Cardiac Manifestation and Clinical Management Strategies in Patients with Fabry Disease and Muscular Dystrophy

by

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Abstract

Background

Fabry disease (FD) and Muscular dystrophy (MD) are hereditary disorders with a high burden of heart disease recognized as a common cause of morbidity and mortality. The routine clinical care management of rare genetic diseases is complicated by extensive multisystem involvement, lack of clinical trials and established guidelines, and limited understanding of underlying characteristics that can predict patient prognosis. Our studies explore the utility of standard cardiac monitoring tools, including 12-lead electrocardiogram (ECG), transthoracic echocardiogram (TTE), and cardiovascular magnetic resonance (CMR) imaging), for phenotypic characterization and disease stratification of FD and muscular dystrophy.

Methods and Results

In Chapter 3, we collected clinical profiles and performed baseline CMR imaging in FD (n=95) patients. We identified clinical factors predictive of increased risk of major adverse cardiac events (MACE) in patients with FD targeted to improve clinical outcomes. Patients were followed over a median of 6.3 years (interquartile range [IQR], 4.5-7.0 years). Twenty-six patients reached the composite endpoint with a high prevalence of heart failure and cerebrovascular events, and zero occurrences of cardiac-related deaths. Patients with MACE had worse health-related quality of life scores. We performed multivariable Cox regression analysis and adjusted for age, and diagnoses dyslipidemia or hypertension. Hypertrophy and presence of myocardial fibrosis increase risk of MACE by 4 to 5 times, and dyslipidemia increases risk of MACE by three times. Early Fabry-specific treatment and close monitoring of comorbidities reduce cardiac complications and mortality. These findings highlight the importance of comprehensive multidisciplinary management to help improve outcomes in FD patients.

In Chapter 4, this study closely followed four adult patients from the Neuromuscular Multidisciplinary Clinic (Alberta, Canada) that presented with X-linked recessive Emery-Dreifuss muscular dystrophy (XLR-EDMD). Clinical status and cardiac function were assessed through clinical history, physical examination, and investigations using a 12-lead electrocardiogram, 24-hour Holter monitor, transthoracic echocardiogram, and plasma biomarkers. Conduction disease, requiring a permanent pacemaker, was prevalent in all patients. With appropriate medical therapy over a median follow-up period of five years, the cardiac status was shown to have stabilized in all the patients. We demonstrate the presence of arrhythmias, conduction abnormalities, and chamber dilation in adult patients with XLR-EDMD.

In Chapter 5, we prospectively enrolled 148 patients with dystrophinopathies (including heterozygotes), limb-girdle MD (LGMD), and myotonic dystrophy (DM1) over 7.7-years in addition to an age-and-sex-matched healthy control cohort (n=50). CMR markers, including 3D strain and fibrosis, were assessed for their respective associations with MACE. The dystrophinopathies and LGMD cohorts experienced reduced left ventricular ejection fraction (LVEF) and high burden of replacement fibrosis. In contrast, DM1 cohort experienced impaired systolic function particularly in patients with left bundle branch block and low ventricular mass. Markers of contractile performance were reduced in all MD groups compared to healthy controls. We followed patients over a median follow-up period of 5.2 years, in which 80 MACE occurred. While LVEF was independently predictive of MACE (adjusted hazard ratio [aHR]: 2.96), peak 3D strain amplitude offers greater predictive value (minimum principal [aHR: 5.48], maximum principal [aHR: 3.25], circumferential [aHR: 3.44], longitudinal [aHR: 3.39], and radial strain amplitude [aHR: 2.96]). The minimum principal strain Cox model was the strongest independent predictor and provided incremental value to LVEF to predict MACE in MD patients.

Conclusions

Cardiac dysfunction is observed across FD and MD subtypes and may benefit from therapeutic strategies centred around managing comorbidities and frequent monitoring with 12-lead ECG, TTE and CMR. CMR imaging was practical to distinguish the unique phenotypic profiles and predict the risk of MACE, particularly, LVH and LGE presence in FD patients, and reduced LVEF and 3D strain amplitudes in MD patients.

Preface

This thesis includes writing and data from published and unpublished manuscripts. Specific contributions to the listed projects are outlined below.

Published manuscript adapted for Chapter 3

Perera K*, Kashyap N*, Wang K, Omar F, Prosia E, Thompson RB, Paterson DI, Fine NM, White JA, Khan A, Oudit GY. Integrating cardiac mri imaging and multidisciplinary clinical care is associated with improved outcomes in patients with fabry disease.: Improving clinical outcomes in patients with fabry disease. *Curr Probl Cardiol*. 2022:101476. doi: 10.1016/j.cpcardiol.2022.101476

* These authors contributed equally to the research article.

Role of N.K.: Obtained and analyzed clinical data, creation of tables and figures, performed statistical analysis, drafted the original manuscript, and incorporated edits from coauthors/reviewers.

Published manuscript adapted for Chapter 4

Kashyap N, Nikhanj A, Gagnon LR, Moukaskas B, Siddiqi ZA, Oudit GY. Cardiac manifestations and clinical management of x-linked emery-dreifuss muscular dystrophy: A case series. *Eur Heart J Case Rep*. 2023;7:ytad013. doi: 10.1093/ehjcr/ytad013

Role of N.K.: Obtained and analyzed clinical data, creation of tables and figures, performed statistical analysis, drafted the original manuscript, and incorporated edits from coauthors/reviewers.

Unpublished data and manuscript adapted for Chapter 5

Kashyap N*, Nikhanj A*, Labib D, Prosia E, Rivest S, Flewitt J, Pfeffer G, Bakal JA, Siddiqi ZA, Coulden RA, Thompson R, White JA†, Oudit GY†. Prognostic Utility of Cardiovascular Magnetic Resonance Based Phenotyping in Patients with Muscular Dystrophy.

* These authors contributed equally to the research article.

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Role of N.K.: Obtained and analyzed clinical data, creation of tables and figures, performed statistical analysis, and incorporated edits from coauthors/reviewers.

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TABLE OF CONTENTS

LIST OF TABLES

List of Figures

Chapter 1. Introduction

1.1 Background

Fabry disease (FD) and muscular dystrophy (MD) are inherited disorders with multisystem involvement and a wide range of phenotypic manifestations. Fabry disease is characterized by progressive multisystem involvement that can lead to angiokeratomas, sensory complications, gastrointestinal problems, neurological complications, renal dysfunction, and severe heart disease (Figure 1.1).[1] Likewise, MD patients are affected by progressive muscle wasting, neurological development delays, respiratory complications, gastrointestinal disruption, and severe heart disease, often requiring multidisciplinary care. Muscular dystrophy is a group of inherited disorders comprised of Emery-Dreifuss muscular dystrophy (EDMD), Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD), and type 1 myotonic dystrophy (DM1). These chronic and debilitating diseases pose significant management and treatment challenges and have a substantial impact on the healthcare system.[2]

Cardiac involvement is a leading cause of morbidity and mortality in both diseases. To improve patient care and outcomes, clinicians and researchers must assess the efficacy and long-term outcomes of commonly used cardiac monitoring modalities, such as a 12-lead electrocardiogram (ECG), transthoracic echocardiogram (TTE), and cardiac magnetic resonance (CMR) imaging. By evaluating the effectiveness of these monitoring modalities, clinicians can optimize patient management and enhance healthcare delivery for individuals with FD and MD. Multidisciplinary care involving cardiology, neurology, and genetics specialists is essential for providing comprehensive care and monitoring these complex and multifaceted disorders.

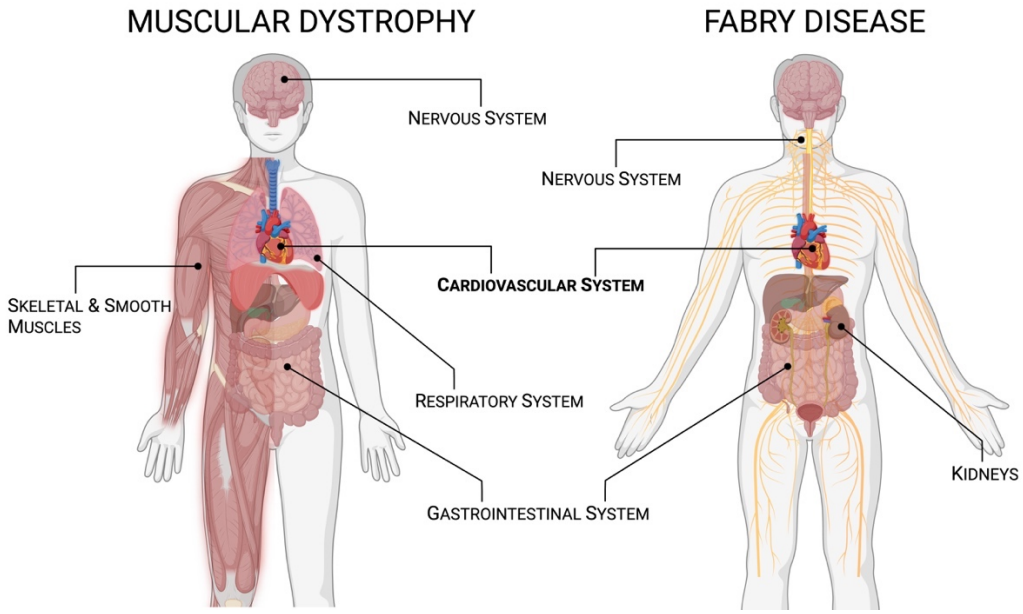

Figure 1.1 Muscular dystrophy and Fabry disease are multisystem diseases that affect multiple organ systems in the body, including the cardiovascular system.

1.2 Genetics, Pathophysiology, and Cardiac Involvement

Fabry disease

Fabry disease is a rare X-linked lysosomal storage disorder that affects approximately 1 in 476,000 to 1 in 117,000 male births annually.[3] The disease is caused by mutations in the *GLA* gene, which leads to the destabilization and dysfunction of the α-galactosidase protein. [4] This protein is crucial for lysosomal degradation of the glycosphingolipid, globotriaosylceramide (lyso-Gb3), by cleaving terminal α-galactose residues from lyso-Gb3 (Figure 1.2).[4, 5] The accumulation of glycosphingolipids in various cells and tissues in FD can compromise several cellular pathways, including inflammatory processes, apoptotic pathways, extracellular matrix remodelling, impaired endocytosis, and autophagy.[6]

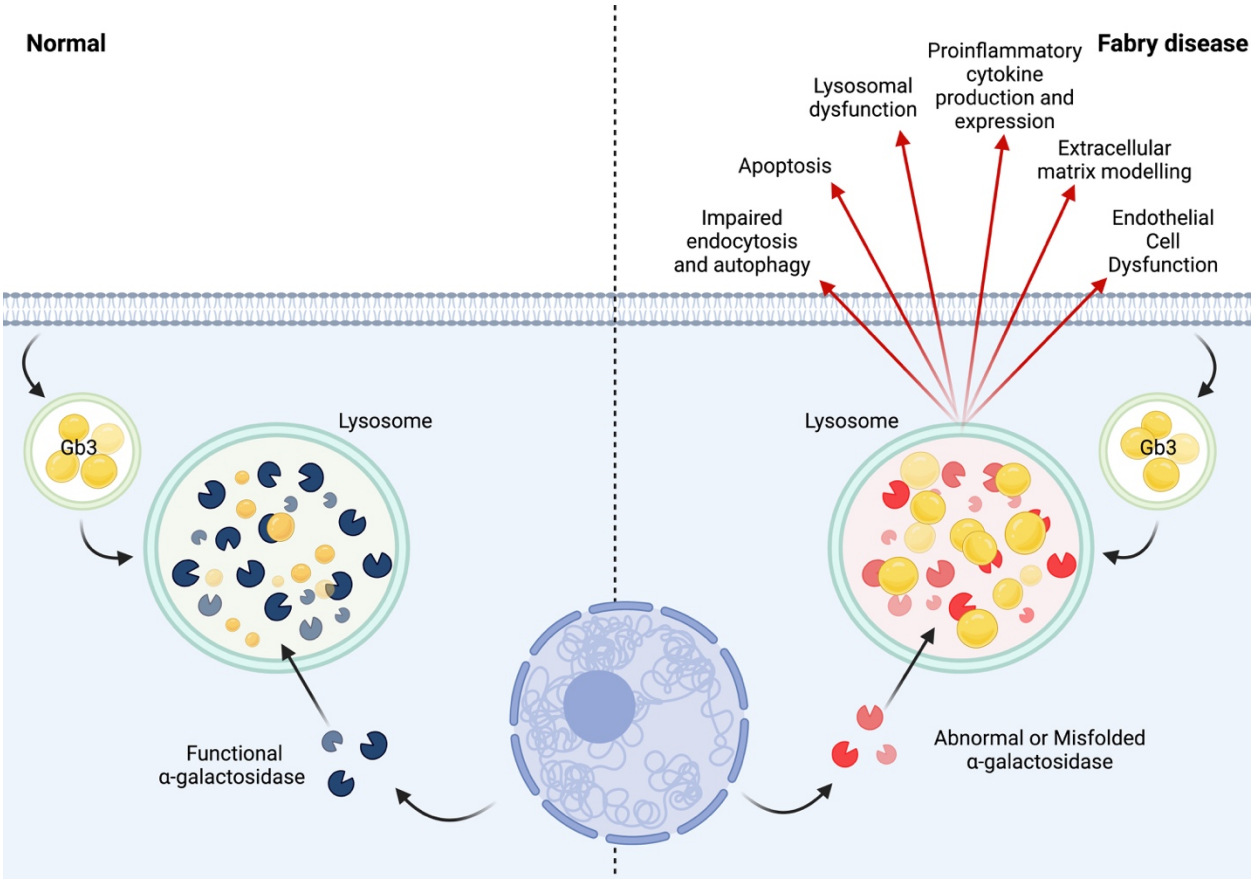

Figure 1.2 Fabry disease pathogenesis originates from abnormal or misfolded lysosomal enzyme, α-galactosidase, with improper degradation of globotriaosylceramide (Gb3) with subsequent malfunctioning of lysosomal and cellular pathways.

Cardiac involvement is a common manifestation of FD, with hypertrophy, myocardial fibrosis, inflammation, and microvascular dysfunction typically observed.[6] However, it is important to note that Gb3 accumulation may not be the primary cause of hypertrophy in FD, as low levels of lyso-Gb3 have been found in cardiomyocytes of some Fabry patients with hypertrophic cardiomyopathy.[7] Cardiac involvement may instead be a consequence of pathways activated by lysosomal/cellular dysfunction. Furthermore, lyso-Gb3 can act as antigens, activating natural killer T-cells and initiating inflammatory pathways.[6] There has been a corresponding increase in inflammatory and remodelling markers, including BNP, TNF, matrix

metalloproteinases, inflammatory cytokines, and galectin-1, in FD patients with hypertrophy or diastolic dysfunction.[8]

In FD, left ventricular hypertrophy (LVH) has been a useful screening tool for identifying patients with unexplained hypertrophy that may require further genetic testing for *GLA* mutations.[9] Regular monitoring by CMR is necessary for the early detection of these markers and the implementation of appropriate interventions.[10] Early diagnosis of FD can allow for early implantation of therapeutics, such as enzyme replacement therapy (ERT) and oral chaperone therapy.[11] Enzyme replacement therapy is an intravenously-administered treatment of recombinant α-galactosidase A that has been demonstrated to decrease/stabilize LV mass, reduce chances of myocardial infarction, heart failure, atrial fibrillation, and significantly improve life expectancy.[10, 12, 13] However, ERT efficacy declines in advanced stages. For instance, it has cardiac fibrosis and has limited capability to prevent further damage or reduce GB3 accumulation in cardiac cells.[12] Chaperon therapy is an orally administered therapy that binds and stabilizes α-galactosidase A in patients by facilitating lysosomal trafficking and restoring the functional use of the enzyme.[10, 14]

Emery-Dreifuss Muscular Dystrophy

Emery-Dreifuss muscular dystrophy is a rare form of MD recognized by the classic phenotypic triad: (1) early joint contractures, (2) slowly progressing muscle weakness and wasting, and (3) cardiac conduction abnormalities.[15] There are seven EDMD genetic subtypes where the majority of known proteins of interest are concerned with the structural and functional integrity of the linker of nucleoskeleton-and-cytoskeleton (LINC) bridging complex located on the nuclear envelope in skeletal and cardiac muscle.[15] The two most common subtypes, EDMD1 and EDMD2, are caused by mutations in the *EMD* and *LMNA* genes, respectively. EDMD1 is an X-linked recessive emerinopathy, and EDMD2 is an autosomal dominant laminopathy (Figure 1.3). Mutant

forms of emerin result in decreased nuclear invagination and abnormalities in Ca^{2+} transients.[16, 17] Mutant lamin A/C results in mishappen nuclei, impaired muscle regeneration and apoptosis in atrioventricular cells, and EDMD2 tends to have a more severe disease course and worse muscle wasting in the peripheral muscles.

Cardiomyopathy manifestations in EDMD consist of conduction abnormalities, supraventricular arrhythmias, chamber dilation and systolic dysfunction.[18] Patients are at risk of sudden cardiac death that can be mitigated through preventative interventions, including cardiac pacing or implanted cardioverter-defibrillator (ICD) devices.[19] Arrhythmias and conduction delays are highly prevalent, and given the rarity of this disease, the patient care process remains poorly defined.

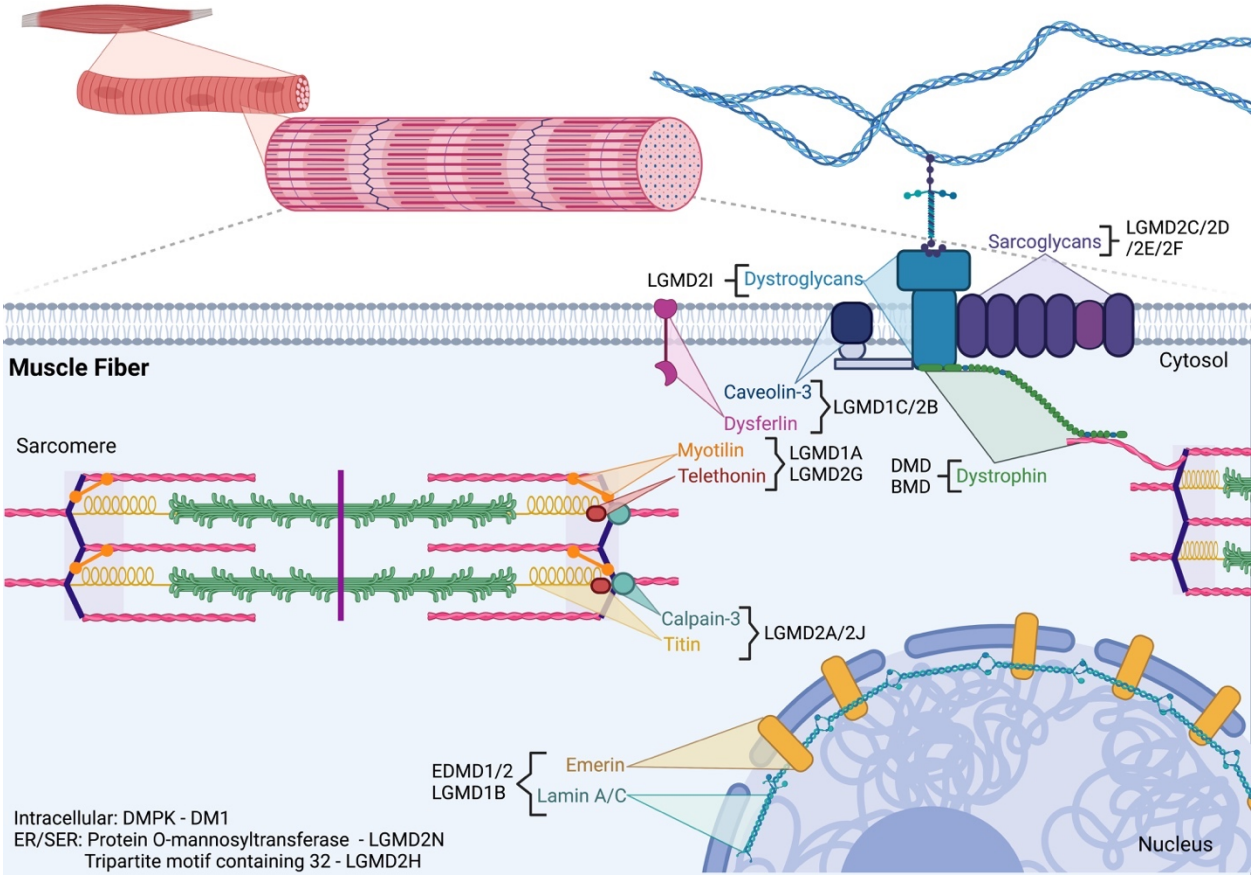

Figure 1.3 Proteins Associated with Muscular Dystrophies

Dystrophinopathies

Dystrophinopathies refer to a group of related diseases, including DMD and BMD, which result from different mutations in the *DMD* gene located on the X chromosome.[20] Duchenne MD affects approximately 4.78 in 100,000 male births due to frameshift mutations. In contrast, BMD affects 1.78 in 100,000 males with in-frame mutations that maintain the reading frame, leading to a truncated, absent, or dysfunctional dystrophin protein.[21] Dystrophin is a subsarcolemmal protein associated with the dystrophin-associated protein complex (DAPC) connecting cytoskeletal F-actin and the extracellular matrix (Figure 1.3).[22] Dystrophinopathies are primarily characterized by systemic muscle wasting and atrophy; however, they differ in disease severity, age-of-onset and rate of progression.[23] Progressive muscle weakness and atrophy can lead to skeletal muscle degradation, reduced respiratory function, gastrointestinal complications, and heart complications. Dystrophin expression and the role of DAPC in the heart is tissue-specific, where dystrophin directly binds to α-actin and interacts with cardioprotective proteins, which prevents cardiac muscle atrophy to the same extent as seen in skeletal muscle.[24, 25] Nevertheless, this link plays an important role in the transmission of mechanical forces to the extracellular matrix.[22] Loss of function of dystrophin leads to instability in the plasma membrane, reactive oxidative species, and inappropriate Ca^{2+} entry leading to ischemic muscle damage and dysfunctional cardiomyocytes.[22, 26]

Cardiomyopathy is a leading cause of mortality in both DMD and BMD.[27] Duchenne muscular dystrophy (DMD) is a severe form of the disease, with symptoms typically appearing in affected males as early as 2-3 years old.[23, 28] These individuals may become reliant on wheelchairs by age 10 and have a life expectancy between 21-40 years old.[23, 29] Diagnosis of DMD is often made through clinical presentation, genetic testing, and muscle biopsy. Ventilatory support and

cardiac intervention are critical in improving life expectancy and quality of life.[27] In contrast, BMD is a milder form of the disease with high phenotypic heterogeneity and later onset. Age of onset of BMD can range from 2-20 years, beginning with musculoskeletal symptoms with muscle weakness in the lower limbs.[30] Age of onset for cardiomyopathy in BMD patients is approximately 29 years; however, cardiomyopathies are frequently undiagnosed due to being asymptomatic.[31] Diagnosis of cardiac involvement can be challenging, as typical signs and symptoms of heart failure can be masked or subtle in patients that are wheelchair-bound or experience respiratory complications.[30, 32] The main cardiac phenotype in dystrophinopathies is dilated cardiomyopathy (DCM) and atrial and ventricular arrhythmias.[30]

Limb-Girdle Muscular Dystrophy

Limb-girdle MD is a highly heterogeneous group of disorders with 30 genetic subtypes encompassing a broad spectrum of symptomology, severity, and age of onset.[33] Limb-girdle MD comprises two major groups of autosomal dominant and autosomal recessive patterns of inheritance caused by defects in proteins associated with molecular pathways and cellular structure proteins (Table 1.1).[33] These proteins play important functional roles in the sarcomere, nucleus, protein assembly and modifications, and the extracellular matrix (Figure 1.3).

