literature on the issue of duty to warn at-risk relatives in the context of clinical genetic testing, little has been written on parallel issues with regard to the management of genetic research results. Some might view this lack as an indication that there is little to discuss in this regard. That is, standard practice is that data obtained through medical research should not be treated as though they are clinically relevant, and this standard should hold for genetic research as well. This paper challenges this conclusion and its underlying assumptions. We argue that the line between genetic research and clinical practice is often ambiguous. In some cases research data gathered from a very small number of subjects could have immediate clinical implications. Hence it is unethical for genetic researchers to absolve themselves of clinical responsibilities for research subjects and/or their families, on the grounds that the data were obtained for research purposes. Indeed, we argue that it could well be unethical to embark on some forms of genetic research unless advance arrangements have been made for genetic counselling and clinical follow-up. Furthermore, in some cases it might be unethical to enrol subjects in studies if the subjects are unwilling to receive their individual results.

5.2 INTRODUCTION

Despite a rapidly expanding literature on the issue of duty to warn at-risk relatives in the context of clinical genetic testing, the situation remains ambiguous. Recent commentary tends to favour considerations of individual autonomy, patient confidentiality, and the individual's right to control his or her genetic information (303-305). However, there are those who argue for an evolving 'duty to warn' which may override considerations of patient confidentiality and the right not to know genetic information (306, 307). Some have reframed the debate as a matter of familial obligation with a 'duty to share' clinically relevant genetic results with relatives (308), supported by an emerging 'principle of mutuality' that supersedes individual autonomy in the genetics era (309). Emerging case law in the U.S. moves to support this principle, as it extends the responsibility of clinicians beyond immediate patients to include family members (310, 311). Meanwhile, broad privacy legislation pushes back in the other direction, as it tends to restrict the sharing of genetic information (303, 312, 313). The predictable result is moral distress for health care professionals (314).

The current focus on the clinical situation with an emphasis on duty to warn, fails to address parallel issues with regard to the management of genetic research results (315). On the face of it this lack may appear understandable, as there is a general proscription against sharing individual results in medical research. This is because the validity of clinical trial information is generally cumulative in nature, such that perceived results with a single research subject cannot, and should not be, generalized. For example, a clinician/researcher may believe that an individual patient has done well in a clinical trial,

and thus might assume he or she has a clinical responsibility to continue the trial medication outside the research protocol. However, in the absence of aggregate trial data the clinician's assumption could well be false. While cumulative evidence is necessary in some types of genetic research such as those designed to study gene-disease associations and gene-environment interactions, there is a broad subset of genetic studies in which this is not the case, particularly with serious monogenic disorders for which a tight linkage assignment has been established. In such cases the researcher may accrue knowledge from a single individual which has immediate clinical relevance not only for that individual, but for other members of the pedigree. To argue that there is no obligation to share this data with individual subjects and/or with those responsible for their clinical follow-up, or to ensure that measures are taken to advise at risk family members of their status on the grounds that *'these are research results and thus should not be considered as clinically relevant,'* is to claim ignorance in the face of knowledge for which the researcher is morally, if not legally, responsible.

5.2.1 Case description

Here we describe our experience conducting genetic research in Newfoundland, Canada, (where it is likely that several serious monogenic diseases remain to be described) on one such disease: autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVC). ARVC is a cause of sudden cardiac death (SCD) in young people.(105, 158) Clinical diagnosis is difficult and is based on observational and descriptive diagnostic criteria (25). ARVC is genetically heterogenous. It is most often inherited as an autosomal dominant disorder, and several genetic loci and cloned genes are known (33, 86, 316). One locus at 3p25 (4) contains an as yet unidentified gene responsible for one

form of ARVC in 16 large families in Newfoundland and Labrador, including up to 1200 individuals over 9 generations in a single family. Analysis of these families has determined the lethal sex-influenced natural history of the disease: 50% of males die in the absence of treatment by 40 years and 80% by 50 years, with corresponding risks for females of 5% and 20% (248). Our original consent form reflected our ignorance of the disease. That document, signed subsequent to genetic counselling, followed common practice with genetic research in that it provided assurances that were a genetic location found, further counselling would occur after molecular testing. At that point the subject would have the option of deciding whether or not to learn his or her DNA results. Subsequent determination of a linked locus at 3p25, and the assignment of a founder DNA haplotype present in all affected subjects across families, meant that DNA testing could define disease status presymptomatically. Effective primary prevention of potentially lethal arrhythmias is available with implantable cardioverter defibrillator therapy (ICD) (248). Hence, although ARVC in Newfoundland and Labrador is a lethal condition with a high recurrence risk and where effective treatment is available, DNA testing remains in the research domain. As a result, several cases have occurred where concerns have arisen regarding: (i) genetic knowledge and clinical responsibilities on the part of genetic researchers; (ii) the rights and responsibilities of research subjects with regard to their genetic information; and, (iii) potential responsibilities to other family members and the general population.

5.2.2 Case 1

A female subject at 50% a priori pedigree risk participated in genetic linkage analysis research. The sex-influenced nature of the disease and inheritance through a female

family member meant there was no immediate family experience of serious sequalae to ARVC, even though multiple SCDs in young people had occurred in the extended pedigree. Research revealed a high-risk DNA haplotype. Nevertheless, this subject refused to learn her DNA results or to receive further clinical testing. The question was raised as to whether we had a duty to warn her eight adult children, including five males aged between 20 and 40 years.

This scenario, although generated through genetic research, could occur following clinical testing, as defining affected status of the subject by any method would place the subject's offspring at 50% risk of a treatable disease causing SCD. In our opinion, clinical testing, regardless of the motivation for the testing (research or clinical), should result in disclosure to the individual. Nevertheless, this still leaves open the ethical question of duty to warn at-risk relatives. Given the lethal and treatable nature of ARVC, even those who support a conservative position with regard to sharing genetic information without patient consent, might support doing so in this situation. Indeed, emerging case law in the United States has already established that in some instances physicians may have a duty to breach the confidentiality of a patient so as to warn family members who are not their patients of their risk of genetic disease (311, 317, 318). Similar provisions are available in Canadian law under the provisions of the so-called *Tarasoff* situation named for the landmark decision of the Supreme Court of California. That case recognizes that: "at some point the need to protect the public from imminent danger becomes paramount, and at that point the doctor's duty of confidentiality ends and is replaced by a duty to warn the person[s] at risk." (319) However, all of the cases cited occur within the clinical context. If the life saving information is unavailable because

genetic researchers have failed to establish working relationships with clinicians, or because researchers restrict disclosure of 'research results', the question of contacting at-risk family members is moot.

5.2.3 Case 2 (a and b)

Two young males at an a priori 50% risk of inheriting ARVC participated in the research. Again, both were recruited prior to our understanding of the natural history of the disease, and thus before we appreciated the true severity of ARVC linked to 3p25 in Newfoundland and Labrador. Both subjects refused to hear their DNA haplotype results. This left the research team in the position of knowing that a serious risk of a potentially lethal but treatable condition was present, yet due to the subjects' refusal to hear their results, the research team was unable to take steps to organize treatment with their clinical care givers.

2a) Despite numerous overtures, the first male steadfastly refused further contact with the research team. He died of a SCD due to ARVC prior to his 30th birthday.

2b) The second male initially refused his haplotype information because he was training for a career for which a high-risk result would curtail his ability to continue. Furthermore, given the potential for SCD there was a possibility that, due to the nature of the job (in transport), members of the public would be put at risk. In this case the individual eventually agreed to receive his results and he was treated successfully. His career plans were subsequently altered.

These, and similar cases, contributed to our consternation with regard to our duties to act upon genetic information irrespective of how the information was obtained. One result

has been a modified consent document so that ARVC study participants are now required to accept that they will receive their results if and when these become available through the course of the research. If a subject does not want to learn his or her result, then the blood sample will simply not be drawn. We are considering a further amendment to the effect that if the research subject is either unwilling or otherwise unable to share clinically relevant results with at-risk family members, steps may be taken by the research/clinical team to ensure that this information is made available to those family members.

5.3 DISCUSSION

The manner in which we frame the distinction between genetic research and clinical genetics will affect our understanding of our duties regarding the management of information accrued through genetic research. First, it is important to contrast the nature of the relationship between the researcher and the subject in genetic research, with that of the clinician and patient in the clinical context. In the clinical context patient autonomy and the assurance of confidentiality in the patient-physician relationship, are often presented as key considerations in arguing against a generalized duty to warn (312, 320). However, insofar as genetic research involves families, the family must remain the primary focus of concern with regard to the management of research results.

The foregoing speaks to the importance of ensuring that genetic research is coordinated with local clinical genetics services, ideally through the mechanism of disease based, health region coordinated, genetic registers that are sensitive to both clinical and research interests. (321-324) Families are the focus of registers, and ongoing clinical care and

management, follow-up of those at risk, and appropriate genetic counselling for serious diseases with high recurrence risks are their major function. When research results are obtained they can be discussed with trained genetic professionals, and issues of potential error in research data can be addressed. Thus, in the absence of an established relationship between genetic researchers and the clinical genetics team, a research project of the nature described above, could well be considered unethical. This last point has particular significance when genetic researchers are from outside the jurisdiction in which clinical services are provided, a not uncommon scenario for genetic research conducted in Newfoundland.

Newfoundland and Labrador's population is ideal for genetic research because of its geographic and genetic isolation, and large family sizes. This has been the impetus for numerous successful collaborative relationships with out-of-province genetic research teams. However, some out-of-province teams have studied Newfoundland families in the absence of local clinical genetics input. In some cases outside researchers have abdicated any responsibility for sharing clinically significant results with research subjects or with local clinicians responsible for their follow-up, on the grounds that research results should not be applied to the clinical context. As a consequence, the province has drafted legislation to ensure that all genetic studies conducted here are subject to local ethics review. For diseases that fit the risk profile of ARVC, local research ethics board approval will occur only when satisfactory arrangements have been made through the clinical genetics service to ensure appropriate follow-up, and for the communication of relevant results with subject/patients and their families. Clinical geneticists working directly with researchers in this manner may be placed under significant burden regarding

potential 'duty to warn' issues that might arise. Although professional clinical guidelines in the USA, UK and Australia permit breaking of confidentiality in 'exceptional circumstances,(325-327) the mechanism by which this would occur remains unclear. One suggestion is that health professionals be required to notify a legislated statutory body, whose remit would be to warn relatives of their risks without identifying the affected proband.(307) Again, however, we note that such guidelines are aimed primarily at the clinical context and do not account for research findings.

The key consideration here is that information obtained through genetic research for serious monogenic disorders (where results from a single individual can have immediate clinical implications) differs from results generated in the course of a standard clinical trial, or in population based, gene association studies. The distinction, therefore, between research results and clinical application, so important in the context of clinical trials, cannot be used as the standard for genetic studies of serious diseases with high recurrence risks, particularly for those with potential ameliorative interventions.

Finally, it is important to mention the privacy paradox. This paradox lies in the fact of increased legislative emphasis on individual privacy protection, even as the advance of genomic medicine necessarily reveals information that is familial in nature. We are sensitive to concerns regarding genetic discrimination, and recognize the need to protect individual and family privacy when appropriate. However, genetic privacy is a somewhat fickle matter, dependent to a large extent on the phenotypic expression of the particular genetic condition. Individuals with achondroplasia, for example, may have concerns about genetic discrimination, but it has nothing to do with the privacy of their genetic

information. Concerns about insurability can also be misleading, as knowledge of serious genetic disease in the family has to be disclosed, regardless of individual disease status. In the case of ARVC, for example, multiple deaths in young people within the extended family will immediately affect insurability. To put it bluntly, individuals at-risk of ARVC have to face the choice of dying with an insurance policy of dubious validity because they failed to disclose relevant family history irrespective of genetic testing, or, to be alive enjoying their loved ones but without insurance.

We acknowledge that ARVC in Newfoundland and Labrador is in some respects an atypical genetic disease. Hence we do not assume that the manner in which we manage research results on this condition is generalizable to all genetic studies. However, the lessons learned from ARVC provide another perspective on the nature and extent of our obligations with regard to genetic information. This behoves genetic researchers in general and research ethics boards in particular, to consider carefully the possible clinical ramifications of research data prior to embarking upon or approving a genetics research project.

We are still in the early days of the genomic era in medicine. It remains unclear whether the new wine of rapidly expanding genetic information can be contained in the old wineskins of principles, policies, and procedures for gathering, storing, and sharing medical information. Our suspicion is that one size simply will not fit all. A more nuanced understanding of the relationship between genetic research and clinical practice is essential as we move forward in this regard.

6 THE CASE FOR CLOSER INTEGRATION OF CLINICAL AND RESEARCH GENETICS PROGRAMS: LESSONS FOR NEWFOUNDLAND AND LABRADOR.

An idealist believes the short run doesn't count. A cynic believes the long run doesn't matter. A realist believes that what is done or left undone in the short run determines the long run.

Sydney J. Harris

Kathy Hodgkinson and Daryl Pullman

6.1 INTRODUCTION

The fundamental difference between genetics and other branches of medicine, whether clinical service or research, is that genetic disease provides medical information pertinent to the relatives of the diagnosed patient. This is particularly true for classic Mendelian diseases where the risks for relatives are high. Optimal management of patients with Mendelian genetic disorders and their at-risk relatives requires both clinical and research expertise, and a systematic approach to the collection, storage and sharing of research and clinical data.

This paper discusses our experience in Newfoundland and Labrador in the management of autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVC). Newfoundland and Labrador is the Eastern most province in Canada. It is known for its founder population and the genetic diseases that result from its historic settlement patterns (250). These diseases provide an excellent resource for research, and a concomitant burden for health care provision (251). ARVC is common in Newfoundland and Labrador, with a prevalence of about 1/3000. We have faced numerous theoretical, practical and ethical challenges in managing ARVC patients while conducting research on this disease, and we have adjusted our policies and practices in light of our evolving knowledge and experience. We believe the lessons learned can inform policy and practice for the management of genetic diseases in all Canadian jurisdictions.

In what follows we begin with some general observations about genetic research with an emphasis on ARVC and its phenotype. We define the structural differences between

clinical genetics and genetic research on the one hand, and their non-genetic counterparts on the other. We then discuss the nebulous distinction between clinical genetics and research genetics, and the implications this has for the management of genetic information. The points raised are illustrated with reference to our own experience in serving patients in our genetic cardiomyopathy clinic even as we conducted research in an attempt to discover the causative gene. Our experience has convinced us that it is not only more pragmatic but also ethically imperative to create genetic registries in order to manage our clinical and research data in a more effective manner. We believe the lessons learned have broad implications for the manner in which genetic data are managed in both clinical and research settings.

6.2 GENETIC RESEARCH IN NEWFOUNDLAND AND LABRADOR

Genetic research has occurred in Newfoundland since the inception of the Faculty of Medicine at Memorial University. (328). Such research is funded through research grants and is at times curtailed because of insufficient funds. In such cases families may be left with no information regarding the research findings despite what could be significant risks associated with their disease. In some cases samples taken from families have been lost, including samples from ARVC families for whom the disease is lethal (248). Many samples taken in the 1970's and 1980's were destroyed in the early 1990's due to a lack of storage facilities (45). A significant number of those samples were from individuals who subsequently died of ARVC. It is a sobering to think that had those early samples been available in the late 1990's, the ARVC gene, found in 2008, (46) might have been found earlier. Our experience with ARVC motivated us to rethink many aspects of the

manner in which we handle genetic information and the patients and families affected by that information.

ARVC in Newfoundland is caused by a missense mutation in a transmembrane protein (TMEM43) of unknown function (46). At least 15 very large ARVC families (up to 1200 people over 10 generations in one family) have been ascertained. ARVC causes sudden cardiac death (SCD) due to tachyarrythmias. If untreated, 50% of young males will be dead by age 40, and 80% by 50 years compared with 5% and 20% of women respectively. Those who survive SCD are still susceptible to heart failure (248, 329). Implantable cardiodefibrilator (ICD) treatment significantly alters survival for males (248).

Given the significant burden of genetic disease and the relatively small genetics research unit at Memorial University, Newfoundland and Labrador, collaboration with external teams is required. However in the early days there were no written agreements regarding provision of DNA results, and no involvement with clinical genetics services. External teams in the past did not require local research ethics board (REB) approval. If treatment/diagnostic options became available, or patent and subsequent royalty payments resulted, there was no guarantee that these would be made available to participating families. As a direct result of the need to protect Newfoundland and Labrador families, recent legislation requires external research teams to undergo Provincial ethics approval. This has the benefit of allowing external collaborators to continue working with local families and research teams, provides local clinical care for families, and allows for equitable benefit sharing arrangements (330). With regard to

ARVC, we were fortunate that one of our external collaborators (Dr. Ludwig Thierfelder's team in Berlin, Germany) was able to provide linkage results for 6 years prior to our local attempt to find the causative mutation.

Local capacity to conduct genetic research is much greater now due to the Atlantic Medical Genomics Genetics Initiative (AMGGI), a large project funded through Genome Canada (331). AMGGI aims to facilitate appropriate local service and support for patients and their families within a framework of research to identify novel disease causing genes in a wide range of genetic conditions prevalent in Atlantic Canada. This ambitious and comprehensive initiative covers the gamut from clinical ascertainment of patients, to research in molecular genetics and the provision of clinical genetics services, culminating with the development and implementation of health policy to ensure the most effective care for our families.

AMGGI provided significant funding for genetic research in Newfoundland and Labrador It also however exacerbated already existing problems with regard to data management. With multiple genetic research projects occurring both locally and in collaboration with external collaborators for many decades, genetic data is often present in various places, and pedigrees exist in various forms. Some pedigrees contain large quantities of information and in some cases irreplaceable samples have been lost. At times family members are seen clinically before a research study is initiated, which means some individuals may be approached numerous times to provide the same information. This is onerous even if there is ongoing clinical management, and an inappropriate burden if there has never been feedback and continuity.

While the double ascertainment of a pedigree is troubling in and of itself, the current environment of stricter ethics protocols complicates matters even more when additional consent is required in order for the clinical genetics team to access pedigree information from the genetics research team or vis-a-versa. We have yet to understand from whom permission should be obtained for a pedigree containing 1200 people when there may be as many as 20 probands. The worst aspect of this system is that data accrued by one set of people is not available to another set, when the families are the same, the disease is the same, and the families themselves perceive that the data is being shared, particularly when that data is essential to the ongoing research or clinical care. High risk individuals who have given their family details many times understandably assume that their disease and risk status is known and being managed appropriately by health care professionals. However, when their status is known only to the research group due to technical, conceptual or procedural barriers, the prospect of clinical information slipping through the cracks is frightening at best.

6.3 CLINICAL GENETICS: WHO IS THE PATIENT?

Clinical genetic service in Newfoundland Labrador has been provided at the tertiary health care centre in St. John's since 1988, with secondary service available in Central and Western health districts. Genetic services provide accurate diagnosis of genetic disease and risk assessment to individuals and families and clinical management (in many cases). Risk depends on a number of factors. The same disease gene can affect individuals differently (variable expression), and the presence of a disease gene may lead to a probability, rather than a certainty, of disease manifestation (reduced penetrance).

One aim of the clinical genetic service is to reduce disease burden through appropriate screening and/or treatment.

Like all clinical care in a complex health care environment, clinical genetics services are provided by a range of medical professionals including physicians, nurses, genetic counsellors, social workers, and others as needed. However, a moment's reflection reveals that clinical genetics dealing with Mendelian disease is distinct from other clinical services in a number of respects. The first key difference is that the primary clinical information for genetic services comes not from the individual patient, but rather from the family pedigree. Even in the current climate of heightened awareness around privacy and confidentiality, genetic health care staff continue to collect and utilize family history information without direct permission from everyone on the pedigree. Although access to a medical record requires permission from the patient or their next of kin, demographic or disease-status information can exist on a pedigree with no permission requested or required from the listed individuals, many of whom may be dead. Without this privileged access to familial information, risk assessment, genetic counselling and in some cases diagnosis would not be possible. This leads to a second key difference between clinical genetics and other clinical services. On the basis of pedigree structure alone genetic risk figures (based on probability) are often available. Thus it is possible for the genetics clinician to provide a probabilistic diagnosis for some family members without even seeing them. If a causative gene is known, then risk information is often concrete and genetic testing can inform individual patients and/or family members of their disease or relative risk status. The familial nature of genetic information thus presents particular

ethical challenges with regard to the clinician's duty to warn at risk relatives who may not even be their patients (276).

6.4 RESEARCH GENETICS: WHO IS THE SUBJECT?

Genetic research is simply an attempt to determine the relationship between genotype and phenotype, looking for genes that cause, or contribute to a disease. The aim is to find the gene(s) and reveal the pathophysiology. The two main methods are family based linkage analysis and population based association studies (332), the latter more usually applied to complex disorders where family history does not follow a Mendelian pattern. Once a major disease gene is discovered, researchers can determine genotype/phenotype associations, genetic and/or environmental modifiers to the expression and penetrance of the disease, and disease epidemiology, including the natural history and the efficacy of potential treatments. Typically such research takes years. The research questions ultimately lead to information that is relevant to the participating individuals, from the initial 'do I have the causative gene?' and 'if I do, how will the disease affect me?' to 'what effective treatments or screening are available?' Such research also informs policy and procedure for provision of evidence based health care.

Newfoundland is known for the willingness of many families with genetic disease to participate in long term research projects, with both clinical and ethical implications for the individuals concerned. The process starts with an individual presenting with a genetic disease and can lead to years of research into the disease and the family. The process of constructing a pedigree will include unaffected family members and others who may be at significant risk for the condition. Genetic researchers may or may not have direct

contact with all of these individuals, but many will be contacted and consented into the study for the purpose of providing additional genetic samples as the research proceeds. Many at risk individuals may not yet be patients in a clinical genetics service, but depending on their risk status it could be imperative that they be referred for clinical follow-up.

While it is standard practice in non-genetic medical research to make a clear distinction between research data and clinical information, this standard is often inappropriate in the context of genetic research. The difference lies in the fact that clinical trial data is aggregate information such that the research result applies not to an individual enrolled in the study but rather to a statistically powered group. Thus it is generally only after a significant number of individuals have been studied in a clinical trial that a valid research result can be generated. For this reason it is inappropriate to make clinical decisions based on preliminary research data and hence research data should not be treated as clinical information. In genetic studies, on the other hand, information acquired from a single individual could have immediate clinical implications for that individual and perhaps to other family members as well. Historically, research teams from other jurisdictions (usually from the USA or mainland Canada) have recruited Newfoundlanders for genetic research of this type, and failed to recognise the clinical significance of their findings. This has sometimes led to morbidity and mortality that could have been avoided.

6.5 GENETIC COUNSELLING AND THE PROVISION OF GENETIC INFORMATION: THE IRRELEVANCE OF THE RESEARCH/CLINICAL DISTINCTION

There is significant overlap between a clinical genetic and a research genetic approach to families with severe Mendelian disease (Table 23 page 189). A coordinated approach to disease management would thus appear sensible. Newfoundland is ideally suited to an overlapping approach primarily because the population is clearly demarked in terms of history and geography, and is served by a single tertiary centre.

If a gene is already known, then counselling and screening can be offered to other at-risk family members. This extended contact is made through the original proband, and family members are asked if they would like an appointment to discuss the issues. If a condition is seen for which the genetic defect is unknown, or where the defect is known but further epidemiology or research into modifying genes needs to occur, the family can be recruited into a genetic research project. Contact with family members occurs the same way as for clinical service, from the original proband, who approaches other family members asking if they agree to be contacted. With initial permission granted in this manner, consent for the research can be obtained from each participant, and information regarding the research progress can be returned as appropriate. To facilitate this process, it is imperative that research teams work in concert with their clinical genetics counterparts who can coordinate clinical management of affected and at risk individuals while providing appropriate support to the family.

If through genetic research a genetic linkage or a causative gene is found, then family members can be notified, and testing offered exactly as it would under clinical conditions where the gene is already known. It matters little to families with an inherited disorder whether they were ascertained via a clinical or research route. Either way, genetic counselling and the provision of genetic information is required, and/or appropriate clinical management instigated at whatever level that information exists. If research provides new information, then individuals who were previously at-risk on the basis of pedigree status may be provided with test information which will place them in a state of near, if not total certainty of whether they have inherited the predisposition. Bear in mind that at some point all known genes were unknown and families had to be recruited via clinical genetics where clinical management was central to the process.

Genetic information is disease and gene specific. Individuals make a decision as to whether they wish to hear the information which is now available. Implications that patients take into consideration include medical matters (the seriousness of the condition, the availability of treatment, etc), and social matters (does the individual have life insurance/mortgages etc. for which a pre symptomatic diagnosis based on genetic information might create difficulties).

The aim of all genetic research should be to benefit patients, and provide information pertinent to their clinical needs. Without research, there would be no progress. For families devastated by severe genetic disease, a move towards amelioration of their condition through research is often desired. It may be argued that there is an ethical imperative to do research into diseases where answers to clinically important questions

are unknown. But ethical problems abound when the ambiguous distinction between research and clinical genetics is maintained or improperly managed (276), and these problems are exacerbated when the genetic condition in question can include early mortality and significant morbidity. A centralized register, allowing access to researchers, and providing robust research information back to those providing clinical care would obviate some of these issues.

6.5.1 Genetic clinical/research registers: a model for Newfoundland

Genetic registers take many forms. In some instances they are primarily research registers, where data is collected from many jurisdictions, in an attempt to find out more about rare disorders than is possible in one locale (333). Other registers collect disease information on a population basis. Finland provides an excellent example of this approach (334, 335). These registers have enormous potential for research into the genetic epidemiology of common, genetically complex conditions such as schizophrenia. (336).

The type of register we advocate for here was recommended already in 1972 by the World Health Organisation (WHO)(322). In those early days WHO advised all medical genetics centres (i.e., a clinical department within a health care setting) to establish registers of high risk genetically determined disease so that genetic counselling could be offered to all those who would benefit. Thus all individuals referred to a clinical genetics program would be placed on a genetic register following consent. In the early 1970s direct mutation testing was not available, yet WHO recognized that with the introduction of linked markers gene tracking through families would be facilitated. The WHO had a

clear remit in proposing this model. They wished to extend counselling from the proband to the at-risk family and maintain long-term contact with the relatives to allow recall for those at-risk for counselling and/or testing. They were clear about the importance of ensuring that appropriate DNA and/or other samples were stored against future need. This was enacted for clinical reasons at a time when few DNA markers were available and few gene locations were known. The final aim of genetic registers was to facilitate research: the recognition that without research, the scope of genetic services, and thus the provision of information to patients would be limited. The total ascertainment of families with a given disorder in the population served by the register was recommended, so that accurate epidemiological studies could occur.

One of the first genetic registers was established in Edinburgh (337), followed by several more UK centres after the Clinical Genetics Society (now the British Society of Human Genetics) working committee report of 1978 (321). In Britain a register covers a Health Service Region with a population of up to 5 million. In Newfoundland one genetic register would be required to cover the province, located in the tertiary centre of St. John's.

The primary clinical benefit of a genetic register system is the improved management of Mendelian genetic disease, with maximum prevention of morbidity and mortality(338). Also of benefit, and related to the above is the availability of a single main pedigree, and a central repository for demographic and medical information. Changes to a pedigree, whether from a researcher or a clinical genetics health care provider, would occur centrally, with only one accurate digital copy of the family tree available for clinical and

research purposes. A register asks whether clinical patients consent to being on the register. If they do, they know they may be called about research. Their consent to participate in any research ethics board (REB) approved project would then be decided between them and the researcher.

Researchers apply for REB approval for each project and permission is requested for access to either minimal (no identifying information) or maximal (identifying information) data. The determination as to whether identifying data is required should be based on the clinical significance of the study. In cases where clinical significance is high, issues of clinical management must be resolved prior to onset of the research, and genetic counsellor support provided. A properly managed registry would assist in identifying where on the spectrum of clinical importance a disease lies, and thus what information should be available from the research team to the patients at any given time: in effect, a triage system. An ongoing challenge in this regard is that research labs generally do not operate to the same standards as do clinical labs, and this has implications for whether it is appropriate to use research data for clinical purposes. Again the triage system that separates diseases on the basis of clinical significance should inform laboratory processes as well. That is, the basic molecular work of a disease with high clinical significance would generally not be entrusted to a junior researcher (e.g. an undergraduate or relatively inexperienced graduate student), and would require close oversight and validation of results by a senior researcher. As is clear to many working in this field, patients often do not make the distinction between researchers and clinical

staff. It thus behoves us to make sure that this regular misunderstanding results in no harm to the patient population.

The costs of running a genetic register should be outweighed by the benefits in health care to the family members in terms of decreased morbidity and mortality. As those deemed not to be at risk are no longer followed and screened, overall health care system costs would hopefully be lowered . Preliminary evidence for this approach is available for the Von Hippel Lindau hereditary tumour syndrome in Newfoundland (339), and elsewhere (340). Appropriate numbers of staff affect the costs required for a register, particularly genetic counsellors who are central to this form of clinical management. The benefits of genetic counselling cannot be overemphasized (341). Staff costs are always the most expensive part of any endeavour: in this type of system, funding from research projects could offset counsellor salaries, and the monies liberated used to fund other counsellors.

Confidentiality issues are always of concern in the provision of any health care, but should be adequately dealt with within a secure computerized network, particularly if housed securely within a health care system. The potential for providing unsolicited information to unsuspecting people that they are at risk of a devastating disease may be exacerbated with a family based register system. This is true of all genetics however, with or without a register. In most cases individuals in a family with a devastating genetic condition are already aware of their risk, although the perception of risk may be overestimated (342) (343). The question has been raised as to whether failure to provide

information pertinent to individuals may be unethical (276). Register systems of this nature have been used in the UK for over 30 years (323, 324, 344).

6.6 DISCUSSION

The boundaries between research and clinical care in genetics are hard to maintain. A register approach helps to make the situation clearer as it ensures more effective clinical management while potentially expediting related research. Given the nature of genetic disease the registry gives priority to clinical care as affected individuals must be managed irrespective of whether causative genes have been identified. Nevertheless, the registry system does not treat research as separate from and incidental to clinical management, but explicitly acknowledges the critical importance of genetic research to that clinical care. When functioning properly the process is something of a non-vicious circle in which patients are seen clinically, their information is ascertained, they become part of a research project, and their research information is fed back to the clinical staff who return it to the family members, who may then choose to become part of the next project.

6.7 SUMMARY

There are many conditions in Newfoundland and Labrador for which gene mapping has yet to be instigated, or is in progress. These include conditions where the clinical picture remains unclear, where the mode of inheritance is unknown as well as those where the genetic cause is well recognised, and yet research is required to determine the clinical and genetic epidemiology and the modifiers of the phenotype. These families still require genetic counselling and risk assessment. In order for both of these to be as accurate as possible, research has to occur. We advocate therefore for an amalgamation of clinical

and research genetics under the remit of a genetic register for Mendelian disease within Newfoundland and Labrador. This approach should be considered in all health regions where genetic programs exist, particularly those where research and clinical endeavours deal with the same individuals.

Table 23 Inherited diseases with high morbidity and/or mortality and high recurrence risk

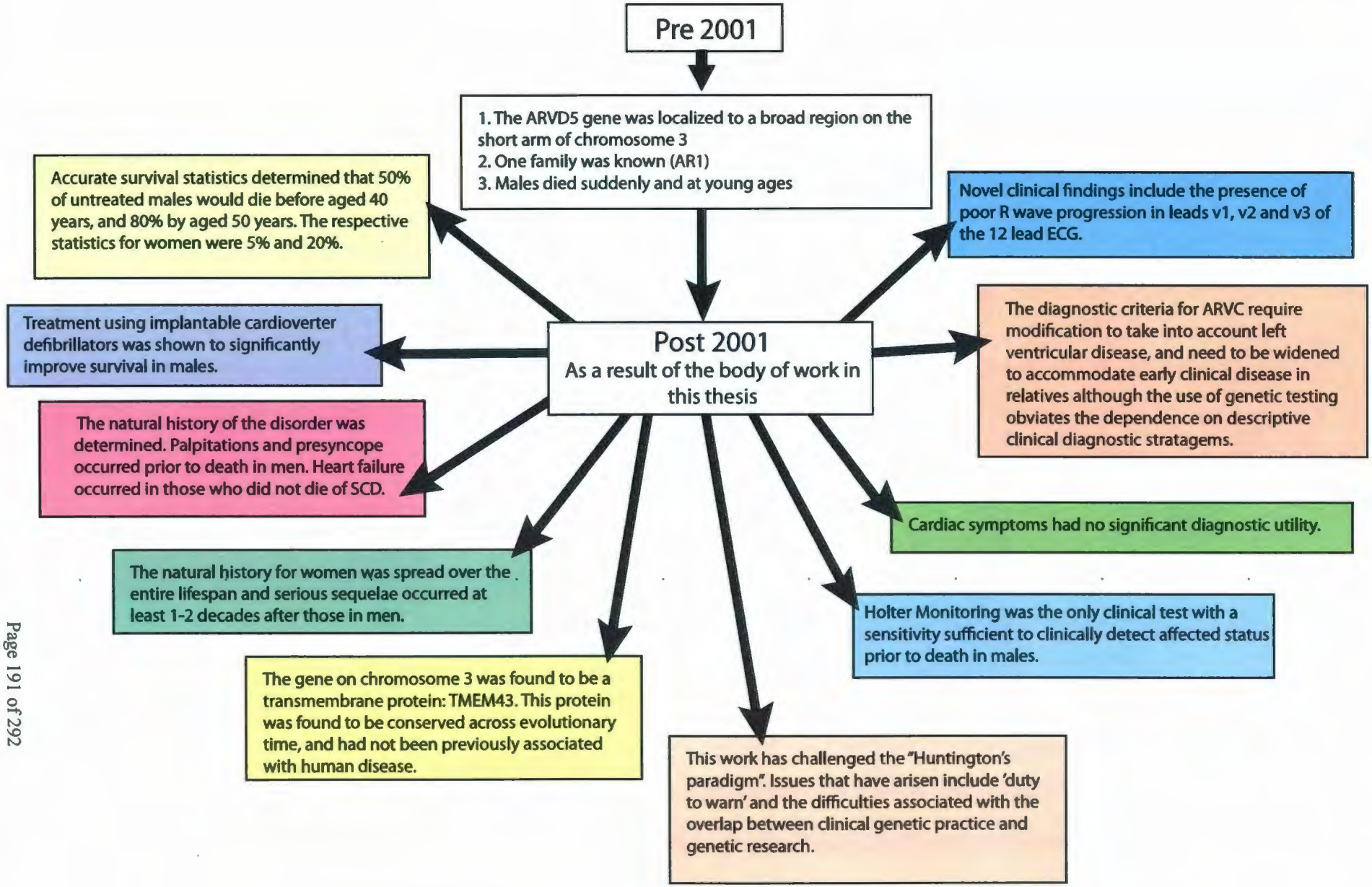
	Clinical Genetics	**Research Genetics**
Ascertainment of family	1. Initial referral of probands 2. Draw family tree 3. Expand contact to other family members via proband	1. Recognition of probands 2. Draw family tree 3. Expansion to other family members via proband
Maintenance of pedigree/pedigree data	Ideally, pedigree drawing software and appropriate associated database	Ideally, pedigree drawing software and appropriate associated database
Primary reason for ascertainment	1. Diagnosis 2. Mutation screening 3. Risk assessment 4. Appropriate clinical follow up	1. Determination of underlying gene /mutation(s) 2. Determination of modifiers 3. Determination of clinical and genetic epidemiology
Clinical importance	Significant regarding risk from pedigree alone. Significant if gene and or mutation known. Significant regarding information on prognosis, screening, management and treatment.	Significant re risk from pedigree alone. Potentially significant if gene and or mutation found as a result of research OR research determines information regarding disease progression and therapeutic outcomes from epidemiological studies.

7 DISCUSSION/CONCLUSION

"One never notices what has been done; one can only see what remains to be done."

Marie Curie

This thesis describes novel clinical, and epidemiological information pertinent to the ARVD5 patient population. It also describes the discovery of a hitherto unknown gene for ARVD5. This work has provided key evidence for the creation of a genetics/cardiomyopathy clinic, has changed perceptions regarding clinical responsibility within genetic research and has led to a health care policy for ARVC in Newfoundland (345). The discovery of the causative gene was positional cloning at its best, using a well ascertained set of families with deep genealogies, with 'across the lifespan' medical information, leading to the ability to define the clinical phenotype, penetrance and expressivity for ARVD5. In this concluding chapter, I briefly expand and amalgamate the multiple conclusions generated throughout the thesis, and discuss some of the pertinent findings in greater detail. I also assess the global advantages and limitations of this study and assess future directions for research.


Pre 2001
1. The ARVD5 gene was localized to a broad region on the short arm of chromosome 3
2. One family was known (AR1)
3. Males died suddenly and at young ages
Accurate survival statistics determined that 50% of untreated males would die before aged 40 years, and 80% by aged 50 years. The respective statistics for women were 5% and 20%.
Novel clinical findings include the presence of poor R wave progression in leads v1, v2 and v3 of the 12 lead ECG.
Treatment using implantable cardioverter defibrillators was shown to significantly improve survival in males.
Post 2001
As a result of the body of work in this thesis
The diagnostic criteria for ARVC require modification to take into account left ventricular disease, and need to be widened to accommodate early clinical disease in relatives although the use of genetic testing obviates the dependence on descriptive clinical diagnostic stratagems.
The natural history of the disorder was determined. Palpitations and presyncope occurred prior to death in men. Heart failure occurred in those who did not die of SCD.
Cardiac symptoms had no significant diagnostic utility.
The natural history for women was spread over the entire lifespan and serious sequelae occurred at least 1-2 decades after those in men.
Holter Monitoring was the only clinical test with a sensitivity sufficient to clinically detect affected status prior to death in males.
The gene on chromosome 3 was found to be a transmembrane protein: TMEM43. This protein was found to be conserved across evolutionary time, and had not been previously associated with human disease.
This work has challenged the "Huntington's paradigm". Issues that have arisen include 'duty to warn' and the difficulties associated with the overlap between clinical genetic practice and genetic research.

7.1 GENETICS

We knew that ARVC worldwide was a genetically and allelically heterogeneous disorder. Our haplotype data however defined ARVC at locus ARVD5 as a founder population. Thus we hypothesised that the gene and the underlying mutation in all 15 ascertained families would be the same. Following a traditional positional cloning approach (utilising linked DNA markers segregating with disease across generations), we identified a missense mutation in a conserved region of a transmembrane protein (TMEM43), a protein with no previous association with human disease, as the cause of ARVD5 in Newfoundland;. Although the positional cloning method generally works efficiently with this type of disorder (Mendelian autosomal dominant), in this case it was made more difficult by the lack of DNA from those who had died suddenly (who were, by our definition, affected, Table 5 page 60), the inherent variable expressivity and reduced penetrance associated with dominant disorders, the sex influenced nature of the disorder, and the potential for misclassifying subjects based on common, rather than ARVC related cardiac anomalies resulting in the inability to unambiguously clinically diagnose subjects. This issue of diagnosis made the search for the ARVD5 gene more challenging, but speaks to the need for detailed clinical assessment and ascertainment, and the requirement for a diagnostic strategy that utilises all available information and is easily applied.

The mutation causing ARVD5 is a mutation at position 358 in *TMEM43* (1073 C-T, S358L). A protein is produced with a leucine instead of a serine within the third transmembrane region of the four transmembrane region protein (Figure 15 page 94). The

presence of a missense mutation (rather than a deletion, or a premature stop codon resulting in an absent protein) is less definitive for predicting disease causality. Indications of causality were thus used to define probably causation. These included i) the gene lies within the original ARVD5 chromosome 3 linked haplotype (4, 248), ii) the gene is expressed in the heart (although this is not obligatory: the gene may be developmentally required for example), iii) the mutation occurs in all affected and obligate carrier subjects from whom DNA has been obtained, iv) the amino acid is conserved throughout evolution (a measure of its biological importance), and v) the mutation is absent/rare in matched population controls and clearly unaffected family members. In addition, the specific base pair change was predicted (by NCBI Build 36.1), to be biologically deleterious. All of those criteria were fulfilled in the case of the 1073 C-T, S358L mutation in *TMEM43*, confirming this as the cause of ARVD5. The production of fat in the myocardium via signalling pathways implicates TMEM43 (MIM 601487) as a regulator of an adipogenic pathway (263). This might explain fibrofatty replacement of the myocardium in ARVC patients if the pathway is deregulated.

That a mutation in *TMEM43* causes of ARVD5 in what is probably the largest population of ARVC in the world may change the perception that ARVC is primarily a disease of the desmosome (74, 243).

7.2 IMPLICATIONS OF THE *TMEM43* MUTATION

7.2.1 ARVC and DCM overlap

The presence of LVE, although noted in other studies, (132, 135, 140, 266, 267) is striking in this cohort and supports placing ARVC within a group called 'arrhythmic cardiomyopathies,' which would take into account various presentations of disease (268). It also speaks to the way the heart is remodelled in the face of disease and how mutations affecting different cardiac proteins and thus different aspects of cardiac morphology work in concert. The only previously known gene with mutations shown to cause both a DCM and ARVC phenotype is desmoplakin, implicated in both autosomal recessive DCM (Carvajal syndrome) and AD ARVC (ARVD9).

How does TMEM43 fit with the other known proteins implicated in ARVC/DCM?

One can speculate that a 'final common pathway' occurs in the development of the cardiomyopathies, with the eventual phenotype influenced by the primary mutation, alongside other proteins that determine cardiac myocyte function. DCM may be considered a non-specific degenerative response as a result of a genetic mutation, a virus, or volume overload, either via several physiological pathways, or combining within one main pathway. As many of the proteins causing DCM are cytoskeletal, one can imagine that contractile force may be impaired from the sarcomere to the extracellular matrix, a mechanism implicated also by the fact that mutations in some sarcomere proteins also cause DCM (94). However, DCM is also often caused by defects in the nuclear membrane protein Lamin AC. These mutations may cause dilatation as a result of impaired force generation from the connections between the Lamins and the cytoskeletal

proteins. Lamin AC is ubiquitous, and is involved in nuclear membrane dissociation during mitosis. The variability of human disease caused by Lamin mutations is wide (346), and includes muscular dystrophies, with or without cardiac involvement. Mice deficient in Lamin AC have problems with formation of the nucleus and their desmin cytoskeletal network; their mechano-transduction is deficient and they have problems with the activation of transcriptional programs responding to mechanical strain (347). However, with the Lamin mutations, it is harder to understand the arrhythmogenic effects that may occur earlier than the structurally apparent defects in DCM (94).

Mutations in phospholamban, the reverse inhibitor of the cardiac sarcomplasmic reticular Ca2+ - ATPase that regulates cardiac contractility, also causes DCM, implicating calcium metabolism as part of the same pathway (348). TMEM43 may fit into this pathway either at the level of the cell membrane or the nuclear membrane, interacting with either the desmosomal/dystrophin-associated complexes or the nuclear membrane proteins.

It is likely that TMEM43 is influenced by regulating factors as yet unknown. It is known that only 2% of genomic DNA codes for proteins, yet there are large tracts of the remaining 98% of DNA that are highly conserved across evolutionary time. They may therefore have a functional role (349).

As was described in the introduction, the majority of proteins implicated in ARVC prior to TMEM43 were the desmosomal proteins. These proteins provide an anchoring and signalling function between and within the cardiac myocyte, and have a link to desmin and other cytoskeletal structures, linking the arrhythmogenic and structural phenotypes. It is likely therefore that ARVC and DCM are diseases on the same pathophysiological

pathway. Emphasising the parallels between these clinical entities are the autosomal recessive disorders of Naxos and Carvajal caused by mutations in plakoglobin and desmoplakin respectively. Both include causing keratoderma and woolly hair in their phenotypes, with an ARVC-like phenotype in the former and a DCM-like phenotype in the latter (77).

RYR2 mutations cause ARVD and catecholiminergic polymorphic ventricular tachycardia (CPVT). Initial reports suggested differences in the positions of these mutations and the predominant phenotype expressed. *RYR2* mutations in the amino cluster cause CPVT and those in the carboxyl cluster cause ARVC. The amino cluster of the RYR2 protein is located in the cytoplasm, whereas the carboxyl cluster contains the transmembrane domains (350) (36) (83) (84) Mutations in the centre of the gene can lead to both phenotypes. It is postulated that all *RYR2* mutations produce the tachycardia, but only the transmembrane domains cause the structural changes seen in ARVC. Modifier genes or environmental influences may also make a difference in the phenotype (94). Functional studies are available for the *RYR2* mutation Arg176Gln (351) where both hypersensitivity and hyperactivation of the RYR2 channel are seen. This leads to excess calcium release from the sarcoplasmic reticulum, leading to calcium overload and delayed afterpolarisations, producing VT (352). The relationship between *RYR2* mutations and ARVC structural defects is unclear.

All the currently separate ARVC/DCM phenotypes will likely be eventually defined by their underlying genes and mutations, each against a background of modifying genes and

environmental factors. Exactly what part TMEM43 will finally play in this orchestra will be the focus of future research.

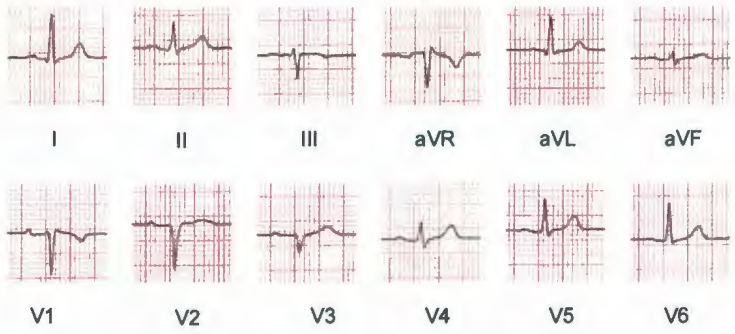
7.2.2 Natural history and phenotype

For the first time, the natural history and phenotype of a genetic subtype of ARVC has been delineated using mutation negative family members as controls. Salient conclusions are i) that the ARVD5 phenotype is lethal, ii) the disease is sex-influenced affecting males significantly more than females, iii) the onset of cardiac symptoms in males is often followed by death, iv) hospitalisation for cardiac disease, heart failure and SCD is significantly more common in mutation positive males than mutation positive females, and occurs at a younger age, v) cardiac symptoms have limited diagnostic utility in determining those with and without the mutation, vi) Holter monitoring for signs of ectopy is the most sensitive diagnostic test (considered the most sensitive test for determining ARVC in Boxer dogs (178)), vii) a late manifestation of the disease in those who do not die is heart failure.

Clinical test abnormalities common in ARVD5 include PVCs and PRWP on 12 lead ECG, delayed LV conduction on SAECG, LVE on echocardiography (with some subjects meeting criteria for DCM) and massive ectopy on Holter monitor at an early age in males. Some ARVC features included in the diagnostic criteria, including epsilon waves and T wave inversion (25)(Figures 2 and 3 page 46), are rarely seen in ARVD5. This must be related to how the underlying mutation affects the myocardium in this genetic subtype. The absence of the epsilon wave in our population may mean that the RV is not affected to the same extent, or that in order to show the epsilon wave properly, a slowed

ECG trace may be required. We have never run this modified 12 lead ECG in Newfoundland. PRWP on ECG (Figure 28 page 198) resembles the pattern seen after an anteroseptal infarct affecting one branch of the left coronary artery. Septal Q waves are a subset of PRWP and occur when all the myocardium at that point is scar tissue. The pattern therefore relates to disruption within the septal region of the heart, in the absence of a significant left axis or LBBB of the heart. As the septum is considered to be spared in 80% of ARVC cases (108) this ECG pattern must tell us something about the 'lack of sparing' of the septum in ARVD5. The SAECG is less diagnostically useful in ARVD5, in contrast to the literature (176).

Figure 28 PRWP in ARVD5: In leads V1, V2 and V3, the R wave is almost non-existant. (See Appendix C for a normal tracing)



Prevalence of ARVD5

Taking all affected subjects alive in 2008 the prevalence of ARVD5 is 1/3000 (Data not included in thesis). This is higher than the oft quoted 1/5000 (110). It is also likely an underestimate.

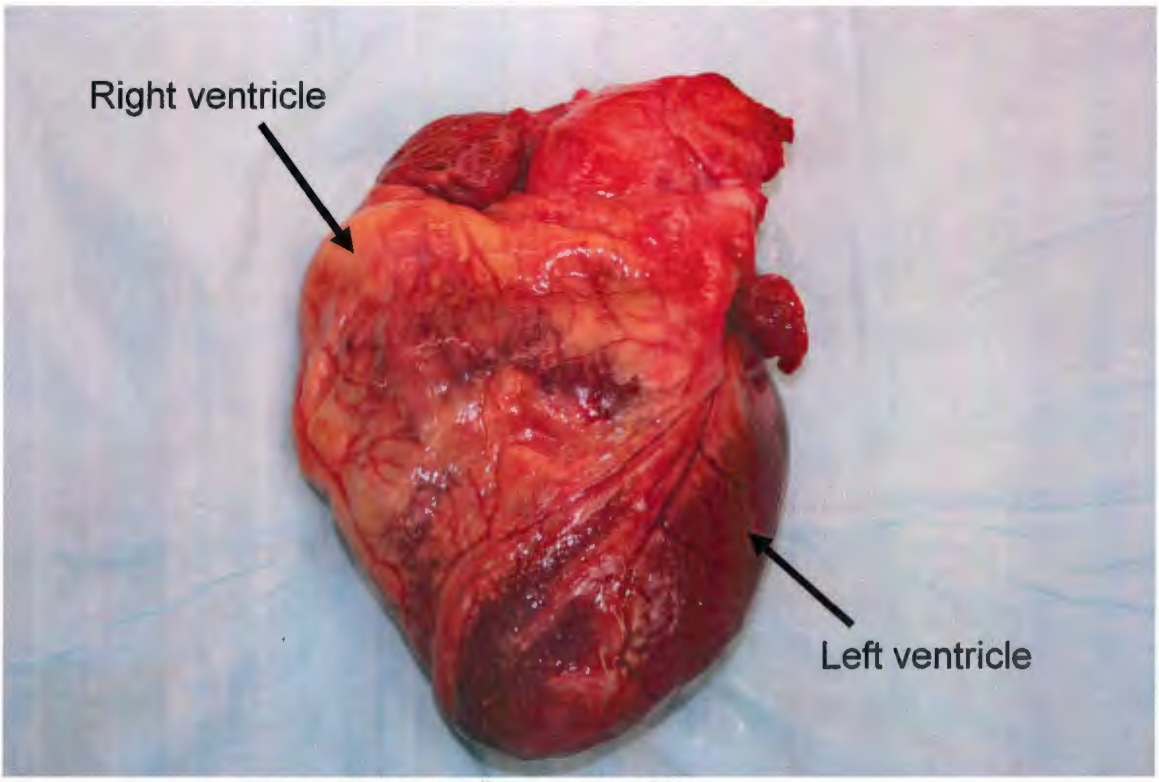
Involvement of the right ventricle

Although in this ARVD5 population the disease appears to affect the LV more than the RV, this almost certainly reflects a 'missing data' bias. We have consistent LV data but

minimal RV data due to the difficulty visualising the RV compared to the LV on transthoracic echocardiography (Appendix C). Like the ECG, echocardiography is available in many non-tertiary centres. Unlike the ECG, it is operator and analyser dependent.

We have some clues that the RV is affected in ARVD5, despite the limitations of the clinical testing. In one case of SCD in a 31 year old (from family AR1), the echocardiogram done 6 months prior to death showed an enlarged LV with a normal RV. On autopsy the RV was massively enlarged. The most recent case was that of an affected male aged 62, who had been maintained on Amiodarone having refused an ICD. Following SCD, his heart was obtained and although his echocardiogram one month earlier was described as 'normal', his RV on autopsy was not, and included many areas of fat (Figure 29 page 200).

Figure 29: Heart post mortem: 62 year old affected male



Note the fat on the RV and the lack of fat on the LV.

Other genetic subtypes of ARVC in the literature appear to be restricted to the RV. The explanation usually provided is that the RV is more susceptible to dilatation following mechanical stress, due to its thinner walls.

ARVC due to the *TMEM43* mutation (ARVD5) is a lethal, sex-influenced, autosomal dominant disorder. It is 100% penetrant. The determination of penetrance allows for the definition of a residual risk of expressing ARVC for at-risk subjects based on clinical testing alone, that is, the remaining likelihood, in the presence of the mutation and the absence of clinical features on clinical testing, of still expressing the disease. This may seem superfluous in the presence of a known mutation detectable on molecular testing, but will be useful for modifying underlying pedigree risk while awaiting molecular test

results, or in jurisdictions where these are not available, or in ARVC families which link to 3p25, but without a known mutation.

The dilemma of diagnosis: how the conclusions from this thesis may inform the diagnostic criteria.

Defining the diagnostic criteria for ARVC depends upon biased ascertainment of those presenting with severe disease. This however is merely one of the multiple problems inherent with the diagnostic criteria for ARVC. Most of the major criteria for ARVC (Table 3 page 57) require tertiary centre type testing or invasive testing. This is true for 5/6 of the major criteria, and up to 3/9 of the minor criteria. Many of our ARVD5 affected subjects presented with death. These individuals could not fulfil current ARVC criteria, as they would meet only one major criterion (autopsy determining evidence of the disease). Even this may not be the case if the pathology was at a unit where little more than a gross assessment was done, and the cause of death was recorded as 'likely arrhythmia'. Additional problems in Newfoundland relate to the limited availability of 'newer' clinical testing compared with larger urban centres. In addition, many subjects live in remote areas of the province, with less access to medical centres larger than a cottage hospital.

Standard ARVC diagnostic criteria (25) had limited usefulness therefore for clinical diagnosis in our Newfoundland families. However, ARVD5 is an autosomal dominant genetic disorder and thus unambiguous obligate carrier status could be assigned. After sudden death under 50 years, the most likely phenotype was serious arrhythmia requiring cardioversion. These three features were used to define affected status in this thesis lised even minimal information.

Table 5 page 60). For epidemiological studies, diagnosis for large populations has to be effective, reproducible, easily defined, and consistent. Our definition fulfilled these requirements.

The issues of clinical diagnosis and presence or absence of symptoms or clinical test findings as diagnostic predictors are common to all diseases that have an underlying genetic cause. The cases that come to initial attention are the tip of the iceberg: those that are serious enough to be recognised or large enough families. The remainder of the iceberg is initially invisible. With 22q11.2 deletion syndrome, a multisystem disorder caused by a common deletion of 3MB on the long arm of chromosome 22, initial ascertainment was through congenital cardiac clinics. The majority of cases thus had congenital heart problems and this feature became closely tied to the diagnosis. With the recognition of the underlying chromosome 22q deletion, the presence of congenital heart defects in those with the deletion is less than 40% (353).

This is analogous to the situation in ARVD5. Using the *TMEM43* mutation to determine those with the genetic susceptibility to develop ARVC allowed us to investigate the efficacy of the current diagnostic criteria. For males with ARVD5 (i.e., those with major clinical sequalea and/or mutation positive and/or obligate carrier status), the diagnostic utility of the 1994 criteria (25) showed 13% sensitivity and 100% specificity. When the modified ARVC criteria were assessed (142), sensitivity and specificity were 43% and 96% respectively. For women the situation was similar, with the original ARVC diagnostic criteria providing a sensitivity of 5% and a specificity of 100% and the modified criteria giving a sensitivity of 60% and a specificity of 97% (data not shown in

the thesis). This is consistent with data from Syrris et al. in 2007 (61) who looked at family members with DSG2 mutations and showed that the penetrance of the disease was 58% if the original Task Force criteria were used (25), and 75% using the modified criteria (142). Data from this paper, and from this thesis underscores the necessity for the ARVC disease diagnostic criteria to be widened.

7.2.3 **Treatment**

We have demonstrated that ICD therapy is life saving in this population with ARVC, using an innovative study design to determine a statistically robust result. This study design had to account for the fact that subjects were from different generations, at different stages in their disease process, some of whom had lived through the application of multiple treatment regimes, and had to use a method to compare those who were treated with ICD to those who were not. The use of the nested case control within the cohort study, with historic controls, was instrumental in obtaining a significant result despite low sample size.

The effectiveness of a cohort study, as in this case, can be enhanced when there is no possibility of doing an RCT. "*Experiments are ethically permissible only when adherence to the scientific protocol does not conflict with the subjects' best interests*" (354). Good observational data are essential (355). Our strategy, which worked well, was to maximize the similarities between this cohort study and a RCT by: (i) defining eligibility criteria analogous to those employed in clinical trials (ii) specifying a zero time (time at which the intervention was applied) for eligibility and modification of baseline differences in prognostic risk, (iii) using the same analyses as a RCT (355).

ARVC in Newfoundland is responsible for many young SCDs and proof of the efficacy of ICD therapy is of immense importance. Given the high prevalence of ARVC in Newfoundland, treatment and follow-up costs alone made it imperative to show that the intervention made a difference. Further research is required to assess the economic impact of ICD use in this population, taking into account all familial, social and medical factors.

7.2.4 **Ethics**

Another first for this thesis was the definition of the privacy paradox: that an emphasis on protecting individual privacy can be harmful when applied to genetic information with clinical repercussions for family members, and also, that the privacy of genetic information is dependent upon the manifestation of disease: a physically obvious phenotype cannot be kept 'private'. ARVC acts in direct contrast to Huntington's disease, which has for many years served as the template against which other genetic disorders are compared. Huntington disease is an autosomal dominant lethal neurological disorder, with no ameliorative treatment or screening. Affected individuals will die, generally 10-15 years post diagnosis, usually in the 4^{th} to 6^{th} decades. Huntington's is however in many respects a most unusual genetic disease. It is deterministic: if you have the gene mutation you will get the disease. It is a trinucleotide repeat expansion resulting in anticipation (the gene size expands during meiosis) when the allele is transmitted from father to child. Disease onset is usually late, with no decrease in reproductive fitness and many disease free years prior to a clinical diagnosis. For affected individuals the only reason for not dying of Huntington's would be death due to another cause, prior to the onset of disease.

Historically, the diagnosis of Huntington's was made following a neurological assessment when the symptoms of the disease were beginning to manifest. But when the Huntington's gene was linked to the short arm of chromosome 4 in 1983 (356) followed a decade later by the cloning of the causative gene (357), it became possible to perform pre-symptomatic testing of this untreatable disorder. Based on family history, at risk individuals could now choose whether they wanted to open 'Pandora's Box' for a look into the future, or keep it closed and take their chances. In contrast to a neurological clinical test which gives information about the neurological state of the patient immediately, getting a DNA result said nothing about the neurological status of the individual on the day of the test.

The advent of the test for Huntington's effected a change in medical practice for this disease as it brought into stark relief the possibility that genetic information could be used in a more insidious manner (358). It was also recognised that insurance companies could use this information to ban someone from coverage based on a disease they would not manifest for years. This led to a reassessment as to the best way to deal with the information accrued. As a direct result, a genetic counselling protocol was established for dealing with the implications (physical, psychological and societal) for each person intending to undergo pre-symptomatic testing for Huntington's (289).

Inasmuch as Huntington's was one of the first genetic tests to become readily available for clinical purposes, it set the standard as to when and how genetic information should be shared with at risk individuals. However, the vast majority of disease genes are not like HD. They are variably expressive and exhibit reduced penetrance, and there is often

screening and the possibility of treatment, if not a cure. The Huntington's paradigm may therefore be the wrong structure upon which to hang the 'standard of care' for other Mendelian disorders. Indeed the question of whether insurance issues should have the stature afforded to them in genetics is itself debatable (359). It may be argued therefore that for many diseases, DNA testing should be considered in the same light as other clinical tests, whether via a research or a clinical route, and the point of consent should thus occur when the test is initiated.

ARVC is almost the opposite end of the spectrum to Huntingtons. Both conditions are autosomal dominant, lethal conditions, but the former has amelioration in the form of ICD treatment. Thus knowing that one is a gene mutation carrier for ARVC allows for the application of prophylactic ICD treatment: considered by most to be a clinical imperative. Insurance issues would also be minimised in ARVC, in the same manner as any lethal clinical condition (whether genetic or not: e.g. diabetes) that requires life saving intervention yet will impact on the ability of the patient to get life insurance.

That patients/subjects/family members not be harmed is a central issue in research. When research has major clinical ramifications (as is the case in many high risk genetic disorders), a mechanism for managing the flow of information is imperative. The solution, in part, is to amalgamate clinical and research genetics under the remit of a genetic register for Mendelian disease within Newfoundland and Labrador.

"*What has been will be again, what has been done will be done again; there is nothing new under the sun.*" - Ecclesiastes 1:9

The concept of a genetic register is not a new concept for Newfoundland. During the writing of this thesis, I discovered this document, written by Clarke Fraser (McGill University, Professor of Medical Genetics) in August 1990, regarding the initial members of the AR1 family

"*A 30 year old Newfoundlander was diagnosed 6 years ago as having a potentially lethal heart condition (arrhythmogenic right ventricular dysplasia ARVD). During the next couple of years, four more cases were identified in various parts of the province. A full compilation of the family trees of these patients was made because in several cases there appeared to be other male family members who had died suddenly while undergoing strenuous exercise, often in the woods. This compilation, made the hard way by interviewing numerous relatives, by searching parish registers etc. revealed that all five patients were related, being connected to a common ancestor over 100 years ago- although they did not realize it. They all had an inherited disease that could be transmitted by male or female but which in the case of the female might never be revealed. Thus to an individual family the condition might not appear inherited and would appear as a heart attack coming out of the blue. Some 30 members of this family had died in this way in the prime of their lives. Having compiled the pedigree and understood the inheritance it is now possible to decide who of the living family members is at risk and to give a degree of risk in each case, and to contemplate the possibility of providing preventative treatment in the form of a drug to reduce the incidence and severity of the arrhythmia. Had there been a familial disease registry with a good collection of family tree information, this compilation of the family tree for these five patients could have been achieved more simply and quickly*".

ARVC continues to support the need for implementation of a genetic registry as the responsibility for information to be passed back to subjects in a clinically appropriate manner is paramount, regardless as to how that information has been derived.

7.2.5 Future research directions

The results presented in this thesis prompt a number of future research directions and remaining questions.

Functional studies

Functional studies are required to determine the role of TMEM43 in the myocyte, and to ascertain how the protein fits into the DCM/ARVC pathways. It will be an interesting journey for the protein chemists. TMEM43 may point to a new pathogenesis. An exciting possibility is that TMEM43 may turn out to have a target that is amenable to drug therapy that could eventually replace or provide key adjunctive support to symptomatic treatment with the ICD.

Modifiers

"A genetic modifier, when expressed, is able to alter the expression of another gene by affecting transcription or by altering the immediate gene transcript or by affecting phenotypes at other levels of organization." (360)

Penetrance and variable expressivity

As defined in the introduction, a common (indeed, almost universal) feature of genetic disease is the situation when a single mutation is not expressed in all subjects who have that mutation, or when it is expressed, it is not always expressed in the same way. The

former is termed penetrance: the proportion of subjects who carry a mutation and express the phenotype. The latter is variable expressivity: the extent to which a mutation shows all, or part of its phenotype in those who have the mutation and/or the variable age at which symptoms develop.

Mendelian diseases, although caused by a single gene mutation, are far from simple (361). Other factors (modifiers) affect the single gene, and these can be genetic or environmental. Genetic factors would include other genes acting as modifiers, either directly on the gene product in question, or somewhere in the pathway. An analogy useful for patients relates to a hockey team. With the abnormal gene (the hockey player who cannot skate or hit the puck), the game would only be salvageable if every other player on the team were an NHL hockey star. If however, the team-mates were a mixture of those who made the junior levels of the NHL plus high school players, the team would struggle. Thus, other genes are the team players, which if strong and protective may have a positive impact on the phenotype dictated by the abnormal gene (incompetent player). The opposite would be true for the non-supportive genes (weak team mates). Environmental issues also may act as phenotypic modifiers. In the hockey analogy these would include ice surface, fan support etc. In diseases, these may include viruses, diet, smoking, drinking, drugs, exercise, work practices, pregnancy, lactation, and the uterine environment affecting early development amongst many others.

Sex differences

ARVD5 has a high variability of expression, the most obvious being the difference between males and females. One is tempted to implicate hormones as a protective

mechanism in females, but there may also be epigenetic changes. Epigenetic changes are those that affect a cell, organ or individual without directly affecting the heritable DNA sequence. Included would be the process of DNA methylation (including the methylation of one of the X chromosomes in females) (362), chromatin remodelling (the structural changes occurring throughout cell division for transcriptional regulation and for chromosome segregation) (363) and gene silencing (the creation of facultative heterochromatin when genes are no longer required in embryogenesis) (364).

One can imagine that a protein that affects TMEM43 coded by a gene on the X chromosome may be more deleterious in a hemizygous male compared with a heterozygous female. Alternatively, the mechanism of methylation of the X chromosome in the female may affect proteins in the pathway implicated in the cardiomyopathies, by affecting the nuclear membrane (the anchor point for the methylated X chromosome).

Some have suggested that the sex differences in ARVD5 may in part be related to work practices. In other forms of ARVC, mechanical stress in athletes on a heart muscle already stressed by an underlying biological defect has been described (365). There is little evidence in the ARVD5 families for such a mechanism. While many men worked physically hard, most of it was not of a cardiovascular nature and appears similar to the physical labours performed by women around the house (the children, the garden, the animals, the housework), particularly in previous generations prior to labour-saving devices.

There is also a possibility that women with ARVD5 may be more seriously affected now than they were in the past. While the data have not been assessed in a systematic manner

to address this question, if such a worsening has occurred, this could not be due to genetic factors and must be related to ascertainment bias or external environmental factors. Exogenous hormones in food, and the introduction 40 years ago of the oral contraceptive pill are possible factors that require further research.

Other variable expression

Variability in ARVD5 is not restricted to differences between the sexes, variability also occurs within each sex. Although few women present early and severely, there are individual examples of this within the Newfoundland pedigrees. Many women are significantly affected with symptoms in their middle years, while others have no signs or symptoms of disease for several decades. Men can present young with heart failure, with SCD or with non-specific symptoms, while still others live well into their middle years with no clinical sequalae. These latter individuals will have clinical features recognisable on clinical testing but will not have brought themselves to medical attention. The most striking example of this is in family AR8 (Figure 8 page 79), where the 19 year old proband died suddenly and the grandfather who transmitted the disease, a man who had never presented for any cardiac testing or treatment, died suddenly at age 72 years. This pedigree pattern might direct us to 'anticipation', a mechanism of variable expression with the worsening of disease across generations. In most conditions in which this occurs, the cause is an expanding DNA tri-nucleotide repeat (TNR), where at meiosis, the number of TNR's may change: usually increasing in number. The gene in effect 'expands' by the addition of copies of the TNR sequence. Diseases exhibiting this 'gross' example of variable expression include Huntington's disease, myotonic dystrophy and Fragile X syndrome. We have not systematically examined clinical anticipation in

ARVD5, i.e., without bias, assigned disease status to all individuals in at least 3 generations within each family and investigated severity of disease and age of onset of key manifestations of disease. However, with the available pedigree data, there does not appear to be consistent worsening of disease across generations, manifested by increasing severity or younger age of onset. Also, the *TMEM43* gene does not contain a trinucleotide repeat sequence, making anticipation an unlikely explanation of variable expression in ARVD5. Investigation of other hypothesized mechanisms of anticipation (366), where there is no identification to date of a specific molecular mechanism, would remain a possible area of future research.

Remaining questions

There are many other questions that remain to be answered for ARVD5. For example, the determination of the location of the ectopy in these families is possible from the morphology of the PVC's observed on the Holter monitor. We have yet to determine if the ectopy is primarily left sided in origin, right sided, or both, or the temporal sequence of ectopy during the disease process. The disease course following ICD therapy also needs to be assessed. Are there prognostic factors that determine those likely to go on to heart transplant after the ICD that are similar to, or different from, the modifiers of the natural disease phenotype? Is there a pattern to the frequency and type of shock from the ICD across time? How would such functional findings relate to the structural findings? These and other clinical questions remain to be answered.

A genetic question remaining to be answered is how the ARVD5 mutation is maintained in the population. The disorder is lethal at early ages in men. Despite the fact that the

majority of women are alive through their reproductive lifespan, one would expect that the removal of 50% of affected men before age 40 years, and 80% before 50 years might have an effect on gene frequency. With a population this large, there is an opportunity to look at the possibility of meiotic drive.

Meiotic drive

The general features of meiosis were understood by the early 20th century, particularly that cell division led to accurate partitioning of nuclear chromosomes with half the parental number in each daughter cell: encompassing Mendel's law of segregation: that each allele in a pair of alleles has an equal chance of being transmitted to the next generation. Meiotic drive is defined as "*any meiotic mechanism that results in the unequal recovery of the two types of gamete produced by a heterozygote*"(367). This was initially assessed as unequal segregation of chromosomes in heterozygous Drosophila melanogaster males (368), later defined as a dominant segregation distorter allele mapping to the centromere of Drosophila chromosome 2. The system is complicated by responder genes and enabling genes. In mice, meiotic drive is obvious with the tail-less 't alleles' which are transmitted in a frequency as high as 99% c.f. wild type. The disease that has been most associated with the potential for meiotic drive in humans is myotonic dystrophy (369-374) although the concept has been dismissed by others (375) (376). Meiotic drive in other diseases has also been implicated (377). Meiotic drive is postulated as a method to maintain disease alleles in a population despite decreased reproductive fitness.

What is the consequence of meiotic drive?

"*Clearly a higher plant species is at the mercy of its pollen grains. A gene which greatly accelerates pollen tube growth will spread through a species even if it causes moderately disadvantageous changes in the adult plant*" (J.B.S. Haldane, 1932)

Haldane recognised that the evolutionary consequences of drive (before it was described) could be considerable: a mutation leading to almost 50% reduction in the fitness of diploid adults could still increase in frequency, or remain the same in the population provided it had a compensatory mechanism, e.g. meiotic drive.

It remains to be seen whether there is any indication that meiotic drive occurs in ARVD5. Of immediate interest is that if one asks of the subject population in general, which parent passed on the ARVD5 gene, the proportions (not presented in this thesis) are 49.9 % fathers and 50.1% mothers. Given the early deaths in males, one might imagine that a greater skew would occur towards females. Whether the presence of more affected males than affected female subjects in this population is related to a bias because of the greater ease of diagnosing males, or a biological indication of some other mechanism (e.g. foetal loss) is unknown. We have an opportunity to further assess this issue in ARVD5.

7.2.6 Study advantages

The homogeneous population

Newfoundland and Labrador is one of the few populations in the world known as a founder population, and is thus of enormous interest to geneticists. Although the history

of the region is known to enhance genetic studies of autosomal recessive conditions where distant cousins are affected in one geographic area due to consanguinity, autosomal dominant conditions also flourish due to founder effects. Newfoundland families have proved to be an invaluable resource in the mapping and cloning of disease causing genes (251). The presence of large families in the province facilitates genetic research, including the genetic and clinical epidemiology of disease.

Other advantages

There are several specific advantages of the studies presented in this thesis, particularly in chapters 2-4. In brief therefore, the main advantages are as follows. The homogeneous population allowed identification of a novel gene on a single background haplotype. This is the largest homogeneous group with ARVC in the world. The ability to define affected status using genetic data, i.e., the *TMEM43* 1073 C>T mutation, or pedigree identification of obligate carriers, provided a diagnostic strategy independent of clinical manifestations. Mutation negative subjects within the family cohort provided a superior control group. The depth and quality of clinical data, including accurate classification of all available medical records on each person born at 50% risk, alive or dead and in the absence of a medical record, the availability of birth and death data, provided advantages for assessment of diagnostic utility and penetrance. The availability of serial testing data across time was advantageous for studying the natural history of the disease from medical records and visits to an at-risk clinic. All of the clinical and genetic data provided essential information for genetic counselling and management of the disease in at-risk individuals.

7.2.7 Study limitations

As with any study, there were several limitations, again presented more fully in the thesis chapters, but briefly revisited here. With respect to study design, the subjects in this cohort study were assessed in both a retrospective and prospective manner. Despite having collected available medical records, there are almost certainly missing data. With respect to diagnosis, we created our own diagnostic strategy which was easy to apply, and straightforward. This was necessary given the inherent limitations in the ARVC task force diagnostic criteria (25), and the lack of anything except very simple clinical testing in many subjects from past generations. Our strategy took into account both a genetic definition, either through the use of a linked haplotype or a mutation, and a clinical definition with the presence of early unexplained death, and the use of cardioversion to alter a lethal rhythm back to sinus rhythm in those aged under 50 years. Bias may thus be present in ascertaining those with more severe disease. However, using only a genetic definition did not change the survival statistics. In addition, we used sibships where over 50% of the sibship had a defined disease status to offset the ascertainment bias inherent in studies of inherited disease. The use of sibships where all of the siblings had a known disease status did not change the survival statistics. However, there may still be ascertainment bias in that there are more male than female affected subjects in the study, as has been mentioned, probably due to the greater ease with which males are diagnosed. One of the greatest limitations for this study is survivor bias. One had to be alive in order for a test to be applied, or to have a medical record other than an autopsy report. However, with the continuation of the prospective study, and the follow-up of those at risk from age 10 years, the remaining questions relating to survivor bias should be

answerable in the future. It is likely that we have failed to ascertain all ARVD5 families in Newfoundland. Families with positive family histories of ARVC could be missed for example if the *TMEM43* mutation is transmitted through several generations of females. Also, time trends, particularly the differences in drug usage across time, pose potential problems. Data have been collected on the use of medical interventions, but the fluidity of drug prescription and the changes in such practices over time make their impact difficult to determine accurately. Other possible time trends in terms of environmental modifiers are also challenging to ascertain and assess. The lack of RV data from echocardiographic assessment limited any conclusions regarding the structure and function of the right ventricle.

7.2.8 Validity and conclusion

This study has excellent internal validity due to the relative completeness of the data and the homogeneity of the population. Of 11 families with ARVC in Nova Scotia where the genetic etiology of their disease is known, nine have the same TMEM43 mutation as the Newfoundland families. One of these nine has no apparent Newfoundland roots (378). In London, England, we have determined that other mutations within *TMEM43* apparently segregate with ARVC disease in UK kindreds (data not shown in thesis). There are thus likely to be individuals with ARVC due to ARVD5 in Canada and the rest of the world for whom the results presented in this thesis are directly applicable. These findings will have external validity for families with the same mutation and may help to inform families with a different mutation in the same gene.

The results from the series of studies presented in this thesis have moved the field of ARVC genetics research forward significantly. The clinical and research implications of this work include families with ARVC who are receiving improved diagnosis, investigation and treatment and researchers with new insights into the variable expressively and pathogenesis of this complex disease. Ethical issues have even broader implications. The future is bright for answering the many exciting research questions arising from this body of work.

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APPENDIX A

Variable Number	Variable Name	Variable Description
1	fid	Family ID
2	gennum	Generation Number
3	index	ID number
4	sibship	Sibship number
5	firstnam	First name
6	lastname	Last name
7	ascert	Ascertainment Status > or = 50%
8	ascer100	Ascertainment status: All of sibship
9	ascer_50	Ascertainment > 50%
10	mcp#	MCP
11	tranpar	Gender of trans. parent
12	gender	Gender
13	dob	Date of Birth
14	accdob	Accuracy of DOB
15	yob	Year of Birth
16	decade	Decade of birth
17	newdeca	DOB (before and after 1950)
18	brn_1920	DOB (Before and after 1920)
19	agelstfu	age at last follow-up
20	agltfunh	Age at last follow-up natural history: taking into account date of defib and date of Ht trans and death
21	YOD2	Year of Death
22	deadnh	Are they dead?. This is censored for date of ICD or trans. If they died after this time, they are considered alive for the analysis
23	var00038	Are they dead?
24	var00042	ID number
25	var00043	First name
26	var00044	Last name
27	datetday	Todays date
28	arvccs	ARVC Clinical Status
29	taskfrc	Do they fulfill task force criteria? NB A no SCD answer is ANY SCD at ANY age (tells why there are no records)
30	tskfmaj	If yes, which criteria do they fill?
31	critmn	Using modified criteria as in Hamid paper 2002, with addition of any relative not just 1st degree
32	dna	DNA Obtained
33	linkage	Linkage Status
34	TMgene	Are they positive for the TM 43 SNP?
35	YOD3	Year of Death
36	alivein96	Were they alive at the begining of 1996 (when Kathy first started to ascertain families)
37	alivein98	Were they alive at the begining of 1998 when the main clinical screening started?
38	Penetrant	Were they penetrant for any of the features listed?

39	penet2	Were they penetrant for any of the features listed (removing those where there was no difference shown between affected and unaffected subjects)?
40	kmPENET	km variable for penetrance. Age at which affected individuals (born at an a priori 50% risk) manifested a feature (for feature list see ARVC datasets file) or last follow up
41	penetfeat	Which feature?
42	penetft2	Which feature (assuming had more than one at the same age)?
43	penetft3	Which feature (assuming had more than 2 at the same age)?
44	penetft4	Which feature (assuming had more than 3 at the same age)?
45	penetft5	Which feature (assuming had more than 4 at the same age)?
46	disease	Disease Status
47	met_diag	Method of Diagnosis: Clinical event = SCD, VT, or transplant under 50
48	diagn_1	One method of diagnosis: hierarchy: Ped Pos: DNA:clinical event
49	var00033	ID number
50	var00022	First name
51	var00023	Last name
52	modepre	Mode of presentation
53	clinic	Have they attended a screening clinic?
54	cht_rev	Was there a retrospective chart review? If Yes: we got chart notes. This includes autopsy reports only.
55	mop_2	Mode of presentation: chart review only or chart review and interview
56	clinprob	Clinical Problem
57	type	Clinical problem type
58	age_1cli	Age at first clinical problem
59	longstan	Longstanding
60	vis_dr	Visit to Doctor
61	age_1vis	Age at first visit to Dr.
62	presyn	Presyncope
63	agepresy	Age @ Presyncope presentation
64	agpresgp	Age group at PS
65	presynkm	KM: time to PS, ICD, Ht trans, death or last f/up
66	diff_ps	Those with clinical event: difference between age at death and age at pre-syncope
67	syncope	Syncope
68	agesynco	Age at syncope presentation
69	agsyngp	Age gp at syncope
70	synkm	KM: time to S, ICD, Ht trans, death or last f/up
71	palpit	Palpitations
72	agepalp	Age at palpitation presentation
73	agpalgp	Age gp at palpitations
74	palpkm	KM: time to Palp, ICD, Ht.trans, death or last f/up
75	chtpain	Chest Pain
76	agecp	Age at chest pain presentation
77	agecpgp	Age group at chest pain
78	chtpnkm	KM: time to CP, ICD, Ht trans, death or last f/up
79	VAR00039	First name
80	VAR00037	Last name
81	hrtfail	Heart Failure
82	agehf	Age at Heart Failure presentation

83	agehfgp	Age group at heart failure
84	NYHF	Functional assessment of heart failure
85	htfailkm	KM: time to HF, ICD, Ht. trans, death or last f/up
86	cardiac	Cardiac Arrest
87	agecard	Age @ Cardiac Arrest
88	cardkm	KM: time to CA, ICD, Ht. trans, death or last f/up
89	hosp	Hospitalization
90	hosp_t	Reason for hospitalisation
91	age_hosp	Age at first Hospitalization
92	hospkm	KM: time to Hosp, ICD, Ht.trans,death or last f/up
93	hoskm2nd	KM: time to Hosp for 2nd reasons, ICD, Ht trans, death or last f/up
94	num_hosp	Number of Hospitalizations
95	days_hos	Days in Hospital
96	var00001	ID number
97	var00019	First name
98	var00028	Last name
99	dead	Are they dead?
100	datedead	Date of Death
101	dodacc	Accuracy of DOD
102	yod	Year of Death
103	dedpre80	Dead before 1980: Class 3 meds not given prior
104	age_dead	Age at Death
105	mortkm	Age at Death or Last follow-up
106	scd	Was it a SCD?
107	cod	Cause of Death
108	describe	Describe (Cause of Death)
109	c1drugs	Class 1 Drugs
110	agec1d	Age at Rx (Class 1 Drug)
111	c1name	Class 1 drug name
112	sotolol	Sotolol
113	agesot	Age at Rx (Sotolol)
114	antiarr	Did they take any antiarrhythmic?
115	ageanti	Age at antiarrythmic
116	var00007	ID number
117	var00024	First name
118	var00030	Last name
119	amiod	Amiodorone
120	ageamiod	Age at Rx. (Amiodorone)
121	betabloc	Betablockers
122	age_beta	Age at Rx. (Betablocker)
123	betaname	Betablockers Name
124	cardmeds	Cardiac Meds
125	age_card	Age at Rx. (Cardiac Meds)
126	cmedname	Cardiac Meds Name
127	var00013	ID number
128	var00025	First name
129	var00026	Last name
130	defib	Defibrillator

131	datdefib	Date of Defib Implant
132	yob2	Year of Birth
133	icdreas	Was the implant for primary or secondary reasons?
134	defiprob	Has there been a problem with the defib?
135	typeprob	Type of problem with the defibrillator
136	age_defi	Age at Rx. (Defibrillator)
137	dat1fir	Date of 1st firing of ICD for an appropriate reason
138	fir1icd	Age of first appropriate firing of the ICD
139	locicd	What type of Loss of consciousness was there?
140	var00020	ID number
141	var00027	First name
142	var00031	Last name
143	rateicd	VT rate at time of ICD fire
144	whyfire	What did it appropriately fire for?
145	causfir	Cause of 1st firing
146	vtdet	Did the ICD detect VT which resolved before a chock was required?
147	vtdet2	What level of VT was it?
148	icdecho	Was there LVE (112%) in the echo closest to the ICD implant date?
149	com_def	Comments (Defibrillator)
150	pacemake	Pacemaker
151	age_pace	Age at Rx. (Pacemaker)
152	com_pace	Comments - Pacemaker
153	cardvers	Cardioversion
154	age_cv	Age at Rx. (Cardioversion)
155	com_cv	Comment - Cardioversion
156	heart_tx	Heart Tx.
157	age_htx	Age at Rx. (Heart Tx.)
158	den_afib	Denovo Atrial Fibrillation
159	agedafib	Age @ Denovo Atrial Fibrillation or Age @ last ECG
160	den_aflu	Denovo Atrial Flutter
161	agedaflu	Age @ Denovo Atrial Flutter or Age @ last ECG
162	den_oar	Denovo Other Atrial Rhythm
163	aged_oar	Age @ Denovo Other Atrial Rhythm @ last ECG
164	den_wpw	Denovo WPW
165	aged_wpw	Age @ Denovo WPW or Age @ last ECG
166	den_vt	Denovo Ventricular Tachy
167	aged_vt	age @ Denovo VT or Age @ last ECG
168	den_tdp	Denovo Torsades de Pointes
169	aged_tdp	age @ Denovo Torsades de Pointes or age @ last ECG
170	den_l_ax	Denovo Left Axis Deviation
171	aged_lax	age @ Denovo Left Axis Deviation or Age @ last ECG
172	den_r_ax	Denovo Right Axis Deviation
173	aged_rax	age @ Denovo Right Axis Deviation or Age @ last ECG
174	den_fdav	Denovo First Degree AV block
175	aged_fav	age @ denovo First degree AV block or Age @ last ECG
176	den_mob1	Denovo Mobitz 1
177	aged_mb1	age @ denovo Mobitz 1 or age @ last ECG
178	den_mob2	Denovo Mobitz 2

179	aged_mb2	age @ denovo mobitz 2 or age @ last ECG
180	den_comp	Denovo Complete Heart Block
181	aged_com	age @ denovo Complete Heart Block or age @ last ECG
182	den_qt_s	Denovo Short QT interval
183	aged_qts	age @ denovo Short QT interval or age @ last ECG
184	den_qt_l	Denovo Long QT interval
185	aged_qtl	age @ denovo long QT interval or age @ last ECG
186	den_pr	Denovo Short PR interval
187	age_d_pr	age @ denovo short PR interval or age @ last ECG
188	denqrs_	Denovo long QRS duration
189	agedqrs_	age @ denovo long QRS duration or age @ last ECG
190	den_eps	Denovo Epsilon Wave
191	aged_eps	age @ denovo Epsilon Wave or age @ last ECG
192	den_prw	Denova Poor R Wave
193	aged_prw	Age @ Denova Poor R Wave or Age @ last ECG
194	den_talr	Denovo Tall R in V1
195	aged_tr	Age @ Denovo Tall R in V1 or Age @ last ECG
196	den_tinv	Denovo T inversion in V2 or V3
197	aged_tin	Age @ denovo T inversion in V2 or V3
198	den_lae	Denovo Left Atrial Enlargement
199	aged_lae	Age @ Denovo Left Atrial Enlargement or age @ last ECG
200	den_rae	Denovo Right Atrial Enlargement
201	aged_rae	Age @ Denovo Right Atrial Enlargement or age @ last ECG
202	den_rvh	Denovo Right Ventricular Hypertrophy
203	aged_rvh	Age @ Denovo Right Ventricular Hypertrophy or age @ last ECG
204	den_lvh	Denovo Left Ventricular Hypertrophy
205	aged_lvh	Age @ Denovo left Ventricular hypertrophy or age @ last ECG
206	denlbbb	Denovo Left Bundle Branch Block
207	aged_lbb	Age @ Denovo Left Bundle Branch Block or age @ last ECG
208	denrbbb	Denovo Right Bundle Branch Block
209	aged_rbb	Age @ Denovo Right Bundle Branch Block or age @ last ECG
210	den_lahb	Denovo Left Anterior Hemi-block
211	aged_lah	Age @ Denovo Left Anterior Hemi-Block or age @ last ECG
212	den_lphb	Denovo Left Posterior Hemi-block
213	aged_lph	Age @ Denovo Left Posterior Hemi-Block or age @ last ECG
214	denpvcs_	Denovo Premature Ventricular Complexes
215	aged_pvc	Age @ Denovo Premature Ventricualr Complexes or age @ last ECG
216	den_infq	Denovo Inferior Q waves
217	agedinfq	Age @ Denovo Inferior Q waves or age @ last ECG
218	den_sepq	Denovo Septal Q waves
219	agedsepq	Age @ Denovo Septal Q waves or age @ last ECG
220	den_latq	Denovo Lateral Q waves
221	agedlatq	Age @ Denovo Lateral Q waves or age @ last ECG
222	den_antq	Denovo Anterior Q waves
223	agedantq	Age @ Denovo Anterior Q waves or age @ last ECG
224	den_peri	Denovo Pericarditis
225	agedperi	Age @ Denovo Pericarditis or age @ last ECG
226	de_std_i	Denovo ST depression >1mm Inferior

227	aged_dpi	Age @ Denovo ST depression >1mm Inferior or age @ last ECG
228	de_std_a	Denovo ST depression >1mm Anterior
229	aged_dpa	Age @ Denovo ST depression >1mm Anterior or age @ last ECG
230	de_std_l	Denovo ST depression >1mm Laterally
231	aged_dpl	Age @ Denovo ST depression >1mm Laterally or age @ last ECG
232	de_ste_i	Denovo ST Elevation <1mm Inferior
233	aged_eli	Age @ Denovo ST Elevation <1mm Inferior or age @ last ECG
234	de_ste_a	Denovo ST Elevation <1mm Anterior
235	aged_ela	Age @ Denovo ST Elevation <1mm Anterior or age @ last ECG
236	de_ste_l	Denovo ST Elevation <1mm Laterally
237	aged_ell	Age @ Denovo ST Elevation <1mm Laterally or age @ last ECG
238	den_twii	Denovo Inferior T wave inversion
239	agedtwii	Age @ Denovo Inferior T wave inversion or age at last ECG
240	den_twif	Denovo Inferior T wave flattening
241	agedtwif	Age @ Denovo inferior T wave flattening or age at last ECG
242	den_twli	Denovo Lateral T wave inversion
243	agedtwli	Age @ Denovo lateral T wave inversion or age at last ECG
244	den_twlf	Denovo lateral T wave flattening
245	agedtwlf	Age @ denovo lateral T wave flattening or age at last ECG
246	den_prsq	Denovo any poor R wave progression or septal Q waves
247	agedprsq	Age @ Denovo any poor R wave progression or septal Q waves or age at last ECG
248	ekgabnan	Any abnormality at EKG on any EKG (poor R wave, pvc's, VT only)
249	PRWPAny	Is there PRWP on any ECG?
250	PVCany	PVCs on any ECG
251	var00008	ID number
252	var00011	First name
253	var00009	Last name
254	ecg	Did they have an ECG?
255	ecgtrace	Is there a trace, or is the report from notes?
256	agegp1	Age Gp @ 1st ecg
257	age_ecg	Age @ first ECG
258	nor_abn1	Was it a Normal ECG?
259	htrate_1	Heart rate first ECG
260	rhyth_1	What is the rhythm on first ECG?
261	axis_1	What is the axis on first ECG?
262	hb_1	Is there Heart Block on first ECG?
263	qt_1	What is the QT interval on first ECG?
264	pr_1	Is the PR interval short on first ECG?
265	qrs_1	QRS duration on first ECG
266	eps_1	Was there an Epsilon wave on first ECG?
267	prwp_1	Was there a poor R wave progression on first ECG(<3mm in V3)?
268	tallr_1	Was there a tall R in V1 on first ECG ?
269	tinv_1	Was there a T wave inversion in V2 or V3 on first ECG?
270	lae_1	Was there left atrial enlargement on first ECG?
271	rae_1	Was there right atrial enlargement on first ECG?
272	lvh_1	Was there Left ventricular hypertrophy on first ECG?
273	rvh_1	Was there right ventricular hypertrophy on first ECG?
274	lbbb_1	Was there left bundle branch block on first ECG?

275	rbbb_1	Was there right bundle branch block on first ECG?
276	lahb_1	Was there left anterior hemiblock on first ECG?
277	lphb_1	Was there left posterior hemiblock on first ECG?
278	pvc_1	ANy PVC's on first ECG?
279	pvc3_1	3 pvc's in a row (NSVT)on first ECG?
280	infq_1	Inferior Q waves on first ECG?
281	septq_1	Septal Q waves on first ECG?
282	latq_1	Lateral Q waves on first ECG?
283	antq_1	Anterior Q waves on first ECG?
284	peric_1	Any pericarditis on first ECG?
285	st_dp_i1	ST depression > 1mm (INFERIOR) on first ECG
286	st_dp_a1	ST depression > 1mm (ANTERIOR) on first ECG
287	st_dp_l1	ST depression > 1mm (LATERALLY) on first ECG
288	st_el_i1	ST elevation >1mm (INFERIOR) on first ECG
289	st_el_a1	ST elevation >1mm (ANERIOR) on first ECG
290	st_el_l1	ST elevation >1mm (LATERALLY) on first ECG
291	tw_inf_1	Inferior T wave on first ECG
292	tw_lat_1	Lateral T wave on first ECG
293	pr1sql_1	Was there any poor R wave progression or septal Q waves on the first ECG
294	var00041	ID number
295	qrs_firs	QRS on first ECG
296	qrs_last	QRS on last ECG
297	qrsl_110	Is the QRS durationN on the last ECG > .110 ms
298	comm_1	Comments on first ECG
299	comment1	Comment on 1st ECG
300	agelast	Age @ last ECG
301	fup_ecgs	How many follow-up ecg's?
302	ecg2	Had a second ECG?
303	ecg2trac	Is there a trace, or is the report from notes?
304	agegp2	Age gp @ 2nd ECG
305	age_2	Age @ 2nd ECG
306	norabn_2	Normal second ECG?
307	hr_2	Heart rate second ECG
308	rhyth_2	What is the rhythm on second ECG?
309	axis_2	What is the axis on second ECG?
310	hb_2	Is there Heart Block on second ECG?
311	qt_2	What is the QT interval on second ECG?
312	pr_2	Is the PR interval short on second ECG?
313	qrs_2	QRS duration on second ECG
314	eps_2	Was there an Epsilon wave on second ECG?
315	prwp_2	Was there a poor R wave progression on second ECG(<3mm in V3)?
316	tallr_2	Was there a tall R in V1 on 2nd ECG ?
317	tinv_2	Was there a T wave inversion in V2 or V3 on 2nd ECG?
318	lae_2	Was there left atrial enlargement on 2nd ECG?
319	rae_2	Was there right atrial enlargement on 2nd ECG?
320	lvh_2	Was there Left ventricular hypertrophy on 2ndECG?
321	rvh_2	Was there right ventricular hypertrophy on 2nd ECG?
322	lbbb_2	Was there left bundle branch block on 2ndECG?

323	rbbb_2	Was there right bundle branch block on 2ndECG?
324	lahb_2	Was there left anterior hemiblock on 2nd ECG?
325	lphb_2	Was there left posterior hemiblock on 2nd ECG?
326	pvc_2	ANy PVC's on 2nd ECG?
327	pvc3_2	3 pvc's in a row (NSVT)on 2nd ECG?
328	infq_2	Inferior Q waves on 2nd ECG?
329	septq_2	Septal Q waves on 2nd ECG?
330	latq_2	Lateral Q waves on 2nd ECG?
331	antq_2	Anterior Q waves on 2nd ECG?
332	peric_2	Any pericarditis on 2nd ECG?
333	st_dp_i2	ST depression > 1mm (INFERIOR) on second ECG
334	st_dp_a2	ST depression > 1mm (ANTERIOR) on second ECG
335	st_dp_l2	ST depression > 1mm (LATERALLY) on second ECG
336	st_el_i2	ST elevation >1mm (INFERIOR) on second ECG
337	st_el_a2	ST elevation >1mm (ANTERIOR) on second ECG
338	st_el_l2	ST elevation >1mm (LATERALLY) on second ECG
339	tw_inf_2	T wave inferior on 2nd ECG
340	tw_lat_2	T wave lateral on 2nd ECG
341	pr2sq2_2	Was there any poor R wave progression or septal Q waves on the 2nd ECG
342	comm_2	Comments on 2nd ECG
343	comment2	Comment on 2nd ECG
344	ecg3	Has a 3rd ecg?
345	agegp3	Age gp @ 3rd ECG
346	age_3	Age @ 3rd ECG
347	ecgtrc_3	Is it a trace or a report from the notes?
348	norabn_3	Normal 3rd ECG?
349	hr_3	Heart rate 3rd ECG
350	rhyth_3	What is the rhythm on 3rd ECG?
351	axis_3	What is the axis on 3rd ECG?
352	hb_3	Is there Heart Block on 3rd ECG?
353	qt_3	What is the QT interval on 3rd ECG?
354	pr_3	Is the PR interval short on 3rd ECG?
355	qrs_3	QRS duration on 3rd ECG
356	eps_3	Was there an Epsilon wave on 3rd ECG?
357	prwp_3	Was there a poor R wave progression on 3rd ECG(<3mm in V3)?
358	tallr_3	Was there a tall R in V1 on 3rd ECG ?
359	tinv_3	Was there a T wave inversion in V2 or V3 on 3rd ECG?
360	lae_3	Was there left atrial enlargement on 3rd ECG?
361	rae_3	Was there right atrial enlargement on 3rd ECG?
362	lvh_3	Was there Left ventricular hypertrophy on 3rd ECG?
363	rvh_3	Was there right ventricular hypertrophy on 3rd ECG?
364	lbbb_3	Was there left bundle branch block on 3rd ECG?
365	rbbb_3	Was there right bundle branch block on 3rd ECG?
366	lahb_3	Was there left anterior hemiblock on 3rd ECG?
367	lphb_3	Was there left posterior hemiblock on 3rd ECG?
368	pvc_3	ANy PVC's on 3rd ECG?
369	pvc3_3	3 pvc's in a row (NSVT)on 3rd ECG?
370	infq_3	Inferior Q waves on 3rd ECG?

371	septq_3	Septal Q waves on 3rd ECG?
372	latq_3	Lateral Q waves on 3rd ECG?
373	antq_3	Anterior Q waves on 3rd ECG?
374	peric_3	Any pericarditis on 3rd ECG?
375	st_dp_i3	ST depression > 1mm (INFERIOR) on 3rd ECG
376	st_dp_a3	ST depression > 1mm (ANTERIOR) on 3rd ECG
377	st_dp_l3	ST depression > 1mm (LATERALLY) on 3rd ECG
378	st_el_i3	ST elevation >1mm (INFERIOR) on 3rd ECG
379	st_el_a3	ST elevation >1mm (ANTERIOR) on 3rd ECG
380	st_el_l3	ST elevation >1mm (LATERALLY) on 3rd ECG
381	tw_inf_3	T wave inferior on 3rd ECG
382	tw_lat_3	T wave lateral on 3rd ECG
383	pr3sq3_3	Was there any poor R wave progression or septal Q waves on the third ECG
384	comm_3	Comments on 3rd ECG
385	comm3	Comment on 3rd EKG
386	ecg4	Has a 4th ECG?
387	agegp4	Age gp @ 4th ECG
388	age_4	Age @ 4th ECG
389	ecgtrc_4	Is it a trace or a report form the notes?
390	nrmabn_4	Normal 4th ECG?
391	hr_4	Heart rate 4th ECG
392	rhyth_4	What is the rhythm on 4th ECG?
393	axis_4	What is the axis on 4th ECG?
394	hb_4	Is there Heart Block on 4th ECG?
395	qt_4	What is the QT interval on 4th ECG?
396	pr_4	Is the PR interval short on 4th ECG?
397	qrs_4	QRS duration on 4th ECG
398	eps_4	Was there an Epsilon wave on 4th ECG?
399	prwp_4	Was there a poor R wave progression on 4th ECG(<3mm in V3)?
400	tallr_4	Was there a tall R in V1 on 4th ECG ?
401	tinv_4	Was there a T wave inversion in V2 or V3 on 4th ECG?
402	lae_4	Was there left atrial enlargement on 4th ECG?
403	rae_4	Was there right atrial enlargement on 4th ECG?
404	lvh_4	Was there Left ventricular hypertrophy on 4th ECG?
405	rvh_4	Was there right ventricular hypertrophy on 4th ECG?
406	lbbb_4	Was there left bundle branch block on 4th ECG?
407	rbbb_4	Was there right bundle branch block on 4th ECG?
408	lahb_4	Was there left anterior hemiblock on 4th ECG?
409	lphb_4	Was there left posterior hemiblock on 4th ECG?
410	pvc_4	ANy PVC's on 4th ECG?
411	pvc3_4	3 pvc's in a row (NSVT)on 4th ECG?
412	infq_4	Inferior Q waves on 4th ECG?
413	septq_4	Septal Q waves on 4th ECG?
414	latq_4	Lateral Q waves on 4th ECG?
415	antq_4	Anterior Q waves on 4th ECG?
416	peric_4	Any pericarditis on 4th ECG?
417	st_dp_i4	ST depression > 1mm (INFERIOR) on 4th ECG
418	st_dp_a4	ST depression > 1mm (ANTERIOR) on 4th ECG

419	st_dp_l4	ST depression > 1mm (LATERALLY) on 4th ECG
420	st_el_i4	ST elevation > 1mm (INFERIOR) on 4th ECG
421	st_el_a4	ST elevation > 1mm (ANTERIOR) on 4th ECG
422	st_el_l4	ST elevation > 1mm (LATERALLY) on 4th ECG
423	tw_inf_4	T wave inferior on 4th ECG
424	tw_lat_4	T wave lateral on 4th ECG
425	pr4sq4_4	Was there any poor R wave progression or septal Q waves on the fourth ECG
426	comm_4	Comments on 4th ECG
427	ecg5	Has a 5th ecg?
428	agegp5	Age gp @ 5th ECG
429	age_5	Age @ 5th ECG
430	ecgtrc_5	Is it a trace or a report from the notes?
431	nrmabn_5	Normal 5th ECG?
432	hr_5	Heart rate 5th ECG
433	rhyth_5	What is the rhythm on 5th ECG?
434	axis_5	What is the axis on 5th ECG?
435	hb_5	Is there Heart Block on 5th ECG?
436	qt_5	What is the QT interval on 5th ECG?
437	pr_5	Is the PR interval short on 5th ECG?
438	qrs_5	QRS duration on 5th ECG
439	eps_5	Was there an Epsilon wave on 5th ECG?
440	prwp_5	Was there a poor R wave progression on 5th ECG(<3mm in V3)?
441	tallr_5	Was there a tall R in V1 on 5th ECG ?
442	tinv_5	Was there a T wave inversion in V2 or V3 on 5th ECG?
443	lae_5	Was there left atrial enlargement on 5th ECG?
444	rae_5	Was there right atrial enlargement on 5th ECG?
445	lvh_5	Was there Left ventricular hypertrophy on 5th ECG?
446	rvh_5	Was there right ventricular hypertrophy on 5th ECG?
447	lbbb_5	Was there left bundle branch block on 5th ECG?
448	rbbb_5	Was there right bundle branch block on 5th ECG?
449	lahb_5	Was there left anterior hemiblock on 5th ECG?
450	lphb_5	Was there left posterior hemiblock on 5th ECG?
451	pvc_5	ANy PVC's on 5th ECG?
452	pvc3_5	3 pvc's in a row (NSVT)on 5th ECG?
453	infq_5	Inferior Q waves on 5th ECG?
454	septq_5	Septal Q waves on 5th ECG?
455	latq_5	Lateral Q waves on 5th ECG?
456	antq_5	Anterior Q waves on 5th ECG?
457	peric_5	Any pericarditis on 5th ECG?
458	st_dp_i5	ST depression > 1mm (INFERIOR) on 5th ECG
459	st_dp_a5	ST depression > 1mm (ANTERIOR) on 5th ECG
460	st_dp_l5	ST depression > 1mm (LATERALLY) on 5th ECG
461	st_el_i5	ST elevation > 1mm (INFERIOR) on 5th ECG
462	st_el_a5	ST elevation > 1mm (ANTERIOR) on 5th ECG
463	st_el_l5	ST elevation > 1mm (LATERALLY) on 5th ECG
464	tw_inf_5	T wave inferior on 5th ECG
465	tw_lat_5	T wave lateral on 5th ECG
466	pr5sq5_5	Was there any poor R wave progression or septal Q waves on the 5th ECG

467	comm5	Comments on 5th ECG
468	ecg6	Has a 6th ecg?
469	agegp6	Age gp @ 6th ECG
470	age_6	Age @ 6th ECG
471	ecgtrce_	Is it a trace or a report from the notes
472	nrmabn_6	Normal 6th ECG?
473	hr_6	Heart rate 6th ECG
474	rhyth_6	What is the rhythm on 6th ECG?
475	axis_6	What is the axis on 6th ECG?
476	hb_6	Is there Heart Block on 6th ECG?
477	qt_6	What is the QT interval on 6th ECG?
478	pr_6	Is the PR interval short on 6th ECG?
479	qrs_6	QRS duration on 6th ECG
480	eps_6	Was there an Epsilon wave on 6th ECG?
481	prwp_6	Was there a poor R wave progression on 6th ECG(<3mm in V3)?
482	tallr_6	Was there a tall R in V1 on 6th ECG ?
483	tinv_6	Was there a T wave inversion in V2 or V3 on 6th ECG?
484	lae_6	Was there left atrial enlargement on 6th ECG?
485	rae_6	Was there right atrial enlargement on 6th ECG?
486	lvh_6	Was there Left ventricular hypertrophy on 6th ECG?
487	rvh_6	Was there right ventricular hypertrophy on 6th ECG?
488	lbbb_6	Was there left bundle branch block on 6th ECG?
489	rbbb_6	Was there right bundle branch block on 6th ECG?
490	lahb_6	Was there left anterior hemiblock on 6th ECG?
491	lphb_6	Was there left posterior hemiblock on 6th ECG?
492	pvc_6	ANy PVC's on 6th ECG?
493	pvc3_6	3 pvc's in a row (NSVT)on 6th ECG?
494	infq_6	Inferior Q waves on 6th ECG?
495	septq_6	Septal Q waves on 6th ECG?
496	latq_6	Lateral Q waves on 6th ECG?
497	antq_6	Anterior Q waves on 6th ECG?
498	peric_6	Any pericarditis on 6th ECG?
499	st_dp_i6	ST depression > 1mm (INFERIOR) on 6th ECG
500	st_dp_a6	ST depression > 1mm (ANTERIOR) on 6th ECG
501	st_dp_l6	ST depression > 1mm (LATERALLY) on 6th ECG
502	st_el_i6	ST elevation > 1mm (INFERIOR) on 6th ECG
503	st_el_a6	ST elevation > 1mm (ANTERIOR) on 6th ECG
504	st_el_l6	ST elevation > 1mm (LATERALLY) on 6th ECG
505	tw_inf_6	T wave inferior on 6th ECG
506	tw_lat_6	T wave lateral on 6th ECG
507	pr6sq6_6	Was there any poor R wave progression or septal Q waves on the 6th ECG
508	comm_6	Comments on 6th ECG
509	var00012	ID number
510	var00014	First name
511	var00015	Last name
512	sa_ecg	Signal average ECG?
513	useinaly	Use in analysis? Has an ECG got BBB?
514	age_secg	Age at first signal averaged ECG

515	sa1agegp	Age group first SA ECG
516	sa1ag40	Above or below 40 years age group on first SA ECG
517	saoldnew	Is it the old or the new SAECG machine?
518	abn_1sa	Is the QRS abnormal on the 1st ECG (>110 on old, >120 on new)
519	totqrs	Total QRS
520	tqrs1110	Total QRS < or = 110 and > 110
521	tqrs1115	Total QRS < or = 115 and > 115
522	tqrs1120	Total QRS < or = 120 and > 120
523	rms	Total rms
524	rms120	Total rms< or = 20 and > 20
525	las	Total las
526	las138	Total LAS > or = 38 and < 38
527	sa1_2pos	Any 2 out of three criteria positive on the first SAECG
528	dn_qrsab	Denovo QRS abnormal?
529	dn_rmsab	Denovo RMS abnormal?
530	dn_lasab	Denovo LAS abnormal
531	dn_23ab	Denovo 2/3 abnormal
532	agednqrs	Age at denovo abnormal QRS or age at last follow-up
533	agednrms	Age at denovo abnormal RMS or age at last follow-up
534	agednlas	Age at denovo abnormal LAS or age at last follow-up
535	agedn23	Age at denovo 2/3 abnormal or age at last follow-up
536	numfsaeg	Number of follow-up SAECGs
537	sa_ecg2	Did they have a second SAECG?
538	saoldne2	Is it the old or the new SAECG machine?
539	usein_2a	Use in analysis? BBB on ECG
540	saage_2	Age @ 2nd SAECG
541	sa2agegp	Age group second SA ECG
542	totqrs_2	Total QRS (2nd saecg)
543	abn_2sa	Is the QRS abnormal on the 2nd ECG (>110 on old, >120 on new)
544	rms_2	Total rms (2nd saecg)
545	rms220	Total rms< or =20 and >20 on 2nd SA ECG
546	las_2	Total las (2nd ecg)
547	las238	Total LAS > or = 38 and < 38 on 2nd SAECG
548	sa2_2pos	Any 2 out of three criteria positive on the second SAECG
549	sa_ecg3	Did they have a third SAECG?
550	saoldne3	Is it the old or the new SAECG machine?
551	usein_3a	Use in analysis? BBBon ECG
552	abn_3sa	Is the QRS abnormal on the 3rd ECG (>110 on old, >120 on new)
553	saage_3	Age @ 3rd SAECG
554	sa3agegp	Age group third SA ECG
555	totqrs_3	Total QRS (3rd ECG)
556	rms_3	Total rms (3rd ecg)
557	rms320	Total rms< or =20 and >20 on 3rd SA ECG
558	las_3	Total las (3rd ecg)
559	las338	Total LAS > or = 38 and < 38 on 3rd SAECG
560	sa3_2pos	Any 2 out of three criteria positive on the third SAECG
561	sa_ecg4	Did they have a fourth SAECG?
562	saoldne4	Is it the old or the new SAECG machine?

563	usein_4a	Use in analysis? BBBon ECG
564	abn_sa4	Is the QRS abnormal on the 4th ECG (>110 on old, >120 on new)
565	saage_4	Age @ 4th SAECG
566	sa4agegp	Age group fourth SA ECG
567	totqrs_4	Total QRS (4th saecg)
568	rms_4	Total rms (4th saecg)
569	rms420	Total rms< or =20 and >20 on 4th SA ECG
570	las_4	Total las (4th saecg)
571	las438	Total LAS > or = 38 and < 38 on 4th SAECG
572	sa4_2pos	Any 2 out of three criteria positive on the fourth SAECG
573	sa_agel	Age @ last saecg (first/last)
574	sa_totql	Total QRS last saecg (first/last)
575	tqrsl115	Total QRS < or = 115 and > 115 (last saecg)
576	sa_rmsl	Total rms last saecg (first/last)
577	sa_lasl	Total las last saecg (first/last)
578	echo	Had a first echo
579	abnecho	Any abnormality on any echo
580	var00010	ID number
581	var00016	First name
582	var00032	Last name
583	echocomm	echo comment
584	numfecho	Number of follow-up echoes
585	age_echo	Age at first Echo
586	agegpech	Age Group @ first echo
587	e_age1	Age (< or >or= 34 yrs)
588	height	Height (inches)
589	height1	Height (cm) needed for Henry
590	weight	Weight (kg)
591	bsa1	BSA (1st echo)
592	e_rvdil	RV dilitation (echo)
593	e_rvabn	RV abnormality (echo)
594	e_lvhyp	LV hypertrophy (echo)
595	e_lvdil	LV dilitation (echo)
596	e_lvsys	LV systolic dysfunction (echo)
597	e_laenl	LA enlargement (echo)
598	e_rwabn	Regional Wall Abnormality (echo)
599	e_lad	Left Atrial Diameter (echo)
600	e_pwtd	Posterior Wall Thickness in Diastole (echo)
601	e_swtd	Septal Wall Thickness in Diastole (echo)
602	e_lvedd	LVEDD (echo)
603	e_lvesd	LVESD (echo)
604	e_fs	Fractional Shortening (echo)
605	FS25	Is fractional shortening less than or equal to 25?
606	e_disk	Diskinesia (echo)
607	e_diskc1	Diskinesia Combined (1st echo)
608	explved1	Expected LVEDD (1st echo)
609	henry1	Henry % (1st echo)
610	ventvol1	Ventricular Volume (1st echo)

611	e_dils1	Dilatation by size (1st echo)
612	e_dilh13	Dilatation by Henry 3 SD 117% (1st echo)
613	e_dilh12	Dilatation by Henry 2 SD 112% (1st echo)
614	dendilh3	Denovo development of LV dilatation 3SD 117% (Henry %)
615	dendilh2	Denovo development of LV dilatation 2SD 112% (Henry %)
616	agdenh3s	Age @ denovo development of LV dilatation 3SD 117% (Henry %)
617	agdenh2s	Age @ denovo development of LV dilatation 2SD 112% (Henry %)
618	agedenh3	Age @ denovo development of LV dilatation. by Henry 3SD 117% (KM) or age at last follow up
619	agedenh2	Age @ denovo development of LV diatation by Henry 2SD 112% (KM) or age at last follow up
620	e_dilvv1	Dilatation by ventricular volume (1st echo)
621	echo2	Did they have a second echo?
622	e_age2	Age @ 2nd echo
623	e_agegp2	Age Group @ 2nd echo
624	height2	Height @ 2nd echo
625	height2e	Height @ 2nd echo (cm)
626	weight2	Weight @ 2nd echo
627	bsa2	BSA (2nd echo)
628	e_rvdil2	RV dilitation (2nd echo)
629	e_rvabn2	RV abnormality (2nd echo)
630	e_lvhyp2	LV hypertrophy (2nd echo)
631	e_lvdil2	LV dilitation (2nd echo)
632	e_lvsys2	LV systolic dysfunction (2nd echo)
633	e_laenl2	LA enlargement (2nd echo)
634	e_rwabn2	Regional Wall Abnormality (2nd echo)
635	e_lad2	Left Atrial Diameter (2nd echo)
636	e_pwtd2	Posterior Wall Thickness in Diastole (2nd echo)
637	e_swtd2	Septal Wall Thickness in Diastole (2nd echo)
638	e_lvedd2	LVEDD (2nd echo)
639	e_lvesd2	LVESD (2nd echo)
640	e_fs2	Fractional Shortening (2nd echo)
641	FS25_2	Is fractional shortening less than or equal to 25 on second echo?
642	e_disk2	Diskinesia (2nd echo)
643	e_diskc2	Diskinesia combined (2nd echo)
644	explved2	Expected LVEDD (2nd echo)
645	henry2	Henry % (2nd echo)
646	ventvol2	Ventricular Volume (2nd echo)
647	e_dils2	Dilatation by size (2nd echo)
648	e_dilh23	Dilatation by Henry 3SD 117% (2nd echo)
649	e_dilh22	Dilatation by Henry 2SD 112% (2nd echo)
650	e_dilvv2	Dilatation by ventricular volume (2nd echo)
651	echo3	Did they have a 3rd echo?
652	e_age3	Age @ 3rd echo
653	e_agegp3	Age group @ 3rd echo
654	height3	Height @ 3rd echo
655	height3e	Height @ 3rd Echo (cm)
656	weight3	Weight @ 3rd echo
657	bsa3	BSA (3rd echo)

658	e_rvdil3	RV dilatation (3rd echo)
659	e_rvabn3	RV abnormality (3rd echo)
660	e_lvhyp3	LV hypertrophy (3rd echo)
661	e_lvdil3	LV dilitation (3rd echo)
662	e_lvsys3	LV systolic dysfunction (3rd echo)
663	e_laenl3	LA enlargement (3rd echo)
664	e_rwabn3	Regional Wall Abnormality (3rd echo)
665	e_lad3	Left Atrial Diameter (3rd echo)
666	e_pwtd3	Posterior Wall Thickness in Diastole (3rd echo)
667	e_swtd3	Septal Wall Thickness in Diastole (3rd echo)
668	e_lvedd3	LVEDD (3rd echo)
669	e_lvesd3	LVESD (3rd echo)
670	e_fs3	Fractional Shortening (3rd echo)
671	FS25_3	Is fractional shortening less than or equal to 25 on third echo?
672	e_disk3	Diskinesia (3rd echo)
673	e_diskc3	Diskinesia Combined (3rd echo)
674	explved3	Expected LVEDD (3rd echo)
675	henry3	Henry % (3rd echo)
676	ventvol3	Ventricular Volume (3rd echo)
677	e_dils3	Dilatation by size (3rd echo)
678	e_dilh33	Dilatation by Henry 3 SD 117% (3rd echo)
679	e_dilh32	Dilatation by Henry 2 SD 112% (3rd echo)
680	e_dilvv3	Dilatation by ventricular volume (3rd echo)
681	echo4	Did they have a 4th echo?
682	e_age4	Age @ 4th echo
683	e_agegp4	Age group @ 4th echo
684	height4	Height @ 4th echo
685	height4e	Height @ 4th echo
686	weight4	Weight @ 4th echo
687	bsa4	BSA (4th echo)
688	e_rvdil4	RV dilatation (4th echo)
689	e_rvabn4	RV abnormality (4th echo)
690	e_lvhyp4	LV hypertrophy (4th echo)
691	e_lvdil4	LV dilatation (4th echo)
692	e_lvsys4	LV systolic dysfunction (4th echo)
693	e_laenl4	LA enlargement (4th echo)
694	e_rwabn4	Regional Wall abnormality (4th echo)
695	e_lad4	Left Atrial Diameter (4th echo)
696	e_pwtd4	Posterior Wall Thickness in Diastole (4th echo)
697	e_swtd4	Septal Wall thickness in Diastole (4th echo)
698	e_lvedd4	LVEDD (4th echo)
699	e_lvesd4	LVESD (4th echo)
700	e_fs4	Fractional shortening (4th echo)
701	FS25_4	Is fractional shortening less than or equal to 25 on third echo?
702	e_disk4	Diskinesia (4th echo)
703	e_diskc4	Diskinesia combined (4th echo)
704	explved4	Expected LVEDD (4th echo)
705	henry4	Henry % (4th echo)

706	ventvol4	Ventricular Volume (4th echo)
707	e_dils4	Dilatation by size (4th echo)
708	e_dilh43	Dilatation by Henry 3SD 117% (4th echo)
709	e_dilh42	Dilatation by Henry 2SD 112% (4th echo)
710	e_dilvv4	Dilatation by ventricular volume (4th echo)
711	echo5	Did they have a 5th echo
712	e_age5	Age @ 5th echo
713	e_agegp5	Age group @ 5th echo
714	height5	Height @ 5th echo
715	height5e	Height @ 5th echo (cm)
716	weight5	Weight @ 5th echo
717	bsa5	BSA (5th echo)
718	e_rvdil5	RV dilatation (5th echo)
719	e_rvabn5	RV abnormality (5th echo)
720	e_lvhyp5	LV hypertrophy (5th echo)
721	e_lvdil5	LV dilatation (5th echo)
722	e_lvsys5	LV systolic dysfunction (5th echo)
723	e_laenl5	LA enlargement (5th echo)
724	e_rwabn5	Regional Wall Abnormality (5th echo)
725	e_lad5	Left Atrial Diameter (5th echo)
726	e_pwtd5	Posterior wall thickness in diastole (5th echo)
727	e_swtd5	Septal Wall thickness in diastole (5th echo)
728	e_lvedd5	LVEDD (5th echo)
729	e_lvesd5	LVEDD (5th echo)
730	e_fs5	Fractional shortening (5th echo)
731	FS25_5	Is fractional shortening less than or equal to 25 on fifth echo?
732	e_disk5	Diskinesia (5th echo)
733	e_diskc5	Diskinesia combined (5th echo)
734	explved5	Expected LVED (5th echo)
735	henry5	Henry % (5th echo)
736	ventvol5	Ventricular Volume (5th echo)
737	e_dils5	Dilatation by size (5th echo)
738	e_dilh53	Dilatation by Henry 3SD 117 % (5th echo)
739	e_dilh52	Dilatation by Henry 2 SD 112% (5th echo)
740	e_dilvv5	Dilatation by ventricular volume (5th echo)
741	echo6	Did they have a 6th echo?
742	e_age6	Age @ 6th echo
743	e_agegp6	Age group @ 6th echo
744	height6	Height @ 6th echo
745	height6e	Height @ 6th echo (cm)
746	weight6	Weight @ 6th echo
747	bsa6	BSA (6th echo)
748	e_rvdil6	RV dilatation (6th echo)
749	e_rvabn6	RV abnormality (6th echo)
750	e_lvhyp6	LV hypertrophy (6th echo)
751	e_lvdil6	LV dilatation (6th echo)
752	e_lvsys6	LV systolic dysfunction (6th echo)
753	e_laenl6	LA enlargement (6th echo)

754	e_rwabn6	Regional wall abnormality (6th echo)
755	e_lad6	Left atrial diameter (6th echo)
756	e_pwtd6	Posterior wall thickness in diastole (6th echo)
757	e_swtd6	Septal Wall thickness in diastole (6th echo)
758	e_lvedd6	LVEDD (6th echo)
759	e_lvesd6	LVESD (6th echo)
760	e_fs6	Fractional shortening (6th echo)
761	FS25_6	Is fractional shortening less than or equal to 25 on 6th echo?
762	e_disk6	Diskinesia (6th echo)
763	e_diskc6	Diskinesia combined (6th echo)
764	explved6	Expected LVEDD (6th echo)
765	henry6	Henry % (6th echo)
766	ventvol6	Ventricular Volume (6th echo)
767	e_dils6	Dilatation by size (6th echo)
768	e_dilh63	Dilatation by Henry 3SD 117 % (6th echo)
769	e_dilh62	Dilatation by Henry 2 SD 112% (6th echo)
770	e_dilw6	Dilatation by ventricular volume (6th echo)
771	var00006	Had a first echo
772	lastecho	Last Echo
773	e_agelas	Age @ last echo
774	e_agegpl	Age group at last echo
775	e_rvdill	RV dilatation (last echo)
776	e_rvabnl	RV abnormality (last echo)
777	e_lvhypl	LV hypertrophy (last echo)
778	e_lvdill	LV dilatation(last echo)
779	e_lvsysl	LV Systolic dysfunction (last echo)
780	e_laenll	LA enlargement (last echo)
781	e_rwabnl	Regional Wall Abnormality (last echo)
782	e_ladl	Left Atrial Diameter (last echo)
783	e_pwtdl	Posterior wall thickness in diastole (last echo)
784	e_swtdl	Septal wall thickness in systole (last echo)
785	e_lveddl	LVEDD (last echo)
786	e_lvesdl	LVESD (last echo)
787	e_fsl	Fractional Shortening (last echo)
788	e_diskl	Diskinesia (last echo)
789	explvedl	Expected LVED (last echo)
790	henryl	Henry % (last ceho)
791	ventvoll	Ventricular Volume (last echo)
792	e_dilsl	Dilatation by size (last ceho)
793	e_dilhl3	Dilatation by Henry 3SD 117% (last echo)
794	e_dilhl2	Dilatation by Henry 2SD 112% (last echo)
795	e_dilvvl	Dilatation by Ventricular Volume (last echo)
796	var00002	ID number
797	var00003	First name
798	var00004	Last name
799	pvcs200	PVCs > or = 200 on any Holter
800	deno200	Denovo PVCs > or = 200
801	aged200	Age @ Denovo PVCs > or = 200 or age @ last holter

802	pvcs1000	PVCs > or = 1000 on any holter
803	deno1000	Denovo PVCs > or = 1000
804	aged1000	Age @ Denovo PVCs > or = 1000 or age @ last Holter
805	holter	Did they have a Holter?
806	abnholt	Abnormality on any Holter: includes PVC's >200 and VT
807	age_holt	Age at first Holter
808	hagegp1	Age gp @ 1st Holter
809	icu_ccu	ICU or CCU Monitoring
810	newpvc1	PVCs on 1st Holter (recoded)
811	h_pvcs	PVCs (24 hrs)
812	pvc200	PVCs > or = 200 (1st Holter)
813	pvc1000	PVCs > or = 1000 (1st Holter)
814	newcoup1	Couplets on 1st Holter (recoded)
815	h_coup	Couplets
816	newbig1	Bigeminy on 1st Holter (recoded)
817	h_bigem	Bigeminy
818	newtrip1	Triplets on 1st Holter (recoded)
819	h_tripl	Triplets
820	h_trig	Trigemeny
821	newrun1	Runs of VT on 1st Holter (recoded)
822	h_runvt	Runs of VT
823	h_sust	Sustained
824	h_idiov	Idioventricular Rhythm on 1st holter
825	numfholt	Number of follow-up holters
826	holter2	Has a 2nd holter
827	h_age2	Age @ 2nd holter
828	hagegp2	Age gp @ 2nd holter
829	newpvc2	PVCs on 2nd holter (recoded)
830	h_pvcs2	PVCs (2nd holter)
831	pvc2_200	PVCs > or = 200 (2nd Holter)
832	pv2_1000	PVCs > or = 1000 (2nd holter)
833	h_coup2	Couplets (2nd holter)
834	h_bigem2	Bigeminy (2nd holter)
835	h_tripl2	Triplets (2nd holter)
836	h_trig2	Trigemeny (2nd Holter)
837	h_runvt2	Runs of VT (2nd holter)
838	h_sust2	Sustained (2nd holter)
839	h_idiov2	Idioventricular Rhythm on 2nd holter
840	holter3	Has a 3rd holter
841	h_age3	Age @ 3rd holter
842	hagegp3	Age gp @ 3rd holter
843	pvc3_200	PVCs> or = 200 (3rd holter)
844	pv3_1000	PVCs > or = 1000 (3rd holter)
845	newpvc3	PVCs on 3rd holter (recoded)
846	h_pvcs3	PVCs (3rd holter)
847	pvcgp3	PVCs <or= 120 and > 120 (3rd holter)
848	h_coup3	Couplets (3rd holter)
849	h_bigem3	Bigeminy (3rd holter)

850	h_tripl3	Triplets (3rd holter)
851	h_runvt3	Runs of VT (3rd holter)
852	h_sust3	Sustained (3rd holter)
853	h_idiov3	Idioventricular Rhythm on 3rd holter
854	holter4	Has a 4th holter
855	h_age4	Age @ 4th holter
856	hagegp4	Age gp @ 4th holter
857	newpvc4	PVCs on 4th holter (recoded)
858	h_pvcs4	PVCs (4th holter)
859	pvc4_200	PVCs > or = 200 (4th holter)
860	pv4_1000	PVCs > or = 1000 (4th holter)
861	h_coup4	Couplets (4th holter)
862	h_bigem4	Bigeminy (4th holter)
863	h_tripl4	Triplets (4th holter)
864	h_runvt4	Runs of VT (4th holter)
865	h_sust4	Sustained (4th holter)
866	h_idiov4	Idioventricular Rhythm on 4th holter
867	holter5	Had a 5th Holter
868	h_age5	Age @ 5th holter
869	hagegp5	Age gp @ 5th Holter
870	newpvc5	PVC's on 5th Holter (recoded)
871	h_pvcs5	PVC's 5th Holter
872	pvc5_200	PVC's > or = 200 (5th Holter)
873	pv5_1000	PVC's > or = 1000 (5th Holter)
874	h_coup5	Couplets (5th Holter)
875	h_bigem5	Bigeminy (5th Holter)
876	h_tripl5	Triplets (5th Holter)
877	h_runvt5	Runs of VT (5th Holter)
878	h_sust5	Sustained (5th Holter)
879	h_idiov5	Idioventricular rhythm (5th Holter)
880	ageh_lst	Age @ last holter
881	hagegpl	Age group @ last holter
882	newpvcl	PVCs on last holter (recoded)
883	h_pvcl	PVCs (last holter)
884	pvcgpl	PVCs <or= 120 and > 120 (last holter)
885	h_coupl	Couplets (last holter)
886	h_bigeml	Bigemeny (last holter)
887	h_tripll	Triplets (last holter)
888	h_runvtl	Runs of VT (last holter)
889	h_sustl	Sustained (last holter)
890	h_idiovl	Idioventricular Rhythm on last holter
891	mri	MRI
892	abnmri	Abnormality on first MRI
893	age_mri	Age at first MRI
894	m_rvdys	RV Dysplasia
895	m_lvdys	LV Dysplasia
896	m_comm	Comment on MRI
897	numfmri	Number of follow-up MRIs

898	m_age2	Age @ 2nd MRI
899	m_rvdys2	RV Dysplasia (2nd MRI)
900	m_lvdys2	LV Dysplasia (2nd MRI)
901	m_comm2	Comment on 2nd MRI
902	var00005	ID number
903	var00017	First name
904	var00018	Last name
905	stress_t	Stress test
906	abnstres	Abnormality on any stress test
907	age_st	Age at first stress test
908	st_resul	Stress Test Result 1st test
909	numfst	Number of follow-up stress tests
910	st_age2	Age @ 2nd stress test
911	st_res2	Stress test result (2nd st)
912	st_age3	Age @ 3rd stress test
913	st_res3	Stress test result (3rd st)
914	ht_cath	Heart Cath.
915	abcthcad	Abnormality (CAD critical) on any heart cath
916	abncth	Abnormality of size of ventricle or global/focal dyskinesia
917	age_hc	Age at first heart cath.
918	hc_cad	Coronary Artery Disease
919	hc_lvedp	LVEDP (heart cath)
920	hc_paps	Pulmonary Artery Pressure systole
921	hc_papd	Pulmonary Artery Pressure diastole
922	hc_mpap	Mean Pulmonary Artery Pressure
923	hc_wp	Wedge Pressure
924	hc_rap	Right Atrial Pressure
925	hc_disk	Diskinesia (heart cath)
926	hc_comm	Comment on cardiac cath
927	numfhc	Number of follow-up heart caths
928	hc_age2	Age @ 2nd heart cath
929	hc_cad2	Coronary Artery Disease (2nd heart cath)
930	hclvedp2	LVEDP (2nd heart cath)
931	hc_paps2	Pulmonary Artery pressure systole (2nd cath)
932	hc_papd2	Pulmonary Artery pressure diastole (2nd cath)
933	hc_wp2	Wedge pressure (2nd cath)
934	hc_rap2	Right Atrial Pressure (2nd cath)
935	hc_disk2	Diskinesia (2nd cath)
936	hc_age3	Age @ 3rd heart cath
937	hc_cad3	Coronary Artery Disease (3rd cath)
938	hclvedp3	LVEDP (3rd cath)
939	hc_paps3	Pulmonary Artery Pressure systole (3rd cath)
940	hc_papd3	Pulmonary Artery Pressure diastole (3rd cath)
941	hc_wp3	Wedge Pressure (3rd cath)
942	hc_rap3	Right Atrial Pressure (3rd cath)
943	hc_disk3	Diskinesia (3rd cath)
944	hc_age4	Age @ 4th heart cath
945	hc_cad4	Coronary Artery Disease (4th cath)

946	hclvedp4	LVEDP (4th cath)
947	hc_paps4	Pulmonary Artery Pressure Systole (4th cath)
948	hc_papd4	Pulmonary Artery Pressure Diastole (4th cath)
949	hc_wp4	Wedge Pressure (4th cath)
950	hc_rap4	Right Atrial Pressure (4th cath)
951	hc_disk4	Diskinesia (4th cath)
952	ep_study	EP Studies
953	age_ep	Age at first EP study
954	ep_iva	Inducible Ventricular Arrhythmia
955	ep_comm	Comment on EP Study
956	gated_sc	Gated Scan
957	age_gs	Age at first gated scan
958	gs_vwabn	Ventricular Wall Abnormalities
959	gs_vwa	Vent. Wall Abn. Category
960	gs_ef	Ejection fraction
961	rheartbi	Right Heart Biopsy
962	age_hb	Age @ heart biopsy
963	lm_hyper	Light Microscopy-hypertrophy (hb)
964	lm_fib	Light Microscopy-fibrosis (hb)
965	lm_fatin	Light Microscopy-fatty infiltration (hb)
966	em_hyper	Electron Microscopy-hypertrophy (hb)
967	em_fib	Electron Microscopy-fibrosis (hb)
968	hb_comm	Comment on Heart Biopsy
969	ventric	Ventriculotomy
970	age_vent	Age @ ventriculotomy
971	explanht	Explanted Heart
972	ageexht	Age @ Explanted Heart
973	eh_htwt	EH-Heart Weight
974	eh_rvdil	EH-RV Dilatation
975	eh_rvhyp	EH-RV Hypertrophy
976	eh_lvdil	EH-LV Dilatation
977	eh_lvhyp	EH-LV Hypertrophy
978	eh_cad	EH-Coronary Artery Disease
979	eh_lmlvh	EH-Light Microscopy LV Hypertrophy
980	eh_lmlvf	EH-Light Microscopy LV Fibrosis
981	eh_lmrvf	EH-Light Microscopy RV Fibrosis
982	var00034	ID number
983	var00035	First name
984	var00036	Last name
985	eh_fatin	EH-Fatty Infiltration
986	autopsy	Autopsy
987	abnaut	Abnormality related to ARVC at autopsy
988	var00021	Case or control
989	age_aut	Age at Autopsy
990	heartwt	Heart Weight (g)
991	rvdilita	RV dilatation
992	rvhyper	RV hypertrophy
993	lvdilita	LV dilatation

994	lvhyper	LV hypertrophy
995	cad	Coronary Artery Disease
996	lmlvhyp	Light Microscopy-LV hypertrophy
997	lmlvfib	Light Microscopy-LV Fibrosis
998	lmrvfib	Light Microscopy-RV Fibrosis
999	fattyinf	Fatty Infilitration
1000	autopcom	Autopsy Comment
1001	oth_ht	Other heart tissue
1002	age_oht	Age at test (other heart tissue)
1003	echo_nm	Did they have an echo with no measurements for Henry?
1004	echo_nma	What age were they?
1005	lvd_nm	Was there LV dilatation recorded?
1006	abn_nm	Was any other malformation on echo found?
1007	abn_tpe	What type of problem?
1008	abn_nme	Any other comment on the echo with no measurements
1009	filter_$	(ascert = 1) & (arvccs = 1 | arvccs = 2) & (gender = 1) (FILTER)

APPENDIX B

Peer reviewed papers and manuscripts

1. Samuels, M.E., Orr, A., Guernsey, D.L., Dooley, K., Riddell, C., **Hodgkinson, K.** Ludman, M., Pullman, D. *Is gene discovery research or diagnosis*? Genetic Medicine 2008 Jun;10 (6):385-90.

2. **Chapter 2**: Nancy D. Merner, B.Sc*, **Kathy A. Hodgkinson**, M.Sc*, Annika F. M. Haywood, Ph.D, Sean Connors, M.D., Vanessa M. French, B.Sc, Jörg-Detlef Drenckhahn, MD, Christine Kupprion, M.D, Kalina Ramadanova, M.D, Ludwig Thierfelder, M.D, William McKenna, M.D., Anne S. Bassett, M.D., Patrick S. Parfrey, M.D., and Terry-Lynn Young, Ph.D **Both authors contributed equally to this manuscript**. *Arrhythmogenic right ventricular cardiomyopathy type 5 (ARVD5) is a fully penetrant, lethal arrhythmic disorder caused by an amino acid substitution in the novel TMEM43 gene* AJHG, April 18 2008.

3. **Chapter 5:** Daryl Pullman and **Kathy Hodgkinson** *Genetic knowledge and moral responsibility: ambiguity at the interface of genetic research and clinical practice.* Clin Genet. 2006 Mar;69 (3):199-203.

4. **Chapter 4: Kathy A. Hodgkinson,** Patrick S. Parfrey, Anne S. Bassett, Christine Kupprion, Jörg Drenckhahn, Mark W. Norman, Ludwig Thierfelder, Susan N. Stuckless, Elizabeth L. Dicks, William J. McKenna, Sean P. Connors. *The impact of implantable cardioverter defibrillator therapy on survival in autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVD5).* Journal of the American College of Cardiology 2005 45(3) 400-408.

Non-peer reviewed publications

1. Elizabeth Dicks PhD, **Kathy Hodgkinson** M.Sc.,Daryl Pullman PhD, Sean Connors MD, DPhil, FRCPC, Carol Tilley RN Doreen Neville PhD, Terry-Lynn Young PhD & Patrick Parfrey MD, FRCPC *Implantable Cardiac Defibrillators for Primary Prevention of Sudden Cardiac Death in High Risk Arrhythmogenic Right Ventricular Cardiomyopathy Patients.* Policy paper for Eastern Health Sept 2007.

Papers in progress

1. **Chapter 3. Kathy A. Hodgkinson**, M.Sc, Sean P. Connors, MD, D.Phil, FRCPC., Nancy Merner, B.Sc. Annika Haywood, Ph.D, Terry Young, Ph.D., Anne S. Bassett, MD, FRCPC, Ludwig Thierfelder, MD, William J. McKenna, MD, FRCP., Patrick S. Parfrey, MD, FRCPC, FACP. *The natural history and phenotypic expression of arrhythmogenic right ventricular cardiomyopathy (ARVD5) caused by a mutation in TMEM43.* Submitted to JACC December 2008.

2. **Chapter 6. Kathy Hodgkinson** and Daryl Pullman. *The case for closer integration of clinical and research genetics programs: lessons from Newfoundland and Labrador.* Submitted to PLoS January 2009.

3. Nancy Merner, **Kathy Hodgkinson**, Annika Hayward, Terry Young. *Missense mutations in TMEM43 cause arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) in the UK population.* In manuscript form.

4. **Kathy Hodgkinson,** Rick Singleton, Coleen Walsh, Daryl Pullman. *The curious case of the disappearing defibrillator*. In manuscript form: to be submitted.

5. Christopher Butt, Richard Neuman, **Kathy Hodgkinson**, Sharon Buehler, John Dean, Trudo Lemmens and Daryl Pullman *Clearing the Fog: Addressing ethical and legal ambiguities at the interface of genetic research and clinical practice*. In manuscript form: to be submitted.

6 **Kathy Hodgkinson** and Daryl Pullman. *Duty to warn in Research Genetics*. In manuscript form: to be submitted.

Poster and platform presentations to learned societies

1. **Kathy A. Hodgkinson**, M.Sc, Sean P. Connors, MD, D.Phil, FRCPC, Nancy Merner, M.Sc., Annika Haywood, Ph.D, Terry Young, Ph.D, Anne S. Bassett, MD, FRCPC, Patrick S. Parfrey, MD, FRCPC. *The phenotype and natural history of ARVC caused by a C>T 1073 mutation in TMEM43*. Presented to the Canadian Cardiovascular Society Congress, Toronto, October 2008

2. T. Young, N. D. Merner, **K. Hodgkinson**, A. F. Haywood, W. McKenna, S. Connors, V. French, L. Thierfelder, P. Syrris, P. Parfrey. *Novel ARVD5 gene causes autosomal dominant sudden cardiac death due to missense mutations in the TMEM43 gene*. Oral presentation to the European Society of Human Genetics, Barcelona, Spain, May 28-30 2008

3. Daryl Pullman, **Kathy Hodgkinson**, Elizabeth Dicks, Fern Brunger, Mark Samuels, Terry Lynn Young. *From Gene Discovery to Health Policy: An ELSI (GE3LS) Success Story in Atlantic Canada.* Presented to the 'ethical legal social' impact of the human genome project meeting, Cleveland, May 2-4 2008.

4. Elizabeth Dicks PhD, , Daryl Pullman PhD, Sean Connors MD, DPhil, FRCPC, **Kathy Hodgkinson M.Sc.**, Carol Tilley BN, RN, Doreen Neville PhD, Terry-Lynn Young PhD & Patrick Parfrey MD, FRCPC. *Gene Discovery to Policy: A Template for Future Health Policy in Newfoundland & Labrador*. Presented to the International GE3LS Symposium 2008 "Navigating the Changing Landscape" Calgary AB, April 28-30th.

5. Andrew Smith BSc , **Kathy Hodgkinson** MSc, Sara MacKay MS, (C)CGC, Fiona Curtis MSc, (C)CGC, Sean Connors MD, DPhil, FCPC, Terry-Lynn Young PhD & Patrick Parfrey MD, FRCPC. *The clinical and genetic epidemiology of familial cardiomyopathy in Newfoundland & Labrador.* Presented to the 1st Annual Canadian Human Genetics Conference, St. Sauveur, QC April 9-12.

6. Daryl Pullman, **Kathy Hodgkinson**, Rick Singleton. *The curious case of the recalcitrant defibrillator.* Podium presentation 18th Canadian Bioethics Society Conference and 3rd International Conference on Clinical Ethics and Consultation (May 30 to June 3, 2007).

7. French, Vanessa (Masters student), **Hodgkinson, Kathy**, Young TL, *Arrhythmogenic Right Ventricular Cardiomyopathy type 5 (ARVD5): Narrowing of the Critical Region*. Canadian Genetic Diseases Network, Montreal, April 2007. Platform and poster presentation.

8. **Kathy Hodgkinson** and Daryl Pullman. *The ethics of human genetic research in Newfoundland and Labrador.* Oral presentation. International Congress of Human Genetics, Brisbane, Australia, August 8 2006.

9. **Kathy Hodgkinson**, Sean Connors, Ludwig Thierfelder, Anne Bassett, Patrick Parfrey. *The natural history of arrhythmogenic right ventricular cardiomyopathy (ARVC) linked to 3p25 in*

Newfoundland. Presented to the Canadian Cardiovascular Society meeting, Montreal, October 2005.

10. **K. Hodgkinson**, T. Young, S. Connors, P. Parfrey, A. Bassett D. Pullman. *Navigating the ethical minefield between genetic research and clinical genetic services.* Presented to the American Society of Human Genetics meeting, Salt Lake City, October 2005.

11. Y. Teplitsky, S. Connors, P. Parfrey, L. Kernaghan, S. MacKay, B. Peddle, P. Ryan, C. Tilley, L. Thierfelder, A. Bassett, T. Young, W. McKenna, **K. Hodgkinson**. *The provision of a multidisciplinary screening, follow-up and research clinic for families with cardiomyopathy in Newfoundland.* Presented to the American Society of Human Genetics meeting, Salt Lake City, October 2005.

12. Terry Young, **Kathy Hodgkinson**, Sean Connors, Ludwig Thierfelder, Anne Bassett, Patrick Parfrey. *The natural history of arrhythmogenic right ventricular cardiomyopathy (ARVC) in a homogeneous Newfoundland population.* Presented to the American Society of Human Genetics meeting, Salt Lake City, October 2005.

13. **Kathy Hodgkinson**, S. Connors, B. Barrowman, L. Thierfelder, T-L Young, P. Parfrey, D. Pullman. *When genetic research findings progress to clinical application.* Oral presentation to Scientific Days, October 2004, Memorial University, Newfoundland.

14. Nancy Whalen, **Kathy Hodgkinson**, Patrick Parfrey, Ludwig Thierfelder, Sean Connors, Terry Lynn Young. *Arrhythmogenic Right Ventricular Cardiomyopathy in Newfoundland: haplotype analysis of the ARVD5 region on chromosome 3.* Poster presentation at Scientific Days, October 2004, Memorial University, Newfoundland.

15. Daryl Pullman, **Kathy Hodgkinson**., Barbara Barrowman. *When genetic research findings progress to clinical application: the ethics of duty to warn and the issues of autonomy.* Oral presentation to the Canadian bioethics society, Calgary, October 2004 and given as an oral presentation to Scientific Days, October 2004, Memorial University.

16. **Kathy Hodgkinson**, Patrick Parfrey, Anne Bassett, Susan Stuckless, Sue Fagan, Ludwig Thierfelder, Sean Connors. *Left Ventricular Enlargement in autosomal dominant Arrhythmogenic Right Ventricular Cardiomyopathy.* Presented to the Canadian Cardiovascular Society, Calgary, October 2004.

17. Cook, G., **Hodgkinson, KA**, Connors, S., Thierfelder, L., Bassett, AS., Parfrey, PS. The Genetic Epidemiology of Arrhythmogenic *Right Ventricular Cardiomyopathy/Dilated Cardiomyopathy in Newfoundland.* Presented to the American Society of Human Genetics meeting, Toronto, October 2004, and to the Scientific Days, October 2004, Memorial University.

18. S. Stuckless, **K. Hodgkinson**, M. Norman, A. Healey, A.M. Whalen, L. Thierfelder, E. Dicks, A. Bassett, P. Parfrey, S. Connors. *The phenotypic expression of poor-R wave progression on ECG in arrhythmogenic right ventricular cardiomyopathy linked to 3p25.* Presented to the American Society of Human Genetics meeting, Los Angeles, November 2003 AJHG Vol 73 (suppl) #5 page 269, abstract 581.

19. D. Pullman, **K. Hodgkinson**, S. Connors, A. Latus, B. Barrowman, L. Thierfelder, A. Bassett, P. Parfrey, J. Dean. *When genetic research findings progress to clinical application: the ethics of duty to warn.* Presented to the American Society of Human Genetics meeting, Los Angeles, November 2003 AJHG Vol 73 (suppl) #5 page 409, abstract 1397.

20. **K. Hodgkinson**, P. Parfrey, S. Stuckless, L. Thierfelder, E. Dicks, A. Bassett, S. Stuckless, S. Connors. *Arrhythmogenic right ventricular cardiomyopathy linked to 3p25: ventricular dysrhythmias and early sudden death, prevented in males with implantable cardioverter*

defibrillator therapy. Presented to the American Society of Human Genetics meeting, Los Angeles, November 2003 AJHG Vol 73 (suppl) #5 page 269, abstract 578.

21. Ravi Tahiliani*, **Kathy Hodgkinson***, Elizabeth Dicks, Susan Stuckless, Ludwig Thierfelder, Patrick Parfrey, Sean Connors. *The Diagnostic Utility of the signal Averaged EKG in Arrhythmogenic Right Ventricular Cardiomyopathy in Newfoundland.* Presented to the Canadian Cardiovascular Conference, Toronto, October 2003. *Joint first authors.

22. **Kathy Hodgkinson**, Susan Stuckless, Ludwig Thierfelder, Anne Bassett, Patrick Parfrey, Sean Connors *Improved survival in males with Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) treated with Implantable Cardioverter Defibrillator (ICD) therapy.* Platform presentation to the Canadian Cardiovascular Conference, Toronto, October 2003.

23. **Hodgkinson, Kathy**. *ARVC in Newfoundland: a unique population*? Presented at Scientific Days, Faculty of Medicine, Memorial University of Newfoundland, Oct 21-23 2002.

24. Stuckless S., **Hodgkinson K.**, Dicks E., Connors S., Thierfelder L., Drenckhahn., Norman M., McKenna W., Parfrey P. *Diagnostic Utility of Premature Ventricular Complexes in a large Arrhythmogenic Right Ventricular Cardiomyopathy (OMIM 60440) population.* Presented at Scientific Days, Memorial University of Newfoundland, Oct 21-23 2002. Also, AJHG, Vol 67 (suppl) #4 page 351 Abstract # 1045 2002.

25. **Hodgkinson K**, Stuckless S., Dicks E., Connors S., Thierfelder L., Drenckhahn J., Norman M., McKenna W., Parfrey P. *Left Ventricular Dilatation in a large Arrhythmogenic Right Ventricular Cardiomyopathy (OMIM#60440) population: prevalence, incidence and diagnostic utility. Presented at Scientific Days*, Memorial University of Newfoundland, Oct 21-23 2002. Also, AJHG, Vol 71 (suppl) #4 page 350. abstract # 1041 2002.

26. E Dicks, **K. Hodgkinson**, S. Connors, L. Thierfelder, A. Bassett, M. Norman, M. Longley, W. McKenna, P.Parfrey. *Initial clinical manifestations of arrhythmogenic right ventricular cardiomyopathy (ARVC).* AJHG 2000 Vol 67 (suppl) #4, 114, abstract # 572

27. **K. Hodgkinson**, E.Dicks, S.Connors, L.Thierfelder, M. Norman, W.McKenna, M. Longley, A.Bassett, P. Parfrey. *Arrhythmogenic right ventricular cardiomyopathy (ARVC) in six Newfoundland families: a sex-limited disease with malignant phenotype in males.* AJHG 2000 Vol 67 (suppl) #4, page 114, abstract # 572.

28. M.W. Norman, M.Longley, M.Seldon, **K.Hodgkinson**, S. Connors, W.J.McKenna, L.Thierfelder. *A founder mutation in a Canadian Cardiomyopathy population can cause a novel variant of Arrhythmogenic Right Ventricular Cardiomyopathy and Dilated Cardiomyopathy.* Presented at the European Society of Cardiology, Amsterdam, August 2000.

29. M.W. Norman, M.Longley, M.Seldon, **K.Hodgkinson**, W.J McKenna. L Thierfelder. *A novel electrocardiographic phenotype identifies a new clinical phenotype of autosomal dominant Arrhythmogenic Right Ventricular Cardiomyopathy.* Presented at the European Society of Cardiology, Amsterdam, Aug 2000. First Prize Winner: Poster Presentation.

Invited presentations

1. Terry Young and **Kathy Hodgkinson**. *The clinical and genetic epidemiology of familial cardiomyopathy in Newfoundland and Labrador.* Canadian Association of Genetic Counsellors/Canadian Association of Medical Genetics Joint Symposium, September 19, 2008. St. John's, NL.

2. **Kathy Hodgkinson.** September 17, 2008. *Ethical dilemmas in genetics*. Canadian Association of Genetic Counsellors short course program. St. John's, Newfoundland.

3. Daryl Pullman and **Kathy Hodgkinson**. *"Personalised medicine: genotype or genohype?"* (using ARVC as one end of a spectrum) Morneau-Sobeco Human Resources Consulting, St. John's, NL, June 8 2008.

4. Terry Young and **Kathy Hodgkinson** Medical Grand Rounds, Faculty of Medicine, May 16, 2008, Memorial University. *On the Hunt for a Killer. The story of ARVD5 gene discovery causing sudden cardiac death in Newfoundland.*

5. **Kathy Hodgkinson**. Invited workshop participant at the Canadian Cardiovascular Society, annual meeting, Quebec City, October 20-24 2007. ARVC workshop with co presenters Dr. Bob Hamilton (Toronto) and Dr. Dan Judge, (John's Hopkins, Baltimore USA).

6. **Kathy Hodgkinson**. '*Human Genetic Research in Newfoundland. The importance of family history*". Mini Medical and Health Sciences School program, October 10 2007.

7. **Kathy Hodgkinson** and Daryl Pullman. *Genetic Research in Newfoundland: ethical challenges.* Presented to The Queensland University of Technology, August 15 2006.

8. **Kathy Hodgkinson** and Daryl Pullman: *Who is fishing in our gene pool*? CIHR invited speaker, Memorial University, The Fluvarium, Sunday, February 5 2006.

9. Sean Connors, **Kathy Hodgkinson**. *ARVC in Newfoundland.* Presented to the Canadian Cardiovascular Conference, Toronto, October 2003.

10. **K.A.Hodgkinson** "*ARVC in Newfoundland*", Oct 28 2003. The Toronto Sick Children's Hospital Department of Genetics.

11. **K.A.Hodgkinson** "*ARVC in Newfoundland: a cause of sudden death in the young*". North York Genetics department. Oct 27 2003.

12. **K.A.Hodgkinson** "*ARVC in Newfoundland, the symbiotic relationship between research and clinical practice*" Oct 4 2003 SAD (Sudden adult death) congress. Toronto. Ontario.

13. **K.A.Hodgkinson** and Sean Connors: "*ARVC in Newfoundland: a unique population*" April 2003 John's Hopkins University, Dept. of Cardiology. ARVD symposium.

14. **Kathy Hodgkinson**. "*ARVC, an under-recognised lethal genetic disorder*". Department of Genetics, Aberdeen, Scotland. November 2002.

15. **K.A Hodgkinson**.and Elizabeth Dicks. *The genetics of arrhythmogenic right ventricular cardiomyopathy.* The Science Congress 2000, St. John's Newfoundland.

16. **Hodgkinson K.A.** *Dominant cardiomyopathies and arrhythmias in the Newfoundland population.* Presented at the Canadian Society of Genetic Counsellors Conference, Memorial University, St. John's, Newfoundland Sept. 1997.

Patents

1. Provisional US patent (#61/016,226) filed February 11, 2008, through Memorial University's Genesis Group. *A mutation in the human gene that causes sudden cardiac death due to ARVC.*

Printed media

1. Macleans Magazine July 7 2008: *When the rhythm goes wrong.* Page 62-63.
2. Throne Speech, March 10, 2008, delivered at the Opening of the First Session of the Forty-Sixth General Assembly of the Province of Newfoundland and Labrador by His Honour, The Honourable John C. Crosbie, Lieutenant Governor of Newfoundland and Labrador.
3. *Scientists identify gene that causes sudden death from heart disorder. Researchers studied Newfoundland families to develop test for lethal DNA glitch.* February 2008 Margaret Munro, Canwest News Service.
4. *Genetic breakthrough: MUN researchers find gene behind prevalent heart problem,* The Telegram, St. John's, NL, Friday, February 29, 2008, Vol. 129, No. 325, front page.
5. MUNMED News, Faculty of Medicine, Vol. 20 No. 2, Spring 2008, cover story. *Gene discovery may lead to cure for broken hearts.*
6. *Newfoundland's sudden-death riddle resolved,* The Globe & Mail, Atlantic Canada Edition, Friday, February 29, 2008, front page.
7. *Is there ever a place for medical maternalism*? SAD (Sudden adult death) newsletter Fall 2007
8. MUNMED: *Sundays at the Fluvarium.* Feb 5 2005.
9. *The First Symptom is Death,* The Telegram, St. John's, NL, June 26, 2006.
10. *Death is first Symptom,* The Gazette, Montreal, Quebec, June 25, 2006, page A13.
11. *Sudden Death First and Only Symptom of Condition. Researchers hot on the trail of gene taking a toll on apparently healthy young men,* Edmonton Journal, Alberta, June 24, 2006.
12. *Carriers Privacy Ethical Issue,* Edmonton Journal, Alberta, June 24, 2006.
13. *Genetic disease attracts attention of German TV. Research heading to small screen.* Jan 24 2002. The Memorial Gazette.
14. *Arrhythmogenic Right Ventricular Cardiomyopathy. Newfoundland families share fatal disease.* MUNMED October 4 2001.

Concerts, performances, exhibitions, commissions & creative works

1. Association of Atlantic Universities, 2008. *The Life Changers-Mending Broken Hearts.* Documentary series featuring cutting edge research undertaken at Atlantic Canada's universities. Featuring ARVC. Scheduled to air on October 5, 2008 (interviewee) http://www.mun.ca/research/changers.php
2. Collaborative documentary July 9 2003 10-40-11.25 as part of the 3-part documentary *Die Gen-Jäger (The Gene Hunters).* Speaking to the issue of the benefits of genetic research as it pertains to ARVC.
3. *The DNA dilemma*: Canadian documentary 2003.Featuring ARVC and ethical issues http://www.telefilm.gc.ca/data/production/prod_2313.asp?lang=en&cat=tv&g=doc&y=2003

Scholarly lectures and other professional presentation

Television/Radio

1. The National, CBC Television, February 29, 2008. *ARVC gene discovery.*
2. NTV Evening News, February 29, 2008. *ARVC gene discovery.*
3. The Current (with Anna Maria Tremonty) Radio: February 6 2006 *Genetic testing panel and Town Hall.* With Daryl Pullman.

Press conferences and releases

1. *Researchers discover gene responsible for deadly heart condition.* Genome Atlantic Press Conference, February 27, 2008.

Relevant community service

2006 **Kathy Hodgkinson**. Invited speaker for 'Sundays at the Fluvarium'lecture series. The purpose of this group of talks was to showcase research at Memorial University for a lay audience.

2008 **Kathy Hodgkinson**. Invited speaker at the Canadian Federation of University Women on Sat Sept. 13 2-4, the EB Foran Room, St. John's City Hall. *ARVD: the search for a killer.*

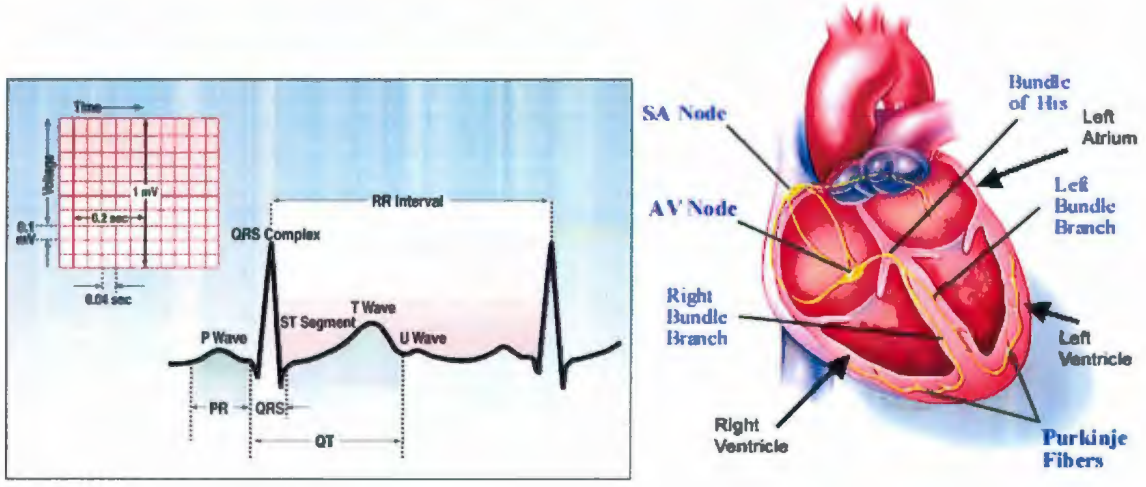
Knowledge transfer activities

1. Quarterly community newsletter for cardiomyopathies.
2. *ARVC (Arrhythmogenic Right Ventricular Cardiomyopathy) in Newfoundland and Labrador, Patient and family Information Guide.* Eastern Health Teplitsky, Y, Young, TL, **Hodgkinson K.A** Patient and Family Information Guide. Eastern Health. 2005.

APPENDIX C

The normal heart beat

Heart beats start at the sinoatrial node in the right atrium where an electrical stimulus is generated by ion transfer (calcium, potassium and sodium) through the myocyte cell membranes, stimulating the atria just before stimulating the ventricles. The electrical impulse travels from the sinus node to the atrioventricular (AV) node, where its rate is regulated, then continues through the conduction pathways via the bundle of His and purkinje fibres into the right and left ventricles. This depolarization and repolarisation results in a coordinated heartbeat at a rate of between 50 and 100 per minute.



The normal heart beat

P wave = atrial depolarization, PR interval = from the onset of atrial activation to onset of ventricular activation, QRS = ventricular depolarization, S-T segment = the ventricles before repolarisation, T-wave = ventricular repolarisation, QT interval = the duration of ventricular activation and recovery (polarisation and depolarisation), RR interval = the time elapsing between two consecutive R waves in the ECG, U wave = repolarisation of the papilliary muscles or Purkinje fibres.

Ventricular Tachycardia (VT)

VT is a complex cardiac rhythm originating in the ventricles and is the presence of multiple consecutive premature ventricular contractions (PVCs). PVCs result from a short circuit of the electrical system in the ventricles, or from an ectopic (out of place) ventricular focus that sends out its own electrical impulse. These beats stimulate the ventricles ahead of the atria, and do not transmit across the ventricles normally, producing an ineffective contraction. They also interfere with the next 'normal' heart beat, often producing the feeling of a 'skipped' beat. The rate is usually between 150 and 200 beats / minute and regular, although VT can produce much faster rates. The rapid rate and the disrupted contraction between the ventricles and the atria may lead to low cardiac output, hypotension, and cardiac arrest. VT is classified as non-sustained (three or more PVCs terminating under 30 seconds) or sustained (VT > 30 seconds or requiring termination in under 30 seconds due to loss of consciousness). The VT is often polymorphic in nature, where no 2 sequential PVCs have the same morphology in one ECG lead or where no morphology lasts for a minimum of 6 beats when at least 3 ECG leads are simultaneously recorded.

Ventricular Fibrillation (VF)

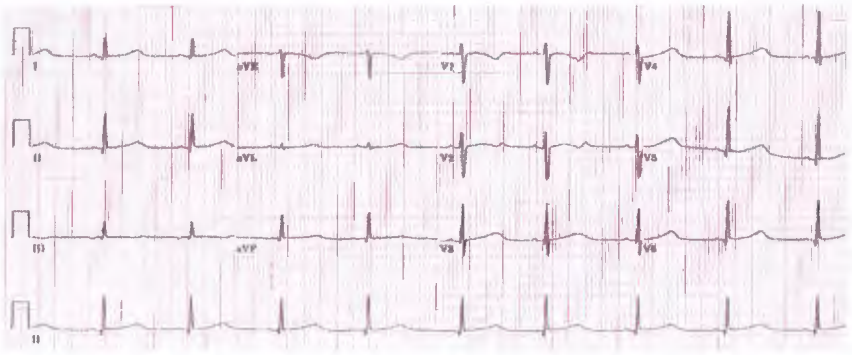
VF is a rapid, chaotic, uncoordinated, ineffective series of ventricular contractions with no QRS complex visible on 12 lead ECG. It is a fatal arrhythmia due to the inability of the heart to perfuse (send blood to oxygenate) body systems. Cardioversion with an external electric shock is the treatment for VF. VF can arise from rapid VT.

The 12 lead Electrocardiogram (ECG)

The 12 lead ECG assesses the electrical impulse across the heart with 6 chest (precordial) leads (V1, V2, V3, V4, V5 and V6) and 6 limb leads (avR, avL, avF, I, II, III) The inferior leads (II, III and aVF) look at the under surface of the left ventricle, the anterior leads (V2, V3, V4) look at the front of the heart viewing the left ventricle and septum the lateral leads (V5, V6, I, aVL) look at the left side of the left ventricle and the posterior

leads (V1, V2, V3) look at the back of the heart. The ECG assesses the electrical activity of the normal heartbeat.

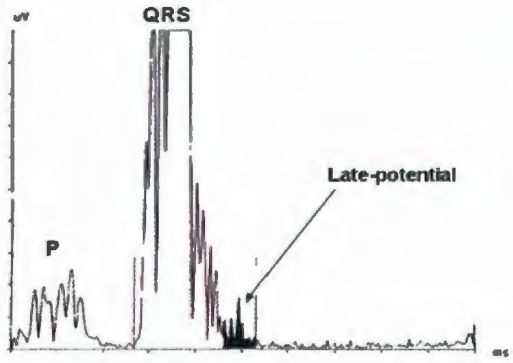
Normal 12 lead ECG



The Signal Averaged ECG (SAECG)

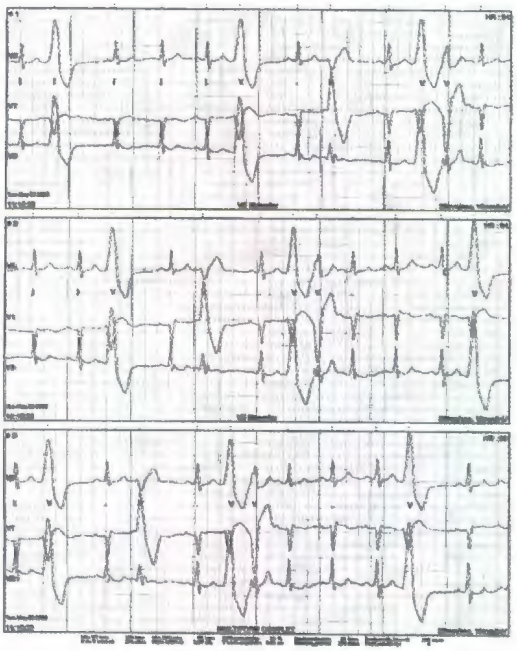
The SAECG 'averages' multiple routine ECGs obtained over a 20-minute period from a horizontal patient to obtain information about ventricular late potentials (VLPs). VLPs are low amplitude high frequency signals at the end of the QRS complex, and are due to the late depolarisation of damaged myocardium. They are known to be present in patients with VT, and assess, in effect, the time required for the electrical signal to pass through the myocardium. They are difficult to elicit as they may be masked by background electromyographic noise, which the SAECG attempts to minimize (379). VLPs on SAECG are classified based on QRS length (filtered QRS duration) >114 ms, LAS (low amplitude signals) >38 ms, and RMS (root mean square voltage of the terminal 40ms of filtered QRS) <20 ms (260).

A SAECG from a patient in family AR1 (Figure 8 page 79)



Holter monitors

Holter monitors (invented by the biophysicist, Norman Holter (1914-1983)) record a continuous ECG over an extended period of time (either 24 or 48 hours) from ECG leads attached to the chest wall. This test documents all heart rhythms, and relates them to the presence or absence of symptoms. The old devices used reel-to-reel tape, or C90 audio cassettes. Modern units record digitally and upload to a computer, which counts ECG complexes, calculates statistics, and highlights areas requiring further study.

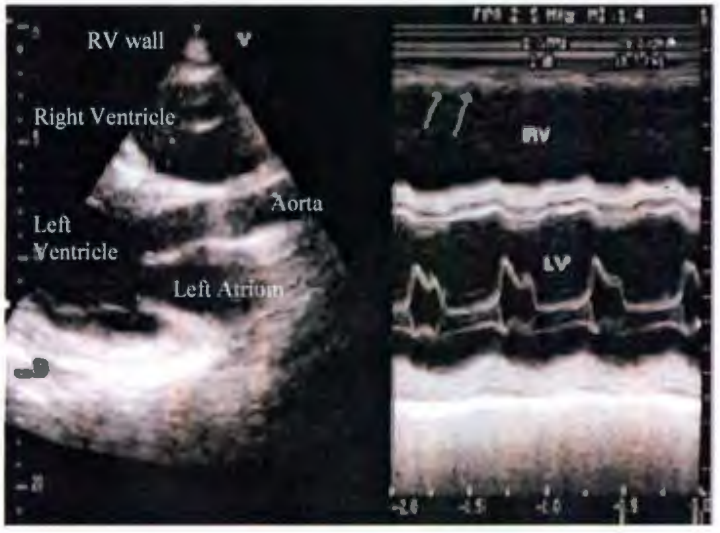


A Holter monitor trace from a patient in family AR1 (Figure 8 page 79)

Echocardiography

A transthoracic (across the chest wall) echocardiogram is an ultrasound of the heart, with a frequency of > 20,000 cycles/sec. The first cardiac ultrasounds were available in the mid 1950's, and up until the mid 1970's, M-mode echocardiography was used, where the ultrasound beam was aimed manually at cardiac structures. M-mode recordings allowed for the measurement of cardiac structures, and an assessment of cardiac motion. ECG and pulse tracings were recorded simultaneously allowing an analysis of time relationships between the different physiological variables. Since the mid 1970's, mechanical or electrical techniques scan the ultrasound beam rapidly across the heart to produce two-dimensional images (2D). Echocardiography which uses both M-mode and 2D recordings is an effective method to determine cardiac output. A clearer view of the heart is obtained by a transoesophageal echo, where the transducer is fed into the oesophagus, and the heart viewed through the thin oesophageal wall. This is however an invasive procedure, requiring, at a minimum, conscious sedation. 3D echocardiography uses a probe with an array of transducers and an analysis system to provide even more detailed views of the cardiac anatomy.

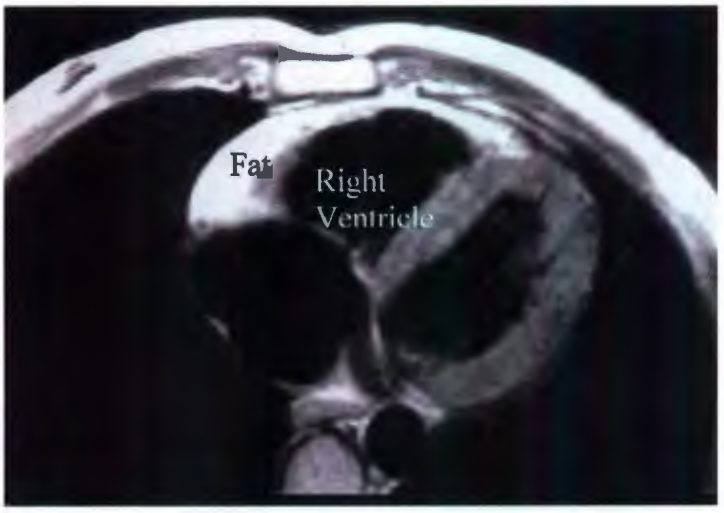
M-mode echocardiography showing thinning and reduced systolic thickening in the right ventricular wall. Both the RV and LV are dilated[10].



[10] http://www.ijri.org/articles/2006/16/1/images/IndianJRadiolImaging_2006_16_1_131_29069_2.jpg

MRI

Magnetic resonance imaging (MRI) is a technique highly adept at visualising bodily structures and functions, particularly soft tissues. This makes it a powerful tool for looking at neurological and cardiovascular structures, and visualising the extent of tumour growth in all body systems. It uses powerful magnetic fields. The magnetic field aligns the nuclear magnetisation of hydrogen atoms contained in the large supply of water in the human body. Radio frequency fields alter the direction of the magnetism which causes the hydrogen nuclei to produce a rotating magnetic field. It is this that the scanner detects and utilises to create an image.



An MRI image of an ARVC subject. Note the white (high contrast) fat in the dilated RV.

Cardiac biopsy

Cardiac biopsy uses a bioptome catheter with jaws at its tip, inserted through a vein or artery. If not part of a catheterization procedure (which uses veins in the groin area), the catheter is usually inserted in the jugular vein. Fluoroscopy (an X-ray that can provide moving images) is used to guide the insertion.

Statistical analyses

Survival analysis

Survival analysis is a time to event (or time to failure) technique. Time is the dependent variable. The status variable is whether the individual has reached the end point. This is often death (hence 'survival'analysis), but it can be any end point of choice. Usually the subjects enter over time and the object of interest is not seen in all subjects: additionally some may just be lost to follow-up. This is known as 'censoring': when individuals within the study do not reach the end point during the time of the study. This is usually the date of analysis if individuals are still alive at that time but censoring can occur if a subject is lost to follow-up, or is moved from the analysis for other reasons. In both cases, the subjects were known to have 'survived' for some amount of time (up until the time they were last seen), but how much longer they might ultimately have survived is unknown. The Kaplan Meier method was developed to use the information contained in those who have survived in the analysis for 'at least this long' This is 'right' censoring which occurs when all subjects in the analysis are accounted for and when the outcome of interest has not been observed for a participant. Left censoring occurs when the outcome of interest has occurred for a subject before the study begins.

Survival analysis can address several questions, including:

i. The amount of time before X displays a change in Y,

or

ii. Given that a change has not been observed in time t, what chances are there it will occur in time t1?

or

iii. What is the difference in outcomes between different subject groups? (the major analyses in this thesis).

Survival analysis is predicated on several assumptions. These are:

i. An identifiable start point is applied uniformly for all subjects (often birth);
ii. There is an identifiable end point: dichotomous and well defined such as death;
iii. Censoring is not related to outcome and
iv. Secular trends are not present (e.g. changes in diagnosis and treatment over the course of a study). This last is the most difficult to achieve in studies which collect information over time.

For the thesis, the Kaplan Meier non-parametric survival model was used. It makes no assumption about the shape of the underlying curve, and is based on the product limit equation. In all the survival analyses, comparing two groups (in the thesis, affected and unaffected: mutation positive, versus mutation negative), the question asked is whether the slopes of the curves generated are the same or different.

Thus the null hypothesis would be H_0: $\lambda A = \lambda UA$, and the alternate hypothesis H_A: $\lambda UA < \lambda A$ where λ is the slope of the curve in a survival analysis defining time to event. The steeper the slope, the higher the rate of the event in a shorter space of time. Thus the null hypothesis for Chapters 2, 3 and 4 states that there is no difference between the slope of the survival curve for affected individuals compared with unaffected individuals, and the alternate hypothesis posits that there is a difference and that the slope of the affected group is steeper than that of the unaffected group.

Sample size/power analysis

No formal assessment of sample size or a power analysis is provided in this thesis. The numbers are defined by the boundaries of the families and the inclusion/exclusion criteria. The sample used is a demarcated population from which an answer will either be statistically valid or not.

Chi Square analysis

Chi square analysis investigates whether distributions of categorical variables (yes/no answers) differ from one another, and is used on actual numbers, not proportions or

means. Initially one calculates the chi-square statistic by finding the difference between each observed and expected frequency (obtained via the null hypothesis) for each possible outcome, squaring them, dividing each by the expected frequency, and taking the sum of the results. The p value is then calculated by comparing the statistic to a chi-square distribution with a specific number of degrees of freedom (the number of possible outcomes, minus 1). Examples of categorical variables used in this thesis include the presence or absence of a symptom or clinical abnormality on a cardiac test.

Cox's proportional regression

Cox's Proportional Hazards regression produces a baseline survival curve with covariate coefficient estimates with their standard errors, a relative (hazards) risk ratio, 95% (or 99%) confidence intervals, and a p value (significance level). Cox's analysis provides information about whether survival is influenced by one or more, categorical or continuous, factors. Cox's analysis allows an assessment of the effect of each factor on the shape of the survival curve. The method computes a coefficient for each factor that indicates the direction and amount of movement it has on the baseline curve. A coefficient of zero indicates that the factor is not a predictor of 'survival' at all compared with a positive coefficient. These coefficient values allow for the construction of a survival curve based on any combination of values, and provide a measure of the sampling error. This analysis method allows an understanding of which variables are significantly related to 'survival'. In this thesis, all Cox's regression analyses are based on a comparison of two groups only.