Preliminary genealogical evidence for the *Plakophilin-2* gene, *PKP2* c.1162C>T founder mutation in cases with Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)

By Tafadzwa Machipisa (MCHTAF001)

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UNIVERSITY OF CAPE TOWN

Supervisors: Dr. Gasnat Shaboodien & Prof. Bongani Mayosi

Cardiovascular Genetics Laboratory, Hatter Institute for Cardiovascular Research in Africa, Department of Medicine, Groote Schuur Hospital and University of Cape Town, Old Groote Schuur Hospital, Groote Schuur Drive, Observatory, 7925, Cape Town, South Africa.

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1. Declaration

I, Tafadzwa Machipisa, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorize the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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2. Table of Contents

2A. List of Figures

2B. List of Tables

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"Weeping may spend the night, but there is joy in the morning'.-Ps.30:5.

4. Abstract

Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a progressive form of inherited heart muscle disease characterized by ventricular arrhythmias and sudden cardiac death. Often the pathogenesis is linked to deleterious mutations in the desmosomal gene plakophilin-2 (*PKP2*). We extended investigations of the pathogenic *PKP2* c.1162C>T founder mutation which had previously been reported to occur within four 'unrelated' probands (6.2%) who self-identified as Afrikaners and who also carried a common haplotype. Common evolutionary history suggests common haplotypes are linked to a common founder and today the Afrikaner populations are a unique ethnic group in South Africa identified with various founder effects for a range of heritable disorders.

Aim

This study aimed to identify the common founder using genealogical and molecular methods for the *PKP2* c.1162C>T mutation in ARVC families of Afrikaner descent in South Africa.

Methods and results

DNA was collected from 46 participants (7 probands and 39 relatives) from the ARVC Registry of South Africa. Probands and relatives were screened for the *PKP2* c.1162C>T mutation using High Resolution Melt and Sanger sequencing. The genetic results indicated that 65.2% (30/46) of the family members harbored this mutation. High Resolution Melt, Sanger sequencing and microsatellite typing were used to create a haplotype which encompassed the c.1162C>T mutation and three microsatellite markers (M1, D12S1692 and M2) spanning the *PKP2* gene. A common haplotype emerged that segregated amongst all of the affected members of the seven Afrikaner families.

Genealogical tracing went back, through multiple generations, into the implicated ancestral lines of the present day Afrikaner families. Four of the seven families attained their 17th century progenitors. Through genealogical analyses of the two largest families, ACM 19 and ACM 38, we identified 116 couples which we reduced to ten candidate South African founder couples who were then subjected to further analyses. After the ACM 12 family was added to the analysis there were five candidate founder couples. Unfortunately, the ACM 71 family did not progress past the 20th century due to tracing difficulties associated with poor record keeping of mixed ancestry data in South Africa and hence, could not be linked back to any other family

tree without finding ACM71.5's grandparents. Additionally, ACM 8 and 57 families were recent finds and completion of their genealogical tracing still has to done.

Conclusions

Our genetic data showed that not only were 30/46 individuals positive for the *PKP2* c.1162C>T mutation but that all 30 individuals also shared the same common haplotype. Our preliminary genealogy tracing data suggests that the *PKP2* c.1162C>T mutation segregates at a higher frequency in the Afrikaner population possibly due to a founder effect. The genealogical evidence supports the hypothesis that the *PKP2* c.1162C>T mutation is a founder mutation and that descendants of the common founders are at risk of developing ARVC. At least three more families need to be recruited to make a clear conclusion and achieve genealogical evidence based saturation, ideally, a common founder.

5. List of abbreviations

ACM- Arrhythmogenic cardiomyopathy

ARVC/D - Arrhythmogenic Right Ventricular Cardiomyopathy / Dysplasia

CASSA- Cardiac Arrhythmia Society of Southern Africa

CVG- Cardiovascular Genetics

DVP- De Villiers/Pama System

DNA- Deoxyribonucleic acid

GISA- Genealogical Institute of South Africa

HICRA- Hatter Institute for Cardiovascular Research in Africa

HRM- High Resolution Melt

HREC- Human Research Ethics Committee

PCR- Polymerase Chain Reaction

PKP2- Plakophilin-2

SAFs- South African Families

SAGs- South African Genealogies

SNP- Single Nucleotide Polymorphism

SCD- Sudden Cardiac Death

TFC- Task Force Criteria

UCT- University of Cape Town

VOC- Dutch East India Company

VT- Ventricular Tachycardia

6. Definitions

Arrhythmia: a condition in which the heart beats with an irregular or abnormal rhythm (NIH, 2011).

Autosome: any of the 22 chromosomes exclusive of the sex chromosomes, X and Y (Strachan and Read, 1999).

Cardiomyopathy: a myocardial disorder that has an abnormal structure and function in heart muscle, in the absence of congenital heart disease, coronary artery disease, hypertension and valvular disease sufficient to cause the observed myocardial abnormality (Elliott et al., 2008).

Compound heterozygote: a genotype with two different mutant alleles at the same locus (Nussbaum et al., 2015).

Desmosomes: intercellular junctions that provide strong adhesion between cells (Garrod and Chidgey, 2008).

Dominant: a human genetics term used for a trait conveyed in a heterozygote (Strachan and Read, 1999).

Double heterozygote: individual, or genotype, with two different mutant alleles, each one at different loci. Not to be confused with compound heterozygote (Nussbaum et al., 2015).

Edict of Fontainebleau (1685): was a decree issued by Louis XIV of France, also known as the 'Revocation of the Edict of Nantes'. It banned Protestant worship and the emigration of Protestants (Mostert, 2013).

Edict of Nantes (1598): a decree that granted the Huguenots the right to practice their religion without persecution from the state by King Henry IV of France (Mostert, 2013).

Gene: a functional DNA unit regulating phenotype which segregates in pedigrees according to Mendelian laws (Strachan and Read, 1999).

Heart Failure: this is clinical syndrome which is characterized by effort intolerance and fluid retention due to cardiac dysfunction (Ntusi and Mayosi, 2009).

Homozygote: a genotype in which the two mutant alleles are identical (Nussbaum et al., 2015).

Huguenots: Calvinist Protestants of France (Mostert, 2013).

Implantable cardioverter defibrillator (ICD): a surgically inserted medical device used for therapy. It shocks the heart when it develops a tachyarrhythmia such as ventricular tachycardia or ventricular fibrillation (Haluska et al., 1989).

Microsatellite: a polymorphic locus consisting of a variable number of tandemly repeated dinucleotide, trinucleotide or tetranucleotide units such as $(TG)_n$, $(CAA)_n$, or $(GATA)_n$; different number of units constitute the different alleles. Often termed 'microsatellite marker' and are used for DNA profiling for kinship. See Single tandem repeat polymorphism (STRP) (Nussbaum et al., 2015).

Mutation: any permanent alteration in genomic DNA that can is heritable. Often leading to a variant, an allele differing from the wildtype (Nussbaum et al., 2015).

Penetrance: the fraction of individuals with a genotype known to cause a disease, with any signs and symptoms of the disease (Nussbaum et al., 2015).

Progenitor: is a direct ancestor. For example, your father's father is your progenitor. Your father's father's brother is not your progenitor. In this study, a progenitor often refers to the original immigrant of a family to arrive in present day South Africa (Cardinal, 2004).

Single nucleotide polymorphisms (SNPs): a change in DNA at a single base pair in DNA that occurs at a frequency of ≥1% in the general population. (Stenson et al., 2009, Nussbaum et al., 2015).

Single tandem repeat polymorphism (STRP): see Microsatellite.

Sudden cardiac death: a sudden death that is thought to be due to a cardiac cause (Chin, 2015).

Syncope: a transient, self-limited loss of consciousness with an inability to maintain postural tone that is followed by spontaneous recovery. This definition excludes seizures, coma, shock, or other states of altered consciousness (Amofa, 2016, Blanc and Benditt, 2003).

7. Introduction

7.1 Cardiomyopathies

Cardiovascular diseases are the leading cause of mortality in the world (Roth et al., 2015). In Sub-Saharan Africa, up to 30% of acute heart failure hospitalizations and early mortality are associated with cardiomyopathies (Sliwa et al., 2005). Cardiomyopathy is a myocardial disorder that is characterized by an abnormal structure and function of the heart muscle, in the absence of congenital heart disease, coronary artery disease, hypertension and valvular disease sufficient to cause the observed myocardial abnormality (Elliott, Andersson et al. 2008). The disease has several known forms, namely arrhythmogenic right ventricular cardiomyopathy (ARVC), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM) and peripartum cardiomyopathy (PCM) (Chin, 2015). Due to the establishment of ARVC registries and advancements in genetic research over the last 20 years, more cases have been observed and knowledge and awareness gradually expanded (Hendricks et al., 2010). We focused primarily on ARVC and its genetic and genealogical links to the founder mutation.

7.2 Natural history of ARVC

The earliest documented account of modern day ARVC is believed to be as far back in records as 1736, documented by the Pope's physician, Giovanni Maria Lancisi, in his book entitled "De Motu Cordis et Aneurysmatibus" (Romero et al., 2013). ARVC accounts were also reported in 1977, during surgery to map and treat ventricular tachycardia at the Hôpital de La Salpêtrière, when the disease was first termed 'arrhythmogenic right ventricular dysplasia' (ARVD) (Fontaine and Chen, 2014). Furthermore, in the late 1980s through autopsy studies, Thiene et al (1988) discovered that ARVC was a major cause of sudden death in the young (<35 years) and in competitive athletes (Turrini et al., 2001, Marcus et al., 2010, Thiene et al., 1988). As a result in 1982, the first comprehensive clinical profile was developed (Turrini et al., 2001). This led to ARVC being included, for the first time, in the World Health Organization (WHO) classification of cardiomyopathies (Fontaine and Prost-Squarcioni, 2004).

Estimating the prevalence of ARVC in the general population is difficult due to the challenging nature of the diagnosis. In European studies, a prevalence of between 0.6 and 4.4 per 1000 has been reported (Quarta and Elliott, 2012). Investigations into the incidence and prevalence of

ARVC have not yet been conducted in African populations (Alfieri et al., 2014). A prevalence of 1:1000 to 1:5000 has been estimated in Europe and the US (McNally et al., 2014).

7.3 Phenotype of ARVC

Arrhythmogenic right ventricular cardiomyopathy is a type of heart muscle disease (cardiomyopathy) characterized by progressive fibro-fatty replacement of the ventricular myocardium (Basso et al., 2008). This causes the walls of the heart chamber to become thin and dilated either focally (i.e., aneurysm) or globally (Figure 1), leading to right ventricular dysfunction and cardiac arrhythmias. It is classically described as a disease of the right ventricle (RV), but left ventricular (LV) involvement is increasingly recognized (Asimaki and Saffitz, 2011, Wilde and Behr, 2013). ARVC often presents with palpitations, syncope, heart failure and sudden cardiac death (SCD) (Teo et al., 2015, Cahill et al., 2013).

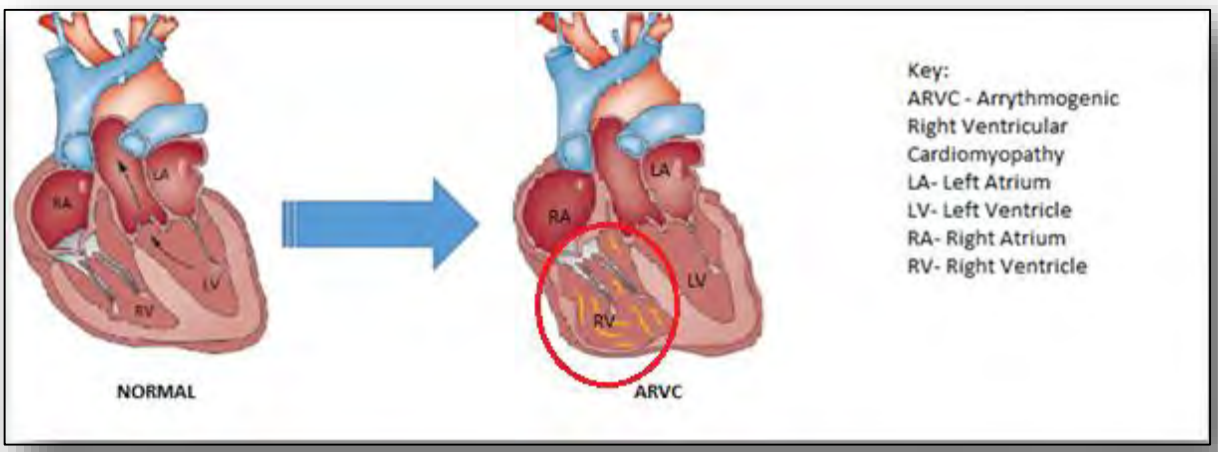


Figure 1: *Variations between a normal heart (left) and an ARVC affected heart (right), with right-sided ventricle involvement only (circled).*

(Adapted and modified from (Wilde and Behr, 2013)).

This disorder is asymptomatic in 40% of the affected, familial in over 50% of cases (Sen-Chowdhry et al., 2010) and currently has no cure. Its complexity lies in it being autosomal dominant with many patients carrying mutations in more than one disease gene region (double or compound heterozygosity) (Watkins et al., 2009). Penetrance, which is age dependent as in other cardiomyopathies, is reduced; it is also thought to be uncommon to find large extended

families with ARVC, and so far, the yield of clinically affected cases through family cascade screening has been limited (Cahill et al., 2013, Quarta and Elliott, 2012, Elliott et al., 2008).

Generally, definitive identification of the diseased phenotype is challenging (Quarta and Elliott, 2012). This is partially due to the "concealed" phase which typically precedes overt cardiomyopathy, the development of heart failure in 10-20% of the cases, and the rare presentation of ventricular systolic dysfunction (Cahill et al., 2013, Elliott et al., 2008). Furthermore, young and active probands often first manifest the disease via a malignant arrhythmia that often causes SCD; hence, most families are screened and recruited after the SCD of a relative (Marcus et al., 2010).

Arrhythmogenic right ventricular cardiomyopathy may be aggravated by endurance exercise, which can lead to SCD. Hence, ARVC has forced some athletes to retire young such as Fabrice Muamba (2012) and James Taylor (2016), or risk joining the unfortunate athletes who died whilst playing for their teams, namely, Antonio Puerta (2007), Matt Gadsby (2009), and Kirk Urso (2012) (Amofa, 2016).

The diagnosis of ARVC is based on the presence of criteria that include structural, histological, electrocardiographic, arrhythmic and genetic factors that are characteristic of the disease (McKenna et al., 1994). These criteria are divided into major and minor criteria, based on their specificity for ARVC. An initial set of task force criteria was presented in 1994 by McKenna and colleagues, and a revision of these criteria was proposed by Marcus and colleagues in 2010 (Appendix 1).

7.4 Genetics of ARVC

Arrhythmogenic right ventricular cardiomyopathy is mostly associated with five desmosomal genes (Figure 2) i.e. desmocollin-2 (*DSC2*), desmoglein-2 (*DSG2*), junctional plakoglobin (*JUP*), plakophilin-2 (*PKP2*) and desmoplakin (*DSP*). The *PKP2* gene is the most common genetic cause of ARVC (Alcalde et al., 2014, Watkins et al., 2009), accounting for up to 70% of mutation-positive cases found in European populations (te Riele et al., 2014, te Riele et al., 2012) .

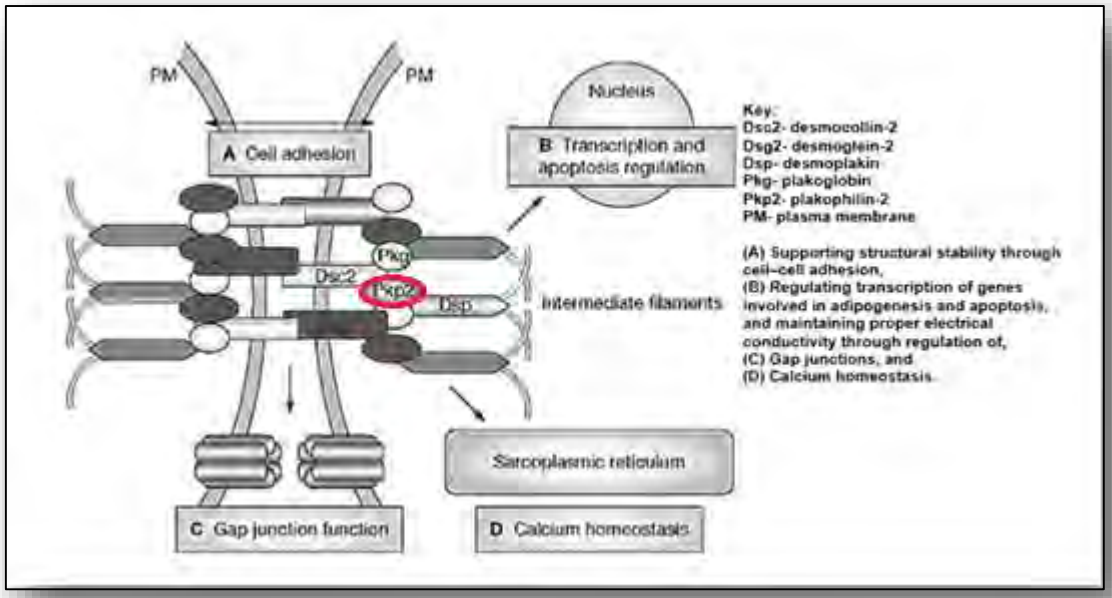


Figure 2: *The cardiac desmosomes and proposed roles of specific desmosomes.*

The desmosomal complex includes: desmosomal cadherins (i.e. desmocollin-2 [DSC2] and desmoglein-2 [DSG2]), the armadillo proteins (i.e. junctional plakoglobin [JUP] and plakophilin-2 [PKP2]), and the plakins (desmoplakin [DSP]). PKP2 is the desmosomal region of interest which is circled in red. (Adapted and modified from :(Marcus et al., 2010)).

In 2004, a South African research programme was launched to study the clinical characteristics and molecular genetics of ARVC in African populations. This was conducted under the auspices of the Cardiac Arrhythmia Society of Southern Africa (CASSA), and the ARVC registry of South Africa, based at Groote Schuur Hospital and the Department of Medicine of the University of Cape Town (UCT). This registry continually records data related to the natural history, prognosis, and efficacy of treatment and the genetics of ARVC in South Africa (Watkins et al., 2009).

In 2009, owing to the referral of over 100 cardiomyopathy patients to the ARVC registry of South Africa, as well as the limited knowledge of cardiomyopathies in Africa, a study was performed to determine the clinical features, survival experience, and profile of the genetics of *PKP2* gene mutations in ARVC (Watkins et al., 2009). They found the *PKP2* gene to harbor the most common disease-causing mutations (13.9%) - the highest in the seven genes screened (5 desmosomal and 2 non-desmosomal genes). They also discovered the *PKP2* c.1162C>T

(p.R388W) mutation in four Afrikaner probands. This, together with the absence of the variant in 241 healthy controls, suggested a founder effect in this population (Watkins et al., 2009).

On further investigation, we found that these probands shared a common haplotype, indicating that the *PKP2* c.1162C>T variant (Figure 3) was indeed a founder mutation. The Afrikaner population is possibly descended from a small pool of predominantly European founders, whose origins will be further explored in this study. Since 2009, the *PKP2* c.1162C>T mutation has also been observed in other individuals of European descent but it is not known whether these individuals are related by descent or state to our South African cohort (Table 1).

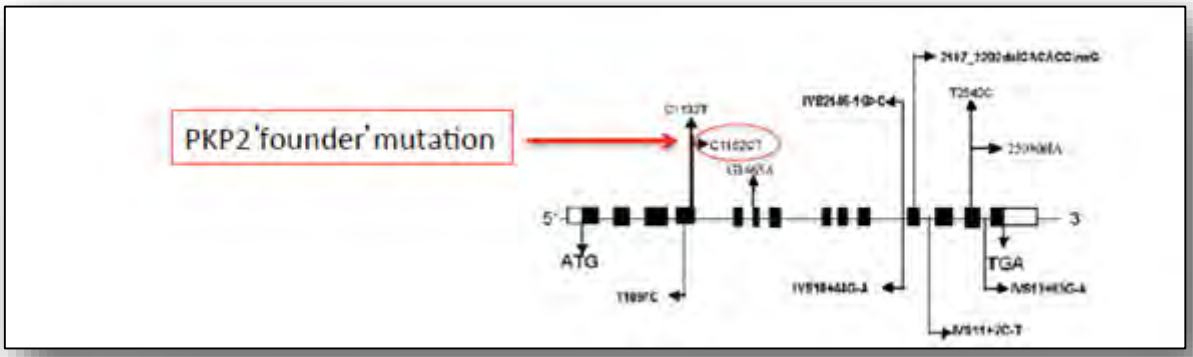


Figure 3: *Schematic representation of the PKP2 gene.*

The location of the PKP2 founder mutation, c.1162C>T (p.R388W) is indicated (circled) with respect to other mutations. Black boxes indicate the exons, with disease causing mutations shown above them, and polymorphisms below. (Adapted and modified from: (Watkins et al., 2009)).

Table 1: *Global distribution of the PKP2 c.1162C>T mutation in individuals of European ancestry.*

Title of paper / thesis/ Unpublished data	**Source**	**Date/Year published**	**Comment**
Unpublished data: Homozygous *PKP2* c.1162C>T	Provided by collaborator Dr Nathalie Roux-Buisson (Laboratoire de Biochimie et Génétique Moléculaire, IBP – DBTP 2ème Etage, CHU de Grenoble, CS10217 – 38043 Grenoble Cedex 9, France)	2016	Collaborators identified an adult female with the first c.1162C>T homozygous *PKP2* mutation, who presented with ventricular arrhythmias. Her mother is a heterozygous mutation carrier.
Unpublished data: Insights into molecular and functional mechanisms behind inherited heart and skin disorders by Nitoiu, Daniela	PhD Thesis: https://qmro.qmul.ac.uk/xmlui/bitstream/handle/123456789/8911/Nitoiu_Daniela_PhD_070415.pdf?sequence=1	2015	Present in Patient RY8012, *PKP2* 4 NM_001005242:c.C1162T: p.R388W Reads (<2% poor, >2% good). These results were observed after Next Generation Sequencing following a targeted-capture array on ARVC patients, i.e., in one patient originating from New Zealand.
Stop-Gain Mutations in *PKP2* Are Associated with a Later Age of Onset of Arrhythmogenic Right Ventricular Cardiomyopathy	(Alcalde et al., 2014)	2014	Present in one Spanish patient.
Unpublished data: Molecular basis for the diagnosis and etiology of genetically related cardiovascular disease as the cause of sudden cardiac death (Molekulare Grundlagen zur Diagnostik und Ätiologie genetisch bedingter kardiovaskulärer Erkrankungen als Auslöser des plötzlichen Herztodes) by Nadine Kiehne (translated from German thesis).	PhD Thesis in German: http://tuprints.ulb.tu-darmstadt.de/2665/1/genehmigte_Dissertation_Nadine_Kiehne.pdf	2011	Present in a patient, Sample BN043; *PKP2* Exon 4, R388W; German patient from the Kerckhoff Clinic group in Germany.
Compound and Digenic Heterozygosity Contributes to Arrhythmogenic Right Ventricular Cardiomyopathy	(Xu et al., 2010)	2010	Present in one North American ARVC patient (Patient 5) aged 16 and male.
Clinical features, survival experience, and profile of plakophylin-2 gene mutations in participants of the Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa	(Watkins et al., 2009)	2009	Present in 4 white South African probands - self identified as Afrikaners.

According to the ARVC registry of South Africa, ARVC cases were observed more commonly in white and coloured than black South Africans possibly due to ascertainment bias caused by differences in access to healthcare.

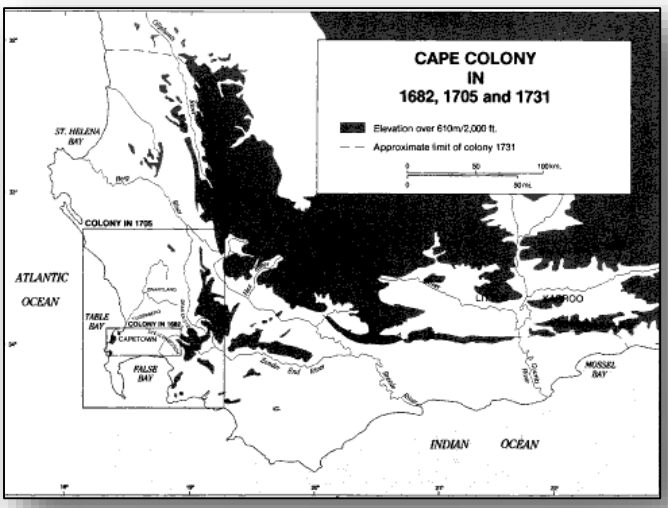
7.5 The founder effect in Afrikaners

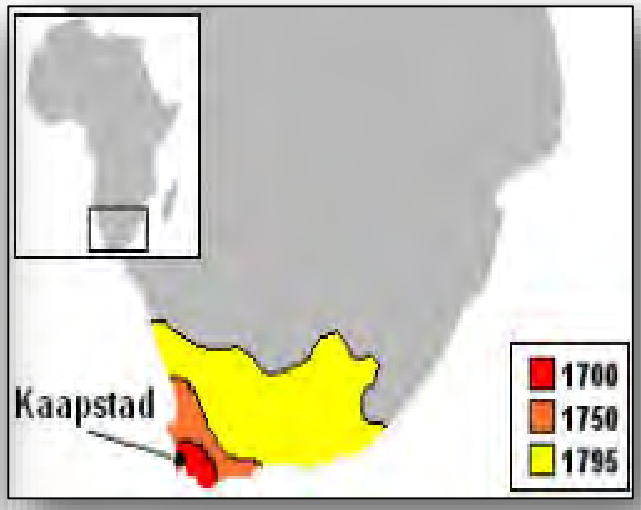
In this study, a progenitor (also section 5) refers to the original immigrant of a family to arrive in present day South Africa (Cardinal 2004); also known as a 'founder'. A founder effect occurs when a small subset of a large population establishes a new population. The new population may differ significantly from the original population, both in terms of its genotypes and phenotypes (Geldenhuys et al., 2014). In the case of South Africa, the first white settlers, led by Jan van Riebeeck (1652), arrived in Table Bay (Figure 4A) onboard three Dutch ships. As a result, the Cape Colony (Figure 4 A & B) was established and governed by the Dutch East India Company (VOC) until 1795. It was established as a re-supply and layover port for vessels of the VOC trading with Asia and later, the rest of the world. During this period, the Dutch Reformed Church (DRC/NGK) was established and began to keep records of congregants (Southey, 2001, Burrows, 1958).

During the VOC rule in 1654 slaves were imported into the Cape Colony; some were later freed and married Dutch settlers or the Hottentots/Khoi Khoi. After the Edict of Fontainebleau or the Revocation (1685), France was in religious turmoil, banning Protestants' religious freedom; this led to 200 French Huguenot (Calvinist Protestants of France) settlers arriving between 1688 and 1689. The Huguenots entered as refugees, had religious similarities with the Dutch and admixture into the modern day Afrikaner population occurred frequently. From 1795-1802, the British occupied the Cape but were constantly at war against the Dutch and from the 1803, the Batavia Republic period commenced in the Cape Colony (Figure 4D). The British and the Dutch rarely admixed due to religious and colonial differences, both wanting to rule the lucrative Cape. In the 1820s, 5000 British settlers entered modern day South Africa, arriving at Port Elizabeth (Figure 4 A & D). In 1848, 163 German (Bergthiel) settlers arrived in Natal (Figure 4D) and also frequently admixed into the Afrikaner population (Southey, 2001, Burrows, 1958).

A

B





C

D

Figure 4: *Maps of South Africa between 1652-1915.*

(A) *A map of the Cape colony 1652-1731 (Adapted from:(Guelke, 1988)).***(B)** *Evolution of the Dutch Cape Colony, 1700-1795. Cape town is highlighted in Dutch/Afrikaans,Kaapstad"(Adapted from:(www.revolvy.com, 2016)).* **(C)** *A Map of migration patterns of Trekboers (Adapted from: (www.revolvy.com, 2016)).* **(D)** *A Map of South African Wars (1879–1915). Footnote: Ethnic, political and social tensions among European colonial powers, indigenous Africans, and English and Dutch settlers led to open conflict in a series of wars and revolts between 1879 and 1915 that would have lasting repercussions on the entire region of southern Africa. Pursuit of commercial empire as well as individual aspirations, especially after the discovery of gold (1886) and diamonds (1867), were key factors driving these developments. (Adapted from:(www.revolvy.com, 2016)).*

In the case of Afrikaners, the disorders that occur at an unusually high frequency may indicate that the original Dutch, French and/or German settlers in the Cape may have carried disease-causing genes, and that consanguineous marriages heightened disease incidence (Geldenhuys et al., 2014). Previous founder studies in the Afrikaner population have demonstrated that founder mutations exist for heart diseases such as hypertrophic cardiomyopathy and long QT syndrome (LQTS) (Brink and Schwartz, 2009).

The founder effect is believed to be predominantly observed in Afrikaner lineages due to geographical isolation (Geldenhuys et al., 2014). From previous studies, Afrikaner ancestors are commonly founded from Dutch (up to one third), German (less than a third) and French (approximately a quarter) roots that migrated to the modern day South Africa (previously known as the Cape of Good Hope) from the 1650s and onwards, in all parts of South Africa (Figure 4C). Furthermore, they have higher frequencies of intermarriages amongst them, which contributed to their genetic homogeneity (Geldenhuys et al., 2014, Greeff, 2007, Southey, 2001, Burrows, 1958). Each modern day Afrikaner's proportions of European and non-European ancestry varies, and so will the segregation of a founder mutation in an isolated, mostly homogeneous population (Geldenhuys et al., 2014, Brink and Schwartz, 2009, Greeff, 2007).

The aim of this research project was to extend the families that had previously been found to harbor the *PKP2* c.1162C>T mutation and to trace the common ancestor who entered modern day South Africa. The tracing method used was tailored for genetic genealogy, which has been a useful tool for human models of risk assessment in genetic health services (Stefansdottir et al., 2013). According to Geldenhuys (2014), ideally, 10 probands are required to find a common ancestor.

The founder research was conducted similarly to the 2009 work done by Brink and Schwartz for the founder families with the long QT syndrome type 1 in South Africa and Geldenhuys' (2014) work on Parkinson's, respectively. The output includes the founder being named, as in the Brink and Schwartz (2009) paper, and a summarized pedigree to show how it was segregated as a human model (Geldenhuys et al., 2014, Brink and Schwartz, 2009).

8. Rationale of the study

We hypothesized that during the 1650s, when the early Dutch or French settlers arrived in the Cape of Good Hope, at least one individual harbored the pathogenic mutation in the *PKP2* gene which was passed on to subsequent generations; this may explain why the *PKP2* c.1162C>T mutation is only found in modern day Afrikaners, who are historically known to marry within the community. A higher prevalence of certain genetic disorders in Afrikaners has been linked to the reduction in genetic variability from geographic isolation and religious and cultural bonds, coupled to rapid population expansion over 10-12 generations (Geldenhuys et al., 2014).

As a result of the Watkins (2009) study, there was a need to validate and expand on the proposed founder effect observed in the Afrikaners, through genealogical tracing of the *PKP2* c.1162C>T mutation positive individuals and their shared common haplotype to find the common founder.

9. Statement of research aim and objectives

9.1 Main aim

The aim of this study is to discover, through genealogical and molecular analysis, the common founder that introduced the *PKP2*, c.1162C>T mutation in the Afrikaner population of South Africa in families diagnosed with ARVC.

9.2 Objectives

1. To use high resolution melt (HRM) analysis to screen for the *PKP2* c.1162C>T mutation in order to identify additional probands and their relatives.

2. To construct the haplotype surrounding the *PKP2* gene using the probands that are positive for the *PKP2* c.1162C>T mutation.

3. To identify the original founder with the *PKP2* c.1162C>T mutation through genealogical tracing of the affected families in the ARVC registry of South Africa.

10. Methods

10.1 Study design

This was a descriptive study of ARVC cases from the ARVC registry of South Africa. The probands and their first degree relatives, who tested positive for the *PKP2* c.1162C>T mutation, were recruited for this molecular and genealogical study of the founder effect. All study family trees of apparently unrelated probands with the *PKP2* c.1162C>T mutation were traced using genealogical methods.

This project forms part of a larger study on the genetics of cardiomyopathy that has been approved by the UCT Human Research Ethics Committee, HREC REF: 518/2016 (which incorporates the ARVC Registry of South Africa).

10.2 Project Workflow

After obtaining DNA from each study participant, laboratory work included the two phases of genotyping, i.e., screening by HRM and microsatellite analysis, then developing a haplotype. This was followed by the tracing of the *PKP2* c.1162C>T mutation positive participants via genealogy (Figure 5).

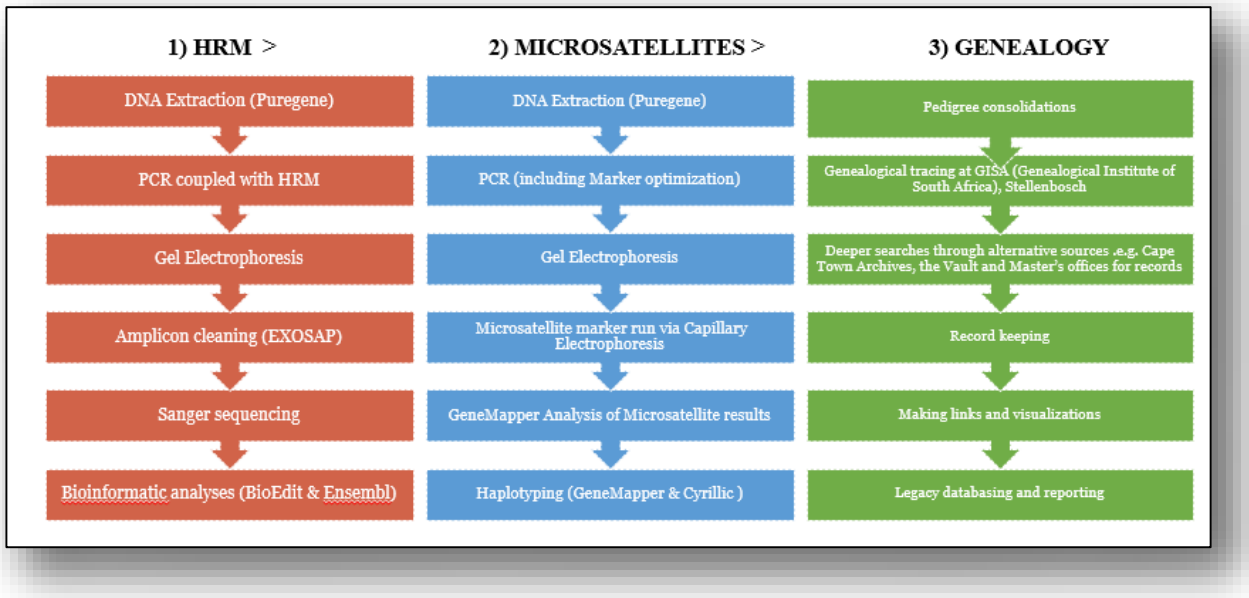


Figure 5: *The three phases of the study's data collection and analyses and reporting (in order).*

Namely, the two genotyping techniques i.e. HRM and Microsatellite, typing and finally, the human model via genealogical tracing and analyses.

In summary, study participants were screened for the proposed founder mutation, c.1162C>T (through the *PKP2* exon 4 genetic marker), and if found positive, were also tested for four other genetic markers spanning the *PKP2* gene. Namely, *PKP2* exon 11 genetic marker (for the Ins/del mutation i.e. c.2197_2202delCACACCinsG, A733fsX740 frameshift mutation (i.e. a deletion of 6 bases and the insertion of one base), and the 3 *PKP2* microsatellite markers M1, D12S1692 and M2 (Blanckenberg, 2011, Watkins et al., 2009). After the genetic screening and shared common haplotypes were discovered, genealogical tracing was performed and to establish the common founder.

10.3 Participant selection and diagnosis

Physicians refer confirmed or suspected cases of ARVC and their first-degree relatives to the Cardiac Clinic, Groote Schuur Hospital (GSH), Cape Town, for consideration for enrolment in the registry.

A standardized case report form was completed for all participants by the physician (as per standard operating procedures of the ARVC Registry of South Africa (Watkins et al., 2009)). All available probands and consenting family members were recruited into the registry after obtaining voluntary, informed consent (Appendix 2). The case report form and the notes of the genetic counsellor contained information on age, gender, ethnicity, presenting symptoms, family history (to commence genealogical tracing), electrocardiographic (ECG) findings, cardiac imaging studies, histology, outcome and genetic counselling (Fish et al., 2016).

All samples were coded to anonymize and safeguard the confidentiality of the patients in the laboratory testing phase.

However, for the genealogical aspect of the study all participants meeting the criteria were contacted again to determine the date of birth, names and locations of ancestors for genealogical tracing purposes.

10.4. Selection criteria

All participants entered into the ARVC registry of South Africa were investigated by a diagnostic panel consisting of a group of cardiologists who determined, by consensus, whether or not patients meet the 2010 ARVC Task Force Criteria (TFC) (Appendix 1). The study

participants were classified as having definite ARVC (if 2010 ARVC Task Force Criteria was met), probable or possible ARVC (if some criteria was met and no alternative diagnosis is found), or not affected with ARVC (if there was no evidence of ARVC and/or an alternative diagnosis present).

10.4.1 Inclusion criteria

Individuals in this study were included under two categories:

A) They were (i) an ARVC patient that has met the 2010 ARVC TFC with the *PKP2* c.1162C>T mutation or (ii) a first degree relative of an ARVC patient with a *PKP2* c.1162C>T mutation, i.e., the 'ARVC at risk' group; OR

B) They carried the c.1162C>T mutation in exon 4 of the *PKP2* gene.

Occasionally, other relations of (A) such as second degree relatives and spouses were included, in the absence of first degree relatives and/or to clarify the lineage the mutation came from.

10.4.2 Exclusion criteria

Arrhythmogenic right ventricular cardiomyopathy patients without the *PKP2* c.1162C>T mutation and individuals not related to the c.1162C>T mutation probands were excluded in the study.

All patients with secondary causes of cardiomyopathy or left ventricular dysfunction (such as valvular heart disease, diabetes, coronary artery disease, or sepsis) were excluded from the study.

10.5. Control selection

Controls were chosen from a South African population comprised of anonymous blood donors. Their bloods were selected by ethnicity, as the c.1162C>T mutation was predominantly seen in those of white South African descent. Also included as controls were populations who self-reported as Cape Mixed Ancestry. Informed consent was obtained from all controls.

10.6 DNA extraction

Written informed consent was obtained from all individuals enrolled in the study. As per the instructions of the manufacturer, the genomic DNA was extracted from 5-10ml EDTA blood

using the PureGeneTM DNA Purification System (Gentra systems, Qiagen, Hilden, Germany) (Fish et al., 2016). Extracted DNA samples were stored in 1.5ml Eppendorf tubes at -80°C, and a working sample of 50ng/ul was used for all downstream applications (Fish et al., 2016).

10.7 Gel electrophoresis

Electrophoresis is a separation technique used for charged particles (compounds) based on their electrical charge. Their size affects the speed of migration. Agarose is commonly used for DNA analysis and separation of Polymerase Chain Reaction (PCR) amplicons. The DNA is visualized in the gel by UV light shining over a fluorescent dye in the UVITec Gel Doc system (Uvitec, Cambridge, UK). This binds strongly to DNA by intercalating between (Hofmann et al., 2014) the bases and is fluorescent (absorbs invisible UV light and transmits the energy as visible light) via transillumination.

Different ladder sizes are used according to the maximum expected size of bands, type of gel, visualization techniques used and the ruler or marker to use to compare or deduce size of a band in base pairs, vary (Hofmann et al., 2014).

In this case, 2% Agarose gels were made by mixing 2 g Agarose powder (Roche) in 100 ml 1X TBE Buffer. It was heated and left to solidify, before immersion in 1X TBE solution in a gel tank. Then a loading dye mix (of Gel Red and Gel Green (Biotium)) was mixed with the Promega 100bp DNA ladder (Appendix 5), 3uL:5uL, respectively and loading dye mix and PCR product 3uL:7uL, respectively, and loaded onto the gel. The gels were electrophoresed at 140 V for an hour and visualized in an UVITec Gel Doc system (Uvitec, Cambridge, UK).

10.8 Primers

Primers are short DNA oligonucleotide sequences (ideally18-25base pairs long) used to initiate annealing (Figure 6) and amplification. They work in the presence of Taq polymerase (the enzyme), $MgCl_2$ (the catalyst) and deoxynucleotides (dNTPs) (the building blocks, i.e. a mix of nucleotides A, C, G and T) in a PCR reaction. Primer pairs are designed so that they bind to DNA encompassing the planned region of amplification. This allows the dNTPs and Taq Polymerase to continue the binding of nucleotides at the 3′ end of the primer to begin elongation.

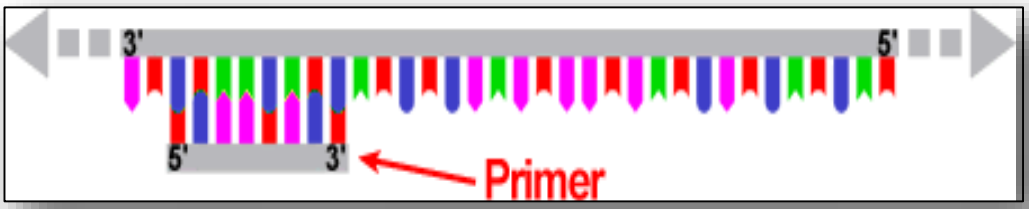


Figure 6: *The exemplary binding of a forward primer; not to scale.*

Adapted from : (NFSTC.org, 2012).

10.8.1 Primer design bioinformatics tools

The primers were designed to span the exon 4 and 11 regions of the *PKP2* gene (Table 2) and the same technique used for the microsatellite markers (in section 10.11 Microsatellites) that span the *PKP2* gene.

The primers were used for combined real-time PCR and HRM analysis as well as for DNA sequencing, while the microsatellite markers were used for PCR and the products used for capillary electrophoresis to investigate microsatellites.

Tools used to design the primers and genetic markers for microsatellites by Mbele and Blackenberg were extracted from their PhD theses methods (Mbele, 2014, Blanckenberg, 2011); which included gene annotation via Ensembl (http://www.ensembl.org/index.html) and perlv5, manually searching for a primer, IDT OligoAnalyser (http://eu.idtdna.com/calc/analyzer) calculations to check ideal primer properties, NCBI primer blasting (http://blast.ncbi.nlm.nih.gov/Blast.cgi) the best sequences (by observing the ideal E values; in the nucleotide blast function) and ordering Custom DNA Oligos from Integrated DNA Technologies (IDT) (IDT, Coralville, Iowa, US).

The following criteria were used to design primers and genetic markers for microsatellites: (1)18-25 base pairs (bps) long, (2) had good sequence diversity with minimal sequence repeats

and secondary structure, (3) melting at a temperature (Tm) of 45°C to 65°C, (4) at a GC content of 45% to 65%, and (5) carrying a G or C clamp at the 3' end.

10.8.2 *PKP2* primers

The *PKP2* primers used in this study were designed by members of the Cardiovascular Genetics Laboratory at the Hatter Institute for Cardiovascular Research in Africa (HICRA), Cardiovascular group (Watkins et al., 2009) and are presented in Table 2.

Table 2: *Primer sequences used for the amplification of PKP2 exons 4 and 11.*

PKP2 **Exon**	**Expected size (bp)**	**Ta, Annealing Temperature (°C)**	**Primer Name and Strand**	**Sequence (5'-3')**
4	291	55	*PKP2* Exon 4F	AGT ATT CGC TGA GTC GTC TCT
			PKP2 Exon 4R	GCA AAG TCA CCA TAA TAG AAG
11	219	55	*PKP2*_Exon11.2_New_F	ACA TCA GTG GCT CAG ACA G
			*PKP2*_Exon11.2_New_R	CCC ATT TCC AGT GCA TCT TTG TG

**Number (e.g. 4) denotes the exon; F= forward primer, R= reverse primer. (Adapted from: (Mbele, 2014)). NB: Exon 11 was also screened in order to validate the Watkins (2009) paper findings.*

10.9 Polymerase Chain Reaction

10.9.1 Principle of PCR

Polymerase Chain Reaction is a molecular method developed by Kary Mullis in the 1980s. It is based on the ability of DNA polymerase to synthesize new strands of DNA complementary to the offered template strand. Due to DNA polymerase's ability to add a nucleotide only onto a preexisting 3'-OH group, it uses a primer to which it can add the first nucleotide. PCR enables a researcher to delineate a specific region of template sequence to amplify. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies (amplicons) (NCBI, 2016).

Deoxyribonucleic acid segments of interest are amplified by means of PCR, to a high copy number, prior to use in downstream molecular applications, such as HRM and Microsatellite analysis in this study. PCR is a laboratory technique used to amplify specific input dsDNA strands by exponential growth. The melting temperature (Tm) of a primer is the temperature at

which one half of the DNA duplex will dissociate to become single stranded DNA (ssDNA) and shows duplex stability (NCBI, 2016).

10.9.2 Polymerase Chain Reaction Protocol

The three phases of PCR are: 1) Denaturation i.e. separation by heat, from a dsDNA strand to a ssDNA strand; 2) Annealing i.e. forward and reverse primers bind to the regions they were designed for; complimentary to the ssDNA; and 3) Extension i.e. elongation of the strand at the complimentary side using Taq Polymerase (a heat- stable polymerase) and dNTPs (NCBI, 2016). The PCR conditions are described in Table 3.

Table 3: *PCR conditions.*

CONDITION	**TEMPERATURE & TIME**	
Initial denaturation	94°C - 4 minutes	
Denaturation Primer Annealing Template Elongation	94°C - 30 seconds * - 45 seconds 72°C - 50 seconds	33 cycles
Final Elongation	72°C - 7 minutes	
Cooling (Optional)	4°C - 30 minutes	

** indicates temperature changes as per primer set*

10.10 High Resolution Melting Analyses

10.10.1 Principle of HRM

High resolution melt characterises nucleic acid samples according to their strand dissociation (melt) behavior, comparing the melt shape and the melt temperature profiles of DNA samples. The HRM process is the warming of the amplicon of DNA from approximately 50°C to approximately 95°C during which detectors monitor the thermally-induced DNA dissociation (Farrar et al., 2009). A high level of fluorescence is normal at the beginning of the HRM process, as there are billions of copies of the amplicon. In the HRM machine, when the melting temperature of the two strands is reached, the individual DNA strands melt apart. Hence, the amount of dsDNA in the solution decreases and as a result, the fluorescence of the solution decreases (Farrar et al., 2009).

The HRM machine measures and records the fluorescence and plots it as a melting curve reflecting the changes in the fluorescence of the solution due to the rising temperature. Similar

to the Tm principle in the PCR procedure, the midpoint of the melting curve, where the rate of change in fluorescence is the greatest, is the melting temperature (Tm) of the particular DNA fragment under analysis. The differences in DNA sequences, GC content and melting properties, also cause a difference in the melting temperature and the melting behavior of a DNA fragment. Sequence differences due to mutations including single nucleotide polymorphisms (SNPs), would thus change the melting temperature and be reflected in the melting curve (Farrar et al., 2009).

For HRM analyses, the amplicon sequences were also restricted to a 250-350bp length, as increased amplicon size is known to lead to decreased resolution in amplicon screening and genetic variant detection with HRM (Farrar et al., 2009).

10.10.2 High Resolution Melting protocol

Genomic DNA was placed into the PCR reagent mix (Table 4), amplified to a PCR product by the HRM conditions in Table 5 by binding with an intercalated dye (EvaGreen (AnaTech)) and then, melted in the RotorGene 6000 (Corbett Life Sciences) at the Cardiovascular Genetics (CVG) Laboratory of the Hatter Institute for Cardiovascular Research in Africa (HICRA) at UCT, as per their standard operating procedures and conditions (Fish et al., 2016). In this case, a RotorGene 6000 condenses the two steps into a single procedure (Table 5).

The HRM method is sensitive enough to detect single base changes (Fish et al., 2016, Fish, 2010). Once a change was identified, the samples were sequenced.

Table 4: *HRM Reagent Mix.*

Reagent (stock concentration)	**Volume per reaction**
Forward Primer (20 µM)	0.5 µl
Reverse Primer (20 µM)	0.5 µl
dNTPs (20 µM) (Bioline)	1 µl
GoTaq Polymerase (5 U/ ul) (Promega)	0.1 µl
GoTaq FlexiBuffer (5X) (Promega)	5 µl
$MgCl_2$ (25 mM) (Promega)	3 µl
EvaGreen dye (1X) (Anatech)	1 µl
DNA (50ng/ µl)	1 µl
Final Reaction Volume (made up to 25 µl with dH2O, distilled water)	25 µl

Table 5: *HRM Conditions*

CONDITION	TEMPERATURE & TIME
Initial denaturation	95°C - 10 seconds
Denaturation Primer Annealing Template Elongation	95°C - 5 seconds 55°C -10 seconds 72°C - 10 seconds 50 cycles
High Resolution Melt	72 - 95°C (0.1°C increments)

10.11 Sanger Sequencing

10.11.1 Principle of Sanger sequencing

Deoxyribonucleic acid sequencing is the reading of the DNA strand, nucleotide by nucleotide. This is the Gold standard of mutation analysis. DNA sequencing was performed on all samples that showed a change in the HRM melting profile, in comparison to the controls.

Sequencing is performed using PCR products as template DNA. During the sequencing reaction, this template is first denatured to form ssDNA. Next, a short oligonucleotide is annealed to the same position on each template strand. The oligonucleotide acts as a primer for the synthesis of a new DNA strand that will be complimentary to the template DNA. The sequencing reactions require the addition of all the components necessary to synthesize and label a target DNA template, a primer, DNA polymerase, 4 dNTPs and 4 ddNTPs (ddG, ddA, ddC, and ddT). The ddNTPs differ from dNTPs in that they lack a 3` hydroxyl group, and thus their incorporation into the chain of nucleotides prevents any further elongation. Because of this, the sequencing reaction produces ssDNA molecules that differ in length by just a single nucleotide; they can be separated from one another using capillary electrophoresis. In automated Sanger sequencing, fluorescently labeled ddNTPs are detected via laser firing. They fluoresce different colors, producing a chromatogram. The results are interpreted by bioinformatic analysis to display the absence or presence of SNP(s) or mutations (Smith-Zagone et al., 2007).

Sequencing was used to investigate PCR amplicons at the nucleotide level, in the samples in which a change in the HRM melting profile was observed in comparison to the controls. This provided additional and more specific genetic information for screening, future haplotyping and association studies. This is in order to primarily see if the relevant proband and its immediate family carried the same genetic SNPs/ mutations in the same exons, as their other family members; common for familial disease types.

10.11.2 Sequencing protocol

The HRM products were first purified (using the. Exonuclease I, Shrimp/ Thermosensitive Alkaline Phosphatase PCR Purification Kit) and prepared for sequencing (via the Big Dye Terminator Sequencing reaction). After the sequencing preparation reactions, the sequencing preparation products were analyzed using capillary electrophoresis at the DNA Sequencing Unit (Department of Genetics, Stellenbosch University), by means of the ABI PRISM® 3130xl Genetic Analyser (Applied Biosystems) or the ABI PRISM® 3730xl Genetic Analyser (Applied Biosystems). The Sequencing Reaction and Analysis protocol were followed (Appendix 6).

The DNA sequences generated in these experiments (.abi files) were aligned to the wildtype sequence that was gene annotated from the Ensembl database (http://www.ensembl.org/) using the BioEdit Sequence Alignment Editor (© Tom Hall). The ClustalW function was used to observe SNPs in Exon 4 and the Ins/Del in Exon 11. The chromatogram was checked for changes at position c.1162, with a C to T change expected in mutation carriers. The changes were analyzed against the Sequencing Reaction and Analysis Protocol (Appendix 6).

10.12 Microsatellites

10.12.1 Principle of microsatellites

Microsatellites are simple sequence tandem repeats. Naturally occurring in both coding and non-coding regions of the DNA; a few human genetic disorders are caused by (trinucleotide) microsatellite regions in coding regions. On each side of the repeat unit are flanking regions that consist of "unordered" DNA. The flanking regions are critical as they allow the development of locus-specific primers to amplify the microsatellites with PCR. The probability of finding an “unordered” particular stretch of DNA (i.e. a combination of simple sequence

tandem repeats and locus-specific flanking regions of DNA, sizing up to 100-500bps) more than once in the genome becomes vanishingly small (Wrent et al., 2016, Gabriel et al., 2002).

The principle of microsatellite typing used short tandem repeat (STR) motifs, consisting of one to six nucleotide repeats (generally di-, tri- tetra- or penta- nucleotides, repeated from 8 to 50 times) present in the genomes of all eukaryotes to map kinship (Wrent et al., 2016). According to Mendelian inheritance patterns, each allele of each microsatellite marker was expected to be inherited once from each respective parent, and only two alleles per marker were expected from each autosomal pair (Gabriel et al., 2002).

10.12.2 Microsatellite protocol

The microsatellites of interest were amplified via PCR (as previously described in Section 10.9) using fluorescently tagged primers.

Polymerase Chain Reaction products were electrophoresed on a 2% agarose gel (Section 10.7) to confirm if the reaction worked and optimized to the right product size and specificity. Subsequently, the PCR product, then underwent capillary electrophoresis and finally, was compared against the GeneScan ROX-labeled GS500 internal size standard (Applied Biosystems), to visualize the microsatellite profile of the three markers Tables 6-9).

The microsatellite markers of interest were: *PKP2*_M1 (tagged with FAM, for dinucleotide CA repeats), *PKP2*_D12S1692 (tagged with HEX, for dinucleotide CA repeats) and *PKP2*_M2 (tagged with HEX, for dinucleotide TG repeats), spanning over the *PKP2* gene region. These identified the number of short tandem repeats in targeted microsatellite regions in the ABI Prism® 3130 XL Genetic Analyzer machine (Applied Biosystems, Foster City, CA, USA) at the Division of Human Genetics, UCT and the output data was analyzed using the GeneMapper version 4.0 software (Applied Biosystems) (Appendix 9).

Table 6: *PCR Reagents, M1 (for FAM labeled markers).*

Reagent	**Volume (ul); n=1**
Forward primer (20uM)	0.5
Reverse primer (20uM)	0.5
dNTPs (20mM)	1
GoTaq Flexi Buffer (5X)	5
GoTaq Polymerase (5U/ul)	0.1
MgCl2 (25mM)	1.5

DNA (50ng/ul)	1
dH20	15.4
Total	25

Table 7: *PCR Reagents, D12S1692 & M2 (for HEX labelled markers).*

Reagent	**Volume (ul); n=1**
Forward primer (20uM)	0.5
Reverse primer (20uM)	0.5
dNTPs (20mM)	1
GoTaq Flexi Buffer (5X)	5
GoTaq Polymerase (5U/ul)	0.1
MgCl2 (25mM)	3
DNA (50ng/ul)	1
dH20	13.9
Total	25

Table 8: *PCR conditions.*

CONDITION	**TEMPERATURE & TIME**
Initial denaturation	94°C - 4 minutes
Denaturation Primer Annealing Template Elongation	94°C - 30 seconds * - 45 seconds 72°C - 50 seconds 33 cycles
Final Elongation	72°C - 7 minutes
Cooling (Optional)	4°C - 30 minutes

** indicates temperature changes as per primer set*

Table 9: *Microsatellite marker sequences used and PCR product sizes for ARVC-9 Locus spanning the pathogenic PKP2 gene.*

Microsatellite Marker	**Chromosomal position (bp)**	**Expected size (bp) and repeats**	**Ta, Annealing Temperature (°C)**	**Fluorescent label and strand**	**Marker Name and Sequence**
PKP2-M1	33,085,390- 33,085,570	181 CA	51	FAM Forward	*PKP2*-M1_CA_1F ctctcaaatagaaataggaagacaa
				None Reverse	*PKP2*M1_CA_1R gggatacagtgtgtagcaattta
D12S1692	32,987,723- 32,987,971 (in intron 7 of gene)	245-261 CA	56	HEX Forward	D12S1692_F ctttgattccataccctcct
				None Reverse	D12S1692_R Gcagcaatttcagacttctc
PKP2-M2	32,849,128- 32,849,468	341 TG	53	HEX Forward	*PKP2*-M2_TG_1F ccaattcctgggctcaatag
				None Reverse	*PKP2*M2_TG_1R tcctcagacatacaggcagaag

**Number (e.g. 4) denotes the exon; F= forward primer, R= reverse primer. (Adapted from:(Blanckenberg, 2011)).*

10.13 Haplotyping

Generally, haplotyping is the observation of alleles on a single molecule. Usually, this is performed in conjunction with genotyping information and microsatellite analysis. This information is merged with relevant pedigrees to indicate patterns of inheritance of genotypes and 'assumed haplotypes' of unscreened parents.

Construction of the most likely haplotype is designed with reference to the gene and STR (short tandem repeat) marker order on the respective chromosome, in the context of each family. The most probable haplotypes are constructed manually based on pedigree data and marker positions. In this study, in the few cases for which only the proband and a parent were available for genotyping, an "assumed haplotype", based on the haplotype identified by family mapping, was generated (Moolman-Smook et al., 1999).

10.14 Genealogy

Genealogy is the study of families and the tracing of their ancestral lineages and history. The methods of genealogy usually include family tree construction, development of an ancestral chart, examination of the family register and use of the family chronicle or history and region/district genealogy (Merwe, 2016). Genealogical data provide unique information for researchers in evolutionary and population genetics, demography and genetic epidemiology (Stefansdottir et al., 2013).

10.15 Pedigree/family tree updates and consolidation

Pedigrees and family trees were initially consolidated with the help of a genetic counsellor (Ms Nakita Verkijk, Division of Human Genetics, University of Cape Town), a physician (Dr Sarah Kraus, Department of Medicine, University of Cape Town) as well as an experienced genealogist (Professor Gerhard Geldenhuys, Genealogical Institute of South Africa, Stellenbosch University). The family histories collected by the Cardiovascular Genetics Laboratory were extended from follow-up interviews with study participants and all known relatives updated and used for genealogical tracing (see Appendix 8).

10.16 Genealogical resources

10.16.1 Western Cape facilities

This study used standard methods for genetic genealogical research in South Africa on hereditary diseases including interviews, genetic analyses, the internet (including online database searches such as familysearch.org) and searches in historical records through sources (diagram of the key genealogical sources used to collect data, Figure 7) such as the Genealogical Institute of South Africa (GISA) in Stellenbosch (hosting microfiche or film of most early Afrikaner records). Furthermore, provincial state archives, Master's offices (in Court), museum records, church records, death, marriage and baptismal registers, immigration records, estate papers, slave records, published genealogies (mostly in de Villiers/Pama (DVP) System), voters' rolls, telephone directories, property records and library records and tombstone inscriptions were used as resources to gather genealogical evidence as reported previously (Merwe, 2016, Geldenhuys et al., 2014).

Finally, contacting or visiting the three mainstream Afrikaans churches, the Nederduitse Gereformeerde Kerk, the Gereformeerde Kerk and the Nederduitsch Hervormde Kerk van Afrika for detailed records of marriages and baptisms, was performed for any record discrepancies, or the provincial national archive were searched as they held the original, edited and most up-to-date copies of records (Merwe, 2016, Geldenhuys et al., 2014).

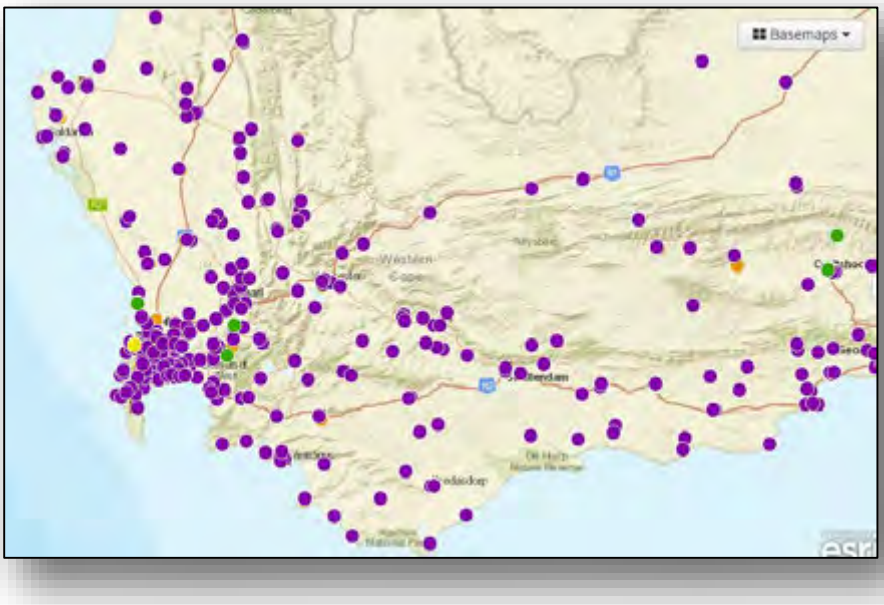


Figure 7: *Western Cape Government facilities: Department of Cultural Affairs and Sport (DCAS) Facilities Web Map with all facilities for Genealogical Resources across the Western Cape Province.*

(Adapted from:(Government, 2016)).

10.16.2 South African Public Records and Archival Considerations

The researcher received training from experienced genealogist, Prof. Gerhard Geldenhuys. Secondly, supplementary training was provided by family search workshops at the Cape Town National Archives conducted by Principal Archivist Jaco van der Merwe. Subsequently, training for alternative methods for mixed ancestry research was provided by Aubrey Springveldt. As a result, the researcher was able to trace most of the family ancestral lines over the course of a year.

Under the Promotion of Access to Information Act (PAIA), which informs much of the advocacy of the Archival Platform, the general public cannot access information, except in the public interest. Furthermore, there is a 100-year Department of Home Affairs document access ban spanning records from 1916-2016. However, for this research, the medical purposes for research were found to comply with the latest amendment of the National Archives of South Africa Act No 43 of 1996. I was trained adequately and hence, granted verbal approval to access some records inclusive of the last 100 years, exclusively for research purposes, as long as they were pre-authorized and signed by the Head of Civil Client services in that archive (Appendix 3).

Finally, the National Archives require a copy of any genealogy or medical research that has used their facilities to be kept in their archive as an archival record. All information regarding individuals in the 100-year ban has been excluded from the archival record, and it will be kept in their respective private collections.

10.16.3 Genealogical software

During tracing, Legacy v8.0 Deluxe Edition Software was used to store, manage, merge, collaborate and analyze the large amount of data generated. For example, each proband had two parents, four grandparents, eight great-grandparents, and this number doubles in each subsequent generation, except when close relatives marry. Several generations in the family trees of the study participants, i.e., over 350 years or up to the earliest recorded migrant into South Africa, were explored and the information gathered to find a common founder (Geldenhuys et al., 2014).

11. Results

11.1 Phenotype

The probands and their families were referred to the Cardiac Clinic at Groote Schuur Hospital where the ARVC Registry was established in 2004. The families were coded in chronological order, according to which proband was recruited first. Due to family members migrating and limited resources, some of the study participants have not been phenotyped by the Diagnostic Panel of cardiologists at the Cardiac Clinic. Some study participants reside in other countries and others are in other provinces, such as Gauteng and the Free State province or have died. However, some were screened by their referring physicians and the screening data was extrapolated from the registry data in order to classify them into definite ARVC, probable ARVC, not affected or other diagnosis. Tables 10 to 16 show the phenotypic profile of 7 families with ARVC due to the founder mutation in *PKP2*.

**It would have been preferred to place the tables and pedigrees after each family subheading but due to the landscape orientation of the tables and to avoid unnecessary repetition of the pedigrees, they were placed at the end of section 11.1 (tables) and section 11.2 (mutation-related pedigrees).*

11.1.1. ACM 5 family

Six of the family members were screened, including the proband; the rest had an unknown phenotype (Table 10). The phenotypic profile of the three affected family members in ACM 5 presented showed moderate disease. The ACM 5 family proband, ACM 5.1, was a competitive marathon runner who presented with symptoms of ARVC at 44 years associated with ventricular tachycardia (VT). Her brother (ACM 5.5) also had adult-onset ARVC. However, the daughter of ACM 5.5 (ACM 5.11) presented with ARVC during her adolescence.

11.1.2. ACM 8 family

Three of the family members, including the proband, were screened, whereas the rest had either an incomplete or unknown phenotype status (Table 11). The phenotypic profile of the two affected family members in the ACM 8 family was consistent with severe early-onset disease in adolescence with VT. The ACM 8 family proband, ACM 8.3, presented at 13 years and received a heart transplant at 23 years. Her younger brother presented at 12 years and died from SCD in 2004 (at 14 years). The mother, ACM 8.2 is symptomatic with definite features of

ARVC (albeit mild) but we have not phenotyped the father who has yet to responded to invitations to visit Groote Schuur Hospital.

11.1.3. ACM 12 family

Only the proband in this family was screened. The proband did not want the rest of the family to be recruited nor their status to be disclosed. This proband presented with moderate, late disease onset (Table 12). ACM 12.1 presented at the age of 45 with VT and was a competitive marathon runner.

11.1.4. ACM 19 family

Seven family members were phenotyped including the proband; the rest had incomplete or unknown phenotypes (Table 13). The phenotypic profile of the three affected family members in the ACM 19 family was of severe disease (ACM 19.1 and ACM19.2, requiring heart transplants during adolescence) and ACM 19.13 with moderate disease, all with VT. The ACM 19.1 and ACM 19.2 siblings presented with an early onset of ARVC during adolescence, aged 14 and 11, respectively. Both parents (ACM19.3 and ACM 19.6) were asymptomatic in their 50s however; their mother (ACM19.6) met borderline ARVC 2010 criteria. The cousin, ACM19.13, from the mother's side, recently presented later with symptoms of ARVC at the age 32.

11.1.5. ACM 38 family

Three family members were phenotyped, including the proband, whereas the rest had an incomplete or unknown phenotype (Table 14). The phenotypic profile of the three affected siblings (ACM 38.3, ACM38.5 and a deceased sister, who died at 8 years from SCD) was that of severe disease before adolescence (from as early as the age of 8 years). ACM 38.3 received a heart transplant at 16 years and ACM 38.5 at 8 years), all with VT. The parents were not phenotyped. It is noteworthy that the mother (ACM 38.2) had a history of recurrent miscarriages.

11.1.6. ACM 57 family

Only the proband in this family was phenotyped. The proband did not want the rest of the family to be recruited nor their status disclosed. This proband presented with adult-onset moderate disease (Table 15). ACM 57.1 presented at the age of 42 years with VT and was a competitive marathon runner.

11.1.7. ACM 71 family

Only the proband in this family was phenotyped (ACM71.1). The rest had an incomplete or unknown phenotype (Table 16). This proband presented at the age of 38 years with moderate disease. ACM 71.1 was a farm worker with a history of hypertension and smoking (inclusive of cannabis).

Table 10: *Phenotypic profile of the ACM 5 family.*

DNA ID	DOB	RESULT (c.1162C>T)	Clinically screened, Year	Symptomatic	Diagnosis by 2010 TFC	Heart transplant	SCD	ARVC confirmed Pathologically
ACM 5.10	1992/10/07	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 5.11	2000/06/26	Positive	Yes-2015	Yes	Possible*	No	No	N/A
ACM 5.12	1953/07/15	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 5.13	1951/03/23	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 5.14	1982/02/01	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 5.15	1974/12/01	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 5.1 (Proband)	1958/07/17	Positive	Yes-2002	Yes	Definite	No	No	N/A
ACM 5.2REN	1981/05/21	Negative	Yes-2010	No (Known with WPW)	No criteria	No	No	N/A
ACM 5.3	1982/06/12	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 5.4	1958/07/17	Positive	Yes-2010	Unknown	Definite*	Unknown	Unknown	Unknown
ACM 5.5	1963/02/22	Positive	Yes- 2013	Yes	Definite*	No	No	N/A
ACM 5.6	1979/06/07	Positive	Yes-2013	No	Possible*	No	No	N/A
ACM 5.7	1961/09/01	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 5.8	1955/09/21	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 5.9	1966/01/26	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown

ACM – CVG database Arrythmogenic Cardiomyopathy coding abbreviation; ARVC- Arrhythmogenic Right Ventricular Cardiomyopathy; DOB- Date of Birth (YYYY/MM/DD); DNA ID- CVG database DNA sample ID; SCD – Sudden Cardiac Death; TFC –Task Force Criteria; WPW- Wolff-Parkinson-White syndrome

2010 ARVC Diagnostic Task Force Criteria Definitions:
Definite – 2 major criteria, or 1 major plus 2 minor criteria, or 4 minor criteria (2010 TFC); Possible- has family history; Borderline – 1 major and 1 minor, or 3 minor criteria (2010 TFC); met some but not all of the 2010 ARVC TFC ; Possible – 1 major criteria, or 2 minor criteria (2010 TFC)
**- Major for family history*

Table 11: *Phenotypic profile of the ACM 8 family.*

DNA ID	DOB	RESULT (c.1162C>T)	Clinically screened, Year	Symptomatic	Diagnosis by 2010 TFC	Heart transplant	SCD	ARVC confirmed Pathologically
ACM8.10	1954/11/17	Positive	No	Unknown	Possible*	Unknown	Unknown	Unknown
ACM8.2	1959/01/22	Positive	Yes-2010	No	Definite*	No	No	N/A
ACM 8.3 (PROBAND)	1988/10/21	Positive	Yes-2001	Yes	Definite	Yes	No	Yes
ACM8.4	1990/10/15	Positive	Yes- 2002	Yes	Definite	No	Yes	Yes
ACM8.9	1986/10/21	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown

ACM – CVG database Arrythmogenic Cardiomyopathy coding abbreviation; ARVC- Arrhythmogenic Right Ventricular Cardiomyopathy; DOB- Date of Birth (YYYY/MM/DD); DNA ID- CVG database DNA sample ID; SCD – Sudden Cardiac Death; TFC –Task Force Criteria; WPW- Wolff-Parkinson-White syndrome

2010 ARVC Diagnostic Task Force Criteria Definitions:
Definite – 2 major criteria, or 1 major plus 2 minor criteria, or 4 minor criteria (2010 TFC); Possible- has family history; Borderline – 1 major and 1 minor, or 3 minor criteria (2010 TFC); met some but not all of the 2010 ARVC TFC ; Possible – 1 major criteria, or 2 minor criteria (2010 TFC)

**- Major for family history*

Table 12: *Phenotypic profile of the ACM 12 family.*

DNA ID	DOB	RESULT (c.1162C>T)	Clinically screened, Year	Symptomatic	Diagnosis by 2010 TFC	Heart transplant	SCD	ARVC confirmed Pathologically
ACM 12.1 (PROBAND)	1953/09/25	Positive	Yes-2003	Yes	Definite	No	No	No

ACM – CVG database Arrythmogenic Cardiomyopathy coding abbreviation; ARVC- Arrhythmogenic Right Ventricular Cardiomyopathy; DOB- Date of Birth (YYYY/MM/DD); DNA ID- CVG database DNA sample ID; SCD – Sudden Cardiac Death; TFC –Task Force Criteria; WPW- Wolff-Parkinson-White syndrome

2010 ARVC Diagnostic Task Force Criteria Definitions:
Definite – 2 major criteria, or 1 major plus 2 minor criteria, or 4 minor criteria (2010 TFC); Possible- has family history; Borderline – 1 major and 1 minor, or 3 minor criteria (2010 TFC); met some but not all of the 2010 ARVC TFC ; Possible – 1 major criteria, or 2 minor criteria (2010 TFC)

**- Major for family history*

Table 13: *Phenotypic profile of the ACM 19 family.*

DNA ID	DOB	RESULT (c.1162C>T)	Clinically screened, Year	Symptomatic	Diagnosis by 2010 TFC	Heart transplant	SCD	ARVC confirmed Pathologically
ACM 19.10	1933/04/07	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 19.11	1988/10/07	Positive	No	No	Unknown	Unknown	Unknown	Unknown
ACM 19.12	1984/10/10	Positive	No	No	Unknown	Unknown	Unknown	Unknown
ACM 19.13	1983/03/10	Positive	Yes-2016	Yes	No criteria	No	No	N/A
ACM 19.1	1991/07/09	Positive (Known to also have the *PKP2* Exon 11 ins/del mutation)	Yes- 2005	Yes	Definite	Yes	No	Yes
ACM 19.2 (PROBAND)	1985/02/13	Positive (Known to also have the *PKP2* Exon 11 ins/del mutation)	Yes-2006	Yes	Definite	Yes	No	Unknown
ACM 19.3	1957/07/18	Negative (Known to also have the *PKP2* Exon 11 ins/del mutation)	Yes- 2006	No	Possible *	No	No	N/A
ACM 19.4	1982/09/08	Positive	Yes- 2008	No	Possible *	No	No	N/A
ACM 19.5	1987/07/13	Negative	Yes- 2009	No	Possible *	No	No	N/A
ACM 19.6	1956/09/14	Positive	Yes-2009	No	Borderline*	Unknown	No	N/A
ACM 19.7	1929/09/26	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 19.9	1958/10/26	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown

ACM – CVG database Arrythmogenic Cardiomyopathy coding abbreviation; ARVC- Arrhythmogenic Right Ventricular Cardiomyopathy; DOB- Date of Birth (YYYY/MM/DD); DNA ID- CVG database DNA sample ID; SCD – Sudden Cardiac Death; TFC –Task Force Criteria; WPW- Wolff-Parkinson-White syndrome

2010 ARVC Diagnostic Task Force Criteria Definitions:
Definite – 2 major criteria, or 1 major plus 2 minor criteria, or 4 minor criteria (2010 TFC); Possible- has family history; Borderline – 1 major and 1 minor, or 3 minor criteria (2010 TFC); met some but not all of the 2010 ARVC TFC ; Possible – 1 major criteria, or 2 minor criteria (2010 TFC)
**- Major for family history*

Table 14: *Phenotypic profile of the ACM 38 family.*

DNA ID	DOB	RESULT (c.1162C>T)	Clinically screened, Year	Symptomatic	Diagnosis by 2010 TFC	Heart transplant	SCD	ARVC confirmed Pathologically
ACM 38.1	1953/08/11	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 38.2	1955/09/21	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 38.3 (PROBAND)	1983/05/06	Positive	Yes-1993	Yes	Definite	Yes-1999	No	Yes
ACM 38.4	1985/12/17	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 38.5	1990/05/04	Positive	Yes- 1997	Yes	Definite	Yes-1998	Alive in 2009	Yes
ACM 38.6	1957/10/25	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 38.7	1954/05/04	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
Sister of Proband	1986/09/18	No DNA	Yes-1990	Yes	Incomplete records	No	Yes- died at 8 years of age	Unknown

ACM – CVG database Arrythmogenic Cardiomyopathy coding abbreviation; ARVC- Arrhythmogenic Right Ventricular Cardiomyopathy; DOB- Date of Birth (YYYY/MM/DD); DNA ID- CVG database DNA sample ID; SCD – Sudden Cardiac Death; TFC –Task Force Criteria; WPW- Wolff-Parkinson-White syndrome

2010 ARVC Diagnostic Task Force Criteria Definitions:
Definite – 2 major criteria, or 1 major plus 2 minor criteria, or 4 minor criteria (2010 TFC); Possible- has family history; Borderline – 1 major and 1 minor, or 3 minor criteria (2010 TFC); met some but not all of the 2010 ARVC TFC ; Possible – 1 major criteria, or 2 minor criteria (2010 TFC)
**- Major for family history*

Table 15: *Phenotypic profile of the ACM 57 family.*

DNA ID	DOB	RESULT (c.1162C>T)	Clinically screened, Year	Symptomatic	Diagnosis by 2010 TFC	Heart transplant	SCD	ARVC confirmed Pathologically
ACM 57.1 (PROBAND)	1962/01/04	Positive	Yes-2004	Yes	Definite	No	No	N/A

ACM – CVG database Arrythmogenic Cardiomyopathy coding abbreviation; ARVC- Arrhythmogenic Right Ventricular Cardiomyopathy; DOB- Date of Birth (YYYY/MM/DD); DNA ID- CVG database DNA sample ID; SCD – Sudden Cardiac Death; TFC –Task Force Criteria; WPW- Wolff-Parkinson-White syndrome

2010 ARVC Diagnostic Task Force Criteria Definitions:
Definite – 2 major criteria, or 1 major plus 2 minor criteria, or 4 minor criteria (2010 TFC); Possible- has family history; Borderline – 1 major and 1 minor, or 3 minor criteria (2010 TFC); met some but not all of the 2010 ARVC TFC ; Possible – 1 major criteria, or 2 minor criteria (2010 TFC)
**- Major for family history*

Table 16: *Phenotypic profile of the ACM 71 family.*

DNA ID	DOB	RESULT (c.1162C>T)	Clinically screened, Year	Symptomatic	Diagnosis by 2010 TFC	Heart transplant	SCD	ARVC confirmed Pathologically
ACM 71.1 (PROBAND)	1971/05/02	Positive	Yes-2010	Yes	Definite	No	No	N/A
ACM 71.2	1967/08/26	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 71.3	1966/06/19	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 71.4	1970/05/10	Negative	No	Unknown	Unknown	Unknown	Unknown	Unknown
ACM 71.5	1946/03/09	Positive	No	Unknown	Unknown	Unknown	Unknown	Unknown

ACM – CVG database Arrythmogenic Cardiomyopathy coding abbreviation; ARVC- Arrhythmogenic Right Ventricular Cardiomyopathy; DOB- Date of Birth (YYYY/MM/DD); DNA ID- CVG database DNA sample ID; SCD – Sudden Cardiac Death; TFC –Task Force Criteria; WPW- Wolff-Parkinson-White syndrome

2010 ARVC Diagnostic Task Force Criteria Definitions:
Definite – 2 major criteria, or 1 major plus 2 minor criteria, or 4 minor criteria (2010 TFC); Possible- has family history; Borderline – 1 major and 1 minor, or 3 minor criteria (2010 TFC); met some but not all of the 2010 ARVC TFC ; Possible – 1 major criteria, or 2 minor criteria (2010 TFC)
**- Major for family history*

11.2 Genotype

11.2.1 *PKP2* c.1162C>T (p.R388W)

A study in 2009 screened the *PKP2* gene and identified a recurrent *PKP2* c.1162C>T mutation in exon 4 in four Afrikaner probands (Figure 8) (Watkins et al., 2009). We extended the study and included not only the four initial probands but their core families as well. We continued screening all new ARVC probands and when a *PKP2* c.1162C>T carrier was found we included them and their nuclear families in our founder study.

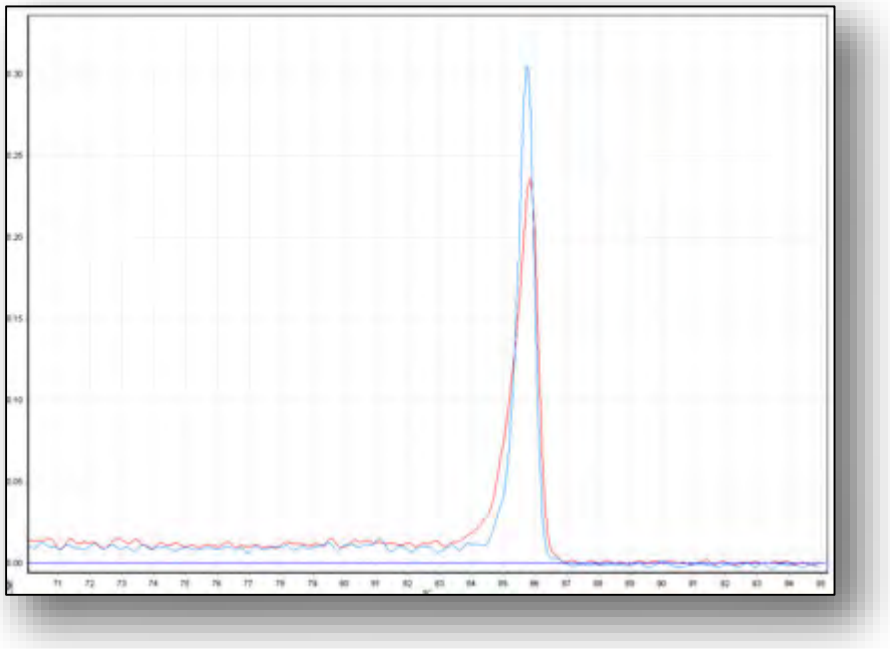


Figure 8: *Mutation peaks from (A) Exon 4; Red is ACM71.1 (mutation positive study participant) and Blue is the control results.*

There were 46 probands and families who underwent molecular screening for the *PKP2* c.1162C>T mutation in 2016 (Table 17).

Table 17: *Study participants screened for PKP2 c.1162C>T change and common haplotype.*

Family ID	No. of study participants screened	*PKP2* c.162C>T Mutation Positive	Common Haplotype
ACM 5	15	10	10
ACM 8	5	4	4
ACM 12	1	1	1
ACM 19	12	8	8
ACM 38	7	3	3
ACM 57	1	1	1
ACM 71	5	3	3
Total Participants	46	30	30

Where possible, we extended and tested the families to include aunts, uncles and cousins that were at risk or those that showed signs and symptoms of heart disease (Figure 9-15).

11.2.1.1 ACM 5

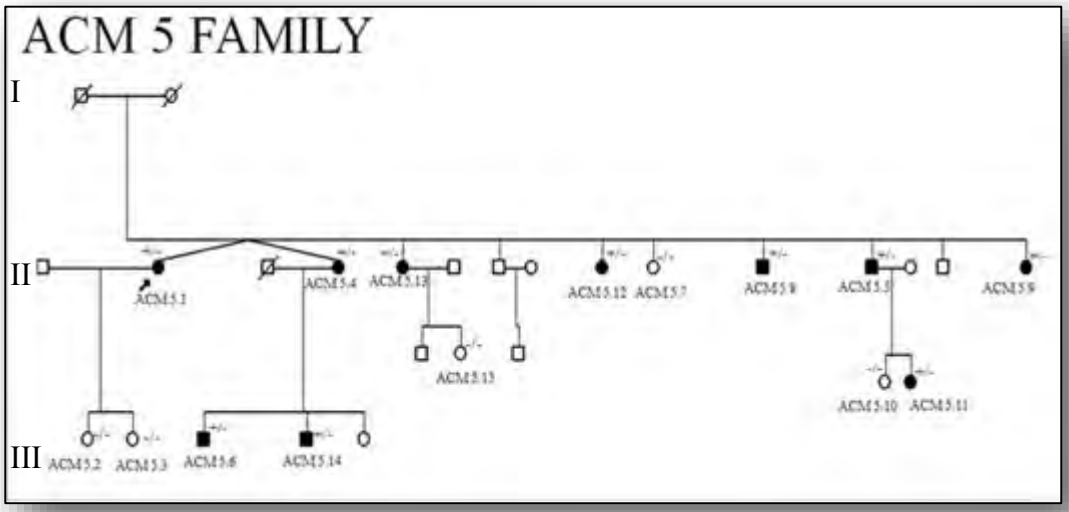


Figure 9: *ACM 5 Family PKP2 c.1162C>T mutation status.*

+ tested positive for the mutation and carries the mutated variant; tested negative for the mutation and does not carry the variant; +/-heterozygous carrier; +/+ homozygous carrier; -/- non-carrier. NB: ACM5.1 has an assumed heterozygous state from the Watkins (2009) paper; supported by the ACM5.4 (ACM5.1 twin's) sample.

11.2.1.2 ACM 8

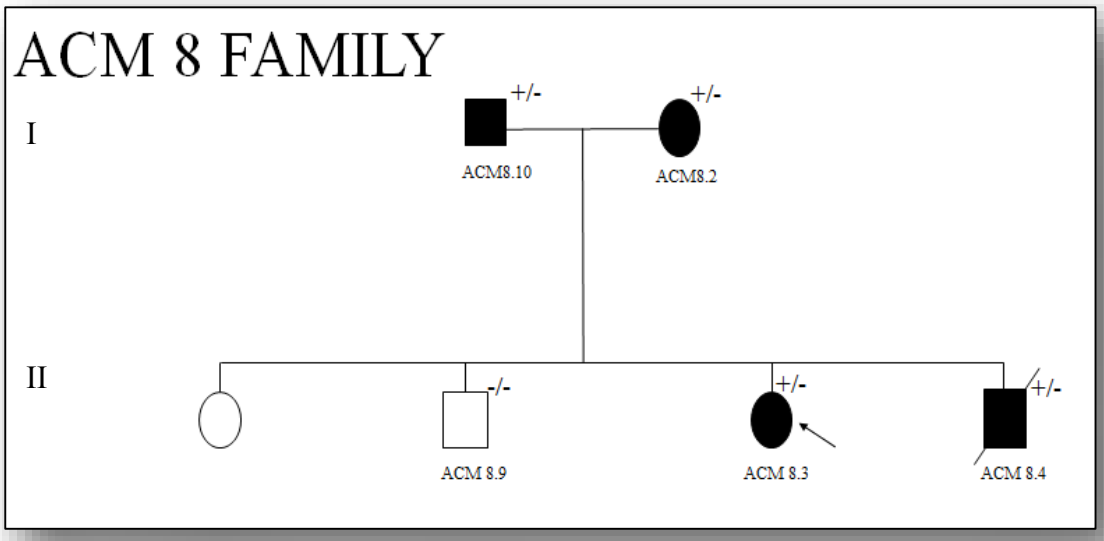


Figure 10: *ACM 8 Family PKP2 c.1162C>T mutation status.*

+ tested positive for the mutation and carries the mutated variant; tested negative for the mutation and does not carry the variant; +/-heterozygous carrier; +/+ homozygous carrier; -/- non-carrier

11.2.1.3 ACM12

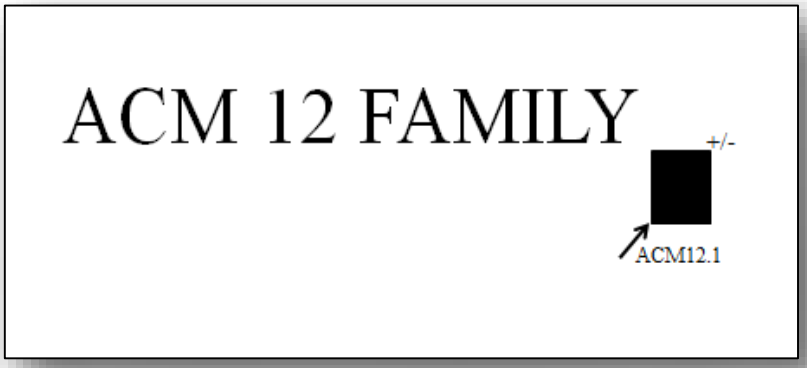


Figure 11: *ACM 12 Family PKP2 c.1162C>T mutation status.*

+ tested positive for the mutation and carries the mutated variant; tested negative for the mutation and does not carry the variant; +/-heterozygous carrier; +/+ homozygous carrier; -/- non-carrier

11.2.1.4 ACM19

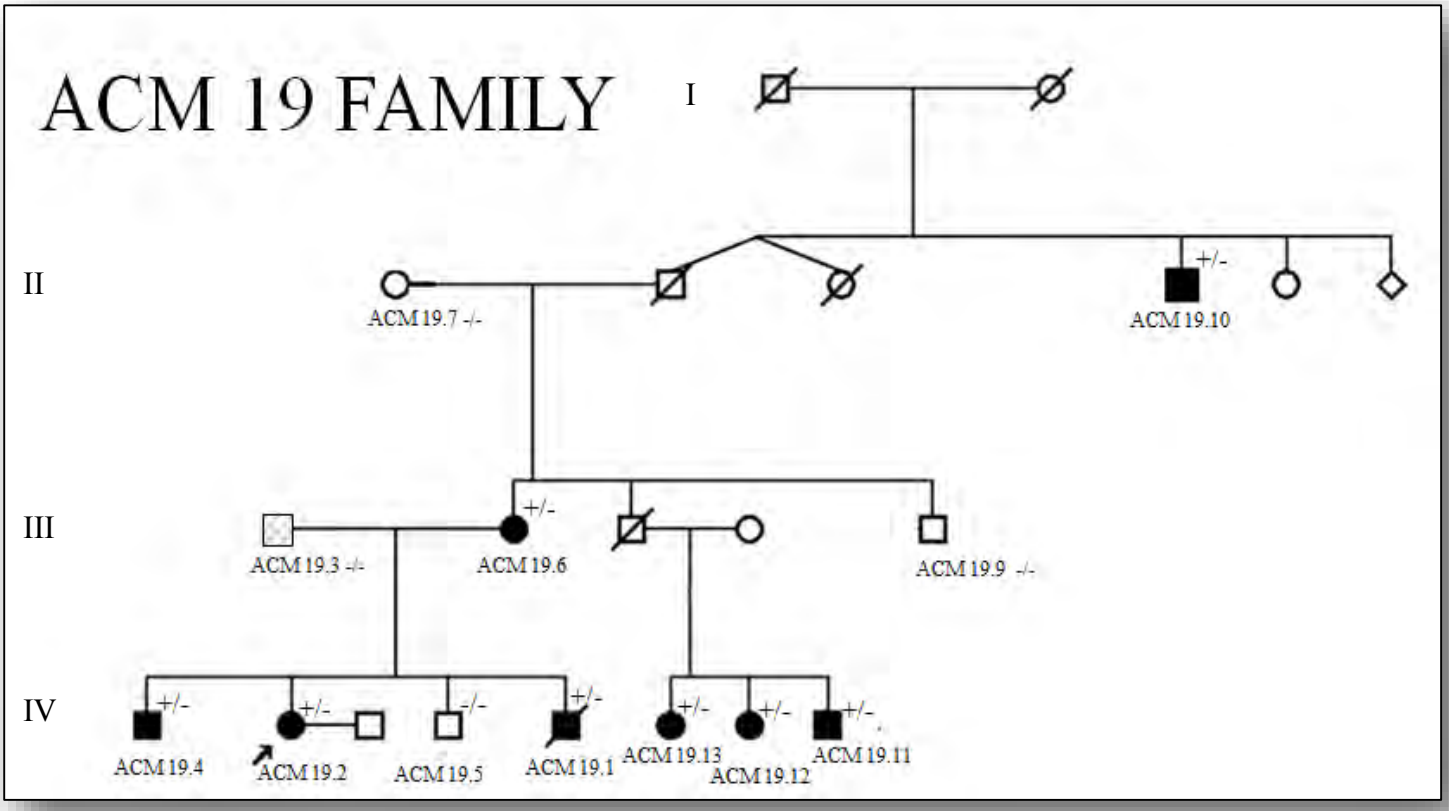


Figure 12: *ACM 19 Family PKP2 c.1162C>T mutation status.*

+ tested positive for the mutation and carries the mutated variant; tested negative for the mutation and does not carry the variant; +/-heterozygous carrier; +/+ homozygous carrier; -/- non-carrier

11.2.1.5 ACM 38

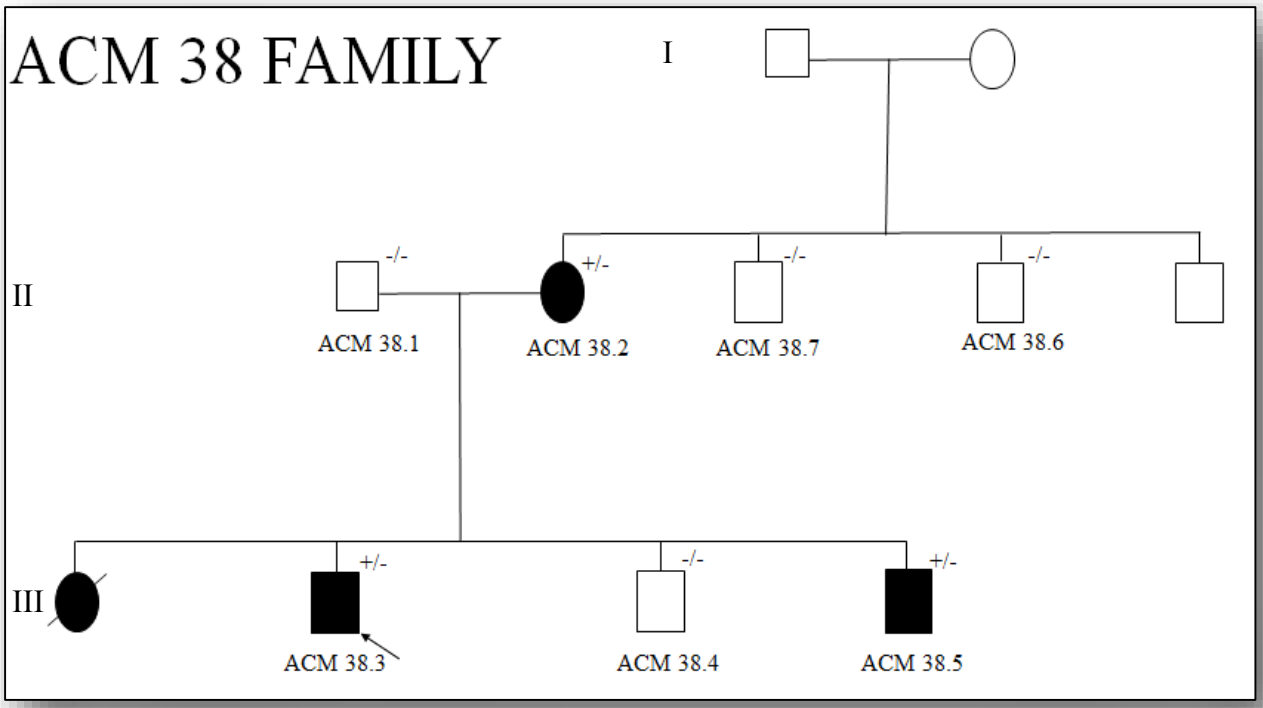


Figure 13: *ACM 38 Family PKP2 c.1162C>T mutation status.*

+ tested positive for the mutation and carries the mutated variant; tested negative for the mutation and does not carry the variant; +/-heterozygous carrier; +/+ homozygous carrier; -/- non-carrier