Table 1.1 Types of Limb-Girdle Muscular Dystrophy and Associated Pathogenic Genetic Variants and Proteins

Type of LGMD	Pattern of inheritance	Gene/Protein	Function
LGMD1A	Autosomal dominant	*TTID*/Myotilin	Structural protein at Z-disc[33, 34]
LGMD1B	Autosomal dominant	*LMNA*/Lamin A/C	Nuclear structural protein.[33]
LGMD1C	Autosomal dominant	*CAV3*/Caveolin 3	Scaffolding protein for caveolar membranes.[33]
LGMD2A	Autosomal recessive	*CAPN3*/Calpain 3	Key regulator of the sarcomere.[35]
LGMD2B	Autosomal recessive	*DYSF*/Dysferlin	Regulation of Ca^{2+} signalling.[36]
LGMD2C, 2D, 2E, 2F	Autosomal recessive	*SCGX*/γ,α,β,δ sarcoglycan	Associated with the DAPC; links actin filaments and extracellular matrix.[33, 37]
LGMD2G	Autosomal recessive	*TCAP*/Telethonin	Sarcomeric structural protein at the Z-disc.[38]
LGMD2H	Autosomal recessive	*TRIM32*/Tripartite motif containing 32	Ca^{2+} movement in the myotubes.[39]
LGMD2I	Autosomal recessive	*FKRP*/Fukutin related protein	Glycosylation of dystroglycan.[33]
LGMD2J	Autosomal recessive	*TTN*/Titin	Tension and stability of the sarcomere.[40]
LGMD2N	Autosomal recessive	*POMT2*/ Protein O-mannosyltransferase	Protein assembly.[33]

Limb-girdle MD are characterized with joint contractures, respiratory failure, gastrointestinal complications, mental disabilities, skeletal muscle weakness and atrophy. The subtypes LGMD1B and LGMD2C-2I are associated with the risk of cardiac involvement.[33, 41, 42] Cardiac complications in LGMD may include DCM, arrhythmias, and atrial conduction defects are the most common cardiac phenotypes exhibited in these patients.[32, 43] The age of onset and cardiac manifestation can vary significantly between subtypes of LGMD, with some individuals experiencing cardiac complications at a young age while others may develop them later in life.[32] In addition to cardiac complications, LGMD can lead to progressive muscle weakness and atrophy, resulting in mobility impairment and reliance on mobility aids or wheelchair assistance for many adult patients.[43] Treatment for LGMD is primarily supportive and focuses on managing symptoms and improving quality of life.[44]

Type 1 Myotonic Dystrophy

Myotonic dystrophies (DM) are the most common type of MD in adults with an autosomal dominant inheritance pattern. Type 1 myotonic dystrophy (DM1) and Type 2 myotonic dystrophy (DM2) are caused by unstable nucleotide CTG/CCTG repeat expansions found in the 3' untranslated regions of the *DMPK* and *CNBP* genes, respectively, resulting in abnormal splicing of downstream effector genes.[45] Myotonic dystrophies are characterized by myotonia, progressive muscle weakness/atrophy, and conduction defects and arrhythmias.[45] The myotonic dystrophies differ in the pattern of muscle involvement, age of onset, and disease severity. In particular, DM1 has earlier disease manifestation and a higher risk of cardiac involvement.[45] Furthermore, the size of nucleotide expansions correlates to worse disease severity and age of onset with the presentation of difficulty eating, respiratory complications and cardiorespiratory complications.[46] Nucleotide expansions increase with age and with each generation exacerbating instability over time and contributing to the progressive nature of this disease.[46] Ribonucleoacid (RNA) transcripts of the variant *DMPK* gene aggregate as nuclear foci that lead to dysregulation of key pathways.[46] There are a few proposed mechanisms for dysfunctional DMPK protein, or nuclear foci, leading to cardiac dysfunction. For instance, an induced pluripotent stem cell model derived from DM1 patients showed that nuclear foci lead to reduced excitability of cardiac Na^+ channels and increased L-type Ca^{2+} channels density resulting in prolonged action potentials and slower conduction velocity.[46, 47] In addition, reduced DMPK levels lead to abnormal cardiomyocyte contractility, increased Ca^{2+} uptake into the sarcoplasmic reticulum and increased basal cytosolic Ca^{2+} levels that may stimulate arrhythmogenic current in cardiomyocytes.[48]

These pathogenic mechanisms may contribute to the high risk of left and right bundle branch block, ventricular tachycardia and sudden cardiac death in DM1 patients.[47] Initial cardiac

manifestation in these patients is characterized by prolonged PR interval and QRS duration.[49] As mentioned, given the progressive nature of DM1, patients are recommended pacemakers and implantable cardioverter-defibrillators when presenting with clinically significant conduction abnormalities or arrhythmias.[49]

Heterozygotes in Fabry disease and Dystrophinopathies

Both FD and dystrophinopathies have an X-linked recessive inheritance pattern; thus, female heterozygotes possess an affected and unaffected copy of the *DMD* and *GLA* genes. Thus, heterozygotes typically exhibit a milder form of the disorder than their hemizygote counterpart. Heterozygotes are undergo X-chromosome inactivation, a natural process during embryonic development to compensate for the extra set of genes females inherit on the X-chromosome, whereby one of the two X chromosomes is randomly silenced and forms a dense structure called the Barr body.[50] The remaining X-chromosome remains functional in some cells while inactivated in others, which can affect the phenotypic variability seen in females carrying X-linked recessive diseases.[51] There can also be preferential inactivation of one X-chromosome over the other, leading to an imbalance in gene expression that can contribute to disease pathogenesis.[52, 53] Many FD and dystrophinopathy heterozygotes are asymptomatic due to X-linked inheritance. In contrast, symptomatic heterozygotes have a broad spectrum of phenotypic manifestations and severity.[14, 53]

Heterozygotes are a frequently clinically overlooked group—dismissed for low prevalence and severity compared to their male counterparts,[54] even though approximately 27% to 47% of dystrophinopathy heterozygotes exhibit some form of cardiac involvement in their adult years.[55, 56] Cardiac phenotype is similar to their male counterparts, such as arrhythmias and cardiomyopathy. It has been hypothesized that even though some muscle cells may possess functional dystrophin in some cardiomyocytes, these cardiomyocytes cannot compensate for the

dysfunctional dystrophin found in other cells leading to the development of DCM.[56] Uneven expression of dystrophin depending on skewed X-inactivation has been associated with the development of cardiac phenotype in dystrophinopathy heterozygotes.[53]

Like the dystrophinopathy heterozygotes, the healthcare field population has devalued the FD heterozygotes.[57] Research typically excluded heterozygotes and may have resulted in an inherent bias associated with FD manifestation derived from male patients, including initial assessments of ERT therapy conducted in only males.[58] Historically, female heterozygotes' manifestations were described as asymptomatic or mild phenotypes, potentially contributing to delayed diagnosis after the onset of symptoms.[57, 59] Most female heterozygotes report clinical features associated with FD, of which neurological, cardiac, and renal involvement were the primary features affecting females.[60, 61] Patients exhibit a wide range of disease severity and phenotypic characterization.[60] Disease severity was also not associated the with α-galactosidase activity levels.[60]

1.3 Rationale and Hypothesis

Muscular dystrophy and FD are hereditary disorders that are vulnerable to morbidity and mortality due to their high burden of heart disease. The routine clinical care management of rare genetic diseases is complicated by extensive multisystem involvement, lack of clinical trials and established guidelines, and limited understanding of underlying characteristics that can predict patient prognosis. To address these challenges, our studies aim to explore the utility of standard cardiac monitoring tools, such as 12-lead ECG, TTE, and CMR imaging, for phenotypic characterization and disease stratification of MD and FD. Using these tools to understand cardiac manifestations of these diseases better, we can develop more accurate prognostic assessments and improve patient outcomes. We hypothesize that features detected by conventional cardiac

monitoring modalities can be used for disease characterization and prognostication in muscular dystrophy and FD.

Chapter 2. Materials and Methods

2.1 Patient Enrollment

In coordination with the Mazankowski Alberta Heart Institute (MAHI) in Edmonton, Canada, 39 patients with Fabry disease (FD) were enrolled over 12-years from May 19, 2010 to June 2, 2022. For the purpose of this thesis, 38 FD patients were included between May 19, 2010 and April 27, 2022. In addition, 61 patients with FD were enrolled in collaboration with the Metabolics and Genetics in Calgary (MAGIC) clinic and the Stephenson Cardiac Imaging Centre in Calgary, Canada. Patients confirmed with genetic assessment for FD diagnosis were enrolled independent of overt cardiac symptoms. Informed consent was obtained the time of clinic visit in respective clinics by supporting clinical staff.

In coordination with the Neuromuscular Multidisciplinary (NMMD) Clinic at the Kaye Edmonton Clinic, 328 patients with neuromuscular diseases were enrolled in the NMMD registry over nine years November 5, 2014 to March 15, 2023. For the purpose of this thesis, neuromuscular disease patients were included between November 5, 2014 to May 6, 2022. Neuromuscular disease patients at the NMMD clinic were comprised of 221 muscular dystrophy (MD) patients (4 EDMD patients, 37 DMD patients, 16 BMD patients, 7 DMD heterozygote patients, 2 BMD heterozygote patients, 47 LGMD patients, 98 DM1 patients, and 10 DM2 patients) and 107 non-MD patients (34 FSHD patients, 35 mitochondrial myopathies, 38 other patients) (Figure 2.1). In addition, 63 MD patients (9 DMD patients, 16 BMD patients, 5 DMD heterozygote patients, 2 BMD heterozygote patients, 10 LGMD patients, and 21 DM1 patients) were enrolled in collaboration

with the Stephenson Cardiac Imaging Center in Calgary, Canada. For the purpose of this thesis, only MD patients were included in our investigations. Muscular dystrophy diagnosis was confirmed with genetic assessment or clinical/familial history.

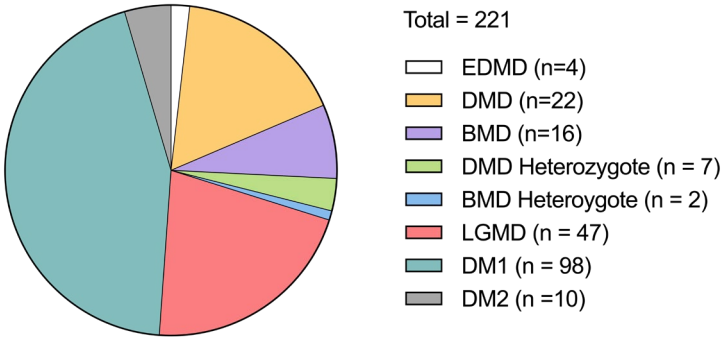

Figure 2.1 Distribution of muscular dystrophy patients enrolled at the Neuromuscular Multidisciplinary Clinic in Edmonton, Canada.

EDMD, Emery-Dreifuss muscular dystrophy; DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy; DM2, type 2 myotonic dystrophy.

2.2 Data Acquisition and Assessment

All patients were enrolled at multidisciplinary clinics and were given informed written consent at initial clinic visit. Baseline clinical assessment was conducted with heart rate, blood pressure, and documentation of pre-existing comorbidities and corresponding diagnostic assessments. Follow-up clinic visits were conducted on a 1-2 years basis. Interpretation and acquisition of 12-lead ECG, TTE, and CMR imaging were performed by an ordering physician and radiologist. Core lab analyses and CMR values for Edmonton and Calgary cohorts were performed by the Department of Cardiac Sciences at the Cumming School of Medicine and Stephenson Cardiac Imaging Center at the Libin Cardiovascular Institute. RedCap Clinical data collected included: laboratory values (ie. low-density lipoprotein [LDL] levels, creatinine,

troponin I), 12-lead ECG, cardiac imaging modalities, and quality of life questionnaires. Recorded parameters for ECG, TTE and CMR included: heart rate, ECG intervals (ie. RR interval, PR interval, QRS duration, QT/QTc interval), ventricular structure and function parameters (ie. left ventricular (LV) and right ventricular (RV) ejection fraction, LV end-systolic and -diastolic volumes and diameters, atrial volumes, LV mass). These values may be indexed to height or body surface area (BSA), of which BSA can be calculated using the equation: $BSA = \sqrt{\frac{weight\ (kg) \times height\ (cm)}{3600}}$.[62] Clinical outcomes will be based on ICD 10 codes collected by the Data Integration and Management Repository (DIMR) analytics branch of Alberta Health Services or through documentation of clinician/emergency department notes collected through chart review via ConnectCare and Netcare.

Chapter 3. Integrating Cardiac MRI Imaging and Multidisciplinary Clinical Care is Associated With Improved Outcomes in Patients With Fabry Disease

3.1 Introduction

Fabry disease (FD) is an X-linked recessive lysosomal storage disorder characterized by a deficiency in alpha-galactosidase A activity leading to accumulation of glycosphingolipids in cardiac cells, myocardial accumulation, microvascular dysfunction, and fibrosis.[5, 14] Cardiac involvement is the primary driver of mortality in patients with FD, accounting for 75% of total deaths in untreated patients.[1, 63, 64]

Cardiac damage can start in early stages of life in FD but early diagnosis can be challenging because of multisystem involvement and variation in symptomology.[14] This often leads to the progression of advanced myocardial phenotypes through structural changes in the heart, including concentric left ventricular remodeling, microvascular dysfunction, and cardiac fibrosis, thereby

increasing mortality risk and major adverse cardiac events (MACE).[14] However, the modern-day clinical care landscape has rapidly evolved to incorporate early diagnostic interventions, better risk stratification, and novel treatment developments. This includes utilizing cardiac magnetic resonance imaging as the preferential diagnostic tool to help characterize heart function by assessing left ventricular hypertrophy (LVH) and myocardial fibrosis.[65] Furthermore, the emergence and usage of enzyme replacement therapy, chaperone therapy, and comorbidity management as early interventions for FD have been crucial in improving disease trajectories.[66] Unfortunately, despite rapid advances in management in FD, there appears to be a paucity of literature that has assessed how early intervention, comorbidity management, and novel treatment regimens have amalgamated to improve outcomes in the context of current treatment guidelines. Given our evolved management and detection of FD, in addition to the increased use of novel Fabry-specific therapies, periodic assessment is crucial in identifying persistent risk factors of poor outcomes that may require additional interventions. Thus, this study aimed to identify clinical and psychosocial risk factors predictive of MACE in patients with FD and determine areas of focus for improving patient outcomes.

3.2 Methods

Patient Cohort

Our data were derived from the Alberta Fabry Disease Registry at the University of Alberta, University of Calgary, and M.A.G.I.C Clinic, a gene-positive FD longitudinal local registry of adult (>18 years of age) outpatient encounters. This cross-sectional retrospective analysis included all baseline clinical data for patients who completed a baseline CMR study in Alberta between July 2006 to February 2022. Exclusion criteria included failure to undergo a baseline CMR and incomplete or missing patient data concerning MACE. The study received

ethics approval from Alberta Research Information Services and obtained written informed consent from all patients.

Clinical Characterization and Outcome Assessment

FD diagnosis was established by genetic testing in conjunction with alpha-galactosidase activity and globotriaosylsphingosine levels.[67] Clinical variables included genotype variant, comorbidities, medical therapy, and use of cardiac devices. Health-related quality of life was assessed using the EQ-5D-3L questionnaire, a 5-item questionnaire assessing: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, with each dimension stratified by levels of severity.[68] The frequency of patient-reported outcome measures (PROMs) at each severity level was available from 81 patients. For each patient, a cumulative PROM score was calculated based on their responses for each outcome where "no problems" = 0, "moderate problems" = 1, and "severe problems" = 2, thus creating a scale from 0 to 10 where 0 is no problems, and 10 is severe problems in all outcomes. The frequency of patients for each cumulative score (0 to 10) was stratified according to no MACE (n=62) and one or more MACE (n=19). In addition, the EQ-5D visual analog scale (VAS) assessed patients' rankings of their current health state on a continuous scale of 0 to 100, with 0 representing the "worst" quality of life and 100 representing the "best" quality of life.[68]

Incidence of MACE was obtained from electronic medical records using each patient's respective baseline CMR as the index date to February 2022 (median study duration, 6.4 years [IQR, 4.5–7.0 years]). The composite end-point was defined as the presence of one or more of the following events: (a) severe heart failure, (b) non-sustained (NS) or sustained ventricular tachycardia (VT); (d) severe bradyarrhythmia, defined as a heart rate below 50 beats per minute requiring device implantation for pacing; (d) atrial fibrillation; (e) cardiac syncope; (f) transient

ischemic attack (TIA) or stroke; (g) hospitalization or emergency room visit for microvascular angina; (h) myocardial infarction (MI); or (i) cardiac death.

Cardiac MRI technique

All CMR imaging was performed according to local institutional practice using either 1.5-T (Siemens Sonata or Avanto, Siemens Medical Systems) or 3.0-T (TRIO or Verio, Siemens Medical Systems) clinical scanners. All sites used standardized imaging protocols including ECG-gated cine imaging using a steady-state free precession pulse sequence in sequential short-axis and conventional long-axis views. The administration of gadolinium-based contrast agents followed this with standard inversion-recovery gradient imaging performed at 10 to 15 minutes in matched imaging views to assess the presence of late gadolinium enhancement (LGE). Phase-sensitive inversion-recovery sequences were adopted as clinically available at sites to assist in the clinical interpretation of LGE images. When not available, all sites chose the appropriate time (longitudinal relaxation time) in accordance with prior published recommendations.

Image analysis was performed using commercially available image analysis software. Each site performed volumetric chamber analysis in accordance with published guidelines of the Society of Cardiovascular Magnetic Resonance.[69] Left ventricular hypertrophy (LVH) was defined as a CMR-derived LVMi greater than 85 g/m^2 for males and 81 g/m^2 for females.[70] Myocardial fibrosis or chronic inflammation on LGE imaging was considered present when extending beyond the ventricular insertion points (isolated RV insertion site fibrosis not included). Corresponding patterns of fibrosis were coded based on visual assessment according to previously published methods. CMR image analysis and interpretations were performed by experienced clinical interpreters, all with level 3 CMR training or equivalent clinical experience.

Statistical Analysis

Descriptive statistics were presented as medians with interquartile ranges (IQR) for continuous data and absolute numbers with percentages for categorical data. Pair-wise comparisons between groups were performed using the nonparametric Mann-Whitney U test, Kruskal-Wallis test, and Wilcoxon sum rank test when appropriate for scalar values and the Pearson's chi square test for nominal values. The primary composite endpoint (all MACE) for patients with LGE present, LVH present, or either LGE or LVH present was assessed using the Kaplan-Meier curve and the log-rank test. Univariable and multivariable Cox proportional hazard model assessed the association between independent predictors (LVH and LGE, and combined LVH or LGE) with MACE in our cohort. Multivariable models adjusted for age, and dyslipidemia were used to evaluate the prognostic utility of LVH and LGE for MACE in our cohort. Covariates were selected based on clinical relevance and statistical significance was determined by univariate analysis and forward selection model identifying (LVH, LGE, age, dyslipidemia, and hypertension). A 2-tailed $p<0.05$ was considered statistically significant, and all statistical analysis was performed using SPSS, version 26 (IBM Corporation, Armonk, NY).

3.3 Results

Clinical and Demographic Characteristics

Ninety-five patients (40 males (42.1%); median age, 47 years [IQR, 38.5–62 years]) were included in the study, where 93 patients were assessed with LGE. There were 63 (76.8%) patients with the classic GLA gene variant (Table 3.1; Table 3.2). Left ventricular hypertrophy was present in 22 (23.1%) while LGE was present in 32 (33.7%) patients. Hypertension (45.7%) was the most common comorbidity in our cohort, followed by dyslipidemia (33.3%), CKD (17.2%), CAD (9.8%), and diabetes mellitus (6.4%) (Table 3.1). Renin-angiotensin system inhibition therapies

(ACE inhibitors or ARBs) and statin therapies were standard, comprising 53.7% and 30.5%, respectively. Enzyme replacement therapy (ERT) and chaperone therapy were reported in 61.5% and 14.3% of patients at follow-up, respectively. The FD cohort was stratified based on LVH absence or presence and LGE absence or presence, respectively, to determine differences between clinical characteristics and management strategies. There was a more significant proportion of males in the LVH present cohort (81.8%) compared to the LVH absent cohort (30.1%, $p<0.001$), contrary to LGE presence, which was comparable between sexes ($p=0.39$). Comorbidities, such as dyslipidemia, CAD, and CKD, were significantly associated with the LVH present cohort.

Table 3.1 Clinical Characteristics and Therapeutic Management for Patients with Fabry Disease

Parameters	All Patients (n=95)	LVH Absent (n=73)	LVH Present (n=22)	P-value	LGE Absent (n=61)	LGE Present (n=32)	P-value
Clinical Parameters							
Age (y)	47 (38.5–62)	47 (36–58)	46 (36–58)	0.06	46 (36–57)	57.5 (44.8–63.3)	0.16
Sex (Male)	40/95 (42.1)	22/73 (30.1)	18/22 (81.8)	0.001	23/61 (37.7)	15/32 (46.9)	0.39
Classic Variant	63/82 (76.8)	48/63 (76.2)	15/19 (78.9)	0.80	39/50 (78.0)	22/30 (73.3)	0.64
Body Mass Index (kg/m^2)	24.7 (22.2–29.7)	25.6 (22.4–30.1)	23.0 (20.9–26.7)	0.13	24.3 (22.1–28.9)	25.8 (22.3–30.9)	0.92
Smoking history	29/95 (30.5)	23/73 (31.5)	6/22 (20.7)	0.71	18/61 (29.5)	11/32 (34.4)	0.63
Hypertension	43/94 (45.7)	30/72 (41.7)	13/22 (59.1)	0.15	23/60 (38.3)	18/32 (56.3)	0.10
Chronic Kidney Disease	16/93 (17.2)	8/72 (11.1)	8/21 (38.1)	0.004	8/59 (13.6)	6/32 (18.8)	0.51
Coronary Artery Disease	9/92 (9.8)	4/71 (5.6)	5/21 (23.8)	0.01	4/58 (6.9)	5/32 (15.6)	0.19
Diabetes mellitus	6/94 (6.4)	5/72 (6.9)	1/22 (4.5)	0.69	4/60 (6.7)	2/32 (6.3)	0.94
Dyslipidemia	31/93 (33.3)	16/72 (22.2)	15/21 (71.4)	0.001	15/59 (25.4)	14/32 (43.8)	0.07
Cardiovascular/Comorbidity Management							
ACE inhibitors/ARBs	51/95 (53.7)	35/73 (47.9)	16/22 (72.7)	0.04	26/61 (42.6)	23/32 (71.9)	0.007
Statins	29/95 (30.5)	15/73 (20.5)	14/22 (63.6)	0.001	14/61 (23.0)	13/32 (40.6)	0.07
Anticoagulants	16/95 (16.8)	6/73 (8.2)	10/22 (45.5)	0.001	5/61 (8.2)	9/32 (28.1)	0.01
Antiplatelet	41/95 (43.2)	27/73 (37.0)	14/22 (63.6)	0.03	20/61 (32.8)	19/32 (59.4)	0.01
Diuretic	21/95 (22.1)	10/73 (13.7)	11/22 (50.0)	0.001	8/61 (13.1)	11/32 (34.4)	0.02
Beta Blockers	25/95 (26.3)	13/73 (17.8)	12/22 (54.5)	0.001	11/61 (18.0)	13/32 (40.6)	0.02
Calcium Channel Blockers	14/95 (14.7)	9/73 (12.3)	5/22 (22.7)	0.23	8/61 (13.1)	5/32 (15.6)	0.74
Cardiac Device	7/95 (7.5)	1/73 (1.4)	6/22 (27.3)	0.001	1/53 (1.9)	6/38 (15.8)	0.01
Fabry Disease Therapies							
ERT							
Baseline	32/91 (35.2)	21/69 (30.4)	11/22 (50.0)	0.09	14/59 (23.7)	16/30 (53.3)	0.005
Follow-up	56/91 (61.5)	37/70 (52.9)	19/21 (90.5)	0.002	28/58 (48.3)	26/31 (83.9)	0.001
Chaperone Therapy							
Baseline	1/95 (1.1)	1/73 (1.4)	0/22 (0.0)	0.58	1/61 (1.6)	0/32 (0.0)	0.47
Follow-up	13/91 (14.3)	9/70 (12.9)	4/21 (19.0)	0.48	5/58 (8.6)	8/31 (25.8)	0.03

Data are represented as either n (%) or median (interquartile range). P values depict the comparison between LVH absent or present and LGE absent or present, respectively. LVH, Left ventricular hypertrophy; LGE, Late gadolinium enhancement; ACE, Angiotensinogen converting enzyme; ARB, Angiotensin receptor blockers; ERT, Enzyme Replacement Therapy.

Table 3.2 Mutations and type of variant in Fabry disease patients

Type of Variant	Mutation	Galactosidase Activity (U/mL)	Total Lyso-GB3 (nmol/L)
Classic	R227X (n=1), R227Q (n=2), R356W (n=8),Y134S (n=5), A143P (n=3), Q386X (n=3), R112C (n=3), S345P(n=10), Q321R (n=1), Q321E, G43V (n=3), W349X (n=1), R112C (n=5), A15E (n=1), c. 622+623 del (n=1), E338K (n=2), R220X (n=2), G261V (n=2), c.1288del (n=2), c.640/801G>A (n=1), Q321E (n=1), R342X (n=2), R342Q (n=2), D313Y (n=1)	1.9 [IQR 0.5–2.7]	44.7 [IQR 15.5–80.6]
Variant	N215S (n=8), A143T (n=3), R112H (n=4), A215S (n=2), X430LysfsX19 (n=1)	1.5 [IQR 1.1–2.8]	6.0 [IQR 1.6–8.8]
Unclassified	c.234delA	-	-

Major Adverse Cardiac Events

Over the median follow-up duration of 6.3 years [IQR, 4.5–7.0 years], there were 59 incidents of MACE, of which 26 patients (27.3%) reached the composite endpoint (Figure 3.1A). Severe HF was the most common type of MACE (n=16), followed by TIA/stroke (n=11), AF (n=9), angina (n=8), severe bradycardia (n=4), cardiac syncope (n=4), MI (n=3), sustained VT (n=2), NSVT (n=2), with zero cardiac deaths. Of twenty-two patients, 63.6% of LVH present patients reached the composite endpoint compared to 15.1% of 73 LVH absent patients ($p \leq 0.001$; Figure 3.1B). Furthermore, 53.1% of 32 LGE present patients reached the composite endpoint compared to 11.5% of 61 LGE absent patients ($p \leq 0.001$). Severe HF (LVH, 45.5%; LGE, 34.4%), TIA/stroke (LVH, 40.9%; LGE, 25.0%), atrial fibrillation (LVH, 22.7%; LGE, 21.9%), and MI

(LVH, 13.6%; LGE, 9.4%), were the most prevalent in both LVH and LGE present groups (Figure 3.1C and 3.1D). Angina (p=0.006) and severe bradyarrhythmia (p≤0.001) are both associated with LVH but not with LGE. Sustained VT and NSVT are both associated with LGE but not LVH (p=0.05).

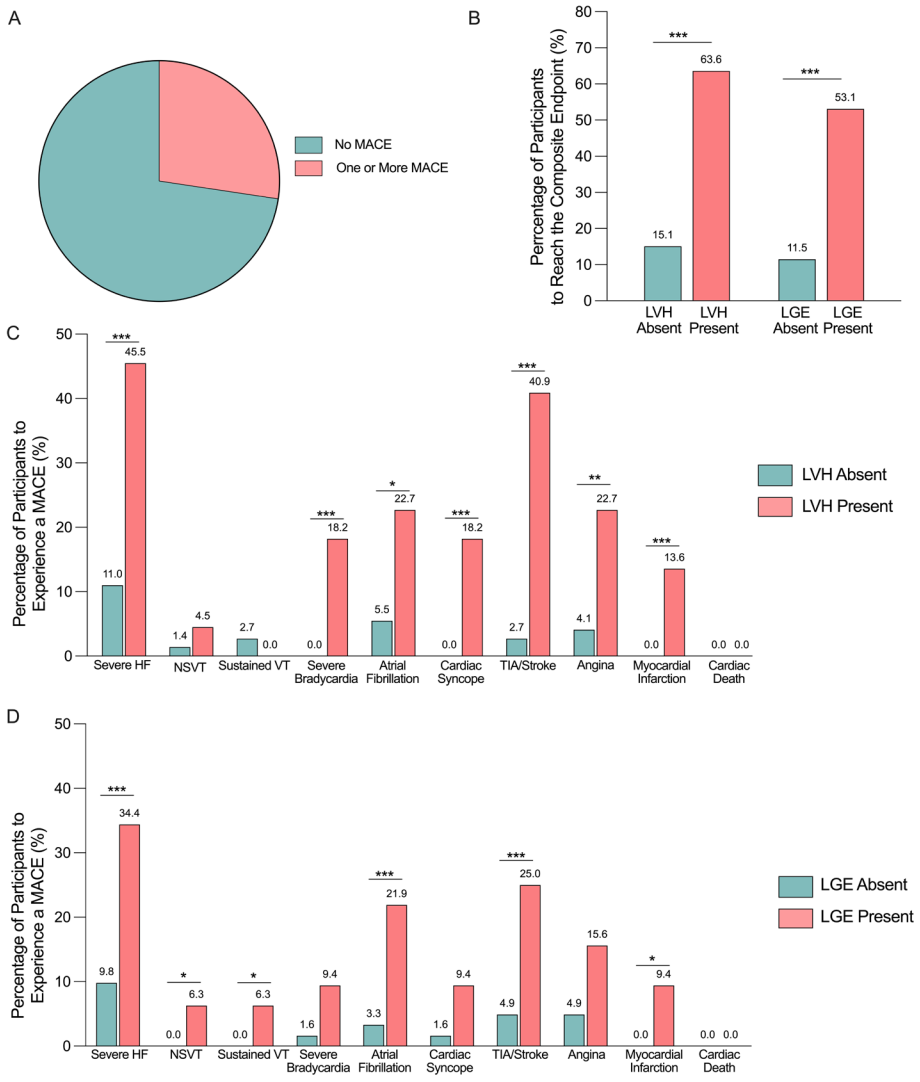

Figure 3.1 Major adverse cardiac events (MACE) and clinical outcomes in Fabry disease patients to experience one or more MACE versus no MACE (A) and stratified between left ventricular hypertrophy (LVH) absent (n=73) versus present (n=22) and late gadolinium enhancement (LGE) absent (n=61) versus present (n=32) groups (B). Furthermore, the

distribution of percentage of patients to experience the various types of MACE in LVH (A) and LGE(B) absent versus present stratified groups.

* p<0.05; ** p<0.01; *** p<0.001.

Health-Related Quality of Life

Health-related quality of life was assessed using the EQ-5D-3L and was obtained for 81 patients. Of these patients, more than 80% indicated no problems with mobility, self-care, and usual activities of living due to their FD, in contrast, the remaining patients indicated moderate to severe issues across these five domains (Figure 3.2A). Moderate or severe pain/discomfort were reported in 37 (45.6%) patients, and 25 (30.9%) patients indicated moderate or severe level of anxiety and depression. There was a higher proportion of patients with one or more MACE with moderate problems (cumulative PROM score ≥ 3), yet none of the patients presented with a severe score (cumulative PROM score ≥ 6) (Figure 3.2B). Likewise, there was a significant difference in quality of life (VAS score ranging from 0-100) between patients no history of MACE (median VAS score, 85 [IQR, 80–90]) compared to patients who had one or more MACE in the past (median VAS score, 70 [IQR, 60–87.5], p=0.003; Figure 3.2C). There was no significant difference in VAS score between LVH absent/present (p=0.08) and LGE absent/present cohorts (p=0.85; Figure 3.3).

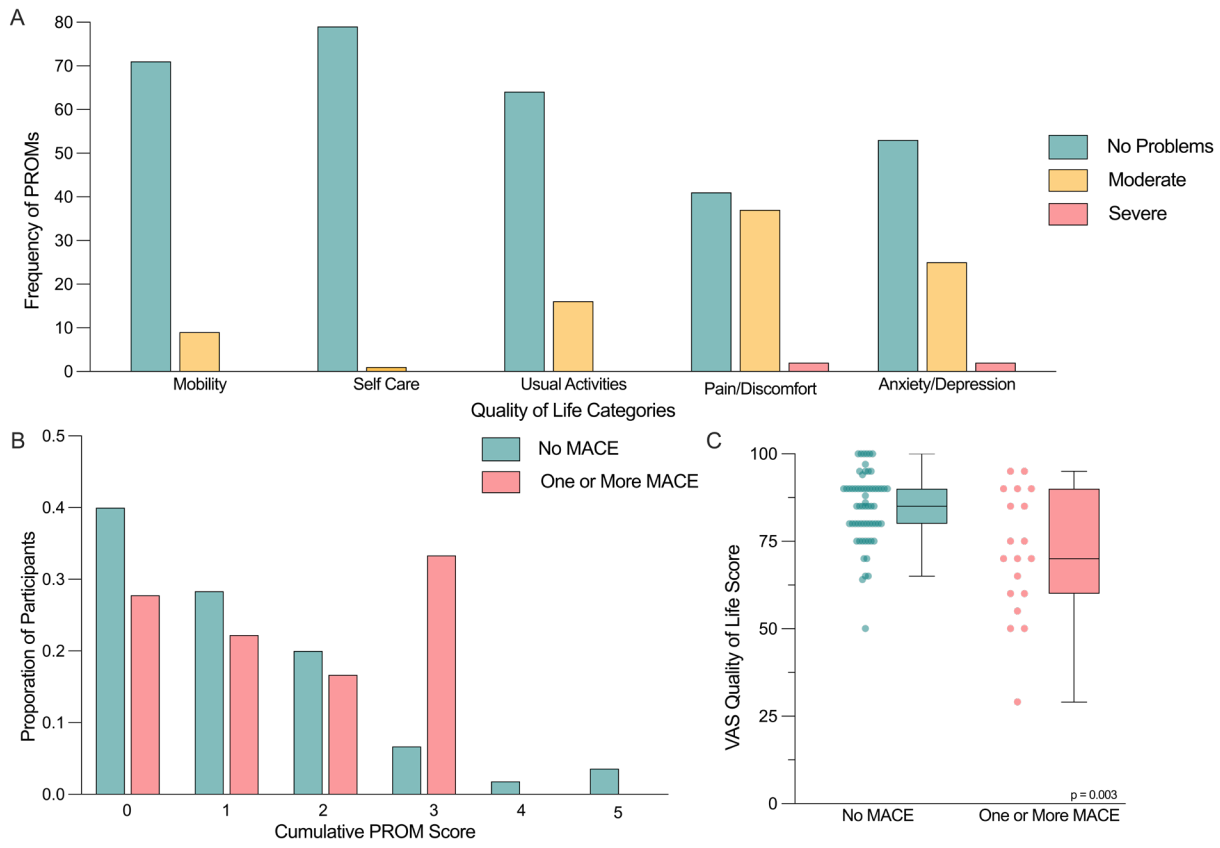

Figure 3.2 Based on the EQ5D-3L questionnaire, the frequency of PROM for mobility, self-care, ability to perform usual activities, pain and discomfort and anxiety and depression were ranked between no, moderate, and severe problem in Fabry disease patients (n=81) (A). The proportion of patients with a cumulative PROM score (out of 15) (B) and VAS quality of life score (p=0.003) (C) were stratified by patients with no MACE (n=62) and one or more MACE (n=19).

PROM, patient-reported outcome measures; MACE, major adverse cardiac events; VAS, visual analog scale.

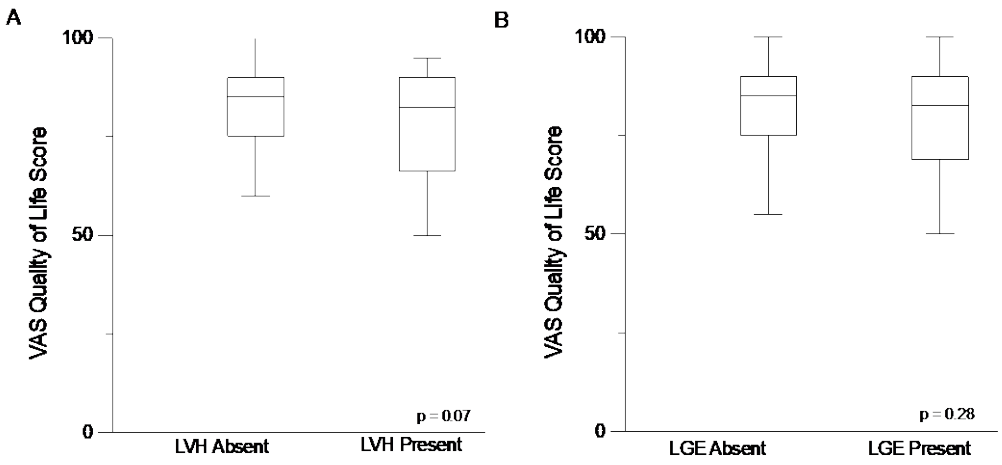

Figure 3.3 Fabry disease patients' (n=81) VAS quality of life scores (out of 100) was stratified based on cardiac magnetic resonance parameters, left ventricular hypertrophy (P=0.08) (A) and late gadolinium enhancement (P=0.85) (B).

VAS, visual analog scale; LVH, left ventricular hypertrophy; LGE, late gadolinium enhancement.

Association between CMR Markers, Clinical Variables, and MACE

In our risk analysis, we assessed the utility of CMR Markers for LVH and LGE (Table 3.3). Patients were classified according to the presence and absence of LVH, LGE, or a composite of LVH or LGE. Our multivariable Cox regression analysis revealed that the presence of LVH (adjusted hazard ratio [aHR], 4.14 [95% CI, 1.78–9.62], p<0.001), LGE (aHR, 4.71 [95% CI, 1.98–11.22], p<0.001), and the combined either LGE or LVH (aHR, 5.90 [95% CI, 2.14–16.32], p<0.001) substantially increased the risk for MACE (Table 3.3). Likewise, the Kaplan-Meier survival probability curve displayed that patients with LVH or LGE had worse event-free survival (p<0.001; Figure 3.4A and 3.4B).

Additionally, dyslipidemia was highlighted as predictive of MACE in the multivariable model for LGE and combined LVH and LGE (aHR, 3.41 [95% CI, 1.32–8.82], p=0.01; Table 3.4).

Patients with cardiovascular risk factors, dyslipidemia, and hypertension, had worse event-free survival (p<0.001; Figure 3.4C and 3.4D).

Table 3.3 Cox regression analysis to assess the prognostic ability of CMR parameters for major adverse cardiac events.

Parameters	Univariable Analysis	
	HR (95% CI)	P value
LVH presence	5.33 (2.46–11.56)	<0.001
LGE presence	5.37 (2.31–12.49)	<0.001
Combined LVH or LGE	7.22 (2.70–19.29)	<0.001
LVMI (5 g/m^2)	1.23 (1.14–1.33)	<0.001
Age (y)	1.04 (1.02–1.07)	0.002
Sex	1.70 (0.78–3.68)	0.18
Classic Phenotype	1.77 (0.53–5.94)	0.36
Smoking History	0.69 (0.29–1.65)	0.40
Hypertension	3.18 (1.40–7.216)	0.006
Dyslipidemia	5.28 (2.28–12.26)	<0.001
Chronic Kidney Disease	3.34 (1.47–7.59)	0.004
Coronary Artery Disease	5.68 (2.21–14.62)	<0.001
ERT	4.00 (1.69–9.44)	0.002
Chaperone Therapy	0.05 (0.00–264280720.00)	0.79

LVH, Left ventricular hypertrophy; LGE, Late gadolinium enhancement; HR, Hazard Ratio; CI, Confidence interval; ERT, Enzyme replacement therapy; ACE, Angiotensinogen converting enzyme; ARB, Angiotensin II receptor blockers.

Table 3.4 Cox regression multivariable analysis to assess the prognostic ability of CMR parameters for major adverse cardiac events.

Parameters	LVH Presence		LGE Presence		Combined LVH or LGE	
	aHR (95% CI)	P value	aHR (95% CI)	P value	aHR (95% CI)	P value
CMR parameter	4.14 (1.78–9.62)	<0.001	4.71 (1.98–11.22)	<0.001	5.90 (2.14–16.32)	<0.001
Age (y)	1.03 (1.00–1.06)	0.10	1.01 (0.98–1.05)	0.39	1.02 (0.99–1.05)	0.19
Dyslipidemia	2.06 (0.78–5.45)	0.15	3.41 (1.32–8.82)	0.01	2.62 (1.03–6.69)	0.04
Hypertension	1.68 (0.67–4.21)	0.27	1.58 (0.57–4.36)	0.38	1.50 (0.57–3.93)	0.41

Data are represented as hazard ratio (95% confidence interval). P values determined with Cox regression analysis for LVH presence, LGE presence, and combined LVH or LGE, respectively. LVH, Left ventricular hypertrophy; LGE, Late gadolinium enhancement; HR, Hazard Ratio; CI, Confidence interval; CMR, Cardiac magnetic resonance.

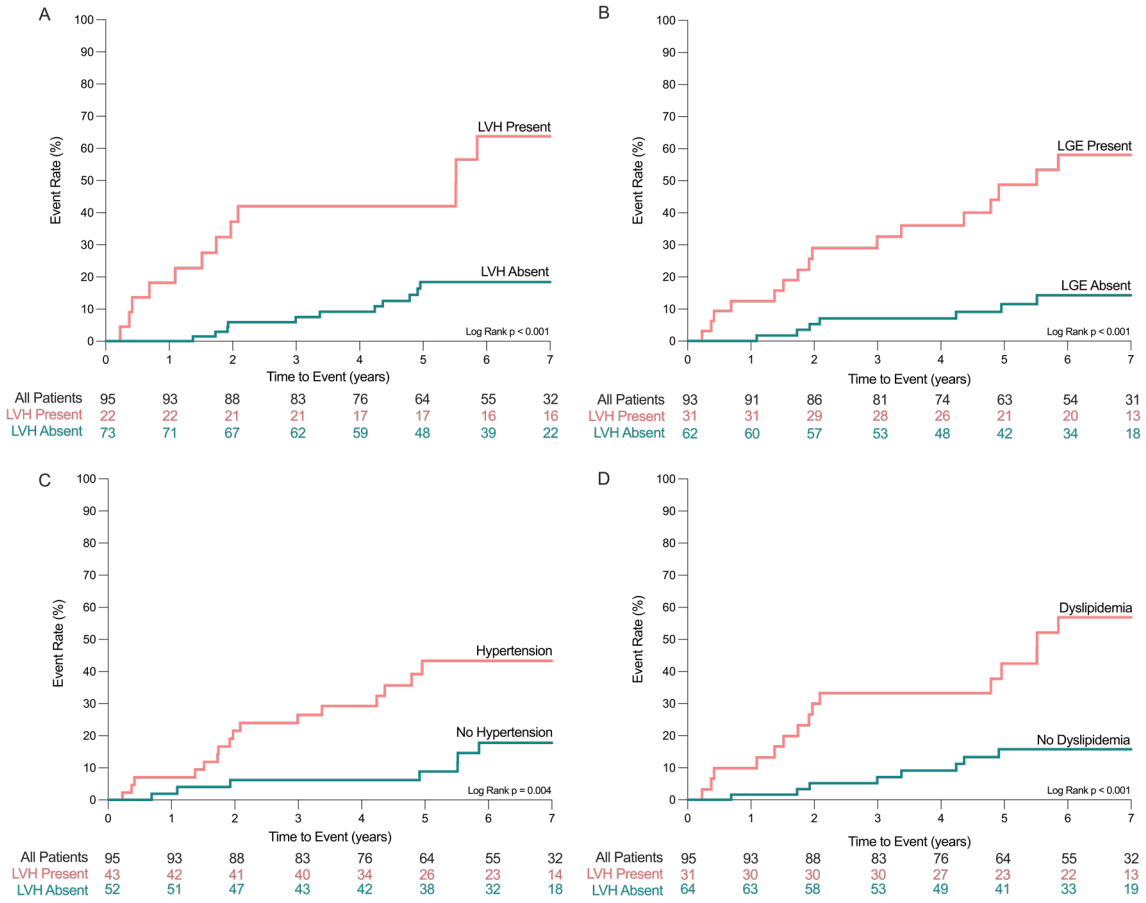

Figure 3.3 Kaplan-Meier analysis of left ventricular hypertrophy (A), late gadolinium enhancement presence (B), hypertension (C), and dyslipidemia (D) to assess the prognostic utility of cardiac magnetic resonance and cardiovascular risk factors for major adverse cardiac events.

LGE, late gadolinium enhancement; LVH, left ventricular hypertrophy.

Clinical Management of Fabry Patients

Clinical outcomes for comorbidities showed no significant difference in systolic blood pressure (sBP) and low-density lipoprotein-cholesterol (LDL-C) levels between baseline and follow-up (Figure 3.5A and 3.5B). There was a significant difference in estimated glomerular filtration rate (eGFR) between baseline and follow-up with the difference in medians is -4.5 mL/min/1.73m^2 (p=0.004; Figure 3.5C). In addition, we assessed the use of FD-specific therapies and determined no significant difference between the age of ERT start in the patients with or without MACE (p=0.08; Figure 3.5D). Notably, there was a more prolonged treatment duration in the cohort with one or more MACE (p=0.005; Figure 3.5E).

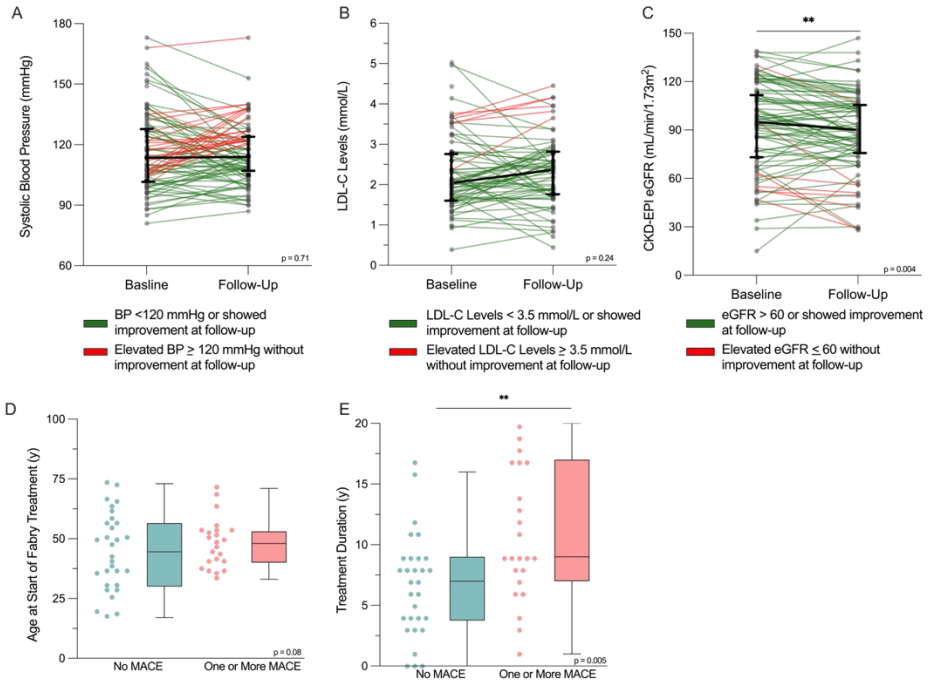

Figure 3.4 Evaluation of clinical management strategies including comorbidity management were assessed by tracking changes between baseline and study end date follow-up for systolic blood pressure (A), LDL-C levels (B), and eGFR (p=0.004) (C). In addition, age at start of Fabry-specific therapies and length of treatment duration (p=0.004) were compared between

no MACE and one or more MACE groups.

BP, blood pressure; LDL-C, low-density lipoprotein-cholesterol; CKD-EPI, chronic kidney disease-epidemiology collaboration; eGFR, estimated glomerular filtration rate; MACE, major adverse cardiac events. ** $p<0.01$.

3.4 Discussion

Our study is the first clinical outcome study to integrate a comprehensive assessment of overall patient health in FD by assessing patient-reported outcomes, comorbidity management, and cardiac function. Life expectancy in FD is markedly lower compared to the general population, with death typically occurring in the fifth decade of life; however, we observed a significant improvement in prognosis and reduced cardiac-related mortality from 6.3% in FD patients.[1, 64] Furthermore, markers of disease severity, including LVH were lower, (LVMi: 65.6 g/m^2 [IQR 52.1–76.3 g/m^2]) compared to several other FD cohorts (LVMi: 78 g/m^2, 82.0 g/m^2, and 89.0 g/m^2).[71-73] In addition, overall health-related quality of life in FD remained high, with patients predominantly affected by pain/discomfort and anxiety/depression. Furthermore, patients with a history of MACE were significantly associated with reduced quality of life, in which self-reported ratings were 15% lower than those without a history of MACE.[74] Finally, patients with cardiovascular risk factors, including dyslipidemia and hypertension, had worse event-free survival and higher risk of MACE, while stable well-controlled blood pressure, lipids, and eGFR did not. Our findings highlight that adhering to guideline-directed therapy, detection of advanced phenotypes with CMR, early ERT initiation, and tight comorbidity management are critical in promoting event-free survival, improved psychosocial health, and reduced mortality.

Several factors may contribute to our cohort's low prevalence of LVH, MACE, and zero cardiac-related deaths.[71, 73, 75] Firstly, ERT regimens were standard in our cohort and are known to

promote reverse cardiac remodeling in FD leading to attenuation or complete cessation of LVM elevation.[64, 66] In our cohort, intervention with cardiac medications and Fabry-specific treatments are commonplace as the number of patients on ERT nearly doubles between baseline and follow-up. Secondly, early genetic screening studies in patients with unexplained LVH and cascade screening in extended families for those diagnosed with FD allowed for earlier medical intervention leading to more optimal outcomes.[9, 76] Thirdly, patients had an annual follow-up in Alberta, with regular CMR, 12-lead ECG, and Holter monitoring, allowing for better monitoring of disease progression. Lastly, managing cardiovascular risk factors in a multidisciplinary setting undeniably attenuates comorbid exacerbation of heart disease.[61, 77-79]

There is an unequivocal association between MACE, poor cardiac outcomes, LVH and LGE.[8, 71, 80-83] Both hypertrophy and myocardial fibrosis were strongly associated with the MACE groups. In our examination of longitudinal trajectories, 53.1% of LGE present patients and 63.6% of hypertrophic patients suffered one or more MACE. The most reported MACE were severe HF, TIA/stroke, atrial fibrillation, and angina, and despite the low burden of hypertrophy (23.2%) in our cohort, LVH increased the risk of MACE by 4.1 times. LVH is associated with the progressive accumulation of glycosphingolipids in cardiomyocytes ultimately leading to the death of engorged myocardial cells.[77] Prehypertrophic patients predominantly experienced heart failure, atrial fibrillation, and angina, while hypertrophic patients experienced a higher proportion of heart failure, cerebrovascular events, conduction abnormalities, angina, and myocardial infarction. These findings were in keeping with other cohorts where cerebrovascular events were markedly higher in proportion in our hypertrophic patients.[84] Likewise, the efficacy of FD-specific therapies depends on disease severity level and age at the start of therapy.[66, 85] Unfortunately, it is likely that ERT only being established in 2001, and our cohort's advancing age, some patients with LVH and

MACE had more severe cardiac involvement at the initiation of ERT therapy. Hence, treatment inefficacy and baseline risk of MACE are likely elevated for certain patients treated later in their disease course.[12]

Late gadolinium enhancement is an established marker for chronic inflammation and cardiac fibrosis in FD and increased the risk of MACE by 4.7 times in our cohort.[8, 86] Heterozygous females are vulnerable to myocardial fibrosis, and LGE presence was evenly distributed across sexes, which emphasizes that LGE presence has important prognostic utility in this population.[61, 71] The LGE presence cohort experienced a higher proportion of heart failure, conduction abnormalities, cerebrovascular events, and myocardial infarction. Accumulating unmetabolized glycosphingolipids activates pathological inflammatory and fibrosis pathways leading to apoptosis, oxidative stress, and endothelial dysfunction.[83, 87] Inflammation, tissue injury, and apoptosis of myocardial cells contribute to the pathological aetiology of conduction system abnormalities.[64] Myocardial fibrosis is irreversible and unmodifiable by ERT, which is likely why the prevalence of LGE presence is comparable to other cohorts.[71]

Cardiovascular risk factors have a pervasive impact on patient outcomes and treatments, including renin-angiotensin system (RAS) antagonism and statins, has been evidenced to attenuate adverse atrial remodeling and LVH.[81, 88-90] Interestingly, despite our cohort having variable frequencies of comorbidities, we found that there was an equal distribution in medications to help manage these comorbid conditions. The strict control of hypertension, lipid levels, and kidney function through renin-angiotensin system (RAS) antagonism and statins are crucial mainstay strategies utilized in our patients to prevent and reduce the burden from comorbidities.[77] Overall, systolic blood pressure and LDL-C levels remained stable, and eGFR showed an age-related decline. Importantly, hypertension is a putative major driver of hypertrophy in sarcomere-negative

hypertrophic cardiomyopathies and is an important target to help reduce cardiovascular events in FD.[81, 91] Furthermore, microvascular dysfunction is a hallmark of early pathophysiology in FD and leads to low-density lipoprotein-cholesterol (LDL-C) uptake in the endothelial wall; henceforth, dyslipidemia may further exacerbate the pathological effects of microvascular dysfunction.[79, 87, 92, 93] Fortunately, statin therapy was often utilized in our dyslipidemia patients. These patients are thought to confer additional benefits from this therapy due to its suspected anti-thromboembolic and anti-inflammatory effects.[94] However, this appears less effective in MACE reduction in those with pre-existing cardiac fibrosis as those with dyslipidemia in the presence of LGE 3.4 times elevated risk of MACE, which emphasizes the necessity to treat and monitor lipid profile early in disease course.[95] Future research aimed to develop novel management strategies, should target patients with established LGE and dyslipidemia to improve outcomes in these high-risk patients.

Our study is not without limitations. The relatively rare prevalence of FD limits sample size and the number of observable MACE in our present cohort, which leaves our analysis vulnerable to survivor bias. In order to account for this limitation, we included a wide range of events that have been used in previous outcome studies and had an extended median follow-up comparatively.[64, 71, 80, 92]

3.5 Conclusion

Our study demonstrates the value of early routinely reported LVH and LGE from CMR predicting MACE in patients with FD, especially those with dyslipidemia. Close monitoring and management of hypertension, dyslipidemia, reduced kidney function, and early intervention with FD-specific therapies may have contributed to improved clinical outcomes in our cohort with an overall reduced prevalence of LVH, low mortality, and fewer incidents of MACE compared to prior studies.

Chapter 4. Cardiac manifestations and clinical management of X-linked Emery-Dreifuss muscular dystrophy: a case series

4.1 Introduction

X-linked recessive (XLR) Emery-Dreifuss muscular dystrophy (EDMD) is the most common EDMD subtype caused by reduced or loss-of-function in the nuclear membrane protein emerin.[96-98] Emerin is associated with gene regulation, stabilization of the nuclear membrane, and plays a role in intercalated disc function in cardiomyocytes.[16, 99] The classic phenotypic EDMD presentation is characterized with early joint contractures, slow progressive muscle wasting and weakness, and cardiac conduction abnormalities.[97] Skeletal muscle involvement typically precedes cardiac involvement.[100] Due to the slow progression of the disease, symptoms are often undetected, allowing for the progression of serious cardiac abnormalities and increased risk of sudden cardiac risk (SCD).[99] There are currently no disease-specific therapies for EDMD therefore management and therapeutic strategies are patient specific. Heart disease, clinical management, and outcomes in *EMD*-associated XLR-EDMD is poorly defined compared to *LMNA*-associated autosomal dominant (AD) EDMD.[101] This case series assesses the disease progression, therapeutic management, and outcomes of four patients with *EMD*-associated XLR-EDMD in a multidisciplinary setting.[102]

4.2 Patient 1

A thirty-seven-year-old male XLR-EDMD patient was referred to the NMMD clinic (Figure 4.1A, Table 4.1). The patient exhibited symptoms of mild joint contractures and generalized weakness

that required the use of a cane to ambulate independently (Table 4.2). From a cardiac perspective, the patient had a history of advanced AV block and permanent atrial fibrillation (AF), for which he had a pacemaker implanted 10 years prior to enrollment. In addition to the patient's pacemaker, he was taking bisoprolol 7.5 mg q.d. for rate control of the underlying permanent AF and enteric-coated acetylsalicylic acid (ECASA) for thromboembolism prophylaxis prevention.

Physical examination demonstrated a regular heart rate of 70 bpm and blood pressure of 102/68 mmHg (Table 4.2). Baseline B-type natriuretic peptide (BNP) and troponin I (TnI), were normal at 23.0 pg/mL and 0.1 ng/mL, respectively.[103] Creatine kinase (CK) was mildly elevated at 345 U/L indicating active muscle damage and inflammation.

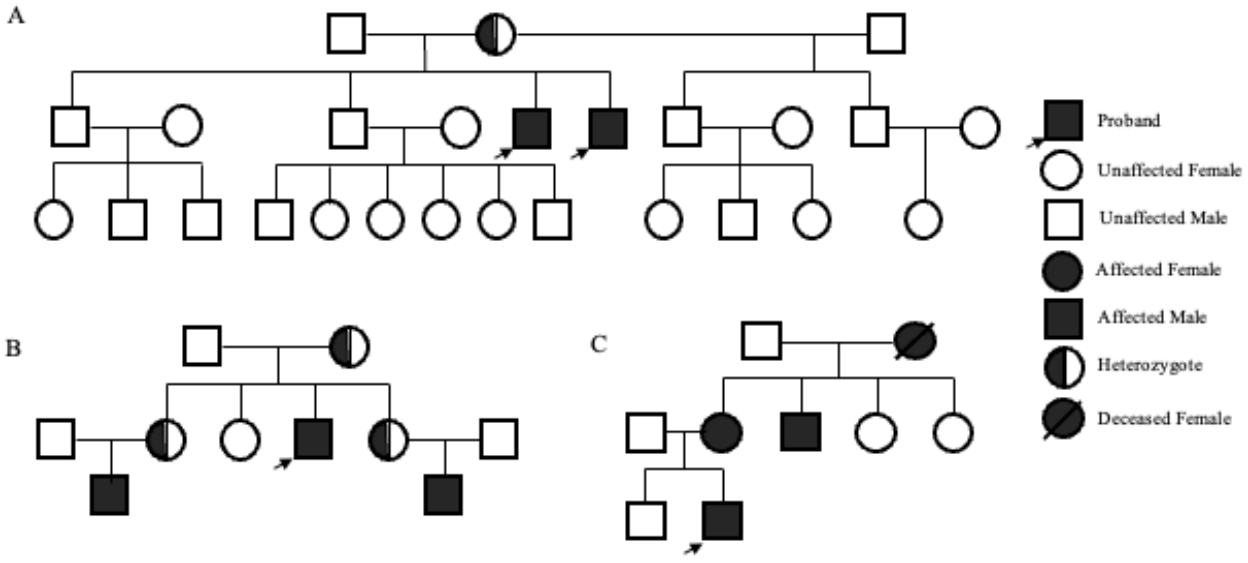

Figure 4.1 Pedigree chart depicting family history of EMD gene-associated in an X-linked recessive mode of inheritance in (a) proband 1 and 2, (b) proband 3, and (c) proband 4. Proband 3 refused to share family history regarding his children.

The 12-lead electrocardiogram (ECG) recorded AF, which was ventricularly paced by a single-chamber pacemaker (Figure 4.2). Electrocardiogram parameters included a prolonged QRS

duration of 148 ms and a QTc interval of 452 ms (Table 4.2). At 4-years and 6-years follow-up, ECG parameters performed were relatively unchanged.

Baseline TTE data showed normal cardiac structure, with no signs of dilation or hypertrophy with left ventricular mass index (LVMI) at 57 g/m^2, low-normal function with a left ventricular (LV) ejection fraction (EF) at 55%, left atrial volume index (LAVI) of 12 mL/m^2, and no signs of valvular disease (Table 4.2). Echocardiogram was performed at 4-year and 8-year follow-up demonstrating stable cardiac function with minimal changes to the cardiac structure (Figure 4.3B).

Table 4.1 Timeline of Clinical Progression in Four Patients with Emery-Dreifuss Muscular Dystrophy.

	Patient 1
23 yr	Genetically confirmed for EDMD
27 yr	Single chamber pacemaker implantation
36 yr	NMMD Clinic Enrollment
36 yr – 44 yr	Advanced AV block and permanent atrial fibrillation. Prolonged QRS duration and QTc interval on 12-lead electrocardiogram.
	Patient 2
20 yr	Genetically confirmed for EDMD
31 yr	Dual chamber pacemaker implantation
39 yr	NMMD Clinic Enrollment
31 yr – 40 yr	Nodal dysfunction treated with pacemaker. Marginal thinning of the myocardium and systolic dysfunction. Managed dyslipidemia with cholesterol-lowering drugs.
	Patient 3
20 yr	Genetically confirmed for EDMD
21 yr	Single chamber pacemaker implantation
50 yr	NMMD Clinic Enrollment
43 yr – 50 yr	Normal left ventricular dimensions with increased ventricular mass. History with permanent atrial fibrillation. History of sleep disordered breathing, and mild pulmonary hypertension.
50 yr	Elevated blood pressure, dilated ventricles and elevated ventricular mass is treated with increased dose of perindopril and discontinued modafinil.
51 yr – 55 yr	Improved ventricular dimensions and mass. Advancing atrial myopathy with increased QRS duration is treated with switch to apixaban.
	Patient 4
6 yr	Genetically confirmed for EDMD
14 yr	Single chamber pacemaker insertion and intraatrial reentrant tachycardia (IART) ablation for AV block and atrial tachycardia.
18 yr	NMMD Clinic Enrollment
18 yr	IART ablation and subcutaneous implantable cardioverter-defibrillator. Started on perindopril.
14 yr - 20 yr	Normal ventricular structure and function.

Table 4.2 Clinical characteristics of patients with Emry-Dreifuss musclular dystrophy.

Patient	Age(y) / Sex / BMI (kg/m^2)	Neuromuscular Symptoms	Cardiovascular Risk Factors	Cardiac Abnormalities	HR (bpm) / sBP (mmHg) / dBP (mmHg)	Baseline ECG Findings (ms)	Baseline TTE Findings	Baseline Medications & Dose (mg)	Cardiac Intervention & Devices	Chamber & Percent Pacing
1	36 / M / 13.6	Elbow and wrist, contractures, Generalized weakness (cane)	None	Permanent AF	64 / 102 / 68	QRS: 148 QTc: 452, AF, ventricular paced	LVEF: 55% LVMI: 57 g/m^2 LVIDd: 40 mm LVIDs: 29 mm LAVI: 12 mL/m^2	Bisoprolol 7.5 mg ECASA 81 mg	Baseline: Single chamber pacemaker	RV: 99.5%
2	39 / M / 25.7	Elbow contractures, Generalized weakness	SDOB (CPAP), dyslipidemia	SA & AV node dysfunction	57 / 159 / 93	PR: 140 QRS: 102 QTc: 452, Multiple PACs	LVEF: 62% LVMI: 80 g/m^2 LVIDd: 46 mm LVIDs: 28 mm LAVI: 23 mL/m^2	Rosuvastatin 10 mg	Baseline: Dual chamber pacemaker	RA: 94.5% RV: 2.0%
3	50 / M / 28.1	Hand weakness Wrist, contractures	Smoker, SDOB (CPAP), hypertension	Permanent AF, severe LVH, Mild RV dilation, Mild mitral & aortic valve regurgitation	55 / 145 / 90	QRS: 102 QTc: 417 AF	LVEF: 50% LVMI: 124 g/m^2 LVIDd: 55 mm LVIDs: 31 mm LAVI: 39 mL/m^2	Perindopril 2 mg, ECASA 81 mg, Modafinil	Baseline: Single chamber pacemaker Follow-up: Perindopril 4 mg, discontinued Modafinil, Switched ECASA to Apixaban.	RV: 48.5%
4	18 / M / 28.1	Scoliosis, leg weakness	None	SA node dysfunction VT, Atrial flutter, Mild RV dilation	51 / 144 / 43	QRS: 94 QTc: 443, Junctional escape rhythm	LVEF: 64% LVMI: 86 g/m^2 LVIDd: 52 mm LVIDs: 29 mm LAVI: 24 mL/m^2	ECASA 81 mg	Baseline: IART ablation, Single chamber pacemaker, Follow-up: Perindopril 2 mg Implanted Subcutaneous ICD	RV: 4.6%

AF, atrial fibrillation; ASA, acetylsalicylic acid; AV, atrioventricular; BSA, body surface area; CPAP, continuous positive airway pressure; HR, heart rate; dBP, diastolic blood pressure; ECG, 12-lead electrocardiogram; sBP, systolic blood pressure; LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVIDd, left ventricle internal diameter end diastole; LVIDs, left ventricle internal diameter end systole; LVMI, left ventricular mass index; MV, mitral valve; PAC, premature atrial contraction; RV, right ventricular; SA, sinoatrial; SDOB, sleep disordered breathing; TTE, transthoracic echocardiogram; IART, intraatrial reentrant tachycardia and ICD=implantable cardioverter defibrillator.

Over the course of 8 years, left ventricular dimensions remained normal with a marginal increase in left ventricular end diastole diameter (LVIDd) from 39 mm to 45 mm and left ventricular end systole diameter (LVIDs) from 29 mm to 30 mm. There was a marginal increase in LVMI at 49 g/m^2 to 66 g/m^2. Qualitative assessment of atria size remained normal. In addition, LVEF remained stable from 55–60% and LAVI increased from 12 mL/m^2 to 29 mL/m^2 (Figure 4.3B). Overall, the patient is predominantly affected by severe conduction deficits that are monitored through periodic assessment at the NMMD clinic and managed with the use of device therapy and medications.

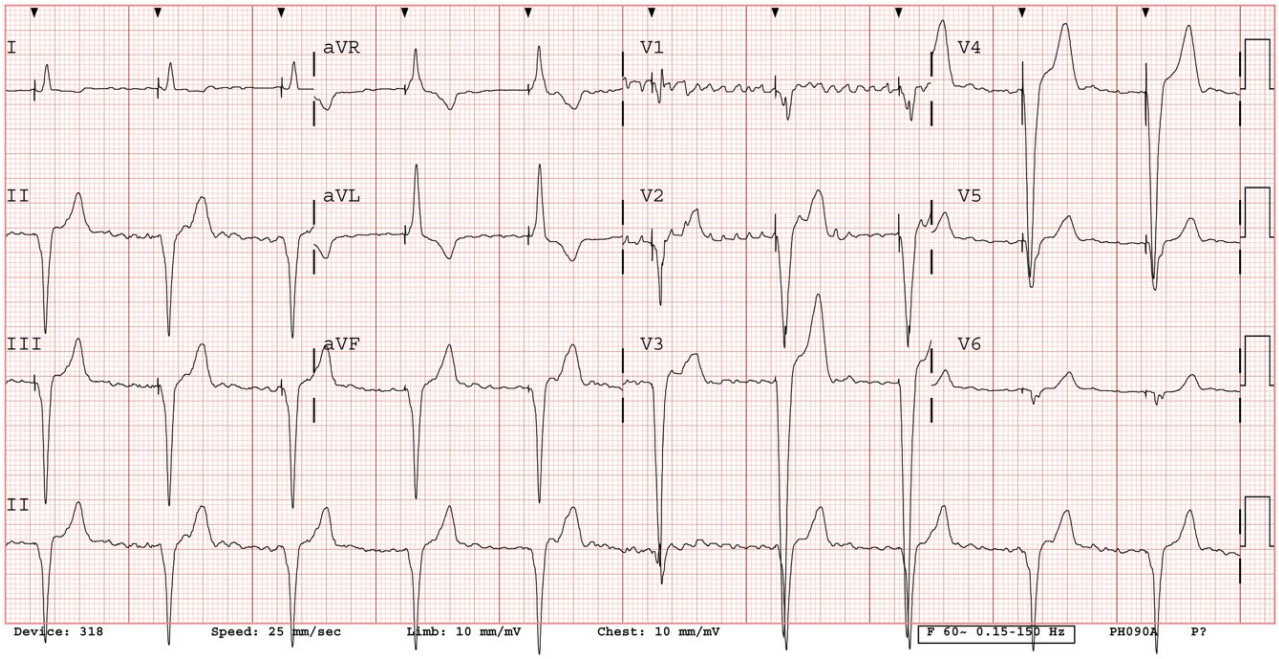

Figure 4.2 Electrocardiograms from patient 1 depicting atrial fibrillation with ventricular paced rhythm at 60 bpm.

Conduction Abnormalities in Cardiac Defibrillator Insertion

Maintained Ejection Fraction, Left Ventricle Mass

- **Holter Monitor**

rhythm and ECG parameters

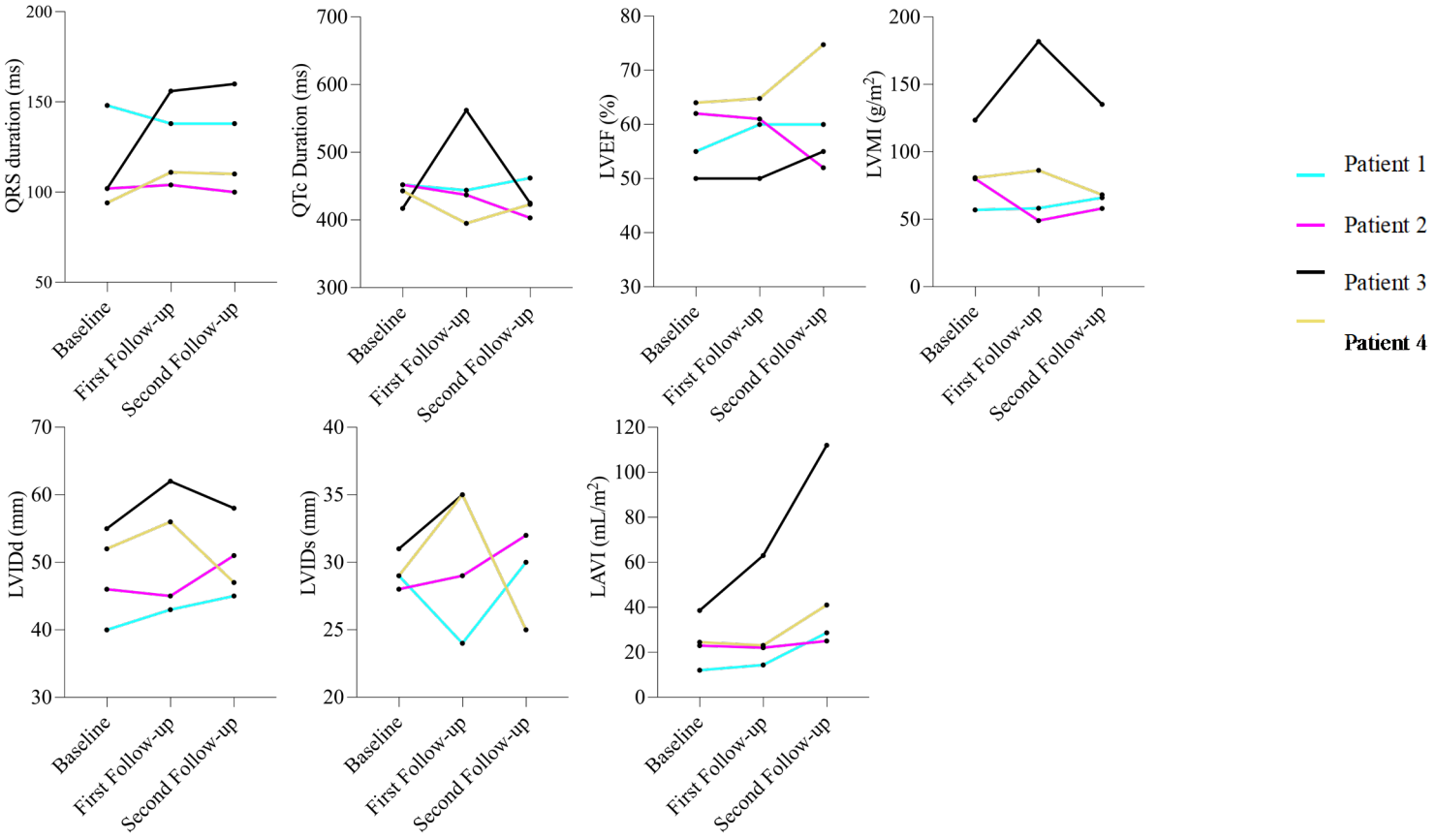

Figure 4.3 (a) Cardiac monitoring and management strategies for X-linked EDMD patients (b) illustrated by 12-lead electrocardiogram and echocardiogram parameters in patients 1-4 to track disease progression from the baseline to median 3-year first follow-up and 5-year second follow-up.

4.3 Patient 2

A thirty-nine-year-old male XLR-EDMD patient, the brother of Patient 1, was referred to the NMMD clinic presented with advanced symptoms including elbow contractures and weak ankles requiring a cane for balance and ambulation (Figure 4.1A). In terms of comorbidities, the patient has a history of sleep disordered breathing (SDOB) for which continuous positive airway pressure (CPAP) was prescribed (but not used on a regular basis due to patient discomfort), and dyslipidemia that was treated with rosuvastatin 5 mg q.d. and maintained his low-density lipoprotein at 3.0 mmol/L (Table 4.2). From a cardiac perspective, the patient had a history of heart failure, syncope, and sinoatrial (SA) and AV nodal dysfunction, for which a dual chamber pacemaker was inserted 8 years prior to clinic enrollment (a generator change was performed seventeen years after insertion) (Table 4.2).

Upon physical exam, the patient was bradycardic with a heart rate of 57 bpm and hypertensive with a blood pressure of 159/93 mmHg; however, no prior history of hypertension was noted, and no additional cardiovascular signs and symptoms were reported (Table 4.1). Creatine kinase levels were elevated at 434 U/L.

Baseline ECG study showed sinus rhythm with a QRS duration of 102 ms and a prolonged QTc interval of 452 ms (Table 4.2). An 8-year follow-up ECG showed the QRS duration remained normal and unchanged and QTc interval incrementally decreased to 437 ms to 403 ms (Figure 4.3B).

Baseline TTE data showed normal cardiac structure, with no evidence of dilation (LVIDd = 46 mm; LVIDs = 28 mm) or hypertrophy (LVMI = 80 g/m^2), normal systolic function (LVEF = 62%), and no signs of valvular heart disease (Table 4.2). Follow-up TTE 2-years and 3-years later demonstrated LV dimensions remained normal (LVIDd = 46 mm and 51 mm; LVIDs = 29 mm and 32 mm) and normal to borderline normal systolic function (LVEF = 61% and 52%) with no signs of hypertrophy (LVMI = 49 g/m^2 and 58 g/m^2) (Figure 4.3B). From baseline, LV mass index in this patient reduced from 80 g/m^2 to 58 g/m^2 (Figure 4.3B). Left atrial volume (LAVI = 22 mL/m^2 and 25 mL/m^2) remained stable and normal (Figure 4.3B). Qualitative assessment of atrial size showed the right atria was initially mild to moderately enlarged that recovered to normal size and the left atria size remained normal and stable. Overall, ECG and TTE parameters remained within the normal range; however, there was marginal thinning of the myocardium and decreased systolic function.

4.4 Patient 3

A fifty-year-old male XLR-EDMD patient was referred to the NMMD clinic and presented with hand weakness and wrist contractures (Figure 4.1B). The patient had a history of smoking and SDOB for which CPAP was used with good compliance and was taking modafinil for daytime sleepiness. He has a history of mild pulmonary hypertension with non-invasive right ventricular systolic pulmonary arterial pressure of 35–40 mmHg. The patient had AF that he received a single chamber pacemaker twenty-nine years prior, which progressed to permanent AF (Table 4.2). Due to the uncertain risk of thromboembolic events from permanent AF, patient was prescribed ECASA 81 mg q.d.

On examination, he was hypertensive and bradycardic with a heart rate of 50 bpm and blood pressure of 145/90 mmHg. Plasma BNP and TnI were within normal ranges, and CK was

elevated at 1695 U/L. Baseline Holter monitor confirmed AF with AV conducted beats and regular junctional tachyarrhythmias.

Baseline ECG study showed AF and ventricular-paced complexes with QRS duration of 102 ms, and QTc interval of 417 ms. At 6-year follow-up, his blood pressure was elevated to 172/94 mmHg, therefore his perindopril was uptitrated from 2 mg q.d. to 4 mg q.d. and modafinil was discontinued. After medication adjustments, follow-up ECG 1-year and 3-years later showed QRS duration increased from 156 ms to 160 ms over 4-year time span (Figure 4.3B). Given his advancing age and progression of atrial myopathy, patient was switched from ECASA to using a direct oral anticoagulant (apixaban 5 mg twice daily).

Baseline TTE demonstrated mild aortic dilation, moderate mitral and tricuspid regurgitation, mild right ventricular (RV) dilation (RVd basal = 5.4 cm) and severely dilated left and right atria, which did not change during follow-up assessments. Patient exhibited mildly reduced LVEF of 51% as well as normal LV dimensions (LVIDd = 55 mm) and elevated ventricular mass (LVMI = 124 g/m^2) (Table 4.1). Echocardiogram showed significant improvements before and after medication adjustments with reduced LV dimensions (LVIDd = 62 mm and 58 mm; LVIDs = 35 mm and 25 mm), and improved hypertrophy (LVMI = 182 g/m^2 and 135 g/m^2) (Figure 4.3B). Systolic function remained steady ranging from 50–55% over the 4-year-period (Figure 4.3B). In addition, LAVI increased from 63 mL/m^2 to 112 mL/m^2 (Figure 4.3B). Overall, LV structure and function improved over the 4-year-period following discontinuation of modafinil and uptitration of perindopril.

4.5 Patient 4

An eighteen-year-old male XLR-EDMD patient referred to the NMMD Clinic presenting with leg and ankle weakness, scoliosis, and a history of falls (Figure 4.1C). The patient experienced

progressive cardiac involvement with hypertension, recurrent atrial tachycardia, SA and AV node dysfunction, and history of non-sustained VT.

Upon enrollment, the patient was bradycardic with a heart rate of 51 bpm and elevated blood pressure of 144/43 mmHg, while prescribed ECASA 81 mg for thromboembolism prophylaxis prevention due to possible increased risk related to atrial abnormalities including atrial standstill.

Pre-device ECG showed a junctional escape rhythm with normal QRS duration of 94 ms, and a prolonged QTc interval of 443 ms (Figure 4.4). The patient received a single chamber pacemaker for AV block. Follow-up monitoring revealed episodes of atrial tachycardia requiring intraatrial reentrant tachycardia (*IART*) ablation. Four years later, a subcutaneous ICD for primary prevention of SCD was inserted due to the potential risk of developing VT given several prior episodes of non-sustain VT. Alternatively, the patient could have been upgraded to a transvenous ICD system. In addition to device therapy, the patient was started on perindopril 2 mg.

Baseline TTE showed normal LV dimensions (LVIDd = 52 mm; LVIDs = 29mm) and normal systolic function at 64%. Patient exhibited mild mitral, tricuspid, and pulmonary valve regurgitation, which progressed to moderate tricuspid regurgitation. Follow-up TTE was performed 2-years and 6-years following pacemaker implantation showed a moderately enlarged right atrium and mildly enlarged left atrium (Table 4.2). Ventricular dimension remained stable (LVIDd = 56 mm to 47 mm; LVIDs = 35 mm to 25 mm), and systolic function remained preserved within normal limits (LVEF = 60–70%) (Figure 4.3B). Overall, LV structure and function remained stable, but the main concern was recurrent atrial tachycardia and nodal dysfunction.

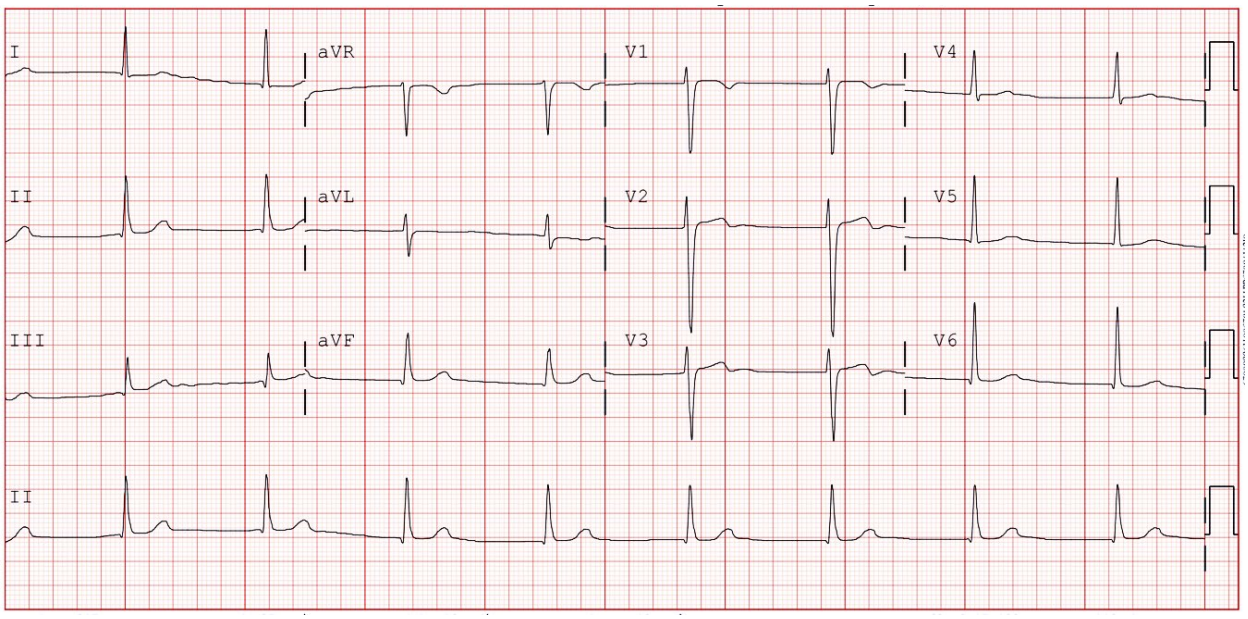

Figure 4.4 Electrocardiograms from patient 4 depicting junction escape rhythm at the rate of 50 bpm with normal axis and no hypertrophy.

4.6 Discussion

We report four cases of XLR-EDMD and a longitudinal assessment of their clinical profile, cardiac outcomes and the therapeutic strategies utilized in clinical management. We discuss the variation in cardiac involvement and evolution of cardiac monitoring parameters over time. Clinical cardiac monitoring for EDMD patients consists of ECG, Holter monitoring, laboratory markers and TTE to monitor conduction abnormalities, arrhythmias, and changes in heart structure and function (Figure 4.3A).[104, 105]

Conduction abnormalities and arrhythmias are the predominant cardiac manifestation in EDMD, presenting as bradycardia, prolonged PR interval, or reduced P wave amplitude on ECG. The incidence of conduction abnormalities and arrhythmias increase with age and include atrioventricular (AV) conduction delays, and atrial and ventricular arrythmias.[106] In XLR-EDMD patients, age of onset for AVB is earlier and there is a high occurrence of AF/atrial flutter compared

to AD-EDMD.[100, 101] In addition, atrial standstill is the primary cause of cardiac death in XLR-EDMD that could be averted with pacemaker implantation.[100, 107] Two patients developed permanent AF, for whom underwent device intervention in the third decade of their life. The other two patients exhibit nodal dysfunction, for whom underwent device intervention in the second decade of their life. All the patients received pacemaker therapy at the first indications of bradycardia, sinus node dysfunction, or as preventative measure. Indeed, due to the prevalence of atrial standstill in EDMD, European Society of Cardiology (ESC) guidelines recommend pacing device intervention at first indication of conduction disturbances or bradyarrhythmias, or before the age of thirty.[108]

Sudden cardiac death and ventricular arrhythmias are associated with AD-EDMD, thus early ICD intervention should be considered along with pacemaker implantation.[101] Despite the rarity of VT in XLR-EDMD, patient 4 had several episodes of non-sustain VT, which emphasizes the importance of consistent follow-up with Holter and cardiac device monitoring. Subsequently, this patient received a subcutaneous ICD as primary prevention of SCD and VT. Cardiac magnetic resonance studies suggest a relationship between remodeling in areas associated with the conduction system to atrial conduction abnormalities and risk of SCD.[109]

Our study is one of few to describe successful atrial ablation procedure in EDMD patients. Patient 4 was diagnosed with recurrent atrial tachycardia detected on a routine ECG and Holter study (Figure 4.3A). Previous case studies have described success with atrial ablation procedure in pediatric and young adult EDMD patients, in which patients present with various types of supraventricular arrhythmias.[110, 111] Butt *et al.* demonstrated a successful approach to atrial ablation using 3D mapping on a twenty-one-year-old male EDMD patient presenting with

sustained VT and atrial flutter.[111] Unsuccessful ablation have been linked to adverse outcomes including systolic dysfunction, embolic stroke, and heart transplant in this cohort.[110, 112, 113]

There is a high prevalence of disabling embolic stroke in EDMD patients untreated with anticoagulation or aspirin treatment.[19, 110, 114] Current guidelines for heart failure cohorts have not been validated in EDMD management nor are they tailored to patients with rare diseases. The CHADSVasc score for AF stroke risk has not been validated in the EDMD population and the use of anticoagulation and antiplatelet therapy in EDMD patients is controversial.[114] Our approach to therapeutics was based on atrial abnormalities and a potential increased risk of thromboembolic events and strokes; we used low-dose ECASA as a preventative measure for patients 1, 3, and 4, as per clinician and patient preference although not evidence-based. In addition, anticoagulation therapy may be preferred treatment option depending on age and severity of atrial myopathy as seen in patient 3. The ideal approach to thromboprophylaxis strategy has not been established, thus further research is needed to assess prophylactic efficacy of anticoagulation therapy.[114]

Muscle weakness and upper limb contractures were the most prevalent neuromuscular symptoms in our cohort. Each case exhibited some degree of skeletal muscle involvement that did not correlate with severity of heart disease. Of the three patients to receive biomarker assessment, all exhibit elevated levels of CK consistent with EDMD-induced scapulohumeroperoneal muscle wasting.[96] In EDMD patients, CK levels can range from normal to 15 times the upper limit and there is no direct link between CK levels and cardiac or skeletal muscle involvement.[99] In addition, cardiac biomarkers, BNP and TnI, were at normal levels in two patients thereby limiting the use of these cardiac biomarkers in these patients.[103] Marchel *et al.* have shown elevated BNP as a predictor of mortality in EDMD patients.[115]

In addition to laboratory markers, our patients were assessed by TTE for cardiac structure and function at baseline and median follow-ups of 3-years and 5-years at the Level III echocardiography laboratory (Figure 4.3A). Systolic function remained preserved with LVEF above 50% for all four patients across all timepoints. Compared to XLR-EDMD, systolic dysfunction is more prevalent in AD-EDMD.[116] EDMD is characterized by a high prevalence of dilated atria and LV.[117] Three patients exhibit dilated atria, and two patients exhibit dilated LV. Dilated cardiomyopathy is rare in XLR-EDMD compared to other X-linked dystrophies; however, AD-EDMD is associated with ventricular dilation.[18] Overall, LV dimensions remained relatively stable over time for three of the four patients, which may be influenced by several factors including the short follow-up, use of medical therapies, and patient lifestyle choices. Patient 3 was the only patient of our cohort to develop eccentric hypertrophy that improved after discontinuation of modafinil and uptitration of perindopril. Modafinil is not recommended in patients with hypertrophy and can induce/worsen hypertension, thus we suspect that the worsening of blood pressure, hypertrophy, and dimensions in patient 3 were associated with the sympathomimetic effects of modafinil. We cannot say whether the improvements were directly linked to modafinil discontinuation or improved control of hypertension, but likely a combination of the two changes.

Family genetic counselling is imperative for diagnosis and early intervention for both AD-EDMD and XLR-EDMD to prevent disease progression. In addition to early intervention and annual monitoring, cardiac devices and pharmacotherapy are foundational aspects of our therapeutic strategy to treat conduction abnormalities and prevent further complications (Figure 4.3A).[101] Several factors may influence our patient outcomes including early pacemaker implantation prior to enrollment at our clinic, the use of CPAP therapy, and extent of conduction abnormalities in our cohort. Thus, cardiac care in a multidisciplinary setting serve an important

role in the management of this disease.[102, 103, 118, 119] The recognition and management of SDOB in patients with EDMD is important since nocturnal hypoxia and hypercapnia secondary to hypoventilation can affect the cardiovascular system and lead to poor outcomes.[120, 121] Monitoring cardiac comorbidities, such as, hypertension, SDOB, and dyslipidemia is an important aspect of our therapeutic approach, thus strict management with statin therapy, positive airway pressure therapy and renin-angiotensin-system blockade prevent further complications. Considering the extent of neuromuscular, cardiovascular, and other comorbidities experienced by the muscular dystrophies, these patients benefit from a multidisciplinary approach to their care management.[102, 103, 118, 119, 122]

Chapter 5. Prognostic Utility of Cardiovascular Magnetic Resonance Based Phenotyping in Patients with Muscular Dystrophy

5.1 Introduction

Heart disease is a leading cause of morbidity and mortality in patients with muscular dystrophy (MD).[123, 124] The major types of MD, including Duchenne muscular dystrophy (DMD), Becker's muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD), and type 1 myotonic dystrophy (DM1), are driven by distinct genetic mutations and pathophysiology.[123, 124] From a cardiac perspective, patients with dystrophinopathies (DMD and BMD) and LGMD are conventionally characterized by mild ventricular dilation, reduced systolic function, and ventricular arrhythmias.[42, 124, 125] In contrast, conduction disease and arrhythmias are more prevalent in patients with DM1, and left bundle branch block (LBBB) correlates with reduced left ventricular (LV) systolic performance.[118, 119]

The accuracy, reproducibility, and versatility of cardiovascular magnetic resonance (CMR) imaging make it a valuable phenotyping tool for patients with MD.[126, 127] Beyond reference

standard volumetric quantification of cardiac chamber structure and contractile function, CMR provides unique insights into myocardial health across the spectrum of diffuse through to regional replacement fibrosis. The versatile delivery of these markers without limitation from poor imaging windows, a common challenge for ultrasound-based assessments in MD patients due to scoliosis, obesity, and lung disease[109, 128, 129], offers strong potential for CMR to deliver personalized phenotype-driven risk modelling in this population. However, the distribution and predictive value of contemporary CMR-based markers in patients with MD has not been well studied to date.

In the current study, we prospectively enrolled MD patients from two independent centers undergoing baseline CMR imaging and comprehensive clinical assessments for MD. Through a systematic comparison of age-and-sex-matched healthy controls, we identified differences in disease phenotype across three major types of MD: dystrophinopathies, LGMD and DM1. This included chamber remodeling, global contractile function, 3D myocardial strain, late gadolinium enhancement (LGE), and tissue mapping. CMR markers associated with future major adverse cardiac events (MACE) were established by longitudinal clinical follow-up allowing us to establish their independent prognostic value in the context of contemporary clinical care.

5.2 Methods

Study Cohort

This was a multi-center, prospective observational cohort study that recruited 148 patients with MD from the Neuromuscular Multidisciplinary (NMMD) clinic at the Kaye Edmonton Clinic, University of Alberta (Edmonton, Canada) and the Cardiovascular Imaging Registry of Calgary (CIROC, NCT04367220), Libin Cardiovascular Institute, University of Calgary (Calgary, Canada) over 7.7-years from 2014 to 2022. Patients were followed over median

5.2 years from the time of CMR. Neurological assessment, muscle biopsy, and genetic testing confirmed the diagnosis of all 148 MD patients, including dystrophinopathies (64 (43.2%) patients; 24 DMD, 27 BMD, 9 DMD carrier, and 4 BMD carrier patients), LGMD (38 (25.7%) patients), and DM1 (46 (31.1%) patients). For this investigation, patients diagnosed with DMD, BMD, and heterozygotes were grouped as "dystrophinopathies" given their similarity in pathogenesis, management practices, and vulnerability to experience MACE, which was confirmed with supplemental testing of clinical features and outcome data stratified based on the types of dystrophinopathies.[130]

Patients were referred to our centers at various stages of their disease and recruited to this study with no bias toward having overt cardiac symptoms. All patients received collaborative multidisciplinary care from specialist physicians that implemented guideline-based medical therapy, including device intervention and follow-up care. All clinical data such as clinical assessment and history, 12-lead electrocardiogram (ECG) parameters, medical therapy including pharmacological therapies and device implantation, and clinical outcomes for this investigation were obtained by electronic chart review. The study was approved by the Health Research Ethics Board at the University of Alberta and University of Calgary, and all patients provided informed and written consent at the time of study enrollment in accordance with the Declaration of Helsinki. On reasonable request, all data regarding this study is available from the corresponding authors.

Healthy Reference Cohort

A healthy reference cohort (n=50) was prospectively recruited through the University of Calgary that were sex (p=0.95) and age (p=0.44) matched based on agreement to median and interquartile range (IQR) to the MD cohort. All healthy controls were recruited from the local community with no history of cancer, cardiovascular disease, moderate or severe obesity (body

mass index ≥35.0 kg/m^2), hypertension, diabetes mellitus, kidney disease, or collagen vascular disease.

Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging was performed at the Mazankowski Alberta Heart Institute (1.5T Aera or Avanto scanner, Siemens Healthcare, Erlangen, Germany) and the Stephenson Cardiac Imaging Centre of the Libin Cardiovascular Institute (3.0T Prisma or Skyra, Siemens Healthcare, Erlangen, Germany, except for two patients scanned at 1.5T Avanto). All patients underwent standardized imaging protocols, including routine cine imaging in sequential short-axis and three long-axis views and slice-matched LGE imaging. The latter was performed 7-10 minutes following administration of intravenous gadolinium contrast (Gadovist 0.1-0.15 mmol/kg, Bayer Canada Inc.).[131, 132] A sub-group of patients (n=46 at 1.5T and n=43 at 3T) underwent pre- and post-contrast T1 mapping using a MOLLI- (n=81) or ShMOLLI- (n=8) based sequence to determine extracellular volume (ECV). Healthy volunteers underwent matched imaging protocols at 3T with 29 participants agreed to contrast administration for the estimation of ECV.

All images were anonymized and digitally transferred to a core laboratory for blinded analysis. Cardiac chamber volumes, systolic and diastolic function, LGE, and ECV were assessed using commercial software (cvi**42** v5.12, Circle Cardiovascular Imaging Inc., Calgary, Canada).[133] Short-axis cine images were used for bi-ventricular volumetric assessments with LV mass inclusive of the papillary muscles. Volume and mass values were indexed by both height and by body surface area (BSA) to assess influence of body composition in this population. Short-axis LGE images were analyzed using a ≥5SD threshold versus normal reference myocardium to estimate total LGE burden. Tissue mapping was added to imaging protocols following their

commercial availability. For patients enrolled following this time point, remote tissue ECV was calculated to assess for diffuse myocardial fibrosis. This was accomplished by segmental ECV calculation at a mid-ventricular slice using a dedicated T1 mapping module, excluding segments with off-resonance artifact or those with objective replacement fibrosis (defined as a segmental LGE burden >10% by volume by signal threshold analysis). The mean ECV value of remaining segments was then reported. Due to inadequate image quality (ie. free of arrhythmia or breath-hold related artifacts), ECV map generation were not performed in 59 patients, pre-contrast T1 not performed in 37 patients, and post-contrast T1 not performed in 59 patients. Deformation analysis was performed using validated 3D strain analysis software, as previously described.[134, 135] This provided measures of 3D global circumferential (3D-GCS), longitudinal (3D-GLS), and radial (3D-GRS) strain in addition to minimum principal strain (3DminPS) and maximum principal strain (3DmaxPS). The latter markers of 3DmaxPS and 3DminPS uniquely describe maximal tissue thickening and shortening occurring in the tissue's local axis of maximal deformation, reflecting the net local direction of myofibril forces.[135-137] This aims to eliminate the inherent "off-axis" error introduced by conventional strain estimates performed using pre-defined axes (i.e., longitudinal, radial, circumferential) that present varying local deviations from helically orientated myofibrils. As such, these measures aim to deliver greater accuracy for the description of tissue deformations.[138] All strain markers were expressed globally and segmentally according to the 17-segment AHA model excluding the apical cap.

Primary Study Endpoints

Major adverse cardiac events were defined as a composite of incident heart failure (HF), composite of atrial or ventricular arrhythmia, need for device implantation, cardiac-related hospitalization, and cardiac mortality. Incident HF was diagnosed based on comprehensive cardiac

assessments, which considered symptoms and signs such as dyspnea, orthopnea, peripheral edema, or abdominal distention. Atrial (ie. atrial fibrillation/flutter) and ventricular tachyarrhythmias (VT) (ie. non-sustained VT and sustained VT) required visual confirmation by 12-lead ECG, Holter monitoring, or device interrogation. Non-sustained VT was defined as three or more consecutive beats with a duration of less than 30 seconds, and sustained VT was defined as ventricular tachycardia with a duration for more than 30 seconds. Holter monitoring, is recommended 1-2 years basis, and 12-lead ECG and device interrogation were recommended on an annual basis; however, this is not mandated as part of our study protocol. Cardiac-related hospitalization includes chest pain/angina, myocardial infarction, hypotension, stroke, and syncope. Cardiac hospitalization, arrhythmias, heart failure symptoms, and cardiac mortality were identified through manual review of provincial electronic health records and death certificates.

Statistical Analysis

Continuous variables were analyzed using a Kruskal-Wallis test, and categorical variables were compared using Pearson chi-square tests summarized as medians and IQRs or percentages. Post-hoc analysis was conducted using a Dunn test for pairwise comparisons of continuous variables. Study follow-up duration was over a median period of 5.2 years from the time of CMR. For risk analysis, patients were stratified based on optimal cut-off values from each CMR-based marker, which were calculated from receiver-operator characteristic curves and corresponding Youden's indices.[139] Left ventricular ejection fraction (LVEF) <55.0% was used to define a diagnosis of cardiomyopathy based on the lower limit of normal (LLN) for our previously published healthy reference population.[135] Univariable Cox regression analysis was used to identify CMR markers with predictive value for MACE; these were included in multivariable Cox regression analysis to derive independent models and respective adjusted hazard ratios (aHR).

Each model was adjusted for age in 5-year increments, assigned-at-birth sex, MD genotype group, presence of respiratory disease, and baseline use of cardiac medications, which were chosen based on clinical or statistical significance. Respiratory disease was defined as respiratory muscle weakness, restrictive lung disease, obstructive pulmonary disease, asthma, or recurrent aspiration pneumonia. Model performance for predicting MACE was assessed using Akaike's Information Criterion (AIC) and concordance index (C-index). In addition, the incremental value of 3D strain to LVEF to predict MACE was evaluated with the likelihood ratio test. Collinearity test determined LVEF and strain markers are independent of each other. In addition, incremental value to LVEF to predict MACE was evaluated All statistical analyses were performed in R v4.0.3 and SPSS statistics software 28.0.1.0, and a p-value<0.05 was considered significant.

5.3 Results

Clinical Characteristics

Baseline characteristics of 148 MD patients revealed a median age of 35 (IQR: 21.0-48.0) years comprised of 51 (34.5%) females (Table 5.1). Within the dystrophinopathies cohort, DMD and BMD patients were exclusively younger males, while carriers were exclusively females, distinguishing this cohort from other types of MD (Table 5.2). Conduction delays were predominantly seen in DM1 patients (Table 5.3). There was a high burden of first-degree atrioventricular block and LBBB in DM1 patients (Table 5.4). Comorbidities, including hypertension, respiratory disease, and sleep-disordered breathing, were prevalent with a comparable high use of respiratory therapies across the various cohorts of MD; in addition, DM1 patients exhibited the lowest need for wheelchair assistance (Table 5.1). The use of cardiac medications at time of CMR was higher among patients with dystrophinopathies while cardiac devices were more prevalent in patients with DM1 (Table 5.1).

Table 5.1 Baseline Clinical Characteristics in Patients with Muscular Dystrophy and Healthy Controls

Characteristic	HC (n=50)	Dystrophinopathies (n=64)	LGMD (n=38)	DM1 (n=46)	*P* Value, MD Cohorts*	*P* Value, All MD vs HC
Males/Females, No.	33 (66.0)/ 17 (34.0)	51 (79.7)/ 13 (20.3)	24 (63.2)/ 14 (26.8)	22 (47.8)/ 24 (52.2) δ	0.002	0.95
Age, Yrs	36.0 (27.0-48.0)	26.0 (19.0-38.8)	39.5 (23.0-54.5) δ	43.0 (34.5-52.5) δ	<0.001	0.44
Height	175.0 (168.0-180.0)	168.0 (153.3-177.0)	172.0 (165.0-178.1)	168.0 (162.6-178.0)	0.08	0.004
Weight	75.0 (66.5-84.8)	75.0 (54.0-83.4)	80.0 (65.8-94.8)	76.0 (58.0-90.0)	0.10	0.77
BSA, m^2	1.9 (1.8-2.1)	1.8 (1.6-2.0)	1.9 (1.7-2.1)	1.9 (1.7-2.1)	0.23	0.19
Current/Former Smoker, No.	-	6 (9.38)	7 (18.4)	6 (13.0)	0.42	-
Ambulation, No.						
Cane/Walker	-	6 (9.38)	3 (7.89)	5 (10.9)	0.90	-
mWC/pWC	-	29 (45.3)	11 (28.9)	2 (4.4) †δ	<0.001	-
Comorbidities, No.						
Dyslipidemia	-	4 (6.25)	5 (13.2)	9 (19.6)	0.09	-
Diabetes	-	2 (3.13)	8 (21.1) δ	4 (8.7)	0.01	-
Hypertension	-	9 (14.1)	9 (23.7)	3 (6.5)	0.08	-
Respiratory Disease	-	29 (45.3)	15 (39.5)	22 (47.8)	0.74	-
Sleep-Disordered Breathing	-	19 (29.7)	9 (23.7)	15 (32.6)	0.66	-
Respiratory Therapies, No.						
Lung Volume Recruitment	-	13 (20.3)	5 (13.2)	14 (30.4)	0.15	-
Mechanical Insufflation-Exsufflation	-	7 (10.9)	1 (2.63)	0	0.13	-
Noninvasive Ventilation	-	18 (28.1)	7 (18.4)	11 (23.9)	0.54	-
Cardiac Therapies, No.						
ACEi/ ARB	-	32 (50.0)	12 (31.6)	5 (10.9) δ	<0.001	-
Beta-Blocker	-	22 (34.4)	8 (21.1)	8 (17.4)	0.10	-
MRA	-	9 (14.1)	4 (10.5)	0	0.56	-
Cardiac Device	-	1ICD (1.6)	2ICD (5.3)	1ICD, 3PM, 2CRT-P (13.0) δ	0.04	-
Vitals, median						
HR, bpm	61.0 (56.0-68.0)	75.0 (62.8-82.8)	72.0 (69.0-85.0)	68.5 (60.0-76.5) †δ	0.02	<0.001
sBP, mmHg	109.0 (101.8-119.0)	108.0 (101.0-124.0)	120.0 (109.0-128.0) δ	111.5 (102.0-119.0)	0.02	0.23
dBP, mmHg	67.0 (56.5-73.0)	68.0 (60.0-74.0)	78.0 (69.0-84.0) δ	70.0 (63.3-76.3)	0.02	0.03

*P value, MD cohorts compares Dystrophinopathies, LGMD, and DM1 cohorts. †$P < 0.05$ vs LGMD; δ$P < 0.05$ vs Dys. HC, healthy control; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy; MD, Muscular dystrophy; BSA, body surface area; mWC, manual

wheelchair; pWC, power wheelchair; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, Mineralocorticoid receptor antagonist; ICD, implantable cardiac defibrillator; PM, dual-chamber pacemaker; CRT-P, cardiac resynchronization therapy-pacemaker; HR, heart rate; sBP, systolic blood pressure; dBP, diastolic blood pressure.

Table 5.2 Baseline 12-Lead Electrocardiogram Characteristics.

Characteristic	**Dystrophinopathies (n = 64)**	**LGMD (n = 38)**	**DM1 (n = 46)**	***p Value***
Heart Rate, bpm	77.0 (62.0-86.3)	68.0 (61.3-80.0)	67.0 (58.0-73.0)	0.01
PR Interval, ms	132.0(117.0-140.0)	158.0 (136.0-163.0)	200.0 (184.0-224.0)	<0.001
QRS Duration, ms	96.0 (88.0-103.0)	97.0 (91.5-108.3)	108.0 (97.0-139.0)	0.002
QT Interval, ms	372.0 (358.0-388.0)	394.0 (364.0-424.5)	422.0 (400.0-443.8)	<0.001
Corrected QT Interval, ms	424.0 (412.3-438.6)	426.4 (406.1-451.6)	447.0 (423.1-457.0)	0.01
Conduction Delay, No.				
1° AVB	0	1 (2.63)	18 (39.1)	<0.001
LAFB	1 (1.56)	0	1 (2.17)	0.81
LBBB	0	0	11 (23.9)	-
RBBB	0	0	0	-

LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy; 1° AVB, first-degree atrioventricular block; LAFB, left anterior fascicular block; LBBB, left bundle branch block; RBBB, right bundle branch block.

Table 5.3 Clinical Characteristics in Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, and Dystrophinopathies Heterozygotes.

Characteristics	Duchenne muscular dystrophy (n=24)	Becker muscular dystrophy (n=27)	Dystrophinopathies Heterozygotes (n=13)	*p* value
Age, years	19 (18-20.25)	33 (22.5-37.5) δ	48 (41-55) †δ	<0.001
Sex, n	24 (37.5)	27 (42.2)	0 (0)	<0.001
Respiratory Disease, n	21 (87.5)	7 (25.9)	1 (7.7)	<0.001
LVEF, %	43.9 (38.7-53.0)	48.8 (42.7-54.9)	56.5 (46.3-61.4)	0.12
LVESV Indexed to BSA, mL/m^2	56.5 (47.3-67.2)	44.8 (41.6-52.6)	35.3 (29.2-53.0) δ	0.04
LVESV Indexed to Height, mL/m	53.2 (35.5-66.3)	51.0 (44.9-59.5)	44.3 (32.5-63.5)	0.63
LVEDV Indexed to BSA, mL/m^2	102.3 (87.0-119.0)	92.0 (86.2-105.0)	92.7 (72.4-100.7)	0.15
LVEDV Indexed to Height, mL/m	93.9 (72.5-111.2)	102.4 (95.7-113.9)	99.5 (84.1-117.4)	0.22
LV Mass Indexed to BSA, g/m^2	47.1 (40.6-53.7)	47.5 (44.2-55.6)	39.4 (36.5-43.23) †	0.01
LV Mass Indexed to Height, g/m	42.9 (37.9-48.9)	57.4 (48.2-65.3) δ	47.5 (41.4-52.0)	0.001
RVEF, %	49.1 (44.2-52.8)	49.7 (43.3-52.8)	54.7 (50.0-57.1)	0.06
Circumferential Strain Amplitude, %	-12.7 (-14.4- -10.8)	-12.8 (-14.5- -10.8)	-13.9 (-15.5- -11.9)	0.52
Longitudinal Strain Amplitude, %	-11.6 (-13.7- -10.5)	-11.5 (-13.8- -10.1)	-13.1 (-15.2- -12.0)	0.12
Radial Strain Amplitude, %	29.7 (24.7-42.6)	37.3 (27.0-45.1)	44.3 (36.1-58.0)	0.10
Minimum Principal Strain Amplitude, %	-22.7 (-25.6- -20.5)	-23.1 (-25.8- -20.8)	-27.4 (-30.6- -21.2)	0.16
Maximum Principal Strain Amplitude, %	48.7 (41.0-59.9)	45.2 (35.3-61.0)	73.2 (58.0-86.6) δ	0.007
LGE presence, n	20 (33.3)	18 (30.0)	4 (15.0)	0.41
MACE, n	8 (33.3)	6 (22.2)	3 (23.1)	0.57
Arrhythmias, n	2 (8.3)	4 (14.8)	1 (7.7)	0.06
Device Implantation, n	1 (4.2)	2 (7.4)	0 (0)	0.90

Cardiac-related hospitalizations, n	3 (12.5)	3 (11.1)	1 (7.7)	0.32
Incident Heart Failure, n	2 (8.3)	1 (3.7)	2 (15.4)	0.49
Cardiac Mortality, n	1 (4.2)	0 (0)	0 (0)	0.43

†p<0.05 vs BMD; δp<0.05 vs DMD. LV, left ventricular; EF, ejection fraction; BSA, body surface area; ESV, end-systolic volume; EDV, end-diastolic volume; RV, right ventricle; LGE, late gadolinium enhancement; MACE, major adverse cardiac events.

Table 5.4 CMR Parameters in Myotonic Dystrophy Patients with and without Left Bundle Branch Block.

Parameter	No LBBB (n=35)	LBBB (n=11)	*P* value
LVEF, %	59.9 (54.6-64.5)	43.3 (39.6-54.4)	<0.001
LVESV Indexed to BSA, mL/m^2	26.2 (22.7-36.2)	32.0 (30.0-45.0)	0.04
LVESV Indexed to Height, mL/m	31.0 (24.2-38.1)	42.4 (33.1-56.5)	0.004
LVEDV Indexed to BSA, mL/m^2	68.2 (57.0-79.3)	69.2 (62.0-73.0)	0.69
LVEDV Indexed to Height, mL/m	75.7 (64.3-89.9)	74.5 (65.7-91.2)	0.73
LV Mass Indexed to BSA, g/m^2	41.5 (37.1-49.8)	39.9 (38.3-42.8)	0.45
LV Mass Indexed to Height, g/m	47.2 (38.2-56.5)	46.6 (41.1-49.9)	0.77
Circumferential Strain Amplitude, %	-14.5 (-15.5- -12.4)	-12.3 (-13.7- -11.1)	0.09
Longitudinal Strain Amplitude, %	-13.3 (-14.8- -11.5)	-12.0 (-13.2- -9.5)	0.18
Radial Strain Amplitude, %	37.9 (31.3-45.7)	33.2 (30.7-38.7)	0.40
Minimum Principal Strain Amplitude, %	-26.8 (-28.8- -25.0)	-22.3 (-24.6- -21.0)	0.006
Maximum Principal Strain Amplitude, %	59.7 (48.1-70.5)	49.3 (42.0-58.2)	0.25
Late Gadolinium Enhancement, n	5 (14.3)	3 (27.3)	0.32

Data are presented as median (interquartile range) or n (%). LBBB, Left bundle branch block; LV, left ventricular; EF, ejection fraction; BSA, body surface area; ESV, end-systolic volume; EDV, end-diastolic volume.

Cardiac Assessment and Differential Remodeling

While all MD groups had lower LVEF relative to healthy controls, systolic function was significantly reduced in dystrophinopathies compared to LGMD and DM1 (p=0.001 and p<0.001, respectively; Figure 5.1A and Table 5.5). Dystrophinopathies exhibited mildly increased chamber volumes versus LGMD and DM1, while the overall MD cohort showed indexed LVESV to be different from healthy controls (Figure 5.1B and 5.1C, and Table 5.5). In addition, recognizing that there is heterogeneity in the dystrophinopathy cohort, we observed the heterozygote population had reduced LVESV indexed to BSA. However, there was mild chamber dilation observed in the dystrophinopathies as whole (Figure 5.1, Table 5.5, and Table 5.2). Dystrophinopathy heterozygotes had markedly lower LV mass indexed to BSA compared to hemizygotes, but this may be indicative of age-and-sex-based differences (Table 5.2). DM1 patients showed smaller chamber volumes and reduced LV mass (Figure 5.1D and Table 5.5), which was maintained when stringent age- and sex-matched health controls were used (Figure 5.2). Left bundle branch block was associated with reduced LVEF in DM1 patients (Table 5.4).

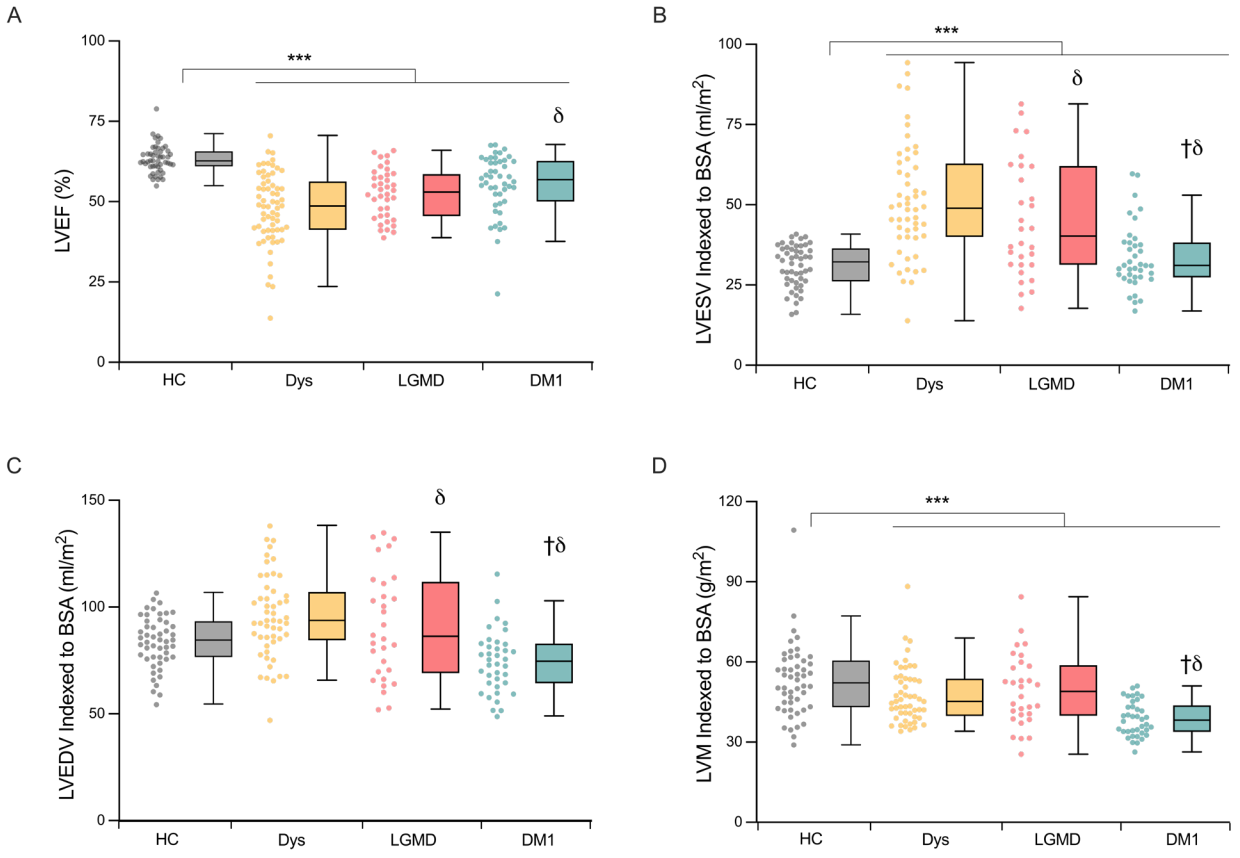

Figure 5.1 Assessment of left ventricular function and structure in muscular dystrophy versus healthy controls.

***$P < 0.001$ for healthy controls versus all muscular dystrophy patients. †P < 0.05 vs LGMD; δP < 0.05 vs Dys. HC, healthy controls; Dys, dystrophinopathies; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; LVM, left ventricular mass.

Table 5.5 Baseline cardiac magnetic resonance (CMR) based markers in patients, stratified by muscular dystrophy subtype.

Characteristic	HC (n=50)	Dystrophinopathies (n=64)	LGMD (n=38)	DM1 (n=46)	*P* Value, MD Cohorts*	*P* Value, All MD vs HC
LVEF, %	62.7 (61.0-65.6)	48.7 (41.2-55.9)	53.0 (45.7-58.2)	56.8 (50.8-62.6) δ	0.001	<0.001
LVESV, mL/m^2	32.2 (26.3-36.2)	48.9 (40.0-62.0)	40.2 (31.4- 61.0)	31.1 (27.5-38.0) †δ	<0.001	<0.001
LVESV, mL/m	35.1 (29.0-39.9)	51.2 (36.6-64.5)	42.2 (30.8-61.9)	33.6 (28.7-42.5) δ	<0.001	<0.001
LVEDV, mL/m^2	84.5 (76.8-92.7)	94.0 (85.1-106.0)	86.3 (70.2-109.8) δ	74.7 (64.8-82.6) †δ	<0.001	0.47
LVEDV, mL/m	92.5 (83.8-105.0)	99.7 (82.3-115.3)	93.9 (72.4-114.4) δ	81.2 (66.0-92.6) †δ	<0.001	0.68
LV Mass, g/m^2	52.2 (43.7-60.1)	45.2 (40.4-53.6)	48.9 (40.4-58.3)	38.2 (34.0-43.2) †δ	<0.001	<0.001
LV Mass, g/m	55.9 (48.7-68.5)	48.8 (41.6-59.2)	50.9 (37.8-62.1)	42.0 (36.7-46.4) †δ	0.004	<0.001
LA Volume, mL/m^2	34.7 (31.1-42.5)	32.1 (26.0-41.5)	29.6 (24.5-42.4)	27.2 (21.5-33.4)	0.20	0.001
LA Volume, mL/m	38.7 (34.3-47.2)	34.3 (25.4-45.6)	30.8 (23.4-41.1)	28.0 (24.2-37.2)	0.33	<0.001
RVEF, %	55.6 (53.1-60.4)	50.0 (44.3-54.7)	49.3 (46.0-54.0)	49.4 (46.2-53.8)	0.96	<0.001
RVESV, mL/m^2	42.1 (34.4-46.0)	40.9 (35.1-53.6)	44.1 (37.4-51.4)	36.3 (32.5-43.2) †	0.03	0.63
RVESV, mL/m	45.0 (38.3-50.7)	46.1 (37.9-55.2)	44.2 (36.1-56.7)	40.8 (34.5-48.6)	0.18	0.98
RVEDV, mL/m^2	95.3 (84.5-101.5)	86.3 (74.6-100.2)	87.3 (74.6-104.5)	79.0 (67.3-85.4) †	0.02	0.004
RVEDV, mL/m	102.4 (92.1-111.5)	93.6 (78.9-109.3)	93.1 (75.3-107.2)	85.0 (67.8-102.4)	0.20	0.001
RA Volume, mL/m^2	40.7 (34.5-98.4)	28.5 (24.2-34.9)	27.2 (22.3-36.0)	26.2 (20.4-37.9)	0.79	<0.001
RA Volume, mL/m	44.0 (38.2-53.1)	30.2 (24.0-38.6)	30.4 (19.7-37.4)	29.1 (21.2-38.9)	0.88	<0.001
LGE Burden (% of LV Mass)	-	12.1 (4.4-25.8)	5.7 (1.7-10.0) δ	1.8 (0.9-4.2) †δ	<0.001	-
Circumferential Strain Amplitude, %	-14.5 (-15.4- -13.0)	-12.7 (-15.2- -11.0)	-14.0 (-16.7- -12.8)	-14.6 (-15.7- -12.5)	0.19	0.01
Longitudinal Strain Amplitude, %	-13.8 (-15.0- -12.5)	-12.0 (-10.8- -14.7)	-13.8 (-15.2- -11.9)	-13.7 (-14.8- -12.1)	0.20	0.007
Radial Strain Amplitude, %	51.3 (41.6-62.1)	30.4 (23.1-52.7)	39.1 (30.1-48.5) δ	39.2 (25.3-47.5) δ	<0.001	<0.001
Minimum Principal Strain Amplitude, %	-27.6 (-29.2- -25.8)	-23.6 (-20.4- -27.8)	-25.4 (-23.2- -27.2)	-25.8 (-27.8- -23.9)	0.07	<0.001
Maximum Principal Strain Amplitude, %	71.9 (57.9-86.4)	51.1 (39.5-67.1)	53.9 (45.6-67.8)	59.1 (42.3-68.7)	0.87	<0.001
ECV, %	26.6 (24.4-27.2)	27.7 (23.6-30.2)	27.1 (24.2-30.0)	26.3 (24.4-28.5)	0.81	0.52

*P value, MD cohorts compares Dystrophinopathies (Dys), LGMD, and DM1 cohorts. †P<0.05 vs LGMD; δP<0.05 vs Dys. Chamber volumes and mass indexed to body surface area and height. HC, Healthy control; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy; MD, Muscular dystrophy; LV, left ventricular; EF,

ejection fraction; ESV, left ventricular end-systolic volume; EDV, end-diastolic volume; LA, left atrial; RV, right ventricular; RA, right atrial; LGE, Late gadolinium enhancement; ECV, Extracellular volume.

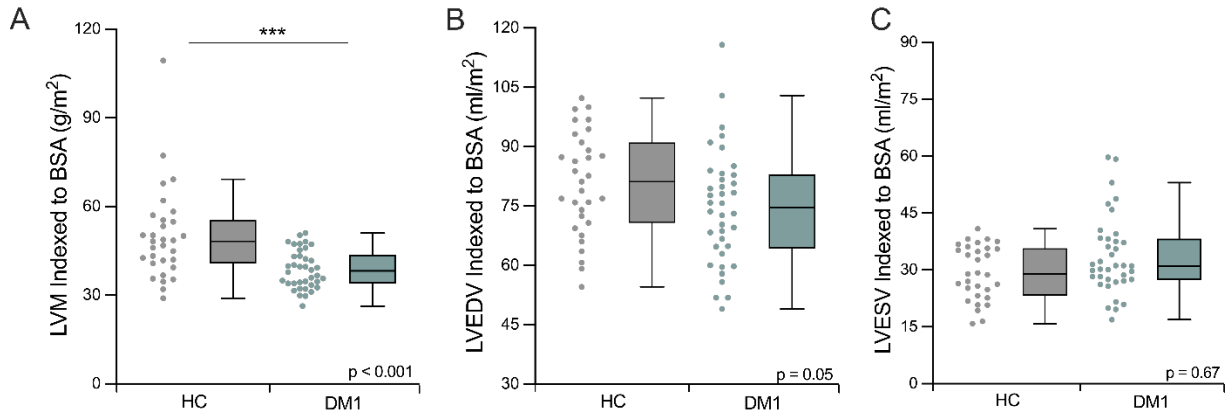

Figure 5.2 Comparison of Ventricular Mass and Volumes in Age-Sex Matched Healthy Controls (n=31) and Myotonic Dystrophy Patients (n=44).

BSA, Body Surface Area; LVM, Left ventricular mass; LVEDV, Left ventricular end-diastolic volume; LVESV, Left ventricular end-systolic volume.

The traditional, geometry-dependent directions of deformation, demonstrated reduced peak strain amplitude and strain rate in all across all MD cohorts compared to healthy controls (Table 5.5, Table 5.6, and Figure 5.3). Radial strain amplitude was the only strain measure that was reduced in dystrophinopathies compared to LGMD and DM1 patients. To gain further insight on the remodeling process, we utilized a novel 3-D representative model to capture the geometry-independent direction of deformation, which reflects local shortening and thickening of local tissue in our cohort (Figure 5.4). In our comparison of healthy control patients and MD cohort, peak 3D principal strain amplitude was reduced across all MD cohorts (Table 5.5, Figure 5.3, and Figure 5.4). Maximum principal strain amplitude was the only strain amplitude that was significantly reduced in the dystrophinopathy hemizygote cohort compared to the heterozygotes (Table 5.2).

Table 5.6 Systolic and Diastolic Strain Rate Assessment.

Data are presented as mean ± SD or n (%). **P* value, MD cohorts compares Dystrophinopathies, LGMD, and DM1 cohorts. Strain rates by three-dimensional myocardial deformation analysis. HC, Healthy control; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy; MD, Muscular dystrophy.

Characteristic	HC (n = 50)	Dystrophinopathies (n = 64)	LGMD (n = 38)	DM1 (n = 46)	*P* Value, MD Cohorts*	*P* Value, All MD vs HC
Analysis, n	48 (96.0)	61 (95.3)	34 (89.5)	38 (82.6)	-	-
Systolic Circumferential Strain Rate, s^{-1}	-1.1±0.2	-0.9±0.3	-1.0±0.2	-1.0±0.2	0.59	0.05
Systolic Longitudinal Strain Rate, s^{-1}	-1.2±0.2	-1.1±0.3	-1.1±0.3	-1.2±0.3	0.08	0.02
Systolic Radial Strain Rate, s^{-1}	3.8±1.2	2.9±1.2	3.0±1.5	3.1±1.0	0.54	<0.001
Systolic Minimum Principal Strain Rate, s^{-1}	-1.7±0.4	-1.6±0.4	-1.6±0.6	-1.7±0.4	0.31	0.24
Systolic Maximum Principal Strain Rate, s^{-1}	4.7±1.4	3.6±1.5	3.7±1.7	3.8±1.2	0.23	<0.001
Diastolic Circumferential Strain Rate, s^{-1}	1.4±0.3	1.2±0.4	1.2±0.3	1.2±0.3	0.92	<0.001
Diastolic Longitudinal Strain Rate, s^{-1}	1.4±0.3	1.2±0.4	1.1±0.2	1.1±0.3	0.78	<0.001
Diastolic Radial Strain Rate, s^{-1}	-6.2±1.9	-4.1±2.2	-3.7±1.3	-4.0±2.0	0.68	<0.001
Diastolic Minimum Principal Strain Rate, s^{-1}	1.7±0.3	1.5±0.5	1.4±0.3	1.9±1.8	0.99	0.006
Diastolic Maximum Principal Strain Rate, s^{-1}	-7.6±2.3	-5.4±2.6	-4.8±1.5	-8.3±-6.5	0.92	<0.001

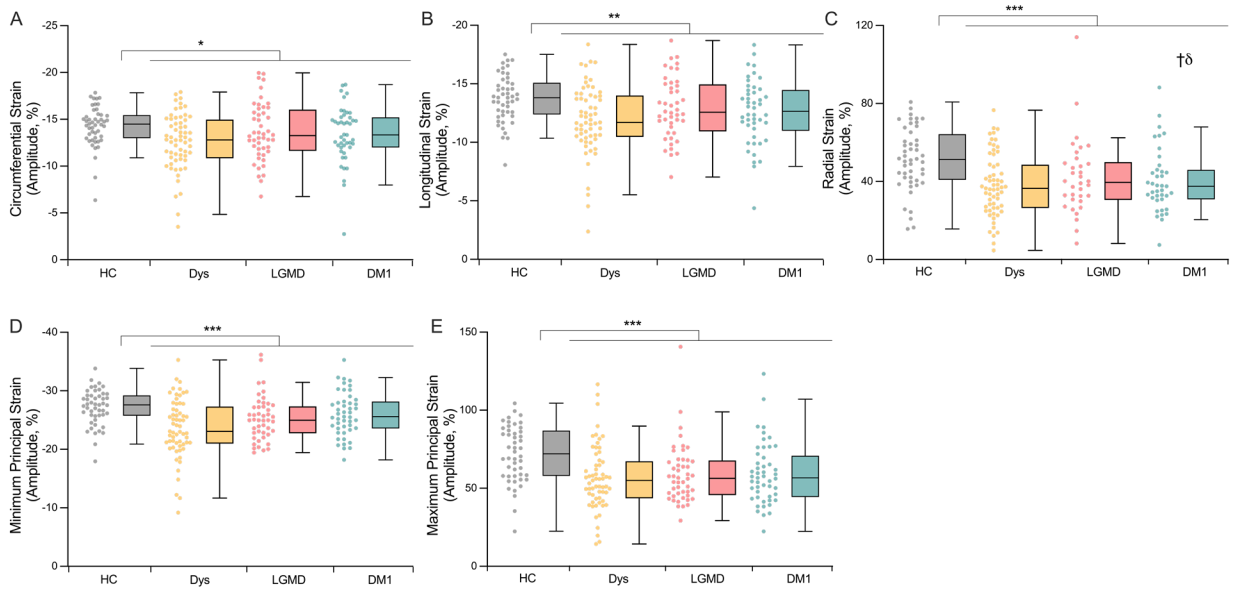

Figure 5.3 Two- and three-dimensional strain analysis assessing systolic strain amplitudes. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for healthy controls versus all muscular dystrophy patients. †P < 0.05 vs LGMD; δP < 0.05 vs Dys. HC, healthy controls; Dys, dystrophinopathies; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy.

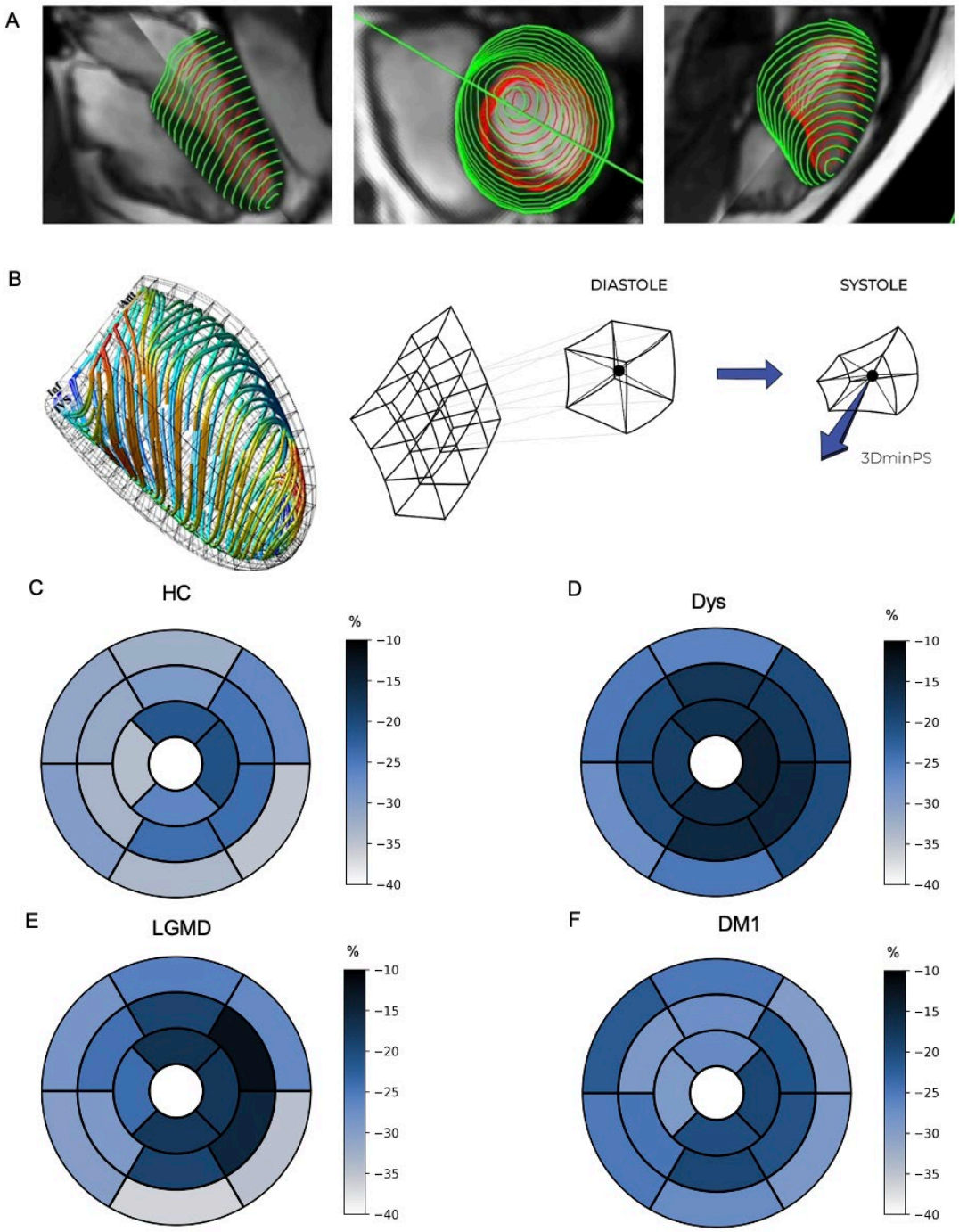

Figure 5.4 3-dimensional myocardial deformation analysis (3D-MDA) for the spatial estimation of 3D minimum principal strain (3DminPS) from routine 2D cine MRI imaging. Multi-planar cine image alignment and chamber segmentation (A) is followed by 3D endocardial and epicardial mesh model deformation by feature tracking techniques. Deformations experienced

by each 3D mesh element are used to estimate local peak strain amplitudes along an intrinsically defined axis of maximal tissue shortening (B). As illustrated by 3D rendered path orientations from 3DminPS, representing the local axes of net fiber shortening, conventional geometry-dependent measures (i.e., longitudinal axis strain) are inherently confounded by a varying degree of off-axis error relative to helically-oriented fiber shortening. Regional AHA 16-segmental distribution of minimum principal strain amplitude values for representative subjects from healthy control (HC) (C), patient with dystrophinopathy (Dys) (D), limb-girdle muscular dystrophy (LGMD) (E), and type 1 myotonic dystrophy (DM1) (F) cohorts are -28.6%, -19.8%, -23.6%, and -24.8%, respectively.

Gadolinium-based contrast agents were administered in 60 (93.8%) dystrophinopathy, 36 (94.7%) LGMD, and 45 (97.8%) DM1 patients. Replacement fibrosis was identified on LGE imaging in a greater proportion (78.3%) of patients with dystrophinopathies, compared to LGMD (44.4%) and DM1 (17.4%) (Figure 5.5A); dystrophinopathies also had higher percent LGE burden by threshold-based quantification (Table 5.5 and Figure 5.5B). Patterns of gadolinium enhancement varied by MD type with dystrophinopathies and LGMD primarily showing subepicardial and patchy mid-wall patterns (Figure 5.5D). Of dystrophinopathies demonstrating LGE, the majority were DMD and showed LGE localized to the anterolateral and inferolateral segments of basal through to apical zones (Figure 5.6). In comparison, DM1 patients had a low prevalence and burden of fibrosis, which was predominantly in a subepicardial pattern (Table 5.5 and Figure 5.5). Extracellular volume values of remote tissue regions were not statistically different between disease cohorts or healthy controls (Figure 5.5C). There was a positive

correlation between the segmental function with myocardial enhancement and diffuse fibrosis localized in anterolateralateral and inferolateral regions (Table 5.7).

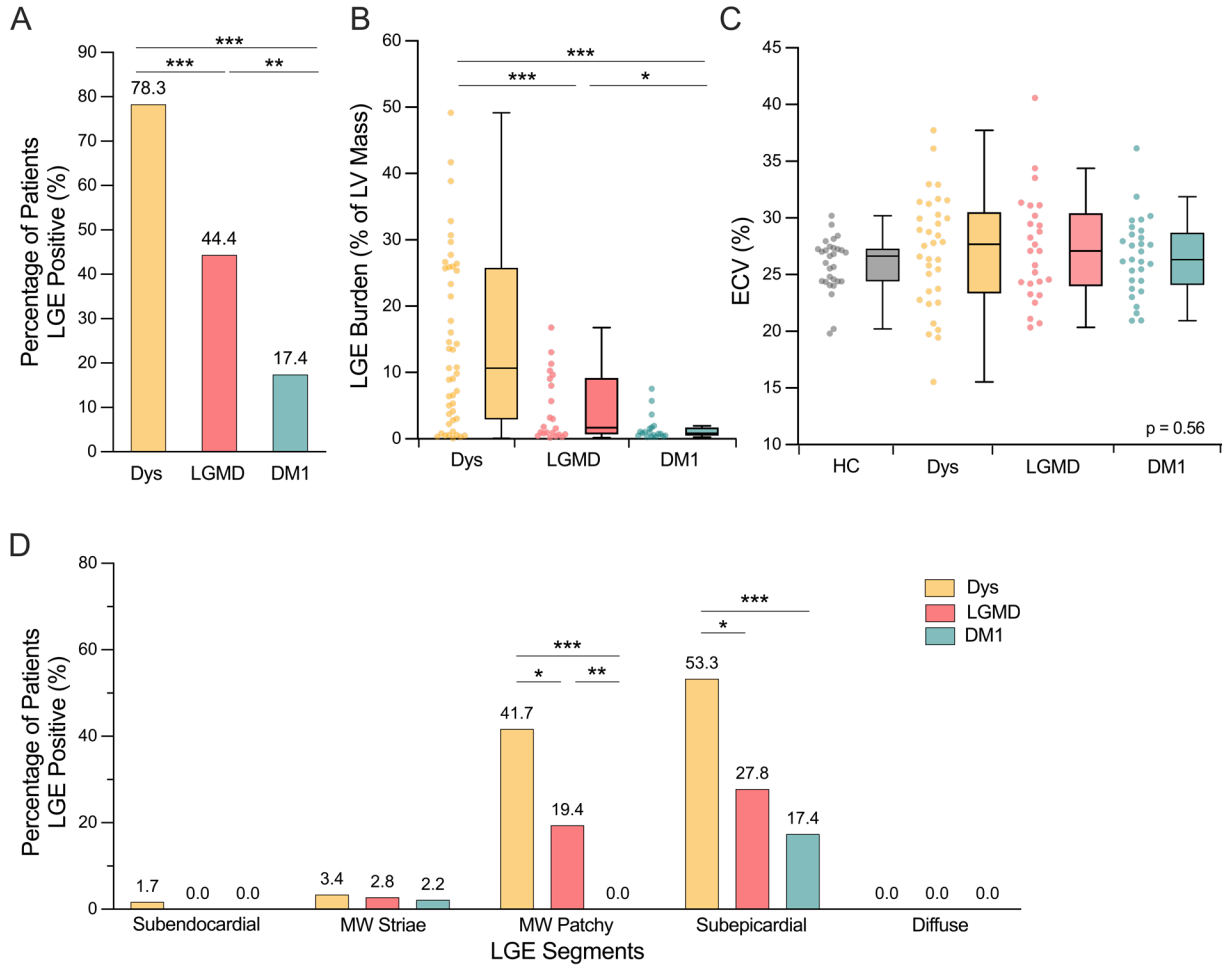

Figure 5.5 Assessment of structural remodeling patterns by late gadolinium enhancement and extracellular volume.

*P < 0.05, ***P < 0.001. 60/64 (93.8%) dystrophinopathies, 36/38 (94.7%) LGMD, and 45/46 (97.8%) DM1 patients received a gadolinium-based contrast agent. HC, healthy controls; Dys, dystrophinopathies; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy; LGE, late gadolinium enhancement; LV, Left ventricular; ECV, extracellular volume; MW, mid-wall.

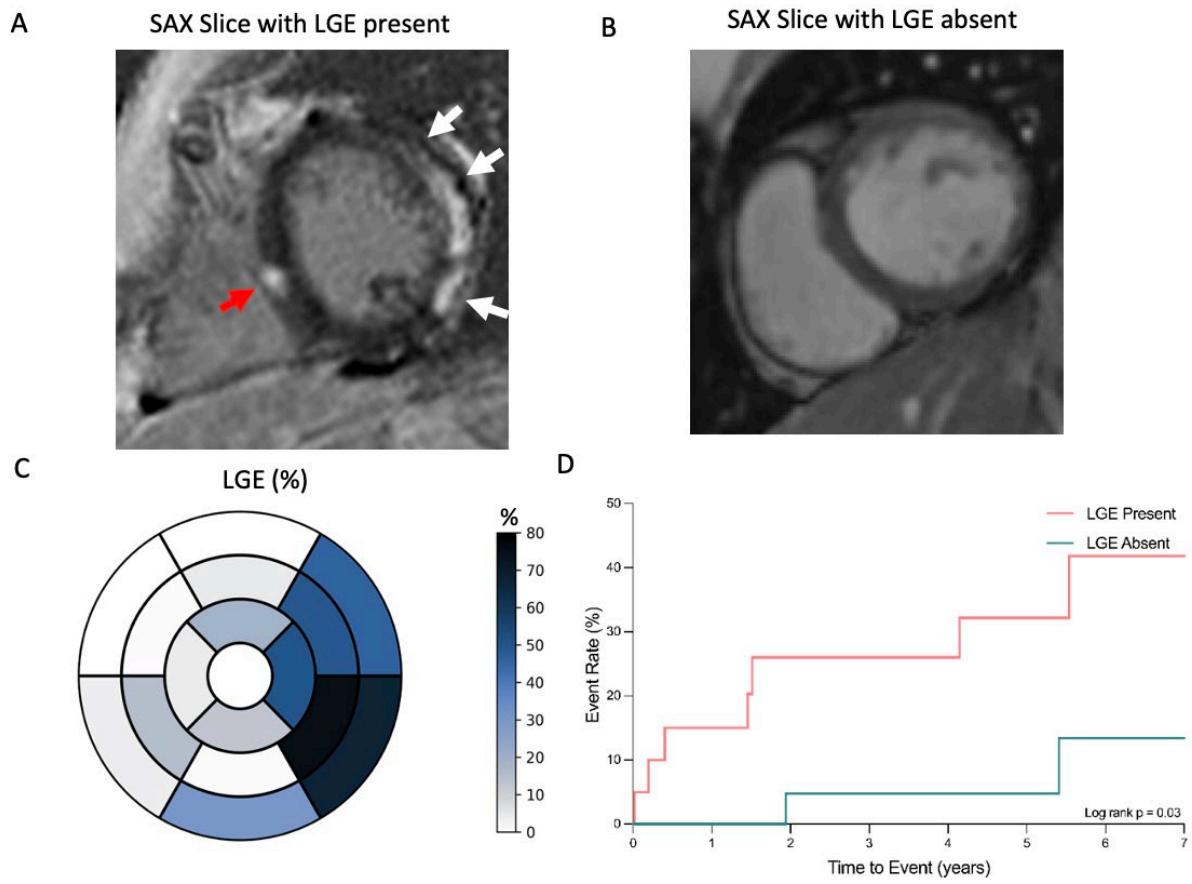

Figure 5.6 Typical CMR findings in a patient with DMD. Subepicardial late gadolinium enhancement (LGE) along the anterolateral and inferolateral segments (white arrows) and right ventricular septum (red arrow) in a mid-chamber short-axis view (A), in comparison to short-axis view of DMD patient with LGE absent (B). Corresponding bulls-eye plot to DMD patient A of segmental percent LGE burden (C). Kaplan-meier analysis in DMD and BMD hemizygotes (n = 47) demonstrates worse event-free survival in patients with LGE presence (excluding patients with subepicardial pattern) (D).

Table 5.7 Association between Segmental Function and Myocardial Fibrosis.

Segment	Location	R-value between LGE and 3DminPS	*P* value	R-value between ECV and 3DminPS	*P* value
1	Basal anterior	0.23	0.01	-	-
2	Basal anteroseptal	0.18	0.04	-	-
3	Basal inferoseptal	0.18	0.05	-	-
4	Basal inferior	0.07	0.39	-	-
5	Basal inferolateral	0.30	<0.001	-	-
6	Basal anterolateral	0.29	<0.001	-	-
7	Mid anterior	0.39	<0.001	0.06	0.62
8	Mid anteroseptal	0.24	0.007	0.04	0.69
9	Mid inferoseptal	0.22	0.01	0.06	0.62
10	Mid inferior	0.25	0.004	0.04	0.76
11	Mid inferolateral	0.35	<0.001	0.33	0.002
12	Mid anterolateral	0.40	<0.001	0.34	0.002
13	Apical anterior	0.17	0.07	-	-
14	Apical septal	0.21	0.02	-	-
15	Apical inferior	0.26	0.004	-	-
16	Apical lateral	0.32	<0.001	-	-

LGE, late gadolinium enhancement; 3DminPS, 3-dimensional minimum principal strain; ECV, extracellular volume.

Prognostic Utility of Cardiovascular Magnetic Resonance Markers

Over a median follow-up of 5.2 (IQR: 1.7-6.3) years, 80 incident MACE occurred in 47 (31.8%) patients. This included 24 cases of arrhythmias (15 atrial flutter or fibrillation and 10 non-sustained and sustained ventricular tachycardia (VT)), 13 device implantations, 23 cardiac-related hospitalization, 15 incident heart failure, and 4 cardiac deaths across the various MD types (Figure 5.7). There was no significant difference in the distribution of primary endpoints in the dystrophinopathies and showed no significant difference in event-free survival over time (Table 5.2 and Figure 5.8). Myotonic dystrophy patients experienced a higher, but not statistically significant occurrence of arrhythmias and device implantations compared to the other MD types (Figure 5.7). Patients who experienced MACE were marginally older with a higher proportion on baseline cardiac medications (Table 5.8). Multivariable risk modelling was performed to assess

the independent predictive utility of all CMR parameters following their assessment by univariable cox regression (Table 5.9).

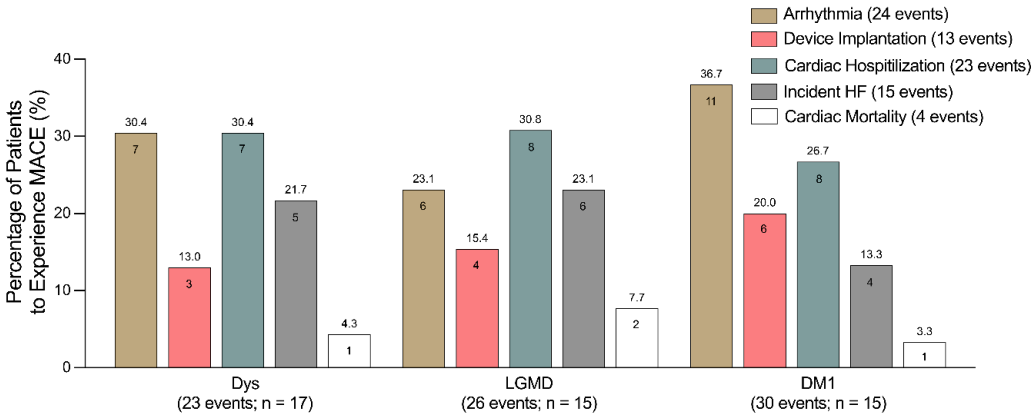

Figure 5.7 Distribution of Major Adverse Cardiac Events across the Muscular Dystrophies shown as Percentages (above bar) and Number of Events (within bar).

MACE, major adverse cardiac events; HF, heart failure; Dys, dystrophinopathies; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy.

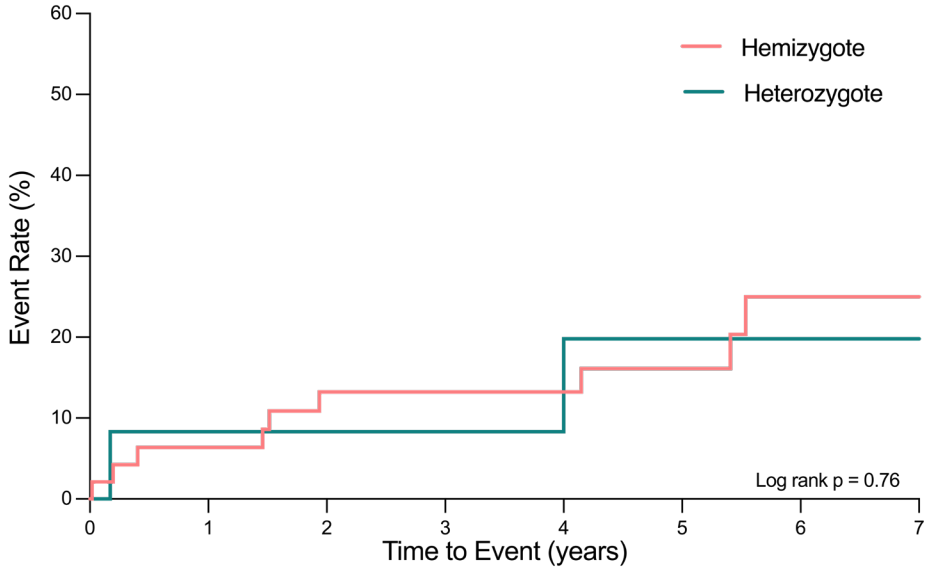

Figure 5.8 Kaplan Meier Curve Depicts Event-free Survival in Dystrophinopathies Hemizygotes.

Table 5.8 Baseline Characteristics in Patients with and without MACE.

	No MACE (n=101)	MACE (n=47)	*P* value
Age	34 (22-48)	42 (23-55.5)	0.09
Sex	62 (61.4)	34 (72.3)	0.19
Respiratory disease	45 (44.6)	21 (44.7)	0.99
Ambulatory Aid			0.38
Cane/Walker	11 (10.9)	4 (8.5)	
mWC/pWC	31 (30.7)	10 (24.4)	
Smoking History	13 (12.9)	6 (12.8)	0.99
Diabetes	7 (6.9)	7 (14.9)	0.12
Dyslipidemia	10 (9.9)	8 (17.0)	0.22
Hypertension	14 (13.9)	7 (14.9)	0.87
Baseline Cardiac Medications	30 (29.7)	28 (59.6)	<0.001
ACEi/ARB	28 (27.7)	20 (42.6)	0.07
Beta-Blocker	18 (17.8)	21 (44.7)	<0.001
MRA	2 (2.0)	6 (12.8)	0.007

MACE, major adverse cardiac events; mWC, manual wheelchair; pWC, powered wheelchair; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, Mineralocorticoid receptor antagonist.

Table 5.9 Univariable Cox-Regression Analysis on the Association Between Clinical Factors and Major Adverse Cardiac Events.

Parameter	HR (95% CI)	*P* value
Age (5y)	1.1 (1.0-1.2)	0.04
Sex	1.5 (0.8-2.8)	0.22
MD group		
Dystrophinopathies (ref)	-	-
Limb-Girdle Muscular Dystrophy	1.6 (0.8-3.2)	0.22
Type 1 Myotonic Dystrophy	1.3 (0.6-2.5)	0.53
Respiratory Disease	1.1 (0.6-1.9)	0.86
Baseline Cