The Rationale, Design and Implementation of the African Cardiomyopathy and Myocarditis Registry

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DEDICATION

I dedicate this work to my mentor and supervisor, the late Professor Bongani Mayosi (28th January 1967 – 27th July 2018).

My mentorship journey with Professor Bongani Mayosi started 8 years ago at the bedside, as a second-year resident in internal medicine. The patient I had presented to him had familial dilated cardiomyopathy. Professor Mayosi shared his passion for inherited cardiac conditions with me that day at the bedside and planted a seed that he nurtured over time, until it grew and became firmly rooted in me. To many, Professor Mayosi was a beacon of hope, the gold standard to which to aspire to. He was a man who not only believed in transformation, but worked tirelessly to achieve it. I am proof that his idea of transformation extended beyond political and social definitions – he believed in the transformation of individuals. He saw potential in me and willingly provided me with the platform on which to explore it. In the book *Outliers, the story of success,* the author, Malcolm Gladwell, makes the argument that success is largely determined by a combination of unique opportunity and hard work. The discipline of hard work was instilled in me by my own father, but the privilege of rare opportunity was provided to me by this formidable man. One of the most fundamental lessons he taught me was that it takes a team to accomplish something of value in medical research. He entrusted me with this project and his team, and gave me the freedom and responsibility to build it in my own way. It was an extraordinary challenge, with tremendous expectation, but it taught me the true extent of what I am capable of. He once told my co-supervisor that I challenged him – I can think of no greater compliment. He encouraged me to be self-sufficient and convinced both his mentor and his mentees to guide me when he was unable. He encouraged me to debate, to express my opinion, to disagree. I am deeply grateful for the experience of knowing him, for the confidence he bestowed and the time he gave me. The imprint of his influence is something that I will carry with me for the rest of my life. Professor Mayosi taught me how to step into the arena and dare greatly.

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I would like to acknowledge the efforts of my team, without them none of this work would have been possible; Unita September (research sister), Veronica Francis (project manager), Marnie van de Wall (echocardiographer), Nakita Laing (genetics counsellor), Amber Ross (genetics counsellor), Tessa Suttle (medical student), Emily Chedwin (medical student), Sinoxolo Bhuvula (research assistant), Shahiemah Pandie (data quality officer) and Lwazi Mhlanti (data manager). I would like to specifically acknowledge the care and support provided to me by Veronica and Unita throughout this journey. I would also like to extend heartfelt thanks to genetics counsellor, Nakita Laing, for sharing her skill, enthusiasm, insight and experience with me over the years. I would like to acknowledge the collaborative efforts made in support of this work by Associate Professor Gasnat Shaboodien and the molecular laboratory team – Dr. Maryam Fish, Dr. Mzwandile Mbele, Ms Tafadzwa Machipisa, Mr Stephen Kamuli, Mr Timothy Spracklen, Dr Babu Muhamed, and Ms Lameez Pearce. I appreciate the clinical

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I am deeply grateful to the patients and families that I have worked with. I celebrate their courage in the face of adversity and thank them for allowing me the privilege of walking alongside them through some of the most challenging moments in their lives.

Most importantly, I would like to thank my parents, Elisabeth and Rodion Kraus, for their unconditional love and support in every endeavour I have pursued, and for teaching me the value of hard work and the importance of having passion for the work that I have chosen to do. Thank you for the example you have set in leadership, humility, kindness, empathy and compassion. A special thanks to my mother for the time she spent reading my drafts. To my siblings and close friends: I salute your patience and resolve in supporting me through this process.

DECLARATION

I, Sarah Kraus, hereby declare that the work on which this dissertation/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.

Signature: Signed by candidate

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LIST OF ABBREVIATIONS AND ACRONYMS

ACE	Angiotensin-converting enzyme
AD	Autosomal dominant
AR	Autosomal recessive
ARB	Angiotensin-receptor blockers
ARVC	Arrhythmogenic right ventricular cardiomyopathy
BSA	Body surface area
CAD	Coronary artery disease
CDH2	Cadherin-2
CI	Confidence interval
CMR	Cardiovascular magnetic resonance
CPR	Cardiopulmonary resuscitation
CRF	Case report form
CVD	Cardiovascular disease
CVG	Cardiovascular Genetics
DCM	Dilated cardiomyopathy
DNA	Deoxyribonucleic acid
DSC2	Desmocollin-2
DSG2	Desmoglein-2
DSP	Desmoplakin
ECG	Electrocardiogram
EMB	Endomyocardial biopsy
EMF	Endomyocardial fibrosis
EPS	Electrophysiology studies
ESC	European Society of Cardiology
EST	Exercise stress test
GSH	Groote Schuur Hospital
HCM	Hypertrophic cardiomyopathy
HF	Heart failure
HFrEF	Heart failure with reduced ejection fraction
HIC	High-income country
HIV	Human immunodeficiency virus
HIVAC	HIV-associated cardiomyopathy

HLA	human leucocyte antigen
HREC	Human Research Ethics Committee
IBM	International Business Machines
ICD	Implantable cardioverter defibrillator
IHD	Ischaemic heart disease
IMHOTEP	Afr***i***can Cardio***m***yopat***h***y and My***o***cardi***t***is R***e***gistry ***P***rogram
IQR	Interquartile range
IVS	Interventricular septum
JUP	Plakoglobin
LBBB	Left-bundle-branch block
LGE	Late gadolinium enhancement
LMIC	Low-to-middle-income country
LMNA/C	Lamin A/C
LV	Left ventricle (or left ventricular)
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVNC	Left ventricular non-compaction
LVOT	Left ventricular outflow tract
MBPC3	myosin-binding C
MRA	Mineralocorticoid receptor antagonists
MRI	Magnetic resonance imaging
MYH7	ß-myosin heavy chain
NCD	Non-communicable disease
NGS	Next generation sequencing
NYHA	New York Heart Association
OHT	Orthotopic heart transplant
PCO	Project Coordinating Office
PKP2	Plakophilin-2
PLN	Phospholamban
PND	Paroxysmal nocturnal dyspnoea
PPCM	Peripartum (or postpartum) cardiomyopathy
RCM	Restrictive cardiomyopathy
RHD	Rheumatic heart disease
RV	Right ventricle (or right ventricular)
RVEF	Right ventricular ejection fraction

SAECG	Signal average electrocardiogram
SCD	Sudden cardiac death
SD	Standard deviation
SOP	Standard operating procedures
SSA	Sub-Saharan Africa
TFC	Task force criteria
TTN	Titin
UCT	University of Cape Town
US/USA	United States of America
VT	Ventricular tachycardia
VUS	Variant of unknown significance
WES	Whole exome sequencing

ABSTRACT

Background. Causes of heart failure in Africa are largely non-ischaemic: hypertension, rheumatic heart disease and cardiomyopathy. Cardiomyopathies pose a great challenge because of poor prognosis and high prevalence in low- and middle-income countries with limited access to specialised care. Little is known about aetiology or outcomes of cardiomyopathy in Africa.

Method. The Afr***i***can Cardio***m***yopat***h***y and My***o***cardi***t***is R***e***gistry ***P***rogram (IMHOTEP) is a pan-African multi-centre, hospital-based cohort study. It aims to describe the clinical characteristics, aetiology, genetics, management and outcome of cardiomyopathies in children and adults. Index patients were recruited as either incident (new) or prevalent (existing) cases, and family screening was conducted in selected cases. Several sub-studies were conducted at the initiating centre, including; outcome studies on prevalent cases incorporated into IMHOTEP, a cardiovascular magnetic resonance (CMR) study on incident cases, and a clinical genetics study on families.

Results. The pilot phase was commenced in Cape Town (February 2015), followed by staggered initiation at 6 further sites. Over 600 index patients have been recruited to the registry to date. Preliminary data on the first 99 incident adult cases recruited at the initiating site, showed that dilated cardiomyopathy (DCM; n=67) was commonest, followed by hypertrophic (HCM; n=13), left ventricular noncompaction (LVNC; n=11), restrictive (RCM; n=4) and arrhythmogenic right ventricular (ARVC; n=4) cardiomyopathies. Cardiomyopathy occurred predominantly in mixed race (46%) and black (41%) Africans, and more frequently in females (54%). Mean age of presentation was 36.8 ±12.5 years. CMR performed in incident cases (67/99, 68%) proved diagnostically useful, however, acute myocarditis was only reported in one individual. In addition, prevalent cases with ARVC and DCM were enrolled from two existing studies at the initiating centre. Except for fewer (24%) genotype positive

(PKP2 20%, CDH2 4%) individuals with ARVC (n=70), the baseline characteristics and diagnostic criteria were similar to what has been reported internationally. Transplant-free survival probability at 1-year, 5-years and 10-years was 98.5%, 90.7%, and 80.8% respectively in ARVC (median survival time 9.0 years) and there were no significant differences in survival between those with and without implantable cardioverter defibrillators [p=0.27]. In the prevalent DCM cohort (n=133), transplant-free survival probability was 93.4%, 82.2% and 73.1% at 1-year, 5-years and 10-years respectively, with a median survival time of 5.3 years. The age of onset (34.8 ±11.0 years) and death (41.5 ±9.6 years) were significantly younger in our DCM patients compared to European cohorts. Thirty-five families were recruited (16 genotype positive, 19 genotype unknown) with autosomal dominant inheritance observed in 94.3% families.

Conclusion. IMHOTEP is the first multi-centre registry for cardiomyopathy in Africa. Preliminary data suggests an earlier age of onset compared to European cohorts and that DCM is the most common form of cardiomyopathy in South Africa. Molecular genetic analysis will provide vital and novel data on the genetic causes of cardiomyopathy in Africans.

CHAPTER 1: Introduction and study rationale

1.1. THE BURDEN OF HEART FAILURE IN AFRICA

Heart failure (HF), the dominant form of cardiovascular disease (CVD) in hospitalised Africans, has great social and economic relevance owing to its high prevalence, mortality and impact on young, economically active individuals.[1,2] According to data from the International Congestive Heart Failure (INTER-CHF) prospective cohort study, despite being the youngest cohort at baseline, African patients had the highest mortality.[3] The younger age of onset of HF and high mortality rates in Africans mirror the findings of the THESIS-HF study.[2,4] In contrast to high income countries, where ischaemic heart disease is predominant, the causes of HF in Africa remain largely non-ischaemic, with hypertension, rheumatic heart disease (RHD) and cardiomyopathy accounting for the majority of cases.[2,3,5] Cardiomyopathy contributes to 20–30% of HF in adults in the African population, and continues to be an important cause of HF in sub-Saharan Africa (SSA) (Figure 1.1.). [2,6-8] According to data from the Global Burden of Disease Study 2013, the number of deaths attributed to cardiomyopathy in SSA has risen, with a 14% change in age-standardised death rate (12.7 per 100 000 in 1990 versus to 14.5 per 100 000 in 2013). [9]

HF poses a major public health challenge globally, affecting 26 million people worldwide, and is associated with substantial healthcare expenditure.[10,11] Development of health policies is reliant on sufficient epidemiological data on incidence, prevalence, determinants and outcomes. Adequate information on cardiomyopathies and myocarditis in the African population is lacking. There is a recognisable need for large, well-designed, epidemiological studies to evaluate the genetic and molecular epidemiology, as well as modifiable risk factors for cardiomyopathy and the impact cardiomyopathy has on the burden of HF in the population.[6,8,12]

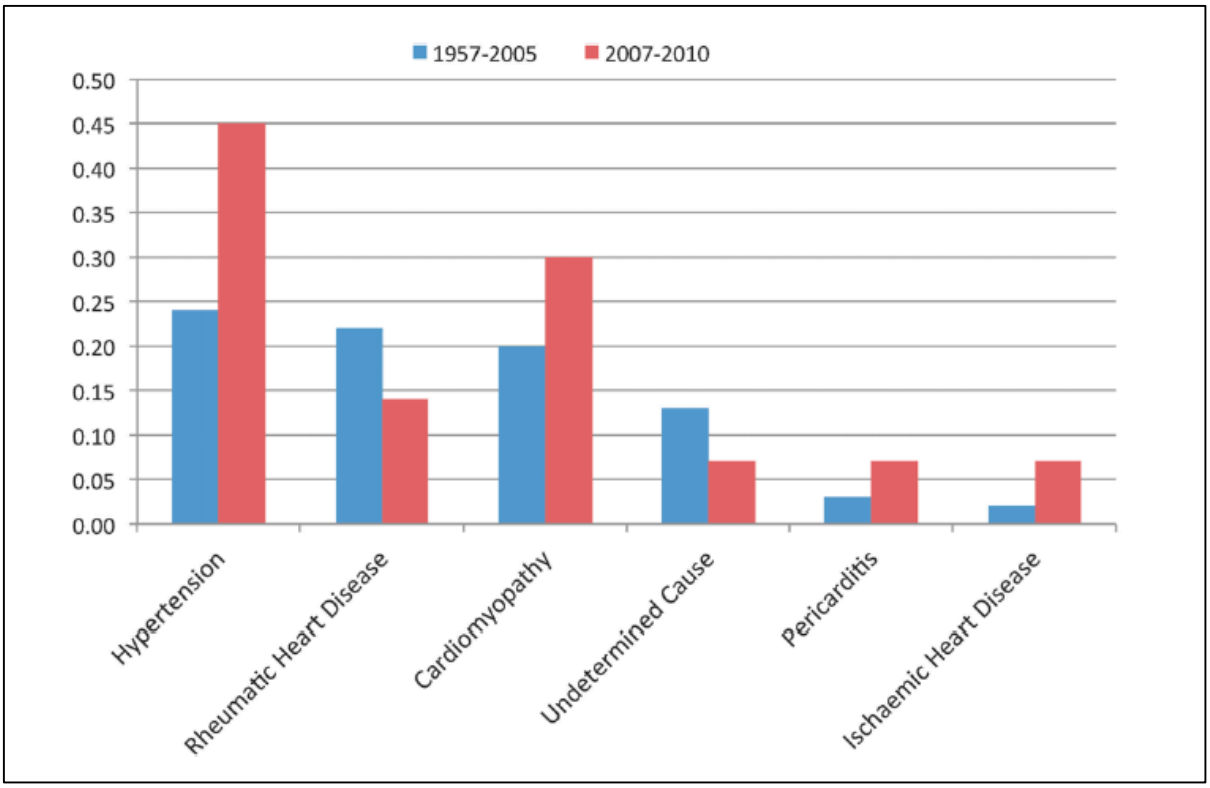


Figure 1.1. Differences in the proportion of the causal factors for heart failure in sub-Saharan Africa during the periods 1957-2005 and 2007-2010.
From: Sliwa K, Mayosi BM. Recent advances in the epidemiology, pathogenesis and prognosis of acute heart failure and cardiomyopathy in Africa. *Heart* 2013;99(18):1317-22[6]

1.2. DEFINITION AND CLASSIFICATION OF CARDIOMYOPATHIES

Cardiomyopathies are a heterogeneous group of familial and acquired myocardial disorders in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease, hypertension, valvular disease, pericardial disease and congenital heart disease sufficient to cause the observed myocardial abnormality.[13] While a number of different classification systems have been published, the European Society of Cardiology (ESC) approach is useful as it classifies cardiomyopathies into different morphological and functional phenotypes - dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC) and unspecified cardiomyopathies, including isolated left ventricular non-compaction (LVNC) and Takotsubo cardiomyopathy. Once a morpho-functional diagnosis is made and alternative

conditions have been excluded, further elucidation is required to identify the specific cause and underlying disease mechanism.[14] Each phenotype is sub-classified as either familial or non-familial (Figure 1.2.). The term 'familial' implies the presence of the same disorder, or a phenotype that is (or could be) caused by the same genetic mutation in at least one other family member. Non-familial cardiomyopathies, which are clinically defined by the presence of a cardiomyopathy in the index patient and the absence of disease in other family members, are subdivided into idiopathic and acquired cardiomyopathies.[13] The relevance of aetiology was demonstrated by Felker *et al.* almost two decades ago, in a study showing that the underlying cause of HF was independently associated with survival among patients with different forms of cardiomyopathy and the differentiation beyond ischaemic versus non-ischaemic causes of HF had prognostic importance (Figure 1.3.).[15]

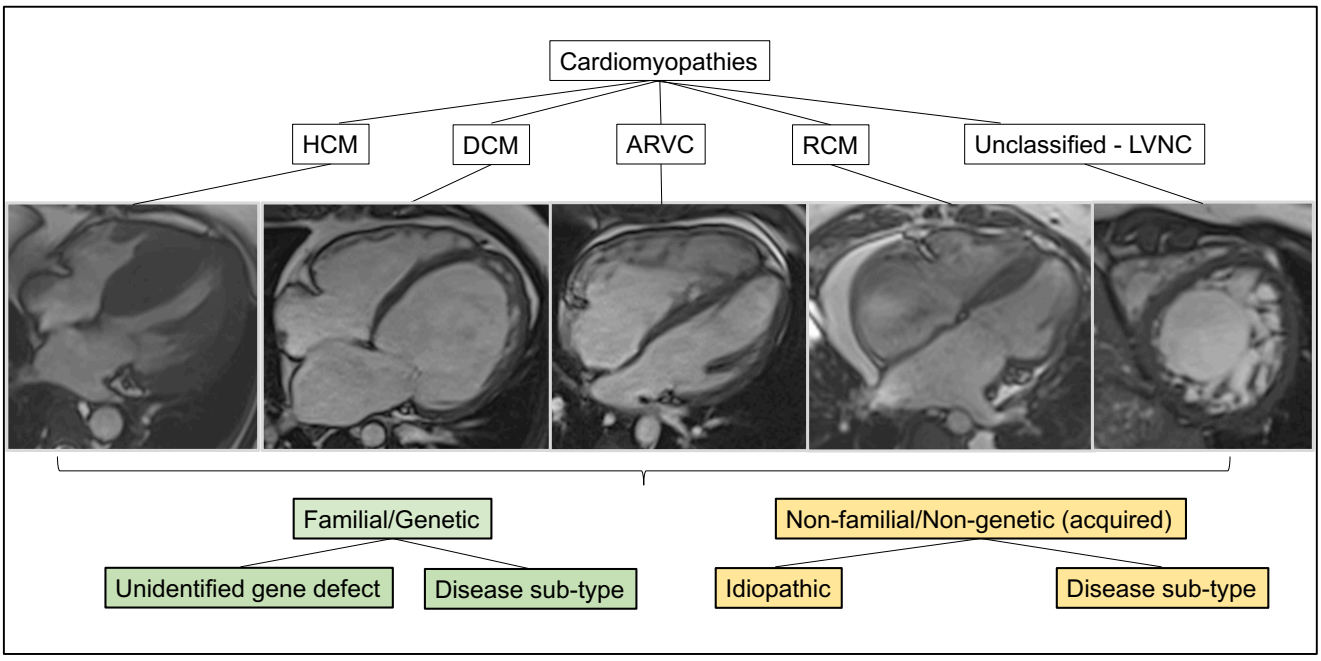


Figure 1.2. Classification of cardiomyopathies
Adapted from: Elliott P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases**.** *Eur Heart J*. 2007;29(2):270-276.[13]
ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; RCM, restrictive cardiomyopathy. Unclassified includes left ventricular noncompaction (LVNC)
Images: Cardiovascular magnetic resonance (CMR) images from IMHOTEP study participants with HCM, DCM, ARVC, RCM, and LVNC

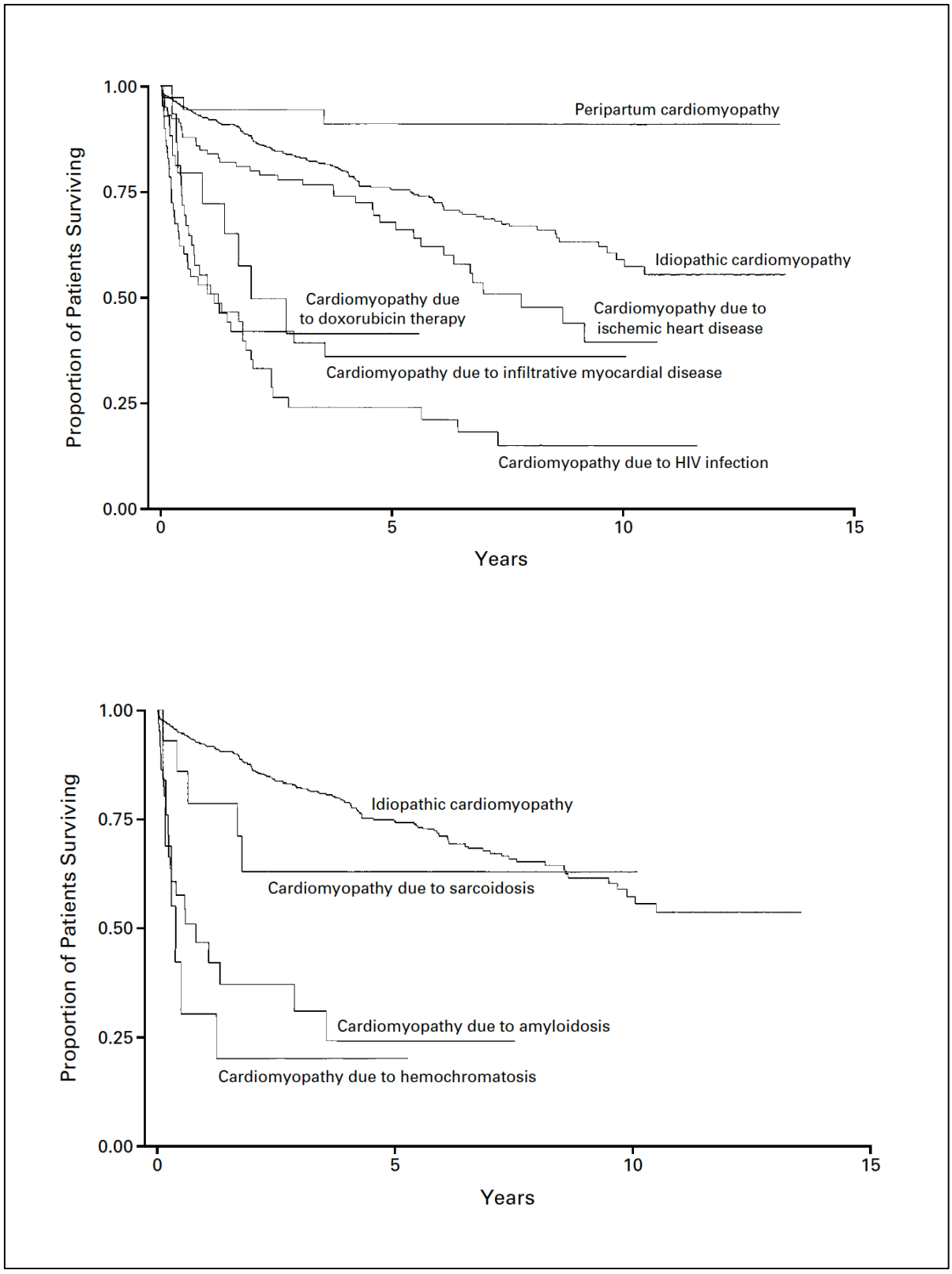


Figure 1.3. Adjusted Kaplan-Meier estimates of survival according to the underlying causes of cardiomyopathy.
From: Felker GM, Thompson RE, Hare JM, et al. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. *N Engl J Med* 2000;342(15):1077-84.[15]

1.3. SPECIFIC CARDIOMYOPATHY PHENOTYPES

1.3.1. Dilated cardiomyopathy (including peripartum cardiomyopathy)

DCM – a primary disorder of the heart muscle that is characterised by left ventricular (LV) dilatation and systolic dysfunction[13] (Figure 1.4.) – is one of the endemic cardiomyopathies in Africa, and accounts for 10–17% of all cardiac conditions at autopsy[16-18] and for 17–48% of patients hospitalised for HF.[2,5,8,19-21] DCM represents a significant health problem as it can lead to progressive refractory HF, requirement for cardiac transplantation and carries a high mortality. Prevalence of DCM in US populations is estimated to be 36.5/100 000,[22] but there have been no population-based studies of the epidemiology of DCM in SSA. DCM can occur at any age, but patients typically present in the third and fourth decade of life and occurs more frequently in men.[8]

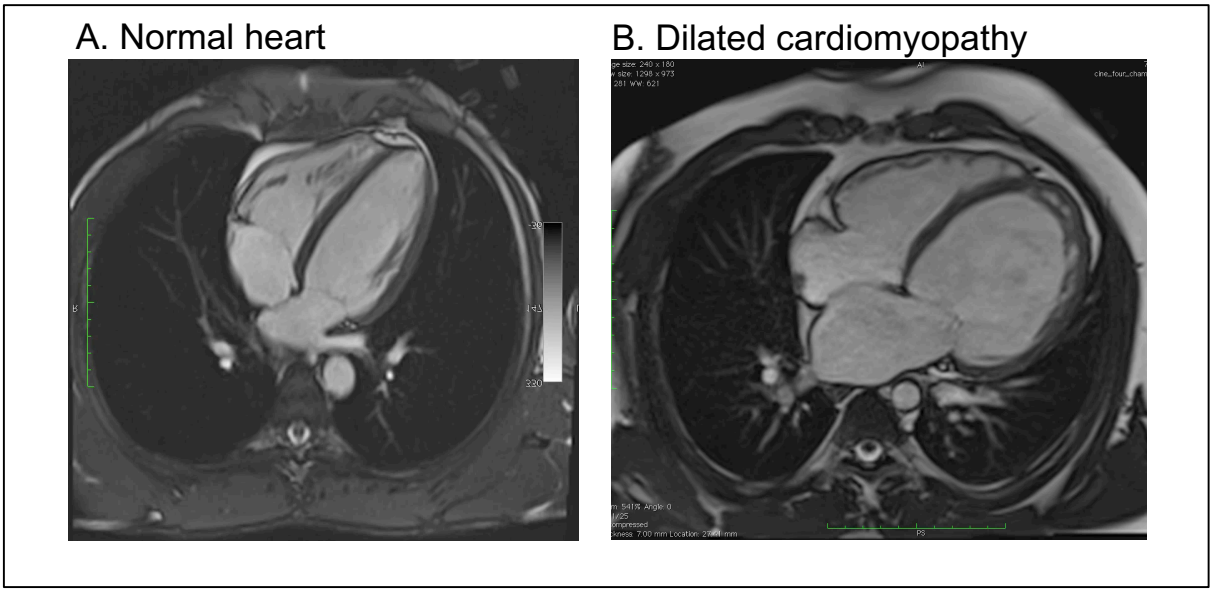


Figure 1.4. A. CMR of a normal heart (4 chamber view), B. CMR of an adult patient with DCM (4 chamber view).

Images: CMR of IMHOTEP study participants

DCM is caused by both familial and non-familial aetiologies, and the exclusion of secondary treatable causes is key in the diagnostic workup (Figure 1.5.). These secondary causes

include: myocarditis (infective, toxic, autoimmune); human immunodeficiency virus (HIV) infection; toxin exposure (chemotherapy, alcohol, illicit drugs such as methamphetamines, cocaine); endocrine conditions (thyrotoxicosis, phaeochromocytoma, acromegaly); nutritional deficiencies (thiamine, carnitine); and tachycardiomyopathy.[13,23] Familial disease accounts for 20–35% of patients with idiopathic DCM in high-income country (HIC) populations.[24,25] In a retrospective hospital-based study conducted at Groote Schuur Hospital (GSH) in South Africa, familial DCM occurred in 26.6% of cases of idiopathic DCM studied.[26]

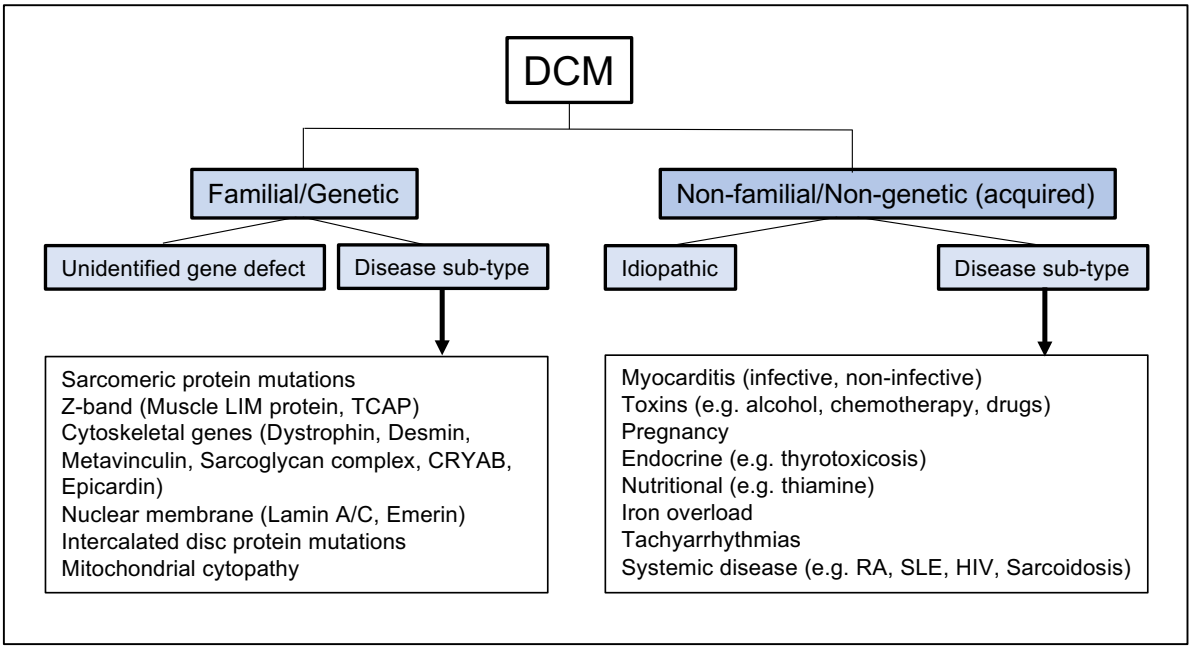


Figure 1.5. Classification and aetiology of dilated cardiomyopathies (DCM)
Adapted from: Elliott P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases**.** *Eur Heart J*. 2007;29(2):270-276.[13]

Diagnosing familial DCM can be challenging. There are few clinical characteristics that reliably distinguish familial from non-familial DCM,[27,28] although familial disease has been associated with a younger age of onset and a male preponderance in some studies.[26,28] Family history by patient report neither proves nor disproves familial disease. *Michels et al.* demonstrated in their original cohort that 20% of patients who had been unselected based on family history were found to have familial disease after investigation of their relatives. Furthermore, in their

follow-up study the index cases of familial disease had increased from 20% to 30% as additional relatives developed the disease.[24,27] Familial DCM genetics are inherently complex. To date there are >30 genes associated with DCM and a genetic cause is identifiable in only 30–35% of familial DCM cases. There is marked variation in age of onset, disease penetrance and clinical severity observed with a single mutation within an extended family or between families with the same variant.[29,30] Very little is known about the genetics of DCM in the African population.

HF is a preventable and treatable condition. Therefore, early diagnosis and treatment of DCM can lead to dramatic decreases in morbidity and mortality. Many patients with DCM (familial or idiopathic) present with advanced disease, but early detection of asymptomatic patients through screening enables presymptomatic intervention that may prevent or ameliorate the progression of advanced disease.[28] Response to medical therapy is variable in DCM. Apparent recovery can occur in a significant proportion (15%) of patients after an adequate period (9±4 months) on optimal medical therapy, and persistent apparent recovery has been demonstrated in approximately 10% of patients with DCM at 8 years.[31] However, 33% of patients who achieved normalisation of their LV dimensions and function at ±19 months had deterioration of LV function subsequently,[31] demonstrating the need for long-term follow-up. A study looking at the long-term progression of idiopathic DCM in paediatric patients suggested that despite having higher baseline LV ejection fractions and similar echocardiographic progression, children had a worse prognosis compared with adult DCM patients.[32] Outcome data available for DCM from South Africa shows a 5-year mortality rate of 40%,[4] but information on long-term outcomes in African patients with DCM, particularly in the paediatric population, is lacking.

Peripartum cardiomyopathy (PPCM) is classified as a subtype of DCM. In order to address the considerable variability in PPCM case definition, in 2010 the Heart Failure Association of the ESC Working Group on PPCM, proposed defining PPCM as an idiopathic cardiomyopathy

presenting with HF secondary to LV systolic dysfunction towards the end of pregnancy or in the months following delivery, where no other cause of HF is found. PPCM is a diagnosis of exclusion. The LV may, or may not, be dilated but the ejection fraction is nearly always reduced to below 45%.[33] PPCM occurs globally and is likely under-reported as a cause of maternal death.[34] Data regarding the incidence of PPCM is limited and highly variable, estimated at 1 in 2500–4000 in the US,[35,36] 1 in 1000 in South Africa,[37] and 1 in 300 in Haiti.[38] The pathophysiology of PPCM has been well studied and a number of contributing factors have been implicated in initiation and propagation of disease.[33,39] Recent experimental studies suggest that the breastfeeding hormone, prolactin, is a key contributor to the development of PPCM. High levels of full-length prolactin have been shown to promote inflammation and the production of a cleaved 16kDa N-terminal fragment of prolactin is implicated in causing endothelial damage and cardiomyocyte dysfunction.[40-42] Furthermore, recent clinical studies demonstrated that the inhibition of prolactin release using the dopamine-D2-receptor agonist, bromocriptine, in conjunction with conventional anti-failure therapy, was associated with higher rates of LV recovery and low morbidity and mortality in PPCM patients.[43]

Familial clustering of PPCM was first described in 1963 by *Pierce et al*.,[44] and familial disease has been shown to occur in 5–10% of cases.[26,45] However, up until recently, the genetic contribution to susceptibility to PPCM was largely unstudied. PPCM and DCM share a number of clinical characteristics, including impairment of systolic function, increased chamber dimensions and nonspecific histological findings. In a recent study, 172 women with PPCM were screened for variants in 43 genes previously associated with DCM. The burden of genetic variants was found to be similar to what has been previously reported in DCM, and 65% of these variants were found in *Titin (TTN).* The authors conclude that while many of these truncating variants lead to a strong predisposition to PPCM, the presence of more common variants is likely not a risk factor for penetrant PPCM and further study is required.[46] It is important to note that these variants were not found in those women with preeclampsia and other forms of pregnancy-induced hypertension.[35] When women present with HF during the

peripartum period, uncertainty often exists as to whether these women are affected with PPCM or have underlying familial DCM that has only become apparent consequent to the haemodynamic stress of pregnancy. There are currently no diagnostic criteria that separate these two entities, although a positive family history can be helpful.[47] PPCM has a higher rate of spontaneous recovery of LV function and has better survival than idiopathic DCM,[15,48] therefore the distinction is important.

1.3.2. Hypertrophic cardiomyopathy

Historically, HCM has been defined by the presence of inappropriate myocardial hypertrophy in the absence of abnormal loading conditions (e.g. hypertension, aortic stenosis) or infiltration (e.g. amyloidosis, sarcoidosis). Traditionally, the term "hypertrophic cardiomyopathy (HCOM)" has been synonymous with the clinically heterogeneous but relatively common genetic form of cardiomyopathy characterised by LV hypertrophy (LVH), classically involving (although not limited to) the interventricular septum (IVS) which can result in LV outflow tract (LVOT) obstruction[13] (Figure 1.6.). Histological findings in this condition include interstitial fibrosis, myocyte enlargement and myocyte disarray.[49,50] It is important to distinguish myocyte hypertrophy, caused by a variety of sarcomeric mutations, from conditions with increased ventricular wall thickness due to interstitial infiltration (e.g. amyloidosis), intracellular accumulation of metabolic substances (e.g. glycogen storage diseases, lysosomal storage diseases, disorders of fatty acid metabolism), or the setting of syndromic conditions (e.g. Noonan syndrome, LEOPARD syndrome, Friedreich's ataxia, Beckwith-Wiedermann syndrome, Swyer's syndrome). In both the American and European classification systems, these non-sarcomeric genetic and acquired infiltrative conditions are classified as 'hypertrophic cardiomyopathy phenocopies'[13,51,52] (Figure 1.7.).

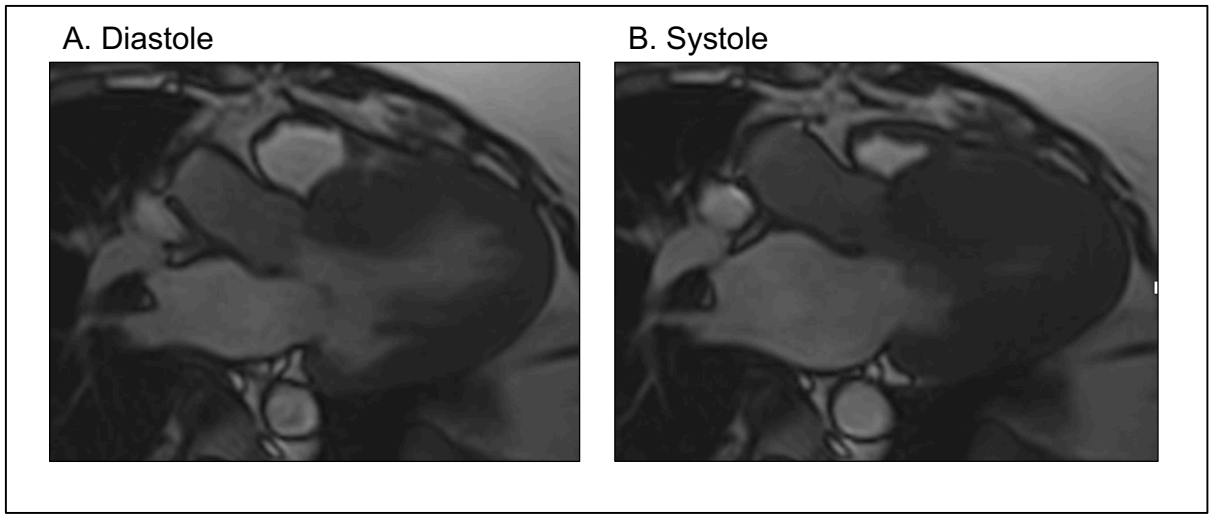


Figure 1.6. CMR of a patient with HCM with LV outflow tract obstruction due to septal hypertrophy and systolic anterior motion of the anterior mitral valve, and mid-cavity obliteration in systole.

Images: CMR of IMHOTEP study participant

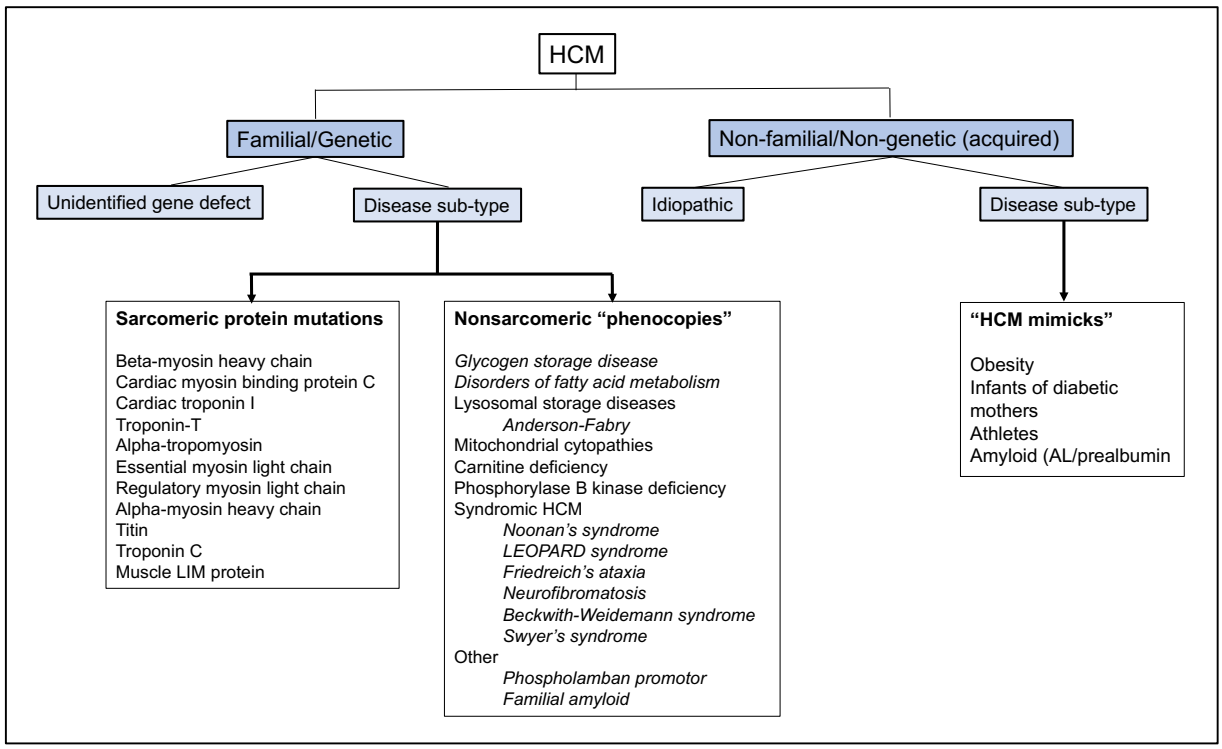


Figure 1.7. Classification and aetiology of hypertrophic cardiomyopathies (HCM)
Adapted from: Elliott P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J*. 2007;29(2):270-276.[13]

Familial HCM is the most common form of genetic cardiomyopathy and has a prevalence of 1 in 500. Prevalence estimates are based on data from the Coronary Artery Risk Development in Young Adults (CARDIA) cohort study published in 1995, and are supported by a number of other phenotype-based echocardiographic studies in different populations.[53,54] While prevalence data in African populations is limited, echocardiographic studies from Ghana and Ethiopia have dispelled the myth that HCM is rare amongst Africans.[21,55] Subsequent genetic population studies suggest that the prevalence of HCM gene carriers is estimated at 1 in 200 or greater.[54] Although all gene carriers may not manifest clinical HCM, these findings suggest that the prevalence of HCM may be higher than previously reported. Additionally, a number of comparative studies have demonstrated that cardiovascular magnetic resonance (CMR) is able to identify LV hypertrophy in regions not reliably visualised with standard echocardiography (namely apical, anterolateral or posterior inferior regions of the LV) and can assist in making the diagnosis of HCM in borderline or ambiguous cases.[56,57] This provides additional support to the suggestion that the prevalence of HCM may have been underestimated by echocardiography-based studies.

Familial HCM is recognised as an autosomal dominant disorder and is primarily a disease of the sarcomere. Numerous mutations encoding various contractile proteins of the cardiac sarcomere have been reported as disease-causing, most commonly in the ß-myosin heavy chain (*MYH7*) and myosin-binding C (*MBPC3*). The yield of screening for causal mutations ranges from 40–70%.[52,58] There are no pre-existing national registries for HCM on the African continent; however, clinical and genetic studies have been conducted on a few single-centre cohorts of patients with HCM in South Africa. Moolman-Smook and colleagues have done pioneering work on the genetics of HCM in South African patients of mixed ancestry and white descent. In one study, 23 (58%) of the 40 unrelated patients' studies were genotype positive, with three founder mutations (*MYH7* Ala797Thr, *TNNT2* Arg92Trp, *MYH7* Arg403Trp) reported in 45% of genotyped patients in that cohort.[59] Up until recently, black Africans were significantly under-represented in these studies. In a recent prospective study on HCM from

Cape Town (total n = 43; mixed ancestry 63%, black Africans 30%, Caucasian 5%, and Indian 2%), comprehensive genetic screening was associated with a 29% yield of causal mutations, all in the *MYH7* and *MBPC3* genes. The authors note that, given the relatively low yield of screening in this study, routine molecular genetic testing in Africans with HCM should not be conducted until there is more data on the full spectrum of causal mutation, and the impact of genetic testing on outcomes is available.[60] Genetic, epigenetic and environmental modifiers of the HCM phenotype are still not well understood, necessitating future research, especially among Africans.

Longitudinal studies from the USA have demonstrated that with contemporary management strategies and interventional therapies, such as implantable cardioverter defibrillators (ICDs) and orthotopic heart transplantation (OHT), patients with HCM have a low disease-related mortality rate of 0.5–1.3% per year.[61] In South Africa, a higher mortality rate of 2.9% per year has been reported.[60] While the risk of SCD is well recognised in HCM, the incidence and mortality from disease progression to a HF syndrome are sometimes underappreciated. Up to a fifth of patients with HCM develop HF at a median age of 48±19 years,[62] and chronic HF as a complication of HCM was observed in 25.6% of patients in the South African cohort.[60]

Although the current clinical guidelines recommend family screening in children from age 10,[58] a recent study showed that a diagnosis in a child first degree relative was made in 8% of families screened, and while the HCM phenotype varies in childhood, severe disease is well described.[63] There is currently no data on the incidence of childhood-onset HCM in the African population.

1.3.3. Restrictive cardiomyopathy

RCM is a rare cardiomyopathy characterised by impaired ventricular filling and diastolic dysfunction with relatively normal wall thickness and systolic function. It has a broad aetiology that includes genetic causes, infiltration, connective tissue disease, glycogen storage disease, drugs and radiation.[13] The prognosis of RCM is poor, particularly in children, with transplant-free survival of 22% in 5 years.[64,65]

Endomyocardial fibrosis (EMF), the most common form of RCM, occurs primarily in tropical and sub-tropical regions worldwide, and is endemic in peri-equatorial regions of Africa. EMF is characterised by ventricular endomyocardial thickening with dense, white fibrous tissue that results in cavity obliteration and impaired ventricular filling[66,67] (Figure 1.8.). The observed geographic restriction and regional variation in countries with high prevalence make accurate estimates of incidence and prevalence of EMF difficult to determine.[68] Population screening for EMF in an endemic region of Mozambique, reported a prevalence of 19.8%, suggesting that it may be a dominant form of cardiomyopathy in that region.[69] Apart from geography, a number of factors have been associated with EMF in Africa – including ethnicity, poverty, diet, age, sex, eosinophilia and infection – but their specific roles in the pathogenesis of EMF remain poorly understood.[67] There is limited compelling evidence to support genetic susceptibility in EMF, although familial occurrence has been reported in case reports from different regions in Africa[70,71] and certain HLA alleles have been associated with EMF in 2 different populations in one study.[72] EMF predominantly affects children and adolescents from poverty stricken communities, and usually presents in the advanced stages of disease. As a result, the early manifestations of this condition are poorly defined. EMF continues to be an important and debilitating condition with a poor prognosis, affecting young individuals from many regions of Africa.[67]

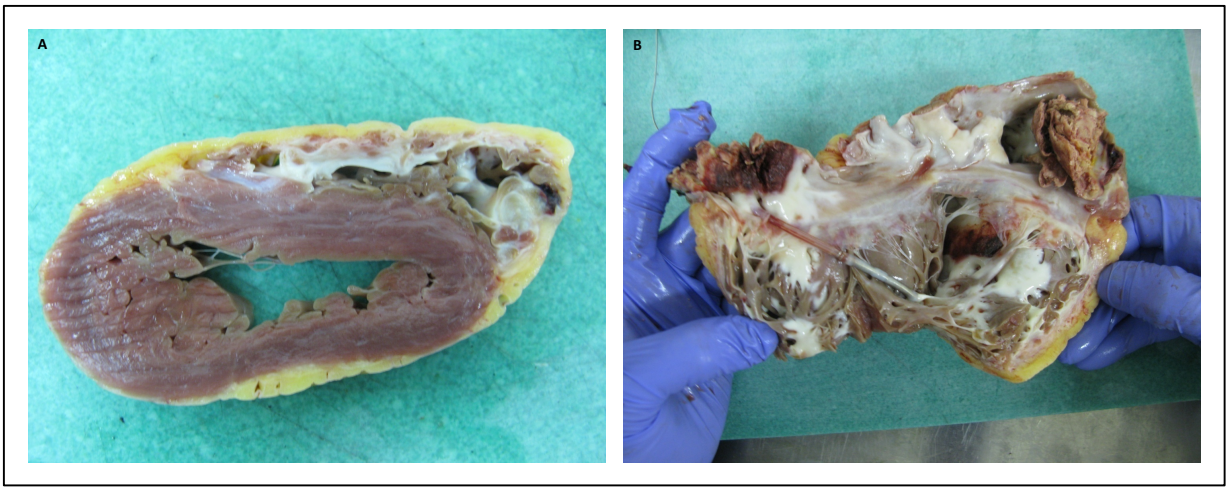


Figure 1.8. Example of isolated right ventricular endomyocardial fibrosis.
Explanted heart of study participant. **A.** Cross-section of right and left ventricles. Endomyocardial fibrosis of right ventricle. **B.** Right atrium and ventricle. Right atrial thrombus, right ventricular endomyocardial fibrosis.
Images provided by H. Wainwright, Department of Pathology, University of Cape Town.

1.3.4. Arrhythmogenic right ventricular cardiomyopathy

ARVC is defined by the presence of right ventricular (RV) dysfunction (global or regional), with or without LV disease, in the presence of histological evidence of fibrofatty replacement (Figure 1.9.) and/or electrocardiographic abnormalities according to published criteria.[13,73] ARVC classically presents with malignant ventricular arrhythmias, which may lead to SCD, with HF occurring in 10–20% of patients. While ARVC is considered uncommon, with an estimated prevalence of 1 in 5000,[13] it is a frequent cause of SCD in young people.[74] The prevalence of ARVC in Africa is undetermined.

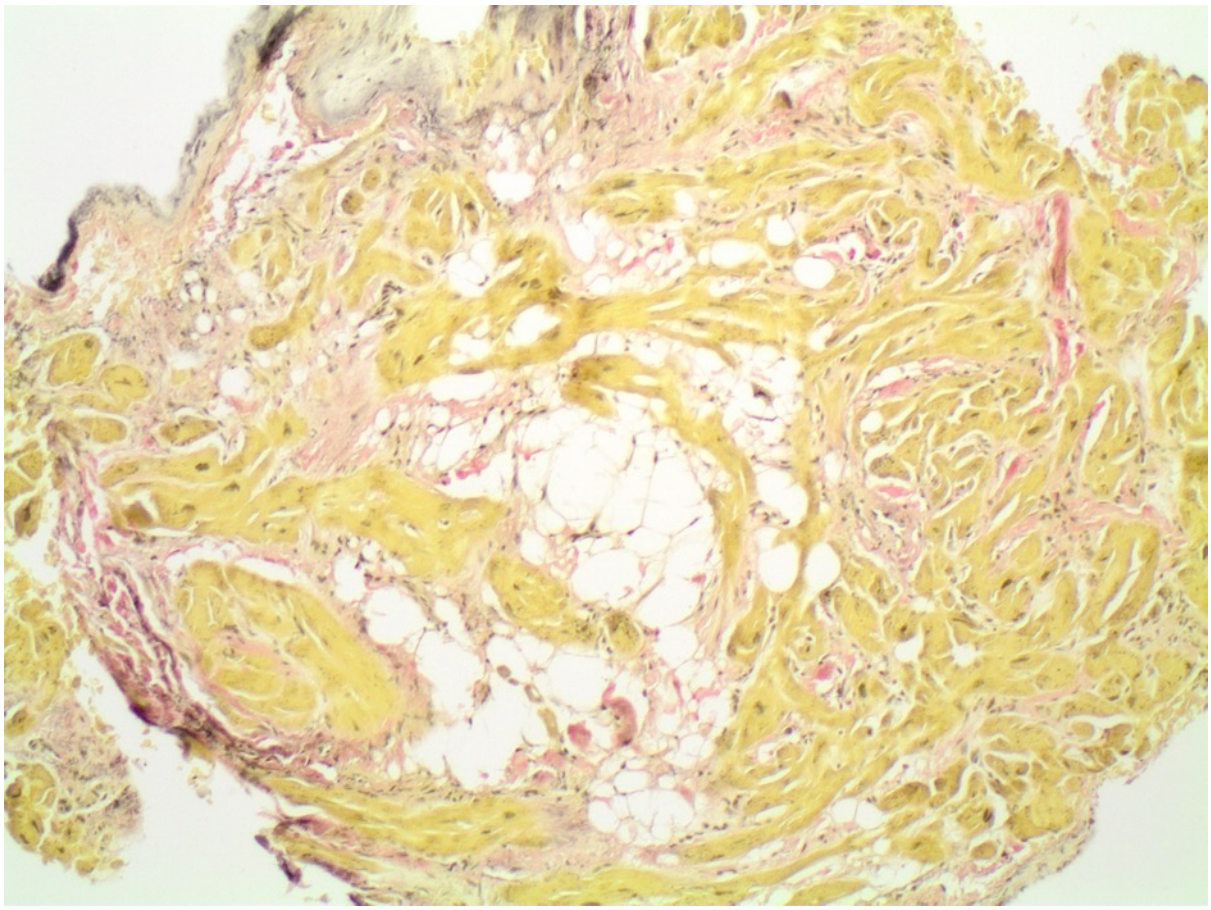


Figure 1.9. Fibrofatty replacement of the myocardium in an IMHOTEP patient with ARVC
From: Mayosi BM, Fish M, Shaboodien G, et al. Identification of Cadherin 2 (CDH2) Mutations in Arrhythmogenic Right Ventricular Cardiomyopathy. *Circ Cardiovasc Genet* 2017;10(2) e001605.[75]

Images provided by H. Wainwright, Department of Pathology, University of Cape Town

ARVC is considered a genetic (sporadic or inherited) form of cardiomyopathy and is familial in 50% of cases. It is usually transmitted as an autosomal dominant (AD) trait with variable penetrance, although autosomal recessive (AR) cardio-cutaneous forms of the condition are well described.[76,77] The discovery of mutations in desmosomal genes in ARVC has been crucial in understanding the underlying mechanisms of disease as desmosomal proteins have an important role in myocyte cell-to-cell adhesion.[78,79] Genetically altered function of cell-adhesion proteins results in the disruption of intercellular junctions, myocyte uncoupling (aggravated by physical exercise)[80] and myocyte death with subsequent fibrofatty tissue replacement. This leads to structural changes within the ventricular wall and electrical

instability with arrhythmias that are thought to occur through a scar-related macro-reentry mechanism.[81,82] Pathogenic genetic variants associated with ARVC have been reported for desmosomal genes encoding plakophilin-2 (*PKP2*),[83] desmoplakin (*DSP*),[84] desmoglein-2 (*DSG2*),[85] desmocollin-2 (*DSC2*),[86] and plakoglobin (*JUP*).[78] In addition, non-desmosomal genes encoding cardiac ryanodine-2 receptor (RYR2),[87] transforming growth factor beta-3 (TGFß3),[88] transmembrane protein 43 (TMEM43)[89] and cadherin 2 (*CDH2*),[75] have also been implicated in the development of ARVC, amongst others.[90]

Following the call for unified international ARVC registries in 2000,[91] a number of large collaborative registries were established. The largest of these are the ESC International Registry[92] – combining cohorts from France, Germany, Italy, Greece, Poland, Cyprus and the United Kingdom – and a collaboration of the John Hopkins University and Dutch Interuniversity Medical Centre's registries.[93] With the knowledge generated from international registries over two decades, the original 1994 diagnostic task force criteria (TFC) were modified in 2010 (Table 1.1.),[73,94] highlighting the important role of collaborative registries for uncommon conditions. The diagnosis of ARVC is reliant on the demonstration of structural, functional and electrophysiological abnormalities that are associated with underlying histological changes. The TFC represent a working framework to improve diagnostic certainty. The diagnosis of ARVC requires a multidisciplinary approach that combines expertise from numerous disciplines including clinical cardiology, electrophysiology, imaging, pathology, and clinical and molecular genetics. By incorporating new knowledge, quantifiable variables, emerging diagnostic modalities such as cardiovascular magnetic resonance (CMR), and advances in the genetics of ARVC, the revised 2010 TFC have been shown to increase diagnostic value, with improved sensitivity without loss of specificity.[73]

The ARVC Registry of South Africa was established in 2003[95] and our group published data on the first 50 participants classified according to the 1994 TFC in 2009. They reported that ARVC occurs in all ethnic groups in South Africa; patients usually presented with symptoms

by the third decade of life; and outcomes were worse than in other parts of the world, with an annual mortality of 2.8% and a 5-year cumulative mortality of 10%.[96] While there are several explanations for the higher mortality, the most likely is that ICDs are underutilised in South Africa due to resource constraints in the public sector. Thirty percent of participants had a family member who was also affected, and genetic analysis revealed that 25% of cases were caused by *PKP2* gene mutations.[96] Additionally, a *PKP2* founder mutation was identified in four unrelated families, and more recently novel *CDH2* mutations were reported in two unrelated families, from this cohort.[75,96] The South African ARVC Registry has provided insight into ARVC in the South African context; however, there are no studies published on this condition from the rest of the African continent.

Table 1.1. Comparison of original and revised ARVC Task Force Criteria

Original Task Force Criteria	Revised Task Force Criteria
I. Global or regional dysfunction and structural alterations*	
Major	
• Severe dilatation and reduction of RV ejection fraction with no (or only mild) LV impairment • Localized RV aneurysms (akinetic or dyskinetic areas with diastolic bulging) • Severe segmental dilatation of the RV	**By 2D echo:** • 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
Minor	
• Mild global RV dilatation and/or ejection fraction reduction with normal LV • Mild segmental dilatation of the RV • Regional RV hypokinesia	**By 2D echo:** • 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 characterization of wall	
Major	
• Fibrofatty replacement of myocardium on endomyocardial biopsy	• 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
Minor	
	• 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. Repolarization abnormalities	
Major	
	• Inverted T waves in right precordial leads (V_1, V_2, and V_3) or beyond in individuals >14 years of age (in the absence of complete right bundle-branch block QRS ≥120 ms)
Minor	
• Inverted T waves in right precordial leads (V_2 and V_3) (people age >12 years, in absence of right bundle-branch block)	• Inverted T waves in leads V_1 and V_2 in individuals >14 years of age (in the absence of complete right bundle-branch block) or in V_4, V_5, or V_6 • Inverted T waves in leads V_1, V_2, V_3, and V_4 in individuals >14 years of age in the presence of complete right bundle-branch block

(*Continued*)

From: Marcus et al. Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: Proposed Modification of the Task Force Criteria. *Circulation* 2010;121:1533-1541[73]

Table 1.1. Comparison of original and revised ARVC Task Force Criteria (continued)

Original Task Force Criteria	Revised Task Force Criteria
IV. Depolarization/conduction abnormalities	
Major	
• Epsilon waves or localized prolongation (>110 ms) of the QRS complex in right precordial leads (V_1 to V_3)	• Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V_1 to V_3)
Minor	
• Late potentials (SAECG)	• 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 μV (low-amplitude signal duration) ≥38 ms • Root-mean-square voltage of terminal 40 ms ≤20 μV • Terminal activation duration of QRS ≥55 ms measured from the nadir of the S wave to the end of the QRS, including R′, in V_1, V_2, or V_3, in the absence of complete right bundle-branch block
V. Arrhythmias	
Major	
	• 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)
Minor	
• Left bundle-branch block–type ventricular tachycardia (sustained and nonsustained) (ECG, Holter, exercise) • Frequent ventricular extrasystoles (>1000 per 24 hours) (Holter)	• 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	
Major	
• Familial disease confirmed at necropsy or surgery	• 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† categorized as associated or probably associated with ARVC/D in the patient under evaluation
Minor	
• Family history of premature sudden death (<35 years of age) due to suspected ARVC/D • Familial history (clinical diagnosis based on present criteria)	• 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

PLAX indicates parasternal long-axis view; RVOT, RV outflow tract; BSA, body surface area; PSAX, parasternal short-axis view; aVF, augmented voltage unipolar left foot lead; and aVL, augmented voltage unipolar left arm lead.

Diagnostic terminology for original criteria: This diagnosis is fulfilled by the presence of 2 major, or 1 major plus 2 minor criteria or 4 minor criteria from different groups. Diagnostic terminology for revised criteria: definite diagnosis: 2 major or 1 major and 2 minor criteria or 4 minor from different categories; borderline: 1 major and 1 minor or 3 minor criteria from different categories; possible: 1 major or 2 minor criteria from different categories.

*Hypokinesis is not included in this or subsequent definitions of RV regional wall motion abnormalities for the proposed modified criteria.

†A pathogenic mutation is a DNA alteration associated with ARVC/D that alters or is expected to alter the encoded protein, is unobserved or rare in a large non–ARVC/D control population, and either alters or is predicted to alter the structure or function of the protein or has demonstrated linkage to the disease phenotype in a conclusive pedigree.

From: Marcus et al. Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: Proposed Modification of the Task Force Criteria. *Circulation* 2010;121:1533-1541[73]

1.3.5. Left ventricular noncompaction

Isolated LVNC is a genetically heterogeneous disease characterised by prominent myocardial trabeculations and deep intertrabecular recesses[13] (Figure 1.10.). The true prevalence of LVNC is unknown, and the reported prevalence in the literature varies considerably. Ritter *et al.* reported a prevalence of 0.05% of all adult echocardiographic examinations in a large institution in North America.[97] Oechslin *et al.* reported a prevalence of 0.014% in patients referred for echocardiography in Switzerland.[98] An epidemiological study of primary cardiomyopathy in Australian children revealed that LVNC accounted for 9.2% of all cases and was identified as the third most common cause of cardiomyopathy, after DCM and HCM.[99] The only prevalence data from Africa is from a single-centre prospective case-control study conducted in South Africa: Peters *et al.* found the prevalence of LVNC in to be 6.9% and observed that patients of African descent frequently had biventricular involvement and pulmonary hypertension.[100]

Our understanding of genotype–phenotype correlations in LVNC is poor and the notion that LVNC is a distinct cardiomyopathy remains to be clarified. There is considerable genetic overlap of different morphological phenotypes of cardiomyopathy, and while a number of genetic mutations associated with LVNC have been reported, a true causal relationship between reported mutations and LVNC is yet to be established.[101] Comprehensive diagnostic assessment – including multimodality imaging, systematic and comprehensive pedigree analysis, family screening and genetic testing – is required to further characterise the morphological expression and myocardial phenotype of genetic mutations. The common complications of LVNC are HF, arrhythmias, SCD, and systemic embolism. Currently there are no specific treatment guidelines for LVNC and further prospective studies are necessary to develop effective treatment strategies.[102]

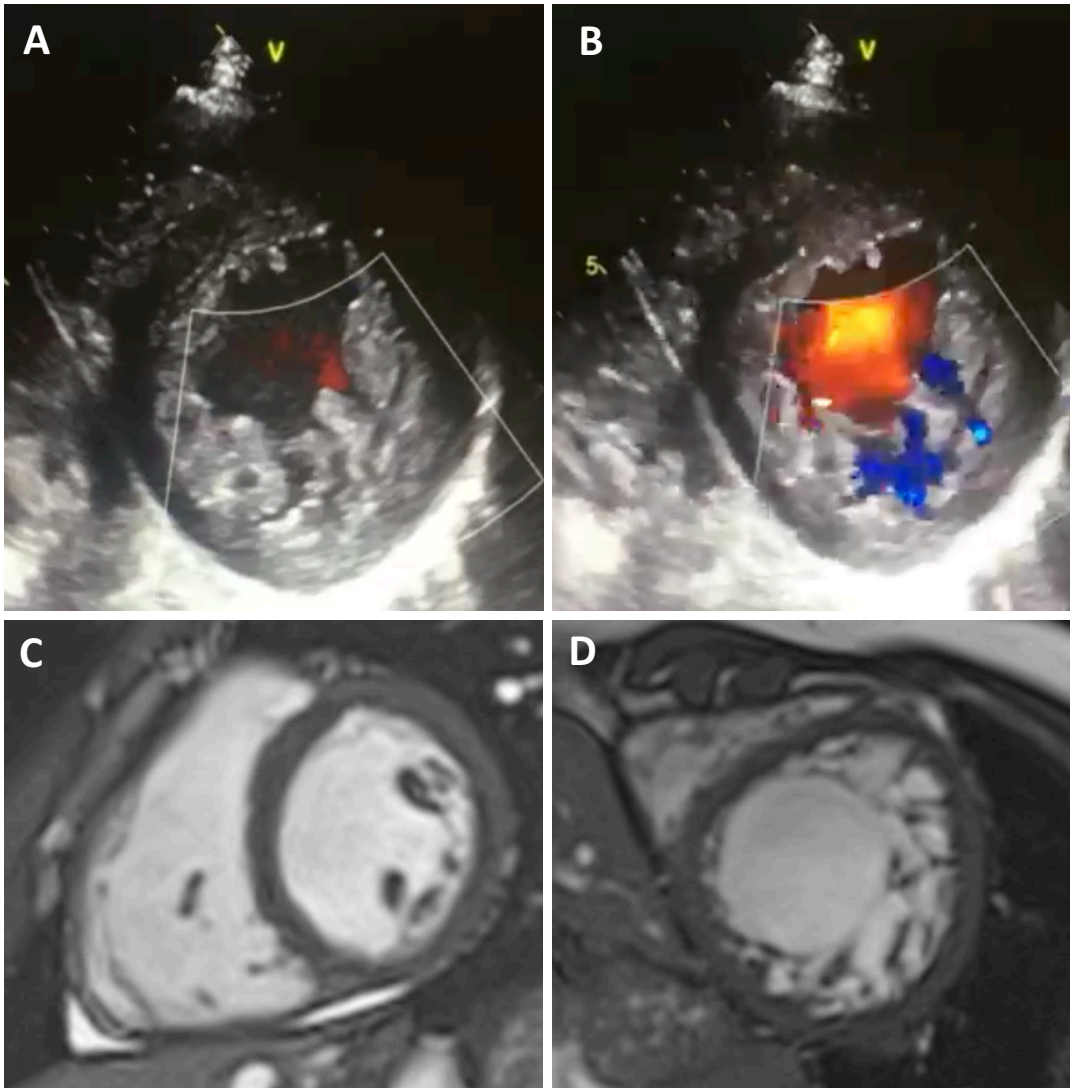


Figure 1.10. Left ventricular non-compaction
A and B. Echocardiogram of an infant with LVNC (short axis view). Short axis view of the left ventricle with colour Doppler demonstrating flow within the intertrabecular recesses**; C.** CMR short axis view of a normal adult heart; **D.** CMR short axis view of a heart of an adult patient with LVNC.

Images: CMR of IMHOTEP study participants; echocardiographic images of IMHOTEP participant provided by G. Comitis, Division of Paediatric Cardiology, Red Cross War Memorial Children's Hospital, University of Cape Town.

1.3.6. Myocarditis

The definition of myocarditis utilised in published studies is variable but the term 'myocarditis' applies to acute or chronic inflammatory disease of the myocardium due to environmental or endogenous triggers. The specific causes of myocarditis are diverse and both infectious (viruses, bacteria, fungi, parasites) and non-infectious (giant cell myocarditis, drug-induced hypersensitivity, organ/tissue/antigen-specific autoimmune conditions such as systemic lupus erythematosus and sarcoidosis) causes of myocarditis have been described.[103] While the diagnosis of myocarditis has traditionally been based on established histological, immunological and immunohistochemical criteria, published CMR criteria for myocarditis are universally accepted,[104] and newer more sensitive and specific CMR sequences have demonstrated improved diagnostic and prognostic value.[105]

The burden of myocarditis as a contribution to prevalent HF varies by age and region and is estimated to range from 0.5% to 4%.[106] Cooper *et al.* highlighted that the lack of substantial data worldwide has hindered epidemiological analysis. The requirement of sophisticated tests, including endomyocardial biopsy (EMB) and CMR, restricts the ability to diagnose myocarditis, and identification of cases in cross-sectional studies is limited.[106]

Myocarditis is an underdiagnosed cause of sudden death and DCM in both adults and children.[106] Data from Western Europe and North America indicate that myocarditis presenting as acute DCM in adults has a high mortality and a low likelihood of complete LV recovery. The prospective US Myocarditis Treatment Trial reported a mortality rate of 20% at 1 year and 56% at 4.3 years.[107] A large single-centre registry of biopsy-proven myocarditis from Boston reported 1 and 5-year survival rates were 79% and 56%, respectively.[108] In Italy, registry data showed that the risk of death or OHT was 27% with an average duration of follow-up of 23 months.[109] In Germany, a series of 203 patients with viral genome-positive cardiomyopathy and a mean follow-up of 4.7 years revealed a 19.2% all-cause mortality, 15% cardiac-related

mortality and 9.9% SCD rate.[110] Myocarditis has been shown to be the most common cause of DCM in children in North America: The Paediatric Cardiomyopathy Registry found that myocarditis was causal in 46% of the 485 children with DCM from a known cause.[111]

Although DCM has been identified as a major cause of HF throughout Africa,[2] there are only a few small single-centre studies looking at the prevalence of myocarditis in African patients. These studies suggest that myocarditis may play a significant role in the pathogenesis of HIV-associated cardiomyopathy and idiopathic DCM in Africa.[112-114] In a study conducted in South Africa, Shaboodien et al. reported that myocarditis was present in 44% of HIV-associated cardiomyopathy cases, 36% of heart transplant recipients, and 25% of participants with idiopathic DCM. While acute myocarditis was present in 50% of HIV- and heart transplant-associated myocarditis cases, it was chronic in all those with idiopathic DCM.[114] In a study looking at 103 HIV-infected individuals without known CVD, Ntusi *et al.* found CMR-detected evidence of subclinical myocardial oedema and an increased incidence of pericardial effusions, providing additional evidence for chronic myocardial inflammation in HIV infected patients.[115] The true incidence of myocarditis in patients presenting with new onset DCM with HF in Africa is unknown.

1.4. ROLE OF CARDIOVASCULAR MAGNETIC RESONANCE IMAGING IN CARDIOMYOPATHIES AND MYOCARDITIS

The fundamental aims of the diagnostic work-up of suspected cardiomyopathy are to accurately define the phenotypic characteristics, identify potentially treatable causes and risk stratification for prognostic purposes, so that directed medical and interventional therapy can be instituted timeously.[14,23] CMR provides key information in a single non-invasive study that includes anatomical dimensions, ventricular function, tissue characterisation (particularly the presence of inflammation), and the presence, extent and location of fibrosis. Cine-CMR provides accurate and reproducible quantification of ventricular volumes, ejection fraction and

LV mass, making it the ideal imaging modality in both the clinical and research settings.[116,117] CMR has emerged as the gold standard for assessing RV volumes and function due to its complex geometry and the recognised limitations of other imaging techniques such as echocardiography and angiography.[118,119] CMR has therefore become the imaging modality of choice in ARVC and is a crucial part of the diagnostic work-up in these patients.

The relative safety of gadolinium agents and the lack of ionising radiation exposure allows for repeated imaging to review response to treatment, conduct periodic family screening and perform serial risk stratification. Additionally, by utilising different imaging techniques, CMR can be diagnostic for certain conditions such as myocarditis, Anderson-Fabry disease, amyloidosis and cardiac iron overload,[104,120-122] and further invasive investigations such as EMB may be deferred. Non-contrast tissue characterisation with T2-weighted imaging can be used to detect acute myocyte oedema and interstitial fluid accumulation.[123] Native T1 mapping has been shown to be significantly more sensitive than T2-weighted imaging and late gadolinium enhancement (LGE) in detecting acute myocarditis, identifying larger extent of myocardial involvement, and diagnosing additional cases with small focal areas of injury.[104,105,124,125] Furthermore, myocardial inflammation can be detected using T2 mapping, as myocardial T2 increases in the presence of oedema, and myocardial extracellular volume (ECV), a surrogate marker of fibrosis (in the absent of confounders such as infiltration), can be calculated using T1 mapping tissue characterisation.[126] LGE is able to detect focal myocardial fibrosis or infiltration, and is a powerful tool for distinguishing between ischaemic and non-ischaemic aetiologies of myocardial dysfunction[127] (Figure 1.11.). Several studies have shown the prognostic significance of LGE in cardiomyopathy.[128] The presence of LGE is an independent predictor for adverse outcomes, including SCD/ventricular tachycardia (VT), hospitalisations and all-cause mortality in DCM,[129] and a 3.4-fold greater risk of major adverse events in HCM, with the risk being proportional with the amount of LGE-detected fibrosis present.[130] LGE has been shown to be a strong predictor of major adverse cardiac events and

mortality in myocarditis.[110,131] Published data on CMR in African patients with cardiomyopathy is extremely limited.

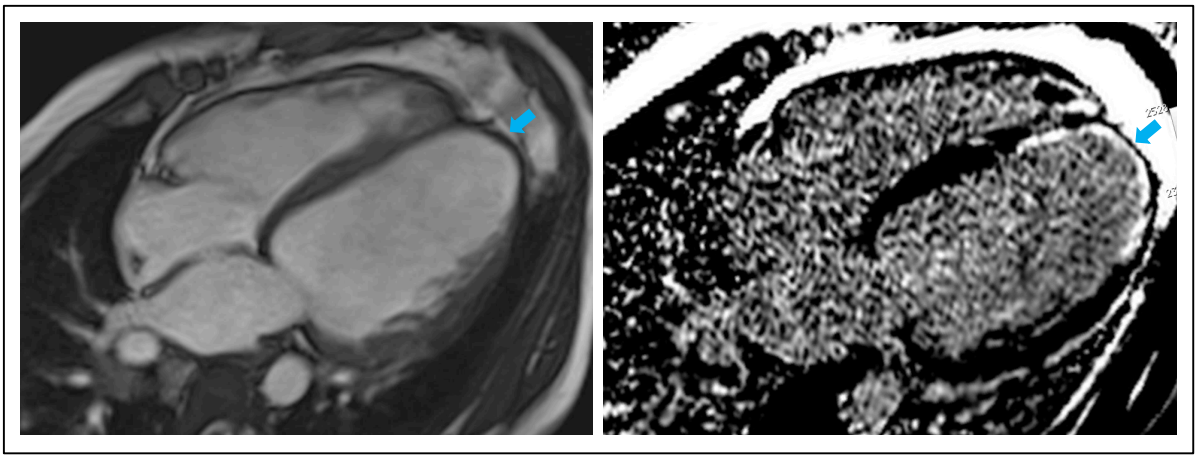


Figure 1.11. CMR of patient excluded from IMHOTEP with ischaemic LV dysfunction

Images: CMR of IMHOTEP study participant

1.5. GENETIC CARDIOMYOPATHIES

Genetic cardiomyopathies warrant close study because they are often present in younger individuals,[26,28] and are an important cause of mortality and morbidity in the young.[27,64] Correctly identifying the definitive disease-causing mutation in an index case potentially affords a gold standard diagnostic marker for the presence or absence of the pathogenic substrate among relatives. If able to identify a pathogenic mutation in an affected individual, family screening provides a unique opportunity to offer early diagnosis, risk stratification and intervention preventatively or early on in the disease process.[58,64,132,133] There is evidence that genotype has a role in risk stratification in both individual and families. For example, Lamin A/C mutation are associated with high penetrance in young asymptomatic genotype-positive relatives, frequent conduction abnormalities and ventricular arrhythmias, and a high (19%) likelihood of requiring cardiac transplantation.[134] In many parts of the world, genetic testing in inherited heart disease has become standard of care,[58,135] but it is not currently available

outside of the research setting on the African continent. The complexity of cardiomyopathy and genetic testing increasingly supports a model of clinical care involving a specialist unit with cardiologists, geneticists and genetic counsellors, able to collectively manage these patients and their families.[14,136]

The genetic complexity underpinning the cardiomyopathies remains a challenge in both research and clinical practice, and molecular genetic results often require nuanced interpretation, rather than definitive labelling of variants as pathogenic or benign.[133] While the cardiomyopathies are classified into different morphological sub-types, there is significant genetic and phenotypic overlap between the different subtypes (Figure 1.12.).[29] Cardiomyopathy-associated mutations are notable for substantial variation in clinical expression. In addition to genetic heterogeneity and allelic variations contributing to variable clinical phenotypes and disease severity – background genomic variation, environmental exposures and lifestyle also account for variable clinical manifestations of disease even within families with identical mutations.[133] Furthermore, next generation sequence analysis of disease and control cohorts have demonstrated that 60-90% of cardiomyopathy mutations are "private" – i.e. unique to a single family.[137,138] These complexities pose significant challenges for clinicians when interpreting genetic results and their application into clinical practise, particularly with regards to risk stratification in family members. While the advancement of sequencing techniques, the development of pathogenicity prediction tools and the building of publicly available resources showing variant frequency in large populations (e.g. The Exome Aggregation Consortium – ExAC) has strengthened our ability to predict pathogenicity,[139,140] it has also cast doubt on some cardiomyopathy-associated variants previously identified by candidate gene analysis.[141] This has led to the reassessment of Mendelian gene pathogenicity showing that rare variation is not always clinically informative, and improved interpretation for specific genes and variant classes is necessary to increase the clinical utility of genetic testing in these conditions.[141] Importantly, the frequency of specific variants appears to differ among

people of different ancestry.[140,142] The interpretation of variants, therefore, relies on the availability of large locally-based population control data.

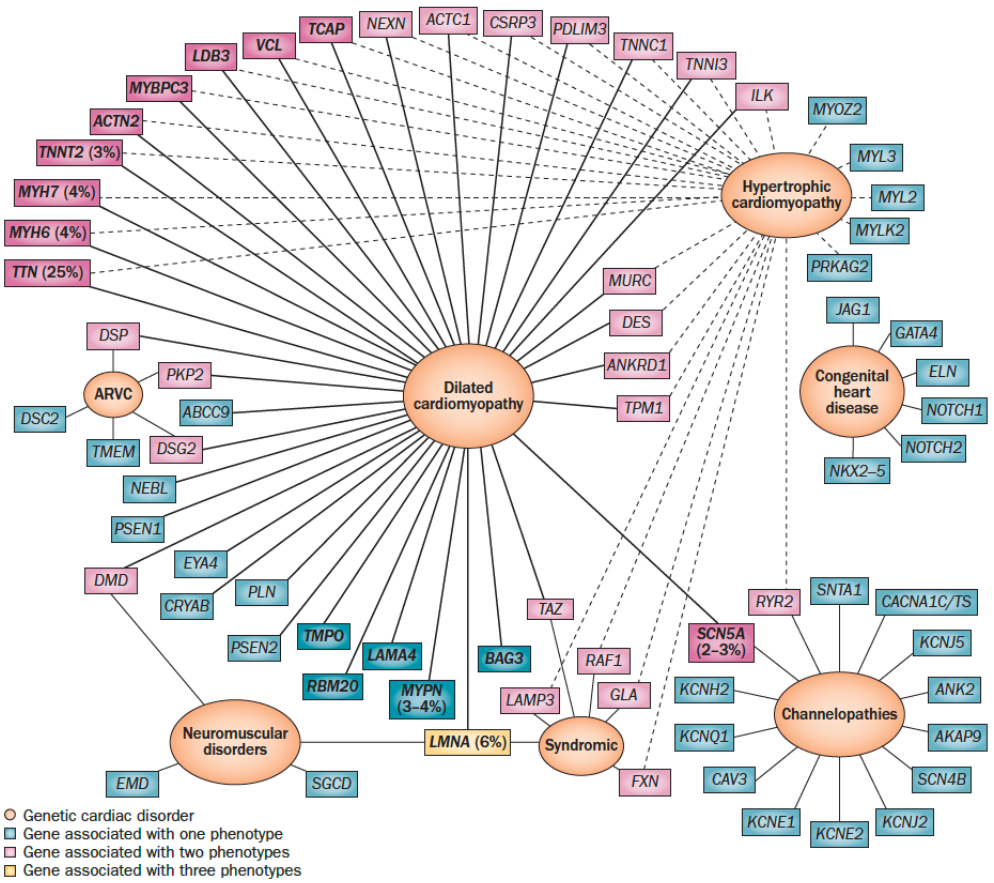


Figure 1.12. Relationship between genes associated with cardiomyopathies and related phenotypes
From: Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: the complexity of a diverse genetic architecture. *Nat Rev Cardiol* 2013; **10**(9): 531-47. [29]

1.6. RATIONALE FOR THIS RESEARCH

There is a need for large population-level studies of the incidence and prevalence of HF and cardiomyopathy in Africans, to influence decision-making for resource allocation for the prevention and treatment of CVD. Along with epidemiological surveys, molecular genetic studies of cardiomyopathy represent a new frontier for cardiovascular research in Africa. The advent of advanced methods of genotyping provide an opportunity for research in the field of

cardio-genetics and the determination of the cost-effectiveness of molecular testing in the diagnosis and management of patients with inherited cardiomyopathies in low-to-middle-income countries (LMICs). We currently do not have sufficient information on the yield of genetic testing within the African population or the impact of genetic testing on outcomes in limited resource settings, to justify the cost of diagnostic genetic testing for cardiomyopathies on the continent. The core of human genetic studies is the careful and comprehensive phenotyping of patients and their family members, and the correlation of those phenotypes with genetic information. CMR provides an abundance of phenotypic information in a single study and is, therefore, the ideal imaging modality to facilitate genotype-phenotype correlations in cardiomyopathy. Furthermore, on a continent where infectious diseases are endemic, little is known about the contribution of infectious aetiologies to the burden of cardiomyopathies in Africans or the interplay between genetic and environmental factors in the natural history of disease in this context. The tissue characterisation capabilities of CMR provides a unique opportunity to study the contribution of inflammatory conditions to the burden of heart muscle disease in our population.

This work represents the building of a clinical research platform that is able to provide information on epidemiology and clinical outcomes in patients with heart muscle disease from different regions of Africa, in addition to providing detailed phenotyping of individuals and families with inherited cardiomyopathies to facilitate molecular genetics research on the continent. The variable clinical expression and age of onset of cardiomyopathies challenges the traditional divide between paediatric and adult clinical care and research, and highlights the necessity for long term follow-up of individuals with cardiomyopathy, and their families. IMHOTEP has therefore been designed to address the deficiencies in our understanding of the epidemiology, aetiology, genetic predisposition and patient outcomes associated with heart muscle disease in both adults and children in Africa, and facilitate the study of families affected by these conditions. The rationale for the design of IMHOTEP is explored further in chapter 3.

1.7. STUDY HYPOTHESES

There are a number of research questions/hypotheses on which this work is based, and while they are not novel, our depth of understanding of these concepts in the African population is limited:

1) Cardiomyopathy is caused by familial (genetic) and non-familial (secondary) factors;
2) There is an overlap in the molecular genetic causes and phenotypic expression of different morpho-functional types of cardiomyopathy;
3) Secondary factors (such as myocarditis, pregnancy, etc.) are present in a significant proportion of cases where they serve as triggering factors in familial cases and causative factors in non-familial cases;
4) The outcome of cardiomyopathy in Africans is poor and is influenced by genetic and non-genetic factors.

1.8. STUDY AIMS

The primary aim of this study was to develop a comprehensive and multi-centre clinical registry and database to systematically capture demographic, clinical, electrocardiographic, imaging, histological, genetic and outcome data on adults and children diagnosed with different morpho-functional types of cardiomyopathy and myocarditis in Africa (the African Cardiomyopathy and Myocarditis Registry Program – IMHOTEP) (Chapter 3).

A number of sub-studies were designed to address the above-mentioned research hypotheses, and were implemented as part of the single-centre pilot phase of IMHOTEP. The aims of these sub-studies were as follows:

1) to reclassify all patients referred to the *Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa* according to the updated 2010 task force

criteria, and report the demographics, clinical and genetic features, and the outcomes in ARVC in South Africa (Chapter 4);

2) to describe the baseline characteristics and vital status of prevalent cases of familial and non-familial forms of DCM incorporated from existing studies into IMHOTEP (Chapter 5);
3) to report preliminary data on the baseline clinical characteristics, CMR features and application of the 3-stage diagnostic approach in adult incident cases recruited to IMHOTEP from the initiating centre, Groote Schuur Hospital (Chapter 6);
4) to describe the inheritance patterns and phenotypic expression in existing and new families with various forms of familial cardiomyopathy incorporated into IMHOTEP (Chapter 7).

CHAPTER 2: Methods

A detailed description of the methods for each study is included in the chapters that follow. A brief overview of the thesis methodology is described below.