11.2.1.6 ACM 57

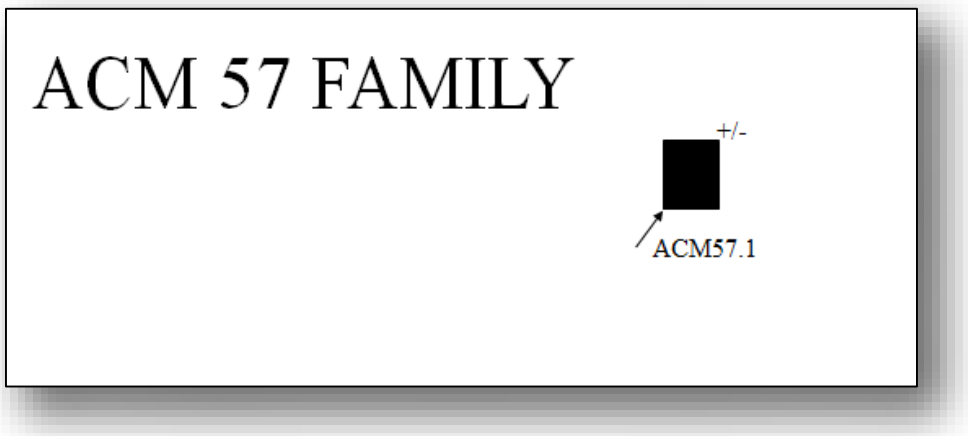


Figure 14: *ACM 57 Family PKP2 c.1162C>T mutation status.*

+ tested positive for the mutation and carries the mutated variant; tested negative for the mutation and does not carry the variant; +/-heterozygous carrier; +/+ homozygous carrier; -/- non-carrier

11.2.1.7 ACM71

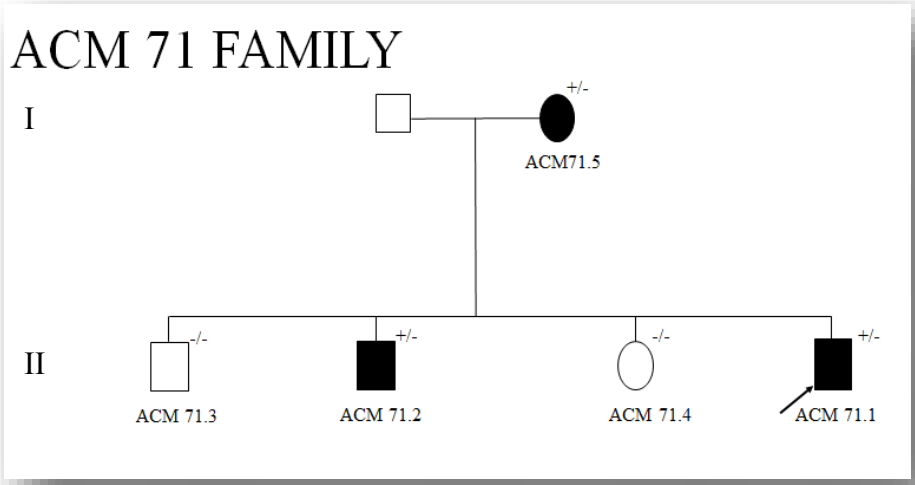


Figure 15: *ACM 71 Family PKP2 c.1162C>T Mutation Status.*

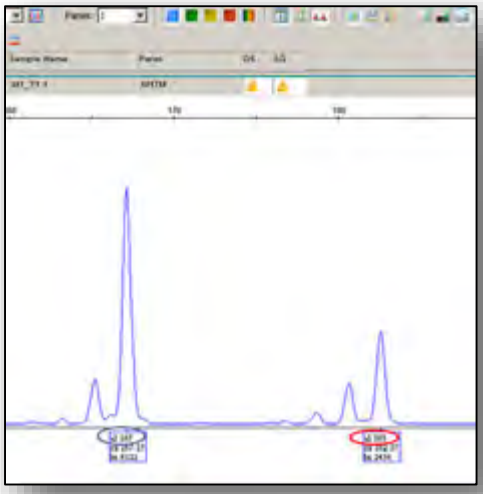
+- + tested positive for the mutation and carries the mutated variant; tested negative for the mutation and does not carry the variant; +/-heterozygous carrier; +/+ homozygous carrier; -/- non-carrier

11.2.2 Microsatellites

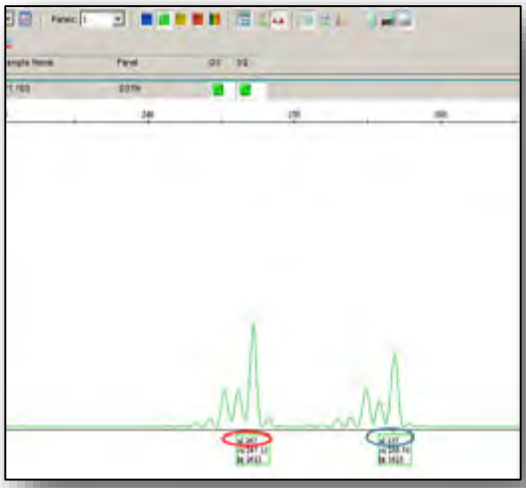
The microsatellite markers used to construct the haplotype were: *PKP2*_M1 (tagged with FAM, for dinucleotide CA repeats), *PKP2*_D12S1692 (tagged with HEX, for dinucleotide CA repeats) and *PKP2*_M2 (tagged with HEX, for dinucleotide TG repeats), spanning the entire *PKP2* gene as well as the 5' and 3' region. Microsatellites were visualized on the ABI 3130XL via stutter peaks; often with the largest peak observed as the "unordered" stretch of DNA's size. They are detected using fluorescence; from the fluorescently labelled tags (HEX (green)/FAM (blue)).

After completing a microsatellite run the peaks were binned and called (Figure 16) for each family member.

A



B



C

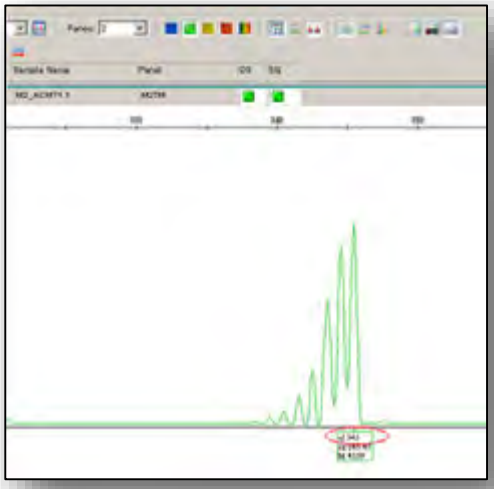


Figure 16: *Microsatellite Analysis of ACM71.1.*

(A) M1 (183), *Peaks sized at 167bps (blue circle) and 183 bps (red circle). M1 common haplotype peak is 183bps,* **(B) D12S1692 (247),** *Peaks sized at 247bps (red circle) and 257 bps (blue circle). D12S1692 common haplotype peak is 247bps,* **and (C) M2 (345),** *Peaks both sized at 345 bps. M2 common haplotype peak is 345bps (red circle).*

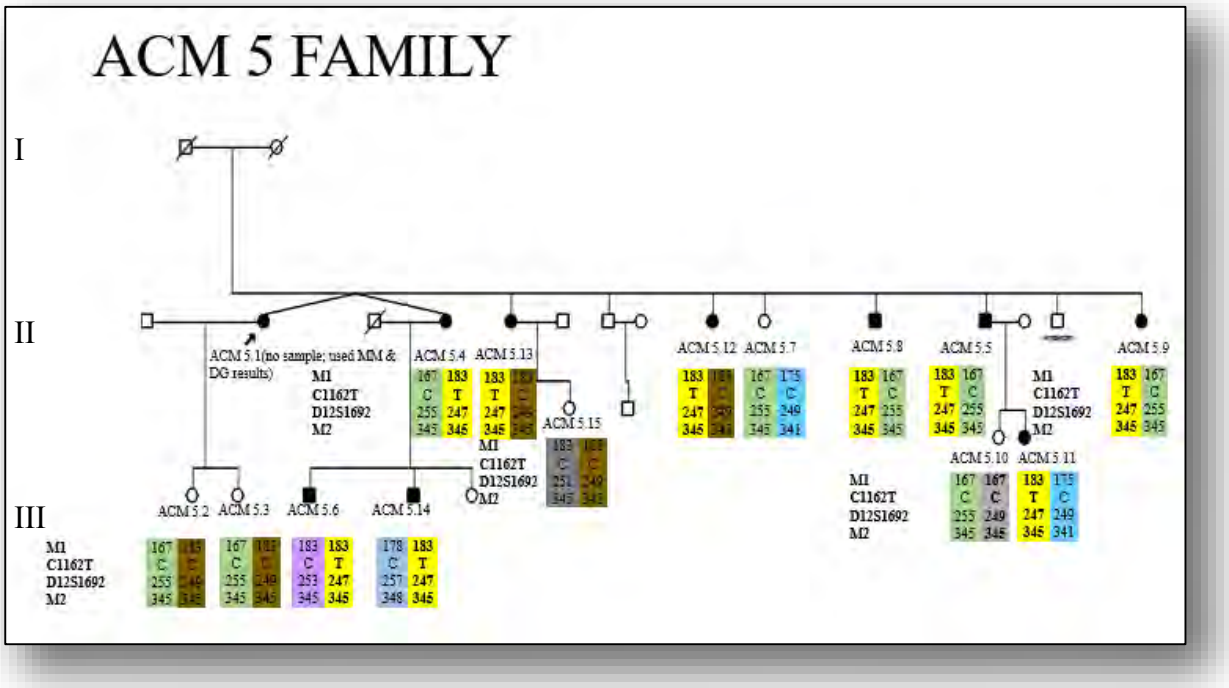
11.2.3 Haplotyping

Merging the genotype and microsatellite typing results enabled us to determine various haplotypes i.e. identifying a group of alleles inherited by progeny from each parent.

Haplotype-based methods offer a powerful approach to disease gene mapping, based on the association between causal mutations and the ancestral haplotypes on which they arose (Gabriel et al., 2002).

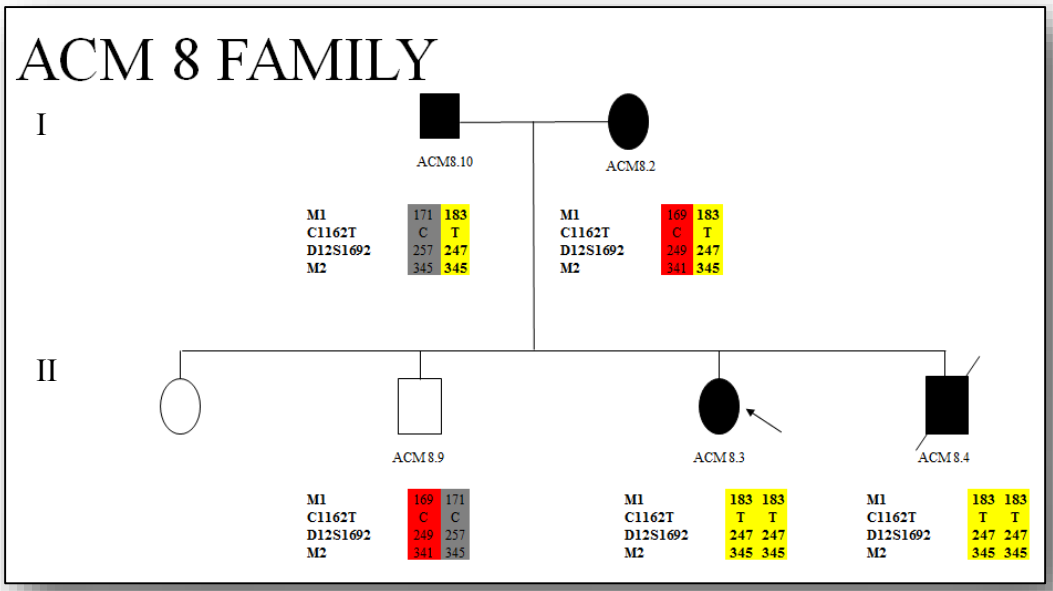
For this study a haplotype was constructed which encompassed three mutations, *PKP2* c.1097T>C, *PKP2* c.1162C>T and *PKP2* c.2197-2202 Ins/Del and three microsatellite markers (M1, D12S1692 and M2) across the *PKP2* gene. The resultant haplotypes underwent intra- and inter-family comparisons, in order to find if a common haplotype emerged for the c.1162C>T positive carriers. A common haplotype (Figure 17 A-G) emerged that segregated amongst all the affected members of the Afrikaner families and asymptomatic mutation carriers.

A



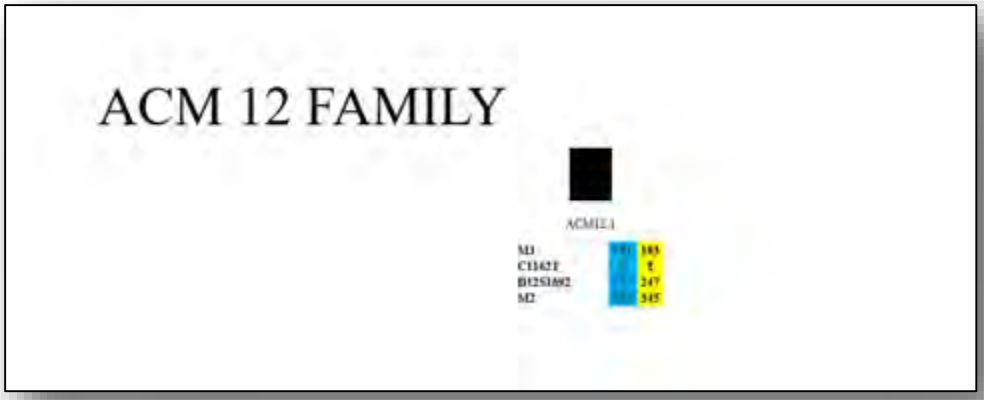
Footnote: *(1)ACM 5.1 had no DNA sample available during DNA halpotyping; however, (MM (Watkins et al., 2009) and DG i.e. experiments run by Dimakatso Gumede in 2014 (unpublished) found a common haplotype in this proband. Due to the twin, also presenting the same c.1162C>T mutation and common haplotype, the proband was shaded to indicate they carry the PKP2 c.1162C>T mutation.(2) Probands of the PKP2 c.1162C>T mutation are identified by arrows. (3)Males are indicated by boxes and females by circles. (4) PKP2 c.1162C>T mutation carriers are shaded and non –carriers are not shaded.(5) Lines on the boxes or circles mean the individual is deceased at time of schematic presentation of pedigree.*

B



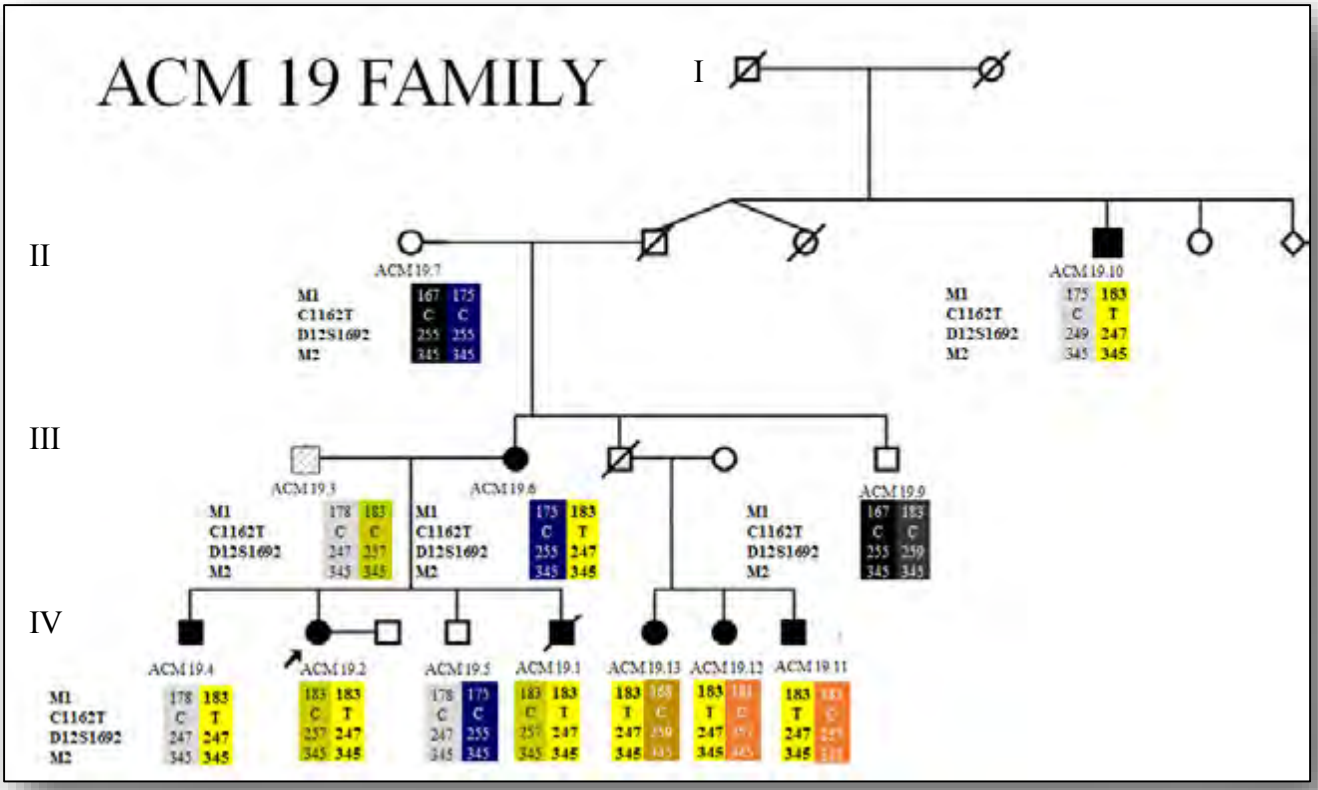
Footnote: *(1) Probands of PKP2 the c.1162C>T mutation are identified by arrows. (2)Males are indicated by boxes and females by circles. (3) PKP2 c.1162C>T mutation carriers are shaded and non –carriers are not shaded.(4) Lines on the boxes or circles mean the individual is deceased at time of schematic presentation of pedigree.*

C



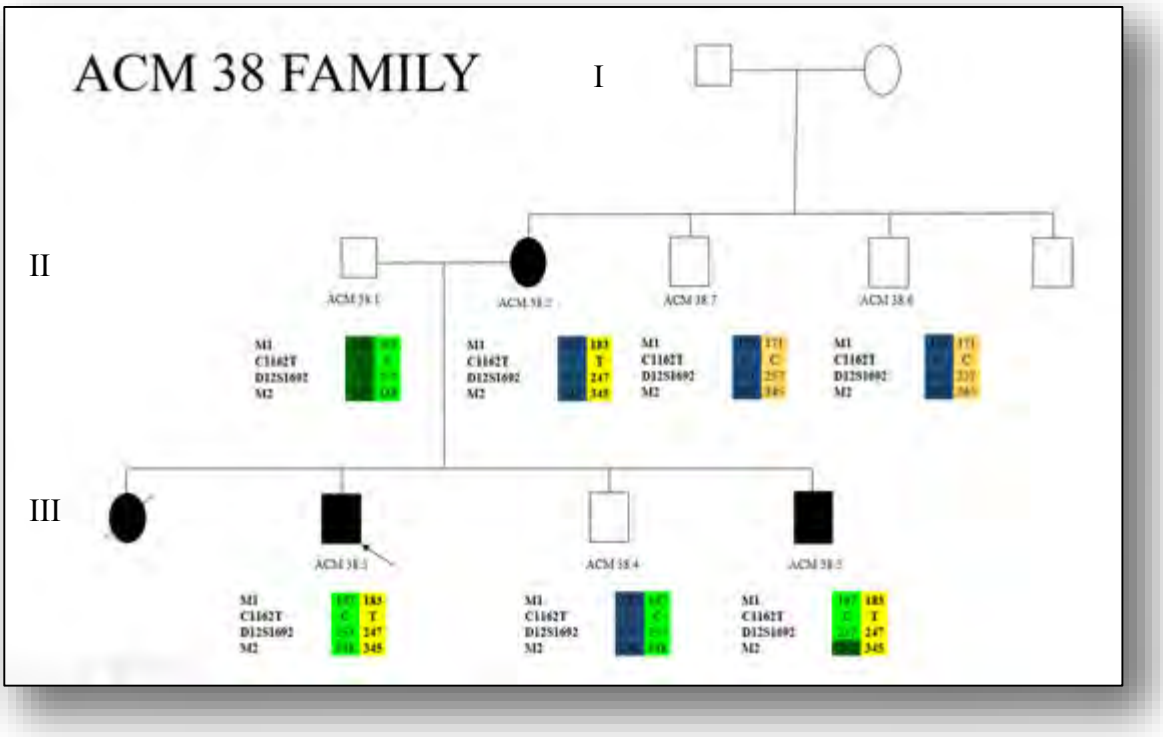
Footnote: *(1) Probands of PKP2 the c.1162C>T mutation are identified by arrows. (2)Males are indicated by boxes and females by circles. (3) PKP2 c.1162C>T mutation carriers are shaded and non –carriers are not shaded.(4) Lines on the boxes or circles mean the individual is deceased at time of schematic presentation of pedigree.*

D



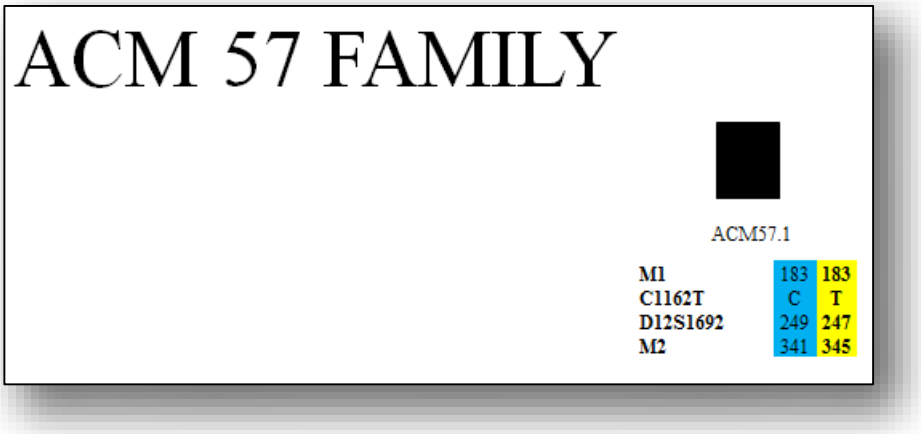
Footnote: *(1) ACM 19.3, the proband's father, has a known PKP2 c.2197_2202delCACACCinsG mutation (Watkins et al., 2009). Shaded box with checked box. (2) Probands of the PKP2 c.1162C>T mutation are identified by arrows. (3)Males are indicated by boxes and females by circles. (4) PKP2 c.1162C>T mutation carriers are shaded and non –carriers are not shaded.(5) Lines on the boxes or circles mean the individual is deceased at time of schematic presentation of pedigree.*

E



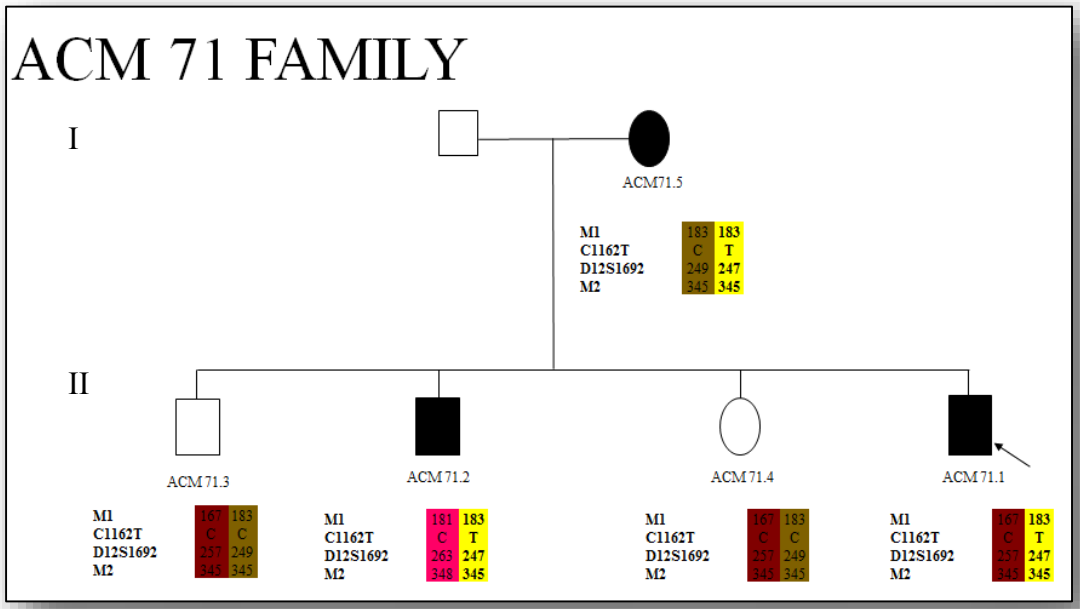
Footnote: *(1) Probands of the PKP2 c.1162C>T mutation are identified by arrows. (2)Males are indicated by boxes and females by circles. (3) PKP2 c.1162C>T mutation carriers are shaded and non –carriers are not shaded.(4) Lines on the boxes or circles mean the individual is deceased at time of schematic presentation of pedigree.(5) The band on ACM 38.5 indicates possible recombination in the M2 marker from 348bps to 345bps; from the father ACM 38.1.*

F



Footnote: *(1) Probands of the PKP2 c.1162C>T mutation are identified by arrows. (2)Males are indicated by boxes and females by circles. (3) PKP2 c.1162C>T mutation carriers are shaded and non –carriers are not shaded.(4) Lines on the boxes or circles mean the individual is deceased at time of schematic presentation of pedigree.*

G



Footnote: (1) *Probands of the PKP2 c.1162C>T mutation are identified by arrows. (2)Males are indicated by boxes and females by circles. (3) PKP2 c.1162C>T mutation carriers are shaded and non –carriers are not shaded.(4) Lines on the boxes or circles mean the individual is deceased at time of schematic presentation of pedigree.*

Figure 17: *Haplotype summary for all study participants; grouped per family (ACM 5 to 71) labelled A to G, respectively.*

11.3. Genealogical tracing

Of interest to us was the oldest family member to pass on the *PKP2* c.1162C>T mutation as this is where we started genealogical tracing-right up until the 17th century or where the first founding ancestors entered.

This study used standard methods for genetic genealogical research in South Africa on hereditary diseases; all resources used are mentioned in section 10.16. The haplotype analyses reports were used to include or exclude specific ancestral lines from genealogical tracing, based on their patterns of inheritance.

We started the genealogical tracing arm of this project by using the most complete family available as this would allow us to identify all possible common founder couples. Location of the families in the past and the present was an important source of information as it dictated the point of origin for data collection which usually started from the repository

(churches/archives/Master's office) in the area where the current descendants were living, baptized or died.

Each mutation positive proband's ancestral lines were traced as far back as the records would allow. Some family lines had complete South African family trees from as early as the 17th century when their ancestors had disembarked from ships on the coasts of South Africa.

The order of the families in this section was determined by the amount of available information in that family and how much they contributed to the identification of the final list of common founder couples.

11.3.1. ACM 38 family

The most complete family traced was ACM 38. With limited information (provided by the genetic counsellor) mostly names, estimated dates of birth (DOB) and places of residence of the proband, their parent and grandparents, we started tracing the ancestral lines of this family. We determined that the ACM 38 family mostly resided in Cape Town and had lost contact with extended family members. We started looking at all the available information (per individual) in the local repositories on this family (section 10.16 on genealogical resources).

Guided by the fact that the genotypes indicated that the *PKP2* c.1162C>T mutation was inherited via the maternal line (ACM38.2), we extended both the maternal and paternal lines of ACM38.3 (Figure 17E). We searched for the maternal grandmother and her parents in the South African Genealogies, SAGs (17 Volumes) (Figure 18) and then the South African Families, SAFs (34 Volumes) manually, using the DVP System (DVP system, 1995). After reaching an impasse we began to search for the grandmother's progenitors in alternative records such as the Microfilmed 1984 South African Voter's Roll at GISA (Figure 19); this confirmed her father's correct DOB and traceable records.

A B

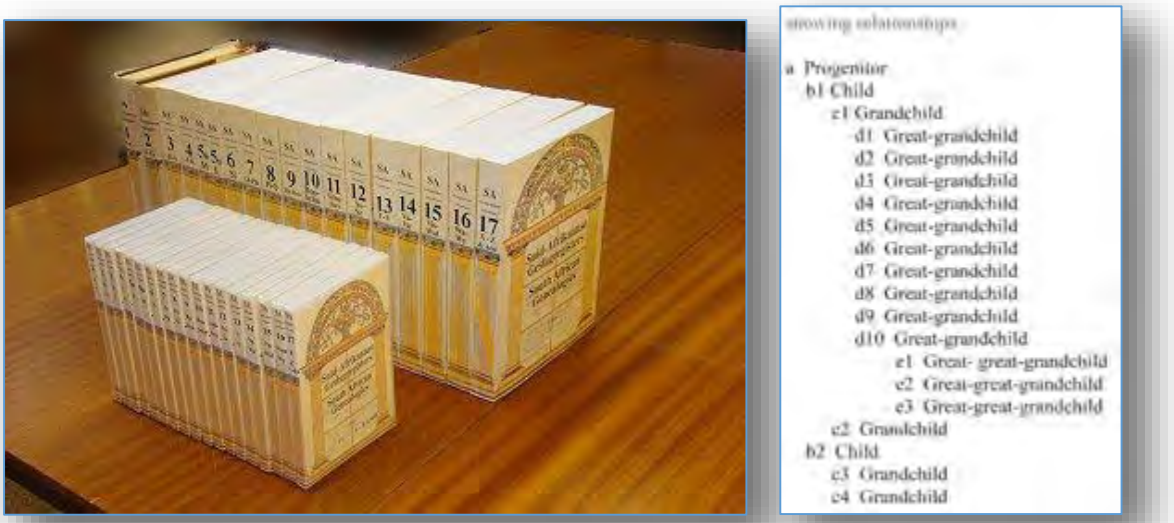


Figure 18: *South African Genealogies (SAGs) and the de Villiers/Pama (DVP) System.*

(A) SAGs (South African Genealogies). *The latest 17 volumes are accessible at GISA. (Adapted from: http://www.gisa.org.za/site/node/12). These volumes are read only using the DVP system;* ***(B)*** *The sample generic chart on the left above is adapted from Genealogical Numbering Systems. Inside each volume page, one must check manually to find the individual of interest, their parents and ancestors, which are usually in earlier lettering. Hence, these books are read from back (large page numbers) to front (smaller page numbers) and each page read from the bottom rows to top the top row. (Adapted from: http://higdonfamily.org/research-tips-for-advanced/numbering-systems-for-genea/descending-numbering-system/de-villierspama-system-1890.html)*

Figure 19: *Microfilm searches at GISA.*

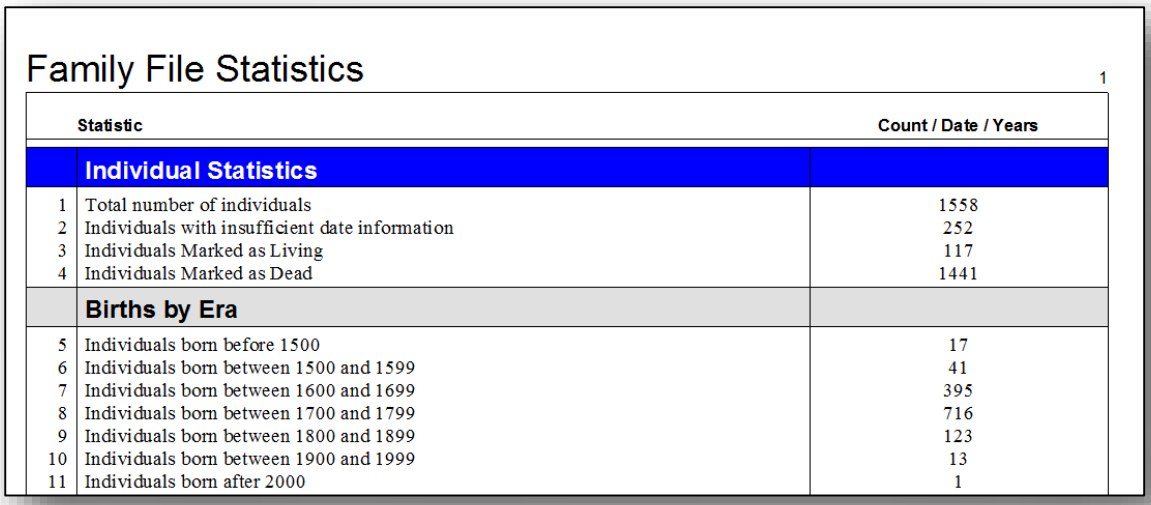
(Adapted from: http://www.gisa.org.za/site/node/12)

Investigation of the paternal line of the proband (ACM38.2) found the South African surname progenitor arriving in the old Cape Province from Netherlands and then marrying a French Huguenot progenitor's offspring in the 17th Century. Most of them were born and resided in the old Cape Province, excluding the few generations that moved to the old Transvaal region and married English spouses. Using the resources in this area lead to a collaboration with a family researcher who had published other sections of his work on this common Dutch family over the past 20 years. The researcher provided the missing family data from his private archive as well as the Pretoria National Archives. I also collected data from the death notices from the old Transvaal death indexes in the Western Cape Archives (only 1910-1943). Some baptismal and marriage records were also traced at GISA on microfilms, referenced per town or city in that region from the earliest town established in 1852 up to 1902; under the changing location names (as per the changed British and VOC leadership after the Anglo-Boer Wars; some regions had names changed).

Records showed that the grandfather of ACM 38.2 returned to the Western Cape and that her maternal line had progenitors from the Netherlands, France and Germany (between the 17th and 18th century), mostly resident in the old Cape Province region. The ancestral lines also showed marriages to 'free' female slaves in the 17th century. In over-all ancestry, a few duplicates were observed as consanguineous marriages occurred- mostly in the 17th and 18th Century.

The results for this family showed South African progenitors dating back more than 10 generations to as early as the 17th century. This family search generated 1588 individuals.

All data was entered into Legacy 8.0 deluxe edition, analyzed manually and the statistics reported (Figure 20).



Family File Statistics 1

	Statistic	Count / Date / Years
	Individual Statistics	
1	Total number of individuals	1558
2	Individuals with insufficient date information	252
3	Individuals Marked as Living	117
4	Individuals Marked as Dead	1441
	Births by Era	
5	Individuals born before 1500	17
6	Individuals born between 1500 and 1599	41
7	Individuals born between 1600 and 1699	395
8	Individuals born between 1700 and 1799	716
9	Individuals born between 1800 and 1899	123
10	Individuals born between 1900 and 1999	13
11	Individuals born after 2000	1

Figure 20: *All relatives genealogically traced of ACM38.2 in a tabular report.*

** As per Legacy v8.0 Deluxe Edition Software output.*

11.3.2. ACM 19 family

With limited information (names, estimated DOB and places of residence of the proband, their parents and grandparents) provided by the genetic counsellor, we started tracing this family. We determined that the ACM 19 family mostly resided in the North West region of South Africa and started using all the available information (per individual) in the local repositories on this family (section 10.16 on genealogical resources).

Guided by the fact that the genotypes indicated that the *PKP2* c.1162C>T mutation was inherited via the maternal line (ACM19.6), we extended both the maternal and paternal lines of the mother's family (Figure 17D). Through ACM19.6 we determined that her father resided in the North West Province but that the grandparents' family resided in the Free State; numerous family members had also relocated to New Zealand. We searched for the paternal family of ACM 19.6 in the SAGs and then the SAFs; manually, using the DVP system.

Parts of the paternal family were previously researched and found in the SAGs and SAFs. We also began to search for the missing paternal line of ACM19.6, namely, her paternal grandmother in alternative records such as the microfilmed baptismal records at GISA (Figure 19) and death notices at the Western Cape archives. This confirmed a lot of the information we had started with and provided the following generation's details and next set of traceable

records- including some eGSSA data sources such as their cemetery project that took pictures of most of the cemeteries.

The paternal line of ACM19.6 was traced back and had the South African surname progenitor arriving in the old Cape Province from Germany and married a Dutch progenitor's daughter, whose mother was a slave in the 17th Century. Most of them resided and were born in the old Cape Province. A few lines of descent were observed in the Eastern Cape and Free State region after the 20th century, that eventually moved to the Free State region and most present day descendants are still in the North West region and sparsely spread around the country, and others have migrated outside of the country.

Other ancestral lines have progenitors that include the Netherlands, France and Germany (between the 17th and 18th Century); mostly resident in the old Cape Province region. The progenitors married English spouses in the 18th and 19th century, and over all the family trees, a few duplicates were observed, due to consanguineous marriages.

The results for this family showed South African progenitors dating back more than 10 generations to as early as the 17th century. From the ACM 19 family alone we managed to trace 2224 individuals. By comparing ACM 38 to the ACM 19 family, we generated 116 South African candidate founding couples.

We entered all the data into Legacy 8.0 deluxe edition, the data was analyzed manually and the statistics reported (Figure 21).

Family File Statistics 1

	Statistic	Count / Date / Years
	Individual Statistics	
1	Total number of individuals	2224
2	Individuals with insufficient date information	135
3	Individuals Marked as Living	7
4	Individuals Marked as Dead	2217
	Births by Era	
5	Individuals born before 1500	0
6	Individuals born between 1500 and 1599	90
7	Individuals born between 1600 and 1699	829
8	Individuals born between 1700 and 1799	996
9	Individuals born between 1800 and 1899	167
10	Individuals born between 1900 and 1999	7

Figure 21: All relatives genealogically traced of ACM19.6's father in a tabular report.

** As per Legacy v8.0 Deluxe Edition Software output.*

11.3.3. ACM 5 family

With information pertaining to names, estimated DOB and places of residence of the proband, their parents, grandparents and great-grandparents (provided by the genetic counsellor) we started tracing the ancestral lines of this family. We determined that the proband (ACM 5.1) resided in the Western Cape Province and her twin sister (ACM 5.4) resided in the Eastern Cape Province; they were the eldest traceable carriers of the *PKP2* c.1162C>T mutation. According to the sources the rest of the ACM 5 family mostly resided in the Free State region of South Africa. With this information we started using all the available information (per individual) in the local repositories on this family (section 10.16 on genealogical resources).

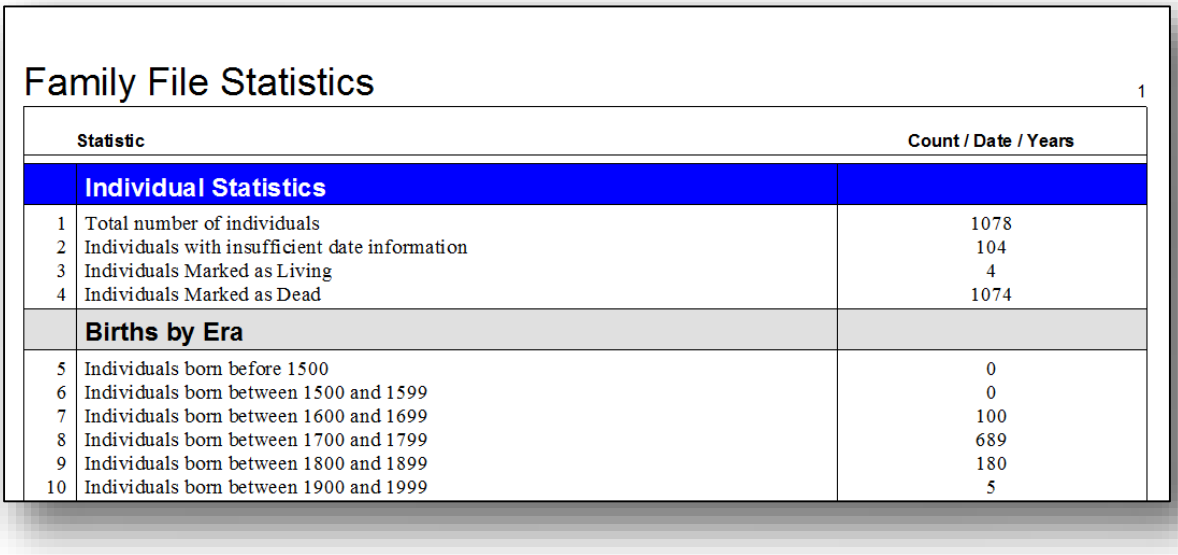
We traced both maternal and paternal lineages of their parents, starting from the Free State region. Numerous living family members have relocated across all South Africa's five Provinces, and outside the country, namely, Amsterdam and Australia. The parents of ACM 5.1 and ACM 5.4 were not found in the SAGs and the SAFs, using the DVP system. Published family books and bibles on the progenitor surnames were first searched for the parents, and parts of the extended family were previously researched, but not the progenitor lines we were searching for. This led to searching the Free State death Index records available at the Western Cape Archives and the Orange Free State (OVS) files at GISA, where we found the marriage register and the death notices of the parents. We also did a search on FamilySearch.org to find the 20th century records, as their original copies are in the Free State. After this discovery, we found the rest of the lineages through collaboration with the Free State Archives (source of all Home Affairs documents), Free State Master of the High Court (MHG) Office (for death notices) and the Free State NGK (Dutch Reformed Church), for baptismal records. The NGK's newly computerized registers made finding the baptismal and marriage registers relatively easy. Some periods still had to be manually checked at GISA and at the Western Cape Archives as their records are not complete and not all ancestors (1) got married in the NGK (2) baptised their progeny in Free State or (3) married spouses from that region. The SAGs and SAFs also complemented a lot of the information as we collected and provided the previous generation's details and next set of traceable records.

The paternal line of ACM5.1 was traced back and had the South African surname progenitor arriving in the old Cape Province from Germany and married a Dutch progenitor's daughter, whose mother was Belgian in the 17th century. Most of them have resided and were born in the old Cape Province and in the old OVS. A few lines of descent were observed in other regions

such as the Eastern Cape region after the 20th century, that eventually moved back to the Free State region and others have migrated outside of the country. Other ancestral lines have progenitors that include the French Huguenots, the English and the Swiss (between the 17th and 18th century), mostly resident in the old Cape Province. Over all the family trees, a few duplicates were observed, mainly due to consanguineous marriages.

The results for this family showed South African progenitors dating over 10 generations back to as early as the 17th century. From the ACM 5 family alone we managed to trace 1078 individuals. By combining the genealogy data for ACM 38, ACM 19 and ACM 5 we generated 10 common South African founding couples.

We entered all the data into Legacy 8.0 deluxe edition, the data was analyzed manually and the statistics reported (Figure 22).



Family File Statistics 1

	Statistic	Count / Date / Years
	Individual Statistics	
1	Total number of individuals	1078
2	Individuals with insufficient date information	104
3	Individuals Marked as Living	4
4	Individuals Marked as Dead	1074
	Births by Era	
5	Individuals born before 1500	0
6	Individuals born between 1500 and 1599	0
7	Individuals born between 1600 and 1699	100
8	Individuals born between 1700 and 1799	689
9	Individuals born between 1800 and 1899	180
10	Individuals born between 1900 and 1999	5

Figure 22: *All relatives genealogically traced of ACM5.1 in a tabular report.*

** As per Legacy v8.0 Deluxe Edition Software output.*

11.3.4. ACM 12 family

With very limited information on names, estimated dates of birth and places of residence of the proband and his parents (provided by the genetic counsellor), we started tracing the ancestral lines of this family. We determined that the proband (ACM 12.1) resided in the Western Cape region. With this information we started using all the available information (per individual) in the local repositories on this family (section 10.16 on genealogical resources).

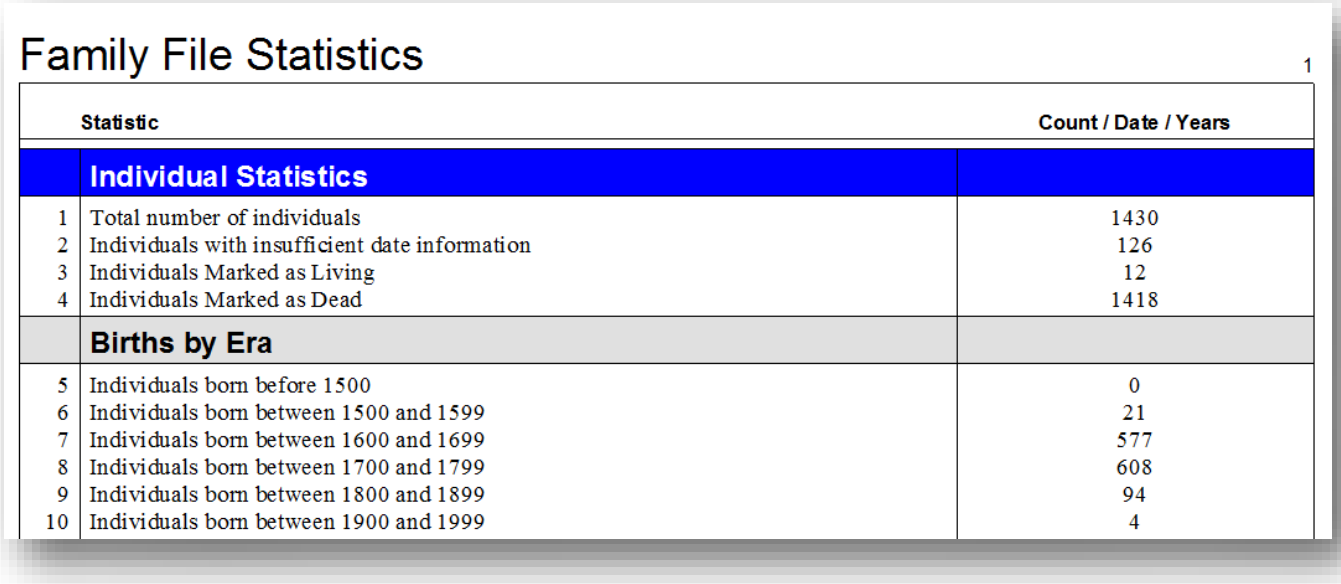
As we did not have the DNA of the proband's parents available for testing we were not able to indicate from which parental line the *PKP2* c.1162C>T mutation was inherited and proceeded to extend both maternal and paternal lineages of the proband. We started from the South African Voter's Roll of 1984 under the Western Cape region which then led to searching the Western Cape Death Index records available at the Western Cape Archives and the Western Cape files at GISA. Here we found the marriage register and the death notices of the parents and other extended family members for the last three centuries.

Our data revealed that the paternal lineage of ACM 12.1 has historically resided in the Western Cape Province, and the progenitor couples were both French Huguenots. The paternal line only mixed with the English from the 20th century and Dutch ancestral lines in the 18th century. The maternal lineage's progenitor came in from France but was of German/Swiss/Jewish (Ashkenazi) origins and was married to a Flemish/Dutch progenitor's daughter. They were the only family members who were screened for the *PKP2* c.1162C>T mutation.

The family of ACM 12.1 is a well-known Huguenot family who had been partially researched from the 18th century and earlier. They had data extracted from the SAGs and the SAFs using the DVP system. Published family books and bibles on the progenitor surnames were also searched and helped start and expand the family tree. Over all the trees, a few duplicates were observed, as cousins inter-married.

The results for this family showed South African progenitors dating back well over 10 generations to as early as the 17th century. This family search generated 1430 individuals. By combining the genealogy data for ACM38, ACM19, ACM5 and ACM 12, we generated 5 candidate South African founding couples.

We entered all the data into Legacy 8.0 deluxe edition, the data were analyzed manually and the statistics reported (Figure 23).



Family File Statistics 1

	Statistic	Count / Date / Years
	Individual Statistics	
1	Total number of individuals	1430
2	Individuals with insufficient date information	126
3	Individuals Marked as Living	12
4	Individuals Marked as Dead	1418
	Births by Era	
5	Individuals born before 1500	0
6	Individuals born between 1500 and 1599	21
7	Individuals born between 1600 and 1699	577
8	Individuals born between 1700 and 1799	608
9	Individuals born between 1800 and 1899	94
10	Individuals born between 1900 and 1999	4

Figure 23: *All relatives genealogically traced of ACM12.1 in a tabular report.*

** As per Legacy v8.0 Deluxe Edition Software output.*

11.3.5. ACM 71 family

With very limited information on names, estimated date of birth and places of residence of the proband, his parents and grandparents provided by the genetic counsellor we started tracing the ancestral lines of this family. The names of this family were recorded with incorrect spellings as it was translated from English to Afrikaans and key family histories such as places of birth were unknown. We determined that the proband (ACM 71.1), his mother (ACM71.5) and maternal grandparents resided in the Western Cape region. With this information we started using all the available information (per individual) in the local repositories on this family (section 10.16 on genealogical resources).

Guided by the fact that the genotypes indicated that the *PKP2* c.1162C>T mutation was inherited via the maternal line; we extended both the maternal and paternal lines of ACM71.5 (Figure 17G). This family is of mixed ancestry and we had to employ alternative methods of tracing after the conventional way used for the other families failed to yield results. The period under investigation included Apartheid government periods where records were separated by racial groupings and often some racial groups had poorly recorded and archived records. Some records in the Western Cape Archives of mixed ancestry individuals indexed did not have first names. After searching all the indexes of towns of interest and their neighboring towns, other

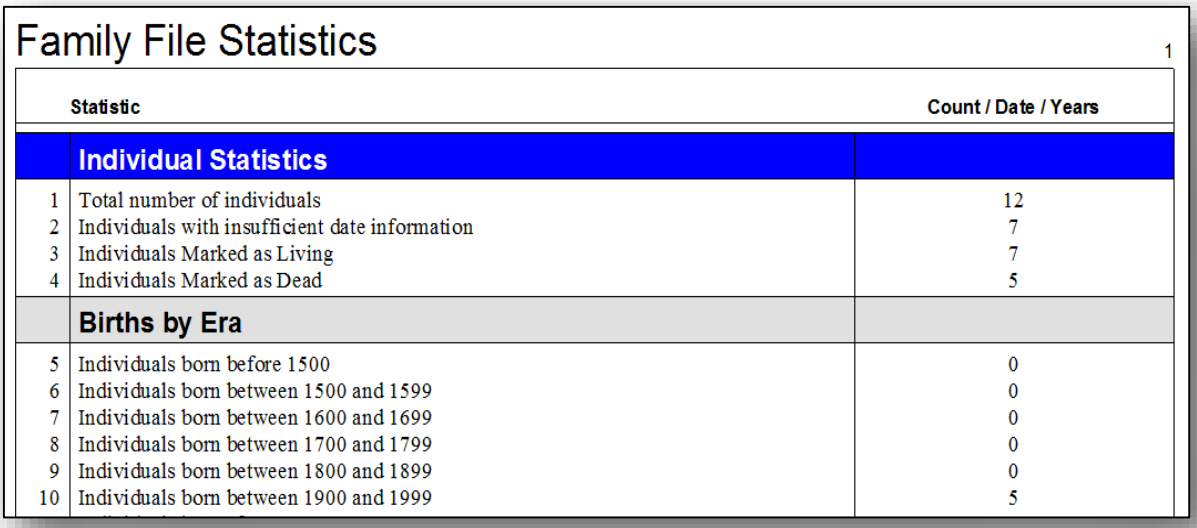
regional registers were added and referenced which could randomly contain the baptismal record of interest. Here we finally found the correct individual but the English name did not match the family's interview details provided. Additionally, the other alternatives had the exact names from the interviews and the same place of baptism but not the same dates of birth. This led to the researcher first searching for ACM 71.5 name from the children's birth records and requesting the research nurse to follow up with the family for further information in order to eliminate the erroneous records.

The correct baptismal record of ACM 71.5 was found and her parents traced but the period and places they were married and born did not have records at the Western Cape Archives, as they were remote farming communities still with the mission churches. The SAGs and SAFs were searched and NGK Stellenbosch archives checked but no new information was found. The respective Bredasdorp and Caledon repositories lacked any baptismal or marriage records of the proband's maternal grandparents and the other repositories contacted including the Department of Home Affairs were not keen to assist, especially for research purposes as some of the records fall under the 100 year ban and are a conflict of interest with their security policy and adherence to the way they use the PAIA Act.

This family search did not proceed any further than 1914, but we determined that the paternal line of ACM 71.5 was Afrikaner. An experienced researcher in mixed ancestry research in the Western Cape was consulted and it was suggested that based on the mother's surname and other researched surname holders in the region, she is of African descent; we were unable to determine if she was of mixed ancestry.

We managed to trace the family back by three generations to the early 20th century. This family search generated 12 individuals, no South African progenitors and no clear South African founding couples.

We entered all the data into Legacy 8.0 deluxe edition, the data were analyzed manually and the statistics reported (Figure 24).



Family File Statistics 1

	Statistic	Count / Date / Years
	Individual Statistics	
1	Total number of individuals	12
2	Individuals with insufficient date information	7
3	Individuals Marked as Living	7
4	Individuals Marked as Dead	5
	Births by Era	
5	Individuals born before 1500	0
6	Individuals born between 1500 and 1599	0
7	Individuals born between 1600 and 1699	0
8	Individuals born between 1700 and 1799	0
9	Individuals born between 1800 and 1899	0
10	Individuals born between 1900 and 1999	5

Figure 24: *All relatives genealogically traced of ACM 71.1 in a tabular report.*

** As per Legacy v8.0 Deluxe Edition Software output.*

11.3.6. ACM 8 family

This family was included during the last phase of the write-up. It has two root individuals due to the homozygous *PKP2 c.1162C>T mutation*s found in both children (Figure 17B). We confirmed via Sanger sequencing that each parent carries a heterozygous mutation in *PKP2*. With very limited information on names, estimated dates of birth and places of residence of the proband (ACM 8.3) and her parents (provided by the genetic counsellor) we started tracing both the lines of this family. We determined that the proband and the parents resided in the Western Cape region. With this information we started using all the available information (per individual) in the local repositories on this family (section 10.16 on genealogical resources).

We completed the name validation phase, searching their town of residence and neighboring towns. With the locally available resources, we reached the 1800s for most of the lines, until we found a database source of the SAGs; which includes the paternal lines of ACM 8.3 (with nearly 34000 individuals to search), including their non-South African progenitors. The maternal line is under investigation as further genealogical tracing still has to be done on this family.

11.3.7. ACM 57 family

This family was included during the last month of the write-up. With very limited information pertaining to names, estimated dates of birth and places of residence of the probands and her parents (provided by the genetic counsellor) we started tracing the lines of this family. The names of this family were recorded with various spellings on the typed and hand written pedigrees. Key family histories such as places of birth were unknown. We determined that the proband (ACM 57.1) and the parents resided in the Western Cape region. With this information we started using all the available information (per individual) in the local repositories on this family (section 10.16 on genealogical resources).

As we had no information of the nuclear family of the proband we begun tracing the maternal and paternal lines of the proband (Figure 17F). We are still in the name validation phase, searching their town of residence and neighboring towns. This was a recent finding and genealogical tracing still has to be done on this family.

11.4 Combined genealogical analysis

In order to determine the common South African founder couple we combined all genealogical data for the four completed families.

During the completion of the largest two families (ACM 38 and ACM 19), there were 116 couples which filtered down to 10 candidate South African founder couples (with the help of the ACM 5 family) that underwent further analyses. After the ACM 12 family was added to the analysis there were five candidate South African founder couples. The ACM 71 family did not progress past the 1900s as we were hampered by the fact that the family could not be linked back to any other family tree without finding the proband's maternal great-grandparents. The ACM 8 and 57 families were a recent finding and completion of the genealogical tracing and the analyses still has to be done.

12. Discussion

During the genetic screening phase of this study we observed the *PKP2* c.1162C>T mutation in 30 individuals in 7 families. Where possible, all probands and at risk family members were clinically phenotyped and screened for the *PKP2* c.1162C>T mutation.

Most individuals carrying the mutation showed symptoms and signs of moderate to severe ARVC. All cases were classified as 'definite' cases according to the 2010 ARVC Task Force criteria. Our results also showed that not all c.1162C>T carriers were symptomatic at the time of phenotyping, which speaks to the incomplete penetrance of ARVC. When we compared the genotype to the phenotype for the individuals carrying the *PKP2* c.1162C>T mutation, we observed two distinct groups: clinically affected individuals that displayed a mild to moderate ARVC phenotype and that usually presented at a more advanced age. Those with a more severe phenotype presented at a much younger age ARVC and their disease appeared to be triggered or exacerbated by a history of long-term endurance exercise.

We identified four families, ACM 5, ACM 12, ACM 57 and ACM 71 that presented with a later age of onset and with a moderate form of ARVC. The proband ACM 5.1, presented at 44 years and was a professional athlete. The proband ACM 12.1, presented at 45 years and was a marathon runner. Proband ACM 57.1, presented at 42 years and was also a marathon runner. Proband ACM 71.1, presented at 38 years and was a farm worker with a history of hypertension and smoking (inclusive of cannabis).

Genetic screening of all four probands showed that they had only carried the pathogenic *PKP2* c.1162C>T mutation. We observed that most of the presenting/ affected family members had an athletic history and suspect that lifestyle, such as a long-term endurance exercise, aggravates or modifies the incomplete penetrance of ARVC, as seen in other ARVC studies (Pescatore et al., 2013, Pasquie et al., 2013). For the ACM71 proband, similar to other studies, the use of drugs and a history of hypertension may be thought to act as confounders (Sen-Chowdhry, 2005).

We identified three families, ACM 8, ACM 19 and ACM 38, who presented with an early age of onset and much more severe ARVC phenotype. The ACM 8 family had two severely affected children; one whom died from an SCD at age 14 (ACM 8.4). Each of the siblings were homozygous for the *PKP2* c.1162C>T mutation. The ACM 19 family had two severely affected siblings that were screened and diagnosed at 12 years and 14 years and required transplantation at ages 15 years and 17 years, respectively. The ACM 38 family had three severely affected

children with the first child having sudden cardiac death at age 8 years, the proband presenting at pre-puberty and requiring a transplant at age 16 years while his brother was transplanted at age 8 years. Genetic screening of the two affected siblings of ACM 19 found a compound heterozygote in *PKP2* (c.1162C>T and c.2197-2202 Ins/Del) (Watkins et al., 2009), resulting in a much more severe phenotype than the cases that had only inherited the c.1162C>T variant on its own. Even though we have identified the c.1162C>T in the two affected siblings of the ACM 38 family (the proband ACM 38.3 and ACM 38.5), we hypothesize, due to (1) the severity and early age of onset and (2) the recombination in ACM 38.5, we can speculate that there is a second mutation, yet to be determined, linked to modifying the ARVC and *PKP2* c.1162C>T mutation seen in this family. Their mother (ACM 38.2) who carries the c.1162C>T mutation is well but has a history of miscarriages. We suspect the affected siblings may have inherited a pathogenic or modifier gene from their father (ACM 38.1) that exacerbates the ARVC phenotype seen in the affected siblings (Figure 17 E). We intend to screen this family using whole exome sequencing to test this hypothesis.

We also observed that all families that carried the *PKP2 c.1162C>T mutation* were of Afrikaner decent and constructed a haplotype to indicate patterns of inheritance of the genotypes in the affected individuals. We found that all individuals positive for the c.1162C>T mutation shared the same haplotype and were thus identical by descent from a common founder (Watkins et al., 2009). We used genealogy tracing to identify the five candidate common founder couples for the pathogenic *PKP2* c.1162C>T mutation.

According to the genealogical results, all the participants who were screened and who self-identified as Afrikaners had genealogical evidence that strongly suggested their Afrikaner descent. Our data is consistent with the possibility that recurrent mutations are identical by descent in all five families. Five of the families carried the mutation from their parent without recombination and we assumed that ACM 12.1 and ACM57.1, with no relatives in the study yet, had the same disease-associated haplotype by descent (as confirmed by the laboratory screening).

Most of the family trees had varying degrees of Dutch, German and French Huguenot ancestry. For example, ACM 12.1 is male and his family carried a common French Huguenot progenitor paternal lineage which contributing to his present day surname. As a result there were significantly more French Huguenot ancestors in his traced lines, in comparison to the others. However, he is still considered to be an Afrikaner because he has the genetic makeup of the

three ancestries that make up the Afrikaners by admixture, marrying Dutch, French and or German spouses. So he rightfully identified as an Afrikaner, and he self-identified by the correct descent. It was also observed that all five Afrikaner families had Western Cape Provincial roots which supports the South African history, whereby almost all progenitors of the Afrikaners stem from the old Cape Province located in the present day Western Cape Provincial area.

Our results also showed that the ACM 38 family was the first and most completely traced family, from ACM 38.2 who presented 332 progenitor individuals and which were made up of 116 progenitor couples. After the second family, ACM 19, was traced, through the paternal line, presenting a number of progenitors and progenitor couples; we manually made a common candidate progenitor couple list (from the Legacy Fan diagrams and Ancestor diagrams; not presented because they contain names, surnames, DOB and other confidential details) to narrow down the search to common candidate progenitor couples. Afterwards, we included the third most complete family, the ACM 5 family (through ACM5.1) and we observed 10 shared common candidate progenitors in the fourth family. ACM 12 further narrowed it down to five common candidate founder couples. The fifth and final family, ACM 71, could not be linked back to the other four trees due to missing data or records. Without finding the baptismal records of the maternal great-grandparents, we encountered an impasse and could not proceed with the elimination process.

Our study consisted of mostly white Afrikaners, except one mixed ancestry family classified as colored and Afrikaner (ACM 71), which is consistent with the international literature also pointing to the *PKP2* c.1162C>T mutation being of European descent in four other international ARVC studies (Table 1). The international literature corroborates our finding that the *PKP2* c.1162C>T founder mutation segregates with European Ancestry in South Africa.

We also observed that Figures 20-23 show a counterintuitive phenomenon where the ancestors increase up till the 1800s but one observes a significantly diminished total number of individuals after the 1800s. This is due to four contributing factors, all mimicked by the diamond theory of ancestors (Appendix 7B): (1) the 100 year ban limiting the records we could access and add to our Legacy database; (2) the tracing method used i.e. it commenced with mostly currently living individuals, hence, those that had provided consent were entered in the 20th century cohort and their deceased direct ancestors (exclusive of siblings) were entered, to avoid violating relatives privacy and the consent; (3) the recurrent/ duplicated ancestors or

names in a single family due to consanguineous marriages, high infant mortality, or renaming practices (Appendix 8.2.e), which were present in all four families independently and (4) the large number of children born in certain generations (1600s to 1800s) and high death rates (although the children's records are well documented and were entered into the database in certain generations).

During the course of this study, we also observed other known Afrikaner founders from other diseases in our family trees, which could be further filtered out by further data collected and more families hence, this small sample size (<10 probands) could not provide conclusive findings for the segregation of only the *PKP2* c.1162C>T mutation (the hypothesis) in a homogenous population group, without increasing the sample size. Further, increasing the sample size, increases the 'unrelated' proband data set (increasing variation in the homogenous group), making the study statistically significant and powerful.

After tracing all the families and eliminating uncommon couples, we found the five different progenitor couples in South Africa (all of Afrikaner descent) that were linked to all four traced trees. However, we do not have sufficient proband families to identify a single founder or founding couple. Our observations propose that Geldenhuys' (2014) observations were correct and that a total of 10 probands may be needed to find only one common couple or ancestor. This study has four traced family lineages to date, and five possible founder couples.

13. Study Limitations

This study was too small to draw a definitive genealogical conclusion (further explained in Appendix 10). This was due to the logistics related to the difficulty in recruiting participants nationwide namely, poor public awareness and the lack of standardized phenotyping. The researcher also encountered genealogical data collection issues such as (1) the 100 year ban associated with the PAIA policy; (2) the unfavorable position the Department of Home Affairs has to take to unrelated data collectors of private records, including researchers, in order to keep the people's records safe from misuse ; and (3) the loss or destruction of the historical documents of some people and communities (often slave, adopted or illegitimate progeny) through time (Myres et al., 2014). Finally, the researcher did not have enough time and resources to visit all the places required to collect data, outside a 100km radius from Cape Town and had to receive training, as the need arose.

14. Conclusion

In this study, we searched for a common founder for the pathogenic *PKP2* c.1162C>T mutation in ARVC families of Afrikaner decent in South Africa. Our probands were recruited from the ARVC Registry of South Africa and after phenotypic and genetic profiling, the families were extended.

During the course of this study, when we correlated the genotype with the phenotype of the families we saw the families grouping into two distinct phenotype categories: moderate (later onset) and severe (early onset). Our phenotyping results showed that family ACM 5, ACM 12, ACM 57 and 71 had moderate (late onset) phenotypes, and ACM 19 and ACM 38 had severe (early onset) ARVC cases.

Genetic screening indicated that 30 out of the 46 family members were positive for the *PKP2* c.1162C>T mutation and that all mutation positive individuals shared a common haplotype.

Genealogical tracing revealed that (1) a single Afrikaner individual descends from hundreds of South African Afrikaner progenitor lines, (2) that relatives have lines in common; often termed as duplicate/shared ancestors, and (3) most of these Afrikaners progenitor lines can chiefly be classified as Dutch, French Huguenot or German. We also realized that homogenous populations may not produce a clear founder with less than ten 'unrelated' probands families, as they have many lines in common. Hence, we may need two additional apparently 'unrelated' probands to help with less common line elimination, in order to find one common founder or a common founding couple.

The preliminary genealogical evidence generated from this study suggests that the *PKP2* c.1162C>T mutation segregates in Afrikaners exclusively and at a higher frequency due to a founder effect. The preliminary genealogical evidence is consistent with the hypothesis that *PKP2* c.1162C>T is a founder mutation and descendants of the candidate common founders are at risk of developing ARVC.

15. Recommendation

We have identified seven probands with eight family lineages that carry the *PKP2* c.1162C>T mutation. Intensive genealogical analysis of 5 of these families has identified five candidate common founder couples for the *PKP2* c.1162C>T founder mutation in Afrikaner families with ARVC. Additional work on the existing families is required to further reduce the number of candidate founder families. It is likely that the recruitment of at least two new cases carrying the recurrent mutation will be needed to conclusively identify the founder couple. We plan to conduct a nation-wide recruitment of new cases of ARVC in order to achieve this objective.

16. References

References

ALCALDE, M., CAMPUZANO, O., BERNE, P., GARCÍA-PAVÍA, P., DOLTRA, A., ARBELO, E., SARQUELLA-BRUGADA, G., IGLESIAS, A., ALONSO-PULPON, L. & BRUGADA, J. 2014. Stop-gain mutations in PKP2 are associated with a later age of onset of arrhythmogenic right ventricular cardiomyopathy. *PloS one,* 9**,** e100560.

ALFIERI, O., MAYOSI, B. M., PARK, S.-J., SARRAFZADEGAN, N. & VIRMANI, R. 2014. Exploring unknowns in cardiology. *Nature Reviews Cardiology,* 11**,** 664-670.

AMOFA, R. 2016. What is ARVC, the disease that ended James Taylor's career, and what other sportsmen have been affected? *The Telegraph*, 12 APRIL 2016

ASIMAKI, A. & SAFFITZ, J. E. 2011. The role of endomyocardial biopsy in ARVC: looking beyond histology in search of new diagnostic markers. *Journal of cardiovascular electrophysiology,* 22**,** 111-117.

BASSO, C., RONCO, F., MARCUS, F., ABUDUREHEMAN, A., RIZZO, S., FRIGO, A. C., BAUCE, B., MADDALENA, F., NAVA, A. & CORRADO, D. 2008. Quantitative assessment of endomyocardial biopsy in arrhythmogenic right ventricular cardiomyopathy/dysplasia: an in vitro validation of diagnostic criteria. *European heart journal*.

BLANC, J.-J. & BENDITT, D. G. 2003. Syncope: definition, classification, and multiple potential causes. *The evaluation and treatment of syncope. A handbook for clinical practice.* Futura Blackwell, Elmsford (NY).

BLANCKENBERG, J. 2011. *Molecular genetics of arrhythmogenic right ventricular cardiomyopathy in South Africa.* University of Cape Town.

BRINK, P. A. & SCHWARTZ, P. J. 2009. Of founder populations, long QT syndrome, and destiny. *Heart Rhythm,* 6**,** S25-S33.

BURROWS, E. H. 1958. A History of Medicine in South Africa up to the End of the Nineteenth Century. *A History of Medicine in South Africa up to the End of the Nineteenth Century.*

CAHILL, T. J., ASHRAFIAN, H. & WATKINS, H. 2013. Genetic Cardiomyopathies Causing Heart Failure. *Circulation Research,* 113**,** 660-675.

CARDINAL, J. 2004. Progenitor. *In:* EASTMAN, D. (ed.) *Encyclopedia of Genealogy.* Eastman's Online Genealogy Newsletter.

CHIN, A. ARVC: Lessons from the ARVC registry of South Africa PASCAR, 2015 Mauritius, 2015.

ELLIOTT, P., ANDERSSON, B., ARBUSTINI, E., BILINSKA, Z., CECCHI, F., CHARRON, P., DUBOURG, O., KÜHL, U., MAISCH, B., MCKENNA, W. J., MONSERRAT, L., PANKUWEIT, S., RAPEZZI, C., SEFEROVIC, P., TAVAZZI, L. & KEREN, A. 2008. Classification of the cardiomyopathies: a position statement from the european society of cardiology working group on myocardial and pericardial diseases. *European Heart Journal,* 29**,** 270-276.

FARRAR, J. S., REED, G. H. & WITTWER, C. T. 2009. High resolution melting curve analysis for molecular diagnostics. *Molecular diagnostics,* 2**,** 229-45.

FISH, M. 2010. *Analysis of desmoplakin in arrythmogenic right ventricular cardiomyopathy.* University of Cape Town.

FISH, M., SHABOODIEN, G., KRAUS, S., SLIWA, K., SEIDMAN, C. E., BURKE, M. A., CROTTI, L., SCHWARTZ, P. J. & MAYOSI, B. M. 2016. Mutation analysis of the phospholamban gene in 315 South Africans with dilated, hypertrophic, peripartum and arrhythmogenic right ventricular cardiomyopathies. *Scientific Reports,* 6**,** 22235.

FONTAINE, G. & CHEN, H.-S. V. 2014. Arrhythmogenic Right Ventricular Dysplasia Back in Force. *The American journal of cardiology,* 113**,** 1735-1739.

FONTAINE, G. & PROST-SQUARCIONI, C. 2004. Implantable Cardioverter Defibrillator in Arrhythmogenic Right Ventricular Cardiomyopathies. *Circulation,* 109**,** 1445-1447.

GABRIEL, S. B., SCHAFFNER, S. F., NGUYEN, H., MOORE, J. M., ROY, J., BLUMENSTIEL, B., HIGGINS, J., DEFELICE, M., LOCHNER, A. & FAGGART, M. 2002. The structure of haplotype blocks in the human genome. *Science,* 296**,** 2225-2229.

GARROD, D. & CHIDGEY, M. 2008. Desmosome structure, composition and function. *Biochimica et Biophysica Acta (BBA)-Biomembranes,* 1778**,** 572-587.

GELDENHUYS, G., GLANZMANN, B., LOMBARD, D., BOOLAY, S., CARR, J. & BARDIEN, S. 2014. *Identification of a common founder couple for 40 South African Afrikaner families with Parkinson's disease*.

GOVERNMENT, W. C. 2016. Western Cape Government facilities: Department of Cultural Affairs and Sport (DCAS) Facilities Web Map

GREEFF, J. M. 2007. Deconstructing Jaco: genetic heritage of an Afrikaner. *Annals of human genetics,* 71**,** 674-688.

GUELKE, L. 1988. The anatomy of a colonial settler population: Cape Colony 1657-1750. *The International Journal of African Historical Studies,* 21**,** 453-473.

HALUSKA, E. A., WHISTLER, S. J., BAKER JR, R. G. & CALFEE, R. V. 1989. Implantable cardiac stimulator for detection and treatment of ventricular arrhythmias. Google Patents.

HENDRICKS, N., WATKINS, D. A. & MAYOSI, B. M. 2010. Lessons from the first report of the arrhythmogenic right ventricular cardiomyopathy registry of South Africa. *Cardiovascular journal of Africa,* 21**,** 129.

HOFMANN, A., SIMON, A., GRKOVIC, T. & JONES, M. 2014. *Methods of molecular analysis in the life sciences*, Cambridge University Press.

MARCUS, F. I., MCKENNA, W. J., SHERRILL, D., BASSO, C., BAUCE, B., BLUEMKE, D. A., CALKINS, H., CORRADO, D., COX, M. G. P. J., DAUBERT, J. P., FONTAINE, G., GEAR, K., HAUER, R., NAVA, A., PICARD, M. H., PROTONOTARIOS, N., SAFFITZ, J. E., SANBORN, D. M. Y., STEINBERG, J. S., TANDRI, H., THIENE, G., TOWBIN, J. A., TSATSOPOULOU, A., WICHTER, T. & ZAREBA, W. 2010. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Proposed Modification of the Task Force Criteria,* 31**,** 806-814.

MBELE, M. 2014. *Molecular genetics of arrhythmogenic right ventricular and dilated cardiomyopathy in South Africans.* University of Cape Town.

MCKENNA, W. J., THIENE, G., NAVA, A., FONTALIRAN, F., BLOMSTROM-LUNDQVIST, C., FONTAINE, G. & CAMERINI, F. 1994. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. *British heart journal,* 71**,** 215.

MCNALLY, E., MACLEOD, H. & DELLEFAVE-CASTILLO, L. 2014. Arrhythmogenic right ventricular dysplasia/cardiomyopathy.

MERWE, J. V. D. ARCHIVES FOR THE PROMOTION OF HUMAN RIGHTS, TRANSPARENCY AND GOOD GOVERNANCE. 2016 NATIONAL ARCHIVES WEEK : 9-13 May 2016 2016.

MOOLMAN-SMOOK, J. C., DE LANGE, W. J., BRUWER, E. C., BRINK, P. A. & CORFIELD, V. A. 1999. The origins of hypertrophic cardiomyopathy–causing mutations in two South African subpopulations: a unique profile of both independent and founder events. *The American Journal of Human Genetics,* 65**,** 1308-1320.

MOSTERT, J. 2013. White cultural identity development in post-apartheid South Africa: A personal narrative. *Canadian Journal of Pentecostal-Charismatic Christianity,* 4**,** 44-60.

MYRES, N. M., WOODWARD, S. R. & HUTCHISON, L. A. 2014. Method and system for displaying genetic and genealogical data. Google Patents.

NCBI. 2016. *PCR* [Online]. Available: http://www.ncbi.nlm.nih.gov/genome/probe/doc/TechPCR.shtml.

NFSTC.ORG 2012. Primer Annealing Temperature.

NIH. 2011. *Types of Arrhythmia* [Online]. Available: http://www.nhlbi.nih.gov/health/health-topics/topics/arr/types#.

NTUSI, N. B. & MAYOSI, B. M. 2009. Epidemiology of heart failure in sub-Saharan Africa. *Expert review of cardiovascular therapy,* 7**,** 169-180.

NUSSBAUM, R. L., MCINNES, R. R. & WILLARD, H. F. 2015. *Thompson & Thompson genetics in medicine*, Elsevier Health Sciences.

PASQUIE, J.-L., HEDON, C., ALFALASI, O., THOMANN, S., MASSIN, F., CRANSAC, F., CUNG, T. & DAVY, J. 2013. Endurance sports precipitate phenotypic expression of Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) in asymptomatic PKP2-mutation carriers. *European Heart Journal,* 34**,** 1901.

PESCATORE, V., BASSO, C., BRUGIN, E., BIGON, L., VETTORI, M., ZANOCCO, A., NOVENTA, D., THIENE, G. & GIADA, F. 2013. Cardiovascular causes of disqualification from competitive sports in master athletes and long term follow-up. *European Heart Journal,* 34**,** 1900.

QUARTA, G. & ELLIOTT, P. M. 2012. Diagnostic Criteria for Arrhythmogenic Right Ventricular Cardiomyopathy. *Revista Española de Cardiología (English Edition),* 65**,** 599-605.

ROMERO, J., MEJIA-LOPEZ, E., MANRIQUE, C. & LUCARIELLO, R. 2013. Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC/D): A Systematic Literature Review. *Clinical Medicine Insights. Cardiology,* 7**,** 97-114.

ROTH, G. A., HUFFMAN, M. D., MORAN, A. E., FEIGIN, V., MENSAH, G. A., NAGHAVI, M. & MURRAY, C. J. L. 2015. Global and Regional Patterns in Cardiovascular Mortality From 1990 to 2013. *Circulation,* 132**,** 1667-1678.

SEN-CHOWDHRY, S., MORGAN, R. D., CHAMBERS, J. C. & MCKENNA, W. J. 2010. Arrhythmogenic cardiomyopathy: etiology, diagnosis, and treatment. *Annual review of medicine,* 61**,** 233-253.

SLIWA, K., DAMASCENO, A. & MAYOSI, B. M. 2005. Epidemiology and etiology of cardiomyopathy in Africa. *Circulation,* 112**,** 3577-3583.

SMITH-ZAGONE, M. J., PULLIAM, J. F. & FARKAS, D. H. 2007. Molecular pathology methods. *Molecular Pathology in Clinical Practice.* Springer.

SOUTHEY, N. 2001. *A dictionary of South African history*, New Africa Books.

STEFANSDOTTIR, V., JOHANNSSON, O. T., SKIRTON, H., TRYGGVADOTTIR, L., TULINIUS, H. & JONSSON, J. J. 2013. The use of genealogy databases for risk assessment in genetic health service: a systematic review. *Journal of Community Genetics,* 4**,** 1-7.

STENSON, P. D., MORT, M., BALL, E. V., HOWELLS, K., PHILLIPS, A. D., THOMAS, N. & COOPER, D. N. 2009. The human gene mutation database: 2008 update. *Genome Med,* 1**,** 13.

STRACHAN, T. & READ, A. 1999. Human molecular genetics. *An overview of mutation, polymorphism, and DNA repair*.

TE RIELE, A. S. J. M., BHONSALE, A., BURT, J. R., ZIMMERMAN, S. L. & TANDRI, H. 2012. Genotype-Specific Pattern of LV Involvement in ARVD/C. *JACC: Cardiovascular Imaging,* 5**,** 849-851.

TE RIELE, A. S. J. M., TANDRI, H. & BLUEMKE, D. A. 2014. Arrhythmogenic right ventricular cardiomyopathy (ARVC): cardiovascular magnetic resonance update. *Journal of Cardiovascular Magnetic Resonance,* 16**,** 50-50.

TEO, L. Y. L., MORAN, R. T. & TANG, W. H. W. 2015. Evolving Approaches to Genetic Evaluation of Specific Cardiomyopathies. *Current Heart Failure Reports,* 12**,** 339-349.

THIENE, G., NAVA, A., CORRADO, D., ROSSI, L. & PENNELLI, N. 1988. Right ventricular cardiomyopathy and sudden death in young people. *New England Journal of Medicine,* 318**,** 129-133.

TURRINI, P., BASSO, C., DALIENTO, L., NAVA, A. & THIENE, G. 2001. Is arrhythmogenic right ventricular cardiomyopathy a paediatric problem too? *Images in Paediatric Cardiology,* 3**,** 18-37.

WATKINS, D. A., HENDRICKS, N., SHABOODIEN, G., MBELE, M., PARKER, M., VEZI, B. Z., LATIB, A., CHIN, A., LITTLE, F., BADRI, M., MOOLMAN-SMOOK, J. C., OKREGLICKI, A., MAYOSI, B. M. & AFRICA, A. R. O. T. C. A. S. O. S. 2009. Clinical features, survival experience, and profile of plakophylin-2 gene mutations in participants of the arrhythmogenic right ventricular cardiomyopathy registry of South Africa. *Heart Rhythm,* 6**,** S10-7.

WILDE, A. A. M. & BEHR, E. R. 2013. Genetic testing for inherited cardiac disease. *Nat Rev Cardiol,* 10**,** 571-583.

WRENT, P., RIVAS, E.-M., PEINADO, J. M. & DE SILÓNIZ, M.-I. 2016. Development of an affordable typing method for Meyerozyma guilliermondii using microsatellite markers. *International Journal of Food Microbiology,* 217**,** 1-6.

WWW.REVOLVY.COM. 2016. *Boers* [Online]. Available: http://www.revolvy.com/main/index.php?s=Boers&stype=topics.

XU, T., YANG, Z., VATTA, M., RAMPAZZO, A., BEFFAGNA, G., PILLICHOU, K., SCHERER, S. E., SAFFITZ, J., KRAVITZ, J., ZAREBA, W., DANIELI, G. A., LORENZON, A., NAVA, A., BAUCE, B., THIENE, G., BASSO, C., CALKINS, H., GEAR, K., MARCUS, F. & TOWBIN, J. A. 2010. Compound and Digenic

Heterozygosity Contributes to Arrhythmogenic Right Ventricular Cardiomyopathy. *Journal of the American College of Cardiology,* 55**,** 587-597.

17. Appendices

Appendix 1: ARVC Task Force Criteria.

I. Global or regional dysfunction and structural alterations	
By 2D echocardiogram: Regional RV akinesia, dyskinesia, or aneurysm and 1 of the following (end diastole): PLAX RVOT ≥32 mm (corrected for body size [PLAX/BSA] ≥19 mm/m^2) PSAX RVOT ≥36 mm (corrected for body size [PSAX/BSA] ≥21 mm/m^2) or fractional area change ≤33% By MRI: Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and 1 of the following: Ratio of RV end-diastolic volume to BSA ≥110 ml/m^2 (male) or ≥100 ml/m^2 (female) or RV ejection fraction ≤40% By RV angiography: Regional RV akinesia, dyskinesia, or aneurysm	By 2D echocardiogram: Regional RV akinesia or dyskinesia and 1 of the following (end diastole): PLAX RVOT ≥29 to <32 mm (corrected for body size [PLAX/BSA] ≥16 to <19 mm/m^2) PSAX RVOT ≥32 to <36 mm (corrected for body size [PSAX/BSA] ≥18 to <21 mm/m^2) or fractional area change >33% to ≤40% By MRI: Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and 1 of the following: Ratio of RV end-diastolic volume to BSA ≥100 to <110 ml/m^2 (male) or ≥90 to <100 ml/m^2 (female) or RV ejection fraction >40% to ≤45%
II. Tissue characterisation of wall	
Residual myocytes <60% by morphometric analysis (or <50% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy	Residual myocytes 60% to 75% by morphometric analysis (or 50% to 65% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy
III. Repolarisation abnormalities	
Inverted T waves in right precordial leads (V1, V2, and V3) or beyond in individuals >14 years of age (in the absence of complete right bundle-branch block QRS ≥120 ms)	Inverted T waves in leads V1 and V2 in individuals >14 years of age (in the absence of complete right bundle-branch block) or in V4, V5, or V6 Inverted T waves in leads V1, V2, V3, and V4 in individuals >14 years of age in the presence of complete right bundle-branch block
IV. Depolarisation/conduction abnormalities	
Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V1 to V3)	Late potentials by SAECG in ≥1 of 3 parameters in the absence of a QRS duration of ≥110 ms on the standard ECG Filtered QRS duration (fQRS) ≥114 ms Duration of terminal QRS <40 mV (low-amplitude signal duration) ≥38 ms Root-mean-square voltage of terminal 40 ms ≤20 mV Terminal activation duration of QRS ≥55 ms measured from the nadir of the S wave to the end of the QRS, including R0, in V1, V2, or V3, in the absence of complete right bundle-branch block
V. Arrhythmias	
Nonsustained or sustained ventricular tachycardia of left bundle-branch morphology with superior axis (negative or indeterminate QRS in leads II, III, and aVF and positive in lead aVL)	Nonsustained or sustained ventricular tachycardia of RV outflow configuration, left bundle-branch block morphology with inferior axis (positive QRS in leads II, III, and aVF and negative in lead aVL) or of unknown axis >500 ventricular extrasystoles per 24 hours (Holter)
VI. Family history	
ARVC/D confirmed in a first-degree relative who meets current Task Force criteria ARVC/D confirmed pathologically at autopsy or surgery in a first-degree relative Identification of a pathogenic mutation categorised as associated or probably associated with ARVC/D in the patient under evaluation	History of ARVC/D in a first-degree relative in whom it is not possible or practical to determine whether the family member meets current Task Force criteria Premature sudden death (<35 years of age) due to suspected ARVC/D in a first-degree relative ARVC/D confirmed pathologically or by current Task Force Criteria in second-degree relative

RV – Right Ventricle; PLAX – Parasternal Long Axis; RVOT – Right Ventricular Outflow Tract; PSAX – Parasternal Short Axis; BSA – body surface area; MRI – Magnetic Resonance Imaging; SAECG – Signal-averaged electrocardiogram; ECG – Electrocardiogram; aVR – Augmented ventricular right; aVL – Augmented ventricular left; ARVC/D – Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia

(Adapted from: (Marcus et al., 2010))

Appendix 2: Informed consent form and Request for Molecular Studies (DNA)

A) Informed Consent Form (English Version)

UNIVERSITY OF CAPE TOWN

Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa

The Cardiac Clinic
E25/87 New Groote Schuur Hospital
Anzio Road, Observatory, 7935, Cape Town, South Africa
Tel: 27-21-447 2777
Fax: 27-21-447 2765

Informed Consent Form

I agree to participate in the study of Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), a heart muscle disease (cardiomyopathy) that predominantly affects the right ventricle and may lead to ventricular arrhythmias and heart failure. I understand that I will be interviewed about my medical history, family history and medications, and that I may undergo further cardiac investigations. However, I will only undergo those investigations necessary to confirm or exclude the diagnosis of ARVC. In addition, I will have a blood sample drawn consisting of 5 tubes for a total of 25 ml of blood. This blood will be used to test for & study the genetic factors that cause ARVC. After my initial consultation, I agree to be followed up annually to assess if there have been any changes in my medical history and medications. As ARVC is a familial disease in 30-50% of people, I agree to have my first-degree relatives contacted for clinical screening & participation in this study. However, each of them will be counselled about ARVC and will also have to sign a consent form before they will be included in this study.

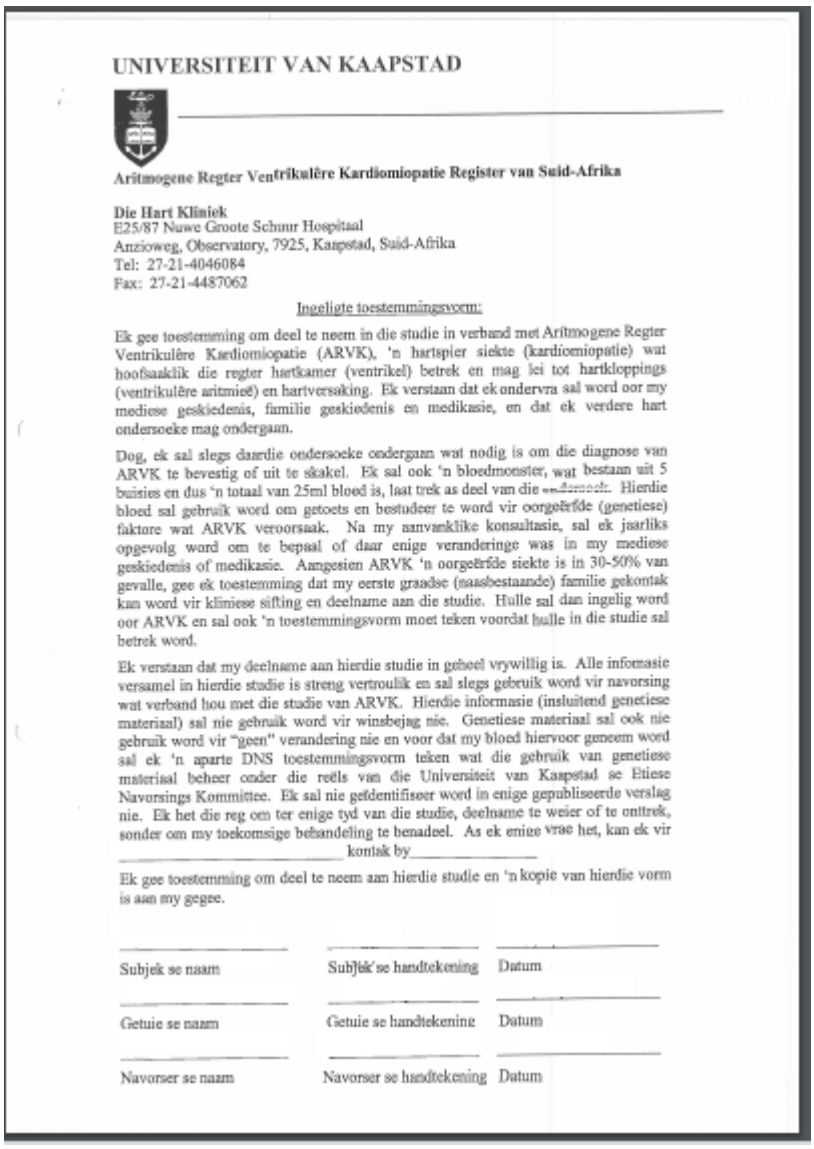
I understand that my participation in this study is entirely voluntary. All information gathered in this study is strictly confidential, and will only be used for research relating to the study of ARVC. This information (including genetic material) will not be used to generate a profit. As well, genetic material will not be used for the purpose of gene alteration, and, prior to blood sampling, I will sign a separate DNA consent form that governs the use of genetic material under the rules of the University of Cape Town Research Ethics Committee. I will not be identified in any published report. I am free to refuse to participate or withdraw from the study at any time, without jeopardising my future care. If I have any questions, I may contact _____ at _____.

I agree to participate in the study and I have been given a copy of this form.

Subject name	Subject signature	Date
Witness name	Witness signature	Date
Investigator name	Investigator signature	Date

ARVCprotocolfeb2009.ver1.0

B) Informed Consent Form (AfrikaansVersion)



UNIVERSITEIT VAN KAAPSTAD

Aritmogene Regter Ventrikulêre Kardiomiopatie Register van Suid-Afrika

Die Hart Kliniek
E25/87 Nuwe Groote Schuur Hospitaal
Anzioweg, Observatory, 7925, Kaapstad, Suid-Afrika
Tel: 27-21-4046084
Fax: 27-21-4487062

Ingeligte toestemmingsvorm:

Ek gee toestemming om deel te neem in die studie in verband met Aritmogene Regter Ventrikulêre Kardiomiopatie (ARVK), 'n hartspier siekte (kardiomiopatie) wat hoofsaaklik die regter hartkamer (ventrikel) betrek en mag lei tot hartkloppings (ventrikulêre aritmieë) en hartversaking. Ek verstaan dat ek ondervra sal word oor my mediese geskiedenis, familie geskiedenis en medikasie, en dat ek verdere hart ondersoeke mag ondergaan.

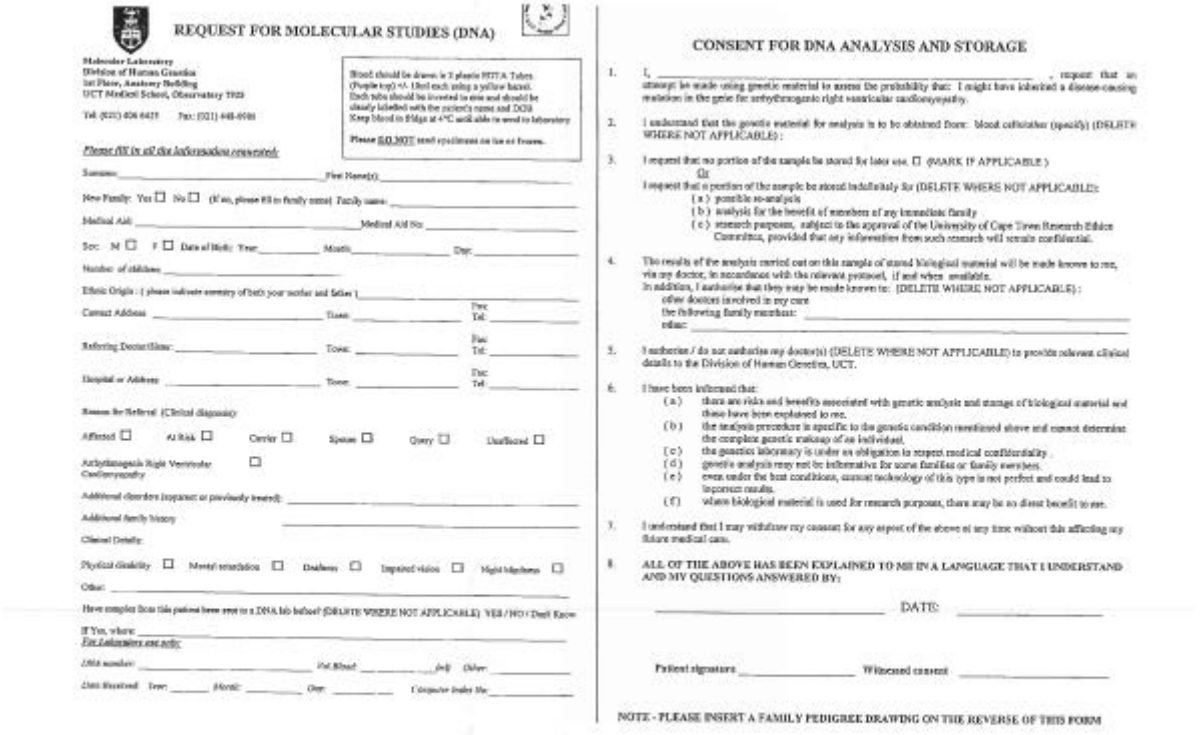
Dog, ek sal slegs daardie ondersoeke ondergaan wat nodig is om die diagnose van ARVK te bevestig of uit te skakel. Ek sal ook 'n bloedmonster, wat bestaan uit 5 buisies en dus 'n totaal van 25ml bloed is, laat trek as deel van die ondersoek. Hierdie bloed sal gebruik word om getoets en bestudeer te word vir oorgeërfde (genetiese) faktore wat ARVK veroorsaak. Na my aanvanklike konsultasie, sal ek jaarliks opgevolg word om te bepaal of daar enige veranderinge was in my mediese geskiedenis of medikasie. Aangesien ARVK 'n oorgeërfde siekte is in 30-50% van gevalle, gee ek toestemming dat my eerste graadse (naasbestaande) familie gekontak kan word vir kliniese sifting en deelname aan die studie. Hulle sal dan ingelig word oor ARVK en sal ook 'n toestemmingsvorm moet teken voordat hulle in die studie sal betrek word.

Ek verstaan dat my deelname aan hierdie studie in geheel vrywillig is. Alle informasie versamel in hierdie studie is streng vertroulik en sal slegs gebruik word vir navorsing wat verband hou met die studie van ARVK. Hierdie informasie (insluitend genetiese materiaal) sal nie gebruik word vir winsbejag nie. Genetiese materiaal sal ook nie gebruik word vir "geen" verandering nie en voor dat my bloed hiervoor geneem word sal ek 'n aparte DNS toestemmingsvorm teken wat die gebruik van genetiese materiaal beheer onder die reëls van die Universiteit van Kaapstad se Etiese Navorsings Kommittee. Ek sal nie geïdentifiseer word in enige gepubliseerde verslag nie. Ek het die reg om ter enige tyd van die studie, deelname te weier of te onttrek, sonder om my toekomsige behandeling te benadeel. As ek enige vrae het, kan ek vir _____ kontak by_____

Ek gee toestemming om deel te neem aan hierdie studie en 'n kopie van hierdie vorm is aan my gegee.

_____	_____	_____
Subjek se naam	Subjek se handtekening	Datum
_____	_____	_____
Getuie se naam	Getuie se handtekening	Datum
_____	_____	_____
Navorser se naam	Navorser se handtekening	Datum

C) Request for molecular studies form (English Version)



REQUEST FOR MOLECULAR STUDIES (DNA)

Molecular Laboratory
Division of Human Genetics
1st Floor, Anatomy Building
UCT Medical School, Observatory 7925

Tel: (021) 406 6425 Fax: (021) 448-6980

Blood should be drawn in 3 plastic EDTA Tubes (Purple top) +/- 10ml each using a yellow barrel. Each tube should be inverted to mix and should be clearly labelled with the patient's name and DOB. Keep blood in fridge at +4°C until able to send to laboratory

Please DO NOT send specimens on ice or frozen.

Please fill in all the information requested:

Surname: First Name(s):

New Family: Yes ☐ No ☐ (If no, please fill in family name) Family name:

Medical Aid: Medical Aid No:

Sex: M ☐ F ☐ Date of Birth: Year: Month: Day:

Number of children:

Ethnic Origin : (please indicate country of birth your mother and father)

Contact Address: Town: Fax: Tel:

Referring Doctor/Clinic: Town: Fax: Tel:

Hospital or Address: Town: Fax: Tel:

Reason for Referral (Clinical diagnosis)

Affected ☐ At Risk ☐ Carrier ☐ Spouse ☐ Query ☐ Unaffected ☐

Arrhythmogenic Right Ventricular Cardiomyopathy ☐

Additional disorders (apparent or previously treated):

Additional family history

Clinical Details:

Physical disability ☐ Mental retardation ☐ Deafness ☐ Impaired vision ☐ Night blindness ☐

Other:

Have samples from this patient been sent to a DNA lab before? (DELETE WHERE NOT APPLICABLE) YES / NO / Don't Know

If Yes, where:

For Laboratory use only:

DNA number: Pat Blood: (ml) Other:

Date Received: Year: Month: Day: Computer Index No:

CONSENT FOR DNA ANALYSIS AND STORAGE

1. I, _____, request that an attempt be made using genetic material to assess the probability that: I might have inherited a disease-causing mutation in the gene for arrhythmogenic right ventricular cardiomyopathy.

2. I understand that the genetic material for analysis is to be obtained from: blood cell/other (specify) (DELETE WHERE NOT APPLICABLE):

3. I request that no portion of the sample be stored for later use. ☐ (MARK IF APPLICABLE)
   Or
   I request that a portion of the sample be stored indefinitely for (DELETE WHERE NOT APPLICABLE):
   (a) possible re-analysis
   (b) analysis for the benefit of members of my immediate family
   (c) research purposes, subject to the approval of the University of Cape Town Research Ethics Committee, provided that any information from such research will remain confidential.

4. The results of the analysis carried out on this sample of stored biological material will be made known to me, via my doctor, in accordance with the relevant protocol, if and when available.
   In addition, I authorise that they may be made known to: (DELETE WHERE NOT APPLICABLE):
   other doctors involved in my care
   the following family members: _____
   other: _____

5. I authorise / do not authorise my doctor(s) (DELETE WHERE NOT APPLICABLE) to provide relevant clinical details to the Division of Human Genetics, UCT.

6. I have been informed that:
   (a) there are risks and benefits associated with genetic analysis and storage of biological material and these have been explained to me.
   (b) the analysis procedure is specific to the genetic condition mentioned above and cannot determine the complete genetic makeup of an individual.
   (c) the genetics laboratory is under an obligation to respect medical confidentiality.
   (d) genetic analysis may not be informative for some families or family members.
   (e) even under the best conditions, current technology of this type is not perfect and could lead to incorrect results.
   (f) where biological material is used for research purposes, there may be no direct benefit to me.

7. I understand that I may withdraw my consent for any aspect of the above at any time without this affecting my future medical care.

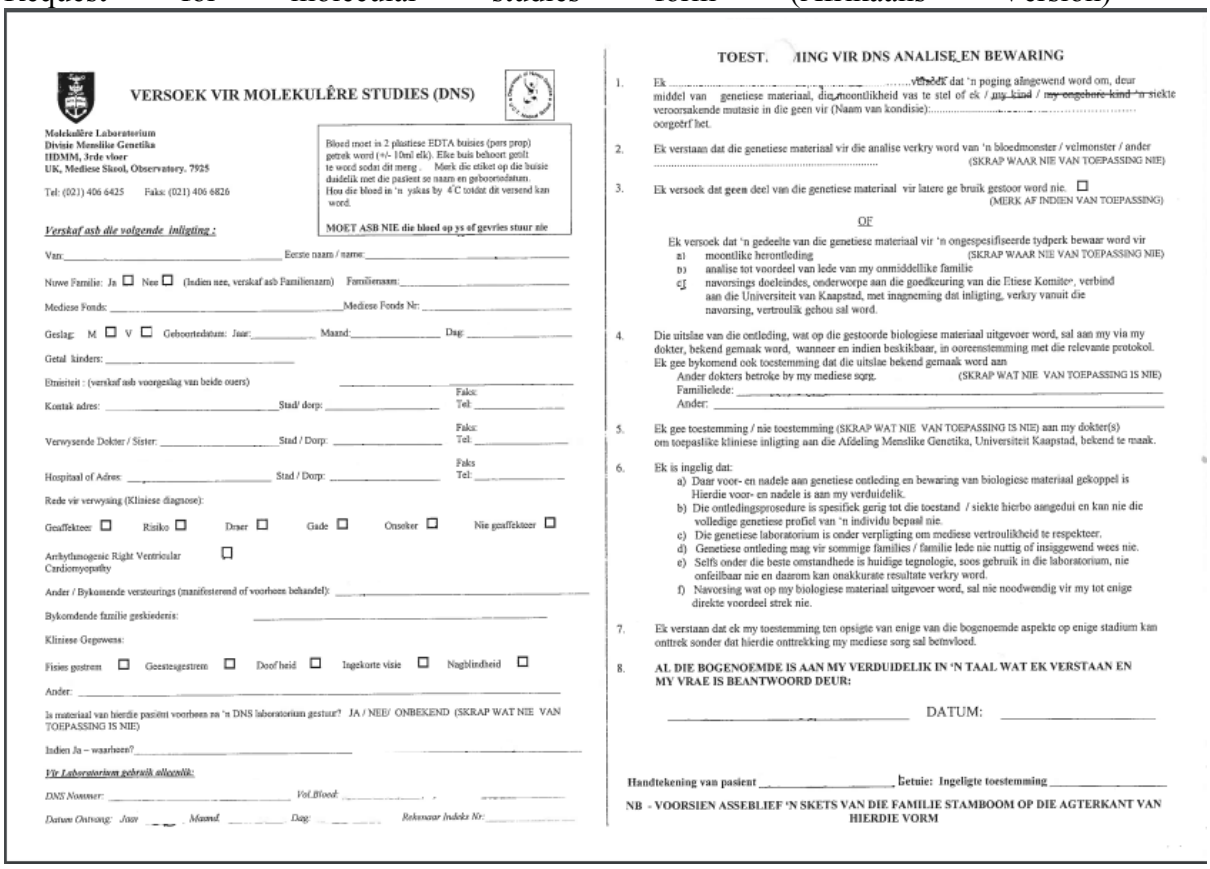
8. ALL OF THE ABOVE HAS BEEN EXPLAINED TO ME IN A LANGUAGE THAT I UNDERSTAND AND MY QUESTIONS ANSWERED BY:

_____ DATE: _____

Patient signature _____ Witnessed consent _____

NOTE - PLEASE INSERT A FAMILY PEDIGREE DRAWING ON THE REVERSE OF THIS FORM

D) Request for molecular studies form (Afrikaans Version)



VERSOEK VIR MOLEKULÊRE STUDIES (DNS)

Molekulêre Laboratorium
Divisie Menslike Genetika
HDMM, 3rde vloer
UK, Mediese Skool, Observatory. 7925

Tel: (021) 406 6425 Faks: (021) 406 6826

Bloed moet in 2 plastiese EDTA buisies (pers prop) getrek word (+/- 10ml elk). Elke buis behoort gelit te word sodat dit meng . Merk die etiket op die buisie duidelik met die pasient se naam en geboortedatum. Hou die bloed in 'n yskas by 4°C totdat dit versend kan word.

MOET ASB NIE die bloed op ys of gevries stuur nie

Verskaf asb die volgende inligting :

Van:_____ Eerste naam / name:_____

Nuwe Familie: Ja ☐ Nee ☐ (Indien nee, verskaf asb Familienaam) Familienaam:_____

Mediese Fonds: _____Mediese Fonds Nr: _____

Geslag: M ☐ V ☐ Geboortedatum: Jaar:_____ Maand:_____ Dag: _____

Getal kinders: _____

Etnisiteit : (verskaf asb voorgeslag van beide ouers) _____

Kontak adres: _____Stad/ dorp: _____ Faks: Tel: _____

Verwysende Dokter / Sister: _____Stad / Dorp: _____ Faks: Tel: _____

Hospitaal of Adres: _____ Stad / Dorp: _____ Faks Tel: _____

Rede vir verwysing (Kliniese diagnose):

Geaffekteer: ☐ Risiko ☐ Draer ☐ Gade ☐ Onseker ☐ Nie geaffekteer ☐

Arrhythmogenic Right Ventricular Cardiomyopathy ☐

Ander / Bykomende verstoorings (manifesterend of voorheen behandel): _____

Bykomende familie geskiedenis: _____

Kliniese Gegewens:

Fisies gestrem ☐ Geestesgestrem ☐ Doofheid ☐ Ingekorte visie ☐ Naglindheid ☐

Ander: _____

Is materiaal van hierdie pasiënt voorheen na 'n DNS laboratorium gestuur? JA / NEE/ ONBEKEND (SKRAP WAT NIE VAN TOEPASSING IS NIE)

Indien Ja – waarheen?_____

Vir Laboratorium gebruik alleenlik:

DNS Nommer: _____ *Vol.Blood:* _____

Datum Ontvang: Jaar ____ *Maand.* _____ *Dag:* ____ *Rekenaar Indeks Nr:* _____

TOEST. ...IING VIR DNS ANALISE EN BEWARING

1. Ek ..... versoek dat 'n poging aangewend word om, deur middel van genetiese materiaal, die moontlikheid vas te stel of ek / my kind / ~~my ongebore kind~~ 'n siekte veroorsakende mutasie in die geen vir (Naam van kondisie):..... oorgeërf het.

2. Ek verstaan dat die genetiese materiaal vir die analise verkry word van 'n bloedmonster / velmonster / ander ..... (SKRAP WAAR NIE VAN TOEPASSING NIE)

3. Ek versoek dat **geen** deel van die genetiese materiaal vir latere ge bruik gestoor word nie. ☐ (MERK AF INDIEN VAN TOEPASSING)

   OF

   Ek versoek dat 'n gedeelte van die genetiese materiaal vir 'n ongespesifiseerde tydperk bewaar word vir
   a) moontlike herontleding (SKRAP WAAR NIE VAN TOEPASSING NIE)
   b) analise tot voordeel van lede van my onmiddellike familie
   c) navorsings doeleindes, onderworpe aan die goedkeuring van die Etiese Komitee, verbind aan die Universiteit van Kaapstad, met inagneming dat inligting, verkry vanuit die navorsing, vertroulik gehou sal word.

4. Die uitslae van die ontleding, wat op die gestoorde biologiese materiaal uitgevoer word, sal aan my via my dokter, bekend gemaak word, wanneer en indien beskikbaar, in ooreenstemming met die relevante protokol. Ek gee bykomend ook toestemming dat die uitslae bekend gemaak word aan
   Ander dokters betroke by my mediese sorg. (SKRAP WAT NIE VAN TOEPASSING IS NIE)
   Familielede: _____
   Ander: _____

5. Ek gee toestemming / nie toestemming (SKRAP WAT NIE VAN TOEPASSING IS NIE) aan my dokter(s) om toepaslike kliniese inligting aan die Afdeling Menslike Genetika, Universiteit Kaapstad, bekend te maak.

6. Ek is ingelig dat:
   a) Daar voor- en nadele aan genetiese ontleding en bewaring van biologiese materiaal gekoppel is Hierdie voor- en nadele is aan my verduidelik.
   b) Die ontledingsprosedure is spesifiek gerig tot die toestand / siekte hierbo aangedui en kan nie die volledige genetiese profiel van 'n individu bepaal nie.
   c) Die genetiese laboratorium is onder verpligting om mediese vertroulikheid te respekteer.
   d) Genetiese ontleding mag vir sommige families / familie lede nie nuttig of insiggewend wees nie.
   e) Selfs onder die beste omstandhede in huidige tegnologie, soos gebruik in die laboratorium, nie onfeilbaar nie en daarom kan onakkurate resultate verkry word.
   f) Navorsing wat op my biologiese materiaal uitgevoer word, sal nie noodwendig vir my tot enige direkte voordeel strek nie.

7. Ek verstaan dat ek my toestemming ten opsigte van enige van die bogenoemde aspekte op enige stadium kan onttrek sonder dat hierdie onttrekking my mediese sorg sal benvloed.

8. **AL DIE BOGENOEMDE IS AAN MY VERDUIDELIK IN 'N TAAL WAT EK VERSTAAN EN MY VRAE IS BEANTWOORD DEUR:**

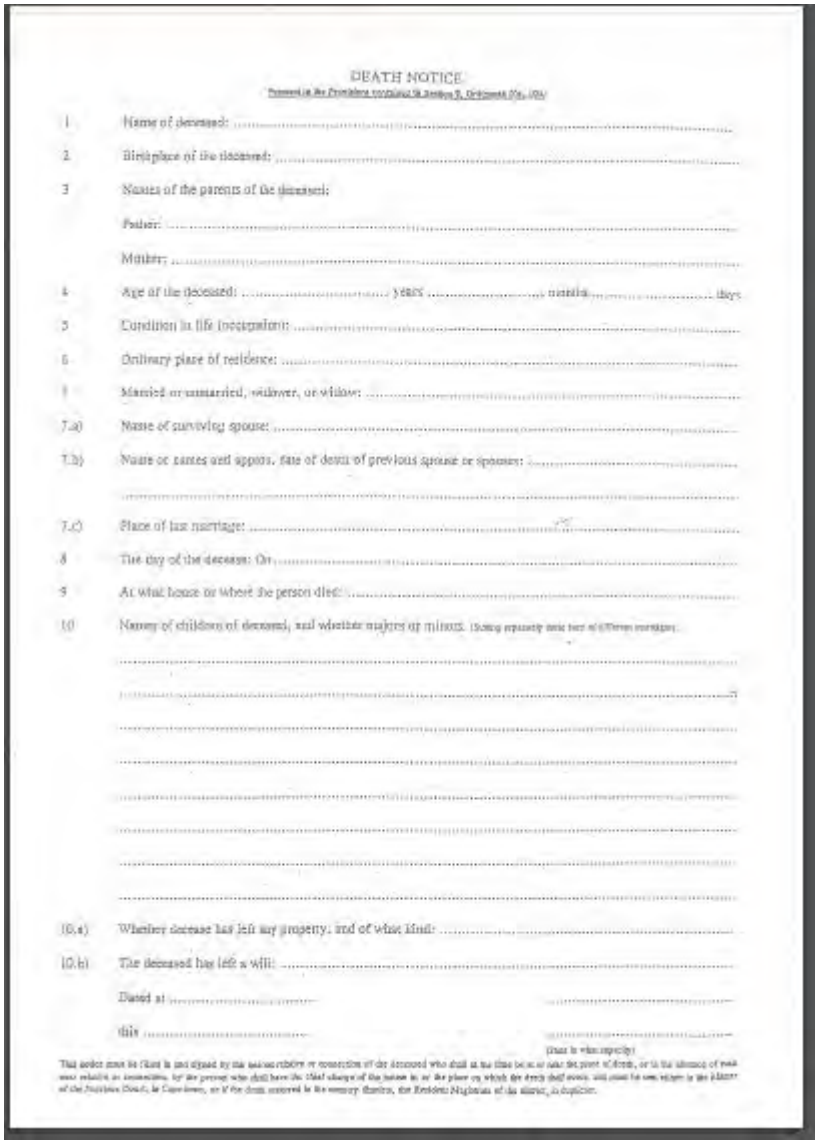
_____ DATUM: _____

Handtekening van pasient _____ **Getuie: Ingeligte toestemming** _____

NB - VOORSIEN ASSEBLIEF 'N SKETS VAN DIE FAMILIE STAMBOOM OP DIE AGTERKANT VAN HIERDIE VORM

Appendix 3: Template Death notice form and Archive requisition forms

A) Death notice form



DEATH NOTICE

1 Name of deceased: .....

2 Birthplace of the deceased: .....

3 Names of the parents of the deceased:

Father: .....

Mother: .....

4 Age of the deceased: ..... years ..... months ..... days

5 Condition in life (occupation): .....

6 Ordinary place of residence: .....

7 Married or unmarried, widower, or widow: .....

7.a) Name of surviving spouse: .....

7.b) Name or names and approx. date of death of previous spouse or spouses: .....

.....

7.C) Place of last marriage: .....

8 The day of the decease: On .....

9 At what house or where the person died: .....

10 Names of children of deceased, and whether majors or minors. (Stating separately sons born of different marriages)

.....

.....

.....

.....

.....

.....

.....

.....

10.a) Whether deceased has left any property, and of what kind: .....

10.b) The deceased has left a will: .....

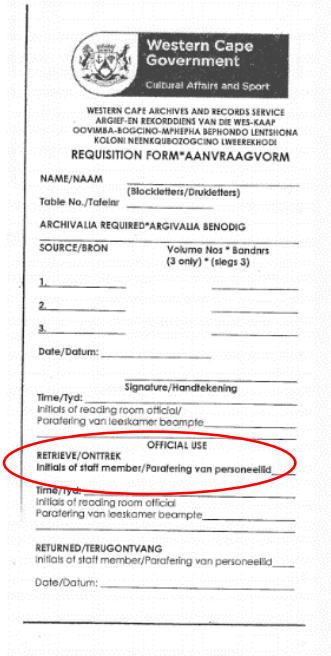
Dated at ....

this ....

(Date in words especially)

This notice must be filled in and signed by the nearest relative or connection of the deceased who shall at the time be at or near the place of death, or in the absence of such near relative or connection, by the person who shall have the chief charge of the house in or the place on which the death shall occur. And must be sent either to the Registrar of the Provincial Court, in Cape Town, or if the death occurred in the country districts, the Resident Magistrate of the district, in duplicate.

B) Requisition forms from the Western Cape Archives (English version). Must be signed in the area circled in red, in order to retrieve documents from the 100 year ban.



Western Cape Government
Cultural Affairs and Sport

WESTERN CAPE ARCHIVES AND RECORDS SERVICE
ARGIEF-EN REKORDDIENS VAN DIE WES-KAAP
OOVIMBA-BOGCINO-MPHEPHA BEPHONDO LENTSHONA KOLONI NEENKQUBOZOGCINO LWEEREKHODI

REQUISITION FORM*AANVRAAGVORM

NAME/NAAM _____
(Blockletters/Druiletters)
Table No./Tafelnr _____

ARCHIVALIA REQUIRED*ARGIVALIA BENODIG

SOURCE/BRON — Volume Nos * Bandnrs (3 only) * (slegs 3)

1. _____ _____
2. _____ _____
3. _____ _____

Date/Datum: _____

_____
Signature/Handtekening

Time/Tyd: _____
Initials of reading room official/
Parafering van leeskamer beampte _____

OFFICIAL USE

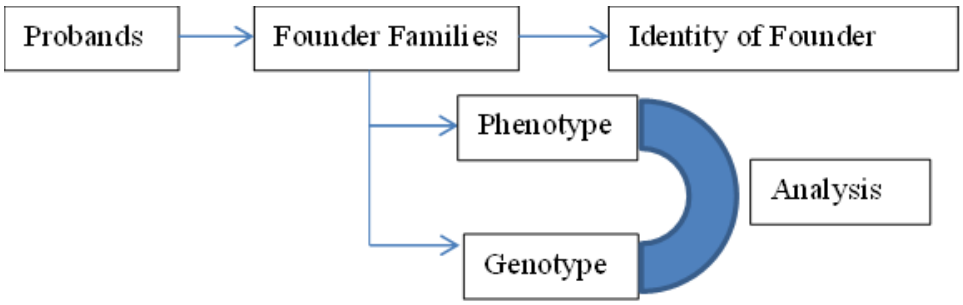
RETRIEVE/ONTTREK
Initials of staff member/Parafering van personeellid ____

Time/Tyd: _____
Initials of reading room official
Parafering van leeskamer beampte _____

RETURNED/TERUGONTVANG
Initials of staff member/Parafering van personeellid ____

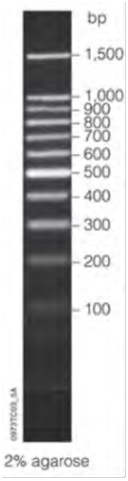
Date/Datum: _____

Appendix 4: Diagrammatic Representation of Research outline



Footnote: The 'Phenotype' indicates the participants clinically screened and classified to be affected by ARVC. They are further genetically screened and satisfy the relevant genotyping diagnostic criteria for the 3 microsatellite markers, M1, D12S1692 and M2, and the 3 exonic regions of interest, *PKP2* Exon 4 and 11; and pedigrees are extended for genealogical tracing, used to track the founder. See more in 'Methods' section 10.

Appendix 5: Gel electrophoresis ladder



100 bp DNA LADDER (PROMEGA)

Appendix 6: Sequencing Reaction and Analysis Protocol Reaction

Reagent	Volume per reaction
Exonuclease I	0.1 µl
SAP (Shrimp Alkaline Phosphatase)	1 µl
HRM Product	5 µl
Final Reaction Volume (made up to 20 µl with dH2O)	20 µl

Table 18: *EXOSAP Reagents Mix.*

CONDITION	TEMPERATURE & TIME
Incubation	37°C – 1 hour
Deactivation	75°C – 15 minutes
Cooling (Optional)	4°C – 30 minutes

Table 19: *EXOSAP PCR Conditions.*

Reagent	Volume per reaction
Forward Primer	0.5 µl
Termination Buffer	2 µl
Sequencing Buffer	0.1 µl
EXOSAP HRM Product	3 µl
Final Reaction Volume (made up to 20 µl with dH2O)	20 µl

Table 20: *Big Dye Terminator Sequencing Reaction Mix.*

CONDITION	TEMPERATURE & TIME
Initial denaturation	96°C - 5 minutes
Denaturation	96°C - 30 seconds
Primer Annealing	50°C - 15 seconds 50 cycles
Extension	60°C - 4 minutes
Cooling (Optional)	4°C - 30 minutes

Table 21: *Big Dye Terminator Sequencing Reaction Conditions.*

DNA Sequencing Analysis

After you get the data from Stellenbosch, open the file and the follow these steps:

1) In your annotation document, select your amplicon from the start of fwd primer to the end of rev primer and copy this to a new word document.
2) Remove all spaces and "^" from the sequence as well as all numbers and rs numbers. (The whole sequence should be just one word).
3) Then copy this sequence to note pad and save it in the same folder as the sequencing file.
4) Open the chromatogram using the **BioEdit Sequence Alignment Editor**. Then open the window called **"DNA Sequence Form"** and click on **"File"** on the top left of the window.
5) Then scroll to import and import your note pad file on the DNA sequence.
6) Then select both (your file and notepad seq) and on top click on "**Accessory Application"** where you will select **ClustalW** multiple alignment.
7) Then click on run **"ClustalW"**. You will get another window which will have both your sequences aligned on each other.
8) Click on **"shade identities"** (icon) to color the sequences so that only changes will be left uncolored, this makes it easy for viewing.
9) In the chromatogram, start looking for changes and find these positions in the annotation file.
10) Open "ensembl" **(http://www.ensembl.org/index.html)** and select "human" and your gene of interest. Once that opens, select the transcript from the list of transcripts. Once you do that, on the left hand pane, you will see option to get cDNA of your gene..
11) In the cDNA sequence, find the change that you see in the chromatogram. Then calculate the cDNA nomenclature (middle line) and name it as c.(number)original base>new base and for the protein change p.(original aa)(position) (new aa).
12) Report rs number (if there is one), there may also be a link to the dbSNP **(http://www.ncbi.nlm.nih.gov/projects/SNP/)**database next to the rs number explanation.
13) If there is not rs number reported in ensemble **(http://www.ensembl.org/index.html)**, check 1000genomes **(http://browser.1000genomes.org/index.html)**, exome variant server **(http://evs.gs.washington.edu/EVS/)**and exac database

(http://exac.broadinstitute.org/) based on the position of amino acid change or cDNA position.

For your convenience, the base changes in the chromatogram are given below:

R- A, G

Y- C, T

M- A, C

K- G, T

S- C, G

W- A, T

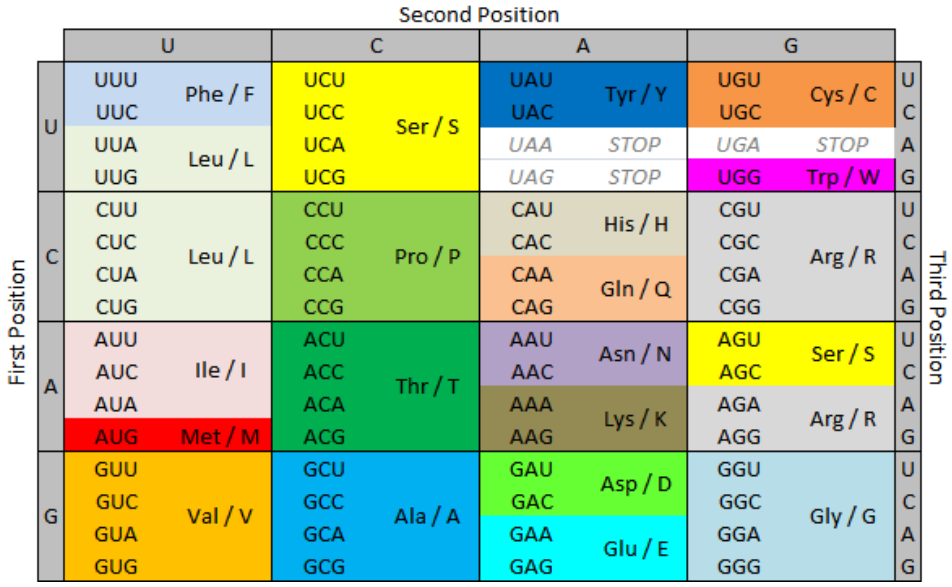
H- A, C, T

B- C, G, T

V- A, C, G

D- A, G, T

N- A, C, G, T



Appendix 7: The two main theories of doubling ancestors

A. Pyramid Theory of Doubling Ancestors

SELF

2 PARENTS

4 GRANDPARENTS

8 GREAT-GRANDPARENTS

16 GREAT-GREAT-GRANDPARENTS

32 GREAT-GREAT-GREAT-GRANDPARENTS

64 GREAT-GREAT-GREAT-GREAT-GRANDPARENTS

128 GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENTS

256 GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENT

512 GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENTS

1024 GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENTS

In this theory the number of ancestors double each generation. It cannot represent the rest of the generations if a population has a higher frequency of homogeneity and so following is the number of theoretical ancestors in each generation, starting at Generation 12 where the figure above leaves off. Depending on the population group, mostly some population start with a few founding members and then grow, so from Geldenhuys' (2014) work in medical genealogy in Afrikaners, often their ancestors begin to mimic the diamond model below (from the 10^{th} generation).

Gen. 12: 2048

Gen. 13: 4096

Gen. 14: 8192

Gen. 15: 16384

Gen. 16: 32768

B. Diamond Theory of Ancestors

In this theory the pyramid begins to narrow beyond the 10th generation; with x representing the number of individuals in each generation. However, in reality, several ancestors will be repeated in Afrikaner families, so they will be counted only once.

SELF
2 PARENTS
4 GRANDPARENTS
8 GREAT-GRANDPARENTS
16 GREAT-GREAT-GRANDPARENTS
32 GREAT-GREAT-GREAT-GRANDPARENTS
64 GREAT-GREAT-GREAT-GREAT-GRANDPARENTS
128 GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENTS
256 GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENT
512 GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENTS
1024 GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENTS
x G-G-GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENTS
x G-G-G-G-G-GREAT-GREAT-GREAT-GREAT-GREAT-GRANDPARENTS
x G-G-G-G-G-G-G-G-GREAT-GREAT-GREAT-GRANDPARENTS
x-G-G-G-G-G-G-G-G-G-G-G-GREAT-GRANDPARENTS

Adapted from: http://www.olivetreegenealogy.com/misc/ancestors.shtml

Appendix 8: South African Genealogical Tracing Protocol

Standard Operating Procedure (SOP) for genetic Genealogy:

1. ***Resources and records available for South African Genealogical Research***

a) Databases; such as

(i) FamilySearch.org: This is an online database/platform provided by a genealogy organization operated by The Church of Jesus Christ of Latter-day Saints. It was previously known as the Genealogical Society of Utah (or "GSU") (established 1894) and is the largest genealogy organization in the world.

(ii) NAAIRS (National Automated Archival Information Retrieval System): This is an online database/platform provided by the Department of Arts and Culture, which helps users of archives to identify and locate archival material in SA. NAAIRS contains only information about archival material, and not the actual texts of the documents. The texts are collected from the respective archives; under the references gathered from NAAIRS.

b) Types of Records Available (restricted by 100 year ban PAIA (for birth records) and 20 year ban (for marriages and death records); (for further details see: Section 10.14 South African Public Records and Archival Considerations)):

(i) Certificates (if first degree relative of person of interest; from the Department of Home Affairs)

(ii) Baptisms (from church of baptism; often have a central archival repository)

(iii) Marriages (from church of marriage; often have a central archival repository)

(iv) Deaths and Burials (first search online, if not online; locate or visit cemetery of burial)

(v) Death Notices (Accessing death notices attached to estate files are dependent on province; for example, in the Western Cape (WC): deaths up till 1979 are in the WC National Archive (records up till 1996 are in transit to the WC National Archives), deaths after 1996 are in the Vault Section of the Master's Office, and deaths after 2008 consult WC Master of the High Court).

(vi) Divorce (often referenced in NAAIRS database)

(vii) Wills (often attached with death notices, in the estate files or include title deeds, which can provide inheritance information of properties such as land property, homes or farms; which can be accessed from the local deeds office for a fee (Source: https://www.westerncape.gov.za/service/title-deeds-proof-property-ownership)

(viii) Inventories

(ix) Passenger Lists (often in the archives private collections, museums or transcribed into books)

(x) Monumental Inscriptions

(xi) Family Bibles (often at GISA)

(xii) Oral History (Often given by informant or reported in media)

(xiii) Church Minute Books

(xiv) Military Records (only available from the military museums and repositories)

(xv) Newspapers (often report personal details and orbituaries)

(xvi) Publications

(xvii) Research Assistance

(xviii) Professional Researchers

(xix) Online Guides and Resources

(xx) Reference Books and Published genealogical tables

(xxi) South African Genealogical Societies

(xxii) Starting dates for South African Church Registers (often in church periodicals or encyclopaedia of SA)

(xxiii) Word list – Glossary (Afrikaans to English) (often in books published after 2013)

2. Preparation steps:

a) Genetically test family of interest to discover eldest probands(s)/progenitor of disease

b) i) Draw family pedigree of all the affected, ii) add patient interview details (including parent, grandparents and further generations) and iii) provide dates and places of birth, christening, marriage and death.

c) In order to get a family search started for research purposes, establish whether or not the family has been researched before. Hence, read the 17 volumes of the SAGs (South African Genealogies) or 34 volumes of SAFs (South African Family Registers).

d) In order to read the South African literature, one must familiarize with:

   i) the Pama/De Villiers System (the South African gold standard for family/ genealogical research.

      For example: In this system the genealogical number of the South African Stamouer/progenitor is "a", and his children are numbered chronologically "b1", "b2", "b3" etc.; his grandchildren are the 'c" generation, great grandchildren "d" and so on. (e.g. b1c3d5e2f5g8 etc.);

   ii) some Afrikaans and Dutch terms (suggested to use Google Translate, until you get the hang of things);

   iii) the Abbreviations and Symbols used in South African Genealogy.

   For example:

1. *Born/Geboorte
2. ≈ Christened or Bapt./Doop
3. † Died/Sterfte
4. Ω Buried/Graf of begrafnis
5. ω Cremated/Veras
6. x Marriage/Huwelik
7. xx Second Marriage, etc/Tweede Huwelik
8. ÷ Divorced/Egskeiding
9. s.o./s.v. Son of/Seun van
10. d.o./d.v. Daughter of/Dogter van
11. wed Widow/ Weduwee
12. wew Widower/Wewenaar
13. ca About Date (Circa)/Ongeveer datum
14. ? Estimated Date/Geskatte datum
15. ≡ Calculated date/Berekende datum

16. < Before date/Voor datum
17. ca.After date/Na Datum
18. / Between dates/Tussen Datums
19. NN Name Unknown/Van onbekend
20. Pn Given names unknown/ Voorname onbekend
21. sp Without descendants/Sonder nasate (sine prole)
22. [ ] Related by marriage/Aangetroudes
23. {Title of person/Titel van person
24. a.Arrival in South Africa/Aankoms in Suid Afrika

e) Learn traditional naming patterns for first names:
Afrikaans families' children often named according to European tradition, including the Netherlands and Germany. This tradition was less so in England. It seems to be most common from about the mid 1700's to the first part of the 20th Century.

For example:
The first Son was named after the Father's father
The second Son was named after the Mother's father
The third Son was named after the Father
The fourth Son was named after the Father's eldest brother
The fifth Son was named after the Mother's eldest brother
The first Daughter was named after the Mother's mother
The second Daughter was named after the Father's mother
The third Daughter was named after the Mother
The fourth Daughter was named after the Mother's eldest sister
The fifth Daughter was named after the Father's eldest sister

Subsequent children were named following the same pattern, being named after the next eldest sibling of the father and mother.
This system can be very useful genealogically. If there is a break in the pattern or the names appear to be out of order, it could indicate that a child has died young. The names were not usually used more than once (see *Note* below), but the system can result in children having the same name, e.g. if the child is the third child, and the father is the first child of a father who was the first child!

Note - if a child died, the name was usually used again, particularly in Afrikaans families and commonly in English families. It was not the name of the dead child that was being re-used but the name of the grandfather or grandmother, etc that was being given again. The system is by no mean invariable, even amongst Afrikaner families.

f) Create or photocopy template documents to copy out data from archived repositories and/ or places where electronic devices such as mobile phones, tablets and cameras are restricted/prohibited.

g) Copy spellings as is.

h) Keep a notepad to keep track of the search. Also, make additional notes and highlight the multiple ways names, surnames and places can be spelt over time and dependent on the scribe of the information .e.g. Stephen (by an English assigned authority for relevant records) and Stephanus (by an Afrikaans assigned authority for relevant records). Or .e.g. variations in spelling for Edmond to Edmund or, etc. Or simply a spelling error .e.g. Petronella spelt as Petronela– in databases such as NAAIRS or family search.org. NB: Before confirming it is the same person, at least 2 other details should match the initial finding such as DOB and place of birth and variations in the name and surname; however, it is advised matched to the SAGs/SAFs lists or family bibles.

i) Get maps and encyclopaedias (at least one old and one new version, of each or find a repository with them) and other genealogical journals and readings to discover the name changes of old or new places; depending on the queries.

j) Find out procedures for each individual repository; before visiting it. *NB*: There are often periodical changes in governmental repositories; as laws and policies change. Stay up-to-date with the changes and fill in the relevant paperwork and produce the relevant documentation required; in order to gain access to the information needed.

k) Get a good genealogical software package that can import or export GEDCOM files.

3. Data Collection Steps:

a) From pedigree and patient interview information obtain the eldest proband.

b) Extract (per individual):

Detail	Description
Name(s) and Surname (if female: maiden and married or if adopted: adoption names and surnames (before and after)).	
Date of Birth (DOB)	*DD/MM/YYYY , place
Date of Baptism	≈ DD/MM/YYYY, place
Name of Parents and DOB/Baptism/Marriage/Death	Father (Full names) x Mother (Full names, with maiden surname)
Date of Marriage	X DD/MM/YYYY, place
Name(s) and Surname(s) of Spouse	
Name(s) and Surname(s) of Spouse's parent's	
DOB/Baptism/Death of Spouse	DD/MM/YYYY, place
Name(s) and Surname(s) of Children and DOB/ Baptism/Death of Children	
Date of Death	† DD/MM/YYYY, place
Record reference number and repository and date collected	

c) Go to the individual's record and begin extracting clues for missing data. Often number the people in a logical pattern.

   Depending on what details you found available, visit a relevant record or resource.

   For example,

   Searching for the Abraham's wife because the pedigree has missing first names one start's by looking for the marriage record or through the children's birth records.

   i. Search SAGs and SAFs for Abraham's family tree.
   ii. Search NAAIRS.
   iii. Search NGK repository.
   iv. Search FamilySearch.org
   v. Add information found for to missing fields.

d) Enter into generational grid (attached page).

e) Enter into Genealogical software (.e.g. Legacy 8.0 deluxe).

f) Look into her parents. Search person by person; do not exclude paternal or maternal lineage; unless genetic testing suggests a logical reason for individual lineages to be excluded. Continue to fill it in until all lines are complete as far back as possible.

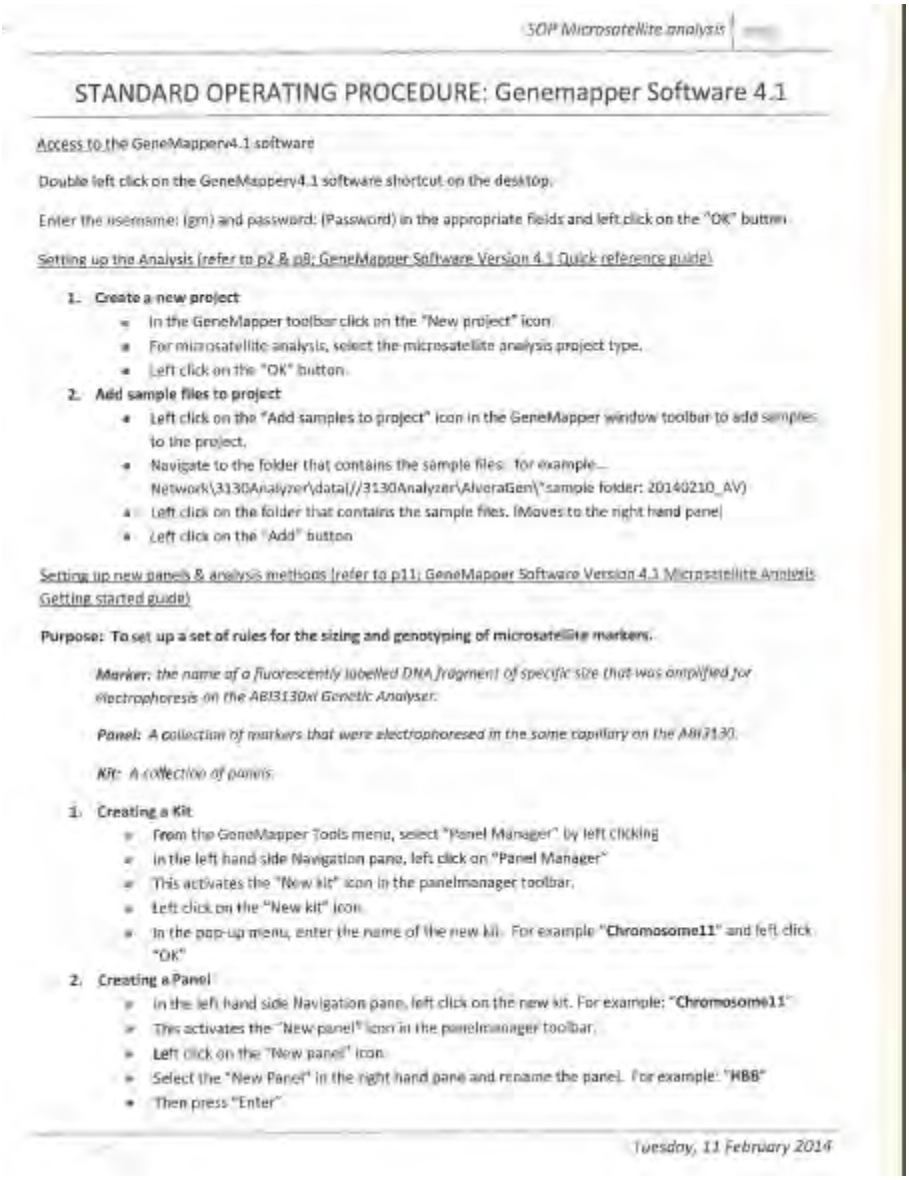
g) Preferred tracing to reach 10-12 generations back; but depends on record completeness and when the first founder of a line entered SA. For example, some families entered in subsequent ships such as the French Huguenots (refer to relevant history periods such as; Section 7.5 The founder effect in Afrikaners).

4. Data Analysis:

a) Print out family chart and manually search for individuals or couples in common in the first two completed family trees.
b) Extend the other trees and begin to eliminate families absent in other family trees and/ or record those also found in other subsequent family lines.
c) It is often useful to do a top-down approach after there are less than 5 common ancestors of couples found and link them from the children's lineage.
d) Report results and collect further historical evidence on the couple such as the context and how they entered South Africa and related family history.

(Adapted and modified from: https://www.geni.com/projects/South-African-Genealogical-Reference-Centre/7572 , Prof. Gerhard Geldenhuys and Western Cape Archives training 2016 by P*rincipal Archivist* Jaco van der Merwe)

Appendix 9: GeneMapper 4.1. Standard Operating Procedure



STANDARD OPERATING PROCEDURE: Genemapper Software 4.1

Access to the GeneMapperv4.1 software

Double left click on the GeneMapperv4.1 software shortcut on the desktop.

Enter the username: (gm) and password: (Password) in the appropriate fields and left click on the "OK" button

Setting up the Analysis (refer to p2 & p8: GeneMapper Software Version 4.1 Quick reference guide)

1. **Create a new project**
   - In the GeneMapper toolbar click on the "New project" icon
   - For microsatellite analysis, select the microsatellite analysis project type.
   - Left click on the "OK" button.
2. **Add sample files to project**
   - Left click on the "Add samples to project" icon in the GeneMapper window toolbar to add samples to the project.
   - Navigate to the folder that contains the sample files: for example... Network\3130Analyzer\data//3130Analyzer\AlveraGen\"sample folder: 20140210_AV)
   - Left click on the folder that contains the sample files. (Moves to the right hand pane)
   - Left click on the "Add" button

Setting up new panels & analysis methods (refer to p11: GeneMapper Software Version 4.1 Microsatellite Analysis Getting started guide)

Purpose: To set up a set of rules for the sizing and genotyping of microsatellite markers.

Marker: *the name of a fluorescently labelled DNA fragment of specific size that was amplified for electrophoresis on the ABI3130xl Genetic Analyser.*

Panel: *A collection of markers that were electrophoresed in the same capillary on the ABI3130.*

Kit: *A collection of panels*

1. **Creating a Kit**
   - From the GeneMapper Tools menu, select "Panel Manager" by left clicking
   - In the left hand side Navigation pane, left click on "Panel Manager"
   - This activates the "New kit" icon in the panelmanager toolbar.
   - Left click on the "New kit" icon
   - In the pop-up menu, enter the name of the new kit. For example "**Chromosome11**" and left click "OK"
2. **Creating a Panel**
   - In the left hand side Navigation pane, left click on the new kit. For example: "**Chromosome11**"
   - This activates the "New panel" icon in the panelmanager toolbar.
   - Left click on the "New panel" icon
   - Select the "New Panel" in the right hand pane and rename the panel. For example: "**HBB**"
   - Then press "Enter"

3. **Creating a New Marker**
   - In the left hand side Navigation pane, left click on the new panel. For example: "**HBB**"
   - This activates the "New marker" icon in the panelmanager toolbar.
   - Left click on the "New marker" icon.
   - In the right hand pane select the new marker and rename. For example: "**D11S1234**"
   - Enter the expected colour of the marker in the specific filterset. For example: "The color of a FAM fluorescent label is blue in filterset D"
   - Also, complete the table by specifying the expected minimum and maximum sizes of the amplifiert product(s).
4. **Creating a new binset**
   - In the left hand side Navigation pane, left click on the new panel.
   - Left click on the "new binset" icon in the panelmanager toolbar.
   - Name the binset. For example: "**BinsetHBB**"
   - Left click "OK"
   - Apply changes and exit Panelmanager.
5. **Creating a new analysis method**
   - Left click on the first row of the Analysis Method column.
   - From the dropdown menu, select New Analysis Method
   - In the New Analysis Method pop-up menu, select Microsatellite as the Analysis type.
   - Left click "OK"
   - In the "General tab" of the Analysis method editor, enter a name for the method. For example: "**MethodHBB**"
   - In the "Allele tab" of the Analysis method editor, left click on the dropdown menu for "Bin set".
   - Select the newly created binset "**BinsetHBB**"
   - Click OK to save the method and close the "Analysis method editor" pop-up menu.

The rest of the settings may be kept as default for the time being. For further user specific settings, refer to p20-p23 of the GeneMapper Software Version 4.1 Microsatellite Analysis Getting started guide.

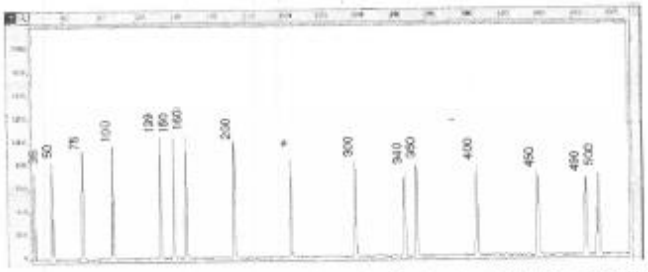
Performing initial analysis on the project (refer to p27 ; GeneMapper Software Version 4.1 Microsatellite Analysis Getting started guide)

Purpose: To size the data for the creation of bins (allele definitions)

1. **Analysing the project for the first time**
   - Left click on the first row of the "Size standard" column in the Samples tab.
     From the dropdown menu, select the **GS500(-250)ROX** size standard
     Note: This sizing method was set up to exclude the 250bp fragment from analysis, since the migration of the fragment on the ABI3130xl is inconsistent.
   - Left click on the first row of the "Panel" column in the Samples tab.
     From the dropdown menu, select the newly created panel. For example: "**HBB**".
   - Left click on the first row of the "Analysis method" column in the Samples tab.
     From the dropdown menu, select the newly created analysis method. For example: "**MethodHBB**"
   - Left click and drag to select the Analysis method, Panel and Size standard columns for all samples.
     Press Ctrl+D to fill down the information to all rows.
   - Left click on the green play button in the Genemapper toolbar to start the analysis of all samples.

- Save the project by naming it with "Today's date". Click "OK".

2. **Review sizing data**
   - Select all samples once analysis is completed by selecting Edit>Select All from the menu in the Genemapper Samples Tab.
   - Left click on the Size match editor icon in the Genemapper toolbar.
   - A normal profile for the **GS500(-250)ROX** size standard should look like this:



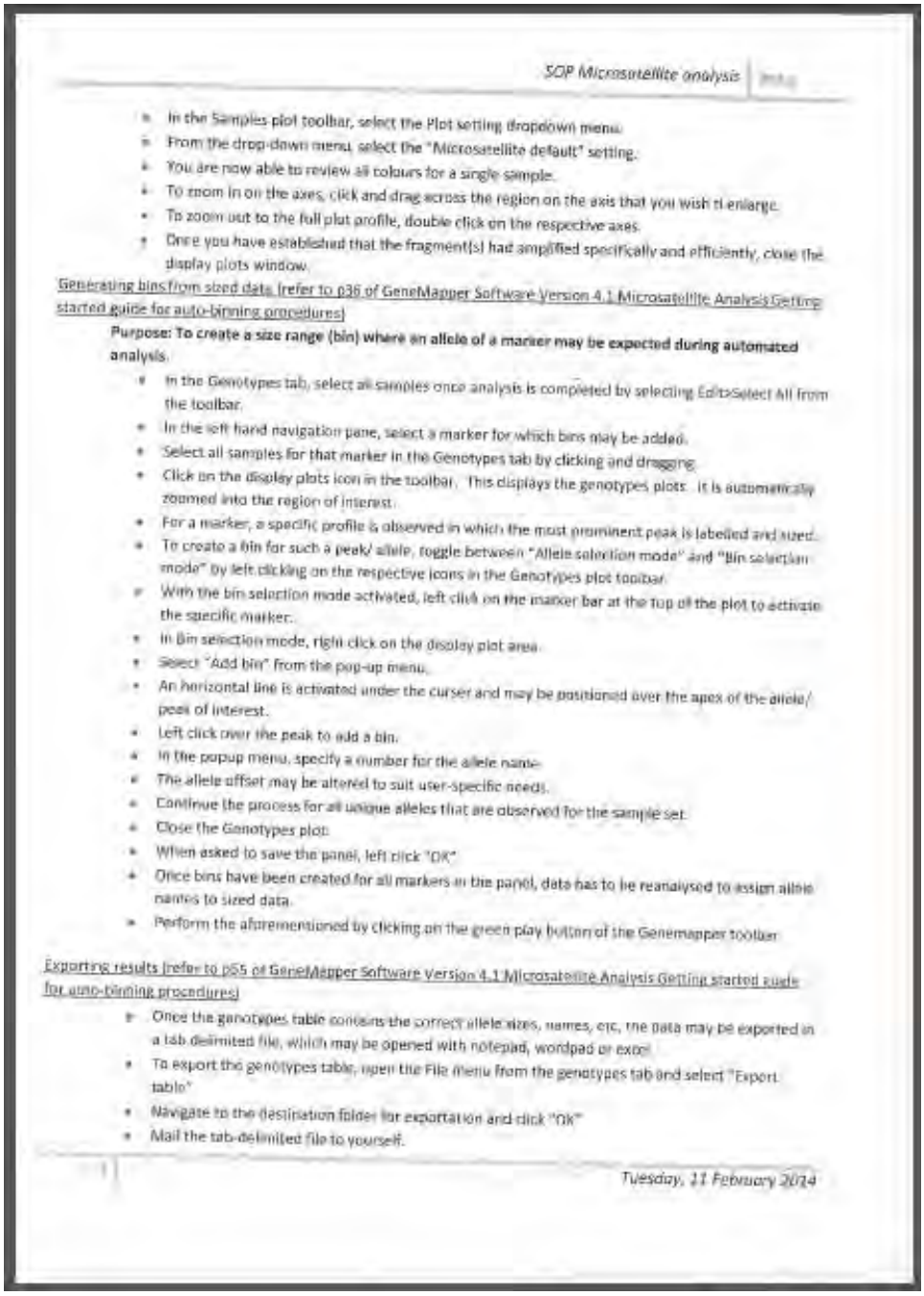
The sizing method may make errors in labelling the peaks of the size standard by either mislabelling or omitting a peak.

- Review each sample to ensure that the sizing has been performed accurately.
- Left click on the sample in the left hand pane of the Size match editor.
- If a peak has not been labelled, left click on the peak in the sizing profile. The selected peak will be blue.
  Right click on the peak and select "Add" from the pop-up menu.
  From the size menu select the appropriate size that was meant to be selected for the peak.
- To correct a mislabelled peak, left click on the peak in the profile.
  Once selected, right click to bring up the pop-up menu.
  From the pop-up menu, select delete to remove the mislabelled peak.
  To correct the size label, right click again to select "add" to re-label the peak with the correct size.
- Repeat the process for all samples to ensure accurate sizing.
- Once all samples have been reviewed, left click on the "Apply" button. Then click OK to return to the Samples tab in Genemapper.
- All edited samples would now be re-activated for re-analysis as their sizing data has manually been altered.
- Left click on the green play button in the Genemapper toolbar to start the analysis of these samples.

3. Examine marker data

Purpose: to assess the specificity and efficiency of the labelled, amplified fragment.

- refer to p34-; GeneMapper Software Version 4.1 Microsatellite Analysis Getting started guide
- In the Samples tab, select all samples once analysis is completed by selecting Edit>Select All from the toolbar.
- Left click on the "Display plots" icon in the Genemapper toolbar.



- In the Samples plot toolbar, select the Plot setting dropdown menu.
- From the drop-down menu, select the "Microsatellite default" setting.
- You are now able to review all colours for a single sample.
- To zoom in on the axes, click and drag across the region on the axis that you wish ti enlarge.
- To zoom out to the full plot profile, double click on the respective axes.
- Once you have established that the fragment(s) had amplified specifically and efficiently, close the display plots window.

Generating bins from sized data (refer to p36 of GeneMapper Software Version 4.1 Microsatellite Analysis Getting started guide for auto-binning procedures)

Purpose: To create a size range (bin) where an allele of a marker may be expected during automated analysis.

- In the Genotypes tab, select all samples once analysis is completed by selecting Edit>Select All from the toolbar.
- In the left hand navigation pane, select a marker for which bins may be added.
- Select all samples for that marker in the Genotypes tab by clicking and dragging.
- Click on the display plots icon in the toolbar. This displays the genotypes plots. It is automatically zoomed into the region of interest.
- For a marker, a specific profile is observed in which the most prominent peak is labelled and sized.
- To create a bin for such a peak/ allele, toggle between "Allele selection mode" and "Bin selection mode" by left clicking on the respective icons in the Genotypes plot toolbar.
- With the bin selection mode activated, left click on the marker bar at the top of the plot to activate the specific marker.
- In Bin selection mode, right click on the display plot area.
- Select "Add bin" from the pop-up menu.
- An horizontal line is activated under the curser and may be positioned over the apex of the allele/ peak of interest.
- Left click over the peak to add a bin.
- In the popup menu, specify a number for the allele name.
- The allele offset may be altered to suit user-specific needs.
- Continue the process for all unique alleles that are observed for the sample set.
- Close the Genotypes plot.
- When asked to save the panel, left click "OK"
- Once bins have been created for all markers in the panel, data has to be reanalysed to assign allele names to sized data.
- Perform the aforementioned by clicking on the green play button of the Genemapper toolbar.

Exporting results (refer to p55 of GeneMapper Software Version 4.1 Microsatellite Analysis Getting started guide for auto-binning procedures)

- Once the genotypes table contains the correct allele sizes, names, etc, the data may be exported in a tab delimited file, which may be opened with notepad, wordpad or excel.
- To export the genotypes table, open the File menu from the genotypes tab and select "Export table"
- Navigate to the destination folder for exportation and click "OK"
- Mail the tab-delimited file to yourself.

(Adapted from: SOP Folder, Department of Human Genetics, UCT)

Appendix 10: Explanations regarding the various study limitations

A10.1 Recruitment and sample size

This study did not have sufficient probands to be able to draw definitive conclusions. Genetic genealogical studies often require 10 or more families to produce a statistically significant and clear genealogically-evidence based conclusion; ideally, a single founding ancestor.

A10.2 Phenotyping

Unfortunately, we were not able to phenotype all, however, at least all probands from each family or at least one clinically affected family representative was screened according to the most recent ARVC TFC, when they were enrolled.

A10.3 Language, culture and Afrikaner history

Some records or hand written information/ data was lost in translation from foreign languages, and I was not familiar with Dutch, German and Afrikaans records. I used Google translate and initially, Afrikaans native speakers' translations; until they were familiar with terms relevant for the research. Common Afrikaner knowledge, undocumented/ verbatim cultural and/or historical knowledge was not included in study as I am a scientist.

A10.4 Genealogical data collection

Unfortunately, we were not able to extend the ACM 71 family beyond three generations as their records were very limited and inaccessible. The gap in some of the records, especially from mixed ancestry and non-Caucasian lineages, were mainly due to the changeover in systems from the Apartheid legislation (1948), that institutionalized (in South Africa) a policy or system of segregation or discrimination on grounds of race. In order to protect person(s) or families (1948-1994; during the Apartheid rule) from being constrained and conditioned by one's designated "racial" group, many records of racial mixes were "lost", unreported or inaccessible. Furthermore, some families that were classified as "white" from all walks of life up to leadership were "denying the colored mother"(Distiller and Samuelson 2005). It is though that some records are hidden privately and some destroyed or so poorly kept that was difficult to decipher.

One example of this situation was that the birth record for the mother was in a repository that had records that were in a very poor state (pages were torn) and because they were hand written

it was difficult to confirm who the parents were. In other instance, the missing records were due to (1) lack of coordination and organization of records or (2) simple rebellion to the NGK by the mission churches (due to 'sins' of the NGK under Apartheid legislation; such as providing the government the records used to keep track of families and racially reclassifying individuals, yearly). This history of their internal feuds due to some underlying social, economic and political issues still lives on especially in small, farming areas. For a time some names were removed or records kept track of 'free' slaves and racial grouping. Hence, they were used by the Apartheid government to monitor and highly tax poor farm workers who were not classified as "white".

Secondly, the other limitation is the central repository for all national records, the Department of Home Affairs (DHA), does not assist researchers. However, they portray mixed messages on the procedures to be followed. Some time was wasted in the beginning waiting for a DHA response (at least 6 weeks).

Also, record mis-referencing, different names and wrong names documented on various documents; caused a few tracing problems, often having to look for more documents to confirm or eliminate the case. An example of mis-referencing was, in the Western Cape Archives, the death notices are referenced by volume number as this repository has at least 27 km of files; alone, excluding what the Western Cape Master of the High court office (MOOC) vault and MOOC office have. A death notice in an estate file had a husband's deceased name and reference in the Western Cape Archive. After requesting the file, with the mis-referenced reference entered into the Archival Requisition form (Appendix 3B), the box of files had another, unrelated person's document at that reference. On querying this matter with the Principal Archivist, the error was attributed to typing error of the data capturer. We searched by date of death, place of death and the surname and then name and the file was found; which matched the search.

An example for different names that occurred during my search, a baptismal register of an Afrikaans child in his mother's parish (the Methodist Church in Transvaal around 1875; because the mother was English) lead the child to be recorded with the three English versions of his first name and the rest of his records named after his Afrikaans version of the names (from marriage register, death notice, estate file and will). We had to trace his sibling and use the SAFs to confirm it was the same person.

Another example, with wrong names occurred with a Free State MHG Free State query, where the spouse of the deceased (Appendix 3A, Section 7b on the form) had the wrong name and surname of the pre-deceased spouse filled out by the current wife of the deceased. The reference was looked into by the researcher and the naming pattern had been thrown off by this error. The researcher had to find the marriage record of the couple and complete list of children with the pre-deceased and witnesses to confirm it was an error on the record and not the researcher's mistake. Fortunately, the second spouse did not have any children. So all the baptismal records and marriage register contained the correct first spouse's name and so did the estate files and will of the deceased; if he had children with the new spouse it may have been more difficult to follow naming patterns.

Finally, time was a major limiting factor as we could have physically visited repositories outside of those within a 100km radius from Cape Town (inclusive of the deeper farming areas; which may still have ACM 71 family records). Also time limited the number of probands we had recruited and could in future, further expand the current families or possibly identified additional families with the *PKP2* c.1162C>T mutation.