2.1. STUDY DESIGN

The African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP) is an on-going, multi-centre, hospital-based longitudinal observational study. The principal aim of IMHOTEP is to define the baseline characteristics and clinical course of the different morphological types of heart muscle disease, with particular focus on primary (genetic) and secondary cardiomyopathies, and myocarditis. IMHOTEP has 2 different arms to facilitate the study of both retrospective and prospective patient cohorts: (1) a prevalent cases arm, based on the inclusion of existing cases of cardiomyopathy and the amalgamation of existing studies (*A Clinical and Genetic Study of Familial Dilated Cardiomyopathy in South Africa HREC REC 197/96; The Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa HREC 047/2003*); and (2) an incident cases arm, which includes new cases of cardiomyopathy and myocarditis recruited based on a new phenotyping and investigative approach. In addition, IMHOTEP has a third arm for 'relatives' to facilitate the study of families with inherited cardiomyopathies.

It should be noted that some participants from existing studies (*A Clinical and Genetic Study of Familial Dilated Cardiomyopathy in South Africa HREC REC 197/96; The Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa HREC 047/2003*) and relatives enrolled in family studies were recruited retrospectively, therefore, the date of initial presentation and death may precede the date of study commencement. This accounts for longer follow-up periods in the outcomes studies presented in chapters 4 and 5.

2.2. STUDY POPULATION

For the purposes of this doctoral thesis, the study population is limited to prevalent cases recruited by the studies mentioned above, newly identified prevalent cases of cardiomyopathy attending follow-up in the Cardiac Clinic at Groote Schuur Hospital, newly diagnosed (incident) cases of cardiomyopathy referred to the Cardiomyopathy Clinic, and relatives of affected index patients. Patient eligibility is discussed in chapter 3.

Additional note: Existing cases with HCM recently reported on by Ntusi *et al*.[60] have been incorporated into IMHOTEP in order to facilitate long-term follow-up; however, this cohort of patients is not included in this thesis.

2.3. DATA COLLECTION AND MEASUREMENTS

The development of the study protocol and data collection tools formed part of the mandate for this doctorate, and included the development of clinical algorithms to ensure precise diagnosis (Appendix G), standard operating procedure (SOP) algorithms (Appendix H), and study tools, including informed consent forms (Appendix B – E), information sheets for participants on genetics research and cardiomyopathies (Appendix F), and case report forms (CRFs) (Appendix I) for data acquisition. Consent forms and information sheets were translated into local languages, including English, Afrikaans, Xhosa, Sesotho, and Portuguese. The development of these tools was conducted by the candidate in consultation with supervisors.

In conjunction with the University of Cape Town (UCT) Clinical Research Centre, an electronic database for IMHOTEP has been developed on the *OpenClinica* platform (https://srvwinocs003.wf.uct.ac.za/OpenClinica/pages/login/login). Patient data (including patient demographics, medical history, co-morbidities, investigations, procedures, adverse

events, management and genetic information) has been collected using this newly developed database, designed to meet the following specifications, as outlined in the study protocol:

- Advanced security features with regard to data storage protected by firewalls, user privileges and access procedures
- Automatic backup systems
- Built-in audit trails
- Data filtering, sanitisation* and data type checking systems
- Data import and export systems
- The ability to hold multiple relational database tables within a single database

The IMHOTEP database became fully operational in 2016 under the governance of the University of Cape Town. The design and content of the IMHOTEP database was led by the candidate.

2.4. STATISTICAL ANALYSIS

International Business Machines (IBM) Statistical Package for Social Sciences (SPSS) 2017 (Version 25.0, Chicago, USA) and Stata/IC (Version 15.1, StataCorp, USA) software were utilised to analyse data. Descriptive statistics were used to describe and draw inferences about the study population and the results have been reported in table format. Categorical data has been reported as number and proportion. Chi-squared test of equal proportions was utilised to determine differences between categorical data. Continuous variables were tested for distribution using a histogram for visualisation and Shapiro-Wilks for test of normality. Normally distributed data has been reported as mean and standard deviation (SD), and Student's t-test (2 samples) and ANOVA (more than 2 samples) were used to determine statistically significant differences between groups. Non-normally distributed data has been

* Data sanitisation is the process of deliberately and irreversibly removing or destroying data stored on a memory device to make it unrecoverable.

reported as median and interquartile range (IQR), and Wilcoxon sum rank (2 samples) and Kruskal-Wallis (more than 2 samples) were used to determine differences. Survival was determined using Kaplan-Meier survival analysis and Cox proportional hazards regression analysis was used to explore the risk factors for composite outcomes. All statistical tests were two-sided, at $\alpha = 0.05$. Statistical analysis was performed by the candidate in consultation with a statistician. Survival analysis was conducted by a statistician.

2.5. ETHICAL CONSIDERATIONS

All research has been conducted in accordance with the Helsinki declaration,[143] with the primary purpose of understanding the causes, development and effects of disease and to improve preventive, diagnostic and therapeutic interventions, as stated in the declaration.

2.5.1. Human Research Ethics Committee approval

Approval from the UCT Human Research Ethics Committee (HREC) for the *Rationale, Design and Implementation of the African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP)* was initially obtained on 21 October 2014 (HREC 766/2014), incorporating the previously approved studies: *A Clinical and Genetic Study of Familial Dilated Cardiomyopathy in South Africa* (HREC 197/96) and *The Arrhythmogenic Right Ventricular Cardiomyopathy and Myocarditis Registry of South Africa* (HREC 047/2003). Annual progress reports have been submitted in accordance with HREC requirements (Appendix A).

2.5.2. Informed consent

All subjects were required to give informed consent for their participation in this study, with the exception of deceased individuals where waver-of-consent has been approved by the UCT HREC. These exceptions primarily relate to the study of familial disease, where clinical information of deceased relatives is collected for the purposes of understanding disease within a family, whereby living affected individuals within the same family have consented to

participate in clinical research. For prospective (incident) participants, informed consent (Appendix B) was obtained at the time of recruitment. In accordance with current practises, a separate consent form was completed and signed prior to the drawing of blood for deoxyribonucleic acid (DNA) analysis and storage. While existing DNA consent forms were initially utilised, newly designed DNA consent forms (Appendix E) and information sheets for participants on cardiovascular genetics research (Appendix F[I]) were created through the course of the study and submitted to HREC for approval. For retrospective participants, informed consent was obtained at the time of recruitment into existing studies (mentioned above) or at the time of DNA specimen collection. Information sheets on the various types of cardiomyopathies have been provided to participants where possible (Appendix F[II-VI]). With the inclusion of minors into IMHOTEP, assent forms for children over the age of 8 years (Appendix C), and illustrated consent forms for younger children were developed (Appendix D).

2.5.3. Other ethical considerations

All investigations and procedures recorded in this study were performed based on clinical indications and published guidelines that comply with current standard of care of cardiomyopathy and myocarditis. No additional costs were incurred by participants, as investigations, treatment and follow-up were conducted according to clinical indications. Although CMR is considered standard of care, it is not a universally available resource in our clinical setting, therefore a number of CMR studies were performed as research-funded studies. Routine informed consent for magnetic resonance imaging (MRI) was taken from all participants who had CMR imaging done as per clinical practice in our institution. In these instances, the results of CMR studies performed were shared with patients' attending clinicians. Participant confidentiality has been maintained through the use of a unique identification number, and study procedures have been conducted in accordance with the Data Protection Act.

2.6. SAFETY

Due to the observational nature of this study, investigations performed were done as part of clinical care and according to clinical indications. Blood specimen collection for DNA was done by a nursing sister (or doctor) trained in phlebotomy and standard safety precautions were adhered to. The Hatter Institute Cardiovascular Genetics Laboratory guidelines for the collection and transfer of blood specimens were followed. Resuscitation equipment was available in all facilities, including the UCT Clinical Research Unit and the Cape University Body Imaging Centre (CUBIC), where participants were seen or imaged.

CHAPTER 3: Rationale and design of the African Cardiomyopathy and Myocarditis Registry Program: The IMHOTEP Study

3.1. INTRODUCTION

Akinkugbe and colleagues observed several decades ago that "the cardiomyopathies pose the greatest challenge of all the CVDs in Africa because of their greater prevalence in societies still plagued by diseases of famine and pestilence; the difficulty in diagnosis, which often requires specialised cardiological investigations that are lacking in resource-poor environments; the lack of access to effective interventions; and the high mortality associated with these often irreversible disorders of heart muscle".[144] Cardiomyopathy contributes 20–30% of cases of HF in adults in Africa.[6] There is, however, limited information on the aetiology, treatment, outcome and prevention of cardiomyopathy in African individuals.[145] The relatively younger age of onset of HF in African patients[2] and higher incidence of PPCM,[146] affecting women of childbearing age, reported in LMICs compared with HICs countries has major economic and social implications.[147]

Over the past 25 years, molecular genetic investigations conducted outside of Africa have identified specific genetic causes of cardiomyopathy, and this information has been applied increasingly in clinical practice to diagnose and manage these conditions.[148] The Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA) have put forward expert consensus recommendations for genetic testing of specific CVDs, using diagnostic, prognostic and therapeutic impact as indicators or contra-indicators for genetic testing.[135] Molecular genetic studies of inherited cardiomyopathies have revealed genetic and allelic heterogeneity which renders the traditional molecular methodologies too labour-intensive and expensive for routine clinical practice.[149] Recent advances in high throughput genotyping and sequencing technologies have facilitated the transition of genetic testing for many

cardiovascular disorders from specialised research to the diagnostic laboratories, as they offer a dramatic increase in the throughput of DNA sequencing, at a reasonable cost.[149-151] Diagnostic genetic screening is not widely available on the African continent, and our knowledge of genetics of cardiomyopathy in Africans is limited to a few small studies.[60,75,96,152-155]

A recent review on myocarditis highlighted the lack of reliable epidemiological and clinical data worldwide. The burden of myocarditis as a percentage of prevalent HF varies from approximately 0.5% to 4%, although it is recognised that this figure is likely an underestimation due to diagnostic difficulty.[106] DCM is a major cause of HF in Africa,[156] but due to the lack of readily available diagnostic tools, myocarditis has rarely been identified and consequently data regarding the contribution of myocarditis to the pathogenesis of DCM are limited.[114] Our understanding of the epidemiology, presentation and natural history of myocarditis comes from Western Europe and North America.[157] A number of studies from different regions of the world report acute myocarditis as an important cause of SCD and chronic DCM in both adults and children.[51,106] The dearth of information on the causes, outcome and treatment of myocarditis in Africa is well recognised.[157]

3.2. RATIONALE

The paucity of data on aetiology, treatment and outcome of cardiomyopathy and myocarditis limits our ability to diagnose, manage and prevent these conditions at population and individual levels in Africa.[145] The IMHOTEP registry has been designed to elucidate the aetiology, clinical features, outcome and management of all forms of cardiomyopathy and myocarditis in children and adults presenting to referral centres from all regions of Africa.

The design of IMHOTEP as a single registry that includes all forms of cardiomyopathies is based on several observations: (1) It is well recognised that there is significant genetic and

phenotypic overlap between the different morphological and functional types of cardiomyopathy,[64] thus raising the possibility that different morpho-functional types of cardiomyopathy may be on a phenotypic and genotypic continuum; (2) cardiomyopathies are caused by both familial (genetic) and non-familial (secondary) aetiologies,[13] but the interplay between genetics and environment is not well understood; (3) myocarditis may be present in a significant proportion of cases where it serves as a causative factor in non-familial cardiomyopathy or a triggering factor for the symptomatic presentation of genetic disease, which may be expected to be more likely in Africa where there is a high prevalence of infectious disease with cardiac involvement, such as HIV infection; (4) EMF is endemic in certain peri-equatorial regions of Africa, including northern Mozambique, where the prevalence is as high as 19.8%, affecting young individuals between the ages of 10 and 19 predominantly[69] and carrying a case fatality rate of up to 75% at 2 years.[158] Although the pathogenesis of EMF is not known, it has been postulated that the conditioning factors may be geography, poverty and diet, the triggering factor may be an unidentified infective agent, and the perpetuating factor is eosinophilia.[8,159,160] Although there have been reports of familial aggregation and putative genetic association in the human leucocyte antigen (HLA) region in EMF, there are no definitive studies of genetic susceptibility and protective factors in this condition.[69-72]

The IMHOTEP study includes adults and children because cardiomyopathies affect individuals of all ages. Certain endemic conditions, such as EMF, affect children and adolescents predominantly.[69] Patients presenting in childhood with severe disease provide a unique opportunity to study both genetic and environmental factors contributing to the development of cardiomyopathy, without the influence of co-morbidities and lifestyle confounders often present in adults.

3.3. STUDY DESIGN, MATERIALS AND METHODS

3.3.1. Study population

IMHOTEP is a pan-African, multi-centre, hospital-based cohort study with two arms: (1) the 'incident cases' arm involves the enrolment of new unrelated cases of cardiomyopathy and myocarditis; and (2) the 'prevalent cases' arm involves the enrolment of existing unrelated cases of cardiomyopathy and myocarditis. The prevalent cases arm of the study includes the integration of existing studies from Cape Town - the Clinical and Genetic Study of Familial DCM in South Africa which was initiated in 1996,[4,26] and the ARVC Registry of South Africa which was initiated in 2004.[95,96] Furthermore, in cases of familial cardiomyopathies, both affected and unaffected relatives of incident and prevalent cases have been recruited. Incident cases, prevalent cases and relatives have been followed prospectively from the time of enrolment in IMHOTEP.

3.3.2. Objectives

The IMHOTEP study has been designed to address the following objectives:

Primary objectives

1. To describe the clinical, electrocardiographic, imaging, histological and genetic characteristics of cardiomyopathy and myocarditis in children and adults in Africa
2. To describe the management of cardiomyopathy and myocarditis in children and adults in Africa
3. To estimate the complications associated with cardiomyopathy and myocarditis in children and adults in Africa
4. To determine the overall survival experience of children and adults diagnosed with cardiomyopathy and myocarditis in Africa

Secondary objectives

1. To identify barriers to the use of evidence-based guidelines in the management of patients with cardiomyopathy and myocarditis in Africa
2. To provide a platform for studies of the pathogenesis, trials of treatment and prevention of mortality and morbidity in patients with cardiomyopathy and myocarditis in Africa

3.3.3. Study eligibility

Patients with known (i.e. prevalent cases) or newly diagnosed (i.e. incident cases) cardiomyopathy or myocarditis who have undergone diagnostic evaluation at a participating centre are eligible for inclusion in IMHOTEP. The ESC definitions and classification of cardiomyopathy have been used as inclusion and exclusion criteria for IMHOTEP (Table 3.1 and 3.2).[13] The clinical definitions of hypertension and coronary artery disease have been adapted to accommodate low resource settings by expanding the clinical definitions of these conditions to guide clinicians in circumstances where sophisticated investigations are not accessible. In keeping with the ESC classification, secondary cardiomyopathies have been included. The diagnosis of myocarditis has been made according to the clinical classification system described in Table 3.3.[161]

Table 3.1. Cardiomyopathy phenotype definitions

Phenotype	Description
DCM	Dilated cardiomyopathy is characterised by the presence of left ventricular dilatation and left ventricular systolic dysfunction in the absence of abnormal loading conditions (hypertension, valve disease) or coronary artery disease sufficient to cause global systolic impairment.* Right ventricular dilation and dysfunction may be present but are not necessary for the diagnosis.
PPCM	Peripartum cardiomyopathy is classified as a subtype of dilated cardiomyopathy; defined as a cardiomyopathy presenting with heart failure secondary to left ventricular systolic dysfunction (LVEF < 45%), with or without LV dilatation, towards the end of pregnancy (last trimester) or in the months following delivery (usually within 5 months postpartum), where no other cause of heart failure is found.[33,162]
HCM	Hypertrophic cardiomyopathy is characterised by the presence of increased ventricular wall thickness or mass, in the absence of haemodynamic stresses sufficient to account for the degree of hypertrophy. This definition includes conditions in which there is myocyte hypertrophy and those in which left ventricular mass and wall thickness are increased by interstitial infiltration or intracellular accumulation of metabolic substrates.
ARVC	Arrhythmogenic right ventricular cardiomyopathy is characterised by presence of right ventricular dysfunction (global or regional), with or without left ventricular disease, in the presence of histological evidence for the disease and/or electrocardiographic abnormalities in accordance with published criteria.[73]
RCM	Restrictive cardiomyopathy is characterised by the presence of restrictive ventricular physiology in the presence of normal or reduced diastolic volumes (of one or both ventricles), normal or reduced systolic volumes, normal ventricular wall thickness, and normal or reduced systolic function.
LVNC	Left ventricular non-compaction is characterised by prominent left ventricular trabeculae and deep inter-trabecular recesses. The myocardial wall is often thickened with a thin, compacted epicardial layer and a thickened endocardial layer. In some patients, LVNC is associated with left ventricular dilatation and systolic dysfunction. Diagnosis should be confirmed by fulfilment of published imaging criteria.[163,164]

* Parameter definitions may vary depending timing of assessment in the natural history of disease and the phenotypic expression. Strict echocardiographic criteria for left ventricular dilatation and systolic dysfunction are: LVEDD >117% of the predicted value corrected for age and BSA, and LVEF < 45% (and/or FS < 25%), respectively.[165]

Definitions adapted from: Elliott P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases**.** *Eur Heart J*. 2007;29(2):270-276.

BSA, body surface area; FS, fractional shortening; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction.

Table 3.2. IMHOTEP inclusion and exclusion criteria

Inclusion criteria: Persons of all ages living in Africa with any of the following:	
Autopsy diagnosis	Cardiomyopathy or myocarditis, based on currently accepted definitions
Idiopathic cardiomyopathies	Aetiology unknown
Familial cardiomyopathies	Hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy/dysplasia, restrictive cardiomyopathy, left ventricular noncompaction, mixed forms of cardiomyopathy
Neuromuscular disorders with cardiac involvement	Disorders that are atypical and/or are genotype negative for classical neuromuscular disorders on genetic screening
Non-familial or secondary causes of cardiomyopathy	Myocarditis (infective/toxin/immune), human immunodeficiency virus (HIV), drugs/toxins, peripartum, endocrine, nutritional, obesity, alcohol, tachycardiomyopathy, eosinophilic, Kawasaki disease, Takotsubo cardiomyopathy, amyloidosis, autoimmune, endomyocardial fibrosis, carcinoid heart disease, Radiation
Myocarditis	Acute or chronic myocarditis
Exclusion criteria: Persons with any of the following	
Systemic arterial hypertension	Blood pressure ≥160/100mmHg documented and confirmed at repeated measures Consider HHD in patients in patients with BP < 160/100: • History of longstanding hypertension with dilated LV and impaired systolic function • High normal (BP ≥ 130/85) or grade 1 hypertension (BP ≥ 140/90) in patients with systolic dysfunction and concentric LVH, particularly if on blood pressure lowering medications • Evidence of hypertensive target organ damage – nephropathy, retinopathy, LVH, small vessel disease
Coronary artery disease (ischaemic heart disease)	Coronary artery obstruction >50% of the luminal diameter of a major branch on coronary CTA or coronary angiography Where coronary CTA or coronary angiography not available, consider CAD in patients with: • History of angina, ACS, or previous MI in a patient with risk factors for CAD, and/or evidence of previous infarction or ischaemia on ECG (Q waves, ST elevation or depression), echocardiogram (regional wall motion abnormalities) and/or CMR (subendocardial or transmural LGE) • Positive exercise stress test
Pericardial diseases	Primary pericardial disease, e.g. pericarditis, pericardial constriction, pericardial effusion (not associated with heart failure)
Congenital heart disease	Ventricular septal defect, atrial septal defect, patent ductus arteriosus, coarctation of the aorta, anomalous origin of the left coronary artery from pulmonary artery, Tetralogy of Fallot, pulmonary or aortic valve stenosis, transposition of the great vessels, hypoplastic right or left ventricle, Ebstein's anomaly, other
Cor pulmonale	Primary pulmonary hypertension, pulmonary disease
Valvular heart disease	Rheumatic heart disease, degenerative valve disease, infective endocarditis, valve prolapse, congenital bicuspid valve, other

LVH, left ventricular hypertrophy; ACS, acute coronary syndrome; HHD, hypertensive heart disease; CTA, computed tomography; CAD, coronary artery disease; ECG, electrocardiogram; CMR, cardiovascular magnetic resonance; LGE, late gadolinium enhancement

Table 3.3. Clinical classification for the diagnosis of myocarditis

Diagnosis	Criteria
Probable acute myocarditis	In the clinical context of possible myocardial injury with cardiovascular symptoms and no evidence of coronary artery disease where the following criteria are met: 1. Biomarkers of cardiac injury raised; and 2. Either of the following: a. Wall motion abnormalities and/or left ventricular systolic dysfunction on echocardiography; or b. Abnormal left ventricular systolic function, left ventricular wall motion abnormalities, and abnormal tissue characteristics on CMR (increased signal intensity ratio on T2-weighted imaging; increased T1 value and increased T2 value on parametric mapping; increased signal intensity on early gadolinium imaging; and/or typical pattern on enhancement on late gadolinium imaging)
Definite myocarditis	Histological or immune-histological evidence of myocarditis

CMR, cardiovascular magnetic resonance

3.3.4. Diagnostic approach

Clinical algorithms, specifically adapted for the limited resource setting, were developed to guide clinicians in the appropriate work-up of patients with suspected cardiomyopathy and myocarditis. These algorithms illustrate a diagnostic approach that has been adopted in Cape Town, based on three stages of investigation: (1) non-invasive stage; (2) invasive stage; and (3) genetic stage (Table 3.4.). This approach was adapted from published standard of care guidelines,[14,52,161,166] and highlights the use of core investigations for all patients and optional extended tests depending on clinical indication and the availability of resources.

CMR was conducted in adult incident cases recruited in Cape Town, where possible. Standardised protocols were utilised to evaluate chamber size and ventricular function, LV mass, strain, haemodynamic assessment, tissue characteristics (including native and post-contrast T1 and T2 mapping techniques), and LGE.[167-172]

Table 3.4. Three stage investigative approach to cardiomyopathy and myocarditis – Stage 1

Stage 1: Non-invasive Confirm diagnosis of cardiomyopathy; morpho-functional phenotype and aetiology; exclude alternative causes (see table 3.2); SCD risk assessment							
	All cardiomyopathies	**DCM**	**HCM**	**ARVC**	**RCM**	**Myocarditis**	**Children**
Core investigations	History Examination Chest X-ray Electrocardiogram Echocardiogram Basic blood investigations: Hb, WCC, renal and liver function	HIV, TSH, CK Ferritin/Iron CMP Glucose (HbA1C) Cholesterol Proteinuria	CK Proteinuria		Ferritin/Iron Eos count Proteinuria	Troponin (CK) CRP/ESR WCC and differential count	HIV TSH Troponin CK CMP Lactate CRP/ESR
Extended investigations	Additional diagnostic investigations done at the physician's discretion according to clinical indications and resource availability	CMR SACE[a] Autoantibodies[b] CRP/ESR[b] Lactate[c] Myoglobinuria[c] Eos count[d] (Thiamine)	CMR Urine and plasma protein immunofixation, free light chains[e], alpha-galactosidase A levels[f]	SAECG Holter (EST) CMR	CMR SACE[a] Urine and plasma protein immunofixation, free light chains[e]	CMR	CMR Viral screen* Metabolic screen** Autoantibodies[b]
	SCD risk assessment	Holter and/or EST according to published guidelines					
	Exclusion of CAD	EST, and/or MIBI, and/or coronary CTA according to published guidelines					

[a]Sarcoidosis suspected; [b]Autoimmune conditions such as systemic lupus erythmatosus or rheumatoid arthritis suspected; [c] Mitochondrial disorder suspected; [d]Hypereosinophilic syndrome suspected; [e]Amyloidosis suspected; [f]Anderson-Fabry disease suspected,

*Viral screen includes coxsackie A/B, parvovirus B19, mumps, rubella, cytomegalovirus, Ebstein Barr virus, echovirus, respiratory viruses, **Metabolic screen includes plasma acylcarnitine, urine reducing substances, urine organic acid, urine ketone

ARVC, arrhythmogenic right ventricular cardiomyopathy; CAD, coronary artery disease; CK, creatinine kinase; CMP, calcium magnesium phosphate; CMR, cardiovascular magnetic resonance; CRP, C-reactive protein; CTA, computerized tomography angiography; DCM, dilated cardiomyopathy; Eos, eosinophil; ESR, erythrocyte sedimentation rate; EST, exercise stress test; HbA1C, glycosylated haemoglobin; Hb, haemoglobin; HCM, hypertrophic cardiomyopathy; HIV, human immunodeficiency virus; LV, left ventricle; LVNC, left ventricular non-compaction; MIBI, myocardial perfusion imaging; RCM, restrictive cardiomyopathy; SACE, serum angiotensin converting enzyme; SAECG, signal average electrocardiogram; SCD, sudden cardiac death; TSH, thyroid stimulating hormone; WCC, white cell count

Table 3.4. Three stage investigative approach to cardiomyopathy and myocarditis – Stages 2 and 3 (continued)

Stage 2: Invasive – Only undertaken at institutions that have the facilities and expertise, in the following circumstances: Diagnostic or etiologic uncertainty, exclusion of CAD, transplantation assessment, SCD risk assessment, arrhythmias		**Not routinely undertaken**
	All cardiomyopathies (as per clinical indications)	**Children**
Extended investigations	**TOE:** Exclusion of left atrial thrombus prior to cardioversion/EPS, or poor transthoracic views where CMR is not available **Cardiac catheterization**: Exclusion of coronary artery disease OR haemodynamic assessment (diagnostic, transplantation) **EMB:** Indications according to published guidelines[173] including: • New onset heart failure of < 2 weeks' duration, a normal sized or dilated LV, and haemodynamic compromise • New onset HF with a dilated ventricle, 2 weeks - 3 months of symptoms, new ventricular arrhythmias or Mobitz type 2 second-degree HB or third-degree HB, or who fail to respond to usual care within 1-2 weeks • Diagnostic purposes that will alter management (e.g. giant cell myocarditis, amyloidosis, cardiac sarcoidosis, ARVC) or post-transplantation (organ rejection) **EPS:** For assessment of arrhythmias or ablation procedure where required **SMB:** If myopathy or muscular dystrophy suspected	Indications must be individualized and according to the attending physicians' discretion **SMB** (myopathy, muscular dystrophy)
Stage 3: Genetics (screening for familial/genetic cardiomyopathy)		
All patients	Family history and basic pedigree	As for adults
Extended investigations	Extended family pedigree (≥ 3 generation) Family screening – indicated in ARVC and HCM, and in DCM and RCM cases with positive family histories Molecular genetic testing	As for adults Screen parents

ARVC, arrhythmogenic right ventricular cardiomyopathy; CAD, coronary artery disease; CMR, cardiovascular magnetic resonance; DCM, dilated cardiomyopathy; EMB, endomyocardial biopsy; EPS, electrophysiology study; HB, heart block; HCM, hypertrophic cardiomyopathy; HF, heart failure; LV, left ventricle; LVNC, left ventricular cardiomyopathy; SCD, sudden cardiac death; SMB, skeletal muscle biopsy; TOE, transoesophageal echocardiogram;

EMB has been recommended in patients with suspected myocarditis based on clinical suspicion, or CMR findings, at centres with appropriate facilities and expertise to conduct EMB safely and pathologists with the requisite experience.

3.3.5. Medical genetics, counselling and genetic testing

Blood samples for DNA analysis were collected and processed using standard protocols from prevalent and incident cases. Patients received basic genetic counselling and were required to sign consent for DNA analysis prior to sample collection. Specimens were transferred to the Cardiovascular Genetics (CVG) Laboratory, Hatter Institute of Cardiovascular Research in Africa, UCT, for DNA extraction, storage and analysis. The molecular genetic approach (not included in this thesis) has been designed to follow three phases (Figure 3.1.). First, all index cases will be screened for known molecular genetic causes of cardiomyopathy using targeted Next Generation Sequencing (NGS). Basic family pedigrees have been constructed at the time of sample collection, and family screening has been conducted in selected cases of unexplained and familial cardiomyopathy, with an emphasis on identifying large multiplex families (>5 affected members) and family trios in children under the age of 12 years (clinical genetics sub-study, Figure 3.2.). Second, genotype-negative probands from multiplex families, and children with severe early-onset cardiomyopathy (in parent-offspring trios), will be subjected to whole exome sequencing (WES) to identify novel genetic causes of cardiomyopathy. We aim to apply this approach to approximately ±10 multiplex families and ±40 parent-offspring trios. Finally, the genotype-negative cases not included in stage two will be screened for the novel genetic mutations that are identified in this and other studies. This approach will provide a comprehensive analysis of established and new genetic causes of cardiomyopathy in Africans.

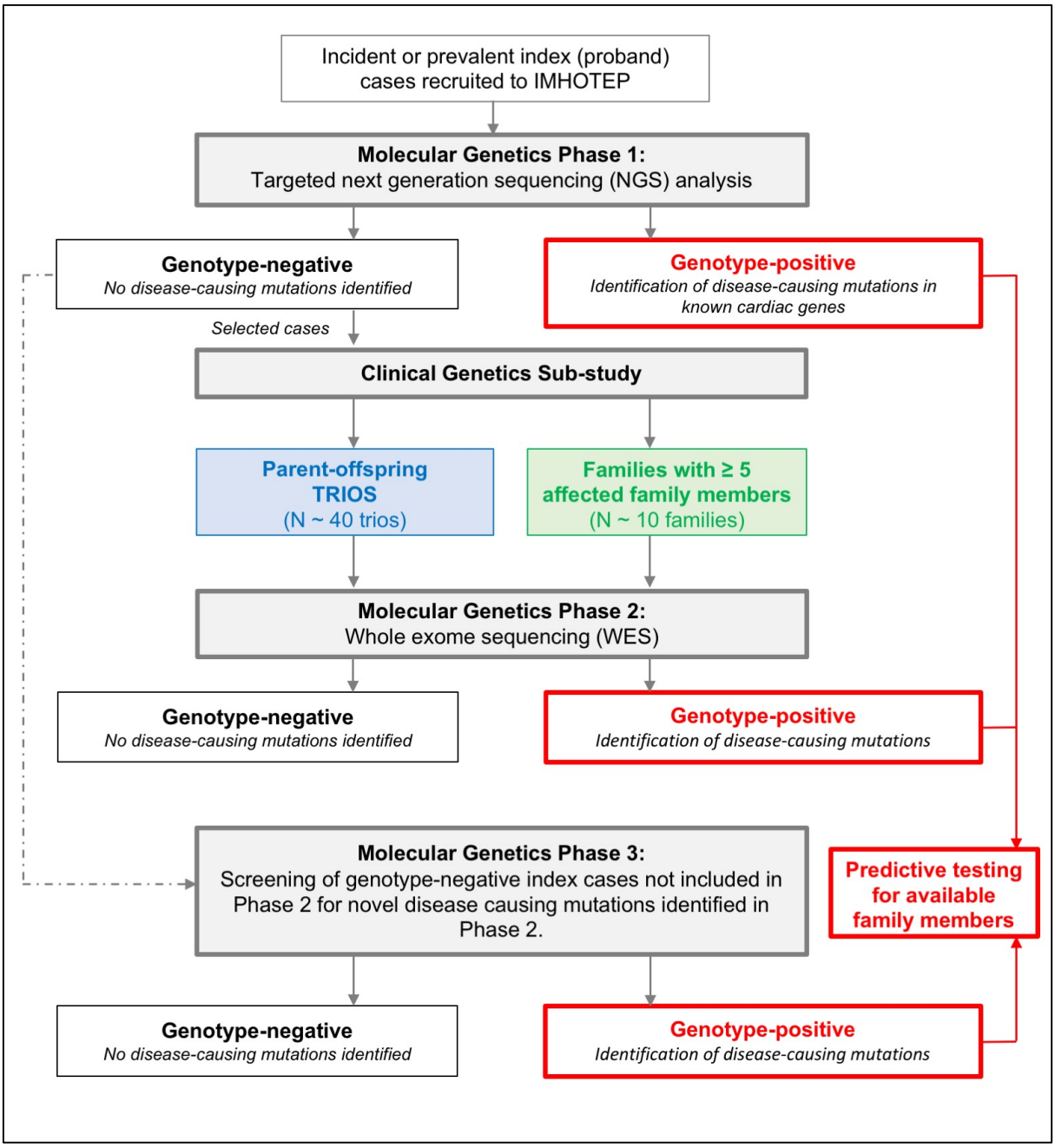


Figure 3.1. Three phase molecular genetics sub-study

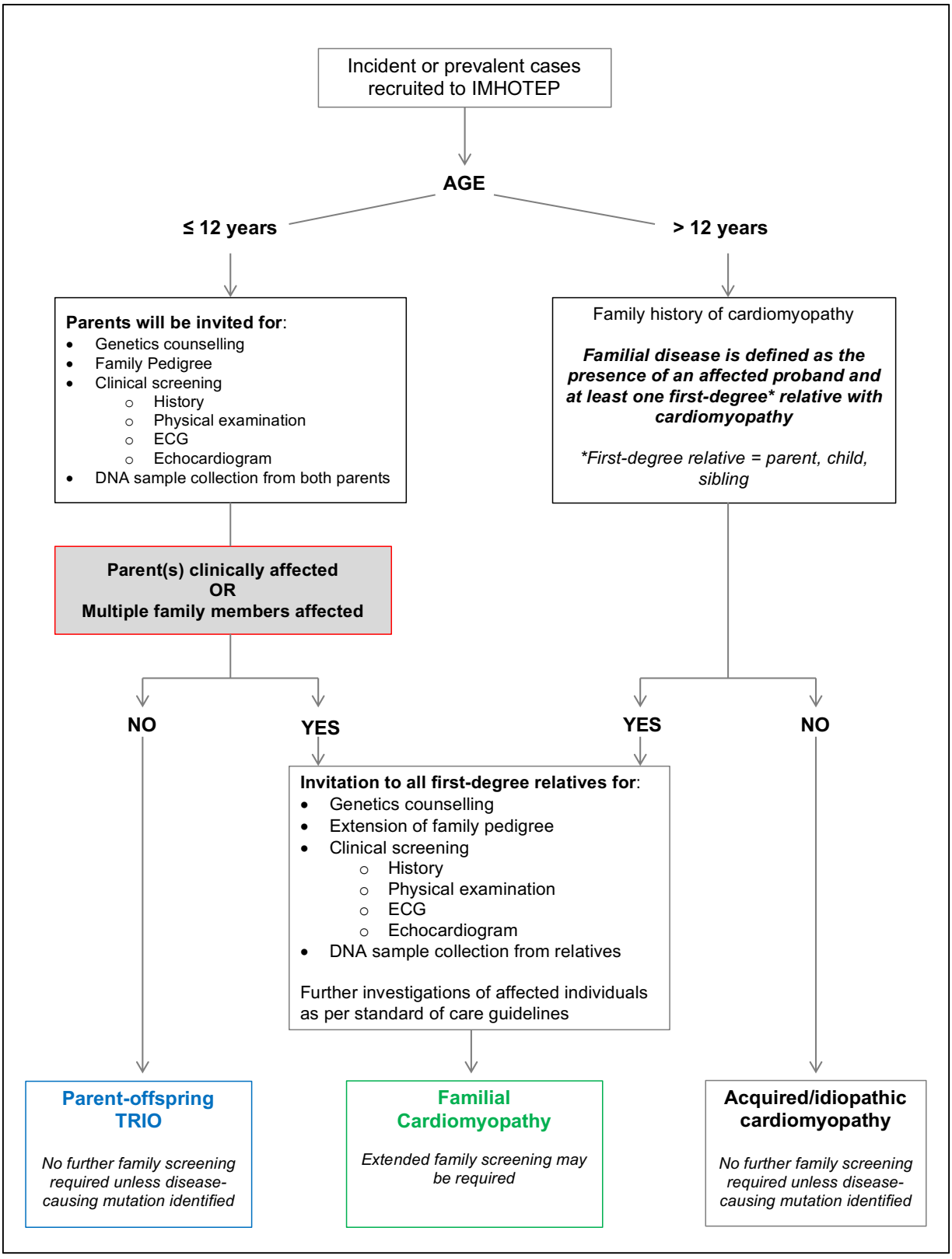


Figure 3.2. IMHOTEP clinical genetics sub-study

3.3.6. Data collection and management

All clinical and demographic information has been captured on a secure database, on the *OpenClinica* platform, accessible to all participating centres via the Internet. Entered data has been stored on a secure server governed by UCT. Access to the database has been controlled by a username and password system. Baseline data has been collected using paper or electronic CRFs at the time of recruitment. Data captured on paper CRFs, has been entered onto the database by a trained data-capturer. Patients have been asked to complete a personal information sheet containing contact details at the time of recruitment. All patient identifiers have been anonymised and recorded separately from clinical and demographic data to ensure the confidentiality of participants. Each participant was assigned a unique identification number at the time of recruitment.

As this is an ongoing longitudinal study of both prevalent and incident patients with cardiomyopathy or myocarditis, managed according to standard of care guidelines at multiple centres with different resource availability, the amount of data recorded varied considerably. Mandatory data required at recruitment includes demographics, medical history and presenting symptoms, physical examination findings, drug therapy, and basic investigations including electrocardiography (ECG) and echocardiography. To accommodate facilities with extended expertise, CRFs for all additional specialised investigations (including signal average electrocardiogram [SAECG], 24-hour ambulatory ECG, exercise stress tests [EST], CMR) and procedures (angiography, EMB, device insertion, electrophysiology studies) have been developed and made available on the database.

Data entry was via voluntary submission by participating centres under the guidance of the local site coordinator. Incentives to clinicians include the use of an established data collection tool for collecting patient information and access to data for their site for purposes of audit and

publication. An active system of data collection instituted by an outreach team has been implemented with the initiation of additional sites.

Source documents have been stored according to the hospital record-keeping protocols at each centre. Informed consent forms, contact information, copied source documents and paper CRFs have been stored in a securely locked area on site. Auditing of randomly selected sample of cases have been conducted to look for data discrepancies and to ensure data quality.

3.3.7. Follow-up

Follow-up clinic visits have been scheduled according to clinical necessity in accordance with standard practice guidelines. Follow-up has been arranged by the attending physician based on the patients' clinical condition. If additional events, investigations or procedures occurred, the attending clinician recorded the data using the relevant CRF(s). IMHOTEP requires an annual follow-up CRF to be completed that can be done at the time of a follow-up visit or by means of a hospital folder and telephonic review to ensure that all symptoms, events, investigations and procedures are recorded, thus enabling review of primary and secondary outcomes on an annual basis (Table 3.5.). A random sample of 10% of the locally adjudicated events will be audited by an independent committee for consistency with the study definitions.

Table 3.5. Primary and secondary outcome measures

Primary endpoint/outcome measures	Secondary endpoints/outcome measures
Death from all causes	Death from cardiovascular disease
Hospitalization for heart failure[a]	Pulmonary embolism
Embolic stroke/transient ischaemic attack	Systemic embolism (other than stroke)
Heart failure (new or decompensated)	
Resuscitated cardiac arrest[b]	
New onset atrial fibrillation	

[a] ***Heart failure*** *(HF) is defined as a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress.*[174]

[b] ***Cardiac arrest*** *is defined as the cessation of cardiac mechanical activity as confirmed by the absence of signs of circulation. Resuscitated cardiac arrest is defined by the restoration of life by establishing or maintaining airway (or both), breathing, and circulation through cardiopulmonary resuscitation (CPR), defibrillation, and other related emergency care techniques.*[175]

3.3.8. Planned genotype analysis

Extracted DNA samples from all index cases have been batched and transferred to Oxford Medical Genetics Laboratory for targeted NGS analysis of the cardiomyopathy related genes (Table 3.6.). Selected genotype-negative probands of multiplex families and children with severe early-onset cardiomyopathy in family trios will be subjected to WES to identify novel genetic causes of cardiomyopathy. The WES analysis will be carried out as previously described by our group[176] and will also be based on the recent guidelines for assigning causality[177,178] and will rely on familial transmission over biological plausibility. We note that for detection of causative mutations in Mendelian disorders, formal statistical testing is often not required; nevertheless, Fisher's exact test will be used to determine the level of significance for mutations detected in any of our 750 cases and not present (or present at much lower frequencies) in ethically matched controls using population data from the genome aggregation database, gnomAD *(https://gnomad.broadinstitute.org).*

Table 3.6. Targeted Next Generation Sequencing for cardiomyopathy related genes*

Abbreviation	Cardiomyopathy-related genes
ACTC1	*α-actin*
ACTN2	*α-actinin 2*
ANKRD1	*ankyrin repeat domain-containing protein 1*
BAG3	*BCL2-associated athanogene 3*
CRYAB	*α-crystallin B chain*
CSRP3	*cysteine and glycine-rich protein 3*
DES	*desmin*
DMD	*dystrophin*
DSC2	*desmocollin 2*
DSG2	*desmoglein 2*
DSP	*desmoplakin*
FHL1	*four-and-a-half LIM domains 1*
FHL2	*four-and-a-half LIM domains 2*
FLNC	*filamin C*
GLA	*α-galactosidase*
JUP	*junction plakoglobin*
LAMP2	*lysosome-associated membrane protein 2*
LMNA	*lamin A/C*
MYBPC3	*myosin-binding protein C*
MYH7	*β-myosin heavy chain*
MYL2	*regulatory light chain of myosin*
MYL3	*essential light chain of myosin*
PKP2	*plakophilin 2*
PLN	*phospholamban*
PRKAG2	*AMP-activated protein kinase*
RBM20	*RNA-binding motif protein 20*
SCN5A	*sodium channel protein type 5 subunit alpha*
TAZ	*tafazzin*
TMEM43	*transmembrane protein 43*
TNNC1	*troponin C*
TNNI3	*troponin I type 3*
TNNT2	*troponin T type 2*
TPM1	*tropomyosin 1*
TTN	*titin*
TTR	*transthyretin*
VCL	*vinculin*

**including, but not limited to*

3.3.9. Statistical analysis

Descriptive statistics were used to describe the study population; all continuous variables were tested for distribution using a histogram for visualisation and Shapiro-Wilks for test of normality. Normally distributed data has been reported as mean and standard deviation. Non-normally distributed data has been reported as median and interquartile range. Categorical data has been summarised in tables and reported as number and proportion. Chi-squared test of equal proportions has been used to determine differences between categorical data.

Wilcoxon sum-rank (2 samples) and Kruskal-Wallis (more than 2 samples) have been used to determine differences between non-normally distributed continuous data. Students' t-test (2 samples) and ANOVA (more than 2 samples) have been used for normally distributed data. Kaplan-Meier survival analysis has been used to explore the survival of cardiomyopathy and myocarditis patients in relation to different treatments. All statistical tests were two-sided, at $\alpha = 0.05$. International Business Machines (IBM) SPSS Statistics 2017 (Version 25.0, Chicago, USA) and Stata/IC (Version 15.1, StataCorp, USA) software was utilised to analyse data.

We aimed to recruit 750 unrelated probands with cardiomyopathy in this pilot study, conducted over a five-year period (2015–2019), involving sites in South Africa and Mozambique. This sample size will be adequate for testing the genetic hypothesis and will provide preliminary data on outcomes. These data will be used to estimate the minimum sample size for future expansion of IMHOTEP to include all the participating centres in IMHOTEP.

3.3.10. Study management

The Project Coordinating Office (PCO) for IMHOTEP is based in the Department of Medicine at UCT. The PCO has been responsible for the management of the registry, the coordination of the different centres, overseeing data collection and quality assurance. A project manager has been appointed to co-ordinate study procedures. The PCO has overseen the development of the CRFs, consent forms, patient information sheets, and management algorithms (Appendix B-I), in addition to the development and maintenance of the web-based database. A data management plan outlining standard operating procedure has been developed, and a data manager and quality assurance officer have been appointed at the PCO to oversee data collection. Additionally, the PCO is the statistical consulting site responsible for analysis of data. Local site investigators have been appointed at each participating site (List of Collaborators – Appendix J). A steering committee (decision-making body) – comprised of the principal and co-investigators – and a scientific panel – comprised of experts in the fields of

cardiomyopathy, cardiovascular imaging, cardiac electrophysiology, histopathology and molecular genetics – has been established. Support from the Pan-African Society of Cardiology (PASCAR), as well as proposed collaborators from other centres was obtained prior to the establishment of this registry (Figures 3.3 and 3.4)

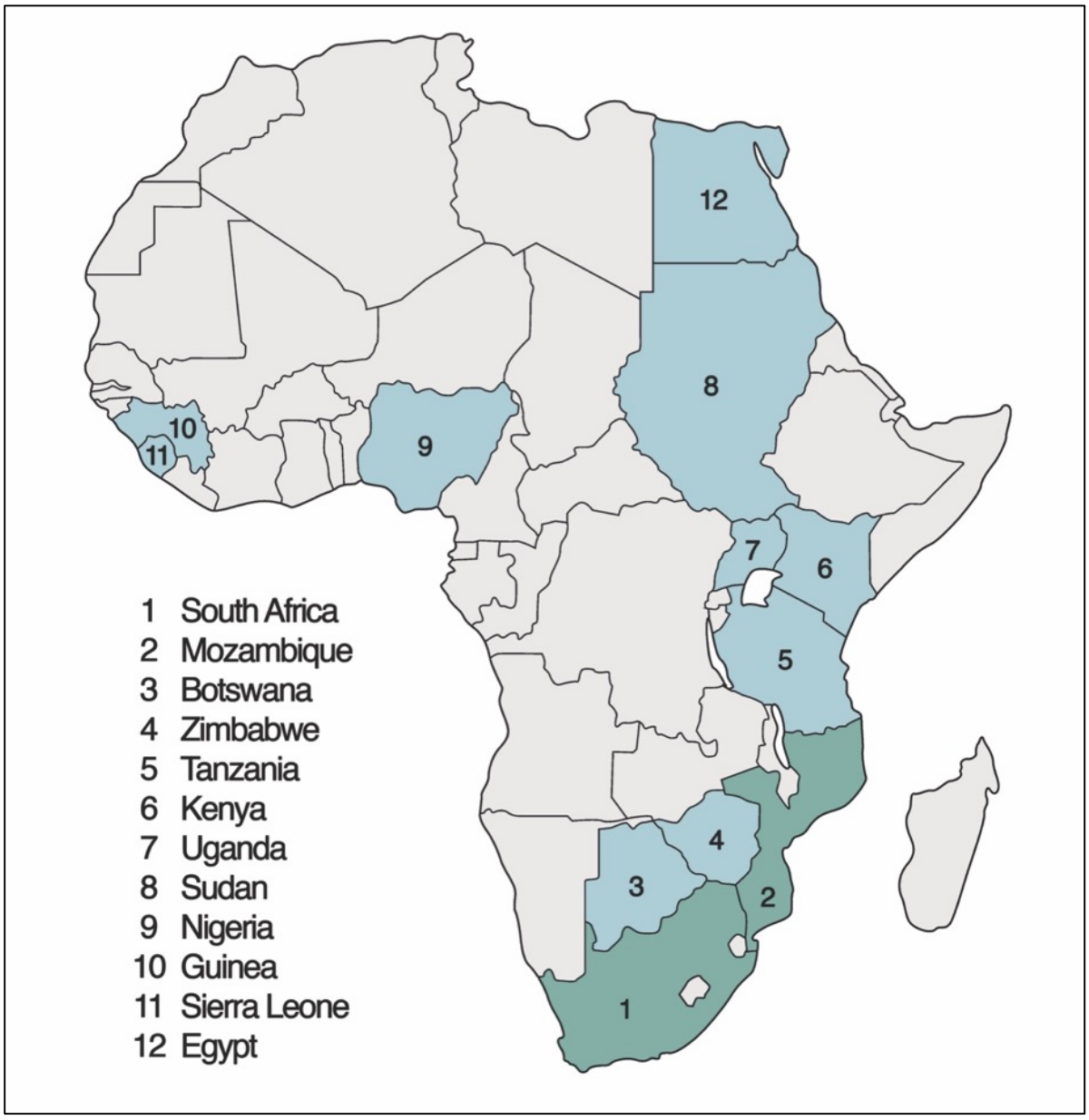


Figure 3.3. IMHOTEP collaborating countries
The pilot phase of the IMHOTEP study will be conducted in (1) South Africa and (2) Mozambique (green). Recruitment sites for the pilot phase include Cape Town (3 sites), Bloemfontein, Port Elizabeth, and Mthatha in (1) South Africa, and Maputo in (2) Mozambique. Following the pilot phase, participating sites from countries 4-12 (blue) will initiate recruitment. Additional collaborating institutions not represented here include Oxford University (United Kingdom), Mayo Clinic (United States) and King Saud Bin Abdalaziz University for Health Sciences (Saudi Arabia).

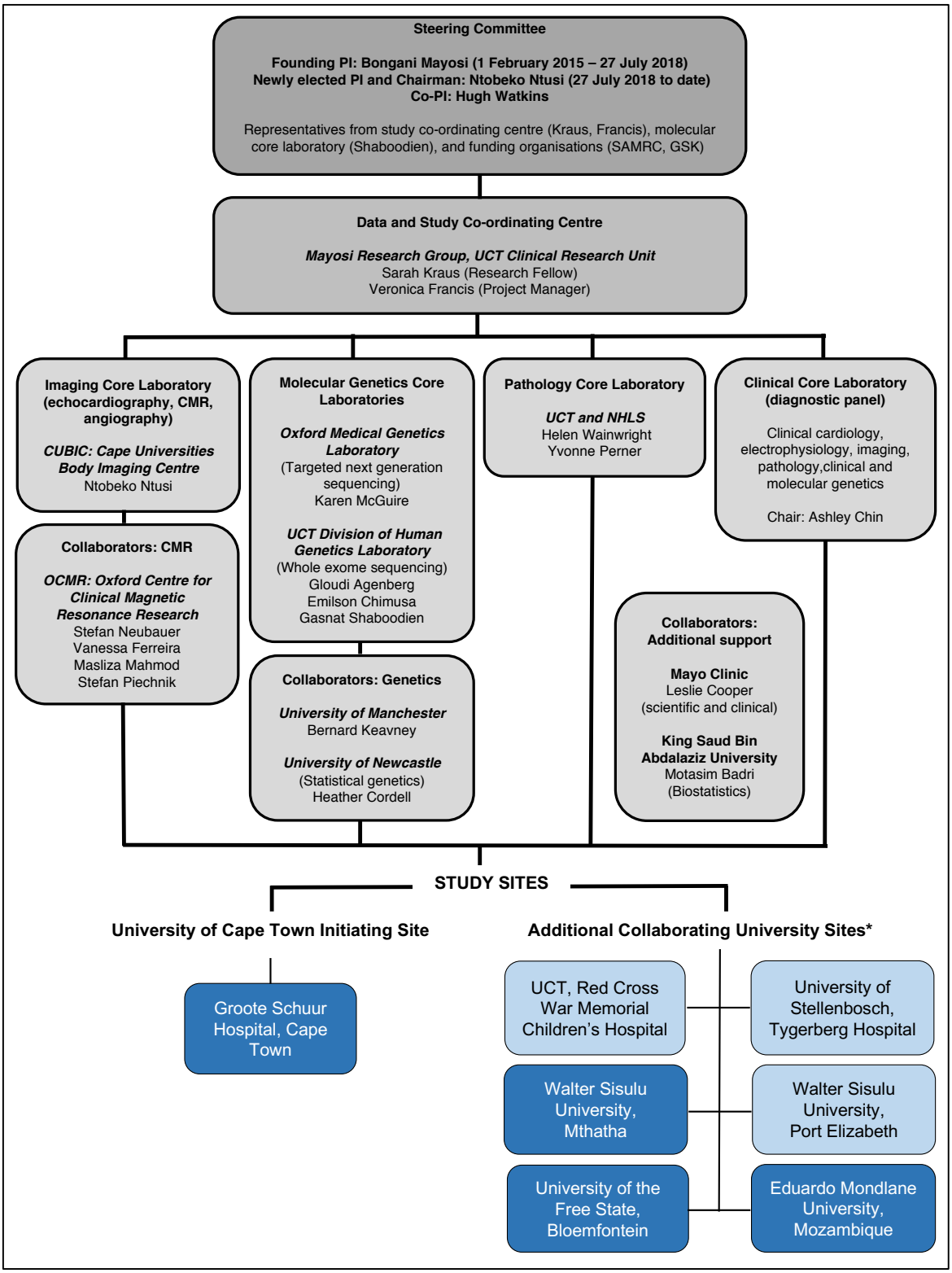


Figure 3.4. Organisational structure of IMHOTEP
Adult sites (dark blue), paediatric sites (pale blue)

CMR, cardiovascular magnetic resonance; NHLS, National Health Laboratory Service, PI, principal investigator; UCT, University of Cape Town

3.3.11. Ethics

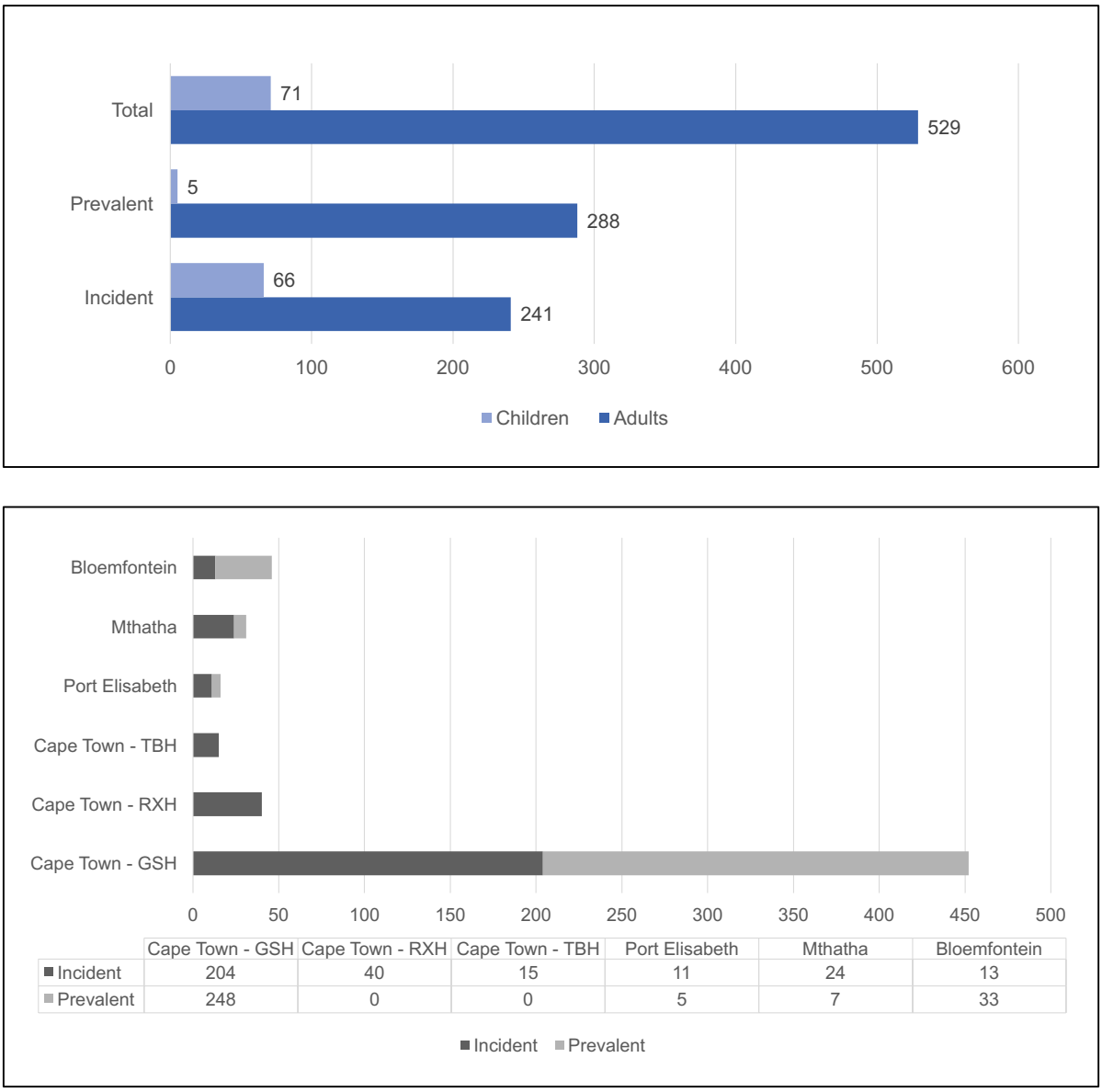
The study has been approved by the UCT Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 766/2014). Participating centres required institutional ethics committee approval prior to being established as part of the registry. For the incident cases, informed consent for inclusion into the registry was obtained from participants prior to enrolment. In the case of minors (under the age of 18 years), the participant's parent/guardian was required to sign consent. Additionally, children aged eight and above could sign an assent form. In accordance with current practices, a separate consent form was required for DNA specimen collection, storage and analysis. Regarding the prevalent case cohort, informed consent for participation in research and DNA analysis was obtained from participants when they were recruited into the previous studies. Detailed information sheets have been developed and were provided to participants.

All invasive investigations have been performed according to prevailing standard of care guidelines for cardiomyopathy and myocarditis,[14,23,52,161,166] or as part of an approved research protocol. There were no additional costs incurred by the participants, as treatment, investigations and follow-up have been conducted according to clinical indications.

3.4. STATUS OF THE STUDY AND STUDY PARTICIPANTS

The pilot phase of the study commenced at Groote Schuur Hospital in Cape Town on February 1, 2015, followed by staggered initiation of additional recruitment sites in Cape Town (Red Cross War Memorial Children's Hospital, Tygerberg Hospital - 2016), Mthatha (2017), Port Elizabeth (2018) and Bloemfontein (2018). Training was conducted at the Mozambican site in November 2018 and recruitment will commence in 2019. A total of 600 index patients have been enrolled into IMHOTEP thus far (20 November 2018) from 6 recruitment sites (Figure 3.5.). We expect that the enrolment of the first 750 participants in the pilot phase of the study

will be complete by 30 September 2019, and 18 months (average) follow-up data will be available by this time. The first phase of the molecular genetics sub-study has been initiated – the first batch of 250 samples have been transferred to Oxford University for targeted NGS and analysis. Preliminary clinical data collected at the initiating centre, Groote Schuur Hospital in Cape Town, will be reported in the chapters to follow.



	Cape Town - GSH	Cape Town - RXH	Cape Town - TBH	Port Elisabeth	Mthatha	Bloemfontein
Incident	204	40	15	11	24	13
Prevalent	248	0	0	5	7	33

Figure 3.5. IMHOTEP recruitment

Total number of adult and children incident and prevalent cases recruited (above). Recruitment according to sites (below).

3.5. DISCUSSION

Cardiomyopathy is an endemic non-communicable disease (NCD) of high importance to the poor majority in Africa, and is a locally relevant unmet need for research.[8,145,159] The IMHOTEP study aims to fill knowledge gaps in our understanding of cardiomyopathy and myocarditis, specifically related to the African population, by delineating clinical features and molecular genetics of the different morpho-functional forms of cardiomyopathy, and obtaining important outcome data. As access to healthcare and the availability of sophisticated investigations and interventions vary considerably across the African continent, IMHOTEP has been designed to record core information required to make a diagnosis of cardiomyopathy or myocarditis and key outcome events over a minimum follow-up period of two years in the pilot phase. Additionally, IMHOTEP has the facility to record detailed reports of specialised investigations and procedures, which can be utilised by those facilities that have the equipment and expertise. We have proposed a standardised three-stage investigative approach to the work-up of cardiomyopathy and myocarditis that can be easily adapted according to resource availability (Table 3.4.).

We postulate that myocarditis is underdiagnosed in Africa, due to the requirement of sophisticated imaging (like CMR) or invasive investigations (specifically EMB) to confirm the diagnosis; by utilising a tailored clinical classification system, we hope to not only improve diagnostic yield, but also facilitate better use of available resources. Infectious diseases contribute significantly to the burden of disease in African countries,[179] highlighting the importance of establishing the role of myocarditis in the aetiology, pathophysiology and outcomes of cardiomyopathies in Africans. IMHOTEP aims to establish the prevalence of myocarditis in patients presenting with new-onset heart failure in Africa, and contribute to the development of innovative diagnostic strategies more suitable for resource-limited settings.

Internationally, there is a recognised need to conduct more extensive genomic studies in much larger cohorts of rigorously phenotyped probands and family members to improve our understanding of the genomic basis of inherited cardiomyopathies.[29,64] By incorporating the prevalent cases that include the amalgamation of several existing studies in which phenotypic information and DNA have been collected over the last 20 years, in addition to the collection of DNA samples in incident cases, IMHOTEP seeks to assemble one of the largest cohorts of cardiomyopathies in African individuals, whereby the genetic origin of disease can be studied. The variable expressivity and penetrance seen in genetic cardiomyopathies suggest that genetic, epigenetic and environmental modifiers influence disease manifestation, in addition to single pathogenic mutations.[64] IMHOTEP will provide the platform to address the environmental modifying factors that alter the natural history of genetic cardiomyopathy. Importantly, IMHOTEP will stimulate the establishment of cardio genetics clinical services in Africa and facilitate the development of cost-effective diagnostic strategies to diagnose genetic cardiomyopathy in a LMIC setting.

3.6. STRENGTHS AND LIMITATION

IMHOTEP is a hospital-based registry and will therefore not address disease burden at the population level. Participating centres will be selected based on the availability of expertise required to diagnose cardiomyopathy and myocarditis and exclude alternative cardiac conditions such as ischaemic heart disease, hypertension, valvular heart disease, congenital heart disease and pericardial disease. This will likely result in an overrepresentation of advanced disease and disease-related adverse events, as patients will be recruited from tertiary centres (selection bias). The pilot phase will provide an opportunity to address operational problems prior to scaling up the study to multiple sites in all regions of Africa (List of collaborators in Appendix J).

3.7. CONCLUSION

Cardiomyopathy and myocarditis contribute significantly to the burden of cardiovascular disease in Africa and result in considerable morbidity and mortality.[2,60,114] Sufficient information on cardiomyopathies and myocarditis in the African population is currently lacking. There is a need for large, well-designed, prospective studies to evaluate the clinical, genetic and molecular epidemiology, as well as modifiable risk factors for cardiomyopathy, and the impact cardiomyopathy and myocarditis have on the burden of heart failure in the population.[3,5] To the best of our knowledge, this is the first multi-national study to record clinical characteristics, adverse events and long-term outcomes of children and adults with heart muscle disease in Africa, with the aim of addressing diagnostic and management deficiencies. The ultimate goal of IMHOTEP is to improve the quality of life and prognosis in affected individuals living in Africa.

3.8. CONTRIBUTIONS AND ACKNOWLEDGEMENTS

B. Mayosi conceived the idea for the IMHOTEP study. S. Kraus, B. Mayosi, and N. Ntusi developed the first protocol of this work and the drafting of the original manuscript for publication. All study tools (included in the body of this thesis and the appendix) were developed by PhD candidate, S. Kraus, in consultation with B. Mayosi and N. Ntusi. Study co-ordination and management has been led by S. Kraus and V. Francis (project manager) under the leadership of B. Mayosi (2014 – July 2018) and N. Ntusi (August 2018 to date). Collaborators are listed in Appendix J. Individuals that have contributed to the revision of the protocol and improved upon the design of this study, and approved the final manuscript have been included in the authorship for publication. Research staff that have contributed to recruitment in the multi-centred pilot as illustrated in Figure 3.5 have been listed in Appendix J. Site initiation and training of co-investigators has been conducted by S. Kraus, V. Francis, G. Shaboodien and N. Laing.

CHAPTER 4: Clinical features, genetics, and outcomes of the patients in the Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa

4.1. INTRODUCTION

ARVC is a genetically determined myocardial disorder characterised pathologically by fibrofatty replacement of the myocardium with myocyte loss and fibrosis, resulting in electrical instability with ventricular arrhythmias and HF, with predominantly RV dysfunction.[73] ARVC is associated with increased risk of premature SCD particularly in athletes, frequently occurring in the early 'concealed' phase of the disease.[74] Disease expression is variable, and the clinical manifestations vary with age and stage of disease.[180] Considerable progress has been made in the understanding of the pathogenesis, clinical manifestations and genetic aetiology of ARVC over the past four decades.

The hereditary nature of ARVC has been well recognised since the time of the first description by Giovanni Maria Lancisi in 1736,[181] with autosomal dominant, and less commonly autosomal recessive inheritance.[76,77,182] A number of ARVC disease-causing mutations in desmosomal and non-desmosomal genes have been identified,[75,78,83-86,89,183,184] and successful genotyping among patients meeting diagnostic criteria is approximately 50% (range 30 – 70%).[93,185-187]

The original 1994 TFC for the diagnosis of ARVC were based on structural, histological, electrophysiological and familial features, and although highly specific, they lacked sensitivity for the detection of early disease.[73,94] Application of the modified international TFC, published in 2010, has been shown to improve diagnostic sensitivity without loss of specificity by providing quantitative criteria, and includes CMR criteria and the presence of disease-causing mutations.[73]

The ARVC Registry of South Africa, established in 2003 by the Cardiac Arrhythmia Society of Southern Africa (CASSA), is the only ARVC registry on the African continent.[95] This report serves as an update on the status of the registry. With the incorporation of the ARVC Registry of South Africa into IMHOTEP, we analysed the entire series of index cases referred with suspected ARVC to the registry since its establishment in 2003. The main aims of the study were: (1) to review the diagnosis of ARVC in all cases referred with suspected ARVC according to the modified 2010 TFC; (2) to describe the clinical characteristics of affected individuals; (3) to report the pathological mutation rate in desmosomal and selected non-desmosomal genes; and (4) to report the outcomes of ARVC in South Africa.

4.2. METHODS

4.2.1. Study population

The study population comprised of 162 unrelated cases that were referred to the ARVC registry of South Africa for evaluation from all regions of South Africa between May 2003 and May 2018. It should be noted that some patients were recruited to the ARVC registry as retrospective cases, therefore the date of initial presentation (ranging from June 1978 to May 2018) and death may precede the date of study commencement. Informed consent for participation in the registry was obtained from participants at the time of referral. Blood samples for DNA were collected and stored in the Cardiovascular Genetics Laboratory at the Hatter Institute for Cardiovascular Research in Africa, UCT. The incorporation of the ARVC Registry of South Africa (HREC: 047/2003) into IMHOTEP (HREC: 767/2014) was approved by the Human Research Ethics Committee of UCT.

4.2.2. Study design and diagnostic evaluation

Following the modification of the TFC, all index cases were reviewed and reclassified according to the 2010 TFC, including those previously reported cases that had been

diagnosed according to the 1994 TFC.[96] The referred cases were reviewed by a diagnostic panel consisting of experts in clinical cardiology, electrophysiology, imaging (echocardiography, angiography and CMR), histopathology, clinical genetics and molecular genetics. The panel reviewed all standard ECG, SAECG, 24-hour ambulatory ECG, EST, electrophysiology studies (EPS) and invasive angiographic images. Echocardiographic images were not available for re-evaluation; however, a standardised protocol for the assessment of the RV was performed on the majority of cases with the establishment of the registry, and the required variables were extracted from those reports. CMR images were reviewed and reported by two trained readers. Morphometric analysis was not routinely performed on EMB specimens prior to the publication of the updated criteria. Histopathology slides were only reviewed for quantification of fibrosis in cases where an additional major or minor criterion for tissue characteristics would alter the 2010 TFC classification, either from borderline to definite, or from possible to borderline or definite. Inclusion and exclusion criteria are outlined in Table 4.1.

Table 4.1. Inclusion and exclusion criteria

Inclusion criteria
A diagnosis of definite or borderline ARVC according to the 2010 TFC,[73] where an alternative pathology could not explain the clinical findings, or pathological confirmation of a diagnosis of ARVC at autopsy or transplant
Exclusion criteria
Either: I. Insufficient criteria to make a diagnosis of definite or borderline ARVC (i.e. possible ARVC or no criteria for ARVC according to the 2010 TFC); or II. Alternative diagnosis to explain clinical findings

ARVC, arrhythmogenic right ventricular cardiomyopathy; TFC, task force criteria

4.2.3. Genotype analysis

Genotyping was performed for index cases where DNA was available for analysis. Mutation analysis for desmosomal genes encoding plakophilin-2 (*PKP2*), desmoplakin (*DSP*),

desmoglein-2 (*DSG2*), desmocollin-2 (*DSC2*), plakoglobin (*JUP*), and non-desmosomal genes encoding phospholamban (*PLN*) and cadherin 2 (*CDH2*) was done for index patients using methods previously reported[75,96,155] at the cardiovascular molecular genetics laboratory, Hatter Institute, UCT (Mbele, M. 2014. Ph.D. Thesis, UCT; Fish, M. 2016. Ph.D. Thesis, UCT; Machipisa, T. 2016. M.Phil. Thesis, UCT, Kamuli, S. 2016. M.Phil. Thesis, UCT). In addition, two participants had genetic screening done at international diagnostic laboratories for the listed mutations (Invitae [https://www.invitae.com/en/licensing/]; Oxford University Hospitals, Genetic Laboratories, National Health Service, Accredited Medical Laboratory Reference No. 0745 [https://www.ouh.nhs.uk/geneticslab]). Classification of variants as disease-causing was re-evaluated according to definitions outlined by the 2010 TFC, recent guidelines for assigning causality[177,178] and public archive, *ClinVar* (https://www.ncbi.nlm.nih.gov/clinvar).

4.2.4. Statistical analysis

Continuous variables were tested for distribution using a histogram for visualisation and Shapiro-Wilks for test of normality, and were summarised as mean and SD or median and IQR depending on distribution. Categorical variables were summarised in tables and reported as number and proportion (%). Median survival from presentation to the time of death was determined by the Kaplan–Meier method, and differences in survival between groups (with and without an ICD, and genotype positive and negative/unknown) were evaluated using the log-rank test. Comparisons between mutation carriers and non-carriers for death or transplantation were performed using Chi-squared test, Fisher exact test, or Student's t-test, and adjusted using *post-hoc* Bonferroni correction. A value of $P < 0.05$ was considered significant. Statistical analysis was performed using statistical software, IBM SPSS Statistics

(Version 25). Survival analysis was done using Stata/IC (Version 15.1, StataCorp, USA) software[2].

4.3. RESULTS

4.3.1. Clinical characteristics

Of the 162 unrelated individuals referred to the ARVC registry of South Africa with suspected ARVC, 153 were evaluated by the diagnostic panel and classified according to the 2010 TFC (Figure 4.1.). Nine patients were not classified due to a lack of sufficient clinical data available for evaluation.

Excluded cases. Eighty-three of the 153 patients evaluated by the diagnostic panel were excluded, in addition to the 9 cases not evaluated (total number of cases excluded, n=92; Table 4.2.). Seven of the excluded patients met *definite* or *borderline* TFC; however, there was compelling evidence supporting alternative diagnoses, including one case of isolated RV endomyocardial fibrosis diagnosed on histology following transplantation, two cases of HCM confirmed on CMR, one case of Brugada syndrome, two cases of cardiac sarcoidosis and one asymptomatic individual with athlete's heart. Twenty-five patients were classified as *possible ARVC* and 51 patients had *no criteria* for ARVC. In the vast majority of cases, an alternative diagnosis was made; 19 individuals were considered to be at risk for ARVC, however there was insufficient clinical evidence to confidently confirm a diagnosis of ARVC at the time of initial evaluation or at follow-up. In addition to standard investigations, CMR was performed in 54/83 (65.1%) of the patients excluded and proved useful in establishing alternative diagnoses outlined in Table 4.2. Table 4.3 outlines investigations performed in excluded patients.

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[2] *The statistical software varied according to preference of the statistician and doctoral student, and the software resources available through the University of Cape Town*

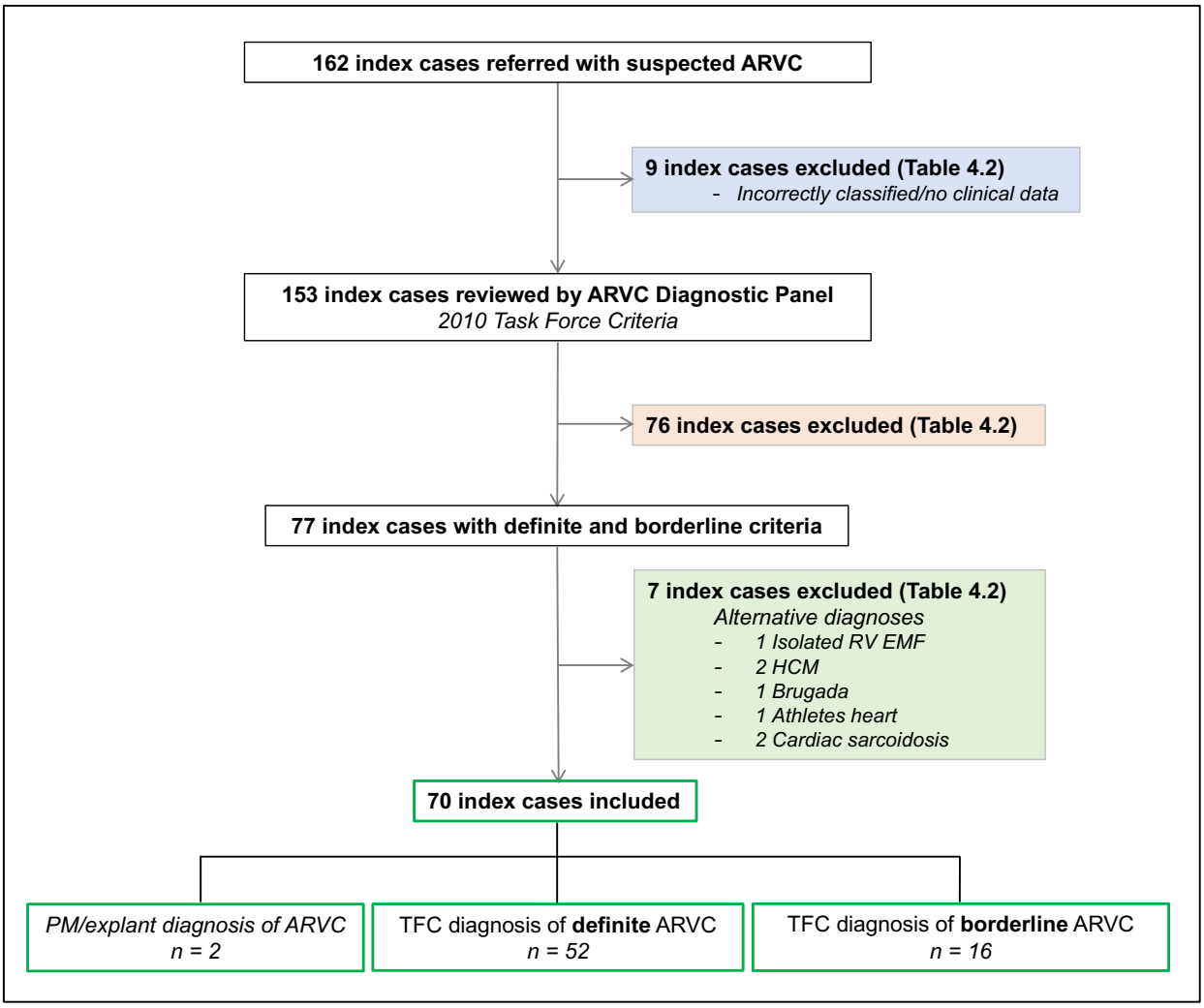

Figure 4.1. Referred cases of suspected ARVC

ARVC, arrhythmogenic right ventricular cardiomyopathy; EMF, endomyocardial fibrosis; HCM, hypertrophic cardiomyopathy; PM, post-mortem; RV, right ventricular; TFC, task force criteria

Table 4.2. Diagnoses of the 92 excluded cases*

	Total number of cases 92 patients n (%)	Classification according to 2010 Task Force Criteria for ARVC			
		'Definite' 4 patients n (%)	'Borderline' 3 patients n (%)	'Possible' 25 patients n (%)	No criteria 51 patients n (%)
At risk for ARVC					
Possible early ARVC but insufficient criteria	13 (14.1)	-	-	10 (40.0)	3 (5.9)
Possible early ARVC or idiopathic RVOT VT	6 (6.5)	-	-	5 (20.0)	1 (2.0)
Idiopathic RVOT VT	12 (13.0)	-	-	-	12 (23.5)
Idiopathic VT/VF/PVC	9 (9.8)	-	-	1 (4.0)	8 (15.7)
Supraventricular tachycardia	5 (5.4)	-	-	-	5 (9.8)
Alternative type of cardiomyopathy (total)	16 (17.4)	4 (100.0)	1 (33.3)	2 (8.0)	9 (17.6)
Dilated cardiomyopathy	5 (5.4)	-	-	-	5 (9.8)
Hypertrophic cardiomyopathy	5 (5.4)	1 (25.0)	1 (33.3)	2 (8.0)	1 (2.0)
Endomyocardial fibrosis	1 (1.1)	1 (25.0)	-	-	-
Left ventricular noncompaction	1 (1.1)	-	-	-	1 (2.0)
Myocarditis	2 (2.2)	-	-	-	2 (3.9)
Sarcoidosis (confirmed on histology)	2 (2.2)	2 (50.0)	-	-	-
Alternative diagnosis (total)	9 (9.8)	-	2 (66.7)	3 (12.0)	4 (7.8)
Brugada syndrome	1 (1.1)	-	1 (33.3)	-	-
Athlete's heart	3 (3.3)	-	1 (33.3)	1 (4.0)	1 (2.0)
Familial heart block	1 (1.1)	-	-	1 (4.0)	-
Other**	4 (4.3)	-	-	1 (4.0)	3 (5.9)
Symptoms only (no cardiac disease)	13 (14.1)	-		4 (16.0)	9 (17.6)
Not classified – no clinical data	9 (9.8)	-	-	-	-

*DNA was available for 83 of the excluded cases. All excluded patients with DNA were genotype negative for known disease causing mutations; **Includes congenital heart disease, chronic lung disease, ischaemic heart disease, TB pericarditis, hypertensive heart disease.
ARVC, Arrhythmogenic right ventricular cardiomyopathy; RVOT, right ventricular outflow tract; VT, ventricular tachycardia; VF, ventricular fibrillation; PVC, premature ventricular complexes.

Table 4.3. Investigations performed in excluded patients*

Investigation	EXCLUDED n = 82 n (%)
Non-invasive investigations	
Electrocardiogram	81 (100)
Signal average electrocardiogram	61 (74.4)
24-hour Holter	34 (41.5)
Echocardiogram	79 (96.3)
Cardiovascular magnetic resonance	52 (63.4)
Invasive investigations	
Right ventriculogram	51 (62.2)
Endomyocardial biopsy	56 (68.3)

* Cases not included in this table: 9 patients not classified due to lack of clinical information, 1 infant that could not be assessed using TFC

Included cases. Seventy of the 153 cases reviewed by the diagnostic panel were considered to have a diagnosis of ARVC; 52 patients met definite criteria, 16 patients met borderline criteria and 2 patients had a pathological diagnosis (1 post-mortem, 1 after transplantation) where preceding clinical investigations were not available. The baseline clinical characteristics including age of onset, gender, race and symptoms at presentation of the 70 affected individuals are summarised and compared with two international series[93,186] in Table 4.4. Of the patients considered to have definite or borderline ARVC, the mean age of onset was 35.4 ± 14.0 years with male predominance (67.1%). Sixty percent of patients were Caucasian, 27.1% were mixed-race, 7.1% were black African, and 5.7% were Indian (South Asian ancestry). The vast majority of patients (95.7%) were symptomatic at presentation. The most common symptoms reported were palpitations (75.7%), followed by presyncope/dizziness (55.7%), chest discomfort (35.7%), syncope (34.3%) and dyspnoea (25.7%). More than half of the cases (54.3%) had documented ventricular tachycardia at the time of presentation. Seven patients (10.0%) presented with cardiac arrest; 6 (8.6%) were resuscitated and survived, and 1 (1.4%) died out-of-hospital and was recruited post-mortem.

Table 4.4. Baseline characteristics of study participants compared to two large international cohorts

Baseline characteristics	SA Registry	International Registries	
	Definite and borderline study participants n = 70	John Hopkins/ Dutch transatlantic cohort[93] n = 439	Canadian registry[186] n = 129
Age at presentation, *years ± SD*	35.4 ± 14.0	36±14	46±17.4
Male gender, *n (%)*	47 (67.1)	282 (64.0)	75 (58.0)
Ethnicity, *n (%)*			
Black African	5 (7.1)	1 (0.2)	-
Caucasian	42 (60.0)	432 (98.0)	-
Mixed Race	19 (27.1)	-	-
Indian	4 (5.7)	-	-
Asian	0 (0.0)	6 (1.0)	-
Symptoms at presentation, *n (%)*			
Symptomatic	67 (95.7)	418 (95.0)	-
Palpitations	53 (75.7)	-	72 (56.0)
Dizziness	39 (55.7)	-	38 (29.0)
Chest pain	25 (35.7)	-	24 (19.0)
Syncope	24 (34.3)	-	33 (26.0)
Dyspnoea	18 (25.7)	-	-
Asymptomatic	1 (1.4)	20 (5.0)	-
Unknown	2 (2.9)	-	-
VT at presentation, *n (%)*	38 (54.3)	220 (50.0)	-
Cardiac arrest at presentation, *n (%)*	7 (10.0)	48 (11.0)	28 (22.0)
Resuscitated and survived	6 (8.6)	25 (5.7)	-
Died	1 (1.4)	23 (5.2)	-

SA, South African; SD, standard deviation; VT, ventricular tachycardia

4.3.2. Investigations and diagnostic criteria

Investigations were performed according to clinical indications and the availability of resources at referring centres. Table 4.5. outlines the investigations performed on study participants. Table 4.6. compares the 2010 TFC in the 70 affected study participants with those of two international series.[93,186]

Table 4.5. Investigations performed for patients with definite and borderline ARVC

Investigation	ALL n = 70 n (%)	Definite n = 54 n (%)	Borderline n = 16 n (%)
Non-invasive investigations			
Electrocardiogram	68 (97.1)	52 (96.3)	16 (100.0)
Signal average electrocardiogram	47 (67.1)	40 (74.1)	7 (43.7)
24-hour Holter	28 (40.0)	19 (35.2)	9 (56.2)
Echocardiogram	59 (84.3)	47 (87.0)	12 (75.0)
Cardiovascular magnetic resonance	29 (41.4)	20 (37.0)	9 (56.2)
Invasive investigations			
Electrophysiological study	50 (71.4)	39 (72.2)	11 (68.8)
Right ventriculogram	44 (62.9)	36 (66.7)	8 (50.0)
Endomyocardial biopsy	36 (51.4)	28 (51.9)	8 (50.0)
Genetic screening	58 (82.9)	46 (85.2)	12 (75.0)

Table 4.6. 2010 task force criteria of study participants compared to two large international cohorts

2010 Task Force Criteria	SA Registry	International Registries	
	South African cohort n = 70 n (%)	John Hopkins/ Dutch cohort[93] n = 416 n (%)	Canadian Cohort[186] n = 129 (%)
Structural abnormalities			
Major criteria (echocardiogram, CMR, and/or RVA)	35 (50.0)	264 (63)	(33)
Minor criteria (echocardiogram, CMR, and/or RVA)	1 (1.4)	50 (12)	(26)
Unknown†	8 (11.4)	-	-
Tissue characteristics			
Major criteria	7 (10.0)	-	-
Minor criteria	5 (7.1)	-	-
Fibrofatty infiltration not quantified	8 (11.4)	-	-
Unknown†	34 (48.6)	-	-
Repolarization abnormalities			
TWI V1-3 (major)	50 (71.4)	308 (74)	(29)
TWI V1-2, or V4-6, or V1-4 with RBBB (minor)	9 (12.9)	72 (17)	(27)*
Unknown†	2 (2.9)	-	
Depolarization abnormalities			
Epsilon wave (major)	4 (5.7)	63 (15)	-
Late potentials or TAD (minor)	36 (51.4)	277 (67)	(43)
Unknown†	2 (2.9)	-	-
Arrhythmias			
VT with LBBB morphology and superior axis (major)§	32 (45.7)	181 (44)	-
VT with LBBB morphology and inferior axis (minor)§	27 (38.6)	268 (64)	-
> 500 PVC/24 hours (minor)	20 (28.6)	205 (49)	-
VT with unknown morphology and axis	5 (7.1)	-	-
Family history			
Disease-causing mutation (performed in 58 patients)	14/58 (24.1)	264 (63)	(34)

†Investigations not performed; *TWI in V2 only, §7 cases had both superior and inferior axes
ARVC, arrhythmogenic right ventricular cardiomyopathy; CMR, cardiovascular magnetic resonance; LBBB, left bundle branch block; PM, post-mortem; PVC, premature ventricular complexes; RBBB, right bundle branch block; SA, South African; TWI, T-wave inversion; VT, ventricular tachycardia

Major and minor structural abnormalities were found in 50.0% and 1.4% cases, respectively. Echocardiograms were performed on the majority (84.3%) of patients; however, diagnostically significant structural changes were only found in 15.3% (9/59) of the studies performed. CMR was performed on 29 (41.4%) of the 70 study participants included (Table 4.7.). Major CMR criteria for ARVC were met in 48.3% of the patients imaged. Mild structural and/or functional abnormalities (not meeting CMR TFC) and/or LGE was observed in a further 41.4% of patients, and only 10.3% of patients were considered to have completely normal studies. Significant differences between patients with definite versus borderline TFC diagnoses were observed for RV end-diastolic volumes corrected to body surface area (RVEDD/BSA 151.8ml/m^2 versus 88.2ml/m^2, p=0.016), RV ejection fraction (RVEF) (36.8% versus 57.4%, p=0.003), LV ejection fraction (LVEF) (51.4% versus 61.8%, p=0.041), tricuspid regurgitation (70.0% versus 11.1%, p=0.003) and LGE of RV septum and/or walls (60.0% versus 14.3%, p 0.045). Evidence of LV involvement on CMR was seen in 75.0% of patients imaged. RV angiograms were performed in 44 (62.9%) of the index cases included, with major diagnostic criteria being met in 52.3% of those imaged.

Major and minor criteria for tissue characterisation were only met in 10.0% and 7.1% of the cohort, respectively. Fibrofatty infiltration on EMB was reported but not quantified in a further 11.4% of cases. EMB was performed in half (51.4%) of patients included with a diagnostic yield of 33.3% (12/36), however, these findings only contributed to changing the TFC diagnosis in 6 (16.7%) of the patients biopsied (possible to borderline/definite ARVC in 2 patients, borderline to definite ARVC in 4 patients).

Electrocardiograms were performed on all patients except 2 cases: one recruited after transplant and one recruited post-mortem. Repolarisation abnormalities were noted in 84% of cases, with 71.4% and 12.9% fulfilling major and minor criteria, respectively. Epsilon waves were only observed in 5.7% of the cohort; however, late potentials on SAECG (or terminal activation of the QRS on standard ECG) were present in 51.4% of patients. Seventy-four

percent of cases had a documented left-bundle-branch-block (LBBB) morphology ventricular tachycardia (VT) with superior (45.7%) and/or inferior (38.6%) axes. Unclassified VT, where the morphology and axis could not be determined, was present in an additional 7.1% of cases. Only one proband met major criteria for family history in the absence of a disease-causing mutation. Two probands that met minor criteria for family history were subsequently found to have disease-causing mutations on genotyping.

Table 4.7. CMR findings for 29 patients with definite and borderline ARVC

CMR findings	ALL n=29 n (%)	Definite n=20 n (%)	Borderline n=9 n (%)	P-value
Normal CMR	3 (10.3)	1 (5.0)	2 (22.2)	0.159
2010 Task Force Criteria for CMR				
Major criteria	14 (48.3)	13 (65.0)	1 (11.1)	0.007
Minor criteria	0 (0.0)	0 (0.0)	0 (0.0)	-
In those that did not meet task force criteria (n = 15)				
RV RWMA only	1/15 (6.7)	1.7 (14.3)	0/8 (0.0)	0.268
Increased right ventricular volumes without RWMA	5/15 (33.3)	3/7 (42.9)	2/8 (25.0)	0.343
RVEF ≤ 45% without RWMA	2/15 (13.3)	2/7 (28.6)	0/8 (0.0)	0.117
Left ventricular involvement*	21/28 (75.0)	15/19 (78.9)	6/9 (66.7)	0.483
Chamber dimensions				
RVEDV/BSA (ml/m^2)[a]	133.0 ±64.4	151.8 ±67.8	88.2 ±18.5	0.016
LVEDV/BSA (ml/m^2)[b]	93.2 ±18.9	94.0 ±19.2	91.3 ±19.4	0.733
RA area (cm^2)[c]	29.5 ±17.1	32.3 ±19.5	22.4 ±4.4	0.171
LA area (cm^2)[d]	23.8 ±6.8	23.6 ±6.7	24.2 ±7.4	0.839
Ventricular function				
RVEF[e]	42.9 ±17.6	36.8 ± 17.3	57.4 ±6.1	0.003
LVEF[f]	54.5 ±13.0	51.4 ±12.2	61.8 ±5.8	0.041
RV aneurysm(s)	5 (17.2)	4 (20.0)	1 (11.1)	0.558
RVOT dimensions	27.6 ±6.4	28.9 ±6.9	24.4 ±3.5	0.122
Tricuspid regurgitation	15 (51.7)	14 (70.0)	1 (11.1)	0.003
Presence of LGE	17/22 (77.3)	12/15 (80.0)	5/7 (71.4)	0.655
LGE of the RV septum and/or walls	10/22 (45.5)	9/15 (60.0)	1/7 (14.3)	0.045
LGE of the LV septum and walls	13/22 (59.1)	9/15 (60.0)	4/7 (57.1)	0.899
Thrombus	1 (3.4)	1 (5.0)	0 (0.0)	0.495

*Unable to access LV in one case

BSA, body surface area; LA, left atrium; LGE, late gadolinium enhancement; LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; RA, right atrium; RV, right ventricle; RVOT, right ventricular outflow tract; RWMA, regional wall motion abnormalities; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction

4.3.3. Outcomes

Outcome data (as of 1 May 2018) is represented in Figure 4.2. Seven cases were lost to follow-up, 12 (17.1%) patients died, and 4 (5.7%) patients were transplanted over a median follow-up period of 9.0 years [IQR 6.1 – 14.4]. The median age of death was 37.9 years [IQR 22.2 – 55.2]. Thirty-six patients had implantable cardioverter defibrillators (ICD) inserted for prevention of SCD. Of those with an ICD, 1 patient was transplanted and 4 died: 1 patient died while swimming, 2 died of HF, and 1 died suddenly after the ICD was turned off at the patient's request. Of those without an ICD, 3 patients were transplanted and 8 patients died: 2 patients died of HF, 4 died suddenly, and 2 died of RV failure following RV disconnection surgery (in 1986 and 1997, respectively). The indication for transplantation was biventricular failure in all cases. Radiofrequency ablation was performed in 15 cases (9 without ICDs, 6 with ICDs), and 4 individuals underwent RV disconnection surgery.

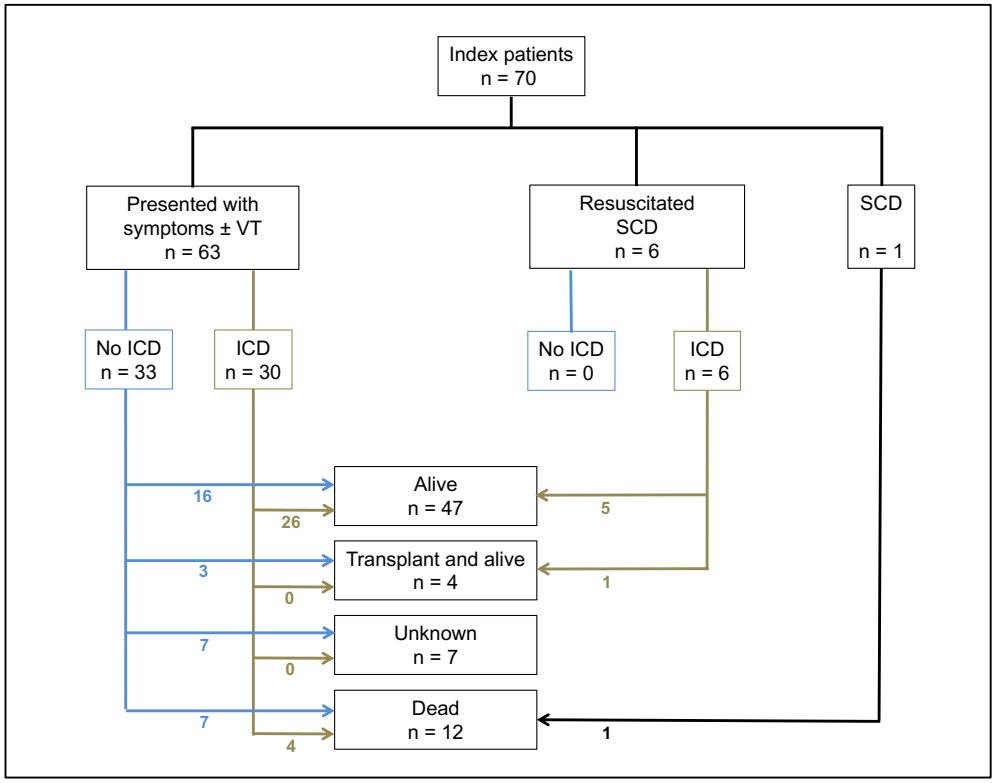

Figure 4.2. Outcomes of patients with ARVC

ICD, implantable cardioverter defibrillator; SCD, sudden cardiac death; VT, ventricular tachycardia

Overall transplant-free survival probability at 1-year, 5-years and 10-years was 99% (confidence interval [CI] 90 – 99.8), 91% (CI 80 – 96), and 81% (CI 68 – 89), respectively, with median survival time of 9.0 years [IQR 6.1 – 14.4]. While SCD was more frequent in patients without an ICD (4/34, 12% versus 2/36, 6%), there were no significant difference in overall transplant-free survival between those with and without devices (p=0.27). Furthermore, there was no significant differences in outcomes between genotype positive and genotype negative/unknown individuals. Figure 4.3. shows the results of the Kaplan–Meier transplant-free survival analysis overall, and comparisons in transplant-free survival in patients with and without ICDs, and genotype positive versus genotype negative/unknown patients.

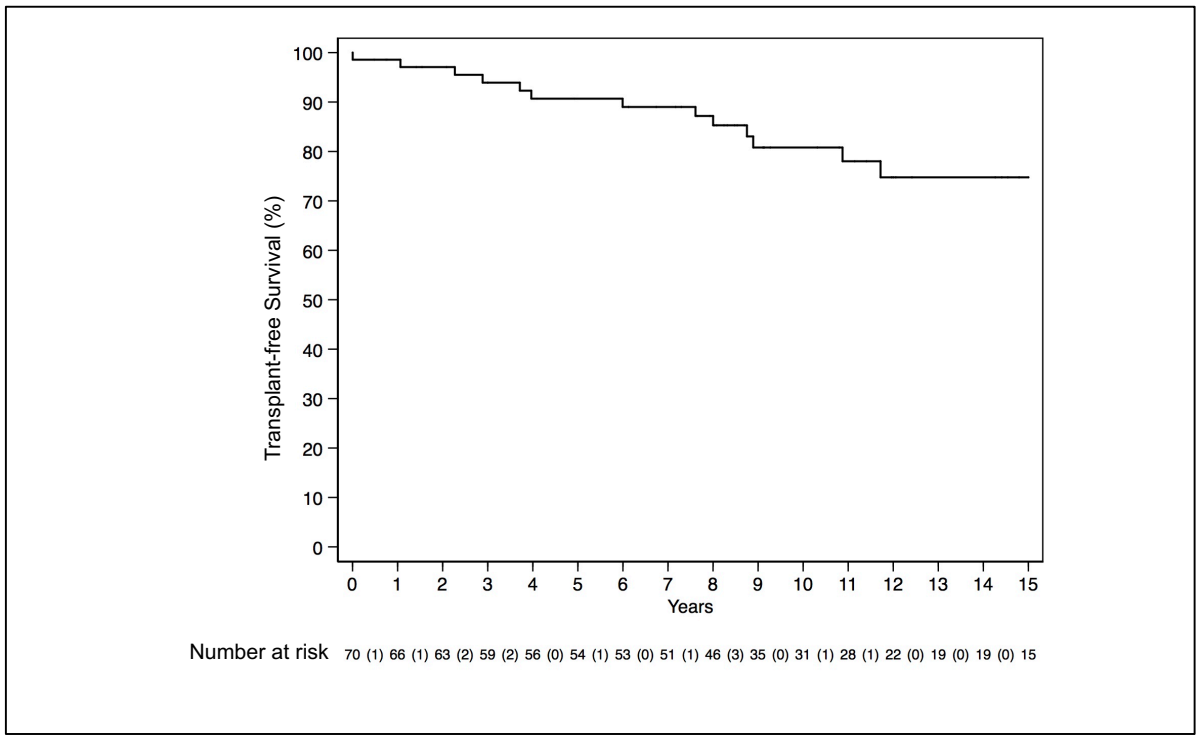

Figure 4.3.1. Kaplan-Meier survival analysis for overall transplant-free survival

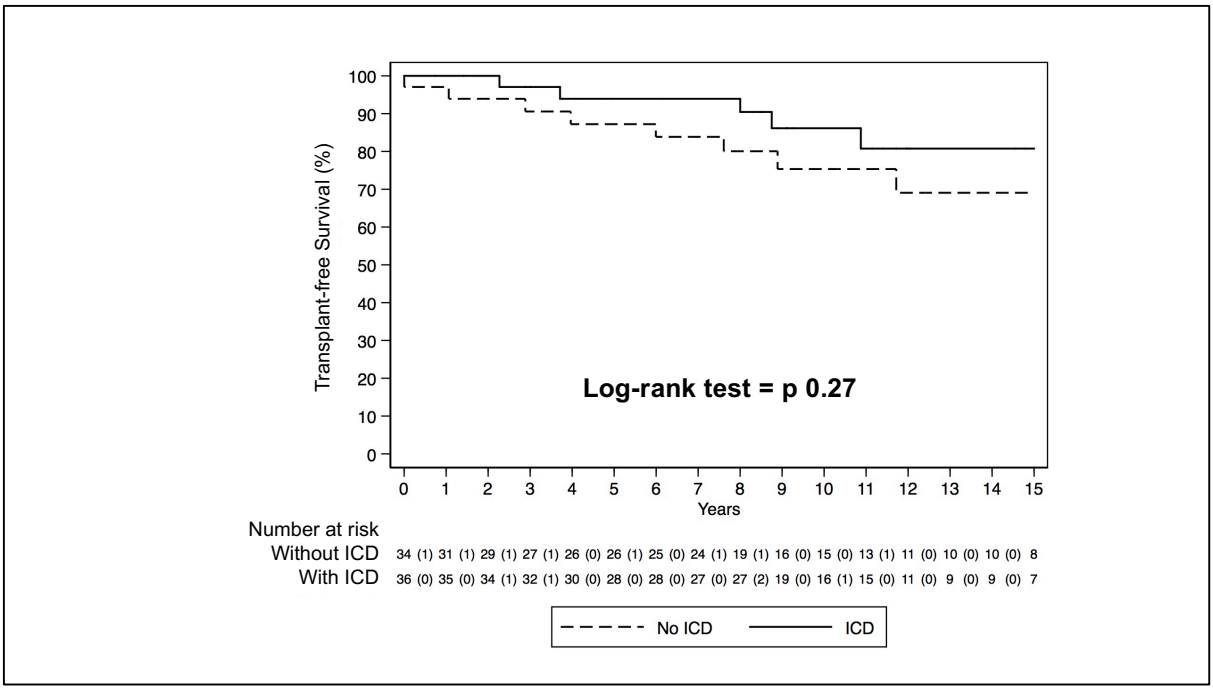

Figure 4.3.2. Kaplan-Meier survival analysis for transplant-free survival in ARVC patients with or without an ICD

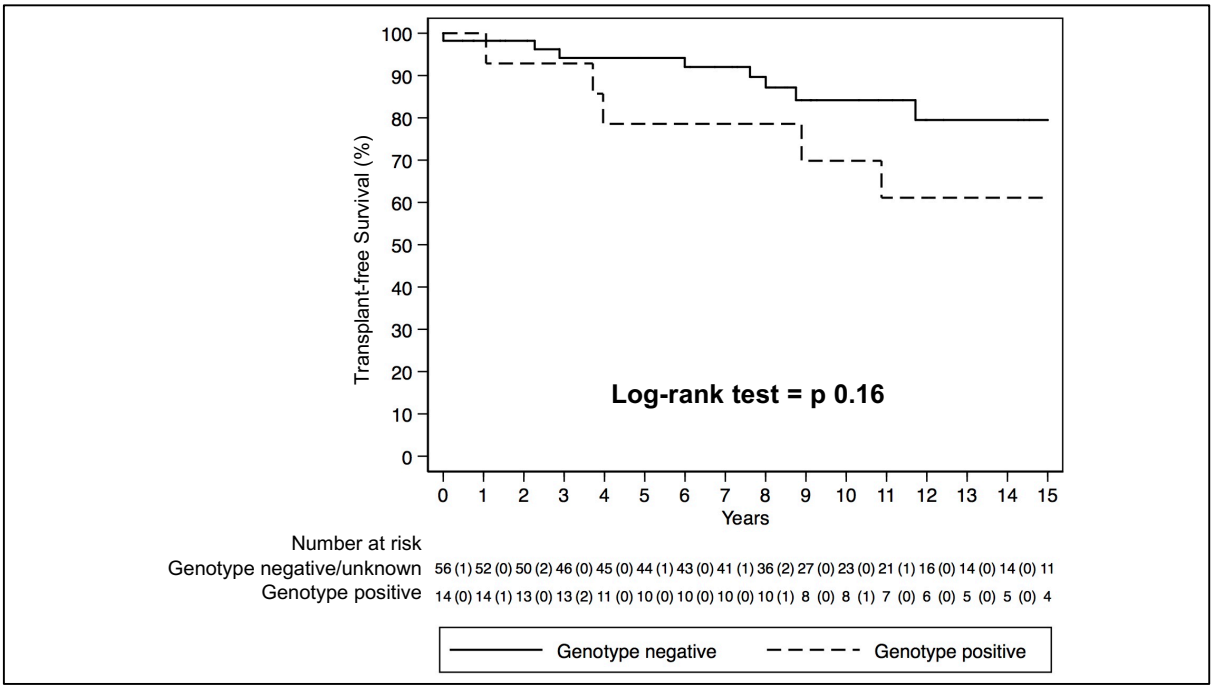

Figure 4.3.3. Kaplan-Meier survival analysis for transplant-free survival in genotype positive and genotype negative/unknown patients with ARVC

4.3.4. Genotypic information

DNA was available for 58 (82.9%) of the 70 unrelated index cases with definite or borderline criteria. Twelve patients were not genotyped; blood specimens were not available in 7 cases (loss to follow-up, n=4; death, n=3) and molecular genetic analysis was incomplete for the remaining 5 cases at the time of writing. Fourteen (24.1%) of the 58 index cases genotyped were found to have disease-causing mutations based on the fulfilment of the following criteria outlined by the 2010 TFC: (1) associated with ARVC, (2) unobserved or rare in large non-ARVC control populations, and (3) alters, or is predicted to alter, the structure or function of the protein, or has demonstrated linkage to the disease phenotype in a conclusive pedigree.[73]

The pathogenic mutations described were previously identified by CVG laboratory-based masters and doctoral students and are referenced and summarised in Table 4.8. All variants described in tables below have been found to be rare in local population controls as described in the referenced work (Mbele, M. 2014. Ph.D. Thesis, UCT; Fish, M. 2016. Ph.D. Thesis, UCT; Machipisa, T. 2016. M.Phil. Thesis, UCT; Kamuli, S. 2016. M.Phil. Thesis, UCT). The *PKP2* c.G1465A variant, classified as a '*variant of uncertain significance' (VUS)* by *ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/)*, was considered '*likely pathogenic'* as segregation with disease has been demonstrated in Family 10 - ACM 39 (Figure 4.4.). In addition, a further 4 *variants of uncertain significance* were observed in this cohort, where only 2/3 of the above criteria were met (Table 4.9.). Although likely pathogenic, further work demonstrating segregation with disease in families or functional studies will be required before these variants can be considered causal. The presence of a disease-causing mutation did not change the diagnostic classification in any of the 14 genotype positive patients as all had a definite diagnosis of ARVC based on other clinical criteria.

Table 4.8. Disease-causing mutation in unrelated index cases in the ARVC Registry of South Africa

Proband	Gene	Exon	Nucleotide change	Amino acid change	Type	Reported	ClinVar	Criteria for pathogenicity according to TFC$^{\Sigma}$
ACM 22.1	PKP2	Intron 10	IVS2146-1G-C c.2146-1G>C	Mutant splice product	Splice site	**Watkins et al., 2009*** Gerull et al., 2004; Syrris et al., 2006; Dalal et al., 2006; den Haan et al., 2009; Fressart et al., 2010; Kant et al., 2016; Svensson et al., 2016	Pathogenic	(1) Reported in multiple individuals with ARVC (2) Conserved. Not observed with any significant frequency in large population cohorts (Lek et al., 2016; 1000 Genomes Consortium et al., 2015; EVS). (3) Supported by functional studies (Groeneweg et al., 2014). Segregation with ARVC in multiple affected relatives in multiple families (Dalal et al., 2006; Svensson et al., 2016).
ACM 19.2$^{\Delta}$	PKP2	11	c.2197-2202del CACACCinsG	A733fsX740	Insertion/ deletion	**Watkins et al., 2009*** Syrris et al., 2006; Dalal et al., 2006; den Haan et al., 2009; Fressart et al., 2010; Tan et al., 2010; Xu et al., 2010; Rajkumar et al., 2012; Baskin et al., 2013; Phillips et al., 2014; Groeneweg et al., 2015	Pathogenic	(1) Reported in multiple individuals with ARVC (2) Conserved. Not observed in ±6,500 individuals of European and African American ancestry in the NHLBI Exome Sequencing Project, nor was it observed in ExAC (3) Predicted to lead to a truncated or absent protein. Segregate with ARVC in six relatives from two different families (Syrris et al., 2006).
ACM 1.2	PKP2	4	c.C1132T	Q378X	Nonsense	**Watkins et al., 2009*** Fressart et al., 2010; Bhonsale et al., 2013; Ohno et al., 2013; Philips et al., 2014; Groeneweg et al., 2015; Torkamani et al., 2016; Walsh et al., 2017; Sonoda et al., 2017; Wada et al., 2017	Pathogenic/ Likely pathogenic	(1) Reported in multiple individuals with ARVC (2) Conserved. Not observed at a significant frequency in large population cohorts (Lek et al., 2016) (3) Predicted to cause loss of normal protein function either by protein truncation or nonsense-mediated mRNA decay

*Cardiovascular Genetics Laboratory, Hatter Institute of Cardiovascular Research in Africa, University of Cape Town
Δ compound heterozygous

Table 4.8. Disease-causing mutation in unrelated index cases in the ARVC Registry of South Africa (continued)

Probands	Gene	Exon	Nucleotide change	Amino acid change	Type	Reported	ClinVar	Criteria for pathogenicity according to TFC
ACM 5.1 ACM 12.1 ACM 19.2$^{\Delta}$ ACM 38.3 ACM 8.3† ACM 57.1 ACM 71.1	PKP2	4	c.C1162T	R388W	Missense	**Watkins et al., 2009*** **The results for individuals, ACM 8.3, ACM 57.1 and ACM 71.1, were reported by Machipisa, T. 2016. M.Phil. Thesis. UCT; Kamuli, S. 2016. M.Phil. Thesis, UCT***	Pathogenic	(1) Reported in multiple individuals and families, founder mutation (2) Conserved. Allele frequency ExAC 0.00001. The NHLBI ESP Exome Variant Server reports Arg388Trp was not observed in approximately 6,400 samples from individuals of European and African American backgrounds (3) Segregates with ARVC in multiple families (Watkins et al., 2009)
ACM 39.5	PKP2	6	c.G1465A	G489R	Missense	**Watkins et al., 2009***	Uncertain significance (however likely pathogenic)	(1) Reported in one individual with ARVC (2) Conserved. Allele frequency ExAC 0.00009 (3) Functional studies not done. **Note: Segregation with ARVC demonstrated in ACM 39 family (see pedigree**)
ACM 51.1	PKP2	13	c.2509delA	S837fs	Deletion	**Watkins et al., 2009*** Gerull et al., 2004, Dalal et al., 2006, Dalal et al., 2006, Dalal et al., 2009, Xu et al., 2010, Antoniades et al., 2006, Fressart et al., 2009, Barahona-Dussault et al., 2010, Den Haan et al., 2009, Tan et al., 2010, Cox et al., 2011, Baskin et al., 2013	Pathogenic/ Likely pathogenic	(1) Reported in multiple unrelated individuals diagnosed with ARVC (2) Conserved. Not detected in more than 1,500 control alleles. ExAC - no frequency reported (3) Predicted to alter PKP2 protein. Reported to segregate with ARVC in a family (PMID: 17010805)

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† homozygous, Δ compound heterozygous

Table 4.8. Disease-causing mutation in unrelated index cases in the ARVC Registry of South Africa (continued)

Probands	Gene	Exon	Nucleotide change	Amino acid change	Type	Reported	ClinVar	Criteria for pathogenicity according to TFC
ACM 136.1	PKP2	5	c.C1237T	R413X	Nonsense	Syrris et al., 2006; den Haan et al., 2009; Unsoeld et al., 2009; Tan et al., 2010; Fressart et al., 2010; Quarta et al., 2011; Philips et al., 2014; Alcalde et al., 2014 **Unpublished result - provided by Shaboodien, G.***	Pathogenic/ Likely pathogenic	(1) Reported multiple times in association with ARVC, with observation in more than ten individuals with ARVC (2) Conserved. Not observed at a significant frequency in large population cohorts (Lek et al., 2016; 1000 Genomes Consortium et al., 2015; EVS). Allele frequency GO-ESP 0.00008, ExAC 0.00002 (3) Predicted to cause loss of normal protein function either by protein truncation or nonsense-mediated mRNA decay. Observed segregation with ARVC in families (Unsoeld et al., 2009, Alcade et al., 2014)
ACM 2.4	CDH2	5	c.A686C	Q229P	Missense	**Mayosi et al., 2017***	Not reported	(1) Reported in one family with ARVC (2) Conserved. Not observed in local population controls (3) Segregation with ARVC demonstrated (Mayosi et al., 2017)
ACM 11.2	CDH2	9	c.G1219A	D407N	Missense	**Mayosi et al., 2017***	Not reported	(1) Reported in one individual with ARVC (2) Conserved. Not observed in local population controls (3) De Novo mutation (trio study) described in chapter 7

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PKP2, plakophilin-2; CDH2, cadherin 2

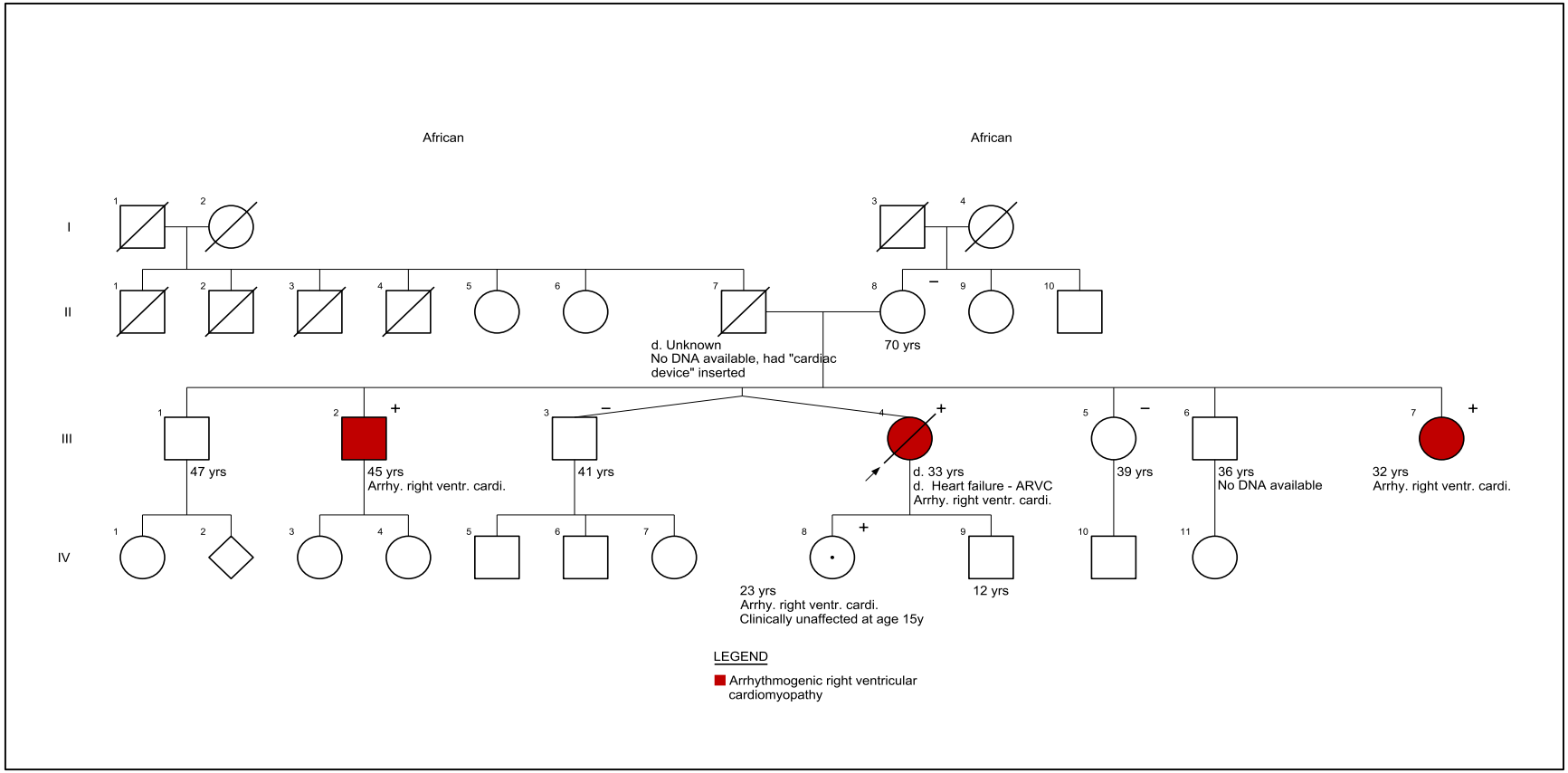

Figure 4.4. Family 10 (ACM 39) pedigree showing segregation of ARVC with *PKP2 c.G1465A* variant (+).
Individuals that tested negative for the *PKP2 c.G1465A* variant are indicated as (-).

Table 4.9. Variants of uncertain significance in unrelated index cases in the ARVC Registry of South Africa

Probands	Gene	Exon	Nucleotide change	Amino acid change	Type	Reported	ClinVar	Criteria for pathogenicity according to TFC
ACM 34.5	PKP2	13	c.T2540C	L847P	Missense	**Watkins et al., 2009***	Uncertain significance	(1) Reported in one individual with ARVC (2) Conserved. Not observed in large population cohorts (Lek et al., 2016; 1000 Genomes Consortium et al., 2015; EVS). (3) Functional effects unknown (Al-Jassar et al.). Silico analysis predicts this variant is probably damaging to the protein structure or function. Linkage analysis not done.
ACM 7.1	DSG2	11	c.A1435G	K479E	Missense	**The result for this individual was reported by Mbele, M. 2014. Ph.D. Thesis, UCT***	Not reported	(1) Not previously reported (novel) (2) Conserved. Not observed in ARVC database, 1000 genomes, NCBI dbSNPs and 232 local population controls* (3) Functional studies and linage analysis not done
ACM 48.1	DSG2	11	c.A1478G	N493S	Missense	Quarta et al., 2011 **This result for this individual was reported by Mbele, M. 2014. Ph.D. Thesis, UCT***	Uncertain significance	(1) Reported as a possible disease-causing mutation in association ARVC (Quarta G et al., 2011) and as an unclassified variant (Van der Zwaag P et al., 2009). (2) Conserved. Not found in ARVC database, 1000 genomes, NCBI dbSNPs and 232 local population controls*, ExAC 0.00004 (3) No functional studies, but predicted as disruptive. Computational prediction tools and conservation analysis do not provide strong support for or against an impact to the protein. No linkage analysis available.

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Table 4.9. Variants of uncertain significance in unrelated index cases in the ARVC Registry of South Africa (continued)

Probands	Gene	Exon	Nucleotide change	Amino acid change	Type	Reported	ClinVar	Criteria for pathogenicity according to TFC
ACM 53.1	DSC2	6	c.G2587A	G863R	Missense	Cox et al., 2011 Kapplinger et al., 2011; Bhonsale et al. 2013; Proost et al., 2017 Elliott et al., 2010 (DCM) **This result for this individual was reported by Mbele, M. 2014. Ph.D. Thesis, UCT***	Uncertain significance	(1) Reported VUS in ARVC and DCM. Previously reported in at least three individuals in association with ARVC however, these individuals all harboured an additional cardio-genetic variant, including two individuals who each harboured a different pathogenic frameshift variant in the PKP2 gene. (2) Not found in 232 population local controls*, G863R variant has been observed in 0.03% alleles from individuals of Non-Finnish European ancestry in large population cohorts (Lek et al., 2016; 1000 Genomes Consortium et al., 2015; Exome Variant Server). Its frequency suggests that it is more likely to be benign and is classified as a variant of uncertain significance by multiple other clinical laboratories (3) Silico analysis predicts this variant is probably damaging to the protein. Computational prediction tools and conservation analysis suggest that it may impact the protein, though this information is not predictive enough to determine pathogenicity. Lacks large segregation studies and functional evidence

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PKP2, plakophilin-2; DSG2, desmoglein-2; DSC2, desmocollin-2

Following the 2009 report of the *PKP2* C1162T founder mutation present in 4 unrelated individuals (ACM 5.1, ACM 12.1, ACM 19.2, ACM 38.3) in this cohort,[96] 3 additional probands (ACM 8.1, ACM 57.1, ACM 71.1) were found to carry the same founder mutation (Machipisa, T. 2016. M.Phil. Thesis, UCT; Kamuli, S. 2016. M.Phil. Thesis, UCT). Cascade genetic and clinical screening was performed for available members of the founder families. A variation in age of onset and severity of clinical phenotype was observed amongst *PKP2* C1162T mutation carriers within these families. The majority of non-proband carriers screened were asymptomatic with minimal clinical manifestations of disease. The index cases (probands) in 4 families (ACM 5, ACM 12, ACM 57, ACM 71) presented with VT in the third and fourth decades of life (ages 38–45 years). Three of these four index cases were competitive marathon runners (ACM 5.1, ACM 12.1, ACM 57.1) and one (ACM 71.1) had additional co-morbidities (hypertension and recreational drug use).

In contrast, probands ACM 8.1, ACM 19.2 and ACM 38.3 presented in childhood or early adolescence with severe disease (Figure 4.5.), all requiring transplantation in the first, second or third decade of life. Homozygosity and compound heterozygosity was associated with severe phenotypes in families ACM 8 (Figure 4.5.A) and ACM 19 (Figure 4.5.B), respectively (Machipisa, T. 2016. M.Phil. Thesis. UCT; Kamuli, S. 2016. M.Phil. Thesis, UCT).[96] The severe phenotype observed in ACM 38 family is unaccounted for; no additional known disease-causing variants have been identified, nor were there any specific environmental cofactors observed. The proband (ACM 38.3) presented at age 9 and required transplantation at age 16. In this family, a female sibling of the proband suffered a sudden death at age 8 with probable ARVC, and a male sibling also diagnosed with ARVC required transplantation at age 8. This observation is important as ARVC is exceptionally rare in children particularly under the age of 10.[93] The most likely explanation for the manifestation of severe disease in these 3 siblings is the presence of a second genetic mutation not yet identified, in addition to the founder variant.

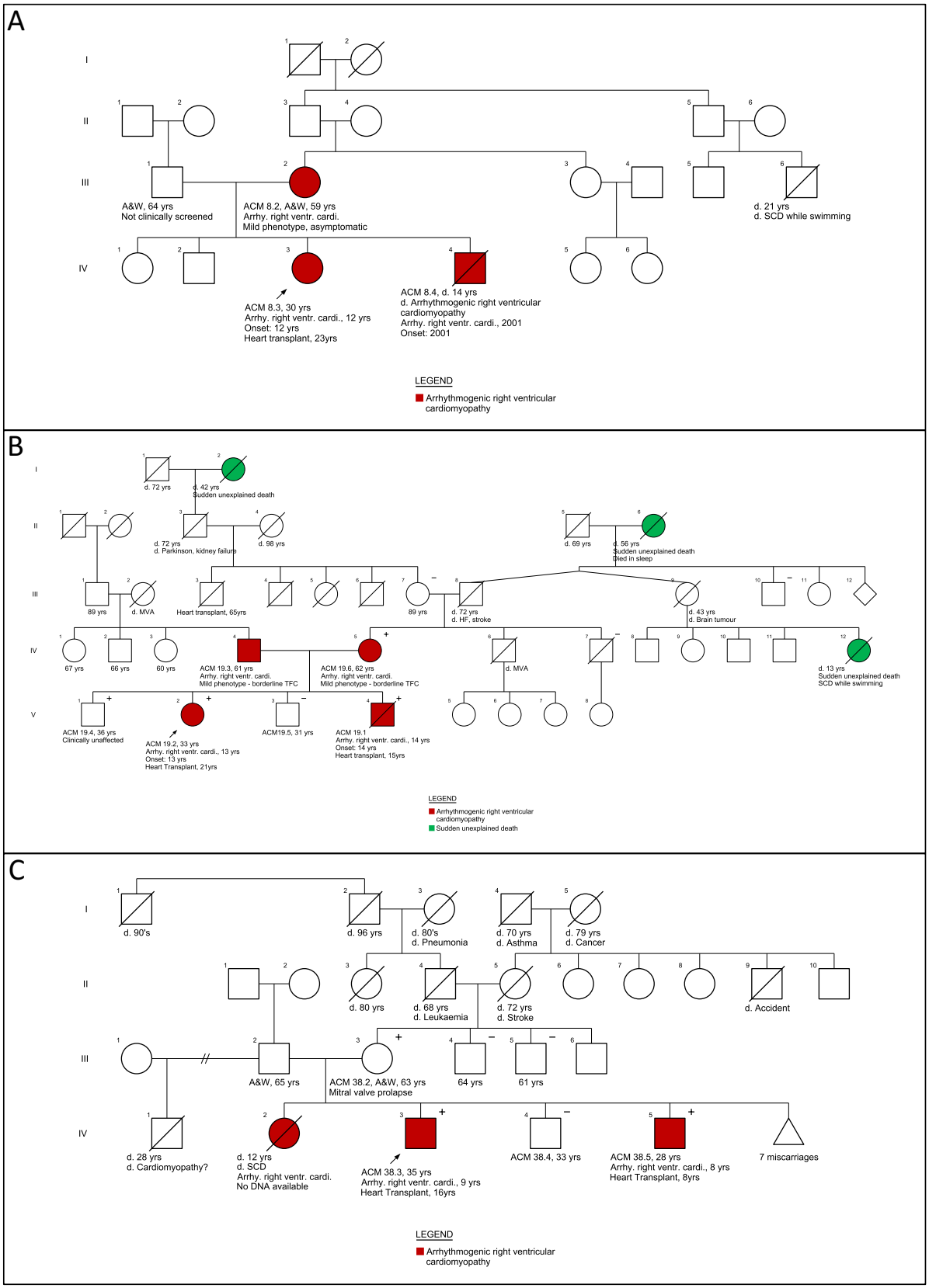

Figure 4.5. Founder families with severe phenotypes
A. Family ACM 8 (Appendix K - Family 4); **B**. Family ACM 19 (Appendix K – Family 7); **C**. Family ACM 38 (Appendix K – Family 9).

4.4. DISCUSSION

In this study, we report the classification of patients referred to the ARVC Registry of South Africa according to the 2010 TFC, and the clinical manifestations, survival and mutation status of patients with ARVC in South Africa. The age of onset (35.4 ± 14.0 years), male predominance (67.1%), and frequency of symptoms (95.7%), VT (54.3%) and cardiac arrest (10.0%) at presentation were similar to what has been reported in international series.[93,186] Although ARVC is seen in all race groups in the South African population, there was a predominance of Caucasian (60.0%) and mixed race (27.1%) participants – likely the result of referral bias and historical racial inequalities in access to tertiary health care services required to make a diagnosis of ARVC, as previously described in this cohort.[96] Similar racial disparities have been described in other areas of health care in South Africa.[188] The true incidence of ARVC in Africa is not known, and African-Americans/Europeans are unrepresented in international cohorts. The role of genetics factors in the underrepresentation of black African individuals in this ARVC cohort remains unclear.

The combined presence of VT (LBBB morphology) and repolarisation changes in the precordial leads on ECG was highly suggestive of the disease (present in 71.2% of cases). Significant structural abnormalities were reported in 51.4% of cases and LV involvement was observed in 75.0% of cases where CMR was performed. Disease-causing mutations were found in 24.1% of patients genotyped. Overall transplant-free survival probability at 1-year, 5-years and 10-years was 99% (CI 90 – 99.8), 91% (CI 80 – 96), and 81% (CI 68 – 89), respectively. While there was a significant difference in age of presentation between genotype positive and genotype negative/unknown individuals (median 25.0 years versus 38.0 years; mean, 27.6 ±13.7 years versus 37.4 ±13.5 years; p 0.018), there was no significant difference in survival between these groups. Although, there was no significant difference in overall transplant-free survival between those with and without ICDs, inferences relating to the clinical

utility of devices in patients with ARVC in SA cannot be made due to the small sample size in this study.

The first objective of this study was to review the diagnosis of all patients referred to the registry; this included those patients diagnosed according to the 1994 TFC and reported on by Watkins and colleagues in 2009 (total number of referred cases, n = 70; cases included in the report with ARVC according to 1994 TFC, n = 50; excluded cases, n = 20).[96] Six patients (6/20, 30%) previously referred but not included in the 2009 report were found to have *definite* or *borderline* ARVC using the modified 2010 criteria. The subsequent inclusion of these cases reflects the improved sensitivity of the repolarisation and arrhythmia criteria. Thirteen cases (13/50, 26%) included in the 2009 report were excluded from this study (Table 4.10.); 1 case had a diagnosis of sarcoidosis made on biopsy at follow-up and 12 cases did not have sufficient criteria for inclusion. We were unable to exclude ARVC with absolute certainty in many of these patients, as they were not available for re-evaluation due to loss to follow-up (n=11) or death (n=2). The changes in diagnosis reflect the advancements in TFC definitions with the inclusion of quantifiable variables for structural abnormalities and histological tissue characterisation, and the utility of modified definitions in the interpretation of the epsilon wave.

Although the impact of the epsilon wave on ARVC diagnosis in large registries has been shown to be low, it can have considerable influence as a major diagnostic criterion and has been identified as a caveat leading to over-diagnosis in patients that do not meet other criteria for ARVC.[189] Inter-observer variability in the interpretation of the epsilon wave has been shown to be high due to the qualitative nature of the original definition, leaving room for subjective interpretation.[189] By utilising the modified definition of an epsilon wave, which quantifies the end of the QRS complex as the latest end of the QRS complex seen in any of the leads V_1, V_2 or V_3,[189] we found that only 5.7% of cases had epsilon waves – a much lower frequency than previously reported in this cohort (56%)[96] and other registries (average 13%, range 1-25%) using conventional methods of interpretation.[96,189] The presence of an epsilon wave did

not influence the diagnosis of ARVC in the four individuals in which it was observed, as they all met sufficient criteria without it, demonstrating once again the association of the epsilon wave with advanced disease.[189] The incorrect interpretation of the epsilon wave has been identified as a major pitfall in over-diagnosing ARVC in our cohort in the past, and largely accounts for the exclusion of cases that may previously have been thought to have the condition.

CMR is regarded as the standard reference for the evaluation of RV function and morphology, and has become the imaging modality of choice in ARVC.[118] While quantification of RV volumes and function has improved the diagnostic sensitivity of this imaging modality, the correct interpretation of images remains subject to the experience and expertise of the reader.[118,190] Although magnetic resonance imaging (MRI) is available in most centres in South Africa, expertise in CMR interpretation is limited to a few select centres. Both the subjective nature of the 1994 TFC for structural abnormalities and the misinterpretation of CMR findings have previously led to over-diagnosis of ARVC in this cohort. Re-evaluation of CMR images by 2 trained readers, without prior knowledge of other clinical criteria, proved useful in both including and excluding referred patients in this study. More than half of the patients with a TFC diagnosis of ARVC did not undergo CMR as part of their work-up; notably, the majority of these patients were referred prior to 2009. This reflects the limited availability of CMR in South Africa, the use of the 1994 TFC in the diagnosis of ARVC prior to 2010 where CMR was not specifically indicated, contraindications to CMR in patients with older devices where MRI was not performed, and lost CMR images. Despite the exclusion of these 13 patients, an additional 33 cases have been recruited into the ARVC Registry of South Africa since 2009, bringing the total number of patients to 70.

Table 4.10. Index cases from the 2009 report that were excluded according to 2010 TFC

Index case	2009 report - 1994 TFC	Updated 2010 TFC classification	Diagnosis	Age	Symptoms at presentations	VT	CMR (original images analysed)	DNA	Desmosomal gene mutation	FU
ACM 49.1	**Definite** Major - Aneurysm - Depolarization/epsilon Minor - Late potentials	**Insufficient criteria** Minor - Late potentials	Undefined	19	Chest pain	None	Very mildly dilated LV and RV with normal function bilaterally. No RWMA. No aneurysm. No LGE	Yes	Negative	LTFU 2008
ACM 13.1	**Definite** Major - Severe dilatation RV - Depolarization/epsilon	**Possible** Major - RV aneurysm on RVA	Possible ARVC	16	Syncope	None	Not performed (ICD incompatible)	Yes	Negative	LTFU 2008
ACM 47.2	**Definite** Major - FFR on histology - Depolarization/epsilon	**No criteria** *FFR not quantifiable*	Probable DCM	37	Survived cardiac arrest (ICD inserted)	RBBB morphology	Suboptimal scan - dilated LV with reduced biventricular function, akinetic LV apex. LGE not done.	Yes	Negative	LTFU 2007
ACM 30.1	**Definite** Major - Depolarization/epsilon Minor - Mild RV dilatation - Late potentials	**Insufficient criteria** Minor - Late potentials	Probable DCM	49	Palpitations	RBBB morphology	Normal LV volume with impaired LV function. Normal RV volume and function. No LGE.	Yes	Negative	LTFU 2008
ACM 29.1	**Definite** Major - Aneurysm - Depolarization/epsilon Minor - Arrhythmia	**Possible** Minor - Repolarization - Arrhythmia	Idiopathic RVOT VT/early ARVC	44	Presyncope. palpitations	LBBB morphology with inferior axis	Suboptimal scan - normal chamber sizes and normal function. No aneurysm. LGE not performed.	Yes	Negative	LTFU 2010

Table 4.10. Index cases from the 2009 report that were excluded according to 2010 TFC (continued)

Index case	2009 report - 1994 TFC	Updated 2010 TFC classification	Diagnosis	Age	Symptoms at presentations	VT	CMR (original images analysed)	DNA	Desmosomal gene mutation	FU
ACM 55.1	**Definite** Major - FFR on histology - Depolarization/epsilon Minor - Late potentials	**Insufficient criteria** Minor - Late potentials *FFR insufficient, non-diagnostic*	Idiopathic PVC	55	Palpitations and dizziness	None	Not performed (not available at the time)	Yes	Negative	LTFU 2006
ACM 31.1	**Definite** Major - Depolarisation/epsilon - FFR on histology Minor - Mild dilatation of RV	**Insufficient criteria** Minor - Late potentials *FFR not significant, non-diagnostic*	Cardiomyopathy unspecified	15	Asymptomatic	None	Increased LV and RV volumes, normal biventricular function. No RV RWMA. No LGE	Yes	VUS (JUP)	LTFU 2016
PN 7	**Definite** Major - FFR on histology - Depolarization/epsilon Minor - Mild RV dilatation - LBBB VT	**Possible** Minor - ±Repolarization (transient TWI V1-2) - Arrhythmia *FFR not sufficient*	Probable idiopathic RVOT VT	38	Palpitation, chest pain, breathlessness	LBBB morphology with inferior axis	Not performed (not available at the time)	No	Unknown	LTFU 1997
PN 9	**Definite** Major - Aneurysm - FFR on histology	**No criteria** *No aneurysm* *FFR not quantifiable*	AF and HHD with heart failure	45	Palpitations and dizziness	None	Not performed (not available at the time)	No	Unknown	Died 1997

Table 4.10. Index cases from the 2009 report that were excluded according to 2010 TFC (continued)

Index case	2009 report - 1994 TFC	Updated 2010 TFC classification	Diagnosis	Age	Symptoms at presentations	VT	CMR	DNA	Desmosomal gene mutation	FU
PN 20	**Definite** Major - Severe RV dilatation - Depolarization/epsilon Minor - Repolarization - Late potentials - Arrhythmia	**Definite** Major - Arrhythmia Minor - Repolarization - Late potentials	Sarcoidosis (tissue diagnosis)	16	Presyncope, palpitations, breathlessness	LBBB morphology, superior and inferior axis	Not performed (not available at the time)	No	Unknown	Died 2000
PN 46	**Definite** Major - Severe RV dilatation - Depolarization/epsilon	**No criteria**	Familial heart block	43	Syncope while running	Polymorphic VT at EPS	Not performed (pacemaker incompatible)	No	Unknown	LTFU 2007
PN 83	**Definite** Major - Aneurysm - Depolarization/epsilon Minor - Late potentials - Arrhythmia	**Possible** Minor - Late potentials - Arrhythmia	Possible ARVC/post-viral myocarditis DCM	46	Palpitations, decline in effort tolerance (marathon runner)	LBBB morphology with inferior axis (EST) EPS inconclusive	Normal LV volume, mildly impaired LVEF, dilated LA, dilated RV, moderately impaired RVEF. No RV RWMA. Circumferential linear mid-wall LGE	No	Unknown	LTFU 2008
PN 117	**Definite** Major - Aneurysm - FFR on histology Minor - PVC	**No criteria** Minor - PVC > 500/24h *FFR insufficient*	Cardiomyopathy unspecified	56	Palpitations	None EPS negative	Normal LV and RV volumes, reduced systolic function bilaterally. No RWMA. LGE not performed	No	Unknown	LTFU 2009

AF, atrial fibrillation; ARVC, arrhythmogenic right ventricular cardiomyopathy; CMR, cardiovascular magnetic resonance; DCM, dilated cardiomyopathy; DNA, deoxyribonucleic acid; EPS, electrophysiology study; EST, exercise stress test; FFR, Fibrofatty replacement (on histology); FU, follow-up, HHD, hypertensive heart disease; ICD, implantable cardioverter defibrillator; LBBB, left bundle branch block; LGE, late gadolinium enhancement; LTFU, lost to follow-up; LV, left ventricle; PVC, premature ventricular contractions; RBBB, right bundle branch block; RV, right ventricle; RVA, right ventriculogram; RVEF, right ventricular ejection fraction; RVOT, right ventricular outflow tract; RWMA, regional wall motion abnormality; TFC, task force criteria; VT, ventricular tachycardia

Despite expanding the genetic screening profile to include 5 desmosomal genes (*PKP2, DSG2, DSC2, DSP, JUP*) and selected non-desmosomal genes (*CDH2 and PLN*), the proportion of genotype positive individuals within this South African cohort is still relatively low (24.1%). Since the 2009 report, we have identified 3 additional unrelated individuals with *PKP2* c.C1162T founder mutations. Cascade genetic screening and clinical correlation of available family members has allowed us to make some key observations regarding variable phenotype expression in patients carrying this mutation. As would be expected with a mutation conserved over many generations, the clinical phenotype associated with the *PKP2* c.C1162T founder mutation is relatively mild – most carriers express minimal clinical signs of disease. Environmental factors, particularly endurance sport, appear to be correlated with the development of symptomatic disease in heterozygous individuals. Severe phenotypes have been associated with compound heterozygosity[96] and homozygosity (Machipisa, T. 2016. M.Phil. Thesis, UCT; Kamuli, S. 2016. M.Phil. Thesis, UCT) in 2 of the founder families, however, the *PKP2* c.C1162T founder mutation alone does not explain the severe phenotype in 3 affected siblings in the ACM 38 family, and further molecular genetic analysis is indicated in this family. Further work, including functional studies and/or family screening to demonstrate segregation with disease, will be required to determine the clinical relevance of the *variants of unknown significance* that have been identified in our cohort.

Routine diagnostic genetic testing is not readily available in South Africa. The relatively low genetic diagnostic yield (24.1%) demonstrated in this cohort indicates that further research is required to better elucidate the genetic causes of ARVC in our population. Although genotyping has not been shown to contribute significantly to confirming the diagnosis of ARVC in index patients in our cohort, identifying a disease-causing mutation has provided an effective screening tool in relatives. Importantly, genetic testing should only be undertaken in index patients where there is sufficient clinical evidence to support the diagnosis of ARVC, or in family members where a genetic diagnosis has been confirmed in the proband.

4.5. LIMITATIONS

Due to the observational nature of this study, the amount of clinical data available for each participant varied considerably, depending on the resource capacity at referral centres and the time period in which the patient was recruited. Participants were therefore not all investigated to the same degree, and thus the prevalence of certain characteristics cannot be determined accurately. Standardised approaches to investigations applied by sufficiently trained individuals appointed to the diagnostic panel were instituted to reduce inter-observer variability in the interpretation of investigative data. Due to social inequalities in access to healthcare that are still present in South Africa, referral bias is likely responsible for the underrepresentation of black African individuals in this cohort. Loss of follow-up, particularly in patients without ICDs may reflect undocumented deaths, therefore, the outcomes data in patients with and without ICDs should be considered with caution. Despite these limitations and the relatively small size of the cohort when compared to international series, this study does contribute to our understanding of ARVC in the South Africa context.

4.6. CONCLUSION

With the exception of the lower mutation rate (24.1%), the clinical presentation and diagnostic criteria correlate with the findings in North American and European studies. While outcomes were poorer than reported in other cohorts, survival was not significantly altered by device therapy or mutation status. The diagnosis of ARVC remains a major challenge, particularly in countries with limited resources and expertise. Reclassification according to updated criteria by a panel of experts in all patients referred, improved the accuracy of clinical diagnosis in those with ARVC, as well as those excluded with other cardiac conditions. The requirement for specialised investigations and multidisciplinary expertise highlights the need for a unified registry, not just in South Africa, but throughout the African continent. The incorporation of the ARVC Registry of South Africa into the newly established African Cardiomyopathy and

Myocarditis Registry Program (IMHOTEP) is an attempt to extend established expertise in the field to other countries lacking in the capacity to support a registry of this nature, and to provide the opportunity for continued research across the continent, whereby the genetic causes of ARVC in African patients can be determined.

4.7. CONTRIBUTIONS AND ACKNOWLEDGEMENTS

The concept and design of this study was adapted to IMHOTEP by S. Kraus from the original ARVC Registry of SA initiated by B. Mayosi. Participant recruitment (retrospective and prospective) was done by S. Kraus. All clinical data was reviewed by S. Kraus and presented to the Diagnostic Panel lead by electrophysiologist, A. Chin. All CMR scans were analaysed by S. Kraus and N. Ntusi. DNA analysis was not done as part of this work, but previously identified variants (referenced) were reclassified by S. Kraus (in consultation with genetics counsellor, N. Laing) according to updated information using available tools. The phenotypic descriptions of the founder families were done by S. Kraus. New patients were recruited by S. Kraus. Data was collected by S. Kraus (assisted by K. Luhkna) and analysed by S. Kraus (in consultation with statistician, K. Manning and W. Basera).

CHAPTER 5: The baseline characteristics and vital status of prevalent cases of dilated cardiomyopathy from the Cape Town cohort

5.1. INTRODUCTION

Dilated cardiomyopathy, associated with LV chamber dilatation and dysfunction, is the dominant form of cardiomyopathy causing heart failure in SSA.[2] It has been shown, that despite guideline-mandated medical therapy, both familial and idiopathic DCM have been associated with a high mortality in the South African population.[4] Most of the information available on the presentation and natural history of cardiomyopathies in Africa has been derived from single-centre cohort studies,[4,37,191,192] and there is limited data describing contemporary management of DCM within a LMIC setting over an extended period of time. In 2011, Ntusi and colleagues reported the clinical characteristics and outcomes of a cohort of 120 patients with idiopathic and familial DCM from Cape Town.[4] Several important observations emerged from that study related to mortality. The presence of symptoms of heart failure was the most important clinical predictor of mortality. Furthermore, digoxin appeared to be a significant predictor of mortality in idiopathic DCM. While this study had important findings for clinical practice and is the largest study to date on DCM in the African population, generalisation of the findings is limited by the relatively small sample size and the retrospective design.[4]

While IMHOTEP is a prospective registry, the inclusion of prevalent cases affords us the opportunity of incorporating retrospective cohorts that have been compiled over decades at our institution to maximise our sample size, improve the statistical power and draw more meaningful conclusions about long-term management and clinical outcomes within our DCM population. In addition, the Cape Town cohort is unique in that DNA was collected and stored

for patients presenting with DCM as part of a previous study, providing an opportunity to examine the genetic contribution to this condition in our population.

The principal aims of the study were to (1) recruit existing cases of DCM where DNA had been collected and sufficient clinical information was available to confirm a diagnosis of DCM, (2) describe the baseline characteristics of prevalent DCM patients and (3) review the long-term overall survival of patients with DCM in the Cape Town cohort.

5.2. METHODS

5.2.1. Study design and population

The prevalent cases arm of the IMHOTEP study involves the recruitment of existing unrelated individuals with a diagnosis of cardiomyopathy at recruiting centres. *A Clinical and Genetic Study of Familial Dilated Cardiomyopathy in South Africa (HREC 197/96)* was an existing study initiated in Cape Town in 1996, in which DNA specimens were collected from patients and relatives with dilated cardiomyopathy, and stored at the Cardiovascular Molecular Genetics Laboratory at the Hatter Institute, University of Cape Town. With the inclusion of prevalent cases into IMHOTEP, patients from this cohort were reviewed and included if they had a valid consent for collection of medical information and molecular genetic analysis, and sufficient clinical information was available to confirm a diagnosis of cardiomyopathy (Appendix H). In addition, existing patients with DCM being followed-up in the Cardiac Clinic at Groote Schuur Hospital, that had not previously been included into the above-mentioned study, were consented and recruited as prevalent cases.

5.2.2. Inclusion and exclusion criteria

Inclusion criteria

I. Informed consent for participation in genetics research.

II. Adults and adolescent minors.

III. A confirmed diagnosis of dilated cardiomyopathy (or postpartum cardiomyopathy) as describe in Table 3.1. (Chapter 3).

Exclusion criteria

I. Consent form not signed/invalid.

II. Insufficient clinical information available.

III. IMHOTEP exclusion criteria as listed in Table 3.2 (Chapter 3).

5.2.3. Data collection and informed consent

Due to the retrospective nature of the study, data was collected by reviewing hospital records, research folders and datasets compiled for by previous investigators (HREC 197/96), and entered into the IMHOTEP *OpenClinica* registry database. As one of the principal aims of IMHOTEP is to conduct molecular genetic analysis on patients recruited, patients were included if they had a valid consent form for DNA collection, storage and analysis, except in specific circumstances where waver of consent was approved by HREC. The original consent forms included permission from participants to collect clinical information from medical records.

5.2.4. Statistical analysis

Descriptive statistics were used to describe the study population. Continuous variables were tested for distribution using the Shapiro Wilks test for normal data and using a histogram for visualisation. Normally distributed data was summarised as mean and standard deviation. Non-normally distributed data was reported as median and interquartile range. Wilcoxon sum

rank and Kruskal-Wallis were used to determine differences between non-normally distributed data. Categorical variables were summarised in tables and reported as number and proportion, and Chi-squared tests of equal proportions were used to determine differences in categorical data. Statistical analysis was performed using SPSS statistical software (SPSS Statistics 25.0). Median survival from presentation to the time of death was determined by the Kaplan–Meier method, and differences in survival between groups were evaluated using the log-rank test, using STATA statistical software (Version 15.1. StataCorp. USA).

5.3. RESULTS

5.3.1. Enrolment and diagnosis

A total of 133 patients with DCM were recruited to IMHOTEP from existing studies conducted at UCT and the Cardiac Clinic at Groote Schuur Hospital over a period of 19 years (1 February 1996 - 1 February 2015) (Figure 5.1). Due to the nature of retrospective recruitment, the timing of initial presentation and diagnosis of DCM varied across the cohort, with onset of disease dating as far back as 1981.

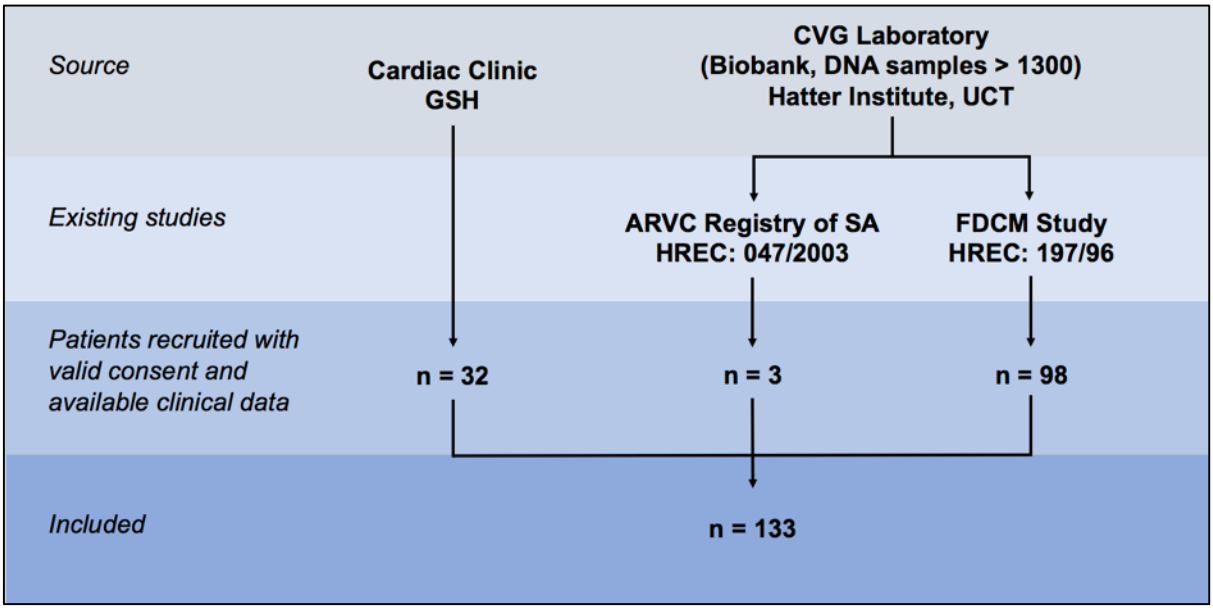

Figure 5.1. Recruitment of existing prevalent DCM cases to IMHOTEP

Idiopathic DCM (33.1%) was the most frequent diagnosis, followed by PPCM (29.3%) and suspected familial DCM (19.5%) (Figure 5.2.). DCM-LVNC overlapping phenotype was noted on CMR in 7.5% of cases. This is, however, not a true reflection of LVNC prevalence in this population as CMR was only performed in 12.8% of cases recruited. Although 16.5% of recruits had co-existing HIV, only 6% were considered to have an HIV-associated cardiomyopathy (HIVAC). Other secondary causes included tachycardia-related cardiomyopathy (n=3), anthracycline-related cardiomyopathy (n=2) and alcohol-related cardiomyopathy (n=1).

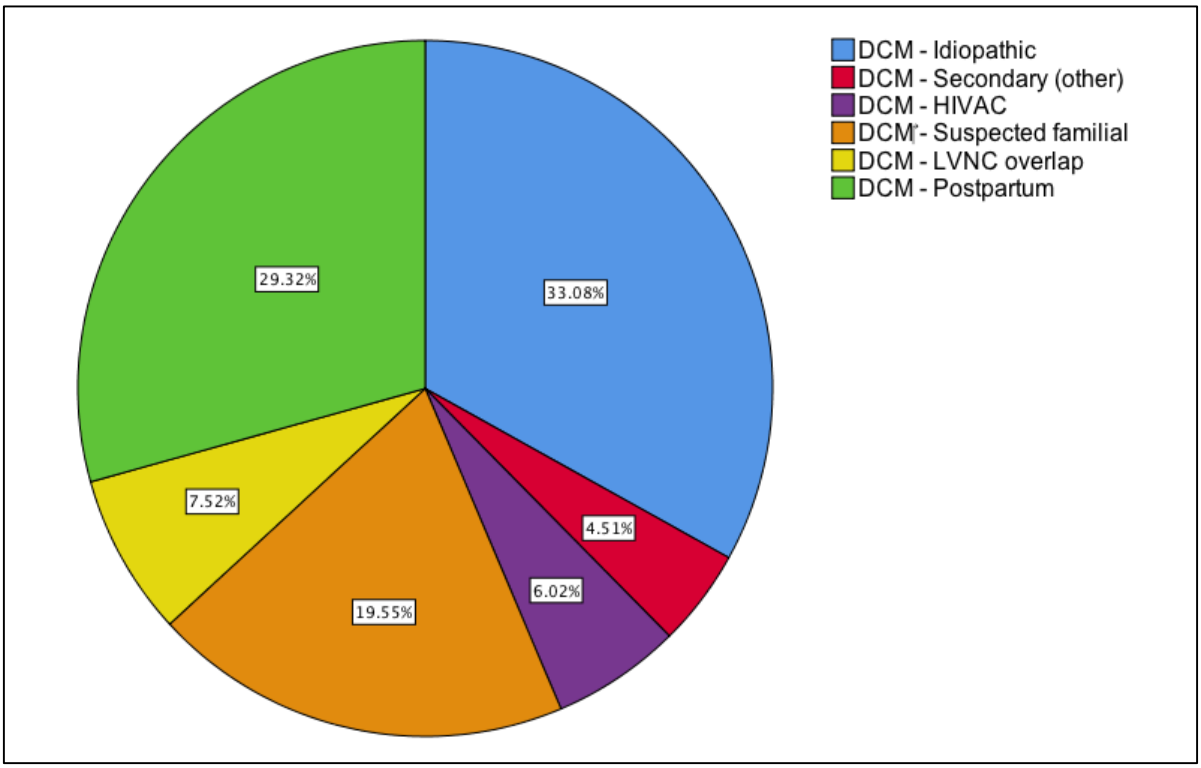

Figure 5.2. Aetiological diagnosis in patients with DCM
DCM – Secondary (other) included tachycardia-related cardiomyopathy, anthracycline-related cardiomyopathy and alcohol-related cardiomyopathy.

DCM, dilated cardiomyopathy; HIVAC, HIV associated cardiomyopathy; LVNC, left ventricular non-compaction

5.3.2. Demographics and baseline clinical characteristics

Table 5.1. represents the baseline characteristics of 133 prevalent DCM cases recruited to IMHOTEP, in comparison to DCM patients recruited to the European Cardiomyopathy Pilot Registry.[193] The vast majority of patients recruited were black African (50.4%) and mixed race (36.1%), with slight female predominance (54.9%). Fifty-nine percent of women presented in the peripartum period. The mean age of presentation was 34.8 ± 11.0 years. Ninety-seven percent of patients were symptomatic at presentation, with 91.0% reporting impaired effort tolerance (NYHA Class II, 44.4%; NYHA Class III, 32.3%; NYHA Class IV, 14.3%). In addition to reduced effort tolerance, the most common symptoms noted at presentation were body swelling/oedema (49.6%), orthopnoea (42.9%), chest discomfort (24.8%) and paroxysmal nocturnal dyspnoea (21.1%). Forty-two percent of patients had clinical signs of congestive heart failure at the time of first enrolment as a research participant. Syncope was infrequently reported (1.5%). Co-morbid chronic illnesses were noted in a third of patients, with HIV (16.5%) being the most frequently encountered co-existing medical condition. Mild hypertension was reported as a co-morbidity in 6.8% of cases but was not considered to be a significant contributor in these cases. A history of atrial fibrillation and embolic stroke/TIA were reported in 9.0% and 4.6% of cases, respectively. Ventricular tachycardia and previous cardiac arrest were reported in 3.0% and 0.8% of cases, respectively.

Table 5.1. Clinical characteristics of prevalent DCM cases recruited to IMHOTEP in comparison with the European Cardiomyopathy Pilot Registry

	IMHOTEP (DCM, n=133)	EURO[193] (DCM, n=1260)
Female, n(%)	73 (54.9)	(25.8)
Ethnicity, n(%)		
Black	67 (50.4)	Not reported
Caucasian	17 (12.8)	
Mixed race (coloured)	48 (36.1)	
Indian	1 (0.8)	
Age at presentation, years		
Mean age ±SD	34.8 ±11.0	-
Median age (Q1-Q3)	34.0 (28.0-40.5)	49.0 (40.0-58.0)
Type of presentation, n(%)		
Cardiac symptoms	129 (97.0)	(81.0)
SCD/cardiac arrest	0 (0.0)	(1.7)
Embolic event	2 (1.5)	-
Incidental finding (asymptomatic)	2 (1.5)	(9.7)
NYHA Class, n(%)		
NYHA Class I	12 (9.0)	(18.9)
NYHA Class II	59 (44.4)	(42.7)
NYHA Class III	43 (32.3)	(30.1)
NYHA Class IV	19 (14.3)	(8.3)
Cardiac symptoms at presentation, n(%)	129 (97.0)	(89.7)
Syncope	2 (1.5)	(8.2)
Presyncope/dizziness	15 (11.3)	-
Chest pain	33 (24.8)	(20.8)
Orthopnoea	57 (42.9)	-
Paroxysmal nocturnal dyspnoea	28 (21.1)	-
Body swelling/oedema	66 (49.6)	-
Palpitations	24 (18.0)	(36.0)
Onset of symptoms peripartum (females), n(%)	43/73 (58.9)	-
Chemotherapy exposure	3 (2.3)	-
Reported lifestyle-social exposures, n(%)		Not reported
Alcohol (>30 drinks/month or binge use)	15 (11.3)	
Illicit drugs: methamphetamines/cocaine	3 (2.3)	
Family history, n(%)		
Heart failure	46 (34.6)	
Cardiomyopathy	19 (14.3)	(25.2)
Sudden cardiac death	19 (14.3)	(11.9)
Co-morbidities present, n(%)	47 (35.3)	Not reported
HIV positive	22 (16.5)	
Hypertension (not considered causal)	9 (6.8)	
Diabetes	3 (2.3)	
Chronic pulmonary disease	5 (3.8)	
Thyroid disease	3 (2.3)	
Cancer previously	4 (2.3)	
History of arrhythmia or stroke reported		
Atrial fibrillation	12 (9.0)	(28.3)
SVT (other than AF)	6 (4.5)	-
Resuscitated cardiac arrest	1 (0.8)	(4.8)
Ventricular tachycardia	4 (3.0)	(13.6)
Stroke	3 (2.3)	(4.5)
Transient ischaemic attack	3 (2.3)	-
Examination at time of enrolment		Not reported
Heart rate (beats/minute), mean ±SD	93 ±20.6	
Systolic blood pressure (mmHg), mean ±SD	112 ±16.5	
Diastolic blood pressure (mmHg), mean ±SD	72 ±12.0	
Clinical signs of congestive heart failure, n(%)	56 (42.1)	

5.3.3. Baseline investigations

The investigative approach varied according to existing guidelines and available investigations at the time of presentation. While the vast majority of cases had diagnostic confirmation on echocardiography, a few of patients were recruited based on histopathology (post-transplant) and/or angiographic findings. Table 5.2. outlines the clinical investigations performed. Electrocardiograms were done in 97.7% of cases (Table 5.3.); 91.5% of patients were in sinus rhythm. Various conduction abnormalities were noted; the most common of which were left bundle branch block (14.6%) and first-degree heart block (13.8%). T-wave inversion was noted in 69.2% of patients. Echocardiograms were available for 98.5% of cases (Table 5.4.). Left ventricular dimensions were increased in the vast majority (90.8%) of patients (mean LV end-diastolic dimension 64 ± 9 mm), with the exception of 12 (9.2%) cases with reduced ejection fraction only (PPCM, n=3; familial DCM, n=2; DCM/LVNC overlap, n=1; HIVAC, n=2; anthracycline-related cardiomyopathy, n=2, idiopathic DCM, n=2). The mean LVEF and fractional shortening were 28 ± 10.9% and 13.4 ± 5.9% respectively. Moderate or severe mitral regurgitation was reported in 45.0% of cases and the mean RV systolic pressure was elevated (40.0 ± 13.0 mmHg).

Table 5.2. Investigations performed in prevalent cases in IMHOTEP compared to European Cardiomyopathy Pilot Registry

Investigation (available for review)	IMHOTEP (n=133) n (%)	EURO[193] (DCM, n = 1260) (%)
ECG	130 (97.7)	(98.5)
Echocardiogram	131 (98.5)	(96.9)
CXR	46 (34.6)	-
CMR	18 (13.5)	(20.6)
Angiogram	43 (32.2)	-
EMB	9 (6.8)	(21.0)
Blood samples for DNA taken	128 (96.2)	(17.9)*

*genetic testing performed

Table 5.3. Electrocardiogram

	Patients with DCM (n = 130)
Rate (beats per minute), *mean±SD*	92 ±22.5
Rhythm	
Sinus rhythm (normal PR interval), *n(%)*	101 (77.7)
Sinus rhythm with 1st degree heart block, *n(%)*	18 (13.8)
Atrial fibrillation, *n(%)*	10 (7.7)
Second degree (Mobitz II) heart block, *n(%)*	1 (0.8)
PR interval (ms), *mean±SD*	165 ±29.6
QRS duration (ms), *mean±SD*	106 ±31.1
QRS morphology	
Normal, *n(%)*	90 (69.2)
Incomplete left bundle-branch-block, *n(%)*	4 (3.1)
Left anterior bi-fascicular block, *n(%)*	1 (0.8)
Left posterior bi-fascicular block, *n(%)*	2 (1.5)
Left bundle-branch-block, *n(%)*	19 (14.6)
Right bundle-branch-block, *n(%)*	5 (3.8)
Non-specific conduction abnormality, *n(%)*	9 (6.9)
T-wave inversion, *n(%)*	90 (69.2)
QTc (ms), *mean±SD*	451 ±41.8
Chamber hypertrophy	
Left atrial hypertrophy, *n(%)*	36 (27.7)
Left ventricular hypertrophy, *n(%)*	48 (36.9)

Table 5.4. Echocardiogram

	Patients with DCM (n = 131)
Left heart study	
Left ventricular dimensions	
LV non-dilated, *n(%)*	12 (9.2)
LV dilated, *n(%)*	119 (90.8)
LVEDD (mm), *mean±SD* (n=127)	64 ±9.0
LV fractional shortening (%), *mean±SD* (n=119)	13.4 ±5.9
LV ejection fraction (%), *mean±SD* (n=127)	28.0 ±10.9
Septal thickness (mm), *mean±SD* (n=115)	9 ±2.2
LV posterior wall thickness (mm), *mean±SD* (n=117)	10 ±2.5
LA dilatation reported, *n(%)*	92 (70.2)
LA dimension (mm), *mean±SD* (n=124)	44 ±7.7
Moderate to severe mitral regurgitation, *n(%)*	59 (45.0)
Right heart study	
Right ventricular dimensions (not routinely measured)	
RV dilatation reported, *n(%)*	37 (28.2)
RV not assessed, *n(%)*	66 (50.4)
RA dilatation reported (not routinely measured)	24 (18.3)
RA not assessed, *n(%)*	78 (59.5)
Moderate to severe tricuspid regurgitation, *n (%)*	35 (26.7)
RV systolic pressure (mmHg), *mean±SD* (n=74)	40.0 ±13.0
Intra-cardiac thrombus reported, *n(%)*	6 (4.6)
Pericardial effusion (≤15mm), *n (%)*	28 (21.4)

LA, left atrium; LV, left ventricle; LVEDD, left ventricular end-diastolic dimension; RA, right atrium; RV, right ventricle

CMR was performed on 18 patients (Table 5.5.). It should be noted that CMR was only performed in selected patients; those with poor recovery of LV function on treatment, PPCM in a subsequent pregnancy after initial recovery, familial DCM, chronic inflammatory conditions (e.g. RA, HIV), tachymyopathies or where there were diagnostic queries. Our findings, therefore, likely reflect the more severe spectrum of disease which may account for the prominence of LGE. Although the numbers were small, CMR proved useful in refining the clinical phenotype, particularly in defining overlapping morphologies (DCM-LVNC overlap, n=8; DCM-HCM overlap, n=1). Subtle differences were noted when comparing those patients with a 'classical DCM phenotype' to those with 'DCM-LVNC overlap'; however statistically significant differences were only noted in LVEF (19.0% versus 27.4%, p 0.04) and minimum wall thickness (6.3±1.1mm versus 4.9±1.3mm, p=0.03) between these two groups. One individual (III.2 - Figure 5.3.) presented with a DCM-HCM overlapping phenotype. It was noted that he had a severely affected sibling (III:5 - Figure 5.3.) with a predominantly dilated phenotype who had presented in childhood and died in adolescence. Although pedigree information was limited, matrilineal inheritance was considered in this family. A skeletal muscle biopsy showed non-specific mitochondrial abnormalities, but the findings were not sufficiently conclusive of a mitochondrial cytopathy.

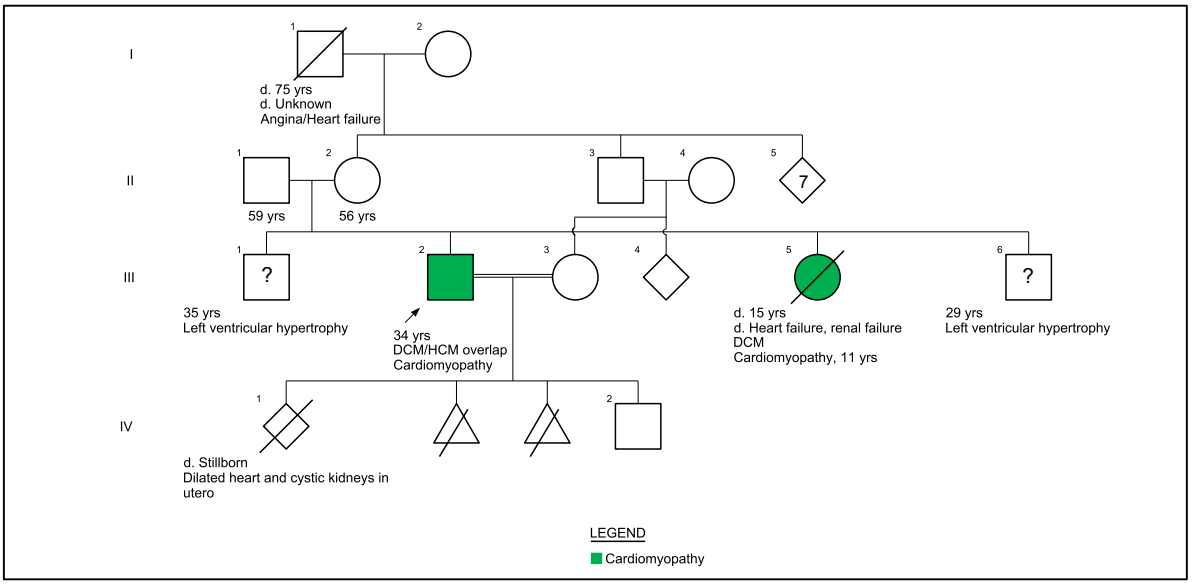

Figure 5.3. Family pedigree for individual with HCM/DCM phenotype (Family 23)

Table 5.5. Cardiovascular magnetic resonance characteristics in 18 patients

	ALL PATIENTS n=18	Specific phenotypes		
		DCM n=9	DCM-LVNC n=8	DCM-HCM n=1
Left heart study				
LVEF, mean±SD	23.8±11.2	18.0±7.4	27.4±9.9	48.0
LVEDV (ml), *mean±SD*	330±105	365±102	298±108	270
LVESV (ml), mean±SD	261±103	306±90	225±101	141
LV mass (g), *mean±SD*	182±57	213±51	145±40	251
LV mass/BSA (g/m^2), *mean±SD*	86±22	97±14	74±23	98
LV minimum wall thickness (mm), *mean±SD*	6.0 ±2.3	6.3±1.1	4.9±1.3	13.0
LV maximum wall thickness (mm), *mean±SD*	8.0 ±2.5	8.1±1.5	7.0±1.8	15.0
LV trabeculation (NC/C ratio), *mean±SD*	-	-	3.6±1.0	-
LA dimension (mm), *mean±SD*	40.1±8.5	40.0±5.2	38.2±10.1	52.0
LA area (cm^2), *mean±SD*	30.3±9.6	30.1±9.5	29.5±10.6	38.0
Right heart study				
RVEF (%), *mean±SD*	36.7±14.2	34.3±20.8	38.3±4.5	43.0
RVEDV (ml), *mean±SD*	176±64	193±61	143±49	284
RVESV (ml), *mean±SD*	115±63.8	134.7±82.3	88±32	163
RA area (cm^2), *mean±SD*	21.8±7.5	26.0±8.9	19.3±6.0	18.0
Pericardial effusion <20mm, *n (%)*	8 (44.4%)	3 (33.3)	5 (62.5)	0 (0.0)
Presence of late gadolinium enhancement, *n (%)*	15/15 (100)	8/8 (100)	6/6 (100)	1/1 (100)
Pattern of LGE, *n (%)*				
Sub-epicardial	-	-	-	-
Mid-wall linear	14/15 (93.3)	8/8 (100)	6/6	-
Mid-wall patchy/diffuse	1/15 (6.7)	-	-	1/1 (100)
Sub-endocardial*	2/15 (13.3)	1/8 (12.5)	1/6 (16.7)	-
Transmural*	1/15 (6.7)	-	1/6 (16.7)	-

*in keeping with an embolic infarct

All scans were performed on the 1.5T Siemens MRI scanner with the exception of one case (done on 3.0 Tesla Siemens MRI Scanner). In all cases, T2-weighted imaging was normal (no myocardial oedema), and T1 mapping was not performed as not available. LGE was not available for 3 patients (inadequate for analysis, n=2; contraindicated, n=1).

Post-hoc tests were not performed as 1 group had fewer than 2 cases.

5.3.4. Drug and interventional therapy

Diuretics were prescribed in 89.8% of participants, with beta-blockers, angiotensin-converting enzyme (ACE) inhibitors (or angiotensin-II receptor blocker - ARB) and spironolactone prescribed in 58.3%, 87.4% and 48.8% of participants. Forty-four percent of patients were prescribed digoxin. Thirteen percent of patients were on anticoagulant therapy. Thirteen percent of patients had devices inserted; cardiac resynchronisation therapy (CRT)

pacemakers (8.3%) for patients with LBBB and poor ventricular function, and ICDs (4.5%) for secondary prevention of SCD. It should be noted that ICDs are not routinely available for primary prevention of SCD in the state health sector in South Africa (Table 5.6.).

Table 5.6. Medical and interventional therapy

	IMHOTEP (n=133) n (%)	EURO[193] (DCM, n=1260) (%)
Medications (reported n=127)		
Diuretics	114/127 (89.8)	71.8
Beta-blockers	74/127 (58.3)	89.7
ACE inhibitors/ARB	111/127 (87.4)	72.8/16.7
MRA	62/127 (48.8)	63.1
Digoxin	56/127 (44.1)	
Amiodarone	4/127 (3.2)	
Ivabradine	2/127 (1.6)	
Anti-platelets	9/127 (7.1)	23.7
Warfarin	17/127 (13.4)	27.7
Devices		
CRT	11 (8.3)	-
ICD	6 (4.5)	31.7
Transplantation	8 (6.1)	-

5.3.5. Survival

Follow-up data was available on 93 (70%) patients; 64 patients were alive at follow-up, 21 patients died, and 8 patients underwent cardiac transplant (7/8 alive; 1/8 died following transplant). Forty (30%) patients were lost to follow-up. The overall transplant-free survival probability at 1-year, 5-years and 10-years was 93.4% (95% confidence interval [CI] 87.3 – 96.7), 82.2% (95% CI 73.3 – 88.3) and 73.1% (95% CI 62.2 – 81.4) respectively. Median survival time was 5.3 years (IQR 2.3 – 8.4) and mean age of death was 41.5 ±9.6 years. Patients with PPCM had better outcomes compared to other forms of DCM (death/transplantation; 26 versus 3, p=0.02) and although not statistically significant, patients on digoxin had higher rates of adverse outcomes (death/transplantation) than those not on digoxin (29 versus 10, p=0.06) (Figure 5.4.).

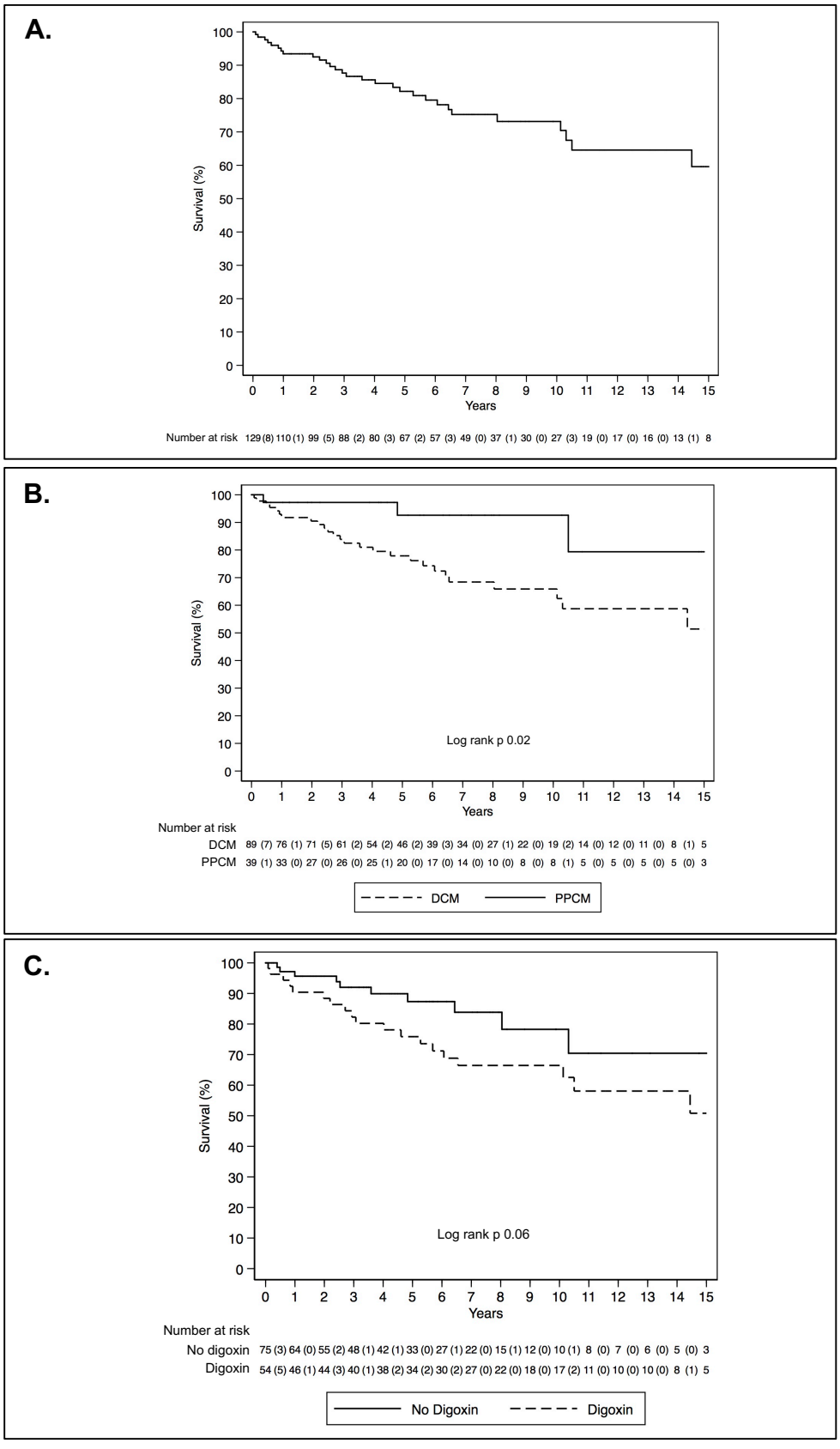

Figure 5.4. Kaplan-Meier survival analysis. A. Transplant-free survival overall. B. Transplant free survival in PPCM compared to DCM (excluding PPCM). C. Transplant-free survival in patients on digoxin compared to those not on digoxin.

5.4. DISCUSSION

In this study, we describe the baseline characteristics of patients with DCM seen at tertiary institution in South Africa over a period of 2 decades. While the numbers do not reflect the full spectrum of patients seen at our institution, they represent a useful resource for genetics research in patients with diverse ethnic backgrounds (black African 50.4%, mixed race 36.1%, Caucasian 12.8% and Indian 0.8%). Due to the retrospective nature of the study, selection of cases was based on the quality of clinical data available to confirm a diagnosis of DCM in patients where DNA has previously been collected and stored at our institution.

Several important observations have emerged; the age of presentation is much younger than has been observed in European cohorts[193] (median IQR of 28.0-40.5 years versus 40.0-58.0 years). With the exception of secondary causes of DCM including tachycardia-related cardiomyopathy, anthracycline-related cardiomyopathy and alcohol-related cardiomyopathy, where the mean age of presentation was 47 ±11.4 years, all other forms of DCM had a much younger age of onset (idiopathic, 37.0 ±12.6 years; familial DCM, 35.8 ±11.2 years; PPCM, 30.9 ±7.5 years; HIVAC, 35.0 ±8.0 years; DCM/LVNC overlap, 30.4 ±9.8 years). With a younger age of onset and a median survival time of 5.3 years, we also observe a younger age of death (41.5 ±9.6 years). These findings have significant social and economic implications for families and communities. Furthermore, in contrast to European cohorts where DCM is predominantly seen in men (74.2%), we have a much more equally weighted cohort with a slight female predominance, which raises concern about the potential impact these conditions have on both maternal health care and child welfare. Majority of patients presented with reduced effort tolerance (NYHA Class II-IV, 91.0%), signs and symptoms of congestive heart failure, dilated LV (90.8%; mean LVEDD 64 ± 9.0mm) and severely impaired systolic function (mean LVEF 28.0 ± 10.9%). As would be expected in a younger cohort of patients, co-morbidities such as diabetes, hypertension and chronic lung disease were infrequent. At

baseline, atrial and ventricular arrhythmias were reported less frequently than in European cohorts.

In comparison to a previous study on idiopathic and familial DCM at our institution where a mortality was reported as 40% at a median follow-up of 5 years,[4] transplant-free survival at 5 years appeared to be better in our cohort (82.2%). This may, in part, reflect the variation in prognosis associated with different aetiologies, and the inclusion of secondary causes of DCM in our cohort, particularly patients with PPCM (29.3%). It is well recognised that PPCM patients have greater rates of recovery and better survival than patients with other forms of DCM,[15] and our findings further support this observation (Figure 5.3. B.). Digoxin has been associated with worse outcomes in HF[194] and has been reported as a significant predictor of mortality in patients with idiopathic DCM.[4] Although not statistically significant, digoxin was associated with reduced survival in our cohort, however, due to the nature of the retrospective observational design of this study, it is not possible to make definitive conclusions related to this observation. On review of the clinical characteristics of those on digoxin compared to those not on digoxin at baseline, we found that there were no significant differences in age, sex, ethnicity, NYHA Class III-IV symptoms, heart rate, blood pressure, QTc, LV dimensions, LV systolic function, or RV pressures. There were, however, significant differences in prescribed medications, with higher numbers of patients on ACE-inhibitors/ARB, MRA and diuretic therapy in the group on digoxin at baseline. The observation likely reflects guideline-based HF management where digoxin is indicated in symptomatic patients already on maximal tolerated doses of ACE-inhibitors, beta-blockers and MRA therapy.[174] While this may suggest more advanced disease, we have not been able to demonstrate any significant differences in any other baseline parameters to support the presence of more severe disease in those on digoxin. Unfortunately, we do not have sufficient data on the clinical management of patients over time to appreciate the relevance of this observation. Our study does, however, show a decline in the percentage of patients on digoxin at our institution, compared to what was reported previously by Ntusi *et al*. The lower rates of beta-blocker therapy overall may be

related to the presence of congestive heart failure, however, when analysed there were no statistically significant differences in NYHA class, diuretic use or the presence of clinical signs of CCF between those on beta-blockers and those not on beta-blockers at baseline. It should be noted that 59% of patients presented prior to the inclusion of MRA therapy into the HF guidelines (Class Ia) in 2012.[195] These observations, while interesting, merely highlight the limitations of retrospective observational studies in making clinically useful inferences.

5.5. LIMITATIONS

This study has all the limitations of a retrospective observational design, including incomplete data, non-uniform assessments, variable degrees of investigation and inclusion bias. The younger age of onset and more severe disease noted in this cohort may be a reflection of selection bias, as patients were enrolled from a previous study that specifically recruited patients with idiopathic and familial DCM at a tertiary centre. Thirty percent of patients were lost to follow-up, and may reflect undocumented deaths. Despite these limitations, inclusion of these patients affords us the opportunity to review outcomes in the context of standard of care within our institution over a prolonged time period and provides important insights into the impact these conditions have on health care and social stability within communities.

5.6. CONCLUSION

While there are a number of clinical questions that remain unanswered, the relative improvement in survival compared to a previous study conducted in our institution is encouraging and may simply reflect the advancements in heart failure over the last 20 years. The age of onset of DCM and age of death are significantly younger in our population compared to European cohorts, with potential health care, social and economic implications. In addition to looking at outcomes, the primary aim of recruiting prevalent cases to IMHOTEP was to increase the number of cases of dilated cardiomyopathy on which to conduct genetic

research. The work done in delineating the underlying cause of dilated cardiomyopathy in our population and refining the phenotypic variation in expression of disease will hopefully prove useful in interpretation of genetic variants that may be identified in this cohort in the future.

5.7. CANDIDATE CONTRIBUTION AND ACKNOWLEDGEMENTS

All patients were retrospectively recruited by S. Kraus. Over 450 unrelated cases of DCM were screened according to the SOP in Appendix H. All clinical data was reviewed by S. Kraus. Data collection was done by S. Kraus with assistance from medical students, T. Suttle and E. Chetwin. Data analysis was done by S. Kraus in consultation with statistician, K. Manning. Vital status follow-up was done by U. September (research nurse) and S. Kraus.

CHAPTER 6: Clinical and cardiovascular magnetic resonance (CMR) characteristics of incident cardiomyopathy patients from Cape Town: application of the 3-stage diagnostic approach

6.1. INTRODUCTION

International registries have played a fundamental role in refining diagnostic criteria and improving clinical care and long-term survival in cardiomyopathy patients, particularly HCM and ARVC.[61,93] While there are a number of single centre studies on the various forms of cardiomyopathy from Africa,[4,60,96,100] there is a paucity of large registry data from the continent. Heart failure studies have identified notable differences in the causes of heart failure in African patients compared to those from high income countries, and have shown that African patients present at a younger age and have a higher mortality.[2,5] While these data are broadly informative on heart failure in Africans, they do not provide sufficient information on the causes, manifestation or outcomes specific to heart muscle disease.

Numerous diagnostic approaches and clinical guidelines for cardiomyopathies and myocarditis have been published, highlighting the degree of complexity underpinning heart muscle disease.[14,23,33,58,73,103,165,196-198] A multidisciplinary approach is recommended with deliberate analysis of every aspect of the clinical phenotype in individuals with cardiomyopathy (and their families), with careful integrated interpretation of cardiac investigations and reanalysis of clinical data throughout the diagnostic, treatment and follow-up process.[14] CMR is recognised as an integral part of the diagnostic work-up of patients with cardiomyopathy, providing reliable and reproducible data pertaining to functional and morphological characteristics of the myocardium.[116,199] Tissue characterisation techniques can be particularly useful in non-invasively determining specific aetiologies. The availability of sophisticated

investigations, specialised management interventions, and clinical expertise remains a challenge in LMICs, and the utility of scarce resources is highly relevant. More information on the manifestation of these conditions in local populations would be highly informative for health care system development and optimisation.

In this chapter, we present pilot phase data on adult patients recruited prospectively to IMHOTEP at the initiating centre, Groote Schuur Hospital, in Cape Town. A primary focus of this study was detailed phenotyping of new cases of cardiomyopathy in order to identify specific aetiologies of heart muscle disease presenting to our tertiary centre. The specific aims of this study were to (1) describe the baseline clinical characteristics of patients with newly diagnosed cardiomyopathy (incident cases) recruited into the IMHOTEP study over a 30-month period; (2) describe the CMR features of patients with newly diagnosed cardiomyopathy and examine the role of CMR in identifying specific aetiologies; and (3) to review the usefulness of the 3-stage diagnostic approach to cardiomyopathy adopted by IMHOTEP and discuss its application in resource restricted environments.

6.2. METHODS

6.2.1. Study population

The study population comprised of unrelated adult patients referred to the Cardiomyopathy Clinic at Groote Schuur Hospital in Cape Town (or directly to the IMHOTEP study) between 1 February 2015 and 31 July 2017.

6.2.2. Study design and diagnostic approach

This sub-study primarily focused on the incident cases arm of IMHOTEP, where patients with newly diagnosed cardiomyopathy were recruited and followed up prospectively. The rational and design of IMHOTEP, and eligibility criteria for inclusion into the study (Chapter 3 – Table

3.2.) have been described in Chapter 3. All patients were evaluated according to the 3-stage diagnostic approach (Chapter 3 – Tables 3.4.1. and 3.4.2.) and the diagnosis of cardiomyopathy was based on published criteria. Core investigations including ECG and echocardiography were required for enrolment. All extended diagnostic investigations and management procedures were done according to the discretion of the attending clinician, however, CMR studies that were not performed as part of the clinical evaluation of the patient, were conducted by the study investigators where possible. Informed consent was obtained prior to recruitment into the registry.

6.2.3. CMR analysis

CMR studies were performed on both 1.5 Tesla and 3.0 Tesla Siemens MRI scanners. Standardised CMR protocols were used to evaluated chamber dimensions, ventricular function, LV mass, haemodynamic assessment, and tissue characteristics (including native and post-contrast T1 and T2 mapping techniques where available) and late gadolinium enhancement (LGE). CMR studies done as part of the clinical evaluation from referral centres were accepted if standardised protocols were followed for image acquisition and image quality was adequate for comprehensive evaluation. All images were reviewed by 2 trained readers and post processing analysis was done using Argus software (Siemens Health Systems, Version VE 11).

6.2.4. Statistical analysis

Descriptive statistics were used to describe the study population. Continuous variables were tested for distribution using the Shapiro-Wilks test for normal data and using a histogram for visualisation. Normally distributed data were summarized as mean and standard deviation. Non-normally distributed data were reported as median and interquartile range. Wilcoxon-sum rank and Kruskal-Wallis tests were used to determine differences between non-normally

distributed data. Categorical variables were summarised in tables and reported as number and proportion and Chi-squared tests of equal proportions were used to determine differences in categorical data. Statistical analysis was performed using IBM SPSS Statistics 2017 (Version 25.0).

6.3. RESULTS

6.3.1. Enrolment

A total number of 314 unrelated patients were reviewed between 1 February 2015 and 31 July 2018. Of the patients seen, 103 patients were newly diagnosed with cardiomyopathy and were enrolled into the incident cases arm of IMHOTEP after initial evaluation (history, examination, electrocardiogram and echocardiography) as per the first stage of the 3-stage diagnostic approach (Chapter 3 – Table 3.4.). Subsequent to enrolment and after further clinical evaluation (including CMR), 4 cases were excluded with alternative diagnoses (Figure 6.1.). Three of the 4 cases excluded had hypertensive heart disease, diagnosed according to the exclusion criteria described in chapter 3 (Table 3.2.). Although these patients had blood pressures <160/100 at enrolment, on further evaluation we found significant hypertensive target organ damage in two of these cases. The third case presented with heart failure and normal blood pressure in the postpartum period. At follow-up, with recovery of her LV function, she was noted to be hypertensive on more than one occasion, requiring multiple anti-hypertensive agents. The fourth patient was diagnosed with LV dysfunction secondary to a left anterior descending artery territory myocardial infarction noted on CMR. The baseline characteristics of the 99 cases of cardiomyopathy included into IMHOTEP are represented in Table 6.1.

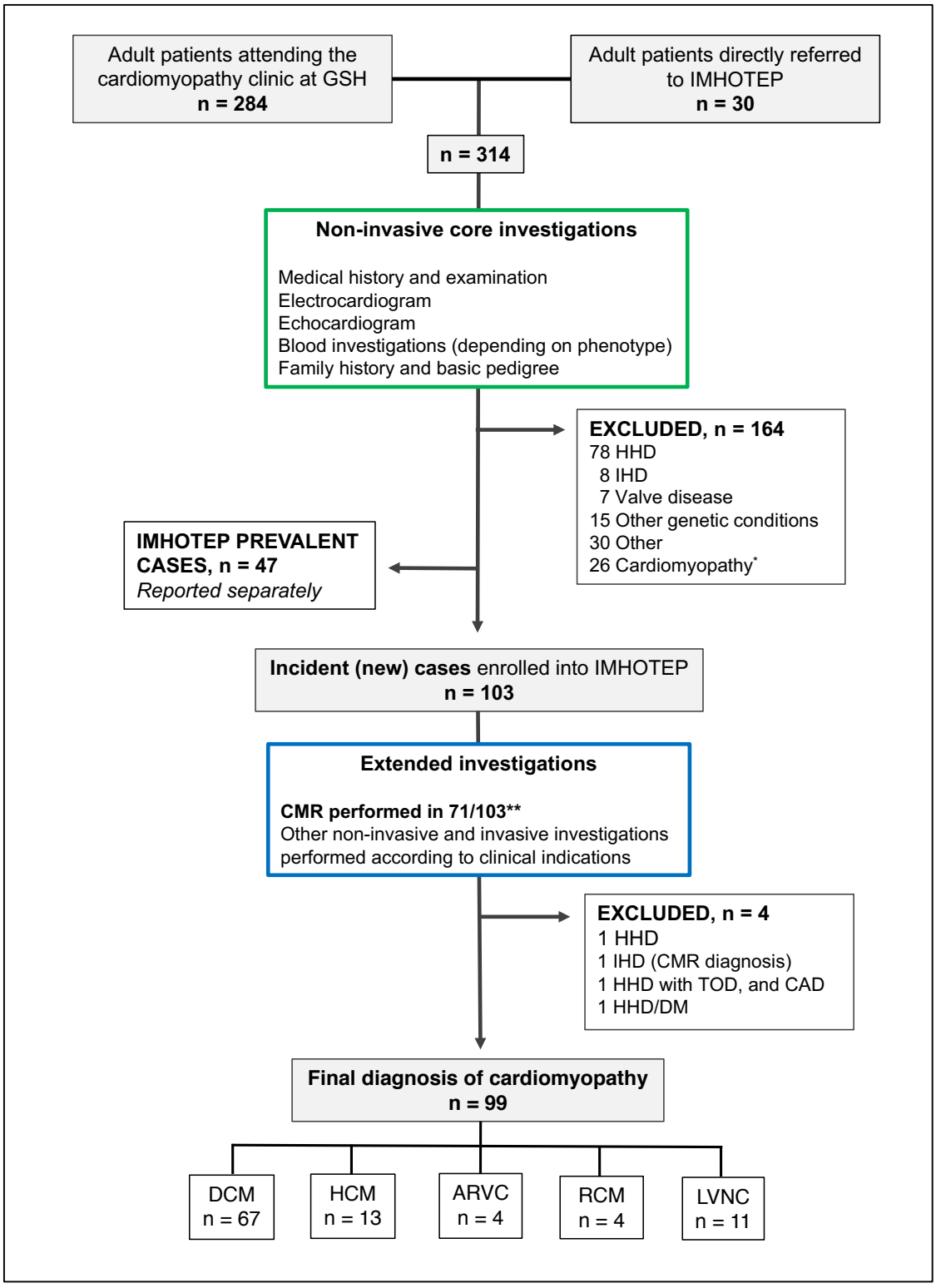

Figure 6.1. Recruitment of incident cases to IMHOTEP

*Cases of cardiomyopathy that had recovered with incomplete baseline data, diagnostic uncertainty, or recruitment not feasible. **CMR was not performed in 32 cases.

ARVC; arrhythmogenic right ventricular cardiomyopathy, CAD; coronary artery disease, CMR; cardiovascular magnetic resonance, DCM; dilated cardiomyopathy, DM; diabetes mellitus, GSH; Groote Schuur Hospital, HHD; hypertensive heart disease, HCM; hypertrophic cardiomyopathy, IHD; ischaemic heart disease, LVNC; left ventricular noncompaction, RCM; restrictive cardiomyopathy, TOD; target organ damage,

Table 6.1. Baseline Characteristics of 99 incident cases recruited

	DCM (n = 67)	HCM (n = 13)	ARVC (n = 4)	RCM (n = 4)	LVNC (n=11)	P value
Female, *n (%)*	36 (53.7)	5 (38.5)	1 (25.0)	3 (75.0)	8 (72.7)	0.301
Ethnicity, *n (%)*						
Black	32 (47.8)	2 (15.4)	0 (0.0)	0 (0.0)	7 (63.6)	<0.001
Caucasian	4 (6.0)	3 (23.1)	3 (75.0)	3 (75.0)	0 (0.0)	
Mixed race (coloured)	31 (46.3)	8 (61.5)	1 (25.0)	1 (25.0)	4 (36.4)	
Age at presentation, *years*						
Mean age ±SD	35.2 ±10.6	49.5±13.6	30.5±16.7	32.8±23.9	35.4±8.0	0.002
Median age (IQR)	34.0 (27.0-43.0)	49.0 (40.0-58.5)	28.0 (16.0-47.5)	25.5 (15.0-57.8)	33.0 (28.0-43.0)	
Age range (minimum – maximum)	16 - 62	25 - 75	15 - 51	13 - 67	25 - 52	
Type of presentation, *n (%)*						
Symptomatic and living	64 (95.5)	10 (76.9)	3 (75.0)	4 (100.0)	10 (90.9)	0.001
Resuscitated (survived cardiac arrest)	1 (1.5)	0 (0.0)	1 (25.0)	0 (0.00)	0 (0.0)	
Embolic event	2 (3.0)	0 (0.0)	0 (0.0)	0 (0.00)	1 (0.0)	
Incidental finding (asymptomatic)	0 (0.0)	3 (23.1)	0 (0.0)	0 (0.00)	0 (0.0)	
NYHA Class, *n (%)*						
NYHA Class I	2 (3.0)	6 (46.2)	4 (100.0)	1 (25.0)	1 (9.1)	<0.001
NYHA Class II	24 (35.8)	5 (38.5)	0 (0.00)	1 (25.0)	3 (27.3)	
NYHA Class III	34 (50.7)	2 (15.4)	0 (0.00)	2 (50.0)	6 (54.5)	
NYHA Class IV	7 (10.4)	0 (0.0)	0 (0.00)	0 (0.0)	1 (9.1)	
Cardiac symptoms at presentation, *n (%)*	66 (98.5)	10 (76.9)	4 (100.0)	4 (100.0)	11 (100.0)	0.007
Syncope	3 (4.5)	3 (23.1)	2 (50.0)	0 (0.0)	0 (0.0)	0.003
Presyncope/dizziness	23 (34.3)	4 (30.8)	2 (50.0)	3 (75.0)	3 (27.3)	0.464
Chest pain	25 (37.3)	5 (38.5)	1 (25.0)	1 (25.0)	2 (18.2)	0.746
Orthopnoea	52 (77.6)	2 (15.4)	0 (0.0)	2 (50.0)	9 (81.8)	<0.001
Paroxysmal nocturnal dyspnoea	47 (70.1)	3 (23.1)	0 (0.0)	2 (50.0)	8 (72.7)	0.002
Body swelling/oedema	34 (50.7)	0 (0.0)	0 (0.0)	1 (25.0)	7 (63.6)	0.002
Palpitations	41 (61.2)	6 (46.2)	2 (50.0)	3 (75.0)	6 (54.5)	0.799
Onset of symptoms peripartum (females), *n (%)*	21/36 (58.3)	0/5 (0.0)	0/1 (0.0)	1/3 (33.3)	3/8 (37.5)	0.104
Viral illness preceding presentation, *n (%)*	17 (40.3)	0 (0.0)	0 (0.0)	1 (25.0)	1 (9.1)	0.416
Previous chemotherapy for cancer, *n (%)*	6 (9.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.007
Lifestyle/substance exposure, *n (%)*						
Alcohol (>30 drinks/month or binge use)	19 (28.4)	1 (7.7)	1 (25.0)	0 (0.0)	2 (18.2)	0.652
Illicit drugs: methamphetamines/cocaine	10 (14.9)	1 (7.7)	0 (0.0)	0 (0.0)	1 (9.1)	0.862

Table 6.1. Baseline Characteristics of 99 incident cases recruited *(continued)*

	DCM (n = 67)	HCM (n = 13)	ARVC (n = 4)	RCM (n = 4)	LVNC (n=11)	P value
Family history, *n (%)*						
Heart failure	9 (13.4)	1 (7.7)	0 (0.0)	0 (0.0)	2 (18.2)	0.954
Cardiomyopathy	2 (3.0)	3 (23.1)	0 (0.0)	0 (0.0)	1 (9.1)	0.480
SCD < 35y	4 (6.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.775
SCD > 35y	3 (4.5)	1 (7.7)	1 (25.0)	0 (0.0)	0 (0.0)	0.541
Co-morbidities, *n (%)*						
HIV (ART naive)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001
HIV (on ART)	7 (10.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	<0.001
Hypertension (not considered causal)	5 (7.5)	8 (61.5)	0 (0.0)	1 (25.0)	2 (18.2)	<0.001
Diabetes	3 (4.5)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0.865
Chronic pulmonary disease	2 (3.0)	0 (0.0)	0 (0.0)	2 (50.0)	0 (0.0)	<0.001
Chronic renal disease	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Connective tissue disease	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0.975
Thyroid disease	3 (4.5)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0.219
Events prior to or at the time of recruitment, *n (%)*						
Embolic stroke/loss of limb	5 (7.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (18.2)	0.451
Atrial arrhythmia	4 (6.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (9.1)	0.434
Ventricular tachyarrhythmia	2 (3.0)	0 (0.0)	4 (100.0)	0 (0.0)	1 (9.1)	<0.001
Second or third degree heart block	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	0.089
Cardiac arrest (survived)	1 (1.5)	0 (0.0)	2 (50.0)	0 (0.0)	0 (0.0)	<0.001
Examination at time of enrolment						
Heart rate (beats/minute), mean ±SD	89 ±22	67 ±13	63 ±14	73 ±20	83 ±12	0.001
Systolic blood pressure (mmHg), mean ±SD	108 ±13	124 ±19	129 ±14	106 ±10	120 ±13	<0.001
Congestive heart failure, *n (%)*	27 (40.3)	1 (7.7)	0 (0.0)	3 (75.0)	2 (18.2)	0.021
Medication at enrolment, *n (%)*						
Diuretics	61 (91.0)	3 (23.1)	0 (0.0)	3 (75.0)	8 (72.7)	<0.001
Beta-blockers	54 (80.6)	8 (61.5)	2 (50.0)	2 (50.0)	10 (90.0)	0.457
ACE inhibitors/ARB	62 (92.5)	4 (30.8)	1 (25.0)	3 (75.0)	7 (63.6)	<0.001
MRA	33 (49.3)	0 (0.0)	1 (25.0)	1 (25.0)	7 (63.6)	0.065
Digoxin	7 (10.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.843
Calcium channel blockers	0 (0.0)	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	0.083
Anti-platelets	10 (14.9)	3 (23.1)	0 (0.0)	0 (0.0)	5 (45.5)	0.397
Oral anticoagulants						
Warfarin	13 (19.4)	0 (0.0)	0 (0.0)	2 (50.0)	3 (27.3)	0.462
Other	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0.001

6.3.2. Diagnosis

The commonest diagnosis was DCM (n=67, 67.7%), followed by HCM (n=13, 13.1%), LVNC (n=11, 11.1%), ARVC (n=4, 4.0%) and RCM (n=4, 4,0%) (Table 6.2.). All patients with ARVC fulfilled definite (n=3) or borderline (n=1) ARVC task force criteria.[73] A diagnosis of LVNC was made if CMR criteria were fulfilled.[164] Ten of the 11 cases (90.1%) with LVNC had overlapping DCM phenotypes with dilatation of the LV chamber and systolic dysfunction. Of the 67 patients with DCM, idiopathic (22.1%) and peripartum cardiomyopathy (18.1%) accounted for the vast majority of cases. Other secondary causes included anthracycline-related cardiomyopathy (6.1%), illicit drug use (methamphetamines and cocaine) (4.0%), HIV-associated cardiomyopathy (4.0%), alcohol (3.0%), thyrotoxicosis (1.0%), tachycardia- or PVC-related cardiomyopathies (2.0%) and rheumatoid arthritis (1.0%). Alcohol was only considered causal where there was significant alcohol intake and other target organ damage was evident (e.g. peripheral neuropathy). Inflammatory causes of cardiomyopathy diagnosed on CMR included 1 case of acute myocarditis (presumed viral, no EMB), 1 case of endomyocarditis associated with hypereosinophilia (EMB non-diagnostic) and 1 case of sarcoidosis (confirmed on EMB). Two male patients (1 Caucasian, 1 black African) presented with a clinical-triad of cardiomyopathy, conduction abnormalities and elevated creatinine kinase levels in the absence of neuromuscular abnormalities. Neither patient had a family history of cardiomyopathy or SCD, however the Caucasian patient presented with a cardiac arrest (survived) at age 17 years.

Table 6.2. Final diagnosis of the first 99 incident cases recruited to IMHOTEP

Final diagnosis	Patients n=99 n (%)
Dilated cardiomyopathy	
Idiopathic dilated cardiomyopathy	22 (22.2)
Familial dilated cardiomyopathy	2 (2.0)
Cardiomyopathy with conduction abnormalities and CK elevation	2 (2.0)
Secondary	
Peripartum cardiomyopathy	18 (18.1)
Myocarditis	1 (1.0)
PVC-related cardiomyopathy	1 (1.0)
Tachycardia-induced cardiomyopathy	1 (1.0)
Sarcoidosis	1 (1.0)
Cardiomyopathy secondary to CTD	1 (1.0)
HIV	4 (4.0)
Thyrotoxicosis	1 (1.0)
Illicit drugs (cocaine, methamphetamines)	4 (4.0)
Alcohol	3 (3.0)
Chemotherapy	6 (6.1)
Hypertrophic cardiomyopathy	13 (13.1)
Arrhythmogenic right ventricular cardiomyopathy	4 (4.0)
Restrictive cardiomyopathy	
Idiopathic	2 (2.0)
Secondary	
Amyloidosis	1 (1.0)
Hypereosinophilia	1 (1.0)
Unspecified	
LVNC-DCM overlap	10 (10.1)
LVNC with heart block (familial)	1 (1.0)

CK, creatinine kinase; CTD, connective tissue disease; HIV, human immunodeficiency virus; PVC, premature ventricular contractions; LVNC, left ventricular non-compaction.

A positive family history of heart failure (12.1%), cardiomyopathy (6.1%), and/or premature SCD (SCD<35 years in 4.0% and SCD≥35 years in 5.1%) was reported in 20.2% of cases. Routine family screening was not routinely conducted due to resource restraints and familial cardiomyopathy, by strict definition of having two or more affected individuals in a family, was only confirmed in 7 cases (DCM n=2, HCM n=2, ARVC n=2, LVNC n=1). Specific examples of familial cardiomyopathy cases are illustrated in Figure 6.2., Figure. 6.3. and Figure 6.4.

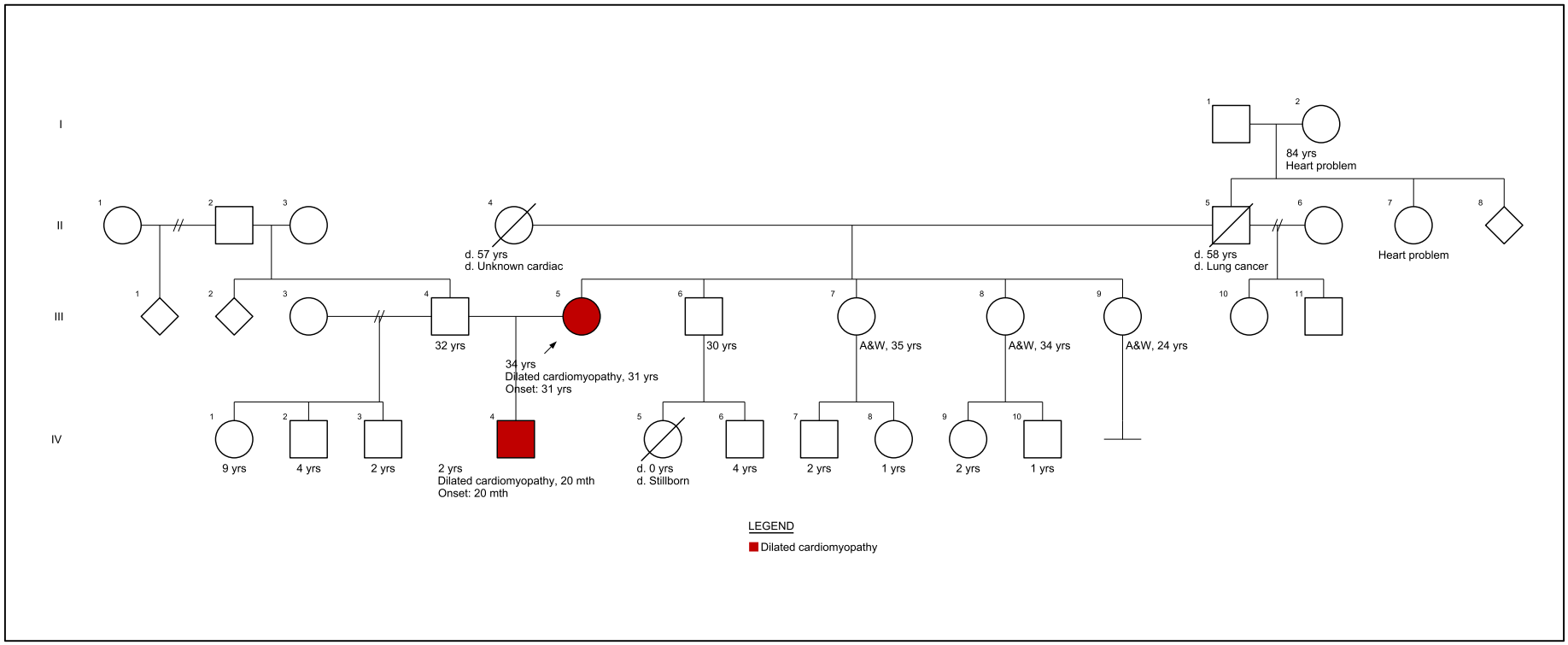

Figure 6.2. Familial DCM (Family 35)

Index patient III:5 was diagnosed with PPCM when she presented with heart failure in her third trimester of pregnancy. Her diagnosis changed to familial DCM after her son (IV:4) presented with heart failure and dilated cardiomyopathy at age 20 months

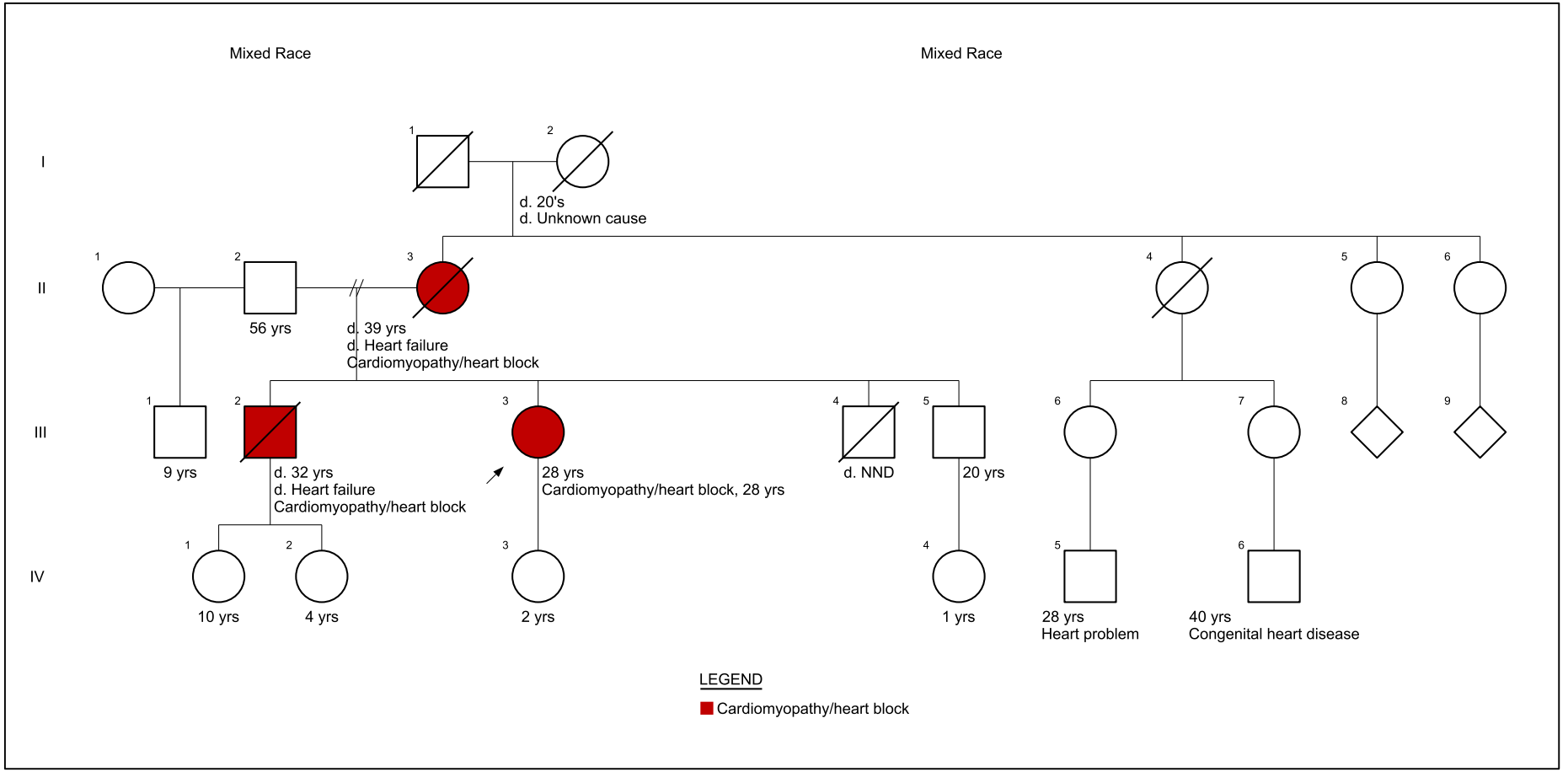

Figure 6.3. Familial cardiomyopathy characterised by LVNC and heart block (Family 28)

Index patient III:3 presented at age 28 years with vague symptoms of palpitations and fatigue. She reported a family history of heart block associated with heart failure and premature death (mother [II:3] and brother [III:2] died at ages 32 and 39, respectively). On evaluation, index patient III:3 had an atrial rhythm and nonspecified conduction abnormalities on ECG. Her echocardiogram showed diastolic dysfunction but normal cardiac dimensions and preserved systolic function. CMR confirmed features of LVNC with linear mid-wall LGE. Shortly after her initial presentation, she developed 2.1 heart block requiring permanent pacing.

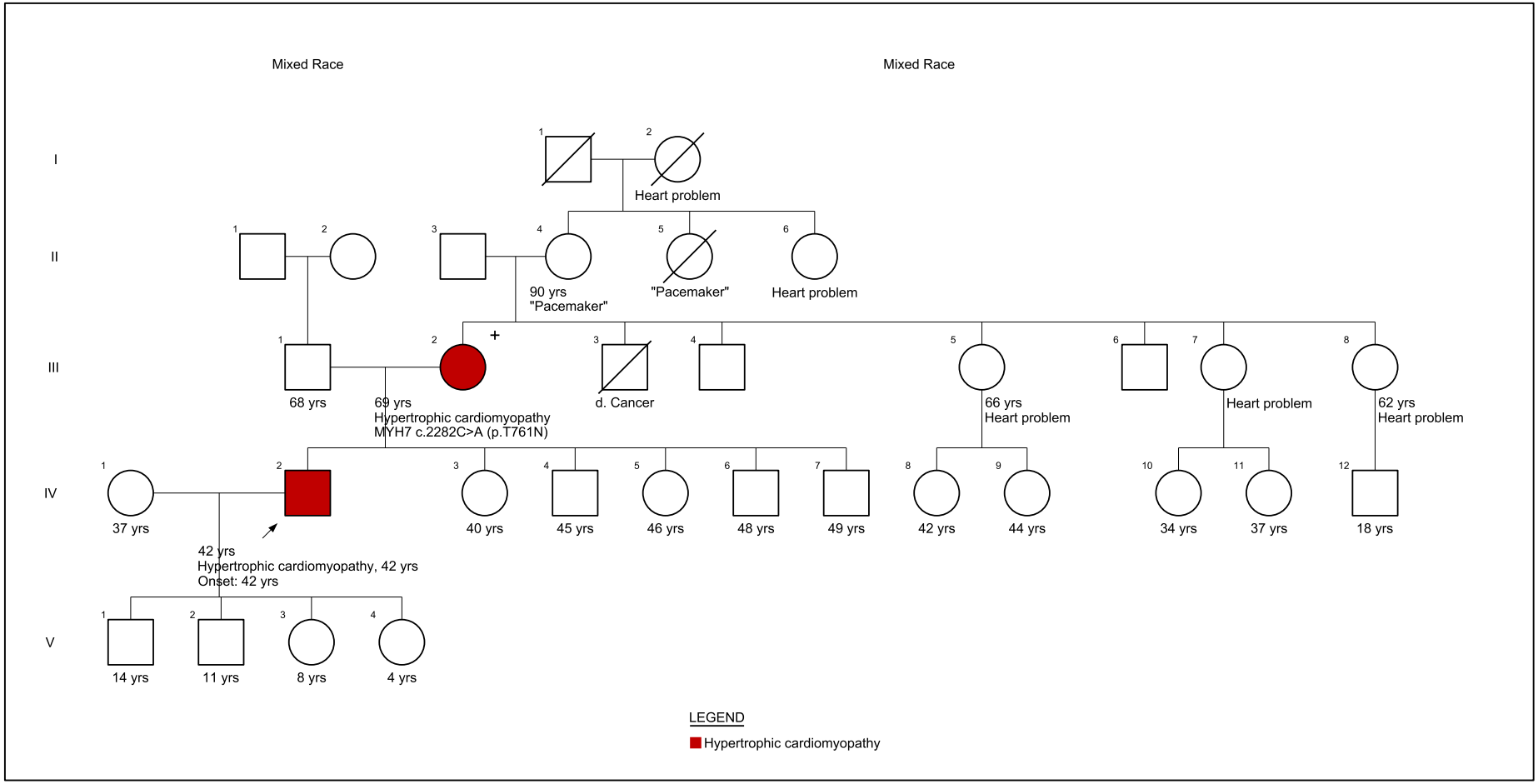

Figure 6.4. Family pedigree for family with HCM and a previously identified sarcomeric mutation MYH7 c.2282C>A (p.T761N) (Family 16)

Index patient IV:2 was recruited to IMHOTEP when he presented with HCM at age 42. On further exploration of the family history, it was discovered that his mother (III:2) had participated in a research study and was reported to have a pathogenic sarcomeric mutation, MYH7 c.2282C>A (p.T761N).[60] Genetic screening for the MYH7 c.2282C>A (p.T761N) in our index case and other family members is still pending.

6.3.3. Demographics

The mean age of diagnosis was 36.8 ± 12.5 years and differed significantly between cardiomyopathies (p=0.002); highest in patients with HCM (49.5 ± 13.6 years) and lowest in patients with ARVC (30.5 ± 16.7 years). A large distribution of age was observed in ARVC and RCM but the numbers were very small in both these groups and the distribution of age was considered normal overall. (Figure. 6.5.). Just over half of the total number of patients recruited were female (n=53, 53.5%). The majority of patients were mixed race (n=45, 45.5%) and black African (n=41, 41.4%), with only a minority of Caucasian patients (n=13, 13.1%) represented.

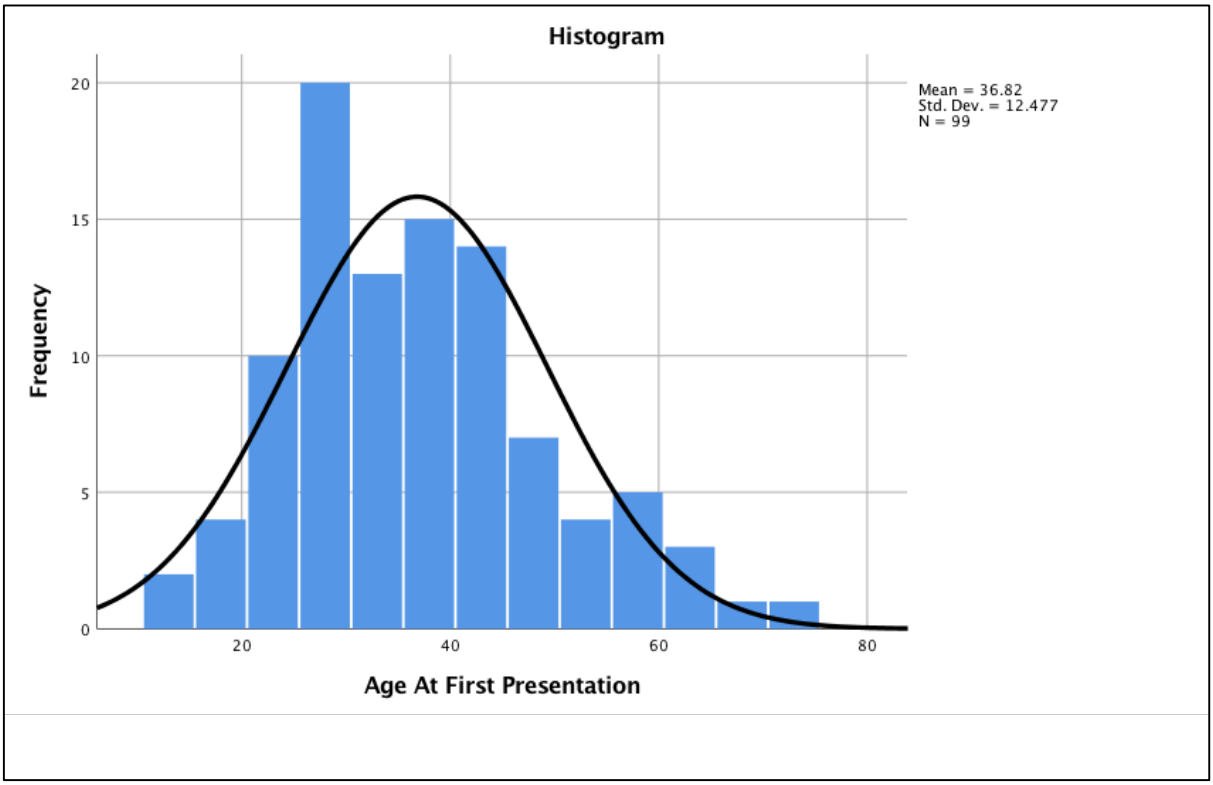

Figure 6.5. Histogram showing distribution of age of presentation

6.3.4. Symptoms, treatment and events at the time of enrolment

Most patients (96.0%) were symptomatic at presentation and there were significant differences between the diagnostic groups for NYHA class, syncope, orthopnoea, paroxysmal nocturnal dyspnoea (PND) and oedema. Syncope was more common in patients with ARVC (50.0%) and HCM (23.1%), whereas moderate to severe effort intolerance (NYHA Class III/IV) was more frequently seen in DCM (61.1%), RCM (50.0%) and LVNC (63.6%). Signs of congestive heart failure (orthopnoea, PND, oedema) were more common in patients with DCM, RCM and LVNC. Clinical features of HF correlated with a significant difference in diuretic use between the groups (DCM 91.0%, RCM 75.0%, LVNC 72.7% compared to HCM 23.1%; and ARVC 0%). Palpitations were frequently reported in all cardiomyopathies and there was no significant difference between the groups. The onset of symptoms in the peripartum period was observed in 25/53 (47.2%) of women, with a final diagnosis of PPCM being made in almost a third of the women enrolled (n=17/53, 32.1%). It should be noted that all cases in the LVNC category, where the onset of symptoms occurred in the peripartum period, the diagnosis of LVNC was made on CMR at least 6 months post-delivery. There were significant differences in baseline heart rate and systolic blood pressure between the different types of cardiomyopathy, with a higher mean heart rate (89 ± 22 beats per minute) and lower mean systolic blood pressure (108 ± 13 mmHg) in DCM patients.

Beta-blockers and ACE-inhibitors (or ARBs) were prescribed in 76.8% and 77.8% of cases respectively, however at the time of recruitment, optimal dosing of these drugs was only achieved 13.2% (beta-blockers) and 14.3% (ACE-inhibitors/ARBs). Mineralocorticoid receptor antagonists (MRA) were most frequently prescribed in DCM (49.3%) and LVNC (63.6%) although the difference between all groups was not considered significant. Calcium channel blockers (verapamil) were only prescribed in 2 patients with HCM. Few patients (7.1%) were on digoxin.

Three (3.0%) patients (ARVC n=2, DCM n=1) had a history of survived cardiac arrest, and 7 (7%) patients had documented ventricular tachyarrhythmia's at the time of enrolment (DCM n=2, ARVC n=4, LVNC n=1). All 4 ARVC patients had implantable cardioverter defibrillators inserted prior to enrolment, however, none of the DCM or LVNC patients had devices at the time of recruitment. One patient (as described in Figure 6.3.) developed 2:1 heart block shortly after recruitment and subsequently had a pacemaker inserted. Atrial fibrillation/flutter was documented in 4 (6%) patients with DCM and 1 patient with RCM (25%). A history of a prior embolic event was observed in 7.5% and 18.2% of patients with DCM and LVNC, respectively. Oral anticoagulation was prescribed in 19.2% of patients in total at enrolment.

6.3.5. Baseline investigations

Investigations performed are represented in Table 6.3. Electrocardiograms and echocardiograms were available for all 99 recruited patients and additional investigations were done according to clinical indications at the attending physicians' discretion. (Table 6.4. and Table 6.5.) Apart from heart rate, there were no significant differences in the ECG findings for the different cardiomyopathies. Most patients (90.9%) were in sinus rhythm (SR) at enrolment (SR with normal PR interval, 81.8%; SR with first degree heart block 9.1%). T- wave inversion was reported in the 67.7% of patients overall. QRS morphology was considered normal in 74.7% of cases. Left bundle branch block was the most frequently occurring conduction abnormality overall but was only observed in DCM and LVNC patients (DCM n=6; 9.0%; LVNC n=2, 18.2%).

As expected, there were differences observed in echocardiographic findings between the different subtypes, specifically for left atrial and ventricular dimensions, LV ejection fraction (LVEF), septal and LV wall thickness, RV systolic pressures and the presence of moderate or severe mitral regurgitation. LV dimensions were increased in both DCM (LVEDD 64.9 ±8.7) and LVNC (LVEDD 63.9 ±8.1). Severely impaired systolic function (mean LVEF 28%) and

diastolic dysfunction was observed in both DCM and LVNC groups. Diastolic dysfunction with preserved mean ejection fraction was seen in patients with HCM and RCM. Mean left atrial size was increased for all subtypes except ARVC. Moderate to severe functional mitral regurgitation was reported in half of the patients with DCM (55.2%) and RCM (50.0%), and over a third of patients with HCM (38.5%) and LVNC (36.4%). RV systolic pressures were significantly higher in RCM, DCM and LVNC subtypes (p 0.012). Intracardiac thrombus was reported in 10.5% of the DCM subtype.

Table 6.3. Investigations done prior to or at the time of enrolment into IMHOTEP

Investigations	DCM (n = 67) n (%)	HCM (n = 13) n (%)	ARVC (n = 4) n (%)	RCM (n = 4) n (%)	LVNC (n = 11) n (%)
ECG	67 (100)	13 (100)	4 (100)	4 (100)	11 (100)
Echocardiogram	67 (100)	13 (100)	4 (100)	4 (100)	11 (100)
SAECG	2 (3.0)	0 (0)	4 (100)	0 (0)	0 (0)
24 hour Holter	1 (1.5)	7 (53.8)	0 (0)	0 (0)	1 (9.1)
Chest X-ray	61 (91.0)	10 (76.9)	1 (25.0)	4 (100)	11 (100)
CMR	40 (59.7)	11 (84.6)	3 (75.0)	4 (100)	11 (100)
Coronary CTA	1 (1.5)	0 (0)	0 (0)	0 (0)	0 (0)
Invasive coronary angiography	7 (10.4)	4 (30.8)	3 (75.0)	3 (75.0)	2 (18.2)
Endomyocardial biopsy	1 (1.5)	0 (0)	1 (25.0)	3 (75.0)	0 (0)
Blood sample for DNA analysis	60 (89.6)	12 (92.3)	4 (100)	4 (100)	11 (100)

CMR, cardiovascular magnetic resonance; CTA, computed tomographic angiography; ECG, electrocardiogram; SAECG, signal average electrocardiogram.

Table 6.4. Electrocardiogram

	DCM (n = 67) n (%)	HCM (n = 13) n (%)	ARVC (n = 4) n (%)	RCM (n = 4) n (%)	LVNC (n = 11) n (%)	P value
Rate (beats per minute), *mean±SD*	93 ±21.2	64 ±15.8	51 ±7.7	83 ±12.4	86 ±17.6	<0.001
Rhythm, *n (%)*						
Sinus rhythm (normal PR interval)	52 (77.6)	12 (92.3)	4 (100.0)	3 (75.0)	10 (90.9)	0.664
Sinus rhythm with 1st degree HB	8 (11.9)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	
Atrial fibrillation	2 (3.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	
Atrial flutter	2 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Atrial ectopic rhythm	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	
QRS duration (ms), *mean±SD*	97 ±25.5	97 ±14.6	98 ±9.6	100 ±0.0	107 ±34.1	0.793
QRS fractionation, *n (%)*	16 (23.9)	5 (38.5)	3 (75.0)	0 (0.0)	4 (36.4)	0.110
QRS morphology, *n (%)*						
Normal	51 (76.1)	9 (69.2)	3 (75.0)	3 (75.0)	8 (72.7)	0.700
Incomplete LBBB	4 (6.0)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	
LBBB	6 (9.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (18.2)	
Incomplete RBBB	2 (3.0)	2 (15.4)	1 (25.0)	1 (25.0)	0 (0.0)	
RBBB	2 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	
Non-specific conduction abnormality	2 (3.0)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	
T-wave inversion, *n (%)*	42 (62.7)	9 (69.2)	4 (100.0)	3 (75.0)	9 (81. 8)	0.435
QTc, *mean±SD*	454 ±41.3	429 ±40.0	413 ± 10.6	455 ±61.3	464 ±29.9	0.081
Chamber hypertrophy, *n (%)*						
Left atrial hypertrophy	11 (16.4)	1 (7.7)	0 (0.0)	1 (25.0)	1 (9.1)	0.274
Right atrial hypertrophy	7 (10.4)	1 (7.7)	1 (25.0)	1 (25.0)	1 (9.1)	0.097
Left ventricular hypertrophy	16 (23.9)	6 (46.2)	0 (0.0)	0 (0.0)	4 (36.4)	0.703
Right ventricular hypertrophy	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.993

Table 6.5. Echocardiogram

	DCM (n = 67) n (%)	HCM (n = 13) n (%)	ARVC (n = 4) n (%)	RCM (n = 4) n (%)	LVNC (n = 11) n (%)	P value
Left heart study						
LV end-diastolic dimension (mm), *mean±SD*	64.9 ±8.7	43.2 ±5.6	52.3 ±7.2	43.6 ±6.9	63.9 ±8.1	<0.001
Left ventricular ejection fraction (LVEF)						
LVEF (m-mode), *mean* (n = 98)	28.4 ±10.8	74.7 ±9.2	55.0 ±2.9	52.5 ±6.6	28.1 ±12.6	<0.001
LVEF (volumetric) *mean* (n = 24)	29.5 ±10.3	70.5 ±10.6	57.0 ±0.0	50.5 ±7.8	26.2 ±5.0	<0.001
Left ventricular hypertrophy reported, n (%)	12 (17.9)	13 (100.0)	0 (0.0)	2 (50.0)	1 (9.1)	<0.001
IVS thickness (mm), *mean±SD* (n=98)	9.6 ±2.3	20.7 ±6.2	10.8 ±1.4	11.4 ±2.1	9.2 ±1.3	<0.001
LVPW thickness (mm), *mean±SD* (n=98)	9.6 ±2.3	15.1 ±5.5	10.9 ±1.0	11.6 ±2.6	9.5 ±2.7	<0.001
E/A ratio, *mean±SD* (n=82)	2.4 ±1.4	1.5 ±0.6	1.5 ±0.2	2.1 ±0.4	2.5 ±1.1	0.088
E/E' ratio, *mean±SD* (n=58)	15.1 ±7.3	15.9 ±6.6	6.0 ±0.8	13.9 ±9.4	10.5 ±3.9	0.210
Deceleration time, *mean±SD* (n=73)	140.1 ±55.8	268.4 ±109.6	255.5 ±6.4	112.3 ±53.3	155.3 ±78.9	<0.001
Left atrial size						
LA dimension (mm), *mean±SD* (n=96)	45.2 ±7.2	42.5 ±8.0	34.0 ±9.4	49.3 ±8.3	41.6 ±7.8	0.020
LA area (cm^2), *mean±SD* (n=33)	23.2 ±4.8	22.5 ±3.1	11.6 ±1.1	26.3 ±4.7	23.2 ±2.6	0.012
LV wall thinning reported, *n (%)*	8 (11.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.264
LV regional wall motion abnormalities, *n (%)*	35 (52.2)	0 (0.0)	0 (0.0)	2 (50.0)	7 (63.6)	0.017
LVNC reported, *n (%)*	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (18.2)	0.121
Mitral regurgitation (moderate – severe), *n (%)*	37 (55.2)	5 (38.5)	0 (0.0)	2 (50.0)	4 (36.4)	0.000
Right heart study						
RA dilatation reported, *n (%)*	26 (38.8)	0 (0.0)	4 (100.0)	3 (75.0)	4 (36.4)	<0.001
RV dilatation reported, *n (%)*	30 (44.8)	0 (0.0)	4 (100.0)	0 (0.0)	4 (36.4)	<0.001
TAPSE (mm), *mean ±SD* (n=62)	16.3 ±4.11	20.8 ±3.1	18.8 ±4.4	16.0 ±7.0	17.7 ±6.3	0.114
Tricuspid regurgitation (mod – severe)	27 (40.3)	0 (0.0)	2 (50.0)	1 (25.0)	2 (18.2)	0.052
RV systolic pressure (mmHg), *mean±SD* (n=74)	32.5 ±11.5	20.3 ±11.0	20.9 ±1.3	36.7 ±5.5	29.1 ±6.6	0.012
Intra-cardiac thrombus						
LA thrombus, *n (%)*	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.970
LV thrombus, *n (%)*	5 (7.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
LV and RV thrombus, *n (%)*	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Pericardial effusion (≤18mm), *n (%)*	13 (19.4)	1 (7.7)	0 (0.0)	2 (50.0)	2 (18.2)	0.634

IVS, intraventricular septum; LA, left atrium; LV, left ventricle; LVNC, left ventricular non-compaction; LVPW, left ventricular posterior wall; RA, right atrium; RV, right ventricle; TAPSE, tricuspid annular plane systolic excursion.

6.3.6. CMR findings

CMR studies were conducted in 71/103 patients initially recruited. Four patients were subsequently excluded with alternative diagnoses. The CMR findings for the remaining 67 cases are represented in Table 6.6. Table 6.7. includes the details of selected cases where CMR contributed to confirming or altering the clinical diagnosis. The data showed significant differences in the means for LV and RV ejection fractions, ventricular volumes and myocardial wall thickness consistent with the different morpho-functional definitions of each subtype. There was no significant difference in LV mass between the groups. Moderate to severe functional mitral and tricuspid regurgitation was observed in 50.7% and 22.4% of cases respectively overall. T1 mapping was only available to those patients (30/67) that were imaged on the 3 Tesla research scanner. Mean T1 times were increased in all subtypes where mapping was performed. Late gadolinium enhancement (LGE) was present in 60/65 (92.3%). Two patients (DCM n=1, ARVC n=1) did not have LGE imaging performed. Linear mid-wall enhancement was frequently observed in the DCM (94.6%) and LVNC (63.6%) subgroup. Three patients (DCM n=2, LVNC n=1) had small sub-endocardial or transmural enhancement patterns consistent with embolic infarcts. Inferolateral epicardial enhancement associated with a corresponding regional wall motion abnormality was reported in one patient with myocarditis. All HCM patients scanned had mid-wall patchy diffuse LGE. Failure to null and markedly increased T1 times consistent with amyloidosis were reported in one case of restrictive cardiomyopathy. One patient initially referred to IMHOTEP with suspected ARVC (definite criteria based on repolarization, depolarization, arrhythmia and structural TFC) had multiple patterns (mid-wall, subepicardial, subendocardial, transmural) of LGE consistent with sarcoidosis, which was subsequently confirmed on histology. A diagnosis of hypereosinophilic endomyocarditis with apical thrombus was made using CMR in a young man who presented with chest pain, a troponin leak and unobstructed coronaries. Images of the above described examples are illustrated in Figure 6.6.

Table 6.6. CMR findings in 67 incident cases

	DCM n = 38	HCM n = 11	ARVC n = 3	RCM n = 4	LVNC n = 11	P value
Left heart						
LVEF						
Mean ±SD	27.4 ±14.9	77.5 ±6.5	55.3 ±4.7	51.5 ±6.6	31.9 16.5	<0.001
Median	25.5	79.0	57.0	50.5	30.0	<0.001
LVEDV (ml), *mean±SD*	292 ±82	152 ±32	215 ±30	106 ± 33	272 ±93	<0.001
LVEDV/BSA (ml/m^2), *mean±SD*	150 ±40	80 ±17	98 ±12	59 ±13	155 ±52	<0.001
LV mass (g), *mean±SD*	161 ±48	190 ±59	162 ±23	125 ±22	151 ±62	0.212
LV mass/BSA (g/m^2), *mean±SD*	82 ±23	102 ±35	74 ±3	71 ±14	88 ±31	0.167
Septal thickness (mm), *mean±SD*	9.0 ±1.8	22.5 ±6.3	11.7 ±1.1	13.8 ±3.6	8.5 ±1.5	<0.001
LV minimum wall thickness (mm), *mean±SD*	6.1 ±1.4	9.5 ±2.8	7.7 ±0.6	10.5 ±3.1	5.5 ±1.6	<0.001
LV maximum wall thickness (mm), *mean±SD*	9.1 ±1.9	22.6 ±6.2	12.0 ±1.0	13.8 ±3.5	8.5 ±1.6	<0.001
LV regional wall motion abnormalities, *n(%)*	12 (31.6)	0 (0.0)	0 (0.0)	1 (25.0)	1 (9.1)	0.121
LV trabeculation (NC/C ratio), *mean±SD*	-	-	-	-	3.6 ±0.8	-
LA dimension (mm), *mean±SD*	40.2 ±6.8	41.1 ±8.6	30.7 ±6.1	46.5 ±13.2	39.6 ±7.3	0.115
LA area (cm^2), *mean±SD*	31.2 ±6.1	31.4 ±8.1	24.3 ±3.2	31.0 ±10.6	29.6 ±8.1	0.562
Mitral regurgitation (moderate-severe), *n (%)*	24 (63.2)	3 (27.3)	0 (0.0)	2 (50.0)	5 (45.5)	0.027
SAM of anterior mitral valve leaflet, *n (%)*	0 (0.0)	5 (45.5)	0 (0.0)	0 (0.0)	0 (0.0)	<0.001
Right heart						
RVEF (%), *mean±SD*	34.1 ±15.3	67.7 ±10.2	22.0 ±14.7	48.5 ±18.1	33.4 ±15.3	<0.001
RVEDV (ml), *mean±SD*	202 ±76	130 ±32	412 ±46	99 ±12.1	182 ±78	<0.001
RVEDV/BSA (ml/m^2), *mean±SD*	104 ±37	68 ±15	189 ±27	56 ±8	106 ±51	<0.001
RV regional wall motion abnormalities, *n (%)*	2 (5.3)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	<0.001
RA area (cm^2), *mean±SD*	25.7 ±8.5	26.2 ±3.5	33.7 ±10.6	31.8 ±9.8	25.1 ±5.0	0.285
Tricuspid regurgitation (mod-severe), *n (%)*	8 (20.8)	0 (0.0)	2 (66.7)	3 (75.0)	2 (18.2)	0.065

Table 6.6. CMR findings in 67 incident cases *(continued)*

	DCM n = 38	HCM n = 11	ARVC n = 3	RCM n = 4	LVNC n = 11	P value
Increased T2-weighted SI (ms), *mean±SD*	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)[e]	0 (0.0)	0.001
Pericardial effusion <20mm, *n (%)*	19 (50)	7 (63.6)	2 (66.7)	3 (75.0)	6 (54.5)	0.957
Intra-cardiac thrombus, *n (%)*	3 (7.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.663
T1 times (ms) *(native T1 time 1100±60ms)*	1288 ±51 (n=21)	1288 ±44 (n=5)	-	1501 (n=1)	1264 ±86 (n=3)	0.005
T2 times (ms)	42.3 ±2.0 (n=19)	40.7 ±2.3 (n=4)	-	45.3 (n=1)	42.5 ±4.0 (n=3)	0.321
Presence of late gadolinium enhancement,* *n (%)*	36/37 (97.3)	11 (100.0)	2/2 (100.0)	4 (100.0)	7 (63.6)	0.004
Pattern of LGE, *n (%)*						
Sub-epicardial	2/37 (5.4)[a,b]	0 (0.0)	0/2(0.0)	0 (0.0)	0 (0.0)	0.816
Mid-wall linear	35/37 (94.6)	0 (0.0)	1/2 (50.0)	0 (0.00)	7 (63.6)	<0.001
Mid-wall patchy/diffuse	1/37 (2.7)[c]	11 (100.0)	1/2 (50.0)	3 (75.0)	0 (0.0)	<0.001
Sub-endocardial	3/37 (8.1)[d]	0 (0.0)	0/2 (0.0)	1 (25.0)[e]	1 (9.1)[d]	0.590
Transmural	2/37 (5.4%)[b,d]	0 (0.0)	0/2 (0.0)	0 (0.0)	0 (0.0)	0.816
Failure to null	0/37 (0.0)	0 (0.0)	0/2 (0.0)	1 (25.0)[f]	0 (0.0)	<0.001
RV wall enhancement	1/37 (2.7)	1 (9.1)	2/2 (100.0)	0 (0.0)	0 (0.0)	<0.001

[a]Myocarditis – localised inferolateral subepicardial enhancement (n=1), [b]Sarcoidosis (n=1), [c]DCM/HCM overlap (n=1); [d]Small embolic infarct (n=2), [e]Hypereosinophilic endomyocarditis - diffuse subendocardial, [f]Amyloidosis, *LGE not done in 2 patients (DCM n=1, ARVC n=1)

BSA, body surface area; LA, left atrium; LGE, late gadolinium enhancement; LV, left ventricle; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; RA, right atrium; RV, right ventricle; RVEF, right ventricular ejection fraction; SAM, systolic anterior motion

Table 6.7. Specific cases demonstrating the diagnostic utility of CMR

Patient	Diagnostic category	Referral diagnosis	CMR features	Specific final diagnosis
1	LVNC	PPCM (LVNC suspected)	NC/C ratio 3.1, LVEDV/BSA 148ml/m^2, LVEF 44%, LGE+	LVNC/DCM overlap
15	LVNC	Idiopathic DCM	NC/C ratio 3.0, LVEDV/BSA 221ml/m^2, LVEF 8%, LGE+	LVNC/DCM overlap
25	LVNC	PPCM	NC/C ratio 3.0, LVEDV/BSA 148ml/m^2, LVEF 20%, LGE+	LVNC/DCM overlap
51	LVNC	PPCM (LVNC suspected)	NC/C ratio 2.5, LVEDV/BSA 150ml/m^2, LVEF 29%, LGE+	LVNC/DCM overlap
58	LVNC	DCM - possible PVC-related cardiomyopathy	NC/C ratio 4.0, LVEDV/BSA 104 ml/m^2, LVEF 46%, LGE-	LVNC/DCM overlap
59	LVNC	Idiopathic DCM	NC/C ratio 4.0, LVEDV/BSA 231 ml/m^2, LVEF 19%, LGE+	LVNC/DCM overlap
69	LVNC	Unspecified familial cardiomyopathy	NC/C ratio 3.4, LVEDV/BSA 64 ml/m^2, LVEF 57%, LGE+	Familial LVNC with heart block
75	LVNC	Idiopathic DCM	NC/C ratio 5.0, LVEDV/BSA 139ml/m^2, LVEF 37%, LGE-	LVNC/DCM overlap
93	LVNC	Idiopathic DCM	NC/C ratio 4.0, LVEDV/BSA 210ml/m^2, LVEF 10%, LGE-	LVNC/DCM overlap
700	LVNC	Idiopathic DCM	NC/C ratio 4.6, LVEDV/BSA 181ml/m^2, LVEF 30%, LGE+	LVNC/DCM overlap
702	LVNC	Idiopathic DCM	NC/C ratio 2.4, LVEDV/BSA 112ml/m^2, LVEF 51%, LGE-	LVNC/DCM overlap
18	ARVC	ARVC – presented with VT	RV RWMA, RVEDV/BSA 204ml/m^2, RVEF 13%, LV and RV LGE+, (LVEDV/BSA 101ml/m^2, LVEF 57%)	Definite ARVC – Major criteria for structural abnormalities, arrhythmia, repolarization. Minor criteria for late potentials
50	ARVC	ARVC – presented with syncope, cardiac arrest	RV RWMA and micro-aneurysms, RVEDV/BSA 158ml/m^2, RVEF 39%, LV and RV LGE+ (LVEDV/BSA 85ml/m^2, LVEF 59%)	Definite ARVC – Major criteria for structural abnormalities, repolarization. Minor criteria for late potentials. Cardiac arrest, VT present but not characterized.
91	ARVC	ARVC – presented with cardiac arrest	RV RWMA and micro-aneurysms, RVEDV/BSA 205ml/m^2, RVEF 14%, LGE not done, (LVEDV/BSA 108 ml/m^2, LVEF 50%)	Definite ARVC – Major criteria for structural abnormalities, repolarization. Minor criteria for late potentials (VF arrest)
49	RCM	Idiopathic RCM	Normal sized LV and RV (LVEDV/BSA 47 ml/m^2, LVEF 60%, RVEDV/BSA 59 ml/m^2, RVEF 66%), dilated atria (LA area 39cm^2, RA area 41cm^2), patchy mid-wall LGE+, T1 time 1340. Pericardial effusion 34mm	RCM – idiopathic

Table 6.7. Specific cases demonstrating the diagnostic utility of CMR (continued)

Patient	Diagnostic category	Referral diagnosis	CMR features	Specific final diagnosis
94	RCM	RCM - suspected amyloidosis	LVEDV/BSA 68 ml/m^2, LVEF 45%, RVEDV/BSA 66 ml/m^2, RVEF 30%, dilated atria (LA area 38cm^2, RA area 26cm^2), septum 19mm, increased T1 time 1501ms. Atrial LGE+, LV failure to null and patchy mid-wall LGE+ consistent with amyloid infiltration. Pericardial effusion 6mm	RCM - amyloidosis
97	RCM	PPCM	LVEDV/BSA 48 ml/m^2, LVEF 53%, RVEDV/BSA 47 ml/m^2, RVEF 36%, dilated atria (LA area 31cm^2, RA area 39cm^2). T1 time 1375ms. Diffuse patchy mid-wall LGE+. Pericardial effusion 21mm	Idiopathic RCM
705	RCM	Chest pain syndrome with troponin leak and unobstructed coronaries, transient eosinophilia	LVEDV/BSA 72 ml/m^2, LVEF 48%, RVEDV/BSA 53 ml/m^2, RVEF 62%, LA area 16cm^2, RA area 21 cm^2, LV endomyocardial enhancement in T2 imaging, endocardial enhancement (apical and mid-cavity) on LGE, LV apical thrombus, apical endomyocardial LGE+	Hypereosinophilic endomyocarditis (necrotic and thromboembolic phase of endomyocardial fibrosis)
24	DCM	Heart failure with severe mitral regurgitation (possible RHD)	LVEDV/BSA 123ml/m^2, LVEF 42%, RVEDV/BSA 78ml/m^2, RVEF 48%, normal mitral valve morphology, severe mitral regurgitation, circumferential linear mid-wall LGE+	Familial dilated cardiomyopathy with severe functional mitral regurgitation
52	DCM	PPCM	LVEDV/BSA 141ml/m^2, LVEF 39%, LV mass/BSA 69g/m^2, septum 13mm, LV walls 6-8mm, increased T1 time 1329ms, patchy diffuse (septum) and circumferential linear mid-wall LGE+	Peripartum presentation with DCM/HCM overlap
90	DCM	Chest pain syndrome with troponin leak and unobstructed coronaries	LVEDV/BSA 100ml/m^2, LVEF 45%, RVEDV/BSA 109ml/m^2, RVEF 42%, RWMA inferolateral wall of the LV with corresponding inferolateral epicardial LGE+	Myocarditis - likely viral aetiology (no EMB)
704	DCM	ARVC – definite diagnosis according to 2010 TFC	Increased RV volumes and RV RWMA, multiple patterns of LGE+ (patchy and linear mid-wall, transmural, epicardial and endocardial) consistent with sarcoidosis	Cardiac sarcoidosis - confirmed histologically
703	**EXCLUDED**	Idiopathic DCM	LAD territory transmural infarction involving the lateral wall, inferior wall and apex	Ischaemic heart disease with LV dysfunction

ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; BSA, body surface area; EMB, endomyocardial biopsy; LGE, late gadolinium enhancement; LV, left ventricle; EDV, end-diastolic volume; LA, left atrium; LVNC, left ventricular non-compaction; NC/C, non-compact/compact; PPCM, peripartum cardiomyopathy; RA, right atrium; RCM, restrictive cardiomyopathy; RHD, rheumatic heart disease; RV, right ventricle; RWMA, regional wall motion abnormalities; TFC, task force criteria; VT, ventricular tachycardia

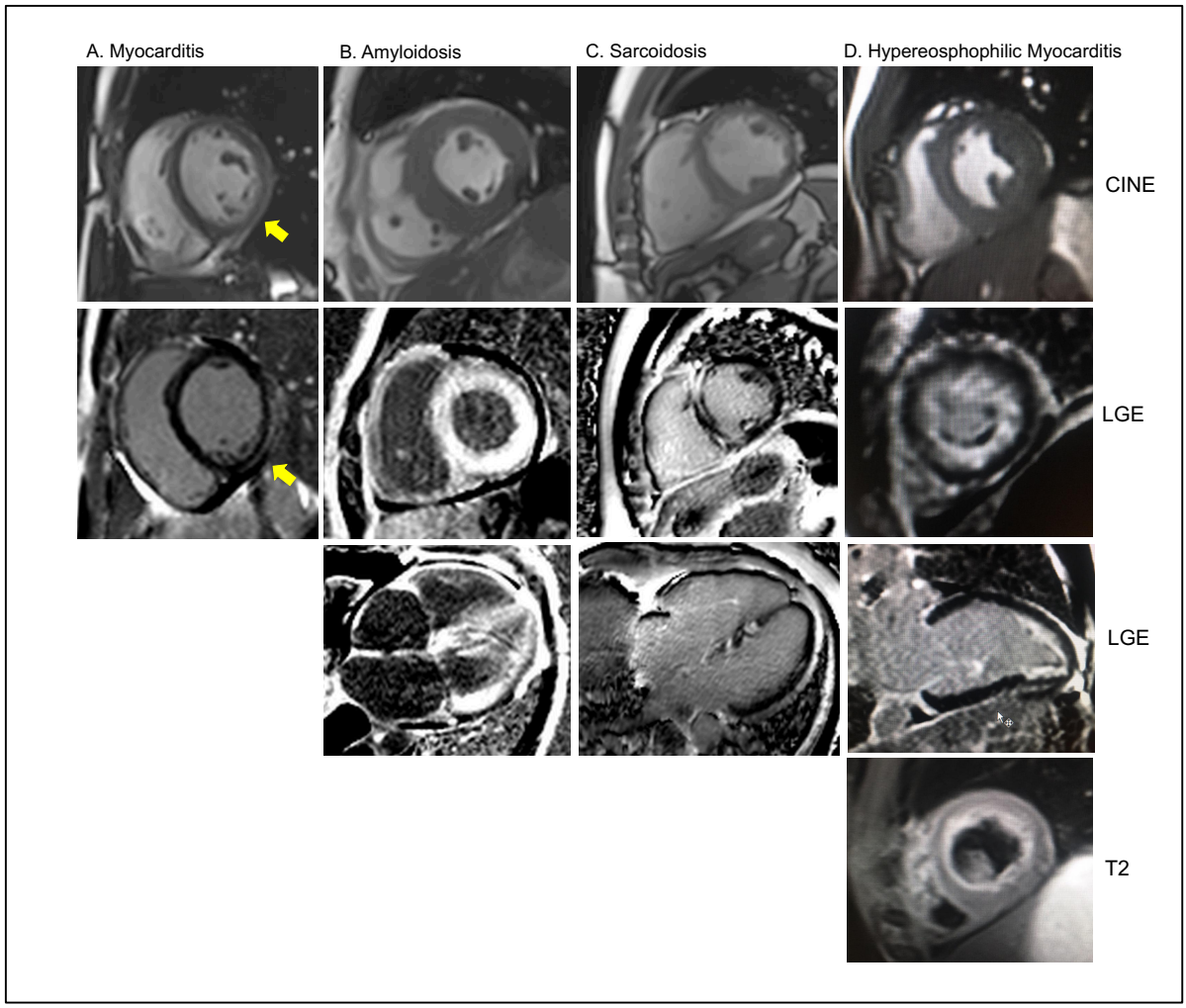

Figure 6.6. Diagnostic CMR images of patients with (A) Myocarditis; (B) Amyloidosis; (C) Sarcoidosis; and (D) Hypereosinophilic Myocarditis

A. Myocarditis – arrows indicate inferolateral regional wall motion abnormality on CINE short axis view with corresponding epicardial LGE
B. Amyloidosis – LV wall thickness 19mm on CINE short axis view with failure to null on LGE imaging
C. Sarcoidosis – increased RV volumes with RV regional wall motion abnormalities on CINE imaging and multiple patterns of LGE
D. Hypereosinophilic myocarditis – apical subendocardial enhancement with thrombus on LGE images and subendocardial oedema on T2 weighted images

Images: CMR of IMHOTEP study participants

6.4. DISCUSSION

IMHOTEP is the first prospective multi-centred cardiomyopathy registry from the African continent. This report includes the first 99 adult incident cases recruited as part of the pilot phase of the study at the initiating centre, Groote Schuur Hospital in Cape Town, and represents real-world contemporary data on the clinical characteristics of patients with different forms of cardiomyopathies referred to a tertiary centre in South Africa.

Contrary to what has been reported by the European registry where HCM is considered the commonest form of cardiomyopathy,[193,200] DCM is by far the most frequent type of cardiomyopathy seen at our institution (accounting for two thirds of the cohort) followed by HCM, LVNC, ARVC and RCM. While we cannot use this data to estimate population prevalence, our findings suggest that DCM is the predominant form of cardiomyopathy presenting to hospital in South Africa. This observation is further supported by a number of HF studies from the African continent where DCM is reported as the third most common cause of heart failure in hospitalised patients.[2,5]

Idiopathic DCM (22.2%) and PPCM (18.1%) accounted for the majority of cases recruited. The increased number of PPCM cases may, in part, reflect referral bias due to clinical and research interest in cardiac disease in pregnancy at our centre. The spectrum of secondary causes of dilated (and restrictive) cardiomyopathy was broad but on an individual level, the delineation of specific causes provided the opportunity to address the underlying cause in more than a third of patients recruited. Examples of potential interventions include, addiction counselling and management in cases of alcohol and drug abuse, antiretroviral therapy in HIV-associated cardiomyopathy, anti-thyroid therapies in thyrotoxicosis-related cardiomyopathy, and immunosuppression for cases of cardiomyopathy due to sarcoidosis, Hypereosinophilic syndromes, and autoimmune connective tissue disorders. Furthermore, bromocriptine has recently been shown be associated with a higher rate of full LV-recovery

and lower morbidity and mortality in PPCM patients.[43] Bromocriptine is easily accessible and its utility in the context of PPCM is highly relevant in our cohort.

A family history of heart failure, cardiomyopathy or sudden cardiac death was reported in 20.2% of patients, however, confirmed familial disease by strict definition, was only diagnosed in 7.1% patients. These figures are likely an underrepresentation of the true prevalence of genetic disease as routine family screening was not conducted in this study and genetic testing has not yet been performed. A previous study conducted in the same institution reported that familial disease was present in 26.6% of patients with DCM,[4] the predominant phenotype in our cohort. Michels *et al*. demonstrated that 20% of patients with a negative family history were found to have familial DCM when family screening was conducted,[24] and with follow-up, the number of cases diagnosed with familial disease increased by 10% as additional relatives developed the condition.[27] The value of follow-up has already been observed in our study by way of 2 examples; an index patient initially recruited as PPCM, was subsequently diagnosed with familial DCM after her son presented with DCM at 20 months of age, and an index case of HCM was found to be the son of a patient reported to have a sarcomeric mutation in a previous study. These examples highlight the usefulness of recruiting both adults and children with cardiomyopathy into the same registry, as well as the inclusion of previous smaller studies on cardiomyopathy from the same institutions. Detailed phenotyping will be important in future genetic analysis, as we intend to test for known mutations in all index patients where DNA has been collected (91/99). A clear understanding of the morpho-functional phenotype and contributing secondary factors (if present), will be essential in determining the significance of any genetic variants identified and may broaden our understanding of the interplay between genetic and environmental factors. While we have not been able to demonstrate familial disease in the two cases presenting with the triad of cardiomyopathy, conduction abnormalities and elevated CK levels, it would be important to exclude Lamin A/C mutations in these patients.

The age at presentation was significantly younger in our cohort than what has been reported in European populations (median age 36.0 years versus 49 years). While the age of presentation of HCM patients was similar to European patients, the mean age of presentation in DCM, ARVC, RCM and LVNC was ≤ 35 years in our cohort. When we excluded PPCM patients from the analysis, the mean age of patients presenting with DCM was 37.5 ± 10.8 years and overall, the mean age of presentation was 38.5 ±12.8 years, therefore the increased numbers of PPCM cases does not solely account for the younger age of presentation in our cohort. This younger age of onset has great social and economic relevance as these conditions are affecting mothers with young children and individuals in the prime of their working life. At the time of recruitment, 43.4 % were employed, 40.4% of patients were unemployed, 6.1% of patients were receiving government disability grants, and 5.1% were students. Of those patients who were unemployed, 60.0% had NYHA class III/IV symptoms. Importantly, more than half of the patients still working had blue collar jobs, which often involves some form of physical labour but only 17.4% of these employed individuals reported normal effort tolerance (NYHA class 1). These observations reflect the social burden cardiomyopathy has on the state, and very few patients with significant symptoms were receiving financially support. The reasons for this are likely multifactorial and further exploration into the social implications of cardiomyopathy in South Africa may be helpful in improving holistic patient care.

Unlike the European registry, IMHOTEP has included LVNC as a separate subtype in accordance with the ESC classification. The only published prevalence data on LVNC from South Africa comes from another tertiary centre, where the estimated prevalence was 6.9%.[100] Although LVNC can be diagnosed on echocardiogram, we only included cases into this category where CMR had been performed confirming the diagnosis of LVNC. While our numbers were small, it is worth noting that the majority of LVNC patients were enrolled as DCM cases initially and presented in a similar manner to those with DCM – with congestive heart failure symptoms, chamber dilatation (mean LVEDD 63.9mm ± 8.1) and reduced systolic

function (mean LVEF 28.1 ± 12.6%). In addition, echocardiographic features of noncompaction were only reported in 2/11 (18.2%) cases with a final diagnosis of LVNC. This would suggest that LVNC may be underdiagnosed in patients presenting with the classical 'DCM phenotype' and CMR is an important investigative modality in this context. While the classification of LVNC as a distinct cardiomyopathy remains unclear, LVNC has been associated with increased risk of embolic events and ventricular arrhythmias,[98] therefore, the finding of LVNC has clinical relevance and may impact decisions regarding primary prevention for stroke and risk assessment for VT/SCD. Of note, the mean ejection fraction was 28% on echocardiogram in this group, however, less than a third of patients were on anticoagulation at the time of enrolment. Although not demonstrated in this study, the confirmation of a diagnosis of LVNC on CMR has the potential to influence clinical decision making with regards prophylactic anticoagulation in patients with LVEF < 40%.[102]

In a clinical context where CMR is not readily available, understanding the diagnostic utility of this modality is important. By conducting CMR studies on as many patients as possible, we were able to make some key inferences. Firstly, the core investigations (history, examination, CXR, basic bloods investigations, ECG, echocardiogram, and family history) are sufficiently reliable in making a diagnosis of cardiomyopathy. This is particularly relevant in IMHOTEP, as majority of recruitment sites do not have access to extended investigations. The exclusion of ischaemic heart disease (IHD) has always been a caveat in studies conducted in resource restricted settings. We found only one case of ischaemic LV dysfunction (misdiagnosed as DCM) after recruitment where an extensive left anterior descending artery territory infarct was found on CMR. While coronary artery disease is not definitively excluded using this imaging modality, CMR was useful in excluding the presence of myocardial infarction as the cause of LV dysfunction in the DCM subgroup. The 3-stage diagnostic approach was developed in an attempt to guide clinicians in resource-restricted environments on how to utilise the cardiomyopathy diagnostic guidelines within the constraints of their clinical service to make the most accurate diagnosis possible. We set out to demonstrate that reliable diagnoses of

cardiomyopathy can be made if the core investigations are done consistently well. In addition, the usefulness of CMR in detailed phenotyping and specific aetiological diagnoses has also been shown, demonstrating that while this is an expensive test, there is both clinical and research value in building a CMR service in Africa.

Secondly, LGE was observed is 92.3 % of patients. In the case of DCM, linear mid-wall and subendocardial patterns of enhancement were seen in 94.6% and 8.1% of patients respectively. These findings differ from what has been reported elsewhere, with linear mid-wall and subendocardial LGE reported in 28% and 13% of DCM, respectively.[127] The mechanisms of mid-wall fibrosis are thought to be multifactorial – genetic predisposition, toxin or pathogen exposure, microvascular ischaemia, abnormal immune or metabolic modulation including the over-activity of the renin-angiotensin aldosterone system.[129] We speculate that the explanation for the higher incidence of LGE in our cohort may be, in part, related to later presentations and suboptimal treatment in our patients. On review of the baseline characteristics in DCM in our cohort compared to the European cohort,[193] although a higher percentage of patients presented with NYHA class III/IV symptoms (61.1% versus 38.4%), LV dimensions and LVEF were similar on echocardiogram (LVEDD 64.9mm versus 64.2mm; LVEF 28.4-29.5% versus 32.5%). When we analysed the time from onset of symptoms to date of recruitment in DCM, a median time of 5.4 months (163 days, IQR 93-310) was calculated. Although 92.5% of patients with DCM had already been started on an ACE-inhibitor (or ARB) therapy at the time of recruitment, only 11.3% (7/62) of those patients were prescribed optimal drug doses. Pathogen exposure and genetic predisposition may also be important factors to consider although currently there is insufficient data available to draw any meaningful conclusions. LGE in DCM has been associated with higher all-cause mortality/hospitalisation and SCD/VT.[129] It would therefore be important to correlate these findings with future outcomes.

Thirdly, CMR is an important tool in detailed phenotyping, and is particularly useful in the context of infiltrative and inflammatory conditions. One of the key aims of IMHOTEP was to establish the contribution of myocarditis to the development of cardiomyopathy in our setting where infectious diseases are more prevalent. We had hypothesised that by conducting CMR studies in patients with new onset heart failure, we would be able to diagnose myocarditis if present. However, we only found acute inflammation in two individuals. As patients were recruited from an outpatient tertiary centre, the delay in referral to us (median 163 days [IQR 93-310] from onset of symptoms to recruitment into IMHOTEP overall) likely accounts for paucity of active myocarditis in our patients. This pilot data indicates that the current study design of IMHOTEP cannot facilitate further understanding of the burden of myocarditis in this context. Inpatient recruitment and earlier imaging would be required to address questions pertaining to myocarditis as a cause of cardiomyopathy and heart failure in our population.

6.5. LIMITATIONS

As this study was conducted at a tertiary centre, these findings reflect the more severe end of the disease spectrum and we are unable to make inferences regarding the prevalence of the different cardiomyopathies at a population level. As patients were recruited from a specialist clinic, the time period from initial symptoms varied, and therefore the baseline data obtained reflects different time points in the natural history of disease. The implications of this limitation mainly relate to our ability to exclude acute myocarditis as a cause of dilated cardiomyopathy, as majority of patients did not have CMR imaging at the time of initial presentation with heart failure. Generalisability of our findings is limited due to the small number of patients in our cohort, however, despite these limitations, we have been able to make some meaningful inferences about the manifestations of heart muscle disease within our local context.

6.6. CONCLUSION

Although this is pilot data with relatively small numbers of patients, we have demonstrated that cardiomyopathy is caused by both familial and non-familial factors in the African population, and that secondary treatable aetiologies are present in a significant proportion of cases. The low rate of acute myocarditis in patients with DCM, is likely related to study design and the delay in clinical presentation and referral to our centre. The utility of CMR in this study demonstrated that the core investigations are reliable in making a diagnosis of cardiomyopathy, and the lack of availability of extended investigations should not limit our ability to conduct meaningful research in this field. The predominant type of cardiomyopathy presenting to hospital in South Africa is dilated cardiomyopathy, most commonly idiopathic DCM and peripartum cardiomyopathy. Most striking, this study shows that the age of onset of cardiomyopathy is significantly younger in South African patients. This has far reaching social and economic consequences, making cardiomyopathy an important public health problem.

6.7. CONTRIBUTIONS AND ACKNOWLEDGEMENTS

The design and implementation of the study was conducted by S. Kraus in consultation with B. Mayosi and N. Ntusi. Patient recruitment, data collection, and data analysis was done by S. Kraus. CMR analysis was done by S. Kraus, under the supervision of N. Ntusi and S. Moosa. P. Samuels and S. Jeremy should be acknowledged for their contributions in CMR image acquisition. N. Laing, U. September and M. Van der Wall assisted in the recruitment of patients.

CHAPTER 7: A clinical genetics overview of families with different morpho-functional types of cardiomyopathy in Africa

7.1. INTRODUCTION

Familial cardiomyopathies are defined by the occurrence, in more than one family member, of either the same disorder, or a phenotype that is (or could be) caused by the same genetic mutation and cannot be accounted for by another acquired cardiac or systemic disease.[13] Familial (genetic) cardiomyopathies merit close study as they provide a unique opportunity for early diagnosis and intervention through clinical family screening, and predictive genetic testing if a pathogenic mutation is identified.[64]

There are two widely adopted approaches for family screening: clinical screening in the absence of a genetic diagnosis in the proband, and predictive molecular genetic testing. Cascade (step-wise) screening using simple non-invasive cardiac screening techniques, is adopted in scenarios where genetic data are not available, either because diagnostic genetic testing is not accessible or where genetic testing has failed to identify a causal mutation. The primary goal of clinical family screening in cardiomyopathy is to identify relatives who may have the same disease as the proband. This approach is justified by the high probability of disease (50%) in first-degree relatives with autosomal dominant inherited cardiomyopathies, and the potential benefit of early diagnosis, risk stratification and treatment implementation. In addition to early diagnosis and risk stratification in relatives, clinical family screening also provides an opportunity to refine the clinical diagnosis in the proband. This can be particularly useful in families with mixed phenotypes, and variable penetrance and expressivity.[58]

The assessment of familial cardiomyopathies requires expertise in clinical phenotyping and a good understanding of inheritance patterns, transmission risk, natural history of disease, and

the implications of a genetic diagnosis for individuals and families.[58] The recognition of mendelian inheritance patterns requires the construction of a multi-generation pedigree through the ascertainment of a reliable and thorough family history. This task requires time and specific expertise as several nuances of genetic cardiomyopathies may potentially confound the interpretation of pedigree data.[132] Confounding factors include incomplete penetrance, variable disease expression, mixed/overlapping phenotypes, phenotypic and genetic heterogeneity, and the influence of environmental factors.[58,64] Furthermore, the diagnostic criteria in familial cardiomyopathies can vary between probands and their relatives.[14]

In many parts of the world, clinical family screening and genetic testing in inherited heart disease has become standard of care[58] and several inherited cardiovascular disease units and registries have been established worldwide. On the African continent, neither family screening nor genetic testing for cardiovascular diseases are readily available to the general population. Apart from a few case reports and small cohort studies,[4,26,59,60,75,96] there is a substantial gap in knowledge on the clinical and molecular genetics of the different forms of cardiomyopathy in African populations.

The advent of advanced methods of genotyping provides opportunities for research in the field of cardio genetics. The adoption of first world diagnostic approaches and guidelines into low-to-middle-income settings remains a challenge, and locally generated research is necessary for determination of the cost-effectiveness of family screening and molecular testing in the diagnosis and management of patients with inherited cardiomyopathies in Africa.

The principal aims of this study were: (1) to establish a standardised approach for identifying and confirming familial cardiomyopathies through family screening and genotyping, applicable to the African research and clinical practice setting; (2) to describe an overview of the clinical genetics, specifically the inheritance patterns and phenotypic variation, in families affected by

different forms of familial cardiomyopathy that have been included into the African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP); and (3) explore the feasibility of family screening and genetic testing in the South African setting.

7.2. METHODS

7.2.1. Study design

The standardised approach used for the clinical genetics component of IMHOTEP (Figure. 7.1.) is an adaptation of published guidelines,[58,135,165] and aims to identify and confirm families affected by different forms of familial cardiomyopathy for future molecular genetics research. It includes a basic guide for baseline clinical screening and follow-up for both genotype positive and genotype unknown families.

7.2.2. Study population

Affected families were identified using the following methods; (1) Existing families were identified from established studies incorporated into IMHOTEP, namely, *the Clinical and Genetic Study of Familial Dilated Cardiomyopathy in South Africa (HREC 197/96)* initiated in 1996[26] and the *Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) Registry of South Africa (HREC 047/2003)* initiated in 2003;[95,96] (2) new families were identified by reviewing the diagnosis and family history of both prevalent and incident index cases of cardiomyopathy enrolled into IMHOTEP between 1 February 2015 and March 2018.

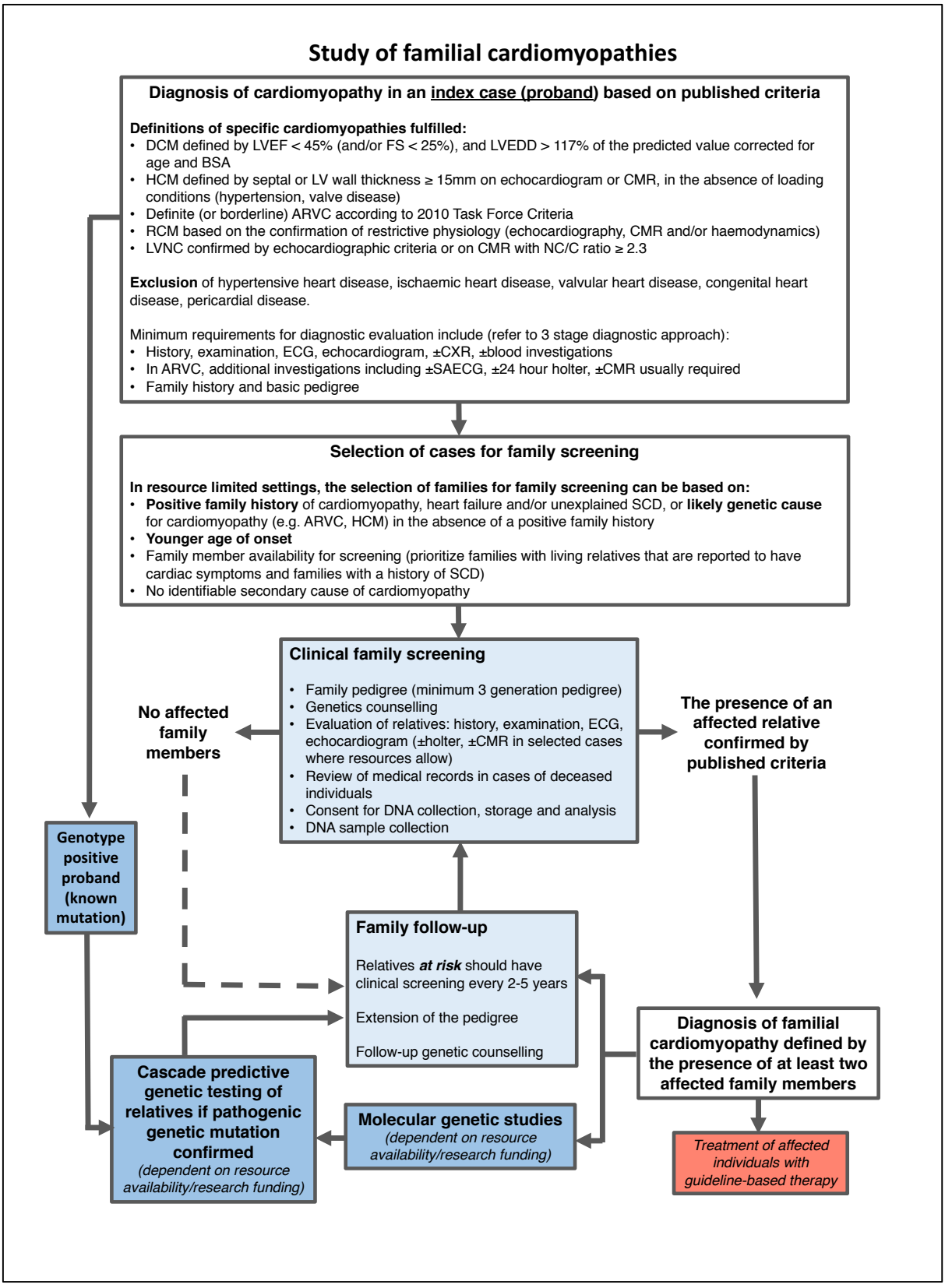

Figure 7.1. Approach to the study of familial cardiomyopathies

7.2.3. Eligibility

The following criteria were required for inclusion: (1) confirmation of a diagnosis of cardiomyopathy by standard definitions in the proband;[13,73,196] (2) informed consent for participation in genetics research from the proband (and family members if enrolled); (3) confirmation of a diagnosis of cardiomyopathy in one or more relatives using published criteria[73,165,196] through clinical evaluation or medical record review, and/or confirmation of a causal genetic mutation in the proband. Genotype positive probands were included, even in the absence of comprehensive family screening for completeness sake.

7.2.4. Clinical genetics

A retrospective review was conducted for existing families identified by *the Clinical and Genetic Study of Familial Dilated Cardiomyopathy in South Africa* and the *Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) Registry of South Africa*. Pedigree information was confirmed by reviewing existing hospital records and previously collected research data. Extension of existing families and repeat screening (as described below) was conducted in families that were available.

For new families, a basic pedigree was constructed for index cases with cardiomyopathy at the time of enrolment into IMHOTEP. In cases where a positive family history was identified, or where the cause for underlying cardiomyopathy was considered most likely genetic (e.g. ARVC, HCM),[58] first degree relatives were invited to come for family screening. A family screening research unit was established, including a physician, genetics counsellor, nursing sister and technologist. At the time of family screening, detailed three to five generation pedigrees were constructed according to standardised human pedigree nomenclature,[201] and genetic counselling was provided. Clinical evaluation of relatives included personal medical history, examination, electrocardiogram and echocardiogram. Extended non-invasive investigations such as SAECG, 24-hour ECG and/or CMR imaging, were done for relatives of

probands affected by ARVC where indicated, or in cases where there was diagnostic uncertainty. Families affected by ARVC were reviewed by the ARVC diagnostic panel, with experts in clinical cardiology, electrophysiology, cardiac imaging, pathology, and genetics.

Existing and new families were included if one or more relatives had a confirmed diagnosis cardiomyopathy according to published criteria.[13,73,165,196] Genotype positive individuals were included if a 2-3 generation pedigree was available for analysis, even in the absence of any affected relatives. Pedigree analysis was performed to determine likely patterns of inheritance and phenotypic expression. Family pedigrees were drawn using *Invitae Family History Tool,* Version 2.15.14. Invitae Corporation, US.

7.2.5. Molecular genetics

The molecular genetics results included in this study have been reported on previously in referenced journals or UCT Masters or PhD dissertations (Table 7.2.). Predictive genetic screening for 1st degree relatives, using previously reported methods,[75,96,155] was performed for genotype positive families if family members were available and willing to submit a blood sample. Classification of variants as disease-causing was evaluated according to definitions outlined by recent guidelines for assigning causality[177,178] and public archive, *ClinVar* (https://www.ncbi.nlm.nih.gov/clinvar).

7.2.6. Statistical analysis

Descriptive statistics were used to describe family pedigree analysis. Categorical variables are represented as number and percentage. Continuous variables were reported as mean and standard deviation, or median and IQR depending on distribution of data.

7.2.7. Ethical considerations

Approval from the University of Cape Town Human Ethics committee was obtained for this study (HREC Ref 766/2014). Informed consent for participation in cardiovascular genetics research was required from all probands, and included a request for permission to invite first-degree relatives for screening. All relatives participating in this research study were asked to sign consent prior to evaluation. Genetics counselling was provided to individuals and families by a genetics counsellor where possible. Information sheets explaining genetics research and cardiomyopathy were provided to patients (Appendix F). Existing families that were enrolled from previously approved studies *(HREC 197/93, HREC 147/2003)*, were only included if consent for genetics studies had previously been obtained. A waver of consent for deceased individuals was granted by the UCT HREC on the premise that the clinical information would be useful in understanding the phenotypic expression of genetic cardiomyopathies within families. All investigations performed were non-invasive and in accordance with standard of care guidelines. Where a diagnosis of cardiomyopathy was confirmed in a family member, with the permission of the participant, the findings were shared with their attending clinicians or an appropriate referral to a cardiologist was arranged for ongoing clinical care.

7.3. RESULTS

7.3.1. Clinical genetics

Pedigree analysis was conducted for 35 families that fulfilled inclusion criteria (Table 7.1.), including 17 families with ARVC, 11 families with DCM, 1 family with DCM associated with atypical muscular dystrophy, 2 families with DCM-LVNC mixed phenotype, 1 family with LVNC and conduction disease, 2 families with hypertrophic cardiomyopathy (HCM), and 1 family with HCM/DCM mixed phenotype. Families were divided into genotype positive and genotype unknown subgroups. Table 7.2. lists the genotype positive families with previously reported

mutations and Table 7.3. lists the genotype unknown families included in this study. Family pedigrees are included in the appendices (Appendix K).

Table 7.1. Clinical genetics of 35 families with familial cardiomyopathy

	Total number of families	Genotype	
		Genotype positive*	Genotype unknown
	n = 35	n = 16	n = 19
Phenotype			
Arrhythmogenic right ventricular cardiomyopathy	17 (48.6)	13 (81.3)	4 (21.1)
Dilated cardiomyopathy (DCM)	11 (31.4)	2 (12.5)	9 (47.4)
Dilated cardiomyopathy with muscular dystrophy	1 (2.9)	0 (0.0)	1 (5.3)
DCM-LVNC (left ventricular noncompaction) overlap	2 (5.7)	0 (0.0)	2 (10.5)
Left ventricular noncompaction with conduction abnormalities	1 (2.9)	0 (0.0)	1 (5.3)
Hypertrophic cardiomyopathy (HCM)	2 (5.7)	1 (6.3)	1 (5.3)
HCM-DCM overlap (possible mitochondrial myopathy)	1 (2.9)	0 (0.0)	1 (5.3)
Mode of inheritance			
Autosomal dominant	33 (94.3)	16 (100.0)	17 (89.5)
Inherited (pedigree analysis or founder mutation)	30 (85.7)	13 (81.3)	17 (89.5)
De Novo	1 (2.9)	1 (6.3)	0 (0.0)
Uncertain (negative family history/family screening)	2 (5.7)	2 (12.5)	0 (0.0)
Uncertain inheritance (possible autosomal recessive)**	1 (2.9)	0 (0.0)	1 (5.3)
X-linked recessive inheritance	1 (2.9)	0 (0.0)	1 (5.3)
Ethnicity			
Caucasian	19 (54.3)	10 (62.5)	9 (47.4)
Mixed ancestry	11 (31.4)	4 (25.0)	7 (36.8)
Black African	5 (14.3)	2 (12.5)	3 (15.8)
Age of diagnosis in the proband, years			
Mean ±SD	28.2 ±26.6	27.1 ±15.3	29.1 ±18.0
Median (IQR)	28.0 (15-43)	28.0 (14-44)	28.0 (15-42)
Proband age of onset < 20 years, n(%)	13 (37.1)	7 (43.8)	6 (31.6)
Families with a family history of SCD ≤ 35 years, n(%)	4 (11.4)	4 (25.0)	0 (0.0)
Families with a family history of SCD > 35 years, n(%)	2 (5.7)	0 (0.0)	2 (10.5)
Clinically affected relatives per family, n(%)			
0 affected relatives	6 (17.1)	6 (37.5)	0 (0.0)
1 affected relative	12 (34.3)	2 (12.5)	10 (52.6)
2 affected relatives	8 (22.9)	4 (25.0)	4 (21.1)
3 affected relatives	5 (14.3)	1 (6.3)	4 (21.1)
4 affected relatives	2 (5.7)	1 (6.3)	1 (5.3)
5 affected relatives	1 (2.9)	1 (6.3)	0 (0.0)
6 affected relatives	1 (2.9)	1 (6.3)	0 (0.0)

**Genotype reported previously, **Unable to extend family screening beyond parents,*
SCD; sudden cardiac death, SD; standard deviation

Nineteen (54.3%) of the families were of European descent, 11 (31.4%) were of mixed ethnicity and 5 (14.3%) were black African families. The median age of presentation of the proband was 28.0 years (IQR 15-43) and there was no significant difference in age of onset between genotype positive and genotype unknown probands. Thirteen (37.1%) probands (7 genotype positive, 6 genotype unknown) presented with severe disease under the age of 20. A family history of SCD was reported in 6 (17.1%) families but was only observed in families with ARVC; SCD under the age of 35 was observed in 4 genotype-positive ARVC families, and SCD ≥ 35 years was observed in 2 genotype unknown ARVC families.

The mode of inheritance was considered autosomal dominant (AD) in 33 (94.3%) of the families. Families with autosomal dominant cardiomyopathies were further subdivided into *inherited*, *de novo* and *uncertain* modes of inheritance based on clinical and molecular genetic findings. Inherited autosomal dominant cardiomyopathy was demonstrated through pedigree analysis (and/or the presence of a known founder mutation) in 30 families (86.7%). One individual had a confirmed *de novo* mutation (described below). Two genotype positive probands were classified as *uncertain* as it was not possible to determine whether the mutation had occurred d*e novo* or had been inherited based on available family data. Autosomal recessive inheritance was considered likely but not confirmed in 1 (2.9%) family (Figure 7.2.). In this family, two affected siblings (III:6, III:7) presented with DCM under the age of 2, both parents (II:6, II:7) were asymptomatic with normal cardiac findings on clinical screening. As this family originated from central Africa, insufficient pedigree information was available to confidently exclude AD inheritance with incomplete penetrance or X-linked recessive inheritance. X-linked recessive inheritance was observed in one family (2.9%) with DCM and associated muscular dystrophy (described below). The number of clinically affected individuals was based on clinical information obtained from medical records or screening conducted in our unit. We did not include relatives with reported 'heart problems' on family history where medical records were not available.

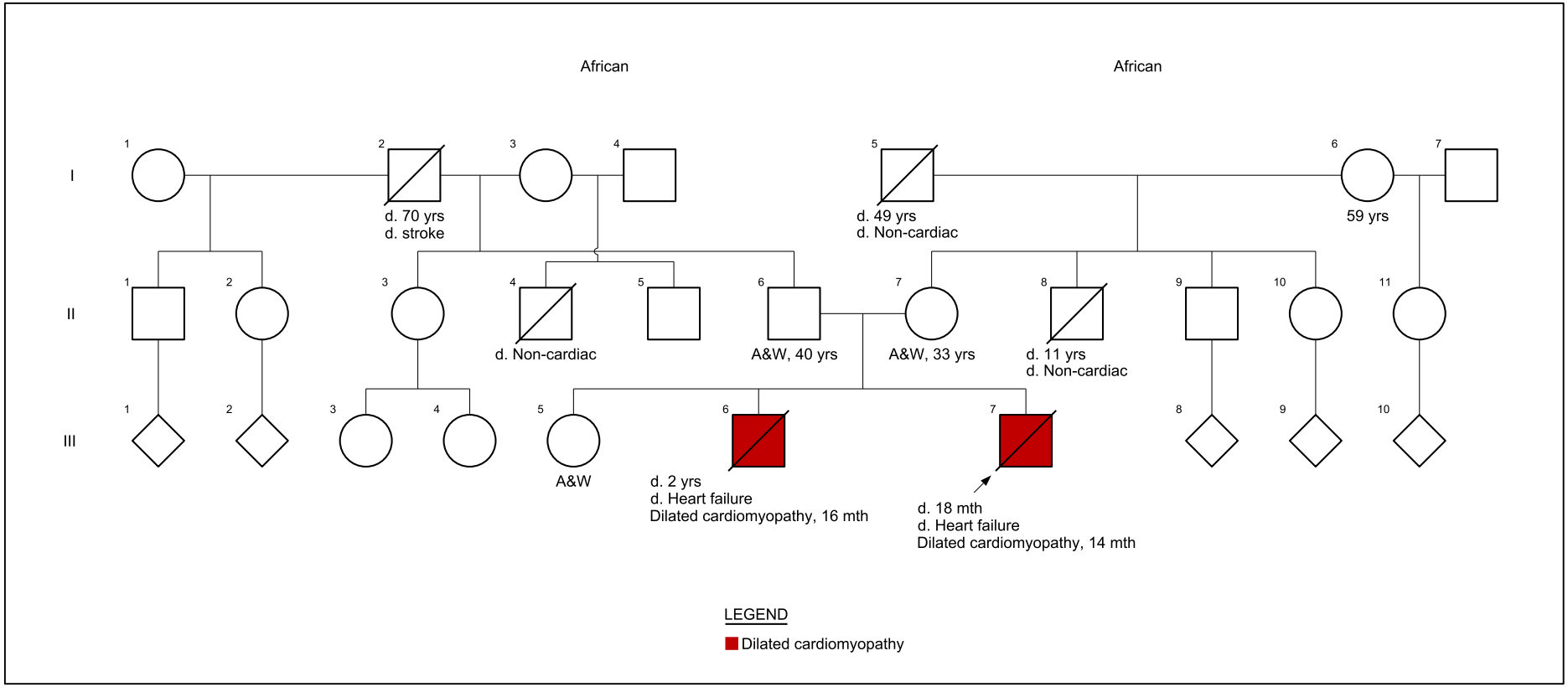

Figure 7.2. Family 29. Dilated cardiomyopathy with probable autosomal recessive inheritance.
A&W, alive and well (clinically unaffected)

Table 7.2. Genotype positive families

Family	Study	Type of CMO	Mode of inheritance	Genetic mutation	Reported	Ethnicity	Proband age of onset	Family history SCD < 35y	Family history SCD ≥ 35y	Number of clinically affected relatives
Family 1 ACM 1	ARVC Registry	ARVC	AD	PKP2 c.C1132T (Q378X)	*Watkins et al. Heart Rhythm 2009*	Caucasian	19	-	-	1
Family 2 ACM 2	ARVC Registry	ARVC	AD	CDH2 c.A686C (Q229P)	*Mayosi et al. Cir Gen 2017*	Caucasian	15	+	-	5
Family 3 ACM 5	ARVC Registry	ARVC	AD	PKP2 c.C1162T (R388W)	*Watkins et al. Heart Rhythm 2009*	Caucasian	44	-	-	2
Family 4 ACM 8	ARVC Registry	ARVC	AD *Homozygous*	PKP2 c.C1162T (R388W) PKP2 c.C1162T (R388W)	*Machipisa, T. 2016. Ph.D. Thesis, UCT*	Caucasian	12	+	-	2
Family 5 ACM 11	ARVC Registry	ARVC	AD *De Novo*	CDH2 c.G1219A (D407N)	*Mayosi et al. Cir Gen 2017*	Caucasian	15	-	-	0
Family 6 ACM 12	ARVC Registry	ARVC	AD	PKP2 c.C1162T (R388W)	*Watkins et al. Heart Rhythm 2009*	Caucasian	44	-	-	0
Family 7 ACM 19	ARVC Registry	ARVC	AD *Compound heterozygous*	PKP2 c.2197-2202del CACACCinsG (A733fsX740) PKP2 c.C1162T (R388W)	*Watkins et al. Heart Rhythm 2009*	Caucasian	13	+	-	4
Family 8 ACM 34	ARVC Registry	ARVC	AD	PKP2 c.T2540C (L847P) (VUS)*	*Watkins et al. Heart Rhythm 2009*	Mixed ancestry	28	-	-	0
Family 9 ACM 38	ARVC Registry	ARVC	AD	PKP2 c.C1162T (R388W)	*Watkins et al. Heart Rhythm 2009*	Caucasian	11	+	-	2
Family 10 ACM 39	ARVC Registry	ARVC	AD	PKP2 c.G1465A (G489R)	*Watkins et al. Heart Rhythm 2009*	Black African	29	-	-	2
Family 11 ACM 57	ARVC Registry	ARVC	AD	PKP2 c.C1162T (R388W)	*Machipisa, T. 2016. Ph.D. Thesis, UCT*	Mixed ancestry	46	-	-	0
Family 12 ACM 71	ARVC Registry	ARVC	AD	PKP2 c.C1162T (R388W)	*Machipisa, T. 2016. Ph.D. Thesis, UCT*	Mixed ancestry	38	-	-	0
Family 13 ACM 136	ARVC Registry	ARVC	AD	PKP2 c.C1237T (R413X)	*Chapter 4*	Mixed ancestry	49	-	-	0
Family 14 DCM 4	FDCM Study	DCM	AD	Troponin T	*Mayosi et al. CJSA 2004;15:237*	Black African	7 months	-	-	6
Family 15 DCM 320	FDCM Study	DCM	AD	PNL c.25C > T (p.R9C)	*Fish et al. Sci. Rep. 6, 22235*	Caucasian	28	-	-	3
Family 16 HCM 4	New family	HCM	AD	MYH7 c.2282C>A (p.T761N)*	*Ntusi et al. Cardiovasc J Afr 2016*	Mixed ancestry	40s	-	-	1

ARVC; arrhythmogenic right ventricular cardiomyopathy, AD; autosomal dominant, CDH2; Cadherin 2, DCM; dilated cardiomyopathy, HCM; hypertrophic cardiomyopathy, JUP; plakoglobin, LVNC; left ventricular noncompaction, PKP2; plakophilin, PNL; phospholamban, SCD; sudden cardiac death. *Probable variant of unknown significance (VUS) although reported as pathogenic in Watkins et al.

Table 7.3. Genotype unknown families

Family	Study	Type of CMO	Mode of inheritance	Genetic mutation	Ethnicity	Proband age of onset	Family history SCD < 35 y	Family history SCD ≥ 35y	Number of clinically affected relatives
Family 17 ACM 6	ARVC Registry	ARVC	AD	Unknown	Mixed ancestry	37	-	+	1
Family 18 ACM 142	New family	ARVC	AD	Unknown	Caucasian	50	-	+	3
Family 19 ACM 145	New family	ARVC	AD	Unknown	Caucasian	19	-	-	1
Family 20 HCM 50	New family	HCM	AD	Unknown	Caucasian	53	-	-	1
Family 21 DCM 343	FDCM Study	DCM/LVNC	AD	Unknown	Caucasian	28	-	-	4
Family 22 DCM 389	New family	DCM	AD	Unknown	Caucasian	44	-	-	3
Family 23 DCM 141	FDCM Study	DCM	AD	Unknown	Mixed ancestry	11	-	-	1
Family 24 DCM 464	New family	DCM	AD	Unknown	Caucasian	71	-	-	1
Family 25 DCM 390	FDCM Study	DCM	AD	Unknown	Mixed ancestry	42	-	-	1
Family 26 DCM 236	FDCM Study	DCM	AD	Unknown	Mixed ancestry	18 months	-	-	2
Family 27 DCM 437	New family	DCM/muscular dystrophy	X-LR	Unknown	Caucasian	15	-	-	2
Family 28 RCM 15	New family	LVNC/conduction disease	AD	Unknown	Mixed ancestry	28	-	-	2
Family 29 DCM 435	New family	DCM	Uncertain (AR)	Unknown	Black African	16 months	-	-	1
Family 30 DCM 3	FDCM Study	DCM	AD	Unknown	Mixed ancestry	25	-	-	3
Family 31 DCM 334	FDCM Study	DCM	AD	Unknown	Caucasian	13	-	-	1
Family 32 DCM 24	FDCM Study	DCM	AD	Unknown	Black African	38	-	-	1
Family 33 DCM 303	FDCM Study	DCM/LVNC	AD	Unknown	Black African	20	-	-	3
Family 34 ACM 149	New Family	ARVC	AD	Unknown	Caucasian	26	-	-	2
Family 35 DCM 458	New Family	DCM	AD	Unknown	Mixed ancestry	31	-	-	1

ARVC, arrhythmogenic right ventricular cardiomyopathy; AD, autosomal dominant; AR, autosomal recessive; X-LR, X-linked recessive; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction; SCD; sudden cardiac death.

7.3.2. Phenotypic description in selected families

Family 21 (Figure 7.3.) – Genotype unknown, AD inheritance

A multi-generation pedigree was constructed for Family 21 (Figure 7.3.). Evidence available supports the diagnosis of dilated cardiomyopathy in 7 individuals over 6 generations, based on information gathered from hospital records dating back to the 1960s and death certificates from as far back as 1920s. The proband (V:8), a 28-year-old Caucasian female, presented for the first time in 2010 with signs and symptoms of advanced heart failure. Investigations, including an electrocardiogram, echocardiography, CMR, haemodynamic study and endomyocardial biopsy, confirmed a diagnosis of dilated cardiomyopathy with severely impaired systolic function (LVEF 20%) complicated by atrial fibrillation. While prominent trabeculations of the left ventricular wall were noted on CMR, the ratio of non-compact to compact myocardium was less than 2.3 and therefore did not fulfil criteria for a diagnosis of LVNC at the time of diagnosis. Although she reported a preceding severe flu-like illness, a familial cardiomyopathy was suspected based on a family history of heart failure in her paternal grandfather (III:3) and his uncle (II:3). Original hospital records confirmed that the proband's paternal grandfather (III:3) died in 1965 of heart failure due to a dilated cardiomyopathy at the age of 29. In addition, individual II:3 underwent orthotopic heart transplantation in 1971 at the age of 59 for dilated cardiomyopathy, confirmed by the pathology report of the explanted heart. He died of organ rejection in the weeks following the procedure. Death certificates were obtained for individuals I:2 and II:5 reporting the causes of death as '*heart failure due to myocardial degeneration'* at age 52, and '*pneumonia and heart failure'* at age 4, respectively. Family screening was initially conducted in 2010 for individuals IV:3, IV:4, IV:5, IV:6 and V:9 with no positive findings. The proband (V:8) responded to medical therapy and follow-up investigations showed sinus rhythm and near normal LV size and function in 2015. In 2017, the proband's daughter (VI:4), age 9 years, presented with acute heart failure and despite maximal supportive therapy, died of multi-organ failure a month after diagnosis.

The echocardiogram at presentation for VI:4 reported a dilated heart with LVEF 15% and features of left ventricular non-compaction which was subsequently confirmed at autopsy. First and second degree relatives (IV:3, IV:4, IV:5, IV:6, V:9, VI:5) and individual V:7 (father of VI:4) were screened for cardiomyopathy in 2017 following the death of VI:4. At repeat screening, the proband's sibling (V:9) was found to have mildly reduced systolic function on echocardiogram and CMR confirmed a reduced left ventricular ejection fraction of 41% with features of LVNC. Individual VI:5 was carefully screened as the pedigree suggests he would be a mutation carrier. His cardiac function and size remained normal, with no features of LVNC on CMR. It should be noted that he was on ACE-inhibitor and beta-blocker therapy for primary hypertension. Individuals IV:3, IV:5, IV:6, VI:5 and V:7 were clinically unaffected.

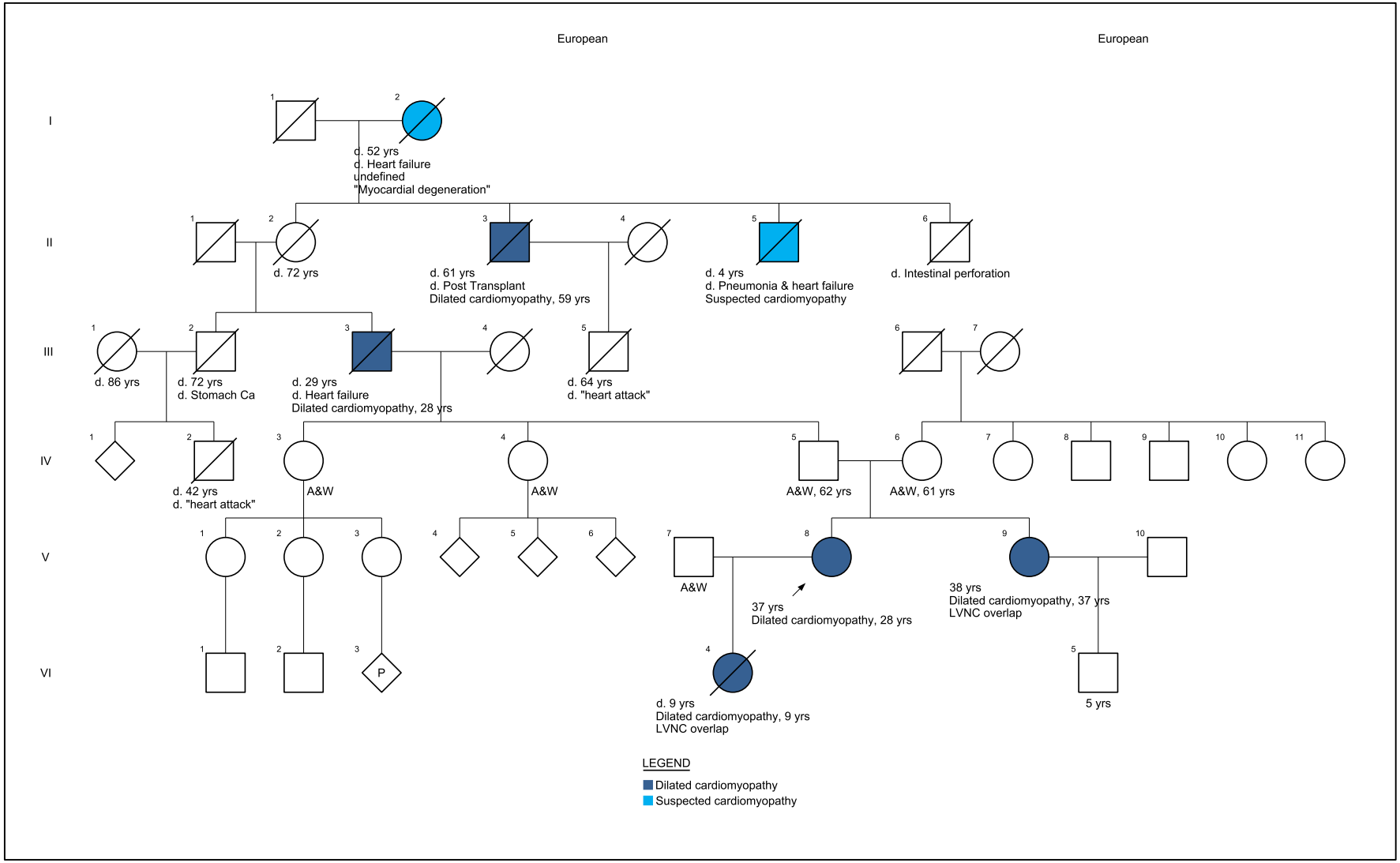

Figure 7.3: Family 21. Dilated cardiomyopathy with left ventricular noncompaction (LVNC) overlap
A&W, alive and well (clinically unaffected)

Family 18 (Figure 7.4.) – Genotype unknown, AD inheritance

A four-generation family pedigree was constructed for Family 18 (Figure 7.4.). The proband (IV:3) was diagnosed with definite ARVC at age 50 following a syncopal episode. The diagnosis of ARVC in the proband was based on significant structural abnormalities of the RV (with LV involvement) on CMR, major repolarisation changes on ECG, late potentials and recurrent ventricular arrhythmias, one episode requiring cardiopulmonary resuscitation. No causal genetic mutation was identified through screened for known pathogenic mutations for cardiomyopathy. On review of the maternal family pedigree, a prominent history of sudden death of family members in the 5th and 6th decade of life was noted. Individual I:5 died suddenly while travelling on a train. Individual II:6 died in his sleep at age 49, with reported chest pain and shortness of breath during the preceding two weeks prior to his death. Individual II:8 reportedly died in her sleep while in hospital for a presumed "heart attack" (pre-ECG era). Individual III:7 suffered a SCD while running but his death was attributed to a myocardial infarction confirmed at autopsy. The proband's younger female sibling (IV:6) subsequently suffered a SCD (age 43) a few months after his initial diagnosis. The autopsy (IV:6) showed some RV dilatation with minimal fatty infiltration, and diffuse mid-wall fibrosis in the LV wall, consistent with early ARVC. Clinical screening was conducted for first degree relatives (III:4, III:5, IV:1, IV:3, V:2, and V:3) of the proband, and available second (III:8) and third (IV:10, IV:11, IV:12, V:12) degree relatives. The mother (III:5) and surviving sister (IV:5) of the proband both had sufficient clinical abnormalities to be considered affected. In addition to earning major criteria for family history of ARVC, both III:5 and IV:5 had minor criteria for late potentials on SAECG, and > 500 PVC's on Holter, as well as evidence of diffuse mid-wall fibrosis within the septum and LV walls on CMR despite normal chamber size and function. Individual III:8 was thoroughly investigated; he had evidence of a previous myocardial infarction (on CMR) with confirmed coronary artery disease requiring previous coronary artery bypass surgery, and interstitial lung disease, but he did not have any findings diagnostic of ARVC. No significant findings were noted in his children that were screened.

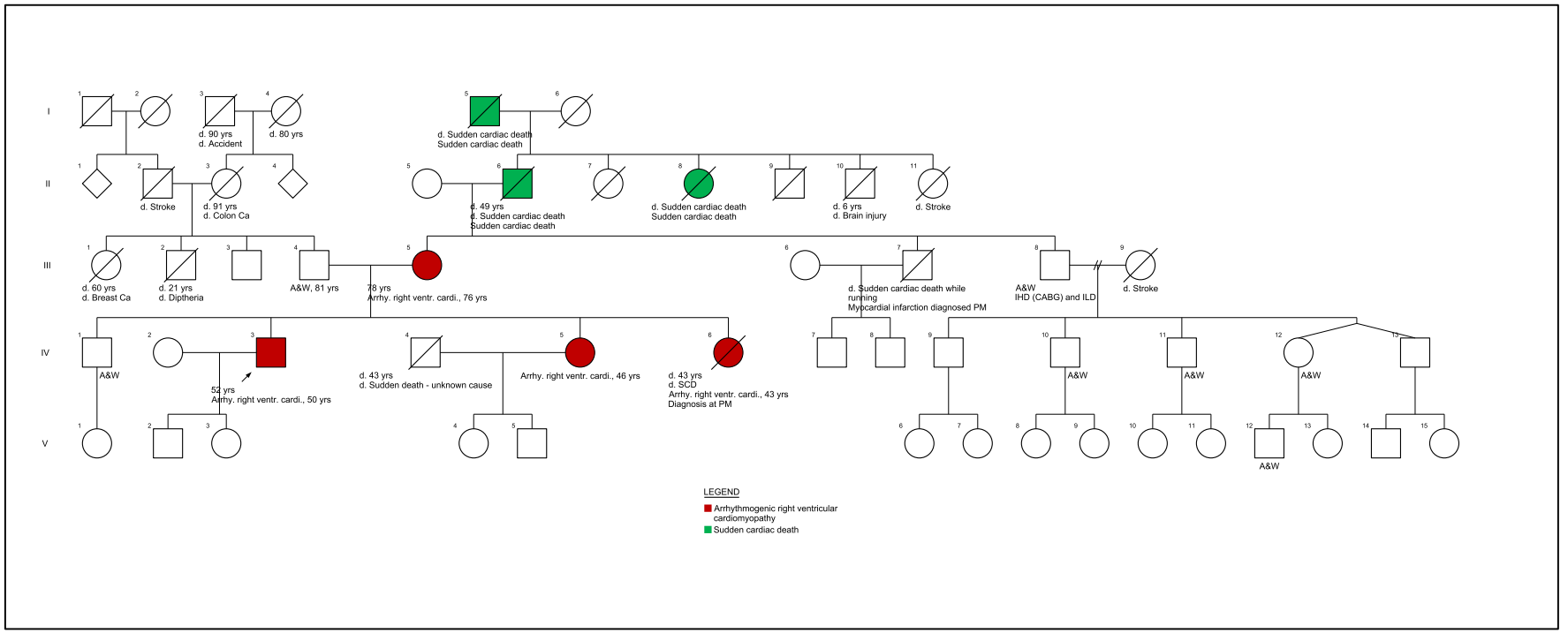

Figure 7.4. Family 18. Arrhythmogenic right ventricular cardiomyopathy

Family DCM 27 (Figure 7.5.) – Genotype unknown, X-linked recessive inheritance

A 3-generation pedigree was constructed for **Family 27** (Figure 7.5.). The male proband (III:8) presented at age 15 with weakness and wasting of the bicep, triceps and peroneal muscles and markedly elevated creatinine kinase (CK) levels (CK 3173 IU/L, normal < 172 IU/L). In addition, he was found to have a dilated cardiomyopathy (LVEF 25-30%) on echocardiogram. A number of diagnoses were considered, including Emery-Dreifuss, Duchenne and Becker's Muscular Dystrophy, but no definitive diagnosis has been confirmed in this patient. Molecular genetic testing for mutations in LMNA/C and exonic deletion and duplication mutations within the dystrophin gene were negative, as was the *cardiomyopathy gene panel* screening done at an international diagnostic genetic screening facility. Subsequent to the proband's diagnosis, his 21-year-old male sibling (III:5) developed acute heart failure following a viral gastrointestinal illness, and required cardiac transplantation within a few months of presentation. The presumed aetiology of "viral myocarditis" was never confirmed as the explanted heart was not sent for histological examination. No neuromuscular abnormalities were documented in his clinical records. At subsequent family screening in our unit, it was noted that this individual had subtle wasting and mild weakness of the peroneal muscles bilaterally and a markedly elevated CK level (CK 2279 IU/L, normal <172 IU/L). Furthermore, the proband's twin sister (III:7), who was 19 years old and 23 weeks pregnant at the time of screening, was found to have a mildly dilated heart with mildly reduced systolic function (LVEF 45%). Although her neuromuscular examination was considered normal, her CK level was mildly elevated (CK 412 IU/L, normal < 146 IU/L). These findings were considered consistent with that of a carrier of an X-linked muscular dystrophy. Both parents (II:3 and II:4) had normal neurological and cardiac examinations, normal cardiac dimensions and systolic function on echocardiogram and normal CK levels.

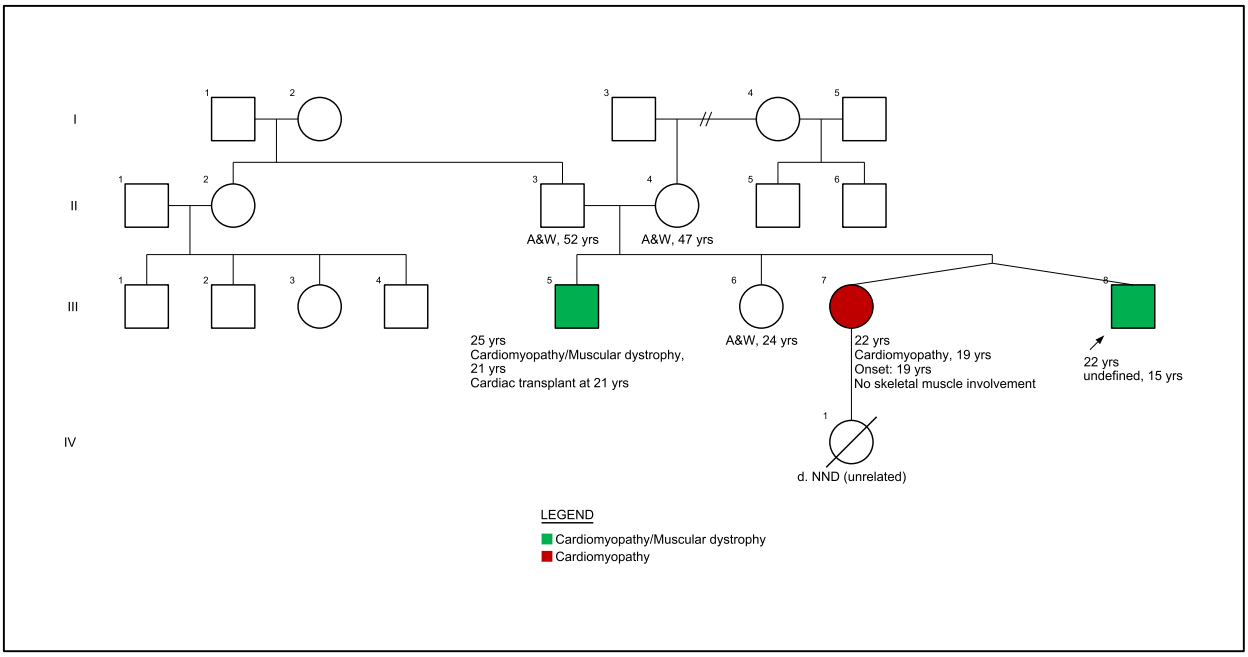

Figure 7.5. Family 27. Dilated cardiomyopathy with associated muscular dystrophy

Family 5 (Figure 7.6) – Genotype positive, *de novo*

Our group (Mayosi *et al*.) recently reported that a genetic mutation in the cadherin-2 (*CDH2*) as a novel genetic cause of arrhythmogenic right ventricular cardiomyopathy (ARVC) in family ACM 2 (Family 2). In addition to the *CDH2* c.686A>C, p.Q229P mutation described in ACM 2 family (Family 2), a second rare mutation in *CDH2* (i.e., *CDH2* c.1219G>A, p.Asp407Asn) was found in another unrelated subject with ARVC, ACM 11.2 (Family 5, III:4).[75] At the time of the original publication, the full clinical and genetic information about the Family 5 was missing. Subsequent additional genetic screening on the Family 5 has demonstrated the absence of this mutation in both asymptomatic parents (II:3 and II:4). Paternity was confirmed with high confidence by means of typing the standard polymorphic microsatellite markers in the father and son. This result confirms that the *CDH2* c.1219G>A, p.Asp407Asn mutation in III:4, which was associated with ARVC, had arisen *de novo (unpublished results provided by Shaboodien, G. CVG Laboratory, Hatter Institute, UCT)*.

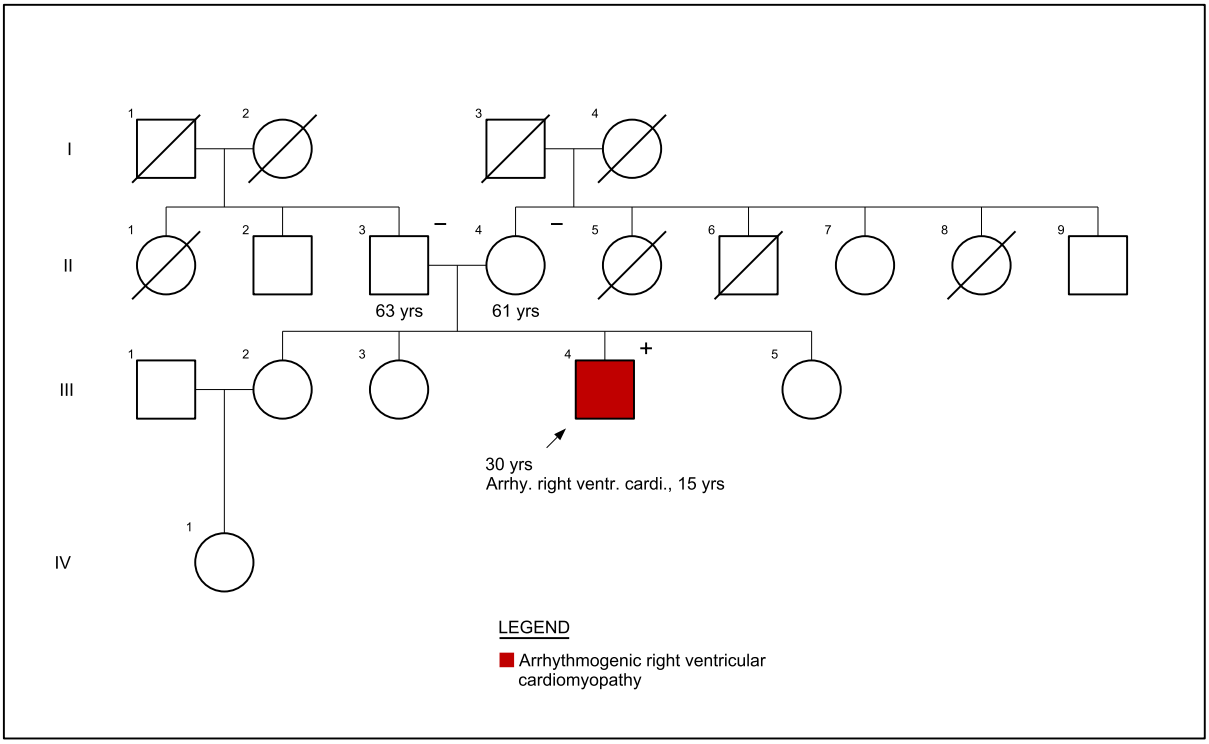

Figure 7.6. Family 5. Arrhythmogenic right ventricular cardiomyopathy

7.4. DISCUSSION

In this study, we describe the inheritance patterns of 35 families recruited to the IMHOTEP study; 16 genotype positive families and 19 genotype unknown families. Autosomal dominant inheritance was found in the vast majority of cases, in keeping with what has been reported in the literature.[4,148,202] Uncertain, although suspected autosomal recessive inheritance was observed in one family, as was X-linked inheritance. The average age of onset of disease in the proband was 28.0 years (ranging from 7 months to 71 years), with 37.1% of probands presenting under the age of 20. More than half the families were Caucasian, with 31.4% and 14.3% represented by mixed ancestry and black African families respectively. The underrepresentation of black African families likely reflects referral bias and societal inequalities related to access to health care that are still in existence as a consequence of the apartheid era in South Africa. Furthermore, the migrant labour system is still observed in South Africa, where individuals move to urban cities such as Cape Town for work opportunities, leaving their families to reside in rural homelands. This social phenomenon greatly impacts the study of families in this ethnic group. In addition, there are a number of foreign nationals from other African countries residing in South Africa, where extended family members have remained behind in their country of origin, limiting our ability to screen these families. Confirmation of cardiomyopathy in relatives from LMICs can be difficult, even when reported by family members, because of variable access to health care, limited availability of sophisticated cardiac investigations and suboptimal record keeping.

The detailed description of selected families illustrates the complexity of phenotyping in cardiomyopathy and the time period required to build multiplex families. In Family 21, clinical evidence of disease only manifested in living relatives 7 years after the initial presentation of the proband. In Families 21, 18 and 27, numerous confounding variables are illustrated – incomplete penetrance; variable age of onset and severity of disease; the uncertainty of the role of environmental influences as precipitating (e.g. viral infections) or protective (e.g.

medications) factors; and variable, overlapping or atypical phenotypes amongst individuals within the same family. Family 21 demonstrates the necessity for tailored serial screening protocols. Family screening in first degree relatives is recommended starting at age 10-12 years,[58] but after the death of the proband's daughter at age 9, clinical screening was advised in all children considered at risk regardless of age. In light of the early age of onset of disease in the daughter and the possibility of compound heterozygosity or homozygosity, her father was also clinically screened (normal findings). CMR was also included in the screening protocol in light of the overlapping phenotype of DCM and LVNC.

A family history of premature SCD was observed in 17.1% of families, with 5.7% occurring under the age of 35 years. The association of premature SCD and cardiomyopathy is well established. *Ranthe* et al. demonstrated that in the general population, a family history of premature death and the risk of cardiomyopathy were most dramatic for familial cardiomyopathy deaths and were much weaker for ischaemic or other cardiac related causes. In that population-based study, the rate of cardiomyopathy increased 100-fold if there was a history of premature death (aged <35 years) in first degree relatives, and increased to more than 400-fold where there were ≥2 premature deaths in first degree relatives within a family.[203] As illustrated in Family 18, SCD can be multifactorial, accounted for by both ARVC and ischaemic heart disease within the same family. Delineation between these two conditions is imperative in determining risk, interpretation of molecular data and the clinical management of the individuals within a family. The observed increases in risk of clinically diagnosed cardiomyopathy in individuals with a family history of premature cardiomyopathy death strongly supports the recommendations of pre-symptomatic family screening.[203]

There are no prospective randomised control trials on the cost-benefit ratio and clinical efficacy of family screening, but the potential benefit for early diagnosis and intervention is well recognised.[58] While we are unable to comment on the outcomes benefit of family screening in this study, the impact of screening can be demonstrated on an individual level in

the families studied. Medical management was initiated and tailored according to the specific findings in each family. By way of examples, in addition to amended screening protocols for relatives and children in Family 21, guideline-based therapy was initiated in the newly diagnosed individual (V:9) prior to the onset of heart failure symptoms, and preconception counselling on the risk of pregnancy and genetic inheritance was provided, altering the decision to go ahead with a planned pregnancy. In Family 18, due to the prominent family history of SCD, in addition to starting beta-blocker therapy in the mildly affected sibling (IV:5), an electrophysiological study was performed (negative) and a loop recorder inserted for this patient. In Family 27, antenatal genetic counselling was provided to the affected pregnant sibling (III:7) regarding the inheritance risk in the foetus (female). In accordance with ESC recommendations,[58,204] she was referred to the combined cardiac-obstetrics clinic, beta-blocker therapy was initiated and she was followed closely throughout her pregnancy, delivery and postpartum. Her cardiac function subsequently normalized on therapy. A number of individuals in the families described required referral for additional psychological support. All families have been enrolled into the molecular genetics research programme.

With complex psychological and genetic underpinning, variable phenotypic expression and rapidly evolving knowledge on disease concepts and management, the need for specialised multidisciplinary services dedicated to the study and management of cardiomyopathies is well recognised.[14,136] When considering genetic testing in cardiomyopathies, the indications may vary according to the complexity and cost of the molecular analysis, the yield of molecular testing, and the impact genetic testing will have on the medical management of individuals and families.[58] Available data from South African cohorts on ARVC and HCM have shown relatively low yield with genetic screening compared to European and Western series.[59,60,96] and very little is known about the genetics of dilated and restrictive cardiomyopathies in African patients. The low yield of genetic screening and paucity of data in the African population supports the necessity for further study on the full spectrum of causal mutations, the search for novel pathogenic variants and investigation into impact of genetic testing on outcomes in

the African context. The potential cost implications of cascade clinical and/or genetic family screening and clinical surveillance as part of standard of care, are considerable. Genetic diagnostic strategies are more likely to be cost-effective than clinical tests alone. Even although the initial costs of genetic testing may be higher, there are potential cost-saving implications of being able to discharge patients without disease-causing mutations from further clinical follow-up.[205] Importantly, the economic decision model utilised to validate the cost-effectiveness of genetic testing in HCM in the UK for example, assumes that the probability of identifying a HCM mutation within a proband is 63%,[205] a significantly higher yield than has been demonstrated in the South African HCM population (29%).[59,60] The cost-effectiveness and the impact of clinical, and/or genetic screening, and clinical surveillance in inherited cardiac conditions in the LMIC setting is yet to be determined.

The challenge faced, beyond the cost and yield of genetic testing, is the correct interpretation of genetic variants – the separation of disease-causing mutations and background variant noise in the genome. The impact of misclassification of benign variant as pathogenic in patients of African descent has been demonstrated in hypertrophic cardiomyopathy. Manrai *et al*. showed that mutations that were most common in the general population were significantly more common among black Americans than white Americans, illustrating the need for sequencing of symptomatic controls and affected patients from diverse populations. In that study, the authors identified multiple persons of African or unspecified ancestry who received positive results based on misclassification of benign variants. Such misclassifications invalidate risk assessments in relatives.[142] In the majority of patients with a definite clinical diagnosis of cardiomyopathy, there is no confirmatory role for routine genetic testing and there are no studies documenting the impact on genetic testing in individuals. The main reason for considering genetic testing is to provide predictive diagnosis in relatives, therefore certainty of pathogenicity of variants is crucial.[58] Routine diagnostic genetic testing in cardiomyopathies in African patients without scrutiny against ethnicity-matched population controls, may yield false positive or negative reports which may in turn have far reaching adverse consequences

for families. Relatives with non-causal variants may be subject to unnecessary repeated clinical evaluations, lifestyle modification (e.g. cessation of sporting activities), therapeutic interventions (e.g. ICD implantation), and the economical and psychological stress associated with an incorrect diagnosis. Relatives without the variant may be falsely reassured that neither they, nor their children, require follow-up screening.

To illustrate the complexity around interpretation further, *Watkins et al.* previously reported the variant *PKP2* c.T2540C L847P in an individual with ARVC from Family 8 (ACM 34). This variant was considered pathogenic as it was not observed in the general population (n=482 local population controls), occurred in positions that were conserved across species and is predicted to be damaging by 4/4 bioinformatics tools.[96] Furthermore, this variant has not been observed in other large population cohorts (Lek et al., 2016; 1000 Genomes Consortium et al., 2015; Exome Variant Server as reported by *ClinVar*). While the *PKP2* c.T2540C L847P variant is rare, segregation with disease has not been demonstrated in the Family 8 due to a lack of sufficient clinical information in relatives, nor is there functional data available on this variant. In addition, the *PKP2* c.T2540C L847P variant was subsequently found in another family (Family 30, DCM 3) with familial DCM, and in this family, it did not segregate with disease (Mbele, M. 2014. Ph.D. Thesis, UCT). Based on current evidence, the *PKP2* c.T2540C L847P variant has been classified as a variant of unknown significance (VUS) by *ClinVar* and cannot be considered predictive of disease until there is more conclusive evidence to support pathogenicity.

Segregation with disease can be difficult to demonstrate in the setting of conditions with low penetrance, such as ARVC, and careful clinical phenotyping of extended family members in conjunction with molecular testing will be required to establish causality of this genetic variant in Family 8. To add to the complexity, a recent study looking at variant non-segregation in LMNA-related DCM challenges the traditional view of cardiomyopathies as strictly monogenic disorders (in particularly DCM) and that multigene causation may account for non-segregation

of variants in families.[206] This observation suggests that non-segregation within a family does not necessarily exclude a variant as disease-causing, and further supports the study of large series of families, deeply phenotyped and genotyped, to better understand clinical variability, non-penetrance and nonsegregation.[207]

The strategy for developing a cardio genetics service in Africa calls for the integration of research and clinical care. Based on the current evidence, routine diagnostic genetic testing in cardiomyopathy is likely to yield more questions than answers, and there is insufficient existing infrastructure to support this kind of service on the continent. This study demonstrates the feasibility of an integrated clinical family screening programme as part of IMHOTEP – where simple and/or more detailed phenotyping (depending on clinical indications and resources) can be done for purposes of risk assessment and initiation of appropriate therapy in relatives and for advancing molecular research on the continent. As has been demonstrated through the establishment of large collaborative registries in ARVC and HCM, knowledge generated from these research programmes influences both clinical and genetic diagnostic and management strategies.[61,73]

In South Africa, we propose the development of an integrated service-delivery and research driven cardiomyopathy family screening programme in tertiary centres in each province, with the aim of providing early diagnosis and risk stratification for relatives at risk as part of clinical care. This service will provide the platform for collaborative molecular research through collective acquisition of clinical data from multiple sites with a centralised molecular genetics research laboratory. With the ethnic diversity observed in South Africa, the building of large ancestry-matched control cohorts to interpret variants found is essential, and should not be limited to one region in South Africa. This approach will not only provide answers to what specific mutations should be tested for in our cardiomyopathy patient population, but also provide information on the impact of family screening and genetic diagnoses on clinical outcomes in our setting. An integrated service-delivery and research programme will facilitate

cost-sharing between government and research funding bodies, making it more financially viable in a resource restricted setting. Clinical and genetic screening has numerous clinical and psychological implications for those diagnosed with disease, and therefore cannot be conducted in isolation of the clinical service platform. Standardised protocols for appropriate counselling and therapeutic interventions will need to be tailored from international guidelines according to resource availability to ensure equity of care in the context of local disease burdens. For example, primary preventative ICDs are not currently available as standard of care in state funded institutions in South Africa, and the additional cost implications of providing (or not providing) this level of care must to be carefully considered. An integrated clinical and research programme ensures collective accountability in both patient care and quality of scientific research, where the ethical implications of genetic testing are carefully considered for the individual, the family and the broader community.

7.5. LIMITATIONS

There are several limitations to this study. The selection of families for screening has been opportunistic and has been largely based on the availability of research funding and clinical resources, as well as the geographic locations and availability of family members. As family screening has not been routinely conducted on all probands enrolled into IMHOTEP, this study does not represent the full spectrum of familial cardiomyopathies in our cohort. The higher frequency of DCM and ARVC reflects the incorporation of existing studies on familial DCM and ARVC at our institution and does not represent the prevalence of these conditions within our population.

7.6. CONCLUSION

In comparison to the international work done in clinical genetics in cardiomyopathies, our findings are consistent with what has been found elsewhere. The relevance of this study is

that it illustrates the feasibility of detailed phenotyping, clinical screening and the potential for molecular genetics research in Africa. It represents the collective efforts of 20 years of work within a single centre in a low-to-middle income country, and the foundation on which an integrated clinical and research cardio genetics service can be built in Africa.

7.7. CONTRABUTIONS AND ACKNOWLEDGEMENTS

The design and implementation of this study was conducted by S. Kraus in consultation with B. Mayosi and N. Ntusi. While a number of families were incorporated from existing studies, all families were revaluated, followed up and extended by S. Kraus. Phenotyping data was collected and analysed by S. Kraus. Pedigrees were constructed and/or updated by S. Kraus, N. Laing and A. Ross. We acknowledge the work done by previous study investigators (referenced in table 7.2) particularly B. Mayosi and A. Wonkam, in building this cohort of families. Recruitment and counselling of families was done by S. Kraus and genetics counsellor, N. Laing, assisted by U. September (research nurse), M. Van der Wall (medical technologist), and A. Ross (genetics counsellor). We acknowledge G. Shaboodien for providing unpublished genetics information for family 5 and family 136.

CHAPTER 8: The vision for IMHOTEP and future research

The overall goal of the IMHOTEP study is to improve the outcomes of African patients and families affected by heart muscle disease. The registry was designed with the intention of providing a platform for development in three major areas: (1) research, (2) clinical practice and (3) education and training, through collaboration and collective expertise (Figure 8.1).

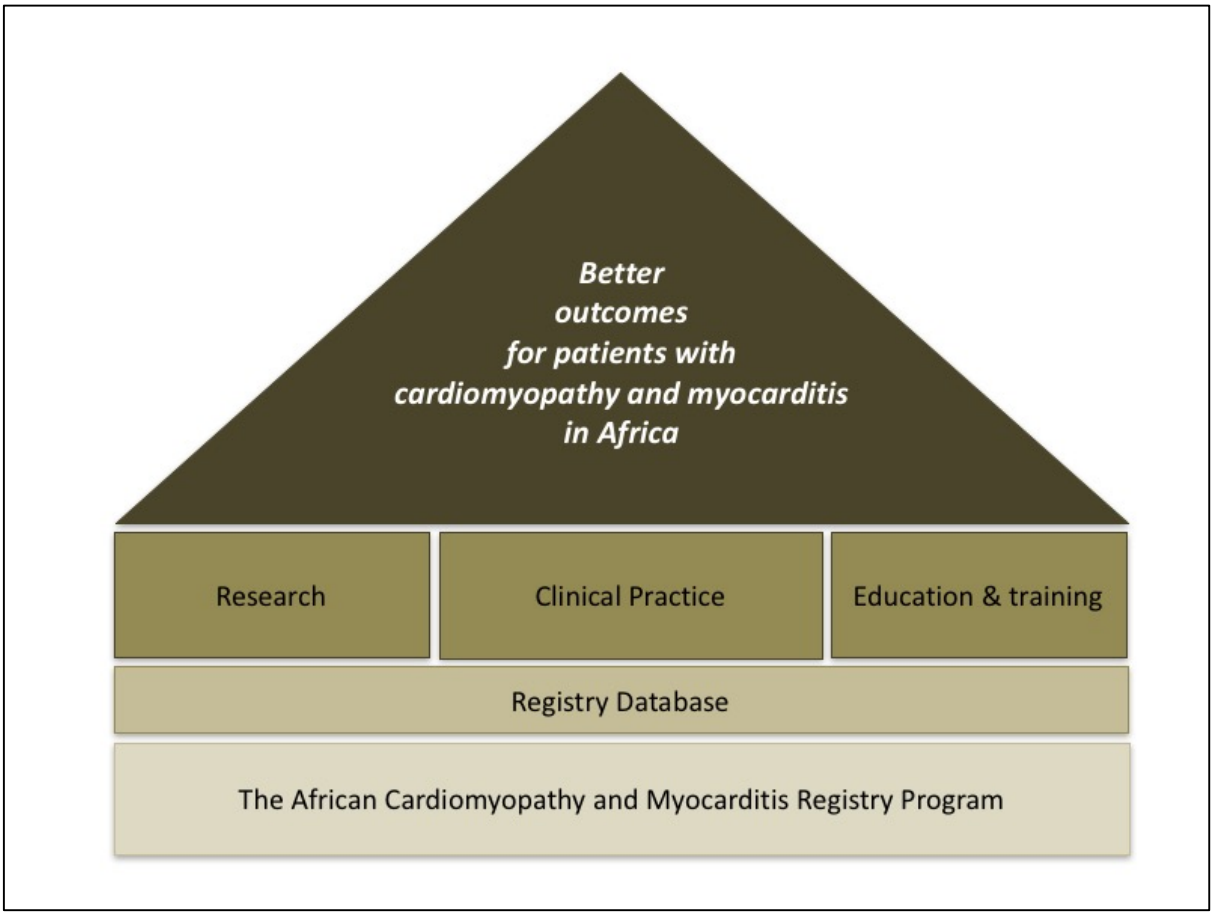

Figure 8.1. The IMHOTEP Study platform

8.1. RESEARCH

8.1.1. Molecular genetics research

In addition to the clinical research described in this dissertation, the IMHOTEP registry has provided the clinical framework on which to build on African-initiated molecular genetics research. One of the principal aims of IMHOTEP is to provide insights into the genetic aetiology of cardiomyopathy in African populations and to facilitate the discovery of novel variants through:

- **Molecular genetics sub-study: phase 1** (as described in chapter 3) - all index cases will be screened for known molecular genetic causes of cardiomyopathy using targeted Next Generation Sequencing (currently underway).

- **Molecular genetics sub-study: phase 2** (as described in chapter 3) **–** genotype-negative probands of multiplex families and children with severe early-onset cardiomyopathy in family trios will be subjected to whole exome sequencing (WES) to identify novel genetic causes of cardiomyopathy.
    - **Multiplex families:** The study of families with cardiomyopathy that are genotype-negative for known pathogenic mutations. Molecular genetic studies are underway for the families described in Chapter 7 by the Cardiovascular Genetics Laboratory at the Hatter Institute for Cardiovascular Research in Africa, University of Cape Town.
    - **Parent-offspring trios:** The study of children presenting under the age of 12 years with severe cardiomyopathy phenotypes that are genotype-negative for known pathogenic mutations. Parent-offspring trios consist of two clinically unaffected parents with a severely affected child. The study of affected children (especially affected infants) using the trio is highly informative in terms of genetic yield, with far greater power to detect causal genes than in autosomal dominant families with

up to 5 affected members. Furthermore, infant and childhood onset cardiomyopathy is rare and the children identified by IMHOTEP make this an internationally competitive resource that will inform genetic aetiology in Africans. Planned postdoctoral work, in collaboration with the University of Oxford, will focus on conducting molecular genetic analysis on parent-offspring trios recruited to IMHOTEP. The specific objectives will be to conduct WES analysis in parent-offspring trios, with functional characterisation of novel mutations identified (e.g. biochemical and functional studies in CRISPR gene edited iPS-derived cardiomyocytes). In addition, we plan to test the contribution of low penetrance rare variants in known genes (and potentially novel genes), and look at modifying influences of low-frequency variants of intermediate size – a unique area of expertise that exists at Oxford. This work will provide exposure to statistical analysis to assign significance to findings and perhaps lead to new ways of individualizing risk. Furthermore, we would like to explore the application of our findings into local clinical practice, particularly with regards to predictive testing in families, evaluating the usefulness of genetic testing in distinguishing early-onset familial cardiomyopathies from myocarditis in children, evaluating the impact of genetic testing on outcomes and the potential development of a targeted NGS diagnostic panel tailored for use in Africa.

- **Future studies:**
  - Planned studies on clinical features and genetics of endomyocardial fibrosis.
  - Planned studies on novel gene modifiers in local patients with HCM.

8.1.2. Clinical research

- **Outcomes sub-study:** IMHOTEP has been created as a clinical registry to facilitate data collection on African patients and families with heart muscle disease over an extended period of time, in order to evaluate the impact of standard of care on patient outcomes.
- **Myocarditis sub-study**: Clinical (including CMR) and multiomic approach to understanding myocarditis amongst Africans.

8.1.3. Planned expansion of IMHOTEP

- We hope to secure funding to expand IMHOTEP to include additional sites in collaborating countries as listed in Appendix J, using a staggered approach (as in the multicentre pilot).
- We hope that IMHOTEP will become a platform that will provide genetic testing opportunities for other African countries.

Candidate's contributions: *S. Kraus intends to continue contributing to the IMHOTEP project. She has a planned postdoctoral fellowship in the Department of Cardiovascular Medicine at Oxford University with the Watkins Research Group, where NGS on IMHOTEP samples is being conducted, and plans to lead the parent-offspring trio sub-study. In addition, she remains involved in the IMHOTEP project and is co-ordinating the outcomes sub-study.*

8.2. CLINICAL PRACTICE

"The prevalence, complexity, clinical importance, heterogeneity and unpredictability of inherited cardiovascular diseases make the development of inherited cardiovascular disease centres an inevitability, with the ultimate goal of reducing the morbidity and mortality associated with these conditions. An inherited cardiovascular disease centre may be seen as

a subunit of a cardiology department, with health professionals specializing in these types of disorders, organised to provide excellence in all related areas, including diagnosis, treatment, follow-up, prevention, risk stratification and prognosis. Among its objectives are the development of action protocols and the creation of databases that enable patients to be included in national and international research networks. To achieve these objectives these centres should include functional units of clinical and basic sciences, research, training and education, acting in harmony in a holistic approach to patients and their families."

Nuno Cardim, António Freitas and Dulce Brito[208]

As described in chapter 7, the goal is to create centres of excellence In Africa that integrate clinical care and research (clinical and scientific) with the intention of providing appropriate and sustainable health care for patients with familial and acquired cardiomyopathies according to resource availability, in addition to the development of expertise in the field of cardio genetics on the African continent. Centres of reference or expertise are made up of the following components: (1) appropriate capacity to diagnose, follow up and manage patients, (2) attractiveness (measured through volume of activity), (3) the ability to provide expert advice on diagnosis and management, (4) the ability to produce and adhere to good practice guidelines, (5) demonstration of a multidisciplinary approach, (6) a high level of expertise and experience demonstrated through publications, grants, honorary positions, (7) teaching and training, and (8) a strong contribution to research.[208] IMHOTEP hopes to clarify the components of what clinical services are required to meet local needs, which may vary across different regions.

Candidate's contributions: *The Cardiomyopathy Specialist Clinic at Groote Schuur Hospital was established by B. Mayosi and K. Sliwa. S. Kraus took over the running of this clinical service for 5 years while conducting her PhD. In addition, she developed the research-based family screening programme (with support from N. Laing) described in chapter 7. A strategy for developing a cardio genetics service in South Africa with potential expansion to other*

regions on the continent, is described in chapter 7 (discussion). The establishment of a cardio genetics service and centres of excellence in cardiomyopathy on the African continent, is the candidates personal ambition. N. Ntusi is involved in CMR research and service development on the continent.

8.3. EDUCATION AND TRAINING

In addition to conducting scientific work that will hopefully broaden our understanding of genetics in African patients with cardiomyopathy, a principal vision of IMHOTEP has been to provide professional development for African based co-investigators and develop collaborative expertise in the field of heart muscle disease on the continent. We have hosted three IMHOTEP investigator meetings (October, 2016 – Cape Town; December, 2017 – Cape Town; November, 2018 – Maputu, Mozambique) in which collaborators have been able to come together and share expertise and experience. Current funding has provided opportunities for studentships in molecular genetics, clinical cardiovascular research and CMR.

Candidate's contributions: *S. Kraus has conducted all site initiation training and given lectures on different aspects of cardiomyopathy at all Investigator Meetings. In addition, she has and will continue to supervise student projects related to IMHOTEP.*

The establishment of an African Myocardial Diseases Working Group would be an important step in developing research, clinical care, and education/training goals discussed in this chapter.

The IMHOTEP Study official logo is shown in Figure 8.2.

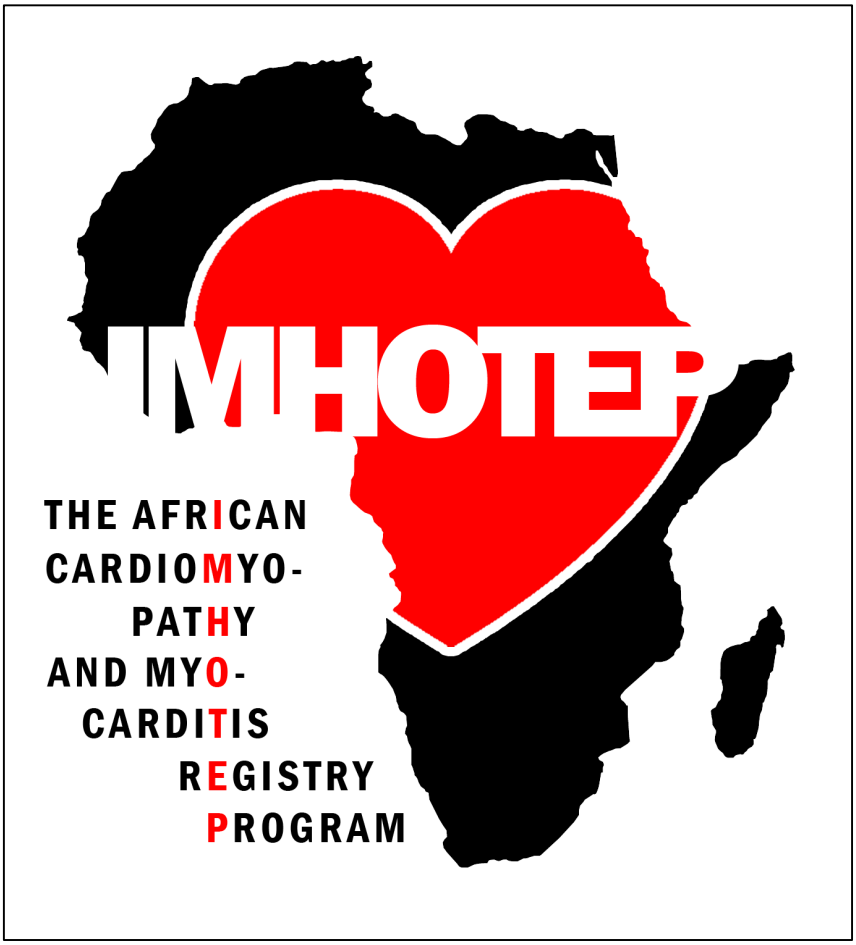

Figure 8.2. The IMHOTEP Study logo. *Designed by Peter Kraus*

CHAPTER 9: Conclusion

Heart failure is an important cause of morbidity and mortality worldwide. Studies have shown that cardiomyopathies contribute significantly to the burden of heart failure in African patients. There is a recognised need for large, multi-centred studies to better understand the genetic underpinnings of cardiomyopathy and the role of non-genetic and environmental factors in the development of heart muscle disease on the continent. The principal aim of this doctorate was to create a collaborative registry applicable to centres within LMIC setting, to systematically capture clinical, genetic, and outcomes data on both adults and children with heart muscle disease from multiple regions on the African continent, over an extended period of time.

We built the foundations for IMHOTEP through the development of study tools and a comprehensive database, and have successfully implemented the initiative. To date, IMHOTEP has recruited over 600 patients of all ages with various forms of cardiomyopathy from 6 different sites in South Africa, and over 35 affected families. We believe that the series of sub-studies presented in this body of work, support our hypotheses that cardiomyopathies are caused by familial and non-familial factors, and that a number of secondary factors (e.g. pregnancy, chemotherapy, HIV, inflammatory conditions, illicit drug and alcohol use) contribute to the development of disease in our population. We suspect that the absence of acute myocarditis in our cohort is a reflection of the study design, specifically the timing of recruitment (median time of recruitment was 162 days after onset of symptoms), rather than a lack of disease. While further work is still required in the molecular genetic aspects of the work, we have described phenotypic overlap between the different morphological types of cardiomyopathies and demonstrated the importance of accuracy of diagnosis, aetiology, and detailed phenotyping in individuals and families affected by heart muscle disease.

One of the key challenges encountered in the creation of this registry has been in the standardisation of case definitions. Morphological and functional parameters vary substantially between the different types of cardiomyopathies. In addition, specific parameter definitions vary according to the age and size of the patient, and whether the patient is a proband or a relative. Furthermore, the influence of medical therapies and interventions on the natural history of disease is difficult to quantify or define. We, therefore, adopted the descriptive definitions provided by the ESC, and developed an adapted 3 stage diagnostic approach to facilitate more accurate diagnoses applicable to all centres regardless of resource availability. By utilising CMR in a significant proportion of incident cases recruited, we have demonstrated that the use of our tailored diagnostic approach in conjunction with the adapted inclusion/exclusion criteria, allow for reliable diagnoses of cardiomyopathy using non-invasive core investigations. While CMR has proved useful in delineating overlapping phenotypes and confirming specific aetiological diagnoses, only 4/103 incident patients recruited were excluded based on CMR and/or other extended (e.g. invasive) investigations after enrolment. We have demonstrated that LV dysfunction secondary to ischaemia in patients presenting with a 'DCM phenotype' is infrequent in our population group and routine angiography is not necessarily indicated unless a patient has specific risk factors. Importantly, hypertension is the leading cause of HF in Africa and therefore hypertensive heart disease with HF with reduced ejection fraction (HFrEF) is the most important differential diagnosis to consider. We have also been able to demonstrate that echocardiographic parameters collected from clinically generated reports correlated with CMR findings fairly accurately (except in the assessment of the right ventricle where CMR is significantly superior). We are therefore confident that this study is feasible in resource restricted environments provided the core investigations (including ECG and echocardiogram) are done well by trained individuals, with the exception of ARVC where more extended investigations are required to confirm the diagnosis.

Although the reclassification of patients referred with suspected ARVC according to the updated 2010 diagnostic criteria was labour intensive, the clinical and research implications of the diagnostic amendments cannot be underestimated, as 92/162 patients referred did not have clinical criteria to support a diagnosis of ARVC. These diagnostic changes reflect the evolution of our understanding of the pathogenesis and genetic aetiology of this condition over 2 decades, the influence of sophisticated diagnostic modalities (e.g. CMR), and clinical expertise in the interpretation of results. The careful review of cases by an experienced diagnostic panel with appropriate expertise is particularly important in ARVC and an essential component of the registry structure. Despite the complexity of the diagnosis of ARVC, we did find that the combined presence of VT with LBBB morphology and repolarisation changes in the precordial leads on ECG were present in 71.2% of patients, therefore, the ECG represents an important screening tool in ARVC and can facilitate appropriate referral of patients to centres that have sufficient expertise to confirm the diagnosis.

While the observational nature of the study limits generalisability of our findings and translation into clinical practice, this work has provided key insights into the demographic and clinical profile of patients affected by these conditions in our setting. Preliminary baseline data on both prevalent and incident cases has shown that in contrast to European cohorts, our patients are significantly younger, there is a slight female predominance overall, and DCM is the predominant form of cardiomyopathy encountered in the hospital setting. The younger age of onset has potentially significant social implications at individual, family, community, and population levels, as economically active individuals and women of child-bearing age are at risk of developing a condition that carries significant morbidity and mortality. The social implications of these conditions have not been explored in this body of work, but the observation is an important one and further exploration into the social impact of these conditions is considered necessary. South African population statistics released in July 2018, estimate that 51% of the population is female and 81% of the population is black African. While our cohort is ethnically diverse, it is likely that the black African population is still

underrepresented. Importantly, the patients described in this thesis come from the Western Cape Province in South Africa, where there is a higher percentage of mixed race individuals, therefore the demographics presented in the prospective arm of the study is representative of the local population. The predominance of Caucasian participants in the ARVC cohort and families study reflects historical referral bias.

CMR has been shown to be diagnostically useful modality in cardiomyopathy. To our knowledge, we have reported the first CMR series of cardiomyopathy patients from Africa. The most notable observation was the high prevalence of LGE in our DCM patients, compared with what has been reported in the literature (linear mid-wall enhancement 94.6% versus 28%). This correlated with elevated T1 times in those cases where T1 mapping was available. The underlying mechanisms of mid-wall fibrosis are multifactorial and the reason for this finding is unclear. LGE has been associated with poorer outcomes and may in part account for the poorer outcomes previously observed in African patients, however, this has not been verified in this study.

Although the IMHOTEP study is ongoing and we have not yet reached our full complement of recruited cases (target n=750), we have made some important observations based on outcomes data from the prevalent DCM and ARVC cohorts. There appears to be an encouraging trend of improvement in survival in DCM compared to a previous study conducted at our institution with transplant-free survival probability of 82.2% at 5 years (compared to 60% previously reported). We have shown that patients with PPCM have a better overall prognosis compared to other forms of DCM. Additionally, pregnancy has been noted to be a precipitating factor in patients with underlying DCM, particularly familial DCM. Whether the decompensation of patients with underlying DCM in pregnancy is also related to abnormal prolactin production remains unclear, and further study is required to establish whether this marker could be used to distinguish PPCM from underlying DCM in postpartum women presenting with HF. We hope that IMHOTEP will provide further insights into the genetic profile

of PPCM, particularly in those that do not recover their LV function on therapy. Our findings support previous reports suggesting that digoxin is an independent predictor of mortality in DCM; however, the reason for this association remains unclear. In the ARVC cohort, we found that there was no significant difference in survival between genotype positive and negative/unknown individuals, nor was there a significant difference in survival between those with or without ICDs although SCD was more common in those without devices. As the numbers in this study are small and there is notable selection bias, these results should be viewed with an appropriate degree of caution.

While the study of families has not provided novel observations in the clinical genetics of cardiomyopathy, we have established a research-based family screening programme that has proven to be feasible in our setting and has provided a platform for molecular genetics research. Each family recruited facilitates novel gene discovery, providing research development opportunities for student scientists on the continent, and the potential to fill the current knowledge gap on the genetics of cardiomyopathy in African populations. The importance of detailed phenotyping in genetics research has been illustrated and while the impact of family screening on overall outcomes has not yet been demonstrated, we have been able to show that it improves the care of individuals within affected families. We have also demonstrated the role of genetic counselling and the adaptation of clinical screening guidelines to the specific needs of families, as well as the necessity for long-term follow-up. We have highlighted the complexity of genetic results interpretation and described some of the challenges associated with application into the clinical setting. Furthermore, we have identified the need for large ethnically diverse African-based population control cohorts, and that expertise in variant interpretation and risk prediction specifically applicable to our population is currently lacking. With the low yield of genotype positive results in patients with ARVC and HCM reported in this dissertation and previous studies conducted in South Africa, and the current deficiencies in knowledge and expertise, we conclude that further research

and capacity building is required prior to initiating a diagnostic genetic service for inherited cardiac conditions on the continent.

While there are a number of study limitations described in the preceding chapters, including notable selection bias particularly in the retrospective cohorts, IMHOTEP does represent a new frontier in cardio-genetics research in Africa. To the best of our knowledge, IMHOTEP is the largest African-based study on heart muscle disease to be conducted to date, and the molecular genetics analysis currently underway will provide vital and novel data on the genetic underpinning of heart muscle disease in Africans. IMHOTEP has provided important insights into the manifestation of these conditions within our population and the feasibility of research comparable to international registries, despite the resource restraints that exist on the continent. It has also highlighted the complexity of these conditions and the requirement for specific expertise, careful investigation and individualized care, in addition to exposing gaps in existing health care practices (e.g. delayed referral to specialist care). By including both adults and children, and building capacity for long-term follow-up, we hope to provide further understanding on how these conditions affect individuals and families over a complete lifespan. We have every expectation that IMHOTEP will continue to expand to include centres across the African continent and develop into network of collective expertise in all forms of heart muscle disease.

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APPENDIX

A. Human Ethics Research Committee (HREC) approval for the IMHOTEP study

B. IMHOTEP informed consent for participation in the registry (adults and minors)

C. IMHOTEP assent for participation in the registry

D. Illustrated consent for children

E. Consent for collection, storage and analysis of DNA samples

F. Information sheets/booklets for patients

   I. Cardiovascular genetics research

   II. Cardiomyopathies in children (Z-pamphlet)

   III. Dilated cardiomyopathy in adults (Z-pamphlet)

   IV. Information booklet on ARVC

   V. Information booklet on HCM

   VI. Information booklet on LVNC

G. Clinical algorithms

   I. Cardiomyopathy - 3 stage diagnostic approach

   II. Myocarditis

H. Standard operating procedures for recruitment

   I. Algorithm for recruitment of prevalent cases from existing studies at Groote Schuur Hospital

   II. Algorithm for recruitment of incident and newly identified prevalent cases to IMHOTEP

I. Case Report Form for baseline core data (adults)

J. List of collaborators

K. Family Pedigrees

APPENDIX A

Human Ethics Research Committee Approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 404 7682 • **Facsimile** [021] 406 6411
Email: nosi.tsama@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

21 October 2014

HREC REF: 766/2014

Prof B Mayosi
Department of Medicine
Old Main Building

Dear Prof Mayosi

PROJECT TITLE: RATIONALE, DESIGN AND IMPLEMENTATION OF THE AFRICAN CARDIOMYOPATHY AND MYOCARDITIS RESGISTRY (ACMR) (incorporating the following studies: A CLINICAL AND GENETIC STUDY OF FAMILIAL DILATED CARDIOMYOPATHY IN SOUTH AFRICA HREC REF 197/96, THE ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY REGISTRY OF SOUTH AFRICA HREC REF 047/2003)(PhD candidate Dr Sarah Kraus)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th October 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please add the PI contact details and the HREC contact details to all the informed consent documents.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

We acknowledge that the PhD student, Dr Sarah Kraus is also involved in this study.

Please quote the HREC reference no in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

APPENDIX B – Informed Consent

IMHOTEP Informed Consent for Adults

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

African Cardiomyopathy and Myocarditis Registry Program
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Email: s.kraus@uct.ac.za
***UCT** HREC REF: 766/2014*
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Informed Consent for Adults

I agree to participate in the study of cardiomyopathy and/or myocarditis. I understand that cardiomyopathy is a heart muscle disease that may lead to heart failure and ventricular arrhythmias, and that myocarditis is inflammation of the heart muscle that can lead to cardiomyopathy. I understand that I will be interviewed about my medical history, family history and medications, and I will be examined. I understand that I may undergo further investigations as per standard of care guidelines for my condition. These investigations will only be done if necessary to confirm a diagnosis of cardiomyopathy and/or myocarditis, or as part of the medical treatment for my condition.

In addition, I will have a blood sample drawn consisting of a total of 25ml of blood (5 teaspoons). This blood will be used to test for and study genetic factors that cause cardiomyopathy. 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. The genetic material will not be used for the purposes of gene alteration. My blood sample will sent to the molecular genetics laboratory at the Hatter Institute, University of Cape Town. The DNA analysis will be done at that laboratory and/or other local and international laboratories that are collaborating in this study.

After my initial assessment, I agree to be followed up annually to access if there have been any changes in my symptoms, medication, or whether I have required any additional investigations, suffered from any complications of the disease or required any procedural interventions. I give permission for the study investigators to contact my attending doctor(s) to access all medical information regarding my condition.

As cardiomyopathy is a familial (genetic) disease in 30-50% of people, I agree to have my first-degree relatives contacted for clinical screening and participation in this study. Each family member will be counseled about cardiomyopathy and will have to sign a consent form prior to being included in the study.

I understand that my participation in this study is entirely voluntary. All information gathered is strictly confidential, and will only be used for research relating to the study of cardiomyopathy and/or myocarditis. This information (including the genetic material) will not be used to generate profit. 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 jeopardizing my future care. All results that are relevant to the medical management of my condition will be made available to my attending doctor(s) and myself. If I have any questions, I may contact _____ at _____.

I agree to participate in the African Cardiomyopathy and Myocarditis Registry and I have been given a copy of this form.

_____	_____	_____
Participant name	Participant signature	Date
_____	_____	_____
Witness name	Witness signature	Date
_____	_____	_____
Investigator name	Investigator signature	Date

IMHOTEP STUDY NUMBER | | | | | | | | |

APPENDIX B – Informed Consent

IMHOTEP Informed Consent for Minors

UNIVERSITY OF CAPE TOWN
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***UCT** HREC REF: 766/2014*
Tel: +2721 4066492

Informed Consent for Minors

I agree to allow my child/ward, _____, to participate in the study of cardiomyopathy and/or myocarditis. I understand that cardiomyopathy is a heart muscle disease that may lead to heart failure and ventricular arrhythmias, and that myocarditis is inflammation of the heart muscle that can lead to cardiomyopathy. I understand that my child/ward and I will be interviewed about his/her medical history, family history and medications, and he/she will be examined. I understand that my child/ward may undergo further investigations (tests) as per standard of care guidelines for his/her condition. These investigations will only be done if necessary to confirm a diagnosis of cardiomyopathy and/or myocarditis, or as part of the medical treatment for their condition.

In addition, my child/ward may have a blood sample drawn consisting of a 5 - 25ml of blood (1 – 5 teaspoons) depending on the size of my child (not more than 3ml/kg for a small child). This blood will be used to test for and study genetic factors that cause cardiomyopathy. 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. The genetic material will not be used for the purposes of gene alteration. My child's blood sample will sent to the molecular genetics laboratory at the Hatter Institute, University of Cape Town. The DNA analysis will be done at that laboratory and/or other local and international laboratories that are collaborating in this study. I can refuse to have blood taken from my child, even if I sign this consent form.

After my child's/ward's initial assessment, I agree to be followed up annually to access if there have been any changes in his/her symptoms, medication, or whether he/she has required any additional investigations, suffered from any complications of the disease or required any procedural interventions. I give permission for the study investigators to contact my child's/ward's doctor(s) to access all medical information regarding his/her condition.

As cardiomyopathy is a familial disease in 30-50% of people, I agree to have my first-degree relatives contacted for clinical screening and participation in this study. Each family member will be counseled about cardiomyopathy and will have to sign a consent form prior to being included in the study.

I understand that my child's/ward's participation in this study is entirely voluntary. All information gathered is strictly confidential, and will only be used for research relating to the study of cardiomyopathy and/or myocarditis. This information (including the genetic material) will not be used to generate profit. I will not be identified in any published report. I am free to refuse to participate or withdraw my child/ward from the study at any time, without jeopardizing his/her future care. All results that are relevant to the medical management of his/her condition will be made available to my attending doctor and myself. If I have any questions, I may contact _____ at _____.

I agree to allow my child/ward to participate in the African Cardiomyopathy and Myocarditis Registry and I have been given a copy of this form. If my child is aged 8 years or older and is able, he/she can sign an assent form, agreeing to participate. If my child/ward refuses to participate, he/she will not be included in the study.

_____	_____	_____
Parent/Guardian's name	Participant signature	Date
_____	_____	_____
Witness name	Witness signature	Date
_____	_____	_____
Investigator name	Investigator signature	Date

IMHOTEP STUDY NUMBER | | | | | | | | |

Version 3; 1 August 2016

APPENDIX C – Assent

IMHOTEP Assent for Children

UNIVERSITY OF CAPE TOWN
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Assent form for children aged 8 and above

We are doctors and nurses from IMHOTEP study, and we are doing a study of the hearts of children and adults with cardiomyopathy. Cardiomyopathy is a problem that affects the heart muscle.

We would like to ask you some questions about your health. This will be very quick and will not hurt at all.

We would like to do some special tests where we take a picture and a video of your heart by using a special machine. This is the same machine that your doctor may have used before to look at your heart. This will not hurt at all.

We would also like to take some blood. The prick of the needle will cause some pain but it will be over quickly. We will take only a few teaspoons of blood (maximum five teaspoons) depending on how big you are. Your blood will be sent to a special laboratory where other doctors and scientists working with your doctors in this study, will be able to do special tests on your blood.

If you agree to take part in this study, you will be helping doctors to learn more about the problem with your heart, and how to treat other children with the same heart problem.

You are allowed to say that you don't want to be in the study. Nobody will be angry with you if you say no.

Before you decide, you can ask questions, or talk to your mother and father about this.

By writing your name, or stamping your fingerprint, on this form, you are saying **yes** to being in our study.

_____		_____
Participant name/fingerprint		Date
_____	_____	_____
Parent/Guardian name	Parent/Guardian signature	Date
_____	_____	_____
Investigator name	Investigator signature	Date

IMHOTEP STUDY NUMBER ☐☐☐ ☐☐☐☐☐

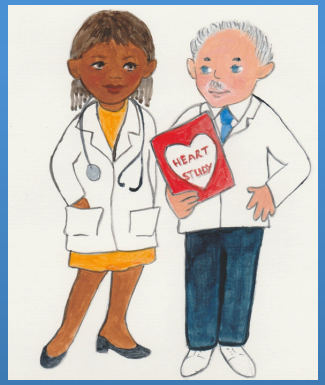
Version 3; 1 August 2016

APPENDIX D – IMHOTEP Illustrated Consent for Children *(illustrated by Dr. Elisabeth Kraus)*

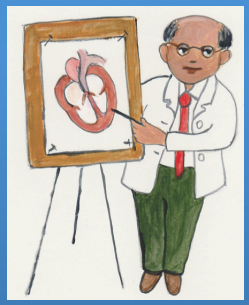
IMHOTEP: The African Cardiomyopathy and Myocarditis Registry Program

HREC REF: 766/2014

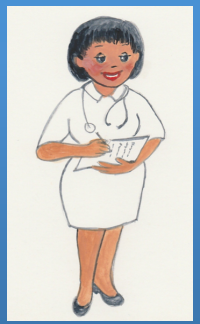
INFORMED CONSENT FOR CHILDREN



Doctors and nurses are doing a study on the hearts of children

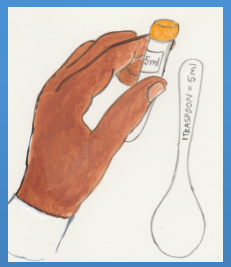


Cardiomyopathy is a problem that affects the heart muscle



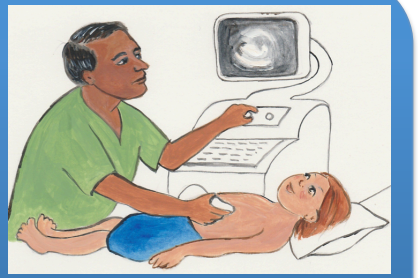
We would like to ask you questions about your health

Do you have any questions for the doctor?

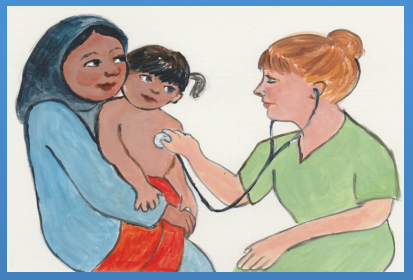


We would also like to take some blood. The prick of the needle will cause some pain but it will be over quickly

We would like to do special tests. This test helps us to make a drawing of the heartbeat. This won't hurt at all



We would like to take a video of your heart. This won't hurt at all



If you agree to be part of our study, you will be helping doctors learn more about the problem with your heart and how to treat children with the same heart problem

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IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

J52 UCT Clinical Research Unit, Old Main Building, Groote Schuur Hospital, Cape Town, Tel: 021 4067674

APPENDIX E – Consent for collection, storage and analysis of DNA samples

REQUEST FOR MOLECULAR STUDIES (DNA)

Molecular Laboratory
Division of Cardiovascular Genetics
4th floor, Chris Barnard Building,
UCT Medical School, Observatory 7925
Tel: (021) 406 6615 Fax: (021) 4478789

Blood should be drawn in plastic EDTA Tubes (Purple top)
20ml of blood in total is required in adults (2-4 EDTA tubes)
Please label blood tubes with the patient's name and DOB.
Place blood in fridge at 4°C until able to send to laboratory
Please DO NOT send specimens on ice or frozen.

Patient details: (or hospital sticker here)

Surname:_____ First Name (S): _____

Hospital folder number: _____

Sex: M ☐ F ☐ Date of Birth (DD/MM/YYYY): _____

Patient address: _____

Contact numbers: _____

Email address: _____

Referring hospital and doctor: _____

Referring doctor's contact details: _____

Clinical information: (PLEASE COMPLETE A FAMILY PEDIGREE OVER THE PAGE)

New Family: Yes ☐ No ☐ **Family name:** _____

Ethnic Origin: Black African ☐, Mixed race ☐, Caucasian ☐, Asian ☐, Other _____

Provisional Diagnosis:

Clinically affected ☐ At Risk (unaffected clinically) ☐ Spouse ☐ Query ☐

Morphofunctional phenotype	**Specific diagnosis**
☐ Dilated cardiomyopathy	☐Familial ☐Idiopathic ☐PPCM ☐Secondary (specify) Specify (e.g. myocarditis)
☐ Hypertrophic cardiomyopathy	☐HCM ☐HCM – phenocopy (specify) Specify (e.g. Noonans)
☐ ARVC	☐Definite ☐Borderline ☐Possible ☐Unconfirmed
☐ Restrictive cardiomyopathy	☐Familial ☐Idiopathic ☐Specific aetiology (specify) Specify (e.g. EMF)
☐ Unspecified cardiomyopathy	☐LVNC ☐Takusubu ☐Other (specify) Specify:
☐ Arrhythmia (with no clinical evidence of CMO)	☐LQTS ☐VT ☐VF ☐SCD ☐Other Specify
☐Marfan Syndrome	☐Confirmed clinically ☐Suspected

Additional disorders (apparent or previously treated): _____

Have samples from this patient been sent to a DNA lab before? ☐YES ☐ NO ☐Don't Know
If yes, please specify lab: _____

For Laboratory use only:

DNA number: _____ *Vol.Blood:* _____*(ml)* *Other:* _____

Date Received (DD/MM/YYYY): _____

Informed Consent

I understand that I have agreed to participate in genetics research that will be conducted at the molecular genetics laboratory at the Hatter Insititute for Cardiovascular Research in Africa, situated at the University of Cape Town in South Africa. This laboratory, in collaboration with other local and international laboratories, is dedicated to doing research related to genetic causes of cardiovascular disease in people living in Africa.

I understand that a blood sample (5-25ml or 1-5 teaspoons of blood) will be collected from me and my genetic material will be extracted from this sample for analysis. In some instances, other samples may be collected depending on the circumstances (please specify if collected): _____

I understand that a portion of my genetic material will be stored at the Hatter Institute for additional research projects approved by the University of Cape Town Human Research Ethics Committee (HREC). My genetic material/information, together with other relevent medical information, may be shared with other researchers and institutions involved in HREC approved studies but my personal identifying information will not be shared. I authorize that my doctors can provide relevent clinical information (medical records) to researchers.

I understand that the nature of research means that I may or may not receive a result from studies performed on my DNA. Although the laboratory will do its best to confirm that the findings relate to my condition, results received from a research laboratory should be confirmed diagnostically. If a genetic cause for my condition is found, the researchers from the Hatter Insitute will do their best to inform me of the results, either via my doctor, a genetics counsellor or in writing, depending on the available resources. If my contact details change, it is my responsibility to inform the laboratory. In the event that I am unavailable or incapacitated, **I do / do not** *(please delete where not applicable)* want my immediate family members to be informed of the results.

I understand that the genetics molecular laboratory is under obligation to respect my confidentiality. I understand that my participation in genetics research is entirely voluntary and that I may withdraw my consent at any time without it affecting my future medical care.

_____	_____	_____
Particpant name	Signature	Date
_____	_____	_____
Doctor/nurse/genetics counsellor	Signature	Date

Study: _____ **HREC REF:** _____ _____
(Study number)

APPENDIX F – Information Sheets for Patients

I. Cardiovascular Genetics Research (page 1)

Investigating the Genetics of Heart Disease
Information Sheet for Patients on Genetic Research

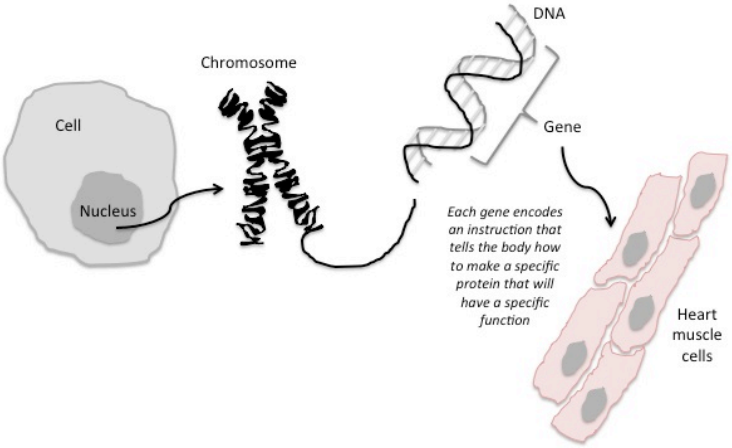
Introduction

You are being asked to participate in a genetic research study because you have been diagnosed with cardiomyopathy. This form provides information about genetics and the risks and benefits of being involved in genetic research. Please read this form and feel free to ask the study doctor or nurse any questions that you may have.

Why are we doing genetic research?

In many cases, cardiomyopathy can be caused by a change (mutation) in a gene within our DNA, which means that this condition can be inherited. In other words, it can affect multiple individuals within a family.

DNA is the code that instructs our bodies how to work and it is organised into sections called genes. Each gene encodes an instruction that tells the body how to make a specific protein that will have a specific function in our bodies. Genes are packaged into bigger structures called chromosomes that are arranged in 23 pairs within every cell (see picture below). One member of each pair of chromosomes is inherited from our mother and from our father. If there is a spelling mistake, or change, within the DNA code within a gene, this means that the instruction will be made incorrectly. A genetic change can be inherited from a parent or it can occur by chance, in an individual, as a new mutation. There could be many different types of changes that can occur within genes, and there could be many different genes that could have these changes in them. Research has allowed us to identify several different genes that are associated with the different types of cardiomyopathy, but there are probably many more genes that have not yet been discovered.



Sometimes a genetic test can be done to try to determine if there is a spelling error in one of your genes. Often these genetic tests cannot look at all the genes in our bodies. Therefore, a negative genetic test does not exclude the diagnosis of inherited (or genetic) cardiomyopathy. Most cardiomyopathies are autosomal dominantly inherited. What that means is that if you inherit one faulty copy of the gene (or it occurs by chance in you for the first time), you will be predisposed to developing cardiomyopathy. If a parent carries a genetic mutation for cardiomyopathy, each child will have a 50% chance of inheriting it from them.

We would like to understand more about what kinds of mistakes (mutations) in the DNA cause cardiomyopathies, particularly in people living in Africa. We would also like to understand what factors cause some people to have more severe heart disease than others.

What will you ask me to do if I want to participate?

Your genetic material (DNA) is in your blood. Therefore we would like to collect some blood samples from you. We will collect a maximum of five tubes of blood. Although this may seem a lot, the total is only about 5 teaspoons (25ml) of blood. In small children, we will only collect one tube of blood (3-5 ml which is about 1 teaspoon of blood). In order for us to know what genetic tests to do on your blood, we need to know exactly what kind of heart problem you have. We therefore need to access your medical records so that we can review your medical history and the specific investigations that have been done.

Version 1: October 2016

APPENDIX F – Information Sheets for Patients

I. Cardiovascular Genetics Research (page 2)

Investigating the Genetics of Heart Disease
Information Sheet for Patients on Genetic Research

What will happen to my samples and information?

Genetics research is done at the Molecular Genetics Laboratory at the Hatter Insititute for Cardiovascular Research in Africa and the UCT Division of Human Genetics Laboratory, situated at the University of Cape Town in South Africa. These laboratories are dedicated to doing research related to genetic causes of disease in people living in Africa. Your genetic material will be stored at the Hatter Institute for additional research projects approved by the University of Cape Town Human Research Ethics Committee (HREC). The genetic laboratory is obligated to respect your confidentiality and all research samples get coded so that individuals working in the laboratory cannot identify who the sample belongs to. Although your genetic material and/or information, together with your medical information, may be shared with other researchers and institutions involved in approved studies, your personal identifying information (name, contact numbers, address etc) will not be shared. It is now common for genetic information to be shared with researchers around the world. The benefit is that many researchers can use the same information for different research projects. We are not always able to do all the tests on the DNA at our laboratories as we may not have the necessary equipment or expertise. For this reason, we sometimes have to ask other institutions and researchers in other parts of the world to help us.

Will I get to know my results?

Due to the nature of research, you may or may not receive a result from studies performed on your DNA. Although the laboratory will do its best to confirm that the findings relate to your heart condition, results received from a research laboratory should be confirmed diagnostically with a confirmatory test. If a genetic cause for your heart condition is found, the researchers from the Hatter Insitute will do their best to inform you of the results, either via your doctor, a genetics counsellor or in writing, depending on the available resources. If your contact details change, it is your responsibility to inform the laboratory. It is important to tell the doctors whether you want your results to be shared with your family in the event that you are unavailable or incapacitated.

What are the risks of being involved in the study and what about confidentiality?

The prick of the needle to take blood can be uncomfortable, but it is not dangerous to take blood. It is possible that someone could find out that you participated in this study. We don't know if this is likely to happen, but we will do our very best to make sure that this does not happen. The genetics molecular laboratory is under obligation to respect and protect your confidentiality.

Are there any benefits to participating in this project?

You will not benefit from participating in this project, and we cannot offer you anything to thank you for participating. Your participation in this project may help us understand why some people develop cardiomyopathy and others do not. This could help us treat other people in the future. If we do find a genetic change (mutation), we may be able to look for the same mutation in other members of your family, and find out who may be a risk of developing the same heart condition as you.

Has this research been approved?

All studies have to be approved by an ethics committee of the University of Cape Town. Research looking at the genetic causes of cardiomyopathies has been approved **(IMHOTEP Study HREC REF: 766/2014)**. The full name of the committee is 'Faculty of Health Sciences Human Research Ethics Committee'. An ethics committee is composed of a group of people who decide whether the study is risky, and whether people should be allowed to participate in it. These people are there to protect research participants like yourself. You can contact the ethics committee if you have any concerns about this study. They are very friendly people and would welcome your questions. Please contact **021 406 6338, 021 406 6626 or 021 406 6496** if there is anything you would like to discuss with the ethics committee.

Do I have to take part and what if I change my mind?

You do not have to take part in this study. You can decide to participate or not. If you decide not to participate then you will still be treated in the same way. You can also decide at any point in time that you do not want your blood samples to be used any longer. Please contact the molecular genetics laboratory at the Hatter Insititute for Cardiovascular Research in Africa if you have any questions or want to withdraw. You can contact us on **021 406 6615**. Alternatively, you can write to us and fax the letter to **021 447 8789** or post it to the **Molecular Laboratory, Divison of Carviovascular Genetics, 4th Floor, Chris Barnard Building, University of Cape Town Medical School, Observatory 7925**. We will destroy your blood samples and genetic material and information. However, once we have shared your genetic information with other researchers, we can no longer destroy it.

Version 1: October 2016

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IMHOTEP
THE AFRICAN CARDIOMYOPATHY AND MYOCARDITIS REGISTRY PROGRAM

The African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP) is a collaborative effort by medical professionals from different African countries to collect information from individuals and families affected by cardiomyopathy, in an attempt to enrich our understanding of the causes of heart muscle disease, and positively impact and improve lives of those living with cardiomyopathy in Africa. By participating in IMHOTEP, you are actively helping the international medical community learn more about this condition

'Cardiomyopathy' is a term that describes a group of conditions that affect the heart muscle. There are many different types of cardiomyopathy

- *Dilated Cardiomyopathy (DCM)*
- *Hypertrophic Cardiomyopathy (HCM)*
- *Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)*
- *Restrictive Cardiomyopathy (RCM)*
- *Left Ventricular Noncompaction (LVNC)*

This pamphlet will explain the types of Cardiomyopathy that affect children most commonly. It will help to explain some of the causes, the investigations required and specific treatment options available for your child.

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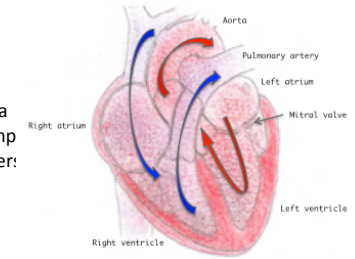
Tel: +27 21 404 7674, Fax: +27 21 406 6711, Email: s.kraus@uct.ac.za
UCT Faculty of Heath Sciences Human Ethics Committee: *HREC REF: 766/2014*
Tel: +2721 4066492

The heart

The heart is a muscular pump with 4 chambers



What is cardiomyopathy?

Your child has been diagnosed with a ***Cardiomyopathy.*** The word "cardiomyopathy" is used to describe a disease that affects the heart muscle, resulting in damage and impairment of the heart's ability to pump blood normally. There are many different types of cardiomyopathies, but the most common form is ***dilated cardiomyopathy (DCM).***

Dilated cardiomyopathy (DCM) is a condition where the heart muscle becomes thinned and stretched (dilated). As a result the heart muscle is weakened and unable to pump blood normally. DCM can be caused by a genetic abnormality or it can be due to direct injury to the heart muscle cells. Injury to the heart muscle cells can be caused by a number of different factors, listed below:

- **Myocarditis** – inflammation of the heart muscle due to an ***infection*** (virus, HIV, bacteria, TB, fungi, parasites), ***toxin***, ***autoimmune condition*** (rheumatoid arthritis, Lupus), or ***Kawasaki disease***
- **Endocrine (hormones) and/or metabolic conditions** – e.g. thyroid disease, abnormal metabolism or impaired energy production in cells (mitochondrial disorders)
- **Nutritional** – deficiencies in thiamine (B1), carnitine, phosphate, selenium
- **Other (less common causes in children):** Iron overload, drugs (e.g. certain chemotherapy agents), alcohol, tachymyopathy (due to a persistent fast arrhythmia)

Your doctor will do specific tests to exclude these causes depending on your child's clinical presentation.

Genetic cardiomyopathies are caused by mutations (mistakes) in the genes that code for a variety of heart muscle proteins that make up the heart muscle cells. As a result, the heart muscle cells are unable to function normally and over time, cardiomyopathy can develops.

Understanding the role of genetics

Our DNA is made up of multiple genes. Each gene codes an instruction that tells the body how to make a specific protein that will have a specific function. Genes are packaged together on chromosomes and each human being has 23 chromosome pairs (46 chromosomes in total). One chromosome from each pair is inherited from your mother, and one from your father. Therefore, you can inherit a genetic mutation from a parent. This is why multiple members of a family can have cardiomyopathy as they share similar DNA. Most cardiomyopathies are autosomal dominantly inherited, which means that if one of your parents carries a genetic mutation for cardiomyopathy, you have a 50% chance of inheriting it from them. Importantly, because you have 2 copies of each gene (one from your mother and one from your father) not everyone who carries a genetic mutation will develop cardiomyopathy but you may become more susceptible to muscle cell damage from other factors listed previously.

Genetic dilated cardiomyopathy can occur in isolation, or occasionally can occur as part of other genetic conditions (e.g. Duchenne Muscular Dystrophy, mitochondrial myopathies, other genetic muscle disorders).

Another type of cardiomyopathy that can occur in children is **hypertrophic cardiomyopathy (HCM).** HCM is a genetic cardiomyopathy where the heart muscle becomes thickened ("hypertrophied") due to an increase in the muscle bulk resulting from excessive contraction of the heart muscle. A thickened heart muscle can also be caused by infiltration of the heart muscle by substances such as fat or glycogen. This occurs if there is a problem with the metabolism of these substances in the body and many organs may be affected. In some cases, hypertrophic cardiomyopathy is associated with other genetic syndromes (e.g. Noonan's syndrome, Friederich's ataxia)

Your doctor will explain to you exactly which kind of cardiomyopathy your child has.

APPENDIX F – Information Sheets for Patients: II. Cardiomyopathies in children (Z-pamphlet) (page 2)

Common symptoms in cardiomyopathy

Heart failure is the most common presentation in children with DCM. The term "heart failure" is used to describe what happens when the heart is unable to pump blood effectively, and can result in shortness of breath and body swelling due to the accumulation of fluid in the lungs and tissues.

Children with heart failure may suffer from breathlessness and/or tiring when doing activities, such as feeding, playing, crawling or walking, This occurs because the heart is not strong enough to keep up with the demands of the activity. Poor weight gain in infants, impaired growth and/or delayed milestones can also occur as a result of heart failure. Due to accumulation of fluid in the lungs, older children may complain of shortness of breath or a heavy feeling over the chest when lying down, and often have to sleep upright. In some cases, children with heart failure may develop leg and/or abdominal swelling, due to the accumulation of fluid in their tissues.

Other symptoms that can occur:

- **Palpitations** – racing, skipping or fluttering sensation in the chest and/or throat
- **Chest discomfort/pain**
- **Dizziness**
- **Syncope** (fainting/blackouts)

If your child has symptoms of palpitations and/or blackouts, it is important to inform your doctor as they may have developed an arrhythmia. An arrhythmia is an electrical problem in the heart where there is an abnormality in the timing, pattern and/or rate of the heartbeat. Arrhythmias occur because of scarring or inflammation within the heart muscle.

What type of arrhythmias occur in cardiomyopathy?

Supraventricular tachycardia (SVT) – SVT causes the heart to beat much faster than normal and can result in worsening heart failure. The heart rate can be slowed with medications.

Ventricular tachycardia (VT) – VT occurs less commonly, but can result in collapse or sudden death. Drugs (like beta-blockers) can be used to prevent VT. In cases where VT occurs recurrently, we sometimes have to insert an Implantable Cardioverter Defibrillator (ICD), which is similar to a pacemaker, to monitor the heartbeat and administer a small shock if VT occurs.

How do we diagnose cardiomyopathy?

The diagnosis of cardiomyopathy is made by taking a history, doing a physical examination and doing some specific investigations that include:

- **Electrocardiogram** – this records the electrical activity of the heart.
- **Echocardiogram** – this is an ultrasound of the heart where we can measure the size and function of the heart.

Neither of these test will hurt your child, however, sedation is sometimes required for small children to keep them still for the duration of the test.

Additional tests may be required to determine the underlying cause of your child's heart condition.

- **Blood tests** for HIV and other viruses, thyroid function, iron levels, cardiac muscle proteins
- **Chest X-ray**
- **Cardiac MRI** – a special scan that allows us to assess the heart size and function more accurately and look for inflammation or scarring within the heart muscle.
- **Cardiac catheterization/angiogram** – it is sometimes necessary to assess the pressures in the heart and lungs, and/or do a small biopsy of the heart muscle. Your child will need to be put to sleep (sedated) for this test.
- **Skeletal muscle biopsy** (taken from the leg)
- If there is concern about an underlying arrhythmia, a 24-hour Holter, exercise stress test and/or electrophysiological study of the heart may be required.

Your doctor will be able to explain these tests to you in more detail if they are required.

How do we treat DCM?

The goals of treatment are to exclude and treat reversible causes, to relieve the symptoms of heart failure, to prevent the progression of disease, and to treat arrhythmias if present. **Medication** is the mainstay of treatment for cardiomyopathy. Each drug has a specific role.

- **Diuretics** ("water tablets") – removes excess fluid in the body to improve symptoms of shortness of breath and body swelling
- **ACE-inhibitors** (e.g. enalapril, captopril) – reduces the workload of the heart and prevents further damage
- **Mineralocorticoid receptor antagonists** (spironolactone) – mild diuretic and protects the heart from further scarring/damage
- **Beta-blockers** – slows the heart beat and prevents further damage to the heart
- **Inotropes** – stimulates heart muscle contraction. Inotropes are usually given IV

Children that are very sick sometimes require admission to intensive care, where intravenous (IV) medication can be given. Some children may require help with their breathing and can be put onto a ventilator to help them to stabilize.

Living with cardiomyopathy

For many parents, this is an unexpected diagnosis and it may take time to psychologically and emotionally come to terms with having a sick child. The doctors, nurses, and other staff such as social workers are here to help you through this process.

While in some cases, the heart is able to recover, in many cases, there is only limited recovery and most children will require regular follow-up visits to the Clinic or Hospital for many years. Many of these children are able to go to school and live fairly normally with their heart condition. Sadly, some children are so sick that their hearts fail and they pass away, despite medical therapy. **Heart transplantation** is sometimes considered in children where the damage to their heart is very severe and permanent. This is not always possible in small children.

APPENDIX F – Information Sheets for Patients: III. Dilated cardiomyopathy in adults (Z-pamphlet) (page 1)

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IMHOTEP
THE AFRICAN CARDIOMYOPATHY AND MYOCARDITIS REGISTRY PROGRAM

The African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP) is a collaborative effort by medical professionals from different African countries to collect information from individuals and families affected by cardiomyopathy, in an attempt to enrich our understanding of the causes of heart muscle disease, and positively impact and improve lives of those living with cardiomyopathy in Africa. By participating in IMHOTEP, you are actively helping the international medical community learn more about this condition

'Cardiomyopathy' is a term that describes a group of conditions that affects the heart muscle. There are many different types of cardiomyopathy

- *Dilated Cardiomyopathy (DCM)*
- *Hypertrophic Cardiomyopathy (HCM)*
- *Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)*
- *Restrictive Cardiomyopathy (RCM)*
- *Left Ventricular Noncompaction (LVNC)*

This pamphlet will explain the type of Cardiomyopathy that affects you (or your family member/friend), the special investigations that may be required to assist us in confirming the diagnosis, and the specific treatment options available for this condition. You have been diagnosed with ***Dilated Cardiomyopathy (DCM)***

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Tel: +27 21 404 7674, Fax: +27 21 406 6711, Email: s.kraus@uct.ac.za
UCT Faculty of Heath Sciences Human Ethics Committee: *HREC REF: 766/2014*
Tel: +2721 4066492

What is dilated cardiomyopathy?

The word **"cardiomyopathy"** is used to describe a disease that affects the heart muscle, resulting in damage and impairment of the heart's ability to pump blood normally. You have been diagnosed with ***dilated cardiomyopathy***. ***DCM*** is a condition where the heart muscle becomes thinned and stretched (dilated). As a result the heart muscle is weakened and unable to pump blood normally. DCM can be caused by a genetic abnormality or it can be due to direct injury to the heart muscle cells. Injury to the heart muscle cells can be caused by a number of different factors, listed below:

- **Myocarditis** – inflammation of the heart muscle due to an ***infection*** (virus, HIV, bacteria, TB, fungi, parasites), ***toxin***, ***autoimmune condition*** (rheumatoid arthritis, Lupus), or ***sarcoidosis***
- **Drugs** – e.g. certain chemotherapy agents, cocaine, methamphetamines (tik)
- **Pregnancy** – also known as ***Peripartum Cardiomyopathy,*** usually develops in the last trimester of pregnancy or in the first 5 months after pregnancy. It is thought to be related to the effects of pregnancy-related hormones on the heart muscle and the increased workload of the heart in pregnancy
- **Iron overload**
- **Endocrine (hormones) conditions** – e.g. thyroid disease
- **Nutritional** – deficiencies in thiamine (B1), carnitine, phosphate, selenium
- **Alcohol**
- **Tachymyopathy** – persistent rapid heart rate due to an electrical abnormality (arrhythmia) in the heart

Your doctor will do specific tests to exclude these causes depending on your clinical presentation.

Genetic or familial dilated cardiomyopathy is caused by mutations (mistakes) in the genes that code for a variety of heart muscle proteins that make up the heart muscle cells. These altered proteins function abnormally, and over time, DCM develops.

Understanding the role of genetics

Our DNA is made up of multiple genes. Each gene codes an instruction that tells the body how to make a specific protein that will have a specific function. Genes are packaged together on chromosomes and each human being has 23 chromosome pairs (46 chromosomes in total). One chromosome from each pair is inherited from your mother, and one from your father. Therefore, you can inherit a genetic mutation from a parent. This is why multiple members of a family can have cardiomyopathy as they share similar DNA. Most cardiomyopathies are autosomal dominantly inherited, which means that if one of your parents carries a genetic mutation for cardiomyopathy, you have a 50% chance of inheriting it from them. Importantly, because you have 2 copies of each gene (one from your mother and one from your father) not everyone who carries a genetic mutation will develop cardiomyopathy but you may become more susceptible to muscle cell damage from other factors listed previously.

Occasionally, dilated cardiomyopathy can occur as part of other genetic conditions (e.g. Duchenne Muscular Dystrophy). If you have such a condition your doctor will have counseled you.

Common symptoms in DCM

Heart failure is the most common presentation in patients with DCM. The term "heart failure" is used to describe what happens when the heart is unable to pump blood effectively, and patients develop shortness of breath and body swelling due to accumulation of fluid in the lungs and tissues.

Patients with heart failure suffer from breathlessness on exertion (e.g. climbing stairs, walking) because the heart is not strong enough to keep up with the demands of the activity. Due to accumulation of fluid in the lungs, patients may develop shortness of breath or a heavy feeling over the chest when lying down, and often have to sleep upright. In some cases, patients will develop leg and/or abdominal swelling, due to the accumulation of fluid in their tissues.

Other symptoms that can occur in DCM include:

- **Palpitations** – racing, skipping or fluttering sensation in the chest and/or throat
- **Chest discomfort/pain**
- **Dizziness**
- **Syncope** (fainting/blackouts)

If you have symptoms of palpitations and/or blackouts, it is important to inform your doctor as you may have developed an arrhythmia. Some patients with DCM develop arrhythmias. An arrhythmia is an electrical problem in the heart where there is an abnormality in the timing, pattern and/or rate of the heartbeat. Arrhythmias occur because of scarring within the heart muscle.

What arrhythmias occur in DCM?

- **Atrial fibrillation (AF)** – AF causes the heart to beat fast and irregularly and can result in worsening heart failure. The heart rate can be slowed with medications. Importantly, AF can cause small clots to develop in the heart, which can cause a stroke or heart attack. To prevent a stroke from happening, all patients with AF should be started on blood thinning treatment (warfarin).
- **Ventricular tachycardia (VT)** – VT occurs less commonly in DCM, but may result in collapse or sudden death. Drugs (such as beta-blockers) can be used to prevent VT. In cases where VT is diagnosed, we often have to insert a Implantable Cardioverter Defibrillator (ICD) which is similar to a pacemaker, that can monitor the heart beat and administer a small shock if VT occurs, to reset the heart to normal.

How do we diagnose DCM?

The diagnosis is made through taking a history, doing a physical examination and doing some specific investigations that include:

- **Electrocardiogram** – this records the electrical activity of the heart.
- **Echocardiogram** – this is an ultrasound of the heart where we can measure the size and function of the heart

Additional tests may be required to determine the underlying cause of your heart failure.

- **Blood tests** for HIV, thyroid function, iron levels, cardiac muscle proteins
- **Chest X-ray**
- **Cardiac MRI** – a special scan that allows us to assess the heart size and function more accurately and look for inflammation or scarring within the heart muscle.
- **Cardiac catheterization/angiogram** – it is sometimes necessary to access the pressures in the heart and lungs, and/or do a small biopsy of the heart muscle.
- If there is concern about an underlying arrhythmia, a **24-hour Holter, exercise stress test** and/or **electrophysiological study** of the heart may be required.

Your doctor will be able to explain these tests to you.

How do we treat DCM?

The goals of treatment are:

1. To exclude and treat reversible causes
2. To relieve the symptoms of heart failure
3. To prevent the progression of disease
4. To treat arrhythmias if present

Medication is the mainstay of treatment for DCM. Each drug has a specific role.

- **Diuretics** ("water tablets") – remove the excess fluid in the lungs and tissues to improve symptoms of shortness of breath and leg swelling
- **Beta-blockers** – slow the heart beat and prevent further scarring and damage to the heart
- **ACE-inhibitors** (enalapril, pharmapress) – reduce the workload of the heart and prevent further damage
- **Mineralocorticoid receptor antagonists** (Spironolactone) – mild diuretic but also protects the heart from further scarring/damage

Patients may become very ill with heart failure, with severe shortness of breath, body swelling and low blood pressure, requiring admission to hospital for intravenous medications and oxygen.

What happens when medical therapy fails?

Heart transplantation is considered in patients who do not respond to medical therapy and/or where the damage to their heart is very severe and permanent.

Some patients with DCM develop a electrical conduction problem called left bundle branch block. This results in dyssynchrony where the right and left sides of the heart beat out of time with each other. In cases such as these, we can insert a pacemaker device that stimulates the right and left sides of the heart to beat in time with each other. This is known as **Cardiac resynchronization therapy (CRT).** It is not a cure, but can help relieve the symptoms of heart failure.

Living with DCM

For many patients diagnosed with DCM, it is an unexpected diagnosis and it may take time to psychologically, physically and emotionally come to terms with having this condition. We recommend that patients with DCM continue to pursue an active lifestyle but avoid high intensity competitive sports. Alcohol, illicit drugs and excessive use of caffeine or other stimulants should be avoided.

Pregnancy can be extraordinarily dangerous in women with heart disease, and is not recommended in patients with impaired heart function. Pregnancy should always be discussed with your doctor. Many of the medications can be dangerous to a developing baby and are contraindicated in pregnancy. Contraception is therefore essential.

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The African Cardiomyopathy and Myocarditis Registry Program
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Email: s.kraus@uct.ac.za
UCT HREC REF: 766/2014

IMHOTEP PATIENT INFORMATION SHEET

IMHOTEP
THE AFRICAN CARDIOMYOPATHY AND MYOCARDITIS REGISTRY PROGRAM

Arrhythmogenic Right Ventricular Cardiomyopathy

'Cardiomyopathy' is a term that describes a group of conditions that affects the heart muscle. This pamphlet has been written to educate patients, their families and their friends about the type of cardiomyopathy that affects them. The African Cardiomyopathy and Myocarditis Registry Program **(IMHOTEP)** is a collaborative effort by medical professionals from different African countries to collect information from individuals and families affected by cardiomyopathy, in an attempt to improve our understanding of the causes of heart muscle disease, and the management of patients' affected with cardiomyopathies. While many aspects of these conditions are frightening and much is still unknown, we would like to invite patients, their families and friends to join us in our quest to build the body of knowledge about these conditions, which will ultimately improve the lives of those living with cardiomyopathy in Africa.

There are different types of cardiomyopathy, namely

Dilated Cardiomyopathy (DCM)
Hypertrophic Cardiomyopathy (HCM)
Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)
Restrictive Cardiomyopathy (RCM)
Left Ventricular Noncompaction (LVNC)

This pamphlet will explain, in detail, the type of cardiomyopathy that affects you (or your family member/friend), the special investigations that may be required to assist us in confirming the diagnosis, and the specific treatment options available for this condition.

You have been diagnosed with ***Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC),*** also known as *Arrhythmogenic Right Ventricular Dysplasia (ARVD).*

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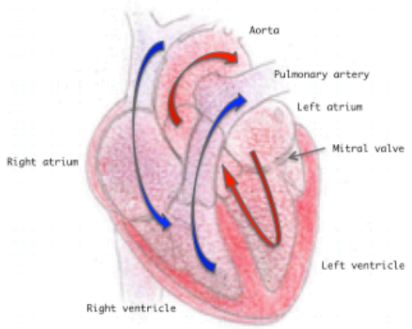
The Heart

The heart is a muscular pump with 4 chambers. There are 2 smaller thin-walled chambers, called the atria, and 2 larger thick-walled chambers called the ventricles. Blood enters the heart from the body through the right atrium, the right atrium contracts and the blood moves into the right ventricle. The right ventricle then contracts and the blood moves into the lungs where it absorbs oxygen. The oxygenated blood then enters the left atrium and is pumped into the left ventricle. The left ventricle forcefully contracts and blood is expelled out of the heart and travels to the organs of the body.

The electrical system of the heart ensures that the right and left atria contract together first, followed by the right and left ventricle, thus allowing blood to move through the heart effectively.



The role of genetics

In many cases, cardiomyopathy is caused by a change or a mistake (mutation) in a gene within our DNA, which means that this condition can be inherited. In other words, it can affect multiple individuals within a family. Our DNA is made up of multiple genes. Each gene encodes an instruction that tells the body how to make a specific protein that will have a specific function. Genes are packaged on chromosomes that are arranged in 23 pairs within a cell. One member of each pair is inherited from our mother and one, from our father. The mutation can, therefore, be inherited from a parent or it can occur by chance, in an individual, as a new mutation. New mutations occur frequently in the eggs and sperm of adults, but it is just by chance that a gene that codes for a heart muscle protein is abnormal. Several genes have been identified as being associated with the different types of cardiomyopathy, but there are probably many more that have not yet been discovered. A negative genetic test, therefore, does not exclude the diagnosis of inherited (or genetic) cardiomyopathy. Most cardiomyopathies are autosomal dominantly inherited. What that means is that you only require one copy of the genetic mutation to develop cardiomyopathy. If one of your parents carries a genetic mutation for cardiomyopathy, you have a 50% chance of inheriting it from them.

APPENDIX F – Information Sheets for Patients: IV. Information booklet on ARVC (pages 3 and 4)

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Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D)

ARVC is a heart muscle disorder where damaged heart muscle cells are gradually replaced by scar tissue and fat. It is now widely recognized that ARVC is often caused by mutations (mistakes) in the genes that code for desmosomal proteins. The desmosome is the mechanical bridge that connects one heart muscle cell to the next. The major proteins that form the desmosome are plakophilin-2 (PKP2), desmoglein-2 (DSG2), desmocollin-2 (DSC2), desmoplakin (DSP) and plakoglobin (JUP). Patients with ARVC commonly have genetic abnormalities in the genes that encode one (or more) of these proteins. As a result of abnormalities in the desmosome, the mechanical bonds (or glue) between the heart muscle cells is defective. Over time, the heart muscle cells are pulled apart and damaged, resulting in scarring and fat replacement. Exercise can result in an increased chance of the heart muscle cells pulling apart, which may explain why ARVC is common among athletes. As the name (ARVC) implies, the right side of the heart is usually predominantly affected, however, the left side of the heart can also be involved. The presence of scar and fat in the heart muscle results in the heart being susceptible to developing ventricular arrhythmias and, over time, can cause impairment of the pump function of the heart resulting in heart failure.

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Common symptoms of ARVC

An ***arrhythmia*** is an electrical problem where there is an abnormality in the timing, pattern or rate of the heartbeat. The heartbeat can be too slow (bradycardia), too fast (tachycardia) or irregular.

Arrhythmias can cause a variety of symptoms that include:

- ***Palpitations*** – racing, skipping or fluttering sensation in your chest and/or throat
- ***Chest discomfort/pain*** – caused by a small drop in blood pressure as a result of the heart pumping less blood to the heart muscle itself
- ***Pre-syncope*** – dizziness, but with no loss of consciousness, caused by a small drop in blood pressure as a result of the heart pumping less blood to the brain
- ***Syncope*** (fainting) – loss of consciousness caused by a large drop in blood pressure as a result of the heart being unable to pump enough blood to the brain
- ***Sudden cardiac death*** – occurs when the heart muscle 'fibrillates' and is unable to pump enough blood to its own muscle and the other vital organs. Without prompt treatment, the heart stops and sudden death may occur.

In ARVC, there are two typical arrhythmias that occur.

- ***Premature ventricular contractions (PVCs)*** – isolated extra irregular heartbeats that occur when the electrical signal starts in the ventricle. Most people are unaware that they are experiencing PVCs, they are not dangerous on their own and can occur in healthy people. PVCs can, however, become bothersome when they occur more frequently and can be associated with other types of sustained rapid heart rhythms, such as ventricular tachycardia
- ***Ventricular tachycardia (VT)*** – a series of rapid heartbeats, where the electrical stimulus originates in the ventricle, usually at a site of scarring. A VT episode may last for just a few beats or may continue for several seconds or minutes. It can lead to life-threatening arrhythmias, such as ventricular fibrillation, where there is complete electrical chaos in the ventricles and the heart is unable to pump blood effectively. VT can stop on its own or it may require medication or an electrical shock to convert the heart back to a normal rhythm.

For additional information on arrhythmias, visit PACE (prevent Arrhythmic Cardiac Events) Clinic at www.paceafrica.org.za

Heart failure usually occurs only later on in life, although it can occur earlier in patients who are more severely affected. Some patients with ARVC never develop heart failure symptoms. The term "heart failure" is used to describe the weakening of the heart muscle and impairment of the heart's ability to pump blood effectively. Heart failure occurs when the heart muscle becomes stretched and thinned. The symptoms of heart failure include:

- ***Reduced effort tolerance*** – fatigue or shortness of breath when exerting one's self (running, climbing stairs, walking, doing housework, dressing). This occurs because the heart muscle is unable to keep up with the oxygen demands of the body during exertion.
- ***Shortness of breath*** on lying flat *(orthopnoea)* or waking up breathless *(paroxysmal nocturnal dyspnoea/PND)* due to fluid build up in the lungs
- ***Swelling*** *(oedema)* of the legs (and body) due to a build up of water in the tissues.

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How is ARVC diagnosed?

There is no single test that can establish or exclude a diagnosis of ARVC. It can be a difficult diagnosis to make because the changes in the heart may be very subtle and are not always picked up with routine tests.

In 1994, international researchers proposed a set of diagnostic criteria that took into account information from a family history and a number of specialized tests. These criteria were revised in 2010 and are used in combination with genetic tests to make a diagnosis. The changes in the heart may only occur later in life, therefore repeat testing and evaluation may be required to make a diagnosis.

There are a number of non-invasive and invasive tests that can be done. Not all tests will be required to make a diagnosis.

Non-invasive cardiac tests include:

- ***Electrocardiogram (ECG)*** – An ECG records the electrical activity of the heart. It is performed lying down, where electrodes attached to wires are placed on the chest. The machine captures the ECG tracing and prints a report that can be analysed by a doctor. Specific changes on the ECG are associated with ARVC.
- ***Signal average electrocardiogram (SAECG)*** – A SAECG in similar to an ECG but it is able to analyse electrical signals that are altered by the presence of scar tissue in the heart.
- ***Holter monitor*** – A holter monitor is a small machine that is able to record a continuous ECG over 24-48 hours while one performs normal daily activities. It can detect abnormalities that are not seen on routine ECG. If one develops symptoms of palpitations, dizziness or chest pain, it allows the doctors to correlate the symptoms you experience with the electrical activity in the heart.
- ***Exercise Stress Test (EST)*** – An EST is a treadmill or bicycle test, where an ECG is done during exercise. It is used to determine whether chest pain, an arrhythmia or an abnormal blood pressure response occurs during exercise.
- ***Echocardiogram ("echo")*** – An echo is an ultrasound of the heart; it allows the operator to measure the size, structure and function of the heart.
- ***Cardiac Magnetic Resonance Imaging (MRI)*** – MRI uses magnetic waves to produce an image of the heart that can distinguish heart muscle from scar tissue and fat. It can also assess the size, structure and function of the heart.

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How is ARVC diagnosed?

Invasive cardiac tests include:

- ***Loop recorder*** – A loop recorder is a small devise that is inserted under the skin on the chest. It is able to record the electrical activity of the heart (ECG) over a long period of time (months or even years). It is used to evaluate whether infrequent heart arrhythmias are occurring.
- ***Electrophysiology study (EPS)*** - A EPS is done to determine whether you are at high risk for developing a serious arrhythmia so that preventative treatment can be provided. During the study, a number of sheaths (thin pipes) are inserted into the large blood vessels in your leg (and/or neck). The cardiologist then inserts wires/catheters through the sheaths and guides them to the heart. This allows us to record the electrical activity of the heart in greater detail. The cardiologist is also able to stimulate the heart to see if arrhythmias occur. During the procedure, you may be sedated and your heart rate and blood pressure will be monitored closely.
- ***Angiography (cardiac catheterization)*** – Angiography is performed in a similar way to an EPS. Contrast dye is injected into the blood to determine the structure and function of the heart, including the right and left ventricles as well as the coronary arteries (the blood vessels that supply the heart muscle with blood). X-ray is used to see how the contrast dye fills the heart.
- ***Cardiac biopsy*** – At the time of angiography or EPS, tiny samples of heart muscle tissue are taken. A pathologist is then able to examine the heart tissue under a microscope to determine whether the heart muscle is abnormal.

Genetic testing

Currently in South Africa, genetic testing for ARVC is only available, in the most part, through research facilities. This means that genetic screening protocols are subject to specific research objectives and funding. In many cases, patients test negative for known mutations, and thus researchers have to look at alternative genes that could cause the disease. This process can take many months or even years. With advancing technology in this field, we hope to find the answers to many of the unanswered questions about the role of genetics in ARVC and that genetic testing will become more widely available in the future.

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How is ARVC treated?

There are two primary goals of treatment:

1. To reduce the frequency and severity of ventricular arrhythmias (VT), and prevent sudden cardiac death
2. To prevent progression of disease that will result in worsening ventricular function and heart failure.

Treatment is based on international research, cardiac test results and your symptoms. The common treatment strategies include: medication, implantable cardiac defibrillators and catheter ablation.

Heart transplantation is considered when all other treatment options have failed.

Medications

Antiarrhythmic medications can be used to decrease the number of episodes and severity of VT by altering the electrical properties of the heart and by blocking the effects of adrenaline on the heart.

Beta-blockers lower the heart rate, blood pressure and the effects of adrenaline, which helps to prevent arrhythmias. If patients continue to experience VT on beta-blocker therapy, other antiarrhythmic drugs, such as amiodarone or sotalol, can be used.

ACE-inhibitors are helpful in reducing the workload of the heart and prevent the development of heart failure.

Diuretics are helpful in reducing salt and water retention that occurs once heart failure has developed. They are effective in reducing symptoms of shortness of breath and leg swelling that results from excess water build up in the body.

Please remember that all medications have potential side-effects.

Implantable cardiac defibrillator (ICD)

ICDs are commonly used to treat patients with ARVC. This small devise is implanted under the skin below the clavicle and wires are inserted into the heart. The device is able to monitor the heartbeat continuously and will automatically deliver a small electrical shock to the heart if a dangerous arrhythmia (such as ventricular tachycardia) occurs. ICDs can also function as pacemakers, stimulating the heart to beat. The device should be checked regularly (3-6monthly) and the battery must be replaced every 4-6 years. These devices do not prevent arrhythmias from occurring; they treat the arrhythmia when it occurs to prevent sudden cardiac death.

Catheter ablation:

Catheter ablation is a procedure whereby the areas of the heart that cause arrhythmias are located and eliminated by cauterization. It is done to reduce the frequency of sustained VT episodes. It is not a curative procedure, and it is important that patients discuss the risks and benefits of catheter ablation with their doctor before having the procedure.

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Living with ARVC

For majority of patients diagnosed the ARVC, it is an unexpected diagnosis. It often helps to explain a long history of symptoms and incidences of sudden death that have occurred within a family. It is not uncommon for patients with this diagnosis to feel a sudden loss of well-being and anxiety about their future. As with any chronic illness, it can take some time to psychologically and emotionally come to terms with having this condition. Family screening for ARVC gives us an opportunity to make a diagnosis in affected family members early on in the disease process, allowing us to plan treatment. It also gives us an opportunity to reassure those family members that are not affected. There are a number of lifestyle adjustments that should be considered.

While the medical community has made great progress in understanding this condition, there are still many questions about how best to treat this condition and what to expect once the diagnosis has been made. We have learnt through studying people who develop ARVC that exercise is a risk factor. Exercise can, not only provoke arrhythmias but can also accelerate progression of disease. We recommend that patients focus of mild aerobic activities such as (walking, golf, Pilates) and avoid competitive high intensity sports (running, rugby, cycling, athletics, competitive swimming, crossfit). Patients are also advised to limit intake of alcohol and caffeine, which may precipitate arrhythmias.

For women with ARVC, contraception must be addressed, although pregnancy is not necessarily contraindicated unless heart muscle function is compromised. It is important to talk to your doctor prior to falling pregnant and the decision to go ahead with a pregnancy must be individualized. Pregnancy puts additional strain on the heart, which can potentially put both mother and child at risk, particularly during the third trimester. Many of the medications used to treat ARVC can be dangerous to the fetus (baby), but the risks associated with stopping these medications must be carefully considered prior to doing so.

For both men and women, it is very important to have genetic counseling prior to considering having a baby, so that you are comfortable with the potential risks to your child of inheriting ARVC.

By participating in the African Cardiomyopathy and Myocarditis Registry, you are actively helping the international medical community to learn more about this condition. With the knowledge gained by studying people with ARVC, we hope to positively impact the lives of those living with this condition.

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Brochure adapted from the John Hopkins Arrhythmogenic Right Ventricular Dysplasia Program

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Hypertrophic Cardiomyopathy

'Cardiomyopathy' is a term that describes a group of conditions that affects the heart muscle. This pamphlet has been written to educate patients, their families and their friends about the type of cardiomyopathy that affects them. The African Cardiomyopathy and Myocarditis Registry Program is a collaborative effort by medical professionals from different African countries to collect information from individuals and families affected by cardiomyopathy, in an attempt to improve our understanding of the causes of heart muscle disease, and the management of patients' affected with cardiomyopathies. While many aspects of these conditions are frightening and much is still unknown, we would like to invite patients, their families and friends to join us in our quest to build the body of knowledge about these conditions, which will ultimately improve the lives of those living with cardiomyopathy in Africa.

There are different types of cardiomyopathy, namely

Dilated Cardiomyopathy (DCM)
Hypertrophic Cardiomyopathy (HCM)
Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)
Restrictive Cardiomyopathy (RCM)
Left Ventricular Noncompaction (LVNC)

This pamphlet will explain, in detail, the type of Cardiomyopathy that affects you (or your family member/friend), the special investigations that may be required to assist us in confirming the diagnosis, and the specific treatment options available for this condition.

You have been diagnosed with ***Hypertrophic Cardiomyopathy (HCM)***, often referred to as *"hocm"*.

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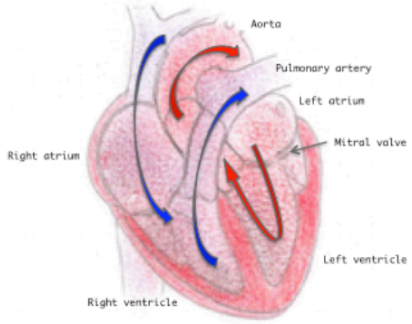
The Heart

The heart is a muscular pump with 4 chambers. There are 2 smaller thin-walled chambers, called the atria, and 2 larger thick-walled chambers called the ventricles. Blood enters the heart from the body through the right atrium, the right atrium contracts and the blood moves into the right ventricle. The right ventricle then contracts and the blood moves into the lungs where it absorbs oxygen. The oxygenated blood then enters the left atrium and is pumped into the left ventricle. The left ventricle forcefully contracts and blood is expelled out of the heart and travels to the organs of the body.

The electrical system of the heart ensures that the right and left atria contract together first, followed by the right and left ventricle, thus allowing blood to move through the heart effectively.



The role of genetics

In many cases, cardiomyopathy is caused by a change or a mistake (mutation) in a gene within our DNA, which means that this condition can be inherited. In other words, it can affect multiple individuals within a family. Our DNA is made up of multiple genes. Each gene encodes an instruction that tells the body how to make a specific protein that will have a specific function. Genes are packaged on chromosomes that are arranged in 23 pairs within a cell. One member of each pair is inherited from our mother and one, from our father. The mutation can, therefore, be inherited from a parent or it can occur by chance, in an individual, as a new mutation. New mutations occur frequently in the eggs and sperm of adults, but it is just by chance that a gene that codes for a heart muscle protein is abnormal. Several genes have been identified as being associated with the different types of cardiomyopathy, but there are probably many more that have not yet been discovered. A negative genetic test, therefore, does not exclude the diagnosis of inherited (or genetic) cardiomyopathy. Most cardiomyopathies are autosomal dominantly inherited, which means that you only require one copy of the genetic mutation to develop cardiomyopathy. If one of your parents carries a genetic mutation for cardiomyopathy, you have a 50% chance of inheriting it from them.

APPENDIX F – Information Sheets for Patients: V. Information booklet on HCM (pages 3 and 4)

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Hypertrophic Cardiomyopathy (HCM)

Hypertrophic cardiomyopathy is a genetic condition that affects the heart muscle. HCM is the most common type of inherited cardiomyopathy, occurring in 1 in every 500 people. HCM is most commonly caused by a mutation (mistake) in the gene that codes for the force-generating structure, known as the **sarcomere,** within the heart muscle. A genetic mutation is only found in approximately 60% of cases so a negative test does not exclude HCM as a genetic condition.

The sarcomere plays a central role in the contraction of the heart muscle and mutations in the genes coding for the sarcomere commonly result in excessive contractility of the heart muscle. As a result, the heart muscle becomes thicker (hypertrophy) and scarred (fibrosis). The thickening of the heart muscle usually affects the septum (the wall between the right and the left side of the heart), but it can involve the other walls of the left ventricle as well (concentric, apical). The thickened muscle can encroach on the blood flow through the heart, most commonly obstructing the flow of blood out of the heart (known as left ventricular outflow tract obstruction). Over time, the pump function of the heart can become compromised resulting in heart failure. The presence of scar tissue results in the heart being susceptible to developing ventricular arrhythmias.

Hypertrophy (thickening of the heart muscle) can also occur as part of other multisystem genetic syndromes such as Noonan Syndrome, Cardio-facio-cutaneous Syndrome, LEOPARD Syndrome, Neurofibromatosis, Costello Syndrome, Beckwith-Weidemann Syndrome, metabolic disease (glycogen storage disease, disorders of fatty acid metabolism), lysosome-associated membrane protein (Danon disease), lysosomal-storage diseases (Fabry disease), and mitochondrial disease. These conditions are uncommon and it is unlikely that you are affected by one of these unless your doctor has indicated otherwise.

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Common symptoms of HCM

Many patients with HCM do not have any symptoms.

An ***arrhythmia*** is an electrical problem where there is an abnormality in the timing, pattern or rate of the heartbeat. The heartbeat can be too slow (bradycardia), too fast (tachycardia) or irregular.

In HCM, there are two typical arrhythmias that occur.

1. ***Ventricular tachycardia (VT)*** – VT is a series of rapid heartbeats, where the electrical stimulus originates in the ventricle, usually at a site of scarring. A VT episode may last for just a few beats or may continue for several seconds or minutes. It can lead to life-threatening arrhythmias, such as ventricular fibrillation, where there is complete electrical chaos in the ventricles and the heart is unable to pump blood effectively. VT can stop on its own or it may require medication or an electrical shock to convert the heart back to a normal rhythm. A patient with VT can experience a variety of symptoms that include:
    - ***Palpitations*** – racing, skipping or fluttering sensation in your chest and/or throat
    - ***Chest discomfort/pain*** – caused by a small drop in blood pressure as a result of the heart pumping less blood to the heart muscle itself
    - ***Pre-syncope*** – dizziness, but no loss of consciousness, caused by a small drop in blood pressure as a result of the heart pumping less blood to the brain
    - ***Syncope*** (fainting) – loss of consciousness caused by a large drop in blood pressure as a result of the heart being unable to pump enough blood to the brain
    - ***Sudden cardiac death*** – occurs when the heart muscle 'fibrillates' and is unable to pump enough blood to its own muscle and the other vital organs. Without prompt treatment, the heart stops and sudden death may occur.

2. ***Atrial fibrillation (AF)*** – AF is a very stable arrhythmia. It occurs due to electrical chaos in the atria of the heart due to excessive stretching and scarring of the atria. Although the ventricles still function in the normal way, because of the electrical chaos in the atria, the ventricles are haphazardly stimulated resulting in fast irregular heartbeat. Many patients who have AF are asymptomatic but they may experience ***palpitations, chest discomfort or dizziness***. Atrial fibrillation can lead to heart failure (described below) due to a persistently fast heart rate, and may cause a stroke. A stroke occurs when blood clots that develop inside the atria due to the abnormal blood flow, dislodge and travel to the brain. Blood-thinning medication is recommended in patients with AF to prevent strokes.

For additional information on arrhythmias, visit PACE (prevent Arrhythmic Cardiac Events) Clinic at ***www.paceafrica.org.za***

APPENDIX F – Information Sheets for Patients: V. Information booklet on HCM (pages 5 and 6)

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Common symptoms of HCM

Heart failure (HF) usually occurs only later on in life, although it can occur earlier in patients who are more severely affected. Some patients with HCM never develop heart failure symptoms. The term "heart failure" is used to describe the impairment of the heart's ability to pump blood effectively.

In HCM, heart failure can occur for a number of different reasons:

- HF can be due to left ventricular outflow tract obstruction where the thickened heart muscle impedes of the flow of blood out of the heart. This can result in ***dizziness, syncope (loss of consciousness)*** or ***chest discomfort/pain*** often precipitated by increased physical activity.
- HF can be due to abnormal relaxation of the heart muscle, making the heart stiff impairing its ability to fill properly with blood (diastolic dysfunction)
- HF can be due to ongoing damage resulting stretching, thinning and weakening of the heart muscle (systolic dysfunction)

The symptoms of heart failure includes some or all of the following:

- ***Reduced effort tolerance*** – fatigue or shortness of breath when exerting one's self (running, climbing stairs, walking, doing housework, dressing). This occurs because the heart muscle is unable to keep up with the oxygen demands of the body during increased activity.
- ***Shortness of breath*** on lying flat or waking up breathless due to fluid build up in the lungs
- ***Swelling*** of the legs (and body) due to a build up of water in the tissues.

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How is HCM diagnosed?

There are a number of non-invasive and invasive tests that can be done to confirm the diagnosis. In the majority of cases, the diagnosis is confirmed on non-invasive imaging (echocardiogram or cardiac MRI) where heart muscle thickness can be measured. Additional tests may be required to access the risk of developing arrhythmias.

Non-invasive cardiac tests include:

- ***Electrocardiogram (ECG)*** – An ECG records the electrical activity of the heart. It is performed lying down, where electrodes ("stickers") attached to wires are placed on the chest. The machine captures the ECG tracing and prints a report that can be analysed by a doctor. Specific changes on the ECG are associated with HCM.
- ***Signal average electrocardiogram (SAECG)*** – A SAECG in similar to an ECG but it is able to analyse electrical signals that are altered by the presence of scar tissue in the heart. This test is not routinely done in HCM.
- ***Holter Monitor*** – A holter monitor is a small machine that is able to record a continuous ECG over 24-48 hours while one performs normal daily activities. It can detect arrhythmias that are not seen on routine ECG. If one develops symptoms of palpitations, dizziness or chest pain, it allows the doctors to correlate the symptoms you experience with the electrical activity in the heart.
- ***Exercise Stress Test (EST)*** – An EST is a treadmill or bicycle test, where an ECG and serial blood pressures are done during exercise. It is used to determine whether chest pain, an arrhythmia or an abnormal blood pressure response occurs during exercise.
- ***Echocardiogram ("echo")*** – An echo is an ultrasound of the heart, it allows the operator to measure the size, structure and function of the heart, as well as measure the exact thickness of the heart muscle
- ***Cardiac Magnetic Resonance Imaging (MRI)*** – MRI uses magnetic waves to produce an image of the heart that can distinguish heart muscle from scar tissue and fat. It can also assess the size, structure and function of the heart, in addition to accurately measuring the heart muscle wall thickness.

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How is HCM diagnosed?

Only selected investigations will be required depending on your clinical presentation. In many cases, invasive investigations are not required.

Invasive cardiac tests include:

- ***Loop recorder*** – A loop recorder is a small devise that is inserted under the skin on the chest. It is able to record the electrical activity of the heart (ECG) over a long period of time (months or even years). It is used to evaluate whether infrequent heart arrhythmias are occurring.
- ***Electrophysiology study (EPS)*** - A EPS is done to determine whether you are at high risk for developing a serious arrhythmia so that preventative treatment can be provided. During the study, a number of sheaths (thin pipes) are inserted into the large blood vessels in your leg (and/or neck). The cardiologist then inserts wires/catheters through the sheaths and guides them to the heart. This allows us to record the electrical activity of the heart in greater detail. The cardiologist is also able to stimulate the heart to see if arrhythmias occur. During the procedure, you will be sedated and your heart rate and blood pressure will be monitored closely.
- ***Angiography (cardiac catheterization)*** – Angiography is performed in a similar way to an EPS. Contrast dye is injected into the blood to determine the structure and function of the heart, including the right and left ventricle as well as the coronary arteries (that supply the heart muscle with blood). X-ray is used to see how the contrast dye fills the heart.
- ***Cardiac biopsy*** – At the time of angiography or EPS, tiny samples of heart muscle tissue are taken. A pathologist is then able to examine the heart tissue under a microscope to determine whether the heart muscle is abnormal.

Genetic testing

Currently in South Africa, genetic testing for HCM is only available, in the most part, through research facilities. This means that genetic screening protocols are subject to specific research objectives and funding. In many cases, patients test negative for known mutations, and thus researchers have to look at alternative genes that could cause the disease. This process can take many months or even years. With advancing technology in this field, we hope to find the answers to many of the unanswered questions about the role of genetics in HCM and that genetic testing will become more widely available in the future.

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How is HCM treated?

There are a few primary goals of treatment:

- To reduce the frequency and severity of ventricular arrhythmias (VT), and prevent sudden cardiac death
- To relieve left ventricular outlet obstruction
- To prevent progression of disease that will result in worsening ventricular function and heart failure.

Treatment is based on international research and guidelines, your individual cardiac test results and your symptoms. The common treatment strategies include: medication, implantable cardiac defibrillators, catheter ablation and surgery.

Heart transplantation is considered when all other treatment options have failed.

Medications

Antiarrhythmic medications can be used to decrease the number of episodes and severity of VT by altering the electrical properties of the heart and by blocking the effects of adrenaline on the heart.

Beta-blockers lower the heart rate, blood pressure and the effects of adrenaline, which helps to prevent arrhythmias. If patients continue to experience VT on beta-blocker therapy, other antiarrhythmic drugs, such as amiodarone or sotalol, can be used.

Calcium-channel blockers (verapamil) lower the heart rate, blood pressure and the effects of calcium on the heart. Calcium plays a central role in heart muscle contraction.

Additional medications (such as disopyramide) may be prescribed if symptoms are not controlled using beta-blockers and calcium-channel blockers.

ACE-inhibitors and ***diuretics*** should only be used in patients who have developed heart failure and have evidence of impaired heart muscle function. They should be used with caution in patients with significant left ventricular outlet obstruction. *ACE-inhibitors* are helpful in reducing the workload of the heart. *Diuretics* are helpful in reducing salt and water retention that occurs once heart failure has developed, and are effective in relieving the symptoms of shortness of breath and leg swelling that results from excess water build up in the body.

Blood-thinning medication (anticoagulation) is recommended in patients with atrial fibrillation to reduce the risk of having a stroke. Both the risks and benefits of this treatment must be discussed with you prior to starting this treatment. The drug most commonly used is called warfarin. Patients on warfarin require regular blood tests to measure the thickness of the blood (prothombin index (PI) or INR) so that the dose of warfarin can be adjusted.

Please remember that all medications have potential side-effects.

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How is HCM treated?

Note: The following invasive treatment strategies have specific indications and are not routinely used in all patients with HCM.

Implantable cardiac defibrillator (ICD) – *not routinely available/required*
ICDs are commonly used to treat patients with HCM. This small devise is implanted under the skin below the clavicle and wires are inserted into the heart. The device is able to monitor the heartbeat continuously and will automatically deliver a small electrical shock to the heart if a dangerous arrhythmia (such as ventricular tachycardia) occurs. ICDs can also function as pacemakers, stimulating the heart to beat if necessary. The device should be checked regularly (3-6monthly) and the battery must be replaced every 4-6 years. These devices do not prevent arrhythmias from occurring; they treat the arrhythmia when it occurs to prevent sudden cardiac death. These devices are very expensive and it is not possible to implant one in every patients living with HCM in Africa. By doing specialized tests, we are able to access whether you are at an increased risk of developing dangerous arrhythmias and motivate for the necessity for an ICD.

Catheter ablation: – *not routinely available/required*
Catheter ablation is a procedure whereby the areas of the heart that cause arrhythmias are located and eliminated by cauterization. It is done to reduce the frequency of sustained VT episodes. It is not a curative procedure, and it is important that patients discuss the risks and benefits of catheter ablation with their doctor before having the procedure.

Percutaneous Intervention: – *not routinely available/required*
Alcohol ablation is a procedure where absolute alcohol solution is injected into the blood vessel that feeds the portion of thickened heart muscle that is obstructing blood flow, resulting in death and shrinkage of those cells.

Surgery: – *not routinely available/required*
In patients who have significant left ventricular outlet obstruction that impairs on the flow of blood out of the heart, surgical myomectomy can be done. Surgical myomectomy is where the portion of thickened heart muscle that is obstructing blood flow is cut out. In some cases, the mitral valve is distorted by the abnormally thickened septum. The mitral valve acts as a door between the left atrium and left ventricle. Normally when the ventricle contracts, the mitral valve closes so that blood moves forward out of the heart and does go backwards into the atrium. If the mitral valve is distorted and doesn't close properly, blood will leak backwards into the atrium when the ventricle contracts. This is known as mitral regurgitation or mitral incompetence. If the mitral valve's function is significantly impaired, it may require repair or replacement with a metal valve.

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Living with HCM

For the majority of patients diagnosed the HCM, it is an unexpected diagnosis. It often helps to explain a long history of symptoms and incidences of sudden death that have occurred within a family. It is not uncommon for patients with this diagnosis to feel a sudden loss of well-being and anxiety about their future. As with any chronic illness, it can take some time to psychologically and emotionally come to terms with having this condition. Family screening for HCM gives us an opportunity to make a diagnosis in affected family members early on in the disease process, allowing us to plan treatment. It also allows us to reassure those family members who are not affected. There are a number of lifestyle adjustments that should be considered.

While the medical community has made great progress in understanding this condition, there are still many questions about how best to treat this condition and what to expect once the diagnosis has been made. We have learnt through studying people who have HCM that exercise is an important risk factor for sudden death. We recommend that patients focus of low-intensity aerobic activities such as (walking, golf, Pilates) and avoid competitive high intensity sports (running, rugby, cycling, athletics, competitive swimming, crossfit). It is also important to avoid dehydration. Recommendations regarding participation in different recreational sport are available. Patients are also advised to limit intake of alcohol and caffeine, which may precipitate arrhythmias. Because coronary artery disease in addition to HCM has a significant impact on survival of HCM patients, it is important to address risk factors such as obesity, diabetes, hypercholesterol and smoking.

For women with HCM, contraception must be addressed, although pregnancy is not necessarily contraindicated unless heart muscle function is compromised. It is important to talk to your doctor prior to falling pregnant and the decision to go ahead with a pregnancy must be individualized. Pregnancy puts additional strain on the heart, which can potentially put both mother and child at risk, particularly during the third trimester. Many of the medications used to treat HCM can be dangerous to the fetus, but the risks associated with stopping these medications have be carefully considered prior to doing so.

For both men and women, it is very important to have genetic counseling prior to considering having a baby, so that you are comfortable with the potential risks to your child.

By participating in the African Cardiomyopathy and Myocarditis Research Program, you are actively helping the international medical community to learn more about this condition. With the knowledge gained by studying people with HCM, we hope to positively impact the lives of those living with this condition.

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Brochure adapted from the John Hopkins Arrhythmogenic Right Ventricular Dysplasia Program

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IMHOTEP PATIENT INFORMATION SHEET

IMHOTEP
THE AFRICAN CARDIOMYOPATHY AND MYOCARDITIS REGISTRY PROGRAM

Left ventricular noncompaction cardiomyopathy

'Cardiomyopathy' is a term that describes a group of conditions that affects the heart muscle. This pamphlet has been written to educate patients, their families and their friends about the type of cardiomyopathy that affects them. The African Cardiomyopathy and Myocarditis Registry is a collaborative effort by medical professionals from different African countries to collect information from individuals and families affected by cardiomyopathy, in an attempt to improve our understanding of the causes of heart muscle disease, and the management of patients' affected with cardiomyopathies. While many aspects of these conditions are frightening and much is still unknown, we would like to invite patients, their families and friends to join us in our quest to build the body of knowledge about these conditions, which will ultimately improve the lives of those living with cardiomyopathy in Africa.

There are different types of cardiomyopathy, namely

Dilated Cardiomyopathy (DCM)
Hypertrophic Cardiomyopathy (HCM)
Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)
Restrictive Cardiomyopathy (RCM)
Left Ventricular Noncompaction (LVNC)

This pamphlet will explain, in detail, the type of Cardiomyopathy that affects you (or your family member/friend), the special investigations that may be required to assist us in confirming the diagnosis, and the specific treatment options available for this condition.

You have been diagnosed with ***left ventricular noncompaction (LVNC) cardiomyopathy.***

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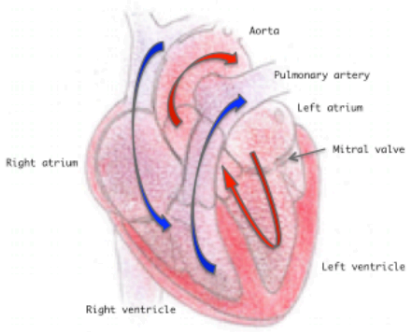
The Heart

The heart is a muscular pump with 4 chambers. There are 2 smaller thin-walled chambers, called the atria, and 2 larger thick-walled chambers called the ventricles. Blood enters the heart from the body through the right atrium, the right atrium contracts and the blood moves into the right ventricle. The right ventricle then contracts and the blood moves into the lungs where it absorbs oxygen. The oxygenated blood then enters the left atrium and is pumped into the left ventricle. The left ventricle forcefully contracts and blood is expelled out of the heart and travels to the organs of the body.

The electrical system of the heart ensures that the right and left atria contract together first, followed by the right and left ventricle, thus allowing blood to move through the heart effectively.



The role of genetics

In many cases, cardiomyopathy is caused by a change or a mistake (mutation) in a gene within our DNA, which means that this condition can be inherited. In other words, it can affect multiple individuals within a family. Our DNA is made up of multiple genes. Each gene encodes an instruction that tells the body how to make a specific protein that will have a specific function. Genes are packaged on chromosomes that are arranged in 23 pairs within a cell. One member of each pair is inherited from our mother and one, from our father. The mutation can, therefore, be inherited from a parent or it can occur by chance, in an individual, as a new mutation. New mutations occur frequently in the eggs and sperm of adults, but it is just by chance that a gene that codes for a heart muscle protein is abnormal. Several genes have been identified as being associated with the different types of cardiomyopathy, but there are probably many more that have not yet been discovered. A negative genetic test, therefore, does not exclude the diagnosis of inherited (or genetic) cardiomyopathy. Most cardiomyopathies are autosomal dominantly inherited, which means that you only require one copy of the genetic mutation to develop cardiomyopathy. If one of your parents carries a genetic mutation for cardiomyopathy, you have a 50% chance of inheriting it from them.

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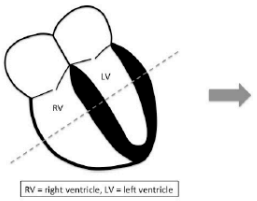
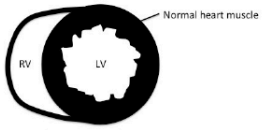
Left Ventricular Noncompaction (LVNC) Cardiomyopathy

Left ventricular non-compaction (LVNC) is a condition that is characterized by sponge-like changes that occur in the heart muscle wall. The left heart muscle wall is often thickened with a thin, compacted ('normal') outer layer and a thickened noncompact ('abnormal sponge-like') inner layer. Due to the changes in the muscle wall, LVNC can be associated with dilatation of the heart ('a big heart') and weakening of the heart muscle, which can impair the heart's ability to pump blood effectively. It is still not clear whether LVNC is a separate cardiomyopathy, or is merely a trait shared by many other distinct cardiomyopathies (examples mentioned on page 1).

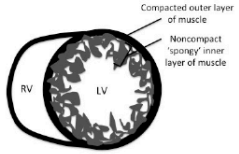
LVNC is thought to result from having a genetic abnormality where there is a change in one of the genes that code for heart muscle proteins resulting in abnormalities in the structure of the heart muscle wall. LVNC can be caused by mutations (mistakes) in the genes that code for a variety of heart muscle proteins, including the sarcomeric proteins, cytoskeletal proteins, mitochondrial proteins, Z-line proteins, just to name a few. It is complex condition to diagnose on DNA testing because so many genes can cause the problem and the mutations are often unique to an individual or family.



RV = right ventricle, LV = left ventricle



Normal heart



LVNC

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Common symptoms of LVNC

Patients with LVNC can present with a number of problems described below.

1. ***Heart failure (HF)*** is the most common presentation in patients with LVNC. The term *"heart failure"* is used to describe the impairment of the heart's ability to pump blood effectively due to weakening of the heart muscle wall. The severity of symptoms depends on the degree impairment/damage of the heart muscle.

   The symptoms of heart failure include some or all of the following:
   - ***Reduced effort tolerance*** – fatigue or shortness of breath when exerting oneself. This occurs because the heart muscle is unable to keep up with the oxygen demands of the body during increased activity. We grade the symptoms according to standardized criteria developed by the New York Heart Association *(Heart 2007;93:476–482)*
     - Class 1: Patients have cardiac disease but have no limitations of physical activity. Ordinary physical activity does not cause undue fatigue or shortness of breath
     - Class 2: Patients have cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue or shortness of breath
     - Class 3: Patients have cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue or shortness of breath
     - Class 4: Patients have cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of fatigue and shortness of breath may be present even at rest. If any physical activity is undertaken, discomfort is increased.
   - *Shortness of breath on lying flat* ***(orthopnoea)*** or *waking up suddenly breathless* ***(paroxysmal nocturnal dyspnea [PND])*** due to fluid build up in the lungs. Patients find that they have to sleep sitting upright to avoid becoming short of breath.
   - *Swelling* ***(oedema)*** of the legs (and body) due to a build up of water in the tissues.

2. Some patients can present with a ***stroke***, often in the form of weakness on one side of the body (e.g. right side of face, right arm and leg). This is caused by a blood clot that forms in the heart and breaks off and travels to the brain where it blocks off a blood vessel supplying a portion of the brain. Unfortunately, blood flowing through the heart gets trapped in the 'sponge-like holes' (noncompact areas) in the heart muscle and small clots develop. We can try and prevent strokes by stopping clot formation in the heart by treating patients with blood thinners (e.g. warfarin). It is recommended that we only treat patients with blood thinners if their heart function is impaired (LVEF < 40%).

APPENDIX F – Information Sheets for Patients: VI. Information booklet on LVNC (pages 5 and 6)

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Common symptoms of DCM *(continued)*

3. An ***arrhythmia*** is an electrical problem where there is an abnormality in the timing, pattern or rate of the heartbeat. The heartbeat can be too slow (bradycardia), too fast (tachycardia) or irregular. Symptoms of an underlying arrhythmia can include some or all of the following:
   - ***Palpitations*** – racing, skipping or fluttering sensation in your chest and/or throat
   - ***Chest discomfort/pain*** – caused by a small drop in blood pressure as a result of the heart pumping less blood to the heart muscle itself
   - ***Pre-syncope*** – dizziness, but no loss of consciousness, caused by a small drop in blood pressure as a result of the heart pumping less blood to the brain
   - ***Syncope*** (fainting) – loss of consciousness caused by a large drop in blood pressure as a result of the heart being unable to pump enough blood to the brain

There are a number of different types of arrhythmias that can occur. Atrial fibrillation and ventricular tachycardia are two important arrhythmias that patients with LVNC can develop.

Atrial fibrillation (AF) – AF is a very stable arrhythmia. It occurs due to electrical chaos in the atria of the heart due to excessive stretching and scarring of the atria. Although the ventricles still function in the normal way, because of the electrical chaos in the atria, the ventricles are haphazardly stimulated resulting in fast irregular heartbeat. Many patients who have AF are asymptomatic but they may experience ***palpitations, chest discomfort or dizziness***. Atrial fibrillation can lead to heart failure due to a persistently elevated heart rate, and may cause a stroke. A stroke occurs when blood clots that develop inside the atria due to the abnormal blood flow, dislodge and travel to the brain. Blood-thinning medication is recommended in patients with AF to prevent strokes

Ventricular tachycardia (VT) – VT is a series of rapid heartbeats, where the electrical stimulus originates in the ventricle, usually at a site of scarring. A VT episode may last for just a few beats or may continue for several seconds or minutes. It can lead to life-threatening arrhythmias, such as ventricular fibrillation, where there is complete electrical chaos in the ventricles and the heart is unable to pump blood at all. VT can stop on its own or it may require medication or an electrical shock to convert the heart back to a normal rhythm. Although VT does not occur in all patients with LVNC, it is a recognized cause for sudden cardiac death in this condition. If you are having symptoms describe above, please consult your doctor. Although there are other causes for palpitations, chest discomfort, dizziness and fainting, you should always go to your closest emergency unit if you have an episode of fainting (collapse with loss of conciousness) so that you can be assessed by a doctor.

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How is LVNC diagnosed?

There are a number of non-invasive and invasive tests that can be done to confirm the diagnosis. In the majority of cases, the diagnosis is confirmed on clinical examination, and non-invasive imaging (echocardiogram and/or cardiac MRI) where heart size and function can be measured. Additional tests may be required depending on your symptoms. Only selected investigations will be required depending on your clinical presentation.

Non-invasive cardiac tests include:

- ***Electrocardiogram (ECG)*** – An ECG records the electrical activity of the heart. It is performed lying down, where electrodes attached to wires are placed on the chest. The machine captures the ECG tracing and prints a report that can be analysed by a doctor.
- ***Signal average electrocardiogram (SAECG)*** – A SAECG in similar to an ECG but it is able to analyse electrical signals that are altered by the presence of scar tissue in the heart. This test is not routinely done in LVNC.
- ***Holter Monitor*** – A holter monitor is a small machine that is able to record a continuous ECG over 24-48 hours while one performs normal daily activities. It can detect arrhythmias that are not seen on routine ECG. If one develops symptoms of palpitations, dizziness or chest pain, it allows the doctors to correlate the symptoms you experience with the electrical activity in the heart. This test is only done if the doctors suspect or want to exclude an arrhythmia and is not routinely require in LVNC.
- ***Exercise Stress Test (EST)*** – An EST is a treadmill or bicycle test, where an ECG and serial blood pressures are done during exercise. It is used to determine whether an arrhythmia or an abnormal blood pressure response occurs during exercise. This is not routinely done.
- ***Echocardiogram ("echo")*** – An echo is an ultrasound of the heart; it allows the operator to measure the size, structure and function of the heart. Some regions of the heart are difficult to visualize using echocardiography and therefore the presence of LVNC can be missed using this modality alone.
- ***Cardiac Magnetic Resonance Imaging (MRI)*** – MRI uses magnetic waves to produce an image of the heart that can distinguish heart muscle from scar tissue and fat. It can also assess the size, structure and function of the heart. MRI is often required to confirm the diagnosis of LVNC because we are able to take clear images of the heart at many different angles allowing us to look at the heart muscle wall in greater details. MRI is the gold standard test for diagnosing LVNC.

APPENDIX F – Information Sheets for Patients: VI. Information booklet on LVNC (page 7 and 8)

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How is LVNC diagnosed?

Only selected investigations will be required depending on your clinical presentation. In many cases, invasive investigations are not required.

Invasive cardiac tests include: (not routinely required in LVNC patients)

- ***Loop recorder*** – A loop recorder is a small devise that is inserted under the skin on the chest. It is able to record the electrical activity of the heart (ECG) over a long period of time (months or even years). It is used to evaluate whether infrequent heart arrhythmias are occurring.
- ***Electrophysiology study (EPS)*** - A EPS is done to determine whether you are at high risk for developing a serious arrhythmia so that preventative treatment can be provided. During the study, a number of sheaths (thin pipes) are inserted into the large blood vessels in your leg (and/or neck). The cardiologist then inserts wires/catheters through the sheaths and guides them to the heart. This allows us to record the electrical activity of the heart in greater detail. The cardiologist is also able to stimulate the heart to see if arrhythmias occur. During the procedure, you will be sedated and your heart rate and blood pressure will be monitored closely.
- ***Angiography (cardiac catheterization)*** – Angiography is performed in a similar way to an EPS. Contrast dye is injected into the blood to determine the structure and function of the heart, including the right and left ventricle as well as the coronary arteries (that supply the heart muscle with blood). X-ray is used to see how the contrast dye fills the heart.
- ***Cardiac biopsy*** – At the time of angiography or EPS, tiny samples of heart muscle tissue are taken. A pathologist is then able to examine the heart tissue under a microscope to determine whether the heart muscle is abnormal.

In addition, specific blood tests may be done to exclude secondary factors that may worsen heart failure, including HIV, haemaglobin levels, iron levels, thyroid function, kidney function etc.

Genetic testing

Currently in South Africa, genetic testing for LVNC is only available, in the most part, through research facilities. This means that genetic screening protocols are subject to specific research objectives and funding. In many cases, patients test negative for known mutations, and thus researchers have to look at alternative genes that could cause the disease. This process can take many months or even years. With advancing technology in this field, we hope to find the answers to many of the unanswered questions about the role of genetics in DCM and that genetic testing will become more widely available in the future.

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How is LVNC treated?

There are a few primary goals of treatment:

- To relieve the symptoms of heart failure
- To prevent stroke
- To treat arrhythmias if present
- To prevent progression of disease that will result in worsening ventricular function and heart failure.

Treatment is based on international research and guidelines, your individual cardiac test results and your symptoms. **Medication is the mainstay of treatment in LVNC. Heart transplantation** is considered when all other treatment options have failed.

Medications

Medications to treat heart failure:

- ***Diuretics*** are helpful in reducing salt and water retention that occurs once heart failure has developed. They are effective in relieving the symptoms of shortness of breath and leg swelling that results from excess water build up in the body.
- ***Beta-blockers*** have important effects on remodeling of the heart muscle and help to prevent progression of disease. Beta-blockers also lower the heart rate, blood pressure and the effects of adrenaline, which helps to reduce the workload of the heart and prevent arrhythmias. Treatment is started at very low doses and the dose is increased slowing over many weeks.
- ***ACE-inhibitors*** are helpful in reducing the workload of the heart and prevent the development of heart failure.
- **Mineralocorticoid receptor antagonists** (Spironolactone) – mild diuretic but also protects the heart from further scarring/damage
- ***Digoxin*** is a very old drug that is still used to treat in heart failure. It has been shown to improve symptoms in patients with heart failure and slows the heart rate.

Medications to prevent stroke:

Blood-thinning medication is recommended in patients with LVNC if their heart function is impaired (LVEF < 40%), or if atrial fibrillation is diagnosed, to reduce the risk of having a stroke. Both the risks and benefits of this treatment must be discussed with you prior to starting this treatment. The drug most commonly used is called warfarin. Patients on warfarin require regular blood tests to measure the thickness of the blood (prothombin index (PI) or INR) so that the dose of warfarin can be adjusted. If the blood is made too thin, you are at risk of bleeding.

Medications to treat arrhythmias:

Antiarrhythmic medications may be indicated if arrhythmias occur. They are used to bring down the heart rate in AF and/or decrease the number of episodes and severity of VT by altering the electrical properties of the heart and by blocking the effects of adrenaline on the heart. If patients experience VT on beta-blocker therapy, other antiarrhythmic drugs, such as amiodarone or sotalol, can be used.

Please remember that all medications have potential side-effects.

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How is LVNC treated?

*Note: The following invasive treatment strategies have **specific indications and are not routinely required** in all patients with LVNC.*

Implantable cardiac defibrillator (ICD) – *not routinely available or required*

ICDs are commonly used to treat patients with HCM. This small devise is implanted under the skin below the clavicle and wires are inserted into the heart. The device is able to monitor the heartbeat continuously and will automatically deliver a small electrical shock to the heart if a dangerous arrhythmia (such as ventricular tachycardia) occurs. ICDs can also function as pacemakers, stimulating the heart to beat if necessary. The device should be checked regularly (3-6monthly) and the battery must be replaced every 4-6 years. These devices do not prevent arrhythmias from occurring; they treat the arrhythmia when it occurs to prevent sudden cardiac death. These devices are very expensive and it is not possible to implant one in every patients living with LVNC in Africa. By doing specialized tests, we are able to access whether you are at an increased risk of developing dangerous arrhythmias and motivate for the necessity for an ICD.

Cardiac resynchronisation therapy (CRT) – *not routinely available and less commonly required*

Ventricular dyssynchrony occurs when the right and left ventricles beat out of time with each other. This happens whens the electrical current to one of the ventricle is interrupted (left or right bundle branch block). This can occur as a complication of DCM and LVNC, and can result in worsening heart failure symptoms because the heart pump is compromised. Cardiac resynchronisation therapy (CRT) is considered in patients who have moderate to severe heart failure symptoms and a bundle branch block on ECG. A pacemaker devise is implanted under the skin below the clavicle. Two wires attached to the device are placed into the heart, one in contact with right ventricle and one in contact with the left ventricle. These wires carry electrical currents that stimulate the ventricles to beat together thus improving the heart pump function. The device should be checked regularly and the battery must be replaced every 5-10 years.

Catheter ablation: - *not routinely available and usually not required in LVNC*

Catheter ablation is a procedure whereby the areas of the heart that cause arrhythmias are located and eliminated by cauterization. It is done to reduce the frequency of sustained VT episodes. It is not a curative procedure, and it is important that patients discuss the risks and benefits of catheter ablation with their doctor before having the procedure.

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Living with LVNC

For the majority of patients diagnosed the LVNC, it is an unexpected diagnosis. It often helps to explain a long history of symptoms. It is not uncommon for patients with this diagnosis to feel a sudden loss of well-being and anxiety about their future. As with any chronic illness, it can take some time to psychologically and emotionally come to terms with having this condition. As LVNC is a genetic condition, family screening (looking at the hearts of other family members) gives us an opportunity to make a diagnosis in affected family members early on in the disease process, allowing us to plan treatment. It also allows us to reassure those family members who are not affected.

There are a number of lifestyle adjustments that should be considered. While the medical community has made great progress in understanding this condition, there are still many questions about how best to treat this condition and what to expect once the diagnosis has been made. We have learnt through studying people who have LVNC that exercise can increase the risk of arrhythmias and put additional strain on the heart. We recommend that patients focus of low-intensity aerobic activities (walking, golf, Pilates) and avoid competitive high intensity sports (long-distance running, rugby, cycling, athletics, competitive swimming, crossfit). Your ability to participate in sporting activities may be limited by your impaired heart function. Patients are advised to limit intake of alcohol and caffeine, which may precipitate arrhythmias and be toxic to the heart muscle cells.

For women with LVNC, **contraception must be addressed** because pregnancy may be contraindicated depending on the severity of your heart muscle impairment. It is important to talk to your doctor prior to falling pregnant and the decision to go ahead with a pregnancy must be individualized. Pregnancy puts additional strain on the heart and can precipitate heart failure, which will put both mother and child at risk, particularly during the third trimester. Pregnancy may also precipitate progression of heart muscle disease resulting is irreversible worsening of the heart muscle function. Many of the medications used to treat LVNC (e.g. ACE inhibitors, warfarin) can be dangerous to the fetus, but the risks associated with stopping these medications have be carefully considered prior to doing so. Impaired heart function may result in poor perfusion of the placenta, which can impair the growth of the baby.

For both men and women who are affected by LVNC, it is very important to have genetic counseling prior to considering having a baby, so that you and your partner are comfortable with the potential risks to your child of inheriting this condition.

By participating in the African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP), you are actively helping the international medical community learn more about this condition. With the knowledge gained through studying people with LVNC, we hope to positively impact the lives of those living with this condition.

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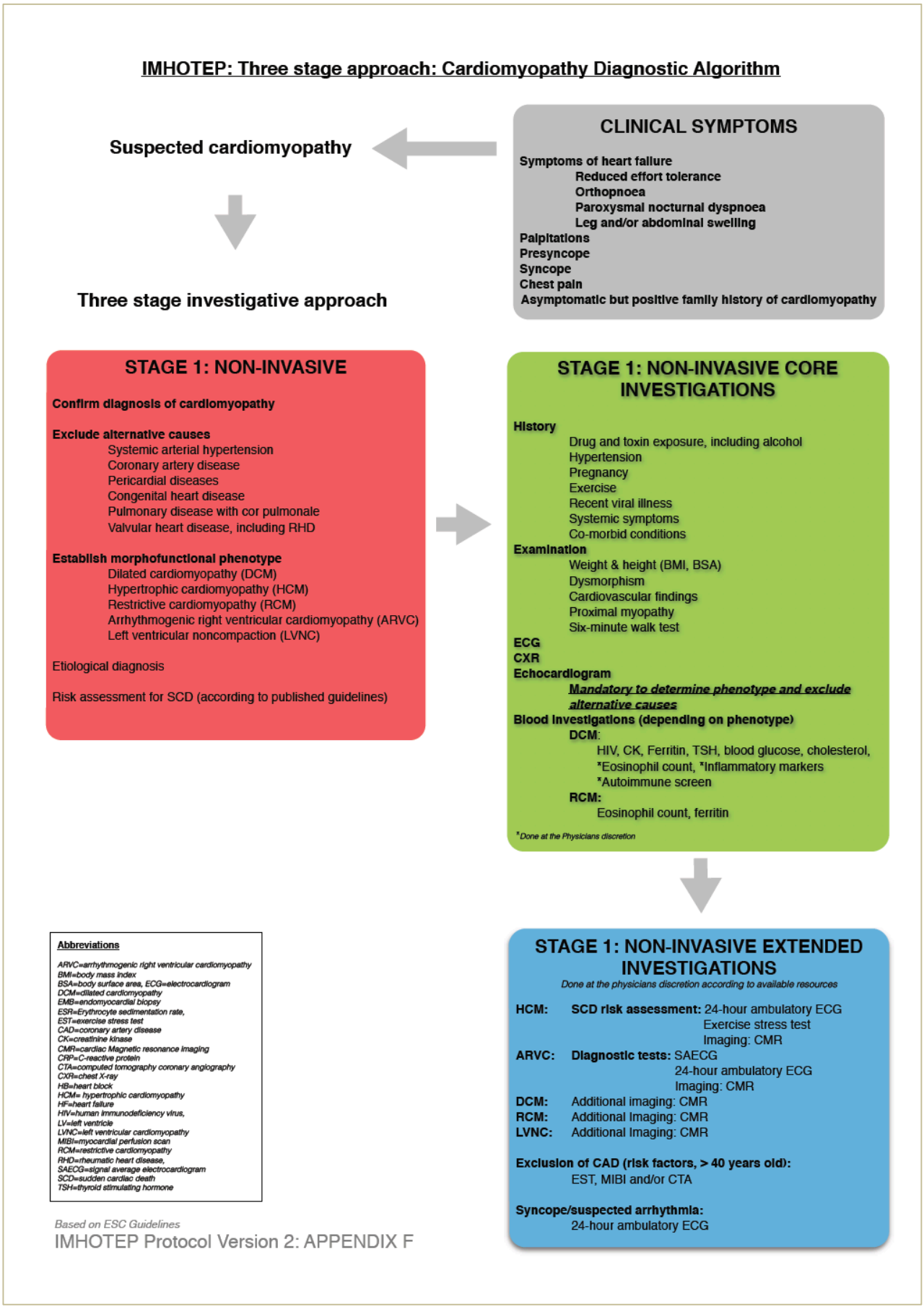
UCT HREC REF: 766/2014

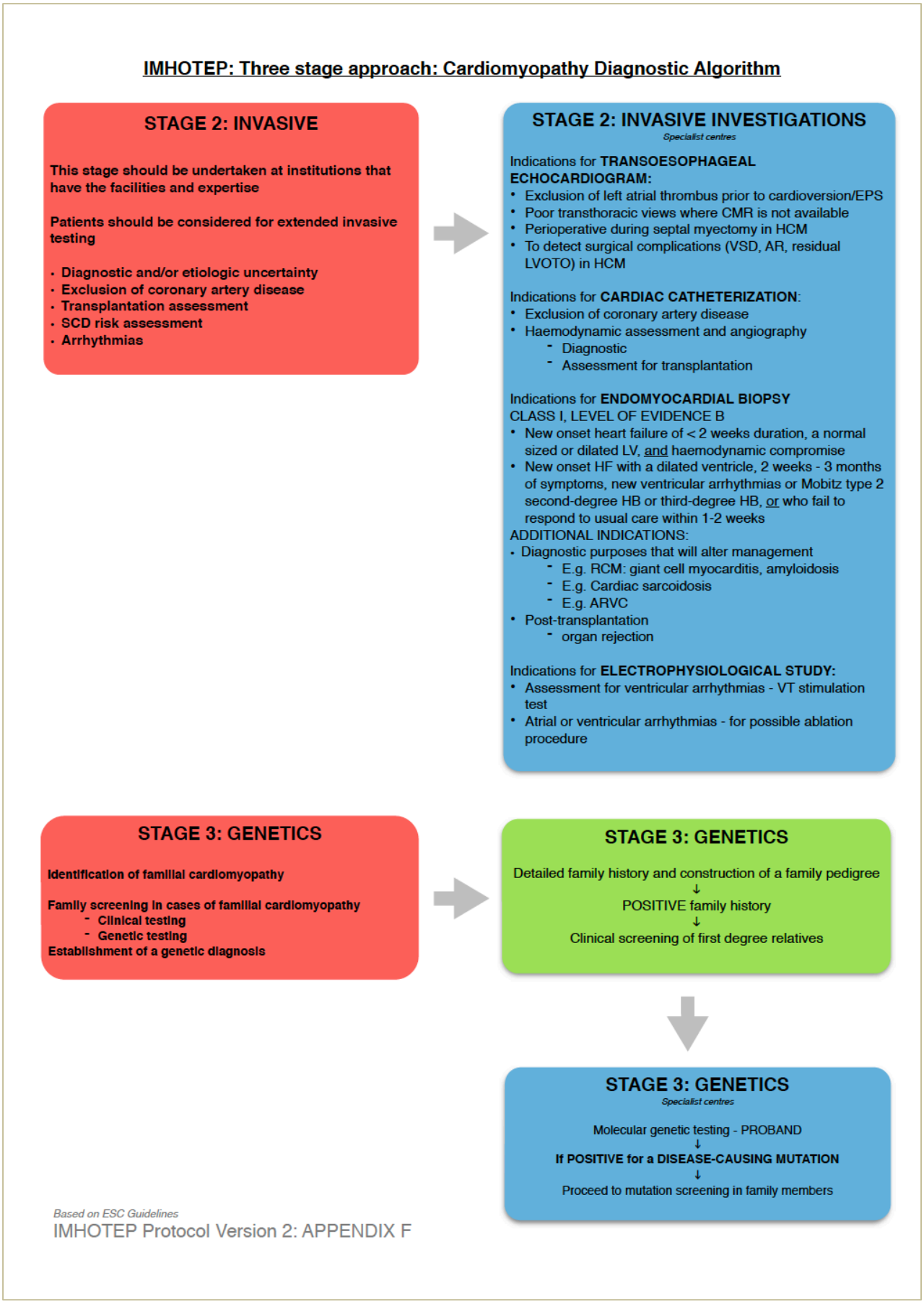
APPENDIX G – Clinical algorithms

I. Cardiomyopathy 3-stage diagnostic approach (page 1)



APPENDIX G – Clinical algorithms

I. Cardiomyopathy 3-stage diagnostic approach (page 2)



APPENDIX G – Clinical algorithms

II. Acute myocarditis

ACUTE MYOCARDITIS ALGORITHM

Clinical features:

Symptomatic
- Heart failure
- Chest pain
- Palpitations
- Syncope (due to arrhythmia or heart block)
- Fatigue
- Cardiogenic shock
- Averted sudden cardiac death

Asymptomatic

With or without a history of a recent flu-like illness

One or more of the following:

Elevated biomarkers
- Troponin, CK, CRP/ESR, WCC, BNP

ECG changes
- Non-specific T wave or ST changes
- Atrial or ventricular conduction delays or arrhythmias

Echocardiogram (any of the following)
- Impaired systolic function
- Regional wall motion abnormalities
- Tissue doppler imaging abnormalities
- Dilated cardiomyopathy phenotype

↓

Possible subclinical acute myocarditis (asymptomatic)
OR
Probable acute or fulminant myocarditis (symptomatic)

↓

Exclusion of acute coronary syndrome
Coronary angiography (gold standard) or clinical assessment

↓

Diagnostic and management approach for myocarditis

↙ ↘

Three stage investigative approach

STAGE 1: NON-INVASIVE

Core investigations
- History and clinical examination
  - Drug and toxin exposure
  - Recent viral illness
  - Systemic symptoms
- Electrocardiogram
- Chest X-ray
- Echocardiogram
- Biomarkers (Troponin, CK, CRP/ESR, BNP)

Blood investigations **At physicians discretion*
- HIV (Human Immunodeficiency Virus)
- *Autoimmune screen, *SACE & calcium

Cardiovascular magnetic resonance (CMR) Imaging

***Should be done within 3 weeks of onset of symptoms*
- T1&T2-weighted images - myocardial oedema
- LGE - Linear mid-wall enhancement

STAGE 2: INVASIVE

Indications for **ENDOMYOCARDIAL BIOPSY (EMB)**
Patients with heart failure and either of the following:
1. a normal sized or dilated LV, < 2 weeks symptoms, and haemodynamic compromise, OR
2. a dilated ventricle, 2 weeks - 3 months of symptoms, new ventricular arrhythmias or Mobitz type 2 second-degree HB or third-degree HB, or who fail to respond to usual care within 1-2 weeks

Cardiac Catheterisation *(see cardiomyopathy diagnostic algorithm)*

STAGE 3: GENETIC

Family history of cardiomyopathy or SCD
↓
If positive, consider myocarditis as a secondary insult in addition to an underlying cardiomyopathy and proceed to family screening and/or genetic testing

Clinical management approach

HAEMODYNAMICALLY UNSTABLE
↓
Resuscitation and supportive management
↓
Refer urgently to cardiology for urgent EMB and assessment for

HAEMODYNAMICALLY STABLE
↓
Stage 1: Non-invasive investigations

LVEF > 45%

Biomarkers - CMR -	Biomarkers + CMR +
↓	↓
Myocarditis unlikely	Myocarditis suspected
↓	↓
HF Treatment	HF Treatment
	↓
	No recovery despite treatment for 2-3 months
	↓
	Stage 2: Invasive investigations
	↓
	EMB

LVEF < 45%

Biomarkers - CMR -	Biomarkers + CMR +
↓	↓
Myocarditis unlikely	Myocarditis suspected
↘	↙
HF Treatment	
↓	
Worsening despite treatment OR No recovery despite treatment in 2-3 months	
↓	
Stage 2: Invasive investigations	
↓	
EMB	
↙	↘
Myocarditis	No myocarditis
↓	↓
Viral studies	HF treatment

ECG=electrocardiogram, EMB=endomyocardial biopsy, ESR=Erythrocyte sedimentation rate, CK=creatinine kinase, CMR=cardiac Magnetic resonance imaging, CRP=C-reactive protein, CXR=chest X-ray, LVEF=left ventricular ejection fraction. HB=heart block. HF=heart failure. HIV=human immunodeficiency virus.

APPENDIX H

I. Algorithm for recruitment of prevalent cases from existing studies at GSH

IMHOTEP: Standard operating procedure for recruitment of prevalent cases from existing studies
Recruitment site: Groote Schuur Hospital, Cape Town

Studies:
ARVC Registry of South Africa
Study of Familial Dilated Cardiomyopathy

STEP 1: Identify index case (proband)

Screening
- CVGpatientdata log (laboratory master log)
- Clinical Study-specific logs

STEP 2: Informed consent

Find DNA consent form by checking:
- Copied DNA consents filed in the research unit
- If no copy on file in the research unit, check laboratory files for consent

If consent is found
- **Check the name and DOB**
- **Check consent form is SIGNED**
- **Copy consent and refile in IMHOTEP folders**

Family pedigree (usually on the back of the consent form)
- Check the pedigree is for the correct patient
- Confirm the patient is the proband
- Copy pedigree and refile in IMHOTEP folder

No signed consent form

EXCLUDE

If proband is included, consider family for suitable for recruitment if:
- Familial disease likely
- Clinical family screening been done
- DNA collected
- Consent from relatives

STEP 3: Clinical data collection

Look for clinical data for the patient by reviewing all the following resources
- Existing research file
- GSH Hospital folder
- Cardiac Clinic Buff
- Contacting referring doctor/institution
- Existing research databases

For suspected ARVC cases, review by diagnostic panel is mandatory

Clinical data must be sufficient to
1. **Confirm the diagnosis of Cardiomyopathy**
2. **Core baseline characteristics available**

Research folders
- Updated research records
- Copy source documents and file in IMHOTEP research folders

No clinical data
No diagnosis

EXCLUDE

ENROLL INTO IMHOTEP

STEP 4: Assign IMHOTEP recruitment number

Assign an IMHOTEP recruitment number according to the next available number on the master log
Add patient personal details (hospital number, contact information) to Recruitment Master Log
Add the corresponding DNA number from the CVGpatientdata log
- Check the name and DOB reconcile
- Filter using first name, surname and DOB to ensure that the individual has not been assigned multiple DNA numbers
- If the individual has multiple DNA numbers, include all DNA numbers on the Recruitment Master Log

STEP 5: Register patient on OpenClinica
- Create subject using primary ID
- Add existing DNA number as secondary ID

STEP 6: Data entry into OpenClinica
- Direct entry from source documents by doctor, **OR**
- Doctor to complete paper CRF to be captured by data-capturer.

APPENDIX H

II. Algorithm for recruitment of incident and newly identified prevalent cases to IMHOTEP

IMHOTEP: Standard operating procedure for recruitment and collection DNA specimens

Prevalent or incident index case with cardiomyopathy or myocarditis

Genetics counselling should be provided prior to consent and specimen collection **Information sheet on Genetics Research must be given to patient to keep.**

STEP 1: Informed consent:
1. IMHOTEP consent
2. **Consent for DNA collection and analysis**

STEP 2: Assign IMHOTEP recruitment number by selecting prepared pack containing **BARCODED STICKERS**, blood tubes, and equipment for taking blood.

STEP 3: Collection of blood specimen:
- Please use disposable gloves when handling blood
- Please ensure blood tubes are adequately filled
- Please ensure that the **blood tubes are inverted 10x** after blood is drawn
- **Label the specimens** with barcoded labels provided
- Place specimens in separate plastic packet provided and then place inside the specimen plastic pack with barcode identifier and remaining unused equipment

EDTA (purple)

Paxgene

STEP 4: Specimen log and labelling

Complete **specimen log**
- **Original to be transferred with blood specimens to the laboratory**
- Copy to be filed at the site

Labeling of documents - Place assigned labels on DNA consent form, IMHOTEP consent form, IMHOTEP participants folder (plastic sleeve), CRF

STEP 5: Transfer of blood specimens to laboratory
- All specimens collected in **EDTA tubes must be stored in the fridge and transferred with a cold pack and must be processed by the laboratory with in 1-3 days**
- Specimens collected in **Paxgene tubes** can be kept at room temperature and must be processed by the laboratory with in 2 weeks
- Specimens must be transported in a styrofoam box
- Original DNA consent forms with corresponding barcoding labels and the specimen log should accompany the blood

On-site buffy coat processing of EDTA specimens may be required at distant sites, as per protocol/training provided by the Hatter laboratory
- 2 x cryvials (with barcoded labels) and pipette provided

To arrange transfer/courier of bloods to the laboratory, please contact Janine Saaiman on +27214066894 or Janine.saaiman@uct.ac.za (alternatively, Dr Gasnat Shaboodien on +27214066615)

STEP 6: Register participant on Open Clinica

Responsibility of the recruiting site research nurse/administrator
1. Add participant to **site log** containing personal identifiers (excel and/or paper log)
2. **Register participant on OpenClinica** (see OpenClinica SOP on how to create a subject)
3. Create **participant folder/sleeve** and file the following:
   - Label folder/sleeve with barcode provided
   - **Original** IMHOTEP consent (copy given to participant) – label with barcode provided
   - **Copy** DNA consent (original sent to laboratory)
   - Patient contact information sheet
   - Baseline clinical information CRF – to be entered onto OpenClinica by site nurse/data capturer (see OpenClinica SOP) – label with barcode provided
   - Copies of source documents (echo report, ECG, ±notes, ±other investigations)

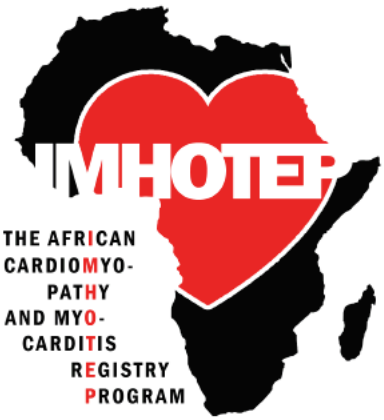
Hatter Institute Molecular Laboratory

(as per standard lab protocols)
- **Specimen log** must be signed by person receiving bloods
- **DNA consent form** processed, scanned and filed
- **Genetics CRF on OpenClinica** – To be completed by the lab team
- Process blood sample (buffy) and store as per laboratory protocols
- Assist sites with reconciliation

Version: July 2017

APPENDIX I – Case Report Form for baseline core data (adults) (page 1)

IMHOTEP BASELINE CRF FOR RECRUITMENT OF ADULTS



Baseline assessment for ADULTS with cardiomyopathy or myocarditis

(individuals ≥ 14 years)

Patient identifier

Study arm: ☐INCIDENT ☐PREVALENT ☐RELATIVE

Diagnostic category: _____

Investigations done prior to, or at the time of recruitment:		
Core investigations (mandatory)	**Extended non-invasive investigations***	**Invasive extended investigations***
☐ Blood investigations	☐ SAECG	☐ Angiography
☐ ECG	☐ Ambulatory ECG (holter)	☐ EP study
☐ Echocardiogram	☐ Exercise stress test (EST)	☐ Cardiac biopsy
☐ CXR	☐ CMR	☐ Skeletal muscle biopsy
☐ Genetic screening	☐ Cardiac CT	
	☐ Radionuclide study	

**Not routinely required*

August 2017

APPENDIX I – Case Report Form for baseline core data (adults) (page 2)

IMHOTEP BASELINE CRF FOR RECRUITMENT OF ADULTS

DEMOGRAPHICS										
Sex	☐Male ☐Female	**Date of birth**	DD/MM/YYYY	**Ethnic group**	B	W	MR	I	A
Education *(highest level completed)*	☐Primary school ☐High school	☐Matric ☐No education	☐Tertiary education ☐Post graduate	Comment					
Occupation	☐White collar job ☐Blue collar job	☐Pink collar job ☐Government grant	☐Unemployed ☐Pensioner	Comment					
	White-collar worker *= professional, managerial, or administrative work* ***Blue-collar worker*** *= manual labour, mining, construction, domestic worker* ***Pink-collar worker*** *= service industry, entertainment, sales*								

CLINICAL PRESENTATION	
Date of recruitment/consent	DD/MM/YYYY
Date of onset of symptoms	DD/MM/YYYY
Date of first presentation to a medical facility	DD/MM/YYYY
Age at first presentation	
Type of presentation	☐ Sudden cardiac death (SCD) ☐ Symptomatic and living (not resuscitated, includes syncope, VT, HF) ☐ Resuscitated (survived cardiac arrest) ☐ Asymptomatic (abnormal test or family screening or incidental finding) ☐ Embolic event (stroke or limb ischaemia)
Provisional diagnosis (select one only) *If a mixed phenotype, select cardiomyopathy –not specified; or other and specify*	☐ Dilated cardiomyopathy ☐ Hypertrophic cardiomyopathy ☐ Arrhythmogenic right ventricular cardiomyopathy ☐ Restrictive cardiomyopathy ☐ Left ventricular non-compaction ☐ Cardiomyopathy – not specified ☐ Peripartum cardiomyopathy - DCM ☐ Peripartum cardiomyopathy – does not fulfill criteria for DCM ☐ Myocarditis with left ventricular dysfunction ☐ Myocarditis without left ventricular dysfunction ☐ Unaffected relative – familial cardiomyopathy ☐ Other – Specify _____

BASELINE SYMPTOMS: INDIVIDUALS ≥ 14 YEARS		
Symptoms at presentation	☐ Asymptomatic	☐ Symptomatic
Symptoms	☐ Syncope ☐ Pre-syncope/dizziness ☐ Chest pain ☐ Palpitations ☐ Orthopnoea	☐ Paroxysmal nocturnal dyspnea ☐ Dyspnoea ☐ Body swelling/oedema ☐ Cough ☐ Fever
NYHA Class	☐ Class I - *Patients have cardiac disease but without the resulting limitations of physical activity. Physical activity does not cause undue fatigue, palpitations or dyspnoea.* ☐ Class II - *Patients have cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Moderate physical activity results in fatigue, palpitations or dyspnoea.* ☐ Class III - *Patients have cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity (washing, dressing, making a bed) causes fatigue, palpitations, or dyspnoea.* ☐ Class IV - *Patients have cardiac disease resulting in inability to carry on any physical activity without discomfort/dyspnoea. Discomfort/dyspnoea may be present even at rest. If any physical activity is undertaken, discomfort is increased*	

IMHOTEP BASELINE CRF FOR RECRUITMENT OF ADULTS

PRECIPITATING FACTORS *(occurring prior to presentation that may have contributed to the development of CMO or progression of disease)*		
History of preceding flu-like illness *Please specify illness if present*	☐Yes ☐No ☐Unknown ☐Not documented	
	☐ Non-specific viral illness	☐ Measles
	☐ Confirmed enterovirus	☐ Viral hepatitis
	☐ Chicken pox	☐ Other - specify _____

PRECIPITATING FACTORS *(continued)*		
Recent Immunizations ☐Yes ☐No ☐Unknown ☐Not documented ***ABBREVIATIONS:*** *T = tetanus* *d or dap = diphtheria* *IPV = inactivated polio vaccine* *aP = acellular pertussis* *HBV = Hepatitis B vaccine* *Hib = haemophilus inflenzae type b* *HPV = Human papillomavirus vaccine*	***If yes, please complete the following:***	
	Date of last immunization	DD/MM/YYYY
	Type of last immunization(s) received *(more than one can be selected)*	
	☐ OPV: Oral polio vaccine	☐ Varicella
	☐ BCG: Bacille Calmette Guerin vaccine	☐ Seasonal Influenza
	☐ HBV	☐ Td vaccine
	☐ RV: Rotavirus vaccine	☐ Tdap-IPV
	☐ Measles	☐ DTaP
	☐ Mumps	☐ DTaP-IPV/Hib
	☐ Rubella	☐ DTaP-IPV-Hib-HBV
	☐ MMR: Measles, mumps and rubella	☐ DTaP-IPV-HBV/Hib
	☐ MCV: Meningococcal	☐ HPV:
	☐ PCV: Pneumococcal conjugated vaccine	☐ Other - specify
	☐ Hib: haemophilus influenzae b	_____
Pregnancy ☐N/A (male sex) ☐Never been pregnant ☐Currently pregnant ☐Previously pregnant	***If currently pregnant or previously pregnant, please complete the following:***	
	Gravity ____ Parity ____	
	Due date OR date of last delivery	DD/MM/YYYY
	Symptoms during last pregnancy	☐Asymptomatic during pregnancy ☐Gestational hypertension but no symptoms of heart failure ☐Gestational hypertension with symptoms of heart failure ☐Symptoms of heart failure only ☐Unknown ☐Not documented
	Onset of symptoms of heart failure within first 5 months postpartum	☐Yes ☐No ☐N/A ☐Unknown ☐Not documented
	Breastfeeding	Months

IMHOTEP BASELINE CRF FOR RECRUITMENT OF ADULTS

TOXIN/DRUG EXPOSURE	
Medication-drug exposure (specifically known to redispose to heart muscle damage) ***If yes, please specify***	☐ Yes ☐ No ☐ Unknown ☐ ND ☐ Anthrocycline-based chemotherapy ☐ Maternal anti-retroviral therapy (antenatal) ☐ Other – specify _____
Alcohol	☐ Never ☐ Current ☐ Former ☐ Unknown ☐ ND *If current or former alcohol use, please specify quantity:* ☐ < 30 units per month ☐ > 30 drinks per month ☐ Binge drinking (≥5 units)
Illicit drugs	☐ Never ☐ Current ☐ Former ☐ Unknown ☐ ND *If yes, specify type of drug* ☐ Cocaine ☐ Methamphetamines ☐ Cannabis ☐ Heroin ☐ Kat ☐ Other - Specify _____
Smoking	☐ Never ☐ Current ☐ Former ☐ Unknown ☐ ND *If current or former smoker, specify number of pack years:*

SOCIAL				
Family History				
Family history of heart failure	☐ Yes	☐ No	☐ Unknown	☐ ND
Family history of cardiomyopathy	☐ Yes	☐ No	☐ Unknown	☐ ND
SCD in a family member <35 years old	☐ Yes	☐ No	☐ Unknown	☐ ND
SCD in a family member >35 years old	☐ Yes	☐ No	☐ Unknown	☐ ND
Family history – other *(please specify)*				
Participation in age-related activities or sport **Social** (e.g. big walks) **Recreational** (e.g. jogging, pilates, yoga, gym, golf, watersports, school sport, social team sports) **Competitive** (regularly competes in events e.g. half or full marathons, cycling, triathlons, Crossfit competitions, university or provincial teams) **Professional athlete**	☐ Never participated in sport ☐ Social sport ☐ Recreational sport ☐ Competitive (provincial/national teams) sport ☐ Professional athlete ☐ Unknown ☐ Not documented			
If competative/professional athlete, please specify sport				

CO-MORBID CONDITION				
HIV infected	☐ Positive	☐ Negative	☐ Unknown	☐ ND
If HIV positive, Antiretroviral Therapy	☐ Yes	☐ No	☐ Unknown	☐ ND
Co-morbid condition	☐ Present	☐ Absent	☐ Unknown	☐ ND
If co-morbid condition present: Diagnosis 1				
Diagnosis 2				
Diagnosis 3				
Diagnosis 4				
Diagnosis 5				
Diagnosis 6				

IMHOTEP BASELINE CRF FOR RECRUITMENT OF ADULTS

MEDICATIONS				
Select category	**Drug name** *(do not use trade names)*		**Dose (mg)**	**Frequency/day**
☐ Diuretics	☐ Furosemide ☐ Hydrochlorothiazide			
☐ MRA	☐ Spironolactone			
☐ Beta-blocker	☐ Carvedilol ☐ Bisoprolol ☐ Atenolol			
☐ Anti-arrhythmics	☐ Digoxin ☐ Amiodarone ☐ Ivabradine			
☐ ACE inhibitors/ARB	☐ Enalapril ☐ Captopril ☐ Losartan			
☐ Calcium channel blockers	☐ Verapamil ☐ Amlodipine			
☐ Anti-coagulation/ anti-platelet	☐ Warfarin ☐ Aspirin			
☐ Other				

IMHOTEP BASELINE CRF FOR RECRUITMENT OF ADULTS

EXAMINATION			Date of examination	D D / M M / Y Y Y Y
GENERAL EXAMINATION	Height (cm)		Weight (kg)	
Signs	☐ Pallor ☐ Jaundice ☐ Clubbing ☐ Rash	☐ Wasting ☐ Arthritis ☐ Cyanosis ☐ Cool peripheries	☐ Dysmorphic ☐ Thyroid goiter ☐ Pedal oedema ☐ Sacral oedema	☐ Anasarca ☐ Lymphadenopathy ☐ Oral candida
	☐ Other – specify			

CARDIOVASCULAR					
Pulse	Rate				
	Rhythm	☐ Regular	☐ Irregular	☐ Unknown	
	Amplitude	☐ Low	☐ Normal	☐ High	☐ Unknown
	All pulses present	☐ Yes	☐ No	☐ Not done	
Blood pressure	Blood pressure	s	d	M	
JVP	JVP	☐Not elevated < 4cm ☐Elevated (> 4cm)	☐Not applicable in children under 5 years ☐Unknown		
	JVP wave	☐Normal ☐CV-waves	☐Canon a waves ☐X-descents	☐Y-descents ☐Unknown	
Precordium	Scars	☐None ☐Sternotomy	☐Thoracotomy	☐ICD	☐PPM ☐Other
	Palpation	☐ Normal ☐ Parasternal heave ☐ Thrill	☐ Epigastric heave ☐ Palpable S1 ☐ Palpable P2		
	Apex beat	☐ Normal ☐ Displaced laterally ☐ Not palpable ☐ Poorly localized	☐ Pressure-loaded but not displaced ☐ Pressure-loaded and displaced laterally ☐ Unknown		
Auscultation	Heart sounds	☐Normal ☐Soft S1	☐Loud S1 ☐Loud P2	☐Loud A2	☐Fixed split S2
	Murmurs *(timing, location, radiation, grade)*				
	Added sounds	☐None ☐S3 (pathological)	☐Physiological S3 ☐S4	☐Diastolic knock ☐Pericardial rub	

OTHER SYSTEMS			
Respiratory examination	☐Clear lung fields ☐Basal crackles consistent with pulmonary oedema (older children) ☐Wheezing ☐Hyperinflation ☐Basal dullness and reduced breath sounds suggestive of a pleural effusion ☐Primary pulmonary disease (please comment)		Comment:
Abdominal examination	☐Normal ☐Hepatomegaly ☐Splenomegaly ☐Ascites		Comment:
Neurological examination	☐Not done ☐Normal ☐Deficit in keeping with a CVA (please comment) ☐Proximal weakness (please comment) ☐Primary neurological disease other than CVA (please comment)		Comment:
SIX-MINUTE WALK	**☐Performed** **☐Not performed**		
Date performed	D D / M M / Y Y Y Y	Distance walked	m

August 2017

APPENDIX I – Case Report Form for baseline core data (adults) (page 7)

IMHOTEP BASELINE CRF FOR RECRUITMENT OF ADULTS

CXR				
Date performed		DD/MM/YYYY	CXR view	☐PA (preferable) ☐AP
Film quality	☐Good ☐Sub-optimal	*If suboptimal, please select:* ☐Mobile AP ☐Over or underpenetrated ☐Rotated film ☐Inadequate inspiration ☐Other _____		
Cardiac	Cardiomegaly		☐Yes ☐No	
	Cardiothoracic ratio on PA film		☐≤50% ☐>50% ☐PA film not done	
Lung fields	Pulmonary oedema/congestion	☐Yes ☐No	*Upper lobe blood diversion (on an erect film) and / or Kerley B lines and / or Confluent alveolar shadowing which spreads out from the hilum ("bats wing" appearance)*	
	Fluid in the fissures	☐Yes ☐No		
	Right pleural effusion	☐Yes ☐No		
	Left pleural effusion	☐Yes ☐No		
	Primary pulmonary pathology	☐Yes ☐No	☐Lobar pneumonia (consolidation) ☐Tuberculosis – cavitating lung disease ☐Tuberculosis – miliary ☐Tuberculosis – pleural disease ☐Post-tuberculosis fibrotic change ☐Interstitial lung disease ☐Isolated lymphadenopathy ☐Lung hyperinflation in children	☐Bronchopneumonia ☐Bronchiolitis ☐Bronchiectasis ☐COPD/emphysema ☐Sarcoidosis ☐Pneumothorax ☐Lung mass
Other	Other – specify			

BASELINE LABORATORY INVESTIGATIONS *NOT ROUTINELY DONE				
Date	HB	MCV	WCC	PLT*
Date	Sodium	Creatinine	eGFR	Potassium
Date	Calcium	Magnesium*	Phosphate*	
Date	ALP	GGT	ALT	AST
Date	T. Bilirubin*	C. Bilirubin*	Albumin*	

CORE INVESTIGATIONS FOR DCM (and RCM) **NOT ROUTINELY DONE – done at the physicians discretion*		***Please note:*** (Not routinely required in HCM, ARVC, LVNC unless clinically indicated) *Ferritin and Eosinophil count should be done in RCM, Troponin T, CK, CRP/ESR, ±Eosinophil count, ±Autoimmune screen to be done if myocarditis is suspected, Skeletal muscle biopsy only indicated if mitochondrial myopathy or muscular dystrophy suspected*		
Date	**HIV serology**	HIV PCR*	CD4 Count*	VL*
Date	**TSH**	T4*	T3*	
Date	**CK**	CK-MB*		
Date	***hs*-Trop T**	Trop T*	Trop I*	
Date	**Ferritin**	Transferrin sats %*		
Date	**Glucose**	HbA1C*		
Date	**Cholesterol**			
Date	**Eosinophils**	Leucocyte*		
Date	CRP*	ESR*		
Date	ANA*	Anti-DS-DNA*	RF*	Anti-CCP*
Date	Pro-BNP*			
Date	Serum ACE*			
Date	Urine dipstick			
Date	Other*			
Date	Skeletal Muscle Biopsy*			

August 2017

APPENDIX I – Case Report Form for baseline core data (adults) (page 8)

IMHOTEP BASELINE CRF FOR RECRUITMENT OF ADULTS

ELECTROCARDIOGRAM				Date performed			DD/MM/YYYY
Rhythm **If ventricular arrhythmia present, complete rhythm and ventricular arrhythmia sections only*	Rate	bpm					
	Rhythm	☐Sinus rhythm ☐Sinus arrhythmia with APC's ☐Sinus pause >1.2 seconds ☐Atrial rhythm ☐Atrial fibrillation		☐Atrial flutter ☐Junctional rhythm ☐First degree heart block ☐Mobitz I heart block ☐Mobitz II heart block ☐Complete heart block		☐2:1 heart block ☐Paced rhythm ☐Ventricular rhythm* ☐Unknown	
PR Interval	PR interval	ms		PR elevation		☐Present	☐Absent
QRS	QRS duration	ms		QRS Axis			Degrees
	QRS Morphology	☐Normal ☐RBBB ☐LBBB ☐LBBB & RBBB ☐Incomplete RBBB		☐Incomplete LBBB ☐Ventricular pre-excitation of WPW ☐Non-specific conduction abnormality ☐Left anterior fascicular block (LAFB) ☐Left posterior fascicular block (LPFB)			
	R wave progression			☐Normal	☐Poor	☐Unknown	
QT interval		QT	ms	RR	ms	QTc	
Depolarization	Epsilon wave	☐Present	☐Absent	☐V1 ☐V2 ☐V3			
	QRS fractionation	☐Present	☐Absent	☐V1 ☐V2 ☐V3 ☐V4 ☐V5 ☐V6 ☐I ☐II ☐III ☐aVR ☐aVL ☐aVF			
	TAD ≥ 55ms	☐Present	☐Absent	☐V1 ☐V2 ☐V3 ☐V4 ☐V5 ☐V6 ☐I ☐II ☐III ☐aVR ☐aVL ☐aVF			
	Low-voltage < 0.5mV	☐Present	☐Absent	☐V1 ☐V2 ☐V3 ☐V4 ☐V5 ☐V6 ☐I ☐II ☐III ☐aVR ☐aVL ☐aVF			
Repolarization	TWI	☐Present	☐Absent	☐V1 ☐V2 ☐V3 ☐V4 ☐V5 ☐V6 ☐I ☐II ☐III ☐aVR ☐aVL ☐aVF			
	T wave alternans	☐Present	☐Absent				
ST changes	ST depression	☐Present	☐Absent	☐V1 ☐V2 ☐V3 ☐V4 ☐V5 ☐V6 ☐I ☐II ☐III ☐aVR ☐aVL ☐aVF			
	ST elevation	☐Present	☐Absent	☐V1 ☐V2 ☐V3 ☐V4 ☐V5 ☐V6 ☐I ☐II ☐III ☐aVR ☐aVL ☐aVF			
Q waves	Pathological Q	☐Present	☐Absent	☐V1 ☐V2 ☐V3 ☐V4 ☐V5 ☐V6 ☐I ☐II ☐III ☐aVR ☐aVL ☐aVF			
Hypertrophy	RAH	☐Present	☐Absent	*(Adults P > 2.5mm tall in Lead II)*			
	LAH	☐Present	☐Absent	*(Adults P ≥ 120ms in Lead II, +/- notched)*			
	RVH	☐Present	☐Absent	*(Adults RVH if 2 or more of the following: V_1R > 6mm, V_1R:S >1, V_6S:R >1, V_1R+V_6S(or V_6S) > 10.5mm, RAD > +90, incomplete RBBB)*			
	LVH	☐Present	☐Absent	Sokolow-Lyon Index (V_1S + V_5R or V_6R)			Mm
				Lead I-R			Mm
				Cornell voltage duration V_3S + aVL-R			Mm
Ventricular arrhythmias	Ventricular arrhythmia	☐Present	☐Absent	***Highest grade observed*** ☐PVCs ☐Torsades de Point ☐NSVT (3-10 beats) ☐VF ☐Sustained VT (>10 beats) ☐Other			
	Morphology	☐RBBB	☐LBBB	☐LBBB & RBBB	☐Polymorphic	☐Unknown	
	Axis	degrees					
	PVC pattern	☐N/A	☐Single	☐Couples	☐Bigeminy	☐Trigeminy	
SAECG	☐Performed	☐Not performed					
No. of beats							
Analysis	Filtered QRS duration	ms		RMS voltage of terminal 40ms			µV
	Terminal QRS < 40 µV	ms		Root-mean-square voltage of total QRS			µV

August 2017

APPENDIX I – Case Report Form for baseline core data (adults) (page 9)

Patient Name:_____

Patient ID:_____

D.O.B$_{dd/mm/yyyy}$**:**____/____/_____ **Sex: M / F**

Mayosi Research Group

IMHOTEP

Adult Echocardiography Report:

Date of Study DD / MM / YYYY	**Equipment:**		**Ht:** m	**Wt:** kg
Indication:		**Research Study:**		
LEFT HEART STUDY		**RIGHT HEART STUDY**		
LEFT VENTRICLE	**Diastole**	**Systole**	**RIGHT VENTRICLE DIMENSIONS AND FUNCTION**	
IVS	cm	cm	RVID(d) (PLAX)	cm
LVID	cm	cm	RVID(d) (base A4C)	cm
LVPW	cm	cm	RVID(s) (base A4C)	cm
LV volumes (EDV/ESV)	ml	ml	RVID(d) (mid A4C)	cm
LVFS		%	RVID(s) (mid A4C)	cm
LVEF (m-mode)		%	Base-to-Apex length (d) (A4C)	cm
LVEF (volumetric)		%	Base-to-Apex length (s) (A4C)	cm
LVEF visual estimation		%	RVOTd PLAX	cm
Comment on systolic function / dysfxn(if any) and size:	_____ _____ _____		RVOTd at AV level PSAX	cm
			RVOTd at PV annulus PSAX	cm
			RVITd PLAX	cm
Hypertrophy: **Y / N**	Pattern(if yes):_____ _____		RVITs PLAX	cm
			RV AREA (d) A4C	cm^2
Wall thinning: **Y / N**	Location(if yes):_____ _____		RV AREA (s) A4C	cm^2
			RV %FAC (RVAd – RVAs) / RVAd x 100	%
Aneurysms / Diast. bulges: **Y / N**	Location(if yes):_____ _____ _____		TAPSE	mm
			Comment on function / dysfunction(if any) and size:	_____ _____ _____
LVNC suspected: **Y / N**	Comment(if yes):_____ _____		**RV WALLS**	
			Free wall thickness (d)	cm
LV Wall motion abnormalities: Y / N (if yes, complete)			Sacculations: **Y / N**	Comment(if yes):_____ _____
Hypokinesia: Y / N Location:				
Akinesia: Y / N Location:			Aneurysms / Bulges: **Y / N**	Location(if yes):_____ _____
Dyskinesia: Y / N Location:				
LEFT ATRIUM			**RV Wall motion abnormalities: Y / N** (if yes, complete)	
LA diameter		cm	Hypokinesia: Y / N Location:	
LA area (A4C)		cm^2	Akinesia: Y / N Location:	
MITRAL VALVE			Dyskinesia: Y / N Location:	
E wave peak velocity		m/s	Septal Flattening: Y / N Systole / Diastole / Both	
A wave peak velocity		m/s	**RIGHT ATRIUM**	
E/A ratio			Area – dilated: **Y / N**	cm^2
E/E' ratio			Minor dimension (diameter)	cm
Deceleration Time		m/s	Major dimension (length)	cm
PHT		m/sec	**TRICUSPID VALVE**	
Diastolic dysfunction: **Y / N**	If yes, grade/findings:_____ _____		TV morphology	
			TV stenosis: **Y / N**	If yes, grade:
MV morphology	_____ _____		TV incompetence: **Y / N**	If yes, grade:
			RV Systolic Pressure	mmHg
MV stenosis: **Y / N**	If yes: MV area:_____ cm^2 Mean Gradient:_____mmHg		**INVERIOR VENA CAVA**	
			Diameter: dilated / non-dilated	cm
MV incompetence: **Y / N**	If yes, grade:_____ _____ _____		Degree of resp collapse:	%
			Estimated RAP Estimated PsAP	_____mmHg _____mmHg

LVOT, AORTIC VALVE and AORTA		PULMONARY VALVE	
AV morphology		PV morphology	
Aortic root diameter	cm	Peak velocity	m/sec
LVOT diameter	cm	Peak gradient	mmHg
LVOT velocity	m/s	PV stenosis: **Y / N**	If yes, grade:
LVOT gradient (rest)	mmHg	PV incompetence: **Y / N**	If yes, grade:_____ PHT:_____m/sec
Aorta peak gradient	mmHg	**PERICARDIUM**	
Aorta mean gradient	mmHg	Pericardial effusion **Y / N**	Measured _____cm Postion _____ Fibrin strands **Y / N**
Aorta peak velocity	m/sec		
AV stenosis: **Y / N**	If yes: Valve area:_____ cm^2		
AV incompetence: **Y / N**	If yes, grade _____ If yes, PHT:_____ m/sec	Pericardial tamponade **Y / N** (if yes, select diagnostic features) ☐RA systolic collapse ☐RV diastolic collapse ☐Increased respiratory flow variation across MV (>25%) ☐Increased respiratory flow variation across TV > 40% ☐Dilated IVC with reduced inspiratory collapse	
Additional aortic dimensions:	Arch: _____ cm Sinus of Valsalva:_____ cm STJ:_____ cm Ascending Aorta:_____ cm		
INTRACARDIAC MASS/THOMBUS		Pericardial thickness ≥ 4mm **Y / N**	
Mass: **Y / N**	Location: ☐LA ☐RA ☐LV ☐RV	Pericardial calcification **Y / N**	
Thrombus: **Y / N**	Location: ☐LA ☐RA ☐LV ☐RV If LV thrombus, specify wall _____	Pericardial constriction **Y / N** (if yes, select diagnostic features) ☐Septal bounce ☐Rapid, early and flat diastolic motion of the LV posterior wall ☐Exaggerate septal shift towards LV with inspiration ☐Dilated IVC with reduced inspiratory collapse (>21mm, <40% collapse) ☐Restrictive filling pattern on Doppler ☐Pre-systolic PV opening ☐Trans-mitral flow, exaggerated respiratory variation of Mitral E vel (>25%) ☐Exaggerated respiratory variation of Tricuspid E velocity (>40%)	
CONGENTIAL ABNORMALITIES			
ASD **Y / N**	If yes, specify		
VSD **Y / N**	If yes, specify		
Other **Y / N**	***Please describe below***		

Additional Comments GENERAL

Reported by:		
Name	**Signature**	**Date** dd/mm/yyyy

APPENDIX J – List of collaborators

Active recruiting sites: collaborating staff

Sites	Staff
Data and study co-ordinating centre, University of Cape Town	S. Kraus, V. Francis, S. Bhovula, M. De Vries, S. Pandie, L. Mhlathi
Cardiovascular Genetics Laboratory, Hatter Institute of Cardiovascular Research in Africa, University of Cape Town	G. Shaboodien, T. Spracklen, S. Khumali, P. Ndibangwi, L. Pearce, K. Brooks
Groote Schuur Hospital, Cape Town	S. Kraus (prevalent and incident cohorts, February 2015 - date), Z. Kerbelker (incident cohort August 2017 – July 2018), J. Cirota (incident cohort, October 2018 - date) and the Mayosi Research Group study team (U. September, M. Van Der Wall, N. Laing, N. Jamieson-Luff)
Red Cross War Memorial Children's Hospital, Cape Town	J. Lawrenson, G. Comitis, T. Aldersley, L. Zulkhe
Tygerberg Hospital, Cape Town	J. Lawrenson, B. Fourie
Mthatha	M. Thomas, K. Thomas, K. Moekestsi, P. Mdlatu, Y. Luzipo
Port Elizabeth	L. Pepeta, N. Makubalo, S. Jiyana, G. Nyengane-Menziwa
Bloemfontein	M. Makotoko, F. Smith, S. Brown, J. Fontein, Y. Tiga, M. Karstens, A. Page, L. Greyling, M. Kautjunga
Mozambique	A. Damasceno, A. Mocumbi, V. Govo, C. Novela, J. Chemane

APPENDIX J – List of collaborators

List of current and future participating sites and investigators by country

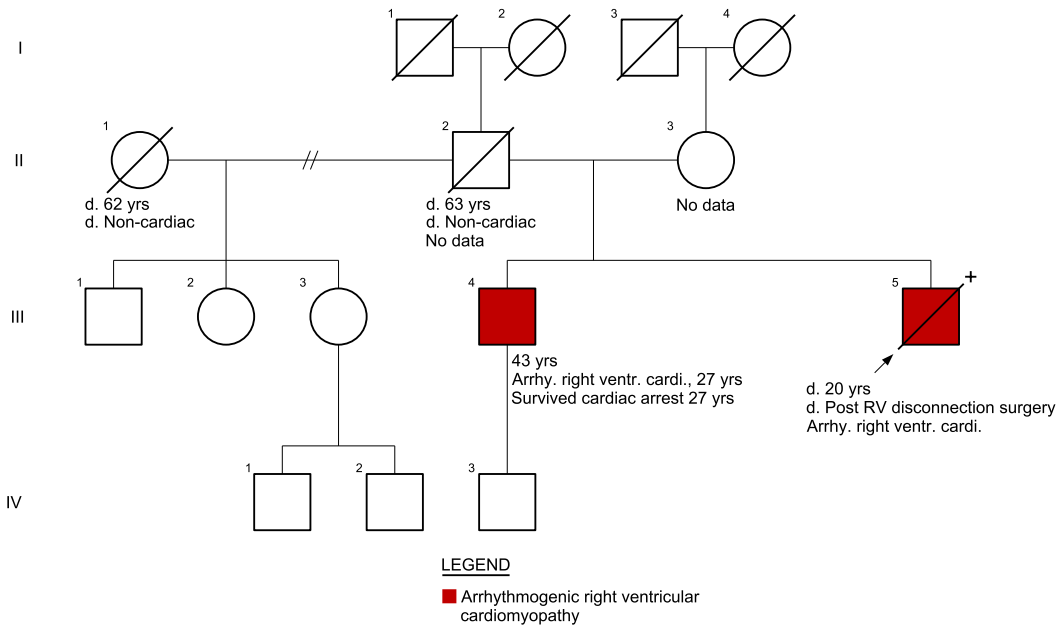
Country	Investigators	Institution
Botswana	Dr. Julius Mwita	University of Botswana, Gaborone
Egypt	Dr. Ahmed El-Guindy, Prof. Sir Magdi Yacoub	Aswan Heart Centre, Aswan
Guinea	Dr. Tolno Sandy Kola	Hospital De L'amitie Sino-Guineenne, Conakry, Guinea
Kenya	Dr. F Ayub Barasa	Moi University, Eldoret
Mozambique	Prof. Albertino Damasceno	Eduardo Mondlane University, Maputo
	Dr. Ana Olga Mocumbi	Instituto Nacional de Saúde, Ministério da Saúde, Maputo
Nigeria	Dr. Okechukwu Ogah	University College Hospital, Ibadan
Saudi Arabia	Prof. Motasim Badri	King Saud Bin Abdalaziz University for Health Sciences, Riyadh
Sierra Leone	Dr. James Russell	Connaught Hospital, Freetown
South Africa	Dr. Ashley Chin, Dr. Sarah Kraus, Prof. Bongani Mayosi, Prof. Mpiko Ntsekhe, Prof. Ntobeko Ntusi, Prof. Karen Sliwa, Prof Ambroise Wonkam, Ms. Nakita Laing, Dr Shaheen Pandie, Dr Blanche Cupido	Groote Schuur Hospital, Cape Town
	Dr. George Comitis, Dr. John Lawrenson, Assoc Prof Rik De Decker, Assoc Prof Liesl Zühlke	Red Cross War Memorial Children's Hospital, Cape Town
	Prof. Paul Brink, Dr. Barend Fourie, Dr. Marshall Heradien	Tygerberg Hospital, Cape Town
	Prof. Lungile Pepeta, Dr. Nomlindo Makubalo, Dr. Mahlubandile Nxele	Dora Nginza Hospital, Port Elizabeth
	Prof. Benjamin Longo-Mbenza, Dr. Khulile Moeketsi, Prof. Baby Thomas, Dr. Kandithalal Thomas	Nelson Mandela Academic Hospital, Mthatha
	Prof. Antoinette Cilliers, Prof. Richard Nethononda, Dr. Hopewell Ntsinjana, Dr. Ferande Peters	Chris Hani Baragwanath Hospital, Soweto
	Prof. Makoali Makotoko, Prof. Stephen Brown	University of the Free State, Bloemfontein
Sudan	Dr. Ahmed Suliman	University of Khartoum, Khartoum
	Dr. Sulafa Ali	Sudan Heart Centre, Khartoum
Tanzania	Dr. Kemilembe Tibazarwa	Muhimbili National Hospital, Dar es Salaam
Uganda	Dr. Charles Mondo	St. Francis Hospital Nsambya, Kampala
	Dr Michael Mungoma	Mulago Hospital, Kampala
United Kingdom	Prof. Bernard Keavney	University of Manchester, Manchester
	Prof. Heather Cordell	University of Newcastle-upon-Tyne, Newcastle
	Dr Vanessa Ferreira, Dr Masliza Mahmod, Prof Stefan Neubauer, Prof Hugh Watkins	University of Oxford, Oxford
United States of America	Prof. Leslie T Cooper	Mayo Clinic, Jacksonville
Zimbabwe	Dr. Ellise Tapiwa Gambahaya	Parirenyatwa Hospital, Harare

APPENDIX K – FAMILY PEDIGREES

1. Family ACM 1 – Genotype positive ARVC
2. Family ACM 2 – Genotype positive ARVC
3. Family ACM 5 – Genotype positive ARVC
4. Family ACM 8 – Genotype positive ARVC
5. Family ACM 11 – Genotype positive ARVC
6. Family ACM 12 – Genotype positive ARVC
7. Family ACM 19 – Genotype positive ARVC
8. Family ACM 34 – Genotype positive ARVC
9. Family ACM 38 – Genotype positive ARVC
10. Family ACM 39 – Genotype positive ARVC
11. Family ACM 57 – Genotype positive ARVC
12. Family ACM 71 – Genotype positive ARVC
13. Family ACM 136 – Genotype positive ARVC
14. Family DCM 4 – Genotype positive DCM
15. Family DCM 320 – Genotype positive DCM
16. Family HCM 4 – Genotype positive HCM
17. Family ACM 6 – Genotype unknown ARVC
18. Family ACM 142 – Genotype unknown ARVC
19. Family ACM 145 – Genotype unknown ARVC
20. Family HCM 50 – Genotype unknown HCM
21. Family DCM 343 – Genotype unknown DCM/LVNC overlap
22. Family DCM 389 – Genotype unknown DCM
23. Family DCM 141 – Genotype unknown DCM/HCM overlap
24. Family DCM 464 – Genotype unknown DCM
25. Family DCM 390 – Genotype unknown DCM
26. Family DCM 236 – Genotype unknown DCM
27. Family DCM 437 – Genotype unknown DCM/muscular dystrophy
28. Family RCM 15 – Genotype unknown LVNC/heart block
29. Family DCM 435 – Genotype unknown DCM
30. Family DCM 3 – Genotype unknown DCM
31. Family DCM 334 – Genotype unknown DCM
32. Family DCM 24 – Genotype unknown DCM
33. Family DCM 303 – Genotype unknown DCM/LVNC overlap
34. Family ACM 149 – Genotype unknown ARVC
35. Family DCM 458 – Genotype unknown DCM

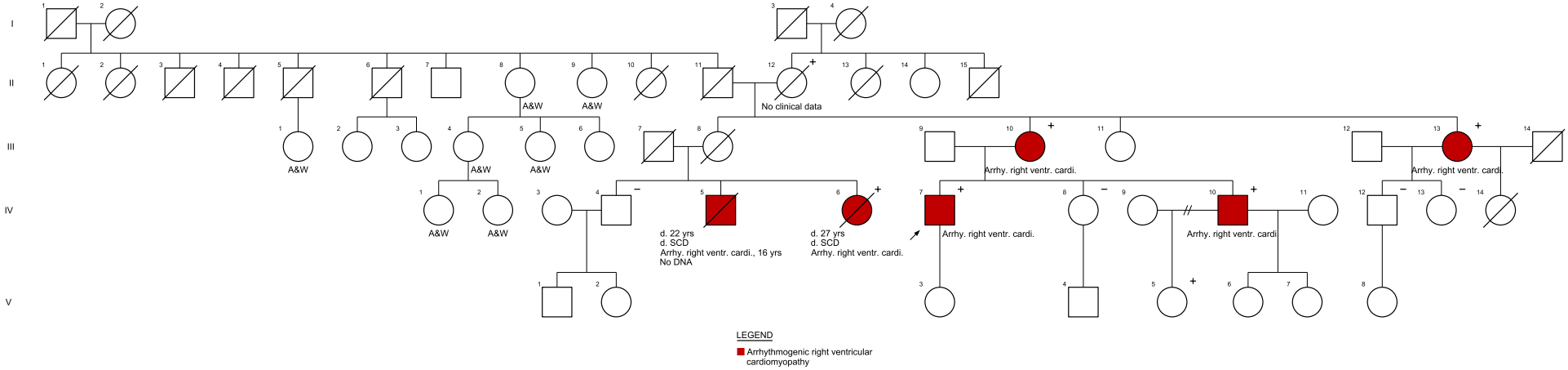
APPENDIX K – FAMILY PEDIGREE

1. Family ACM 1 [+PKP2 c.C1132T]



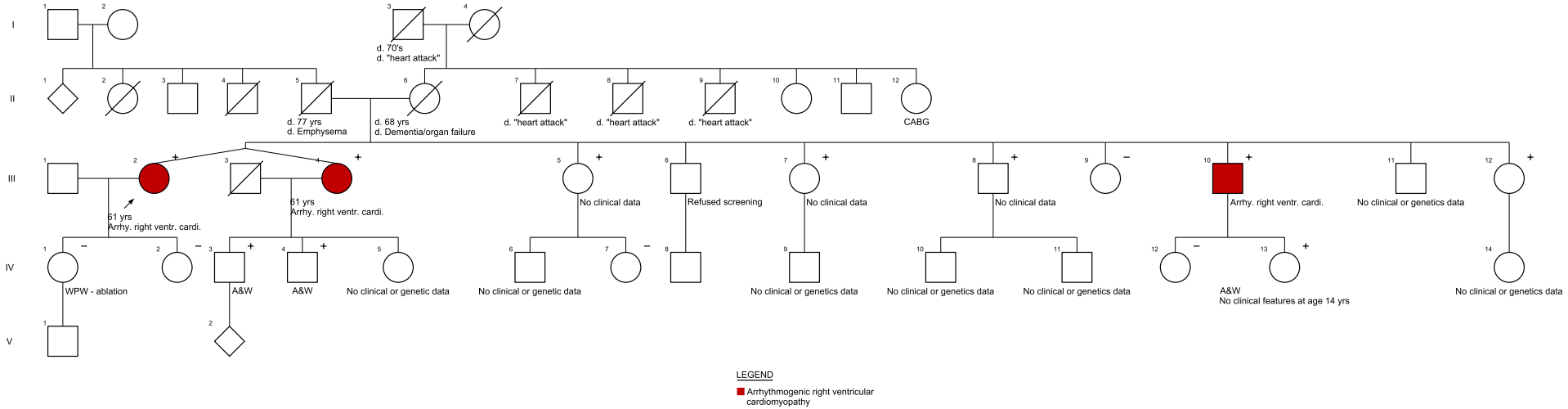
APPENDIX K – FAMILY PEDIGREE

2. Family ACM 2 [+CDH2 c.A686C]



APPENDIX K – FAMILY PEDIGREE

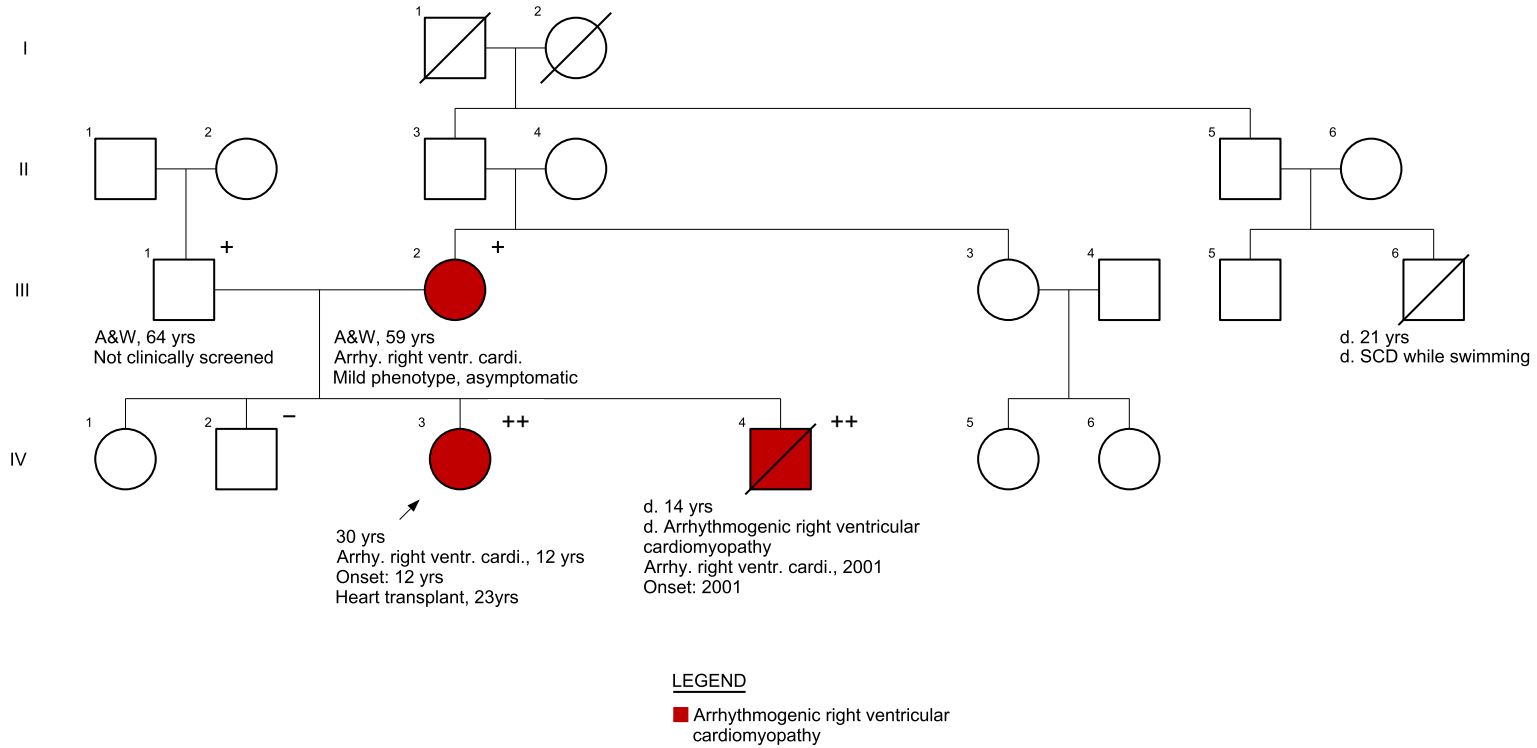
3. Family ACM 5 [+PKP2 c.C1162T]



APPENDIX K – FAMILY PEDIGREE

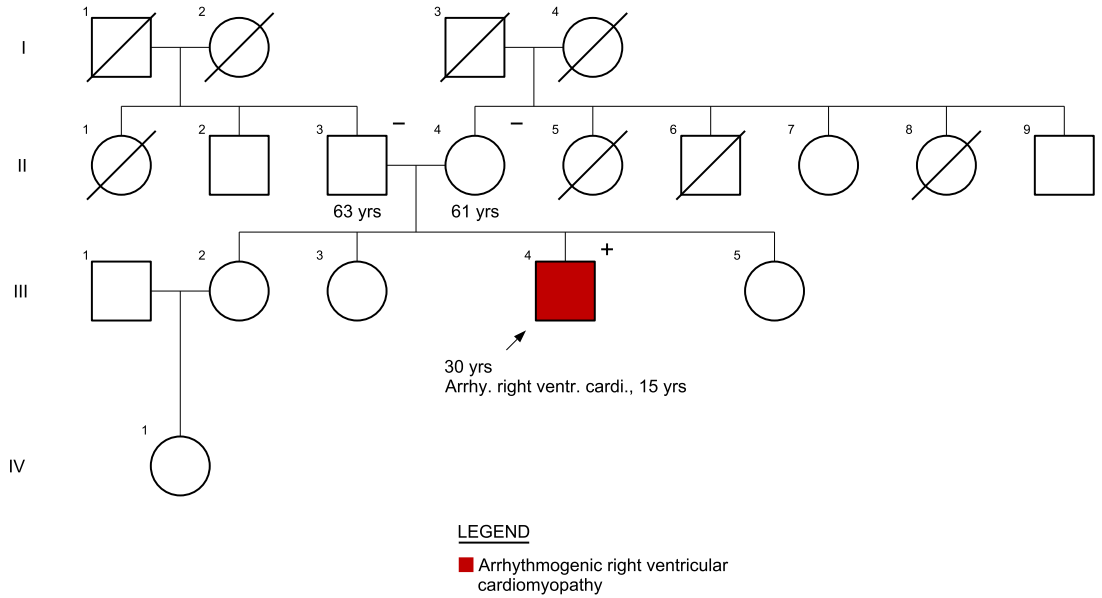
4. Family ACM 8 [+PKP2 c.C1162T]

Individuals IV:3 and IV:4 - homozygous



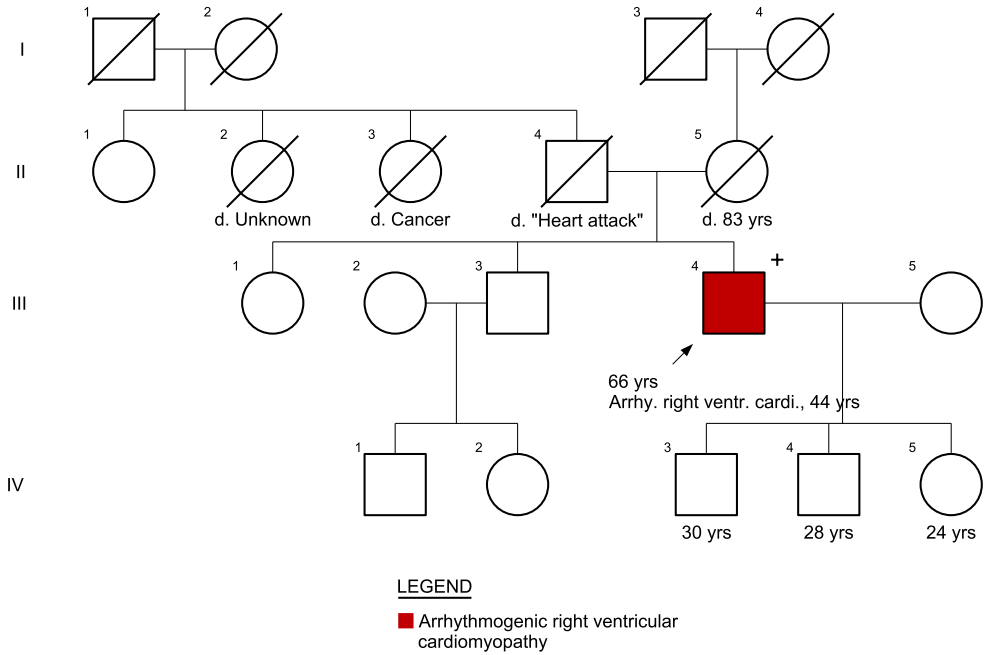
APPENDIX K – FAMILY PEDIGREE

5. Family ACM 11 [+CDH2 c.G1219A]



APPENDIX K – FAMILY PEDIGREE

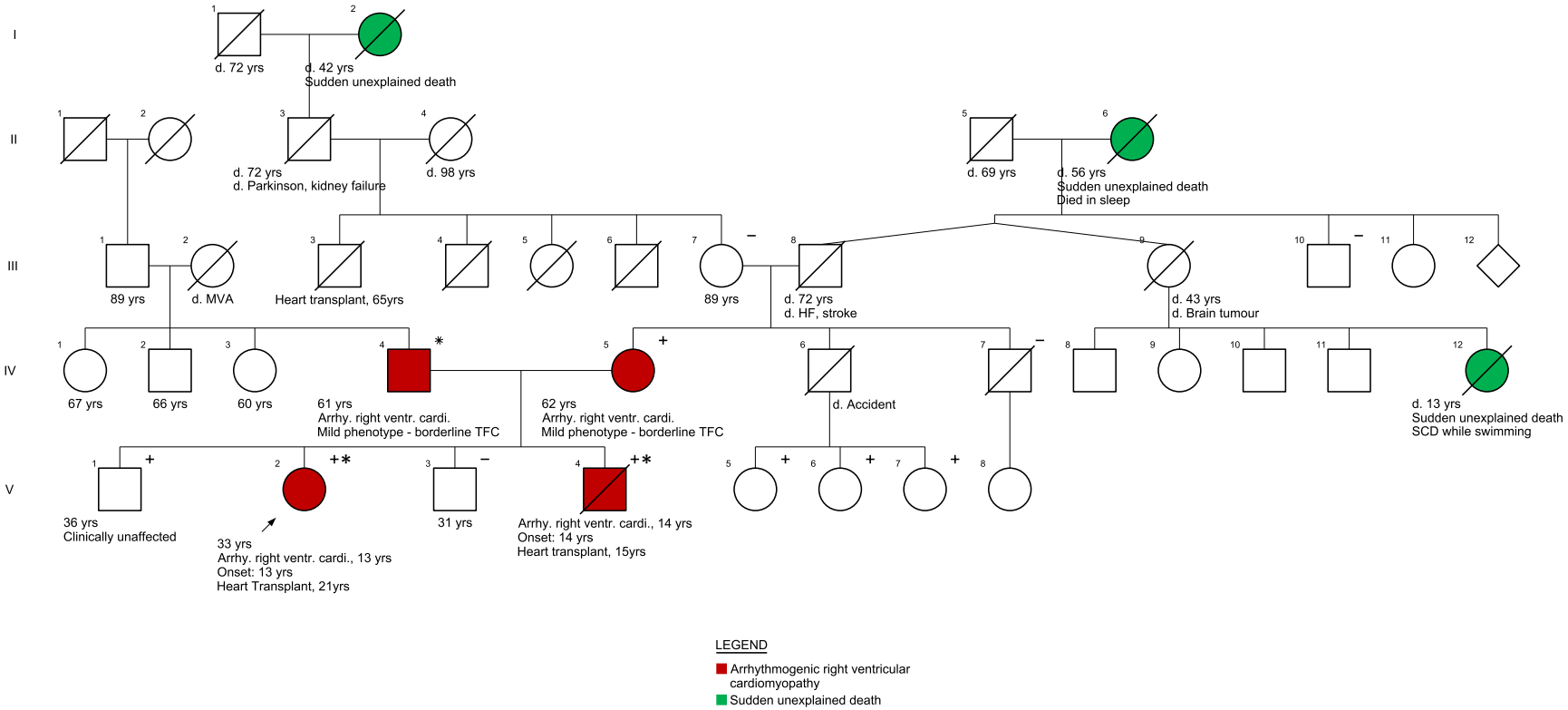
6. Family ACM 12 [+PKP2 c.C1162T]



APPENDIX K – FAMILY PEDIGREE

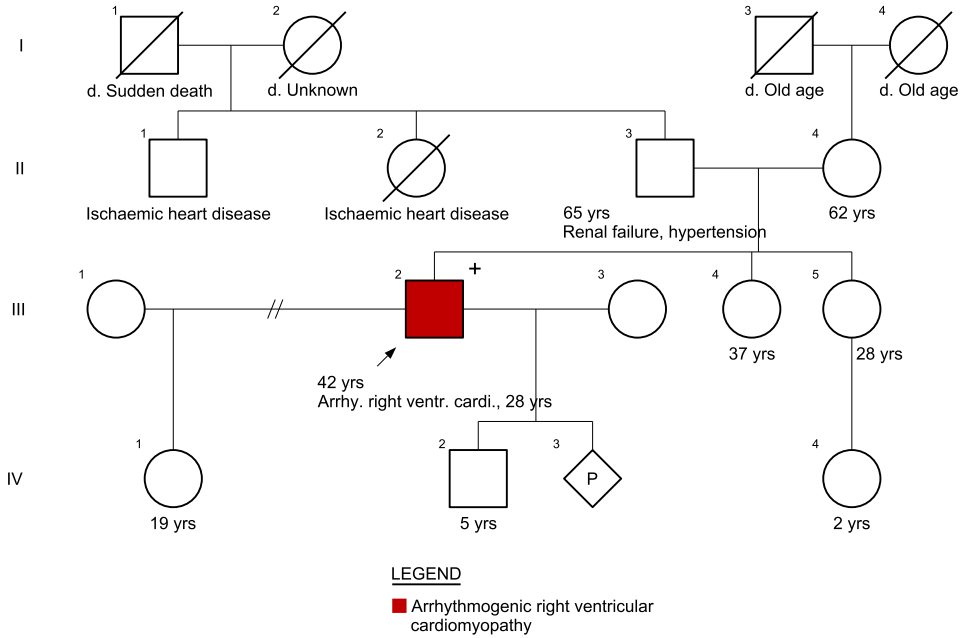
7. Family ACM 19 [+PKP2 c.C1162T; *PKP2 c.2197-2202del CACACCinsG]

Individuals V:2 and V:4 – compound heterozygous



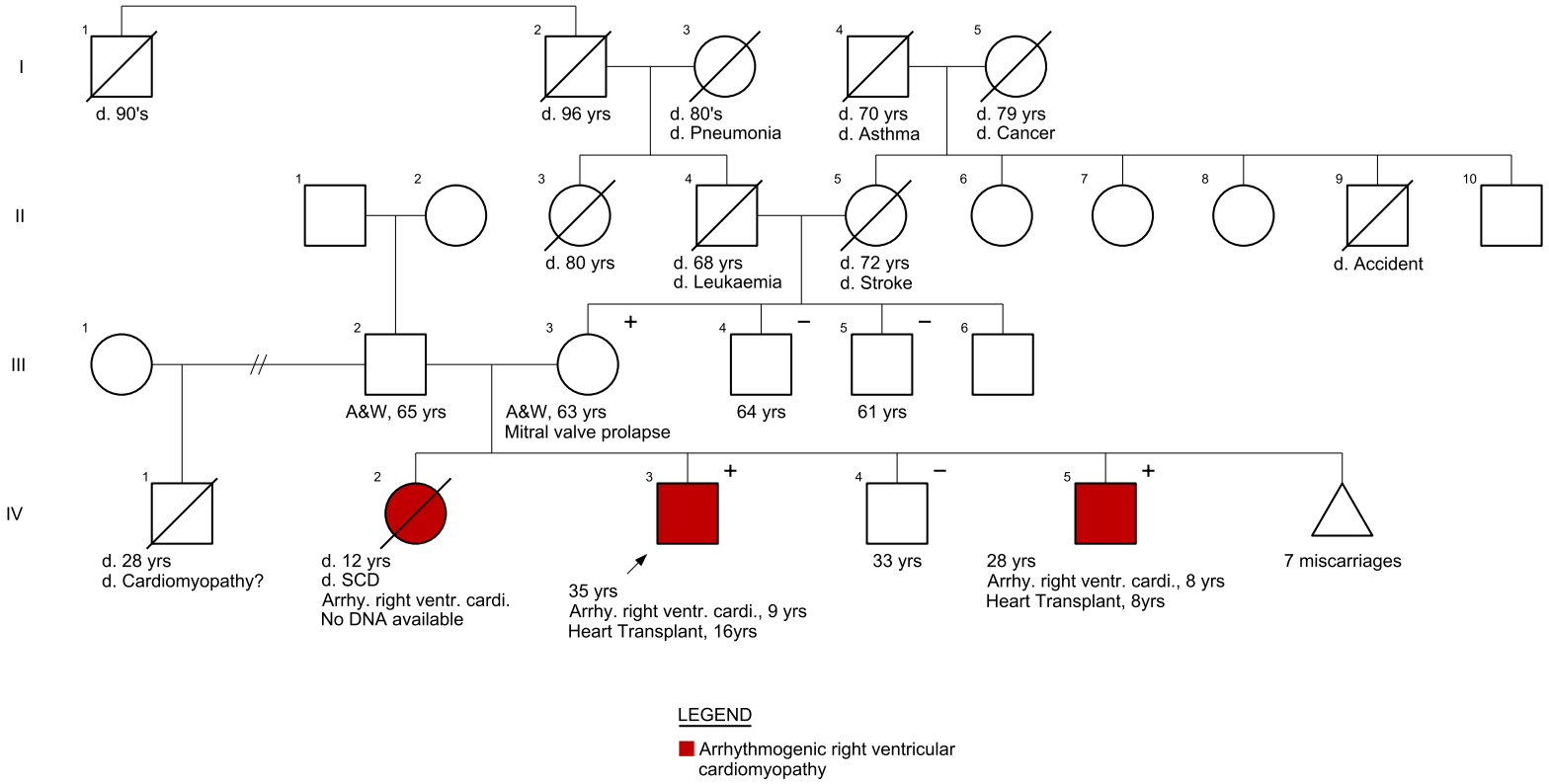
APPENDIX K – FAMILY PEDIGREE

8. Family ACM 34 [+PKP2 c.T2540C]



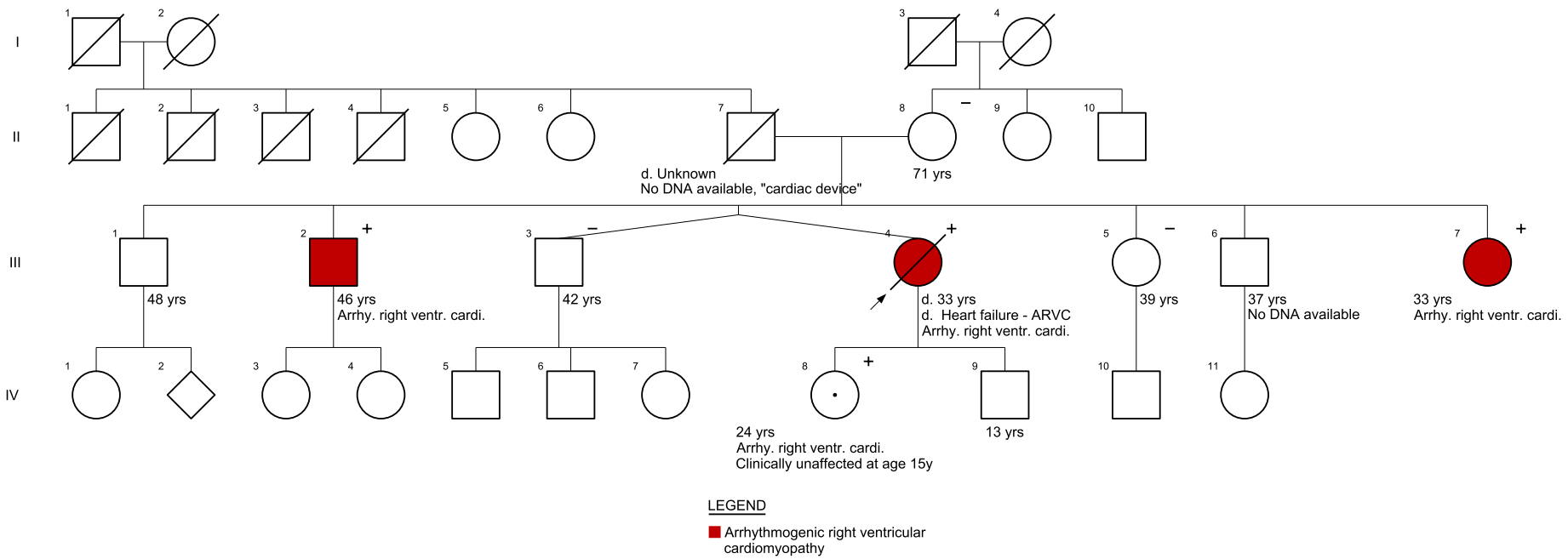
APPENDIX K – FAMILY PEDIGREE

9. Family ACM 38 [+PKP2 c.C1162T]



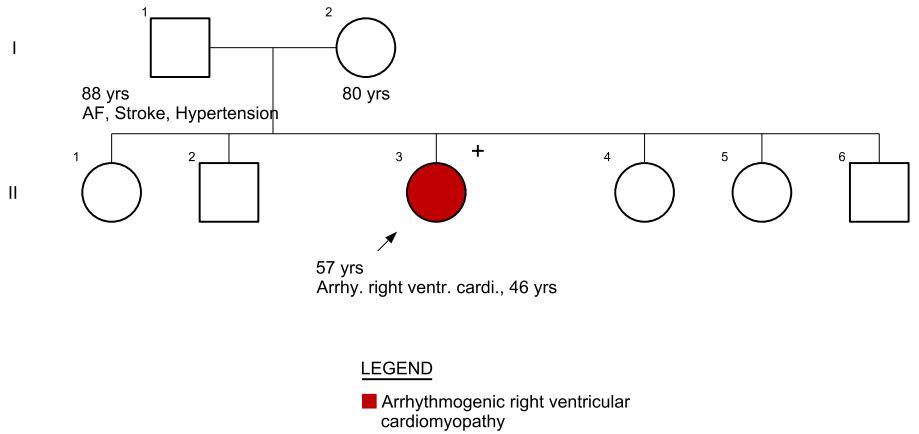
APPENDIX K – FAMILY PEDIGREE

10. Family ACM 39 [+PKP2 c.G1465A]



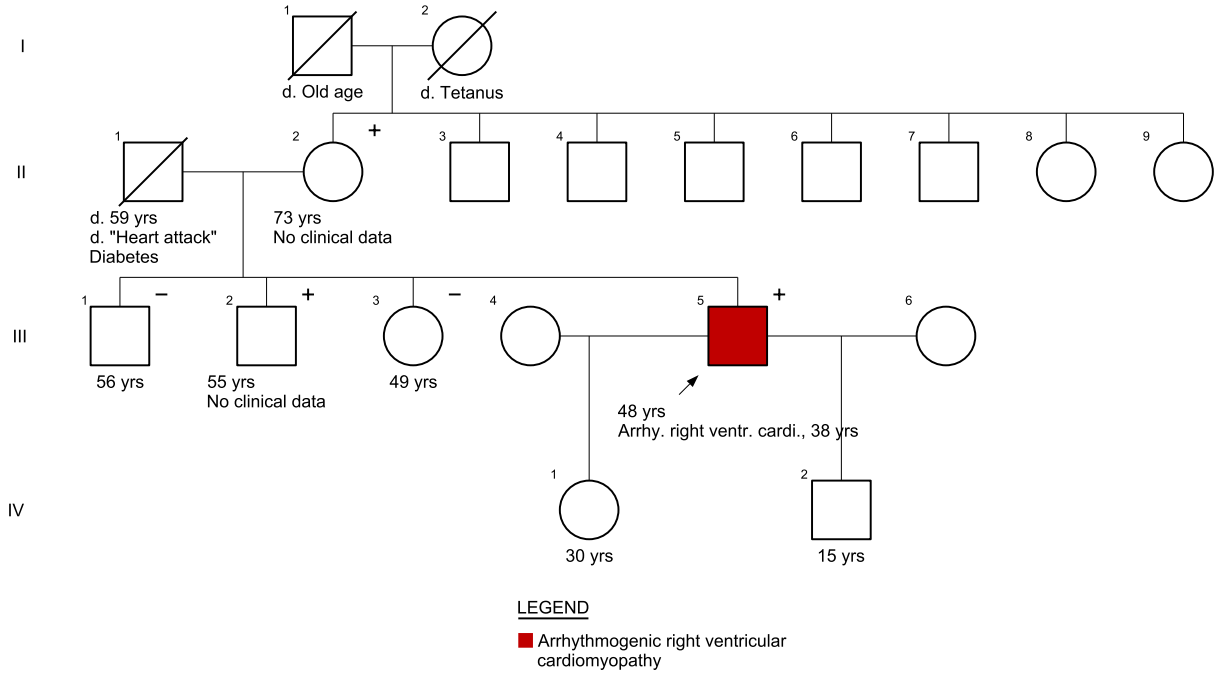
APPENDIX K – FAMILY PEDIGREE

11. Family ACM 57 [+PKP2 c.C1162T]



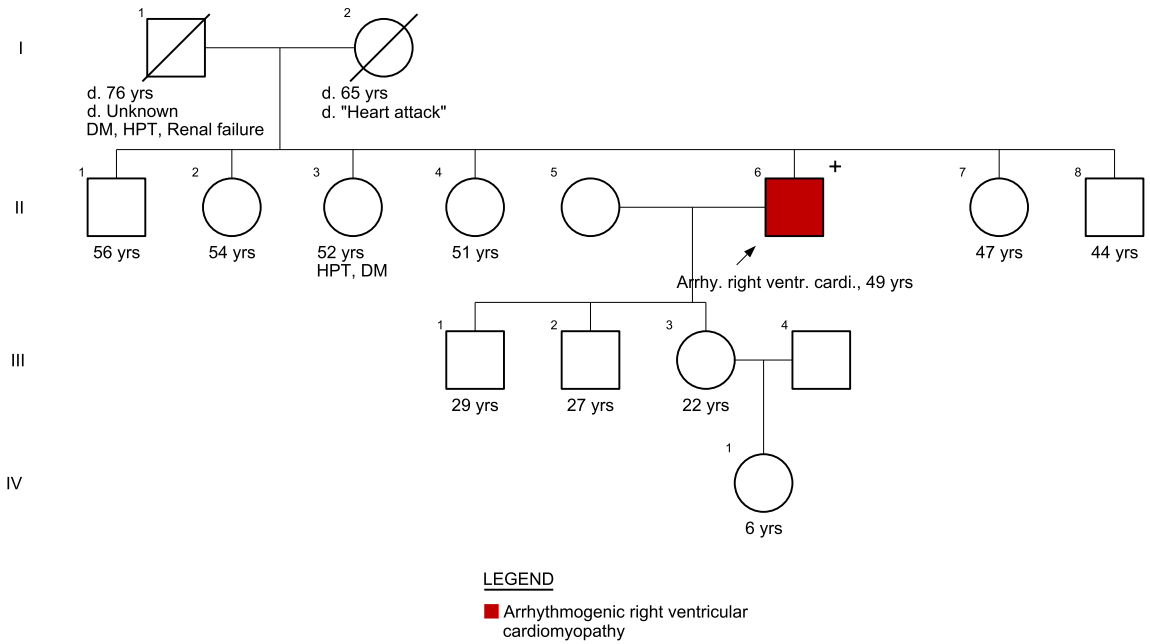
APPENDIX K – FAMILY PEDIGREE

12. Family ACM 71 [+PKP2 c.C1162T]



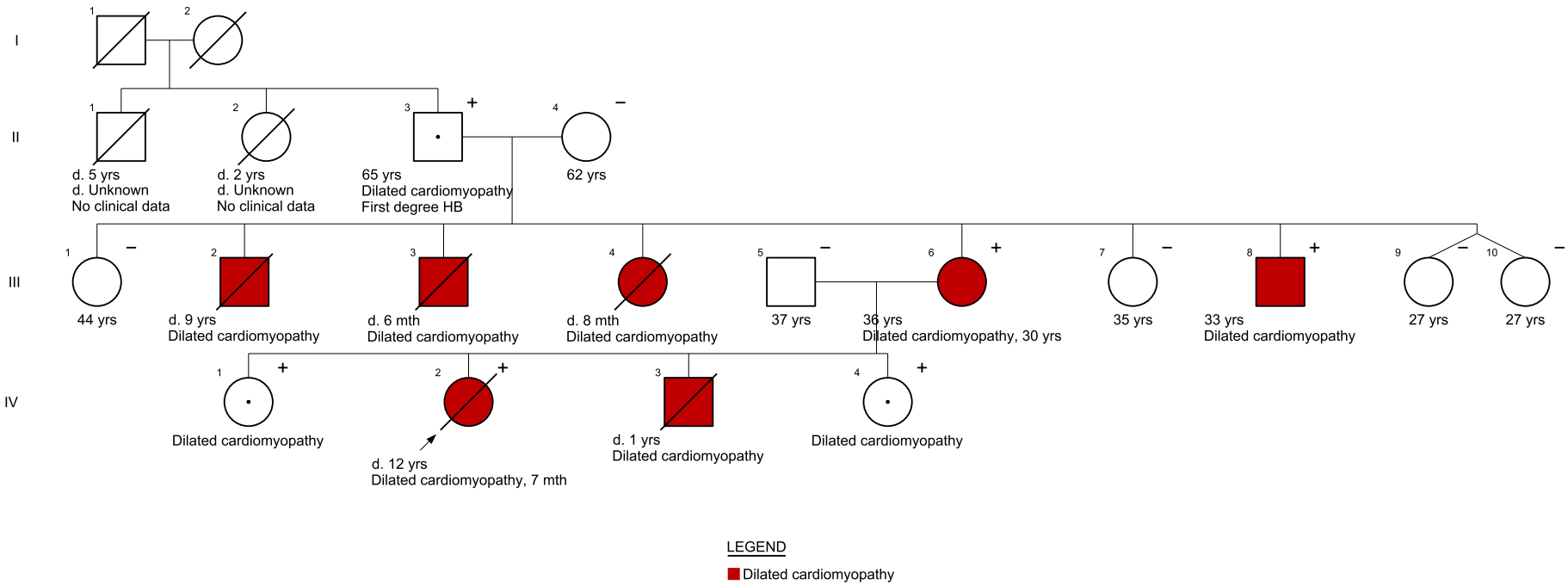
APPENDIX K – FAMILY PEDIGREE

13. Family ACM 136 [+PKP2 c.C1237T]



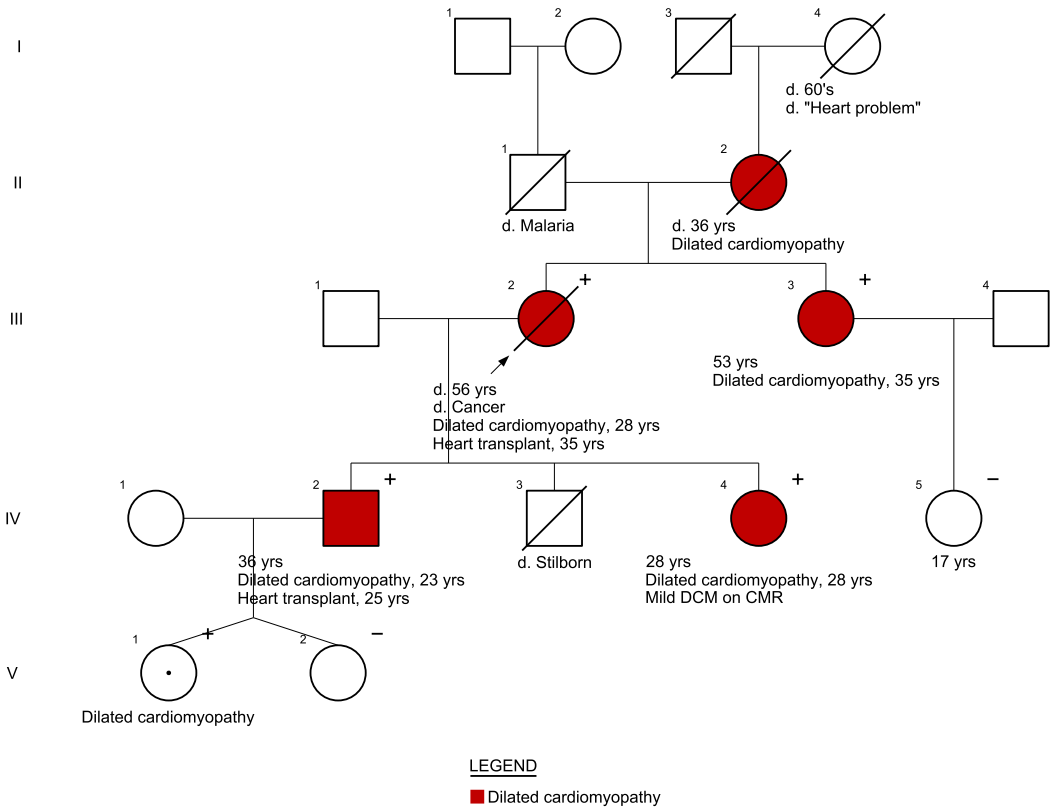
APPENDIX K – FAMILY PEDIGREE

14. Family DCM 4 [Troponin T]



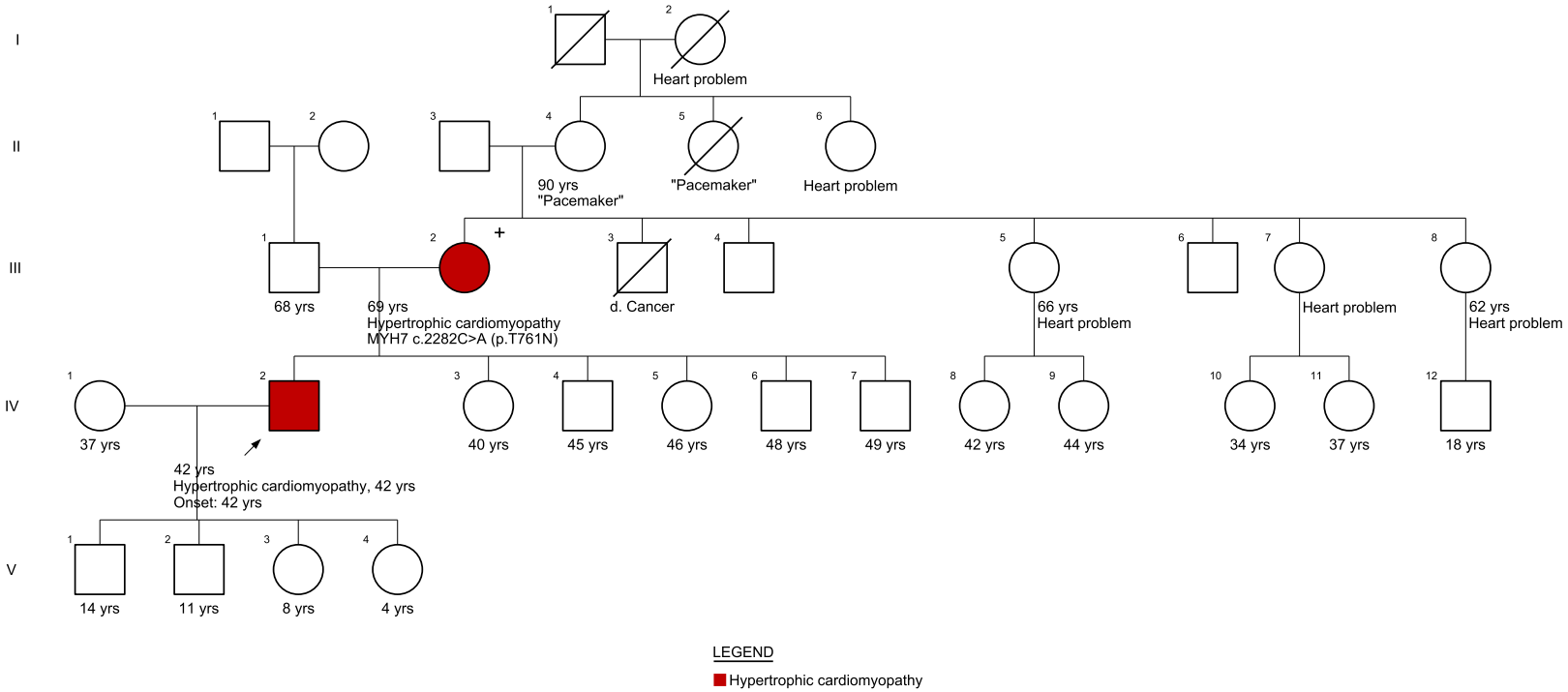
APPENDIX K – FAMILY PEDIGREE

15. Family DCM 320 [+PLN c.25C>T (p.R9C)]



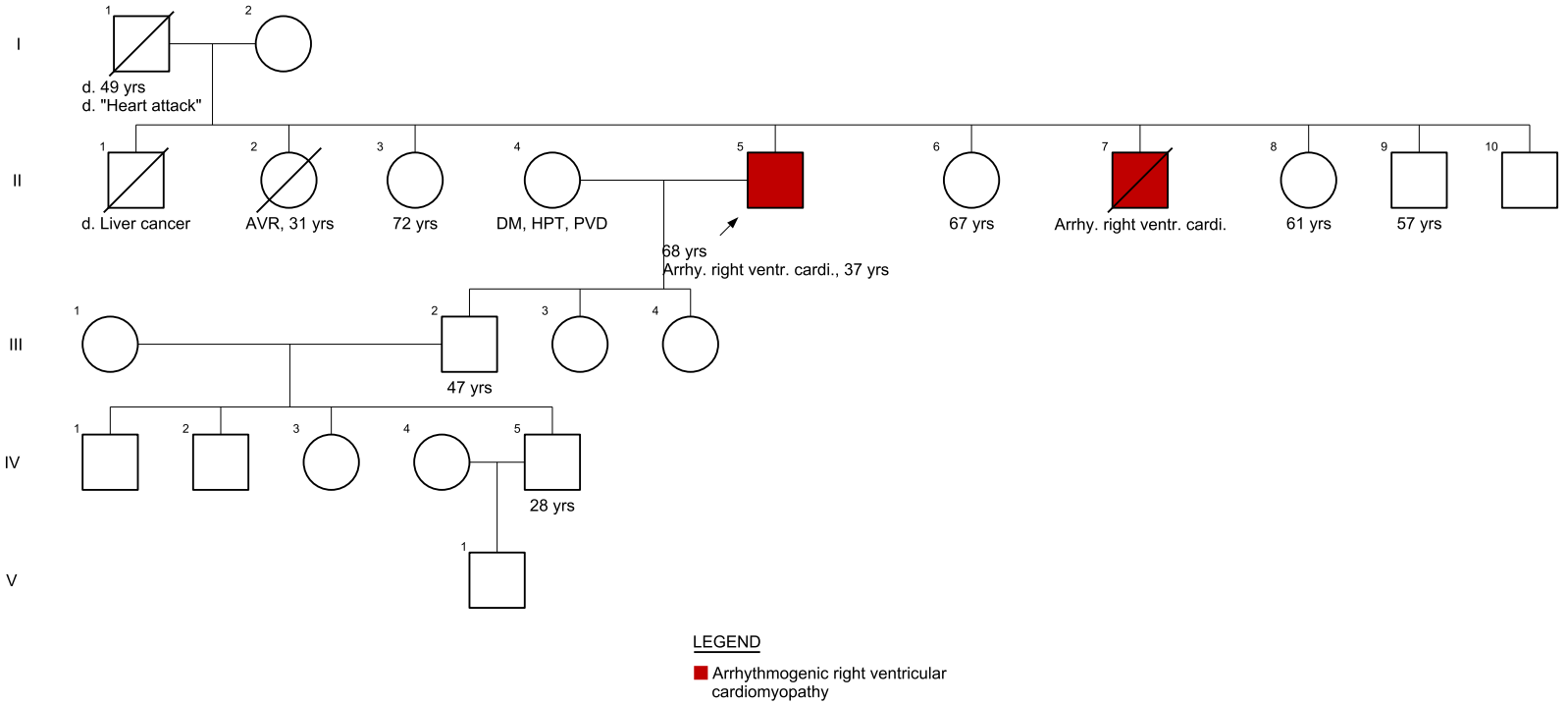
APPENDIX K – FAMILY PEDIGREE

16. Family HCM 4 [+MYH7 c.2282C>A]



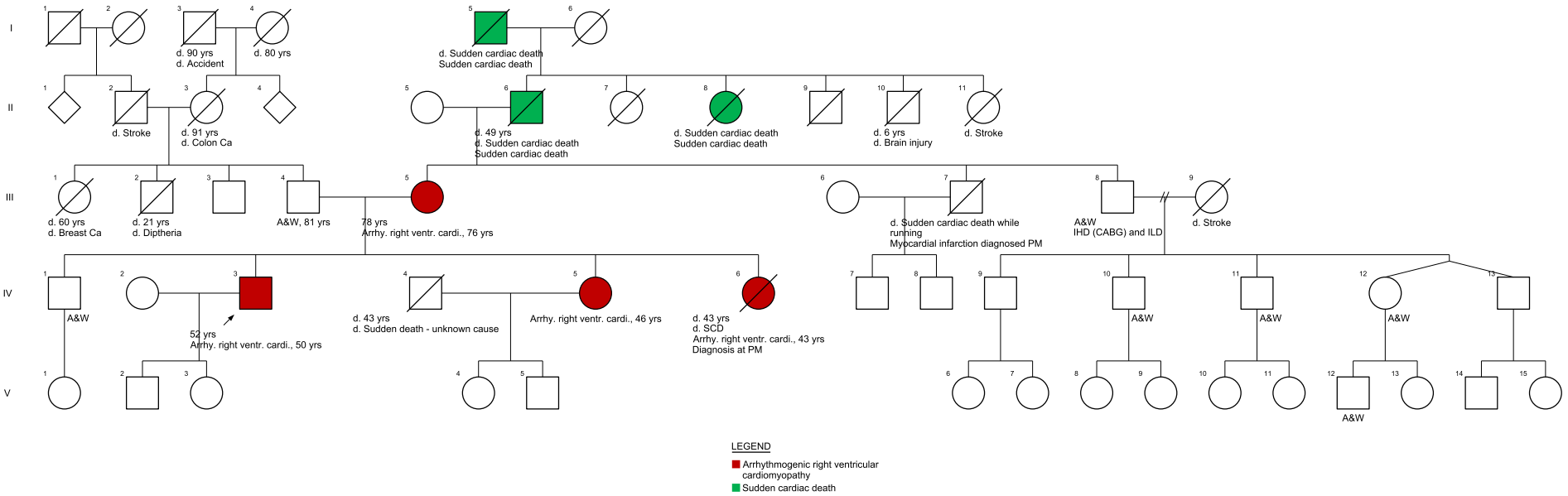
APPENDIX K – FAMILY PEDIGREE

17. Family ACM 6



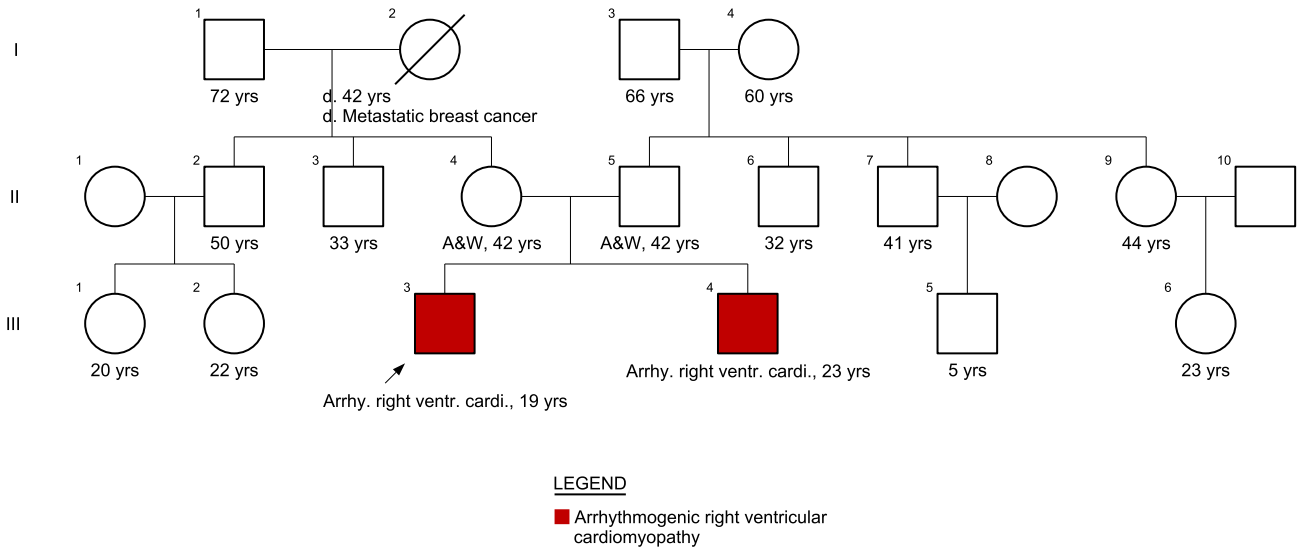
APPENDIX K – FAMILY PEDIGREE

18. Family ACM 142



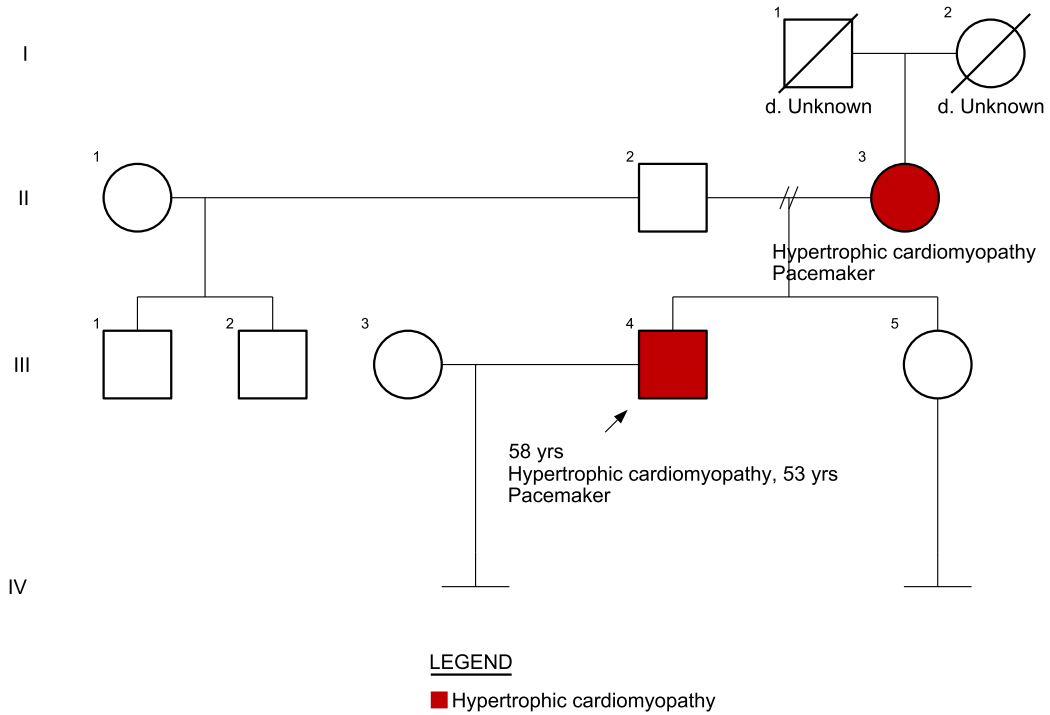
APPENDIX K – FAMILY PEDIGREE

19. Family ACM 145



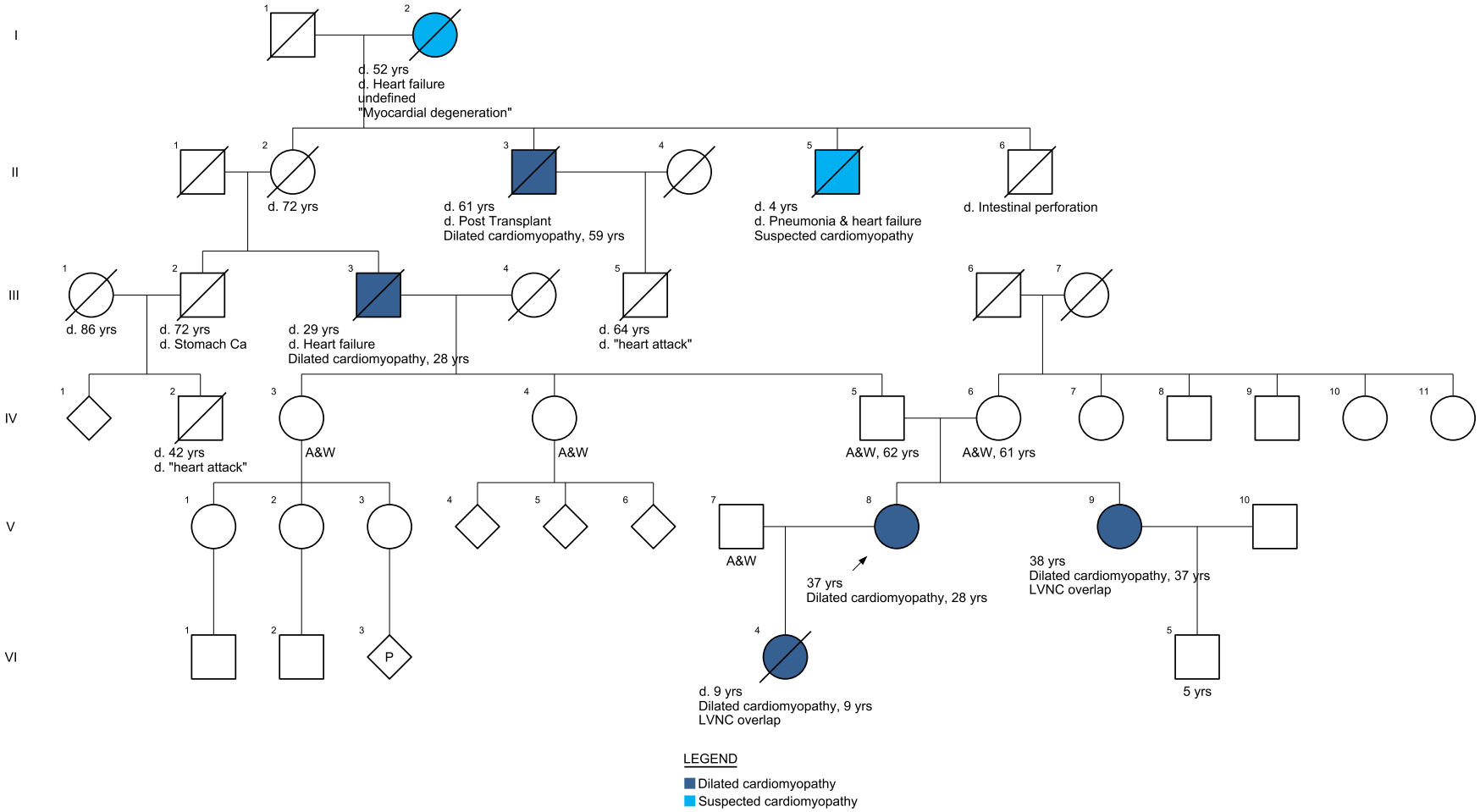
APPENDIX K – FAMILY PEDIGREE

20. Family HCM 50



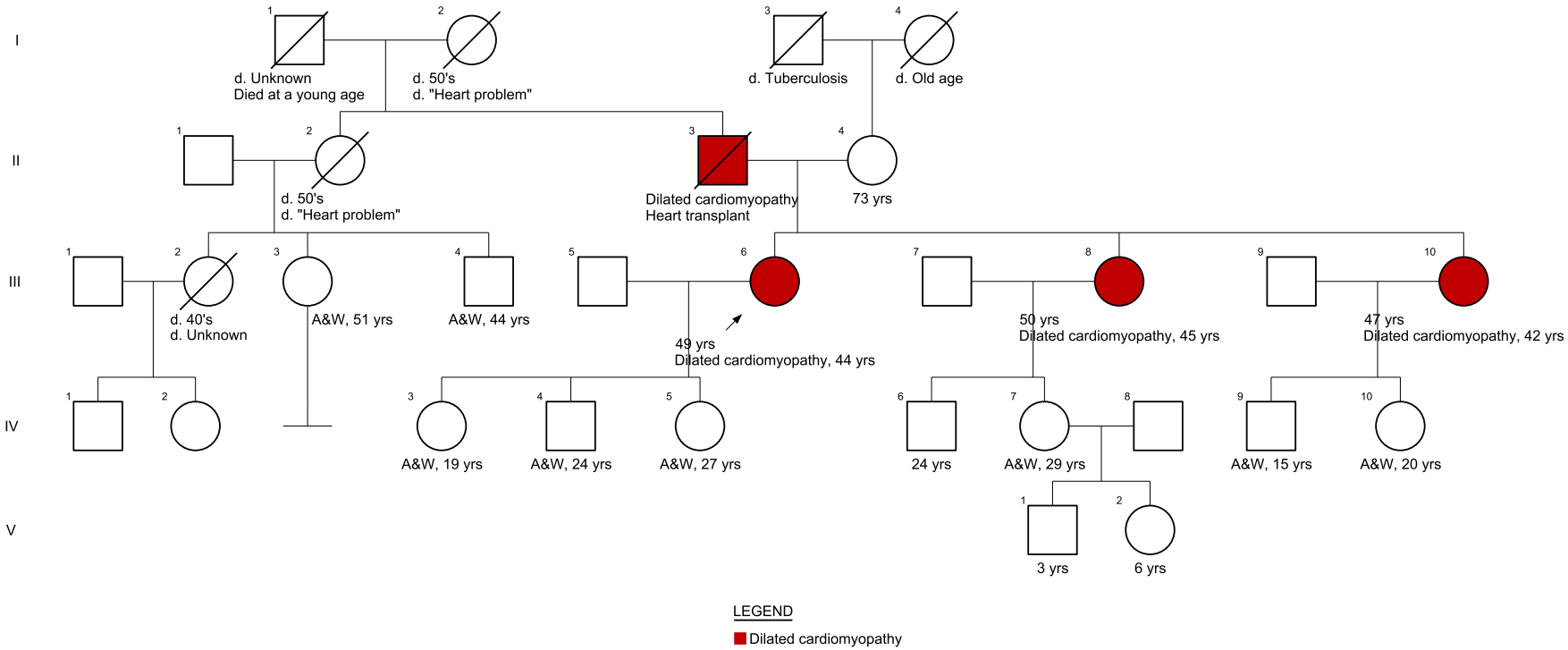
APPENDIX K – FAMILY PEDIGREE

21. Family DCM 343



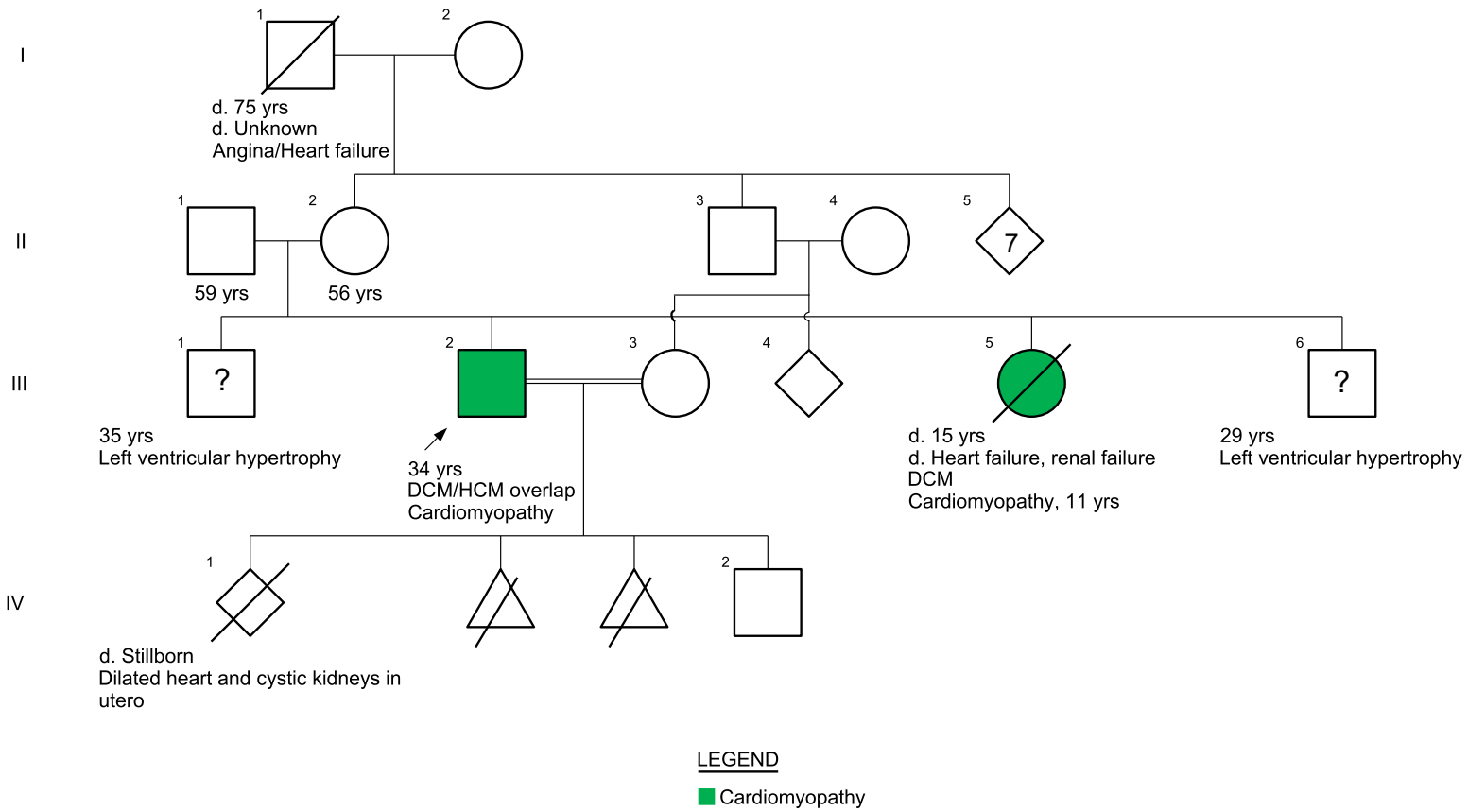
APPENDIX K – FAMILY PEDIGREE

22. Family DCM 389



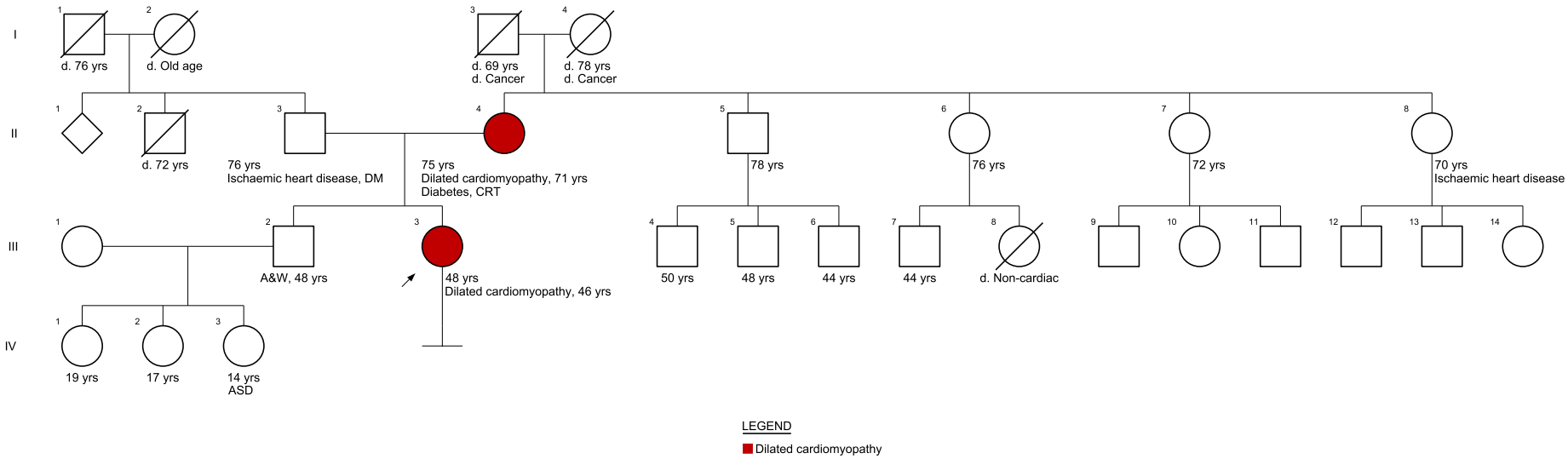
APPENDIX K – FAMILY PEDIGREE

23. Family DCM 141



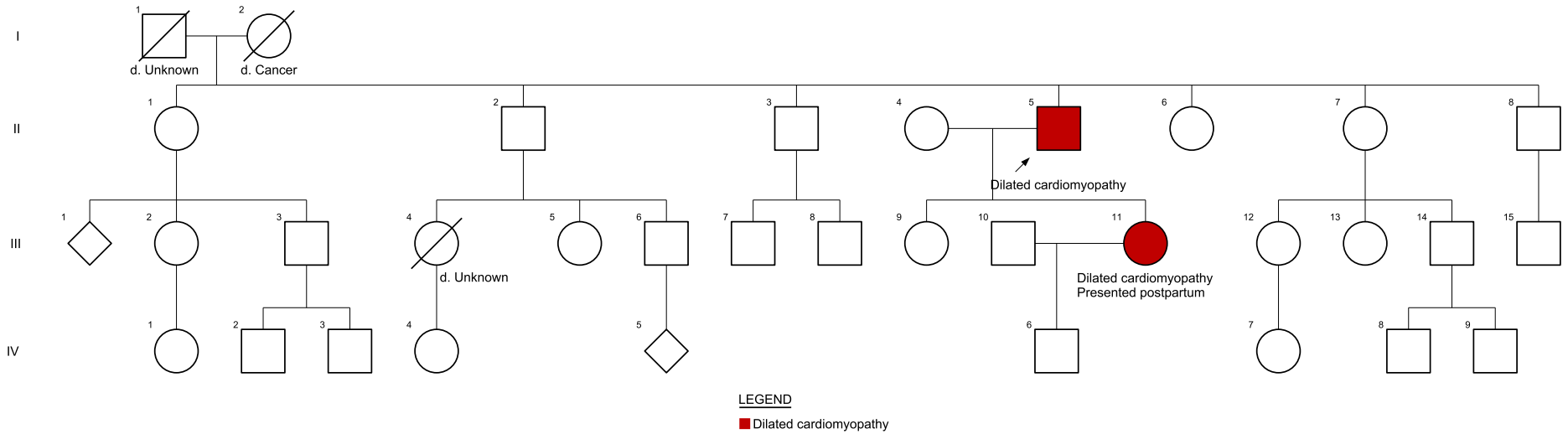
APPENDIX K – FAMILY PEDIGREE

24. Family DCM 464



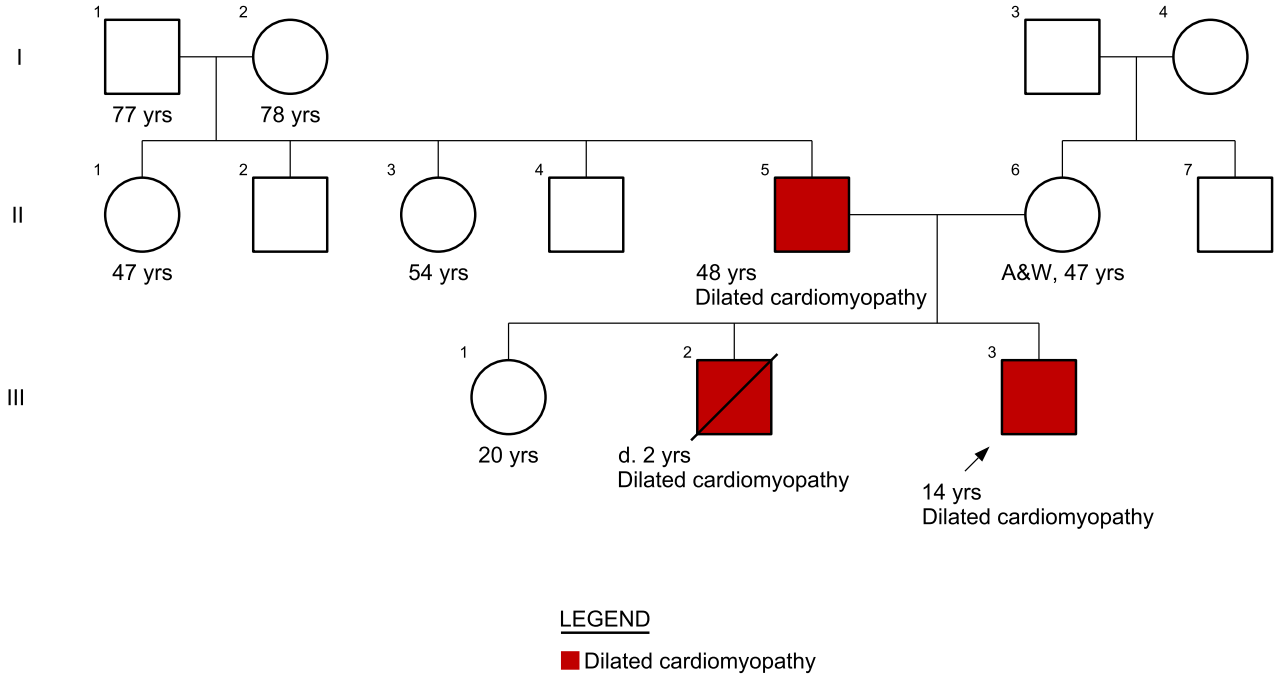
APPENDIX K – FAMILY PEDIGREE

25. Family DCM 390



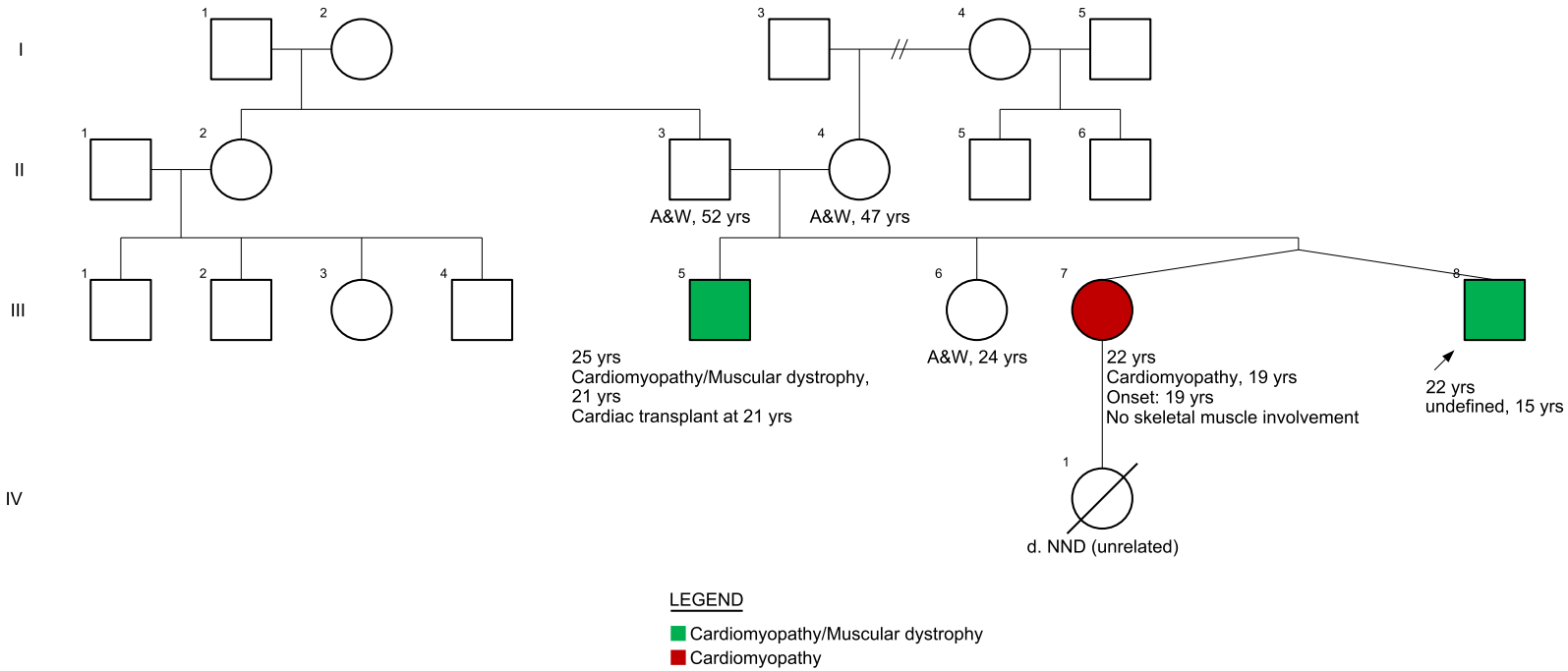
APPENDIX K – FAMILY PEDIGREE

26. Family DCM 236



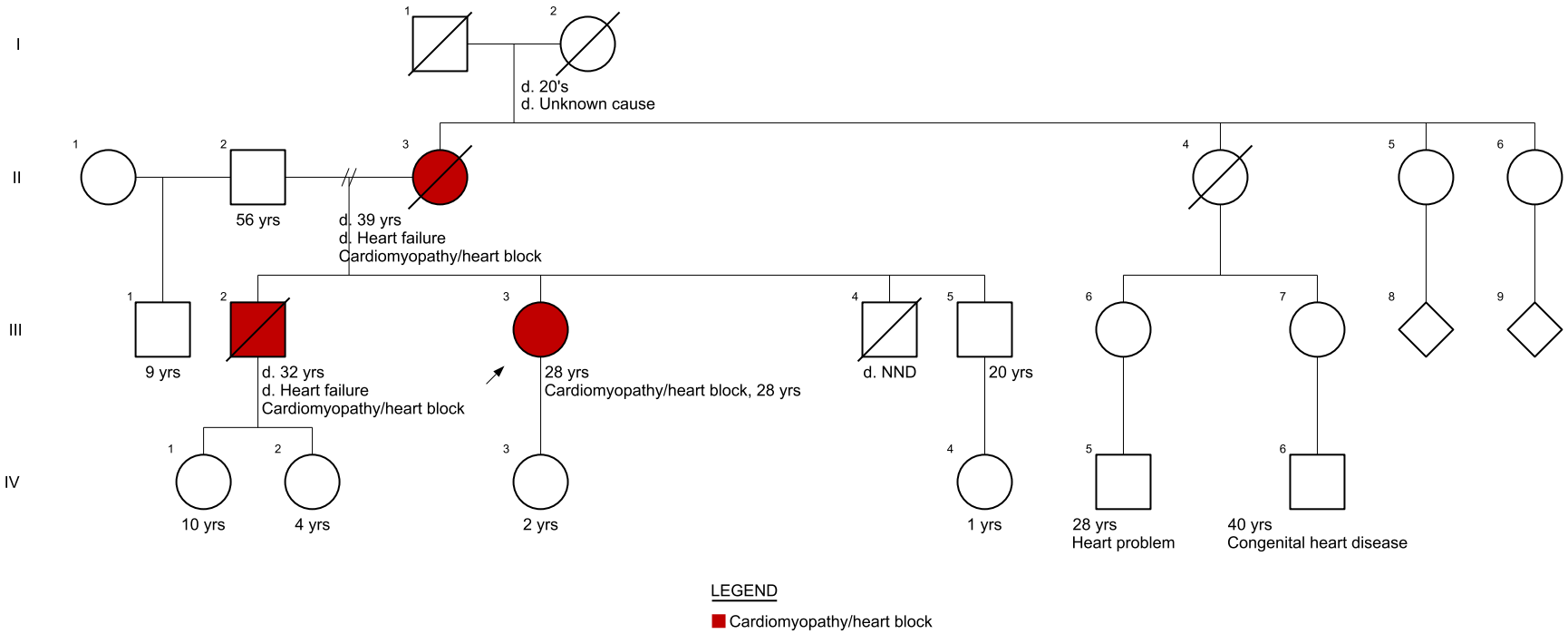
APPENDIX K – FAMILY PEDIGREE

27. Family DCM 437



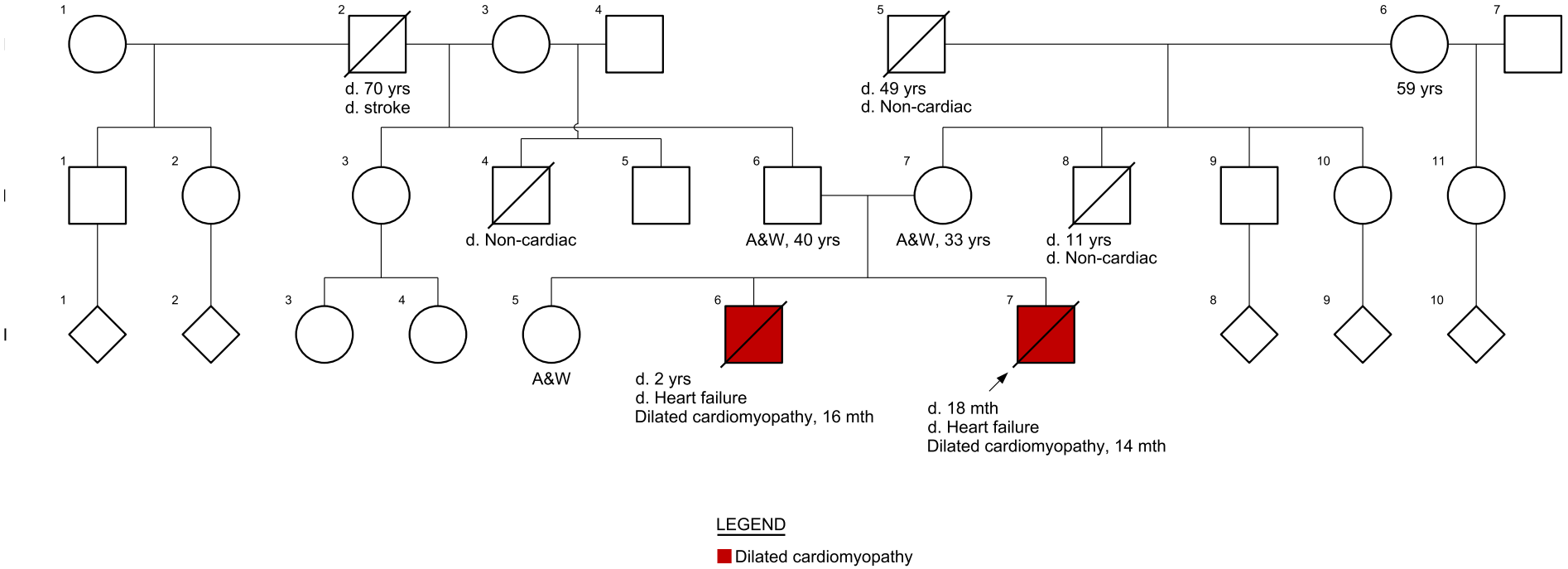
APPENDIX K – FAMILY PEDIGREE

28. Family RCM 15



APPENDIX K – FAMILY PEDIGREE

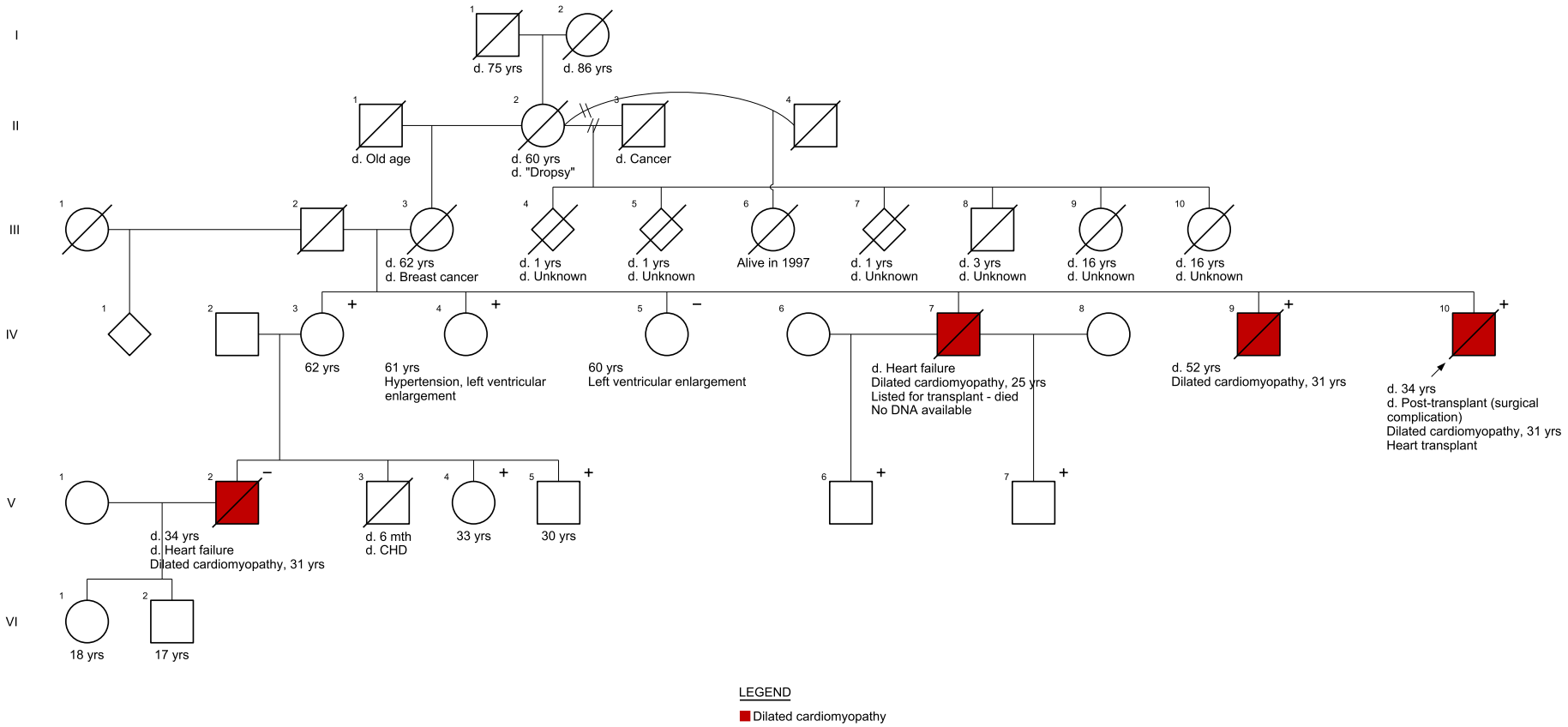
29. Family DCM 435



APPENDIX K – FAMILY PEDIGREE

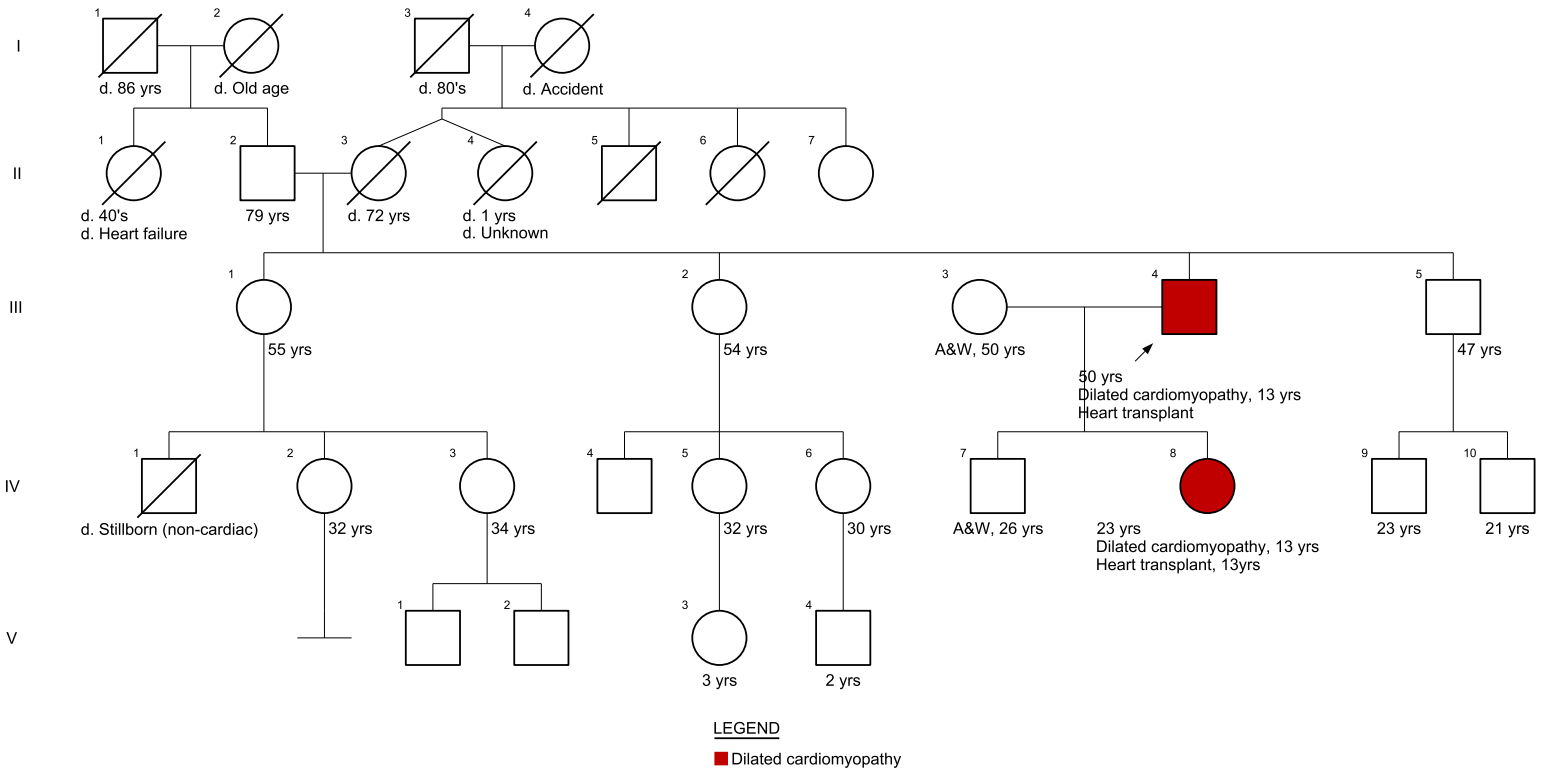
30. Family DCM 3

Variant of unknown significance (VUS), +PKP2 c.2540T>C, <u>does not segregate with disease</u> (Mbele, M. 2014. Ph.D. Thesis, UCT)



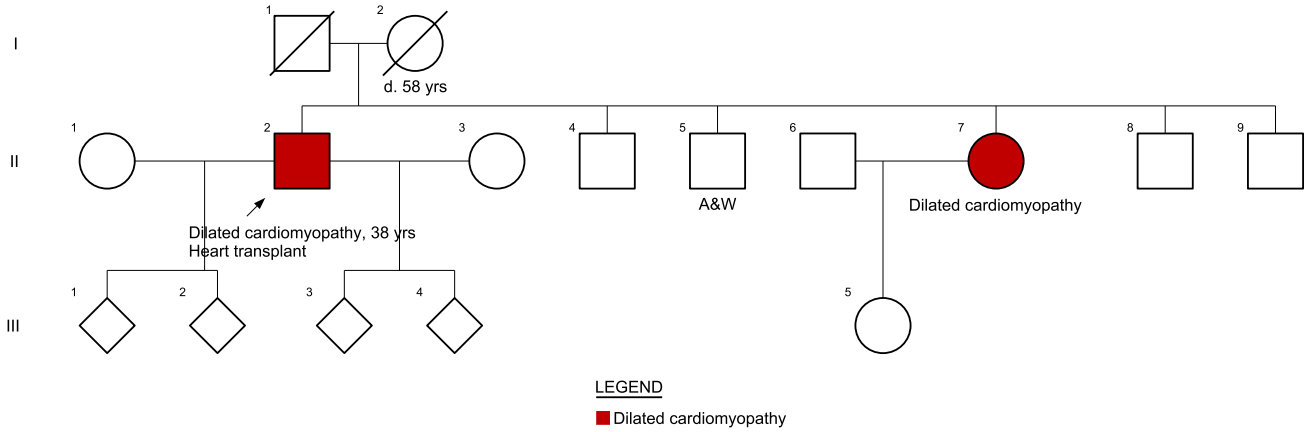
APPENDIX K – FAMILY PEDIGREE

31. Family DCM 334



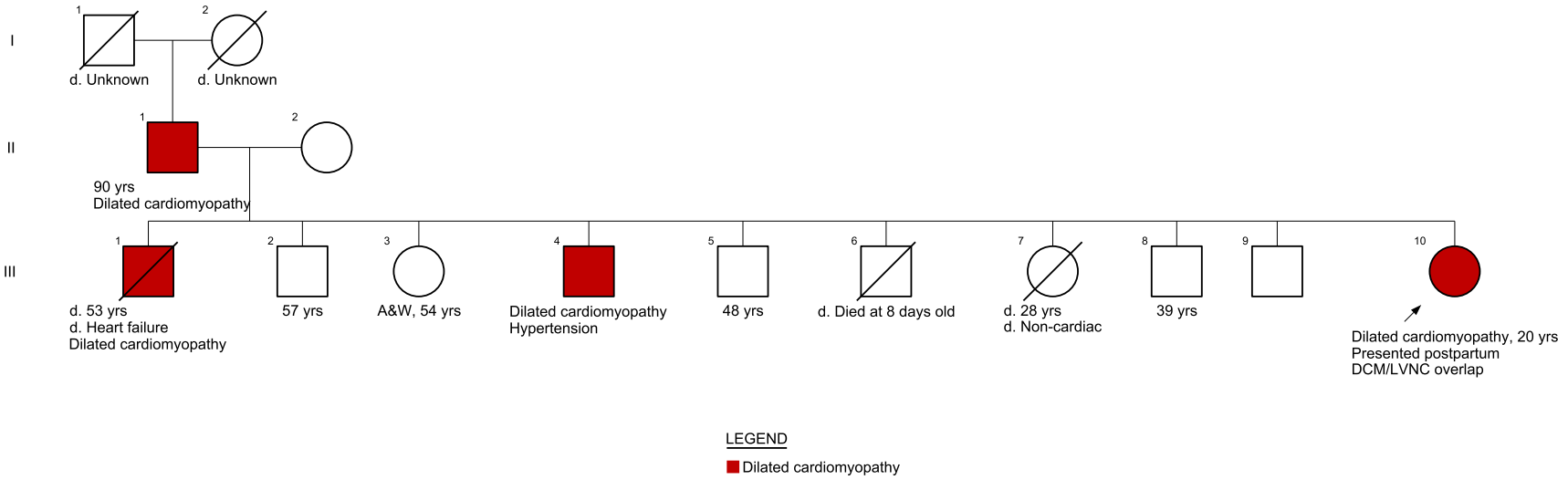
APPENDIX K – FAMILY PEDIGREE

32. Family DCM 24



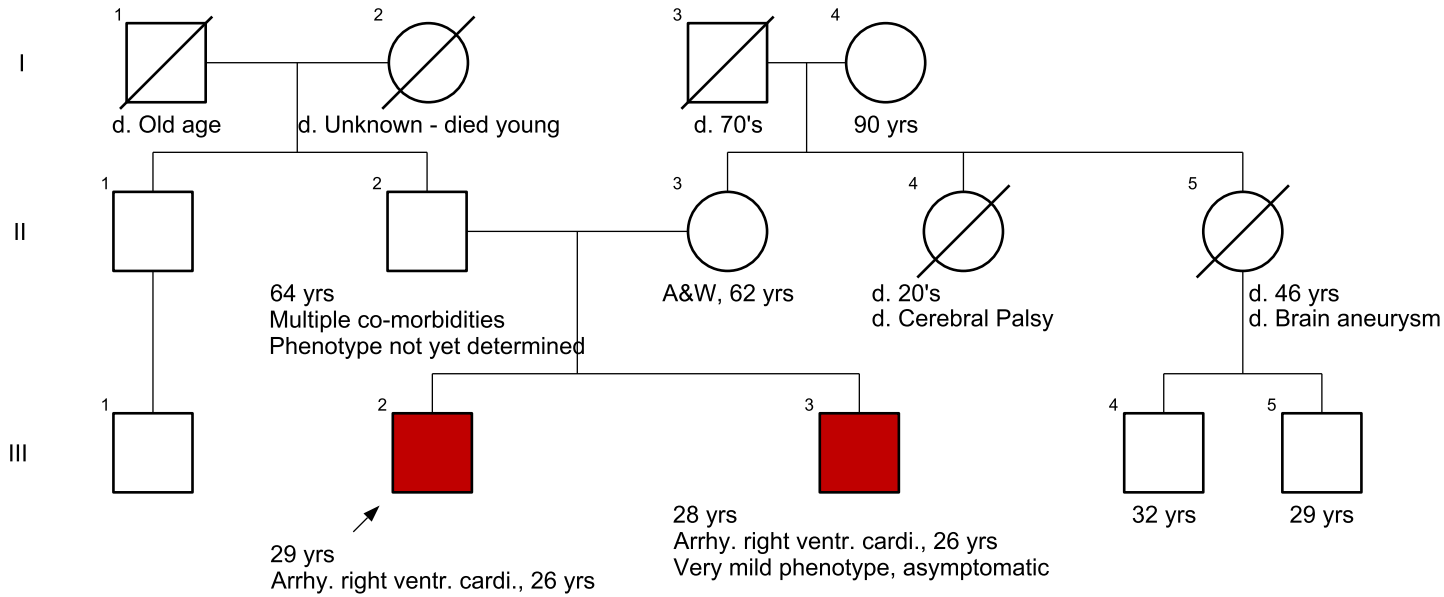
APPENDIX K – FAMILY PEDIGREE

33. Family DCM 303



APPENDIX K – FAMILY PEDIGREE

34. Family ACM 149



APPENDIX K – FAMILY PEDIGREE

35. Family DCM 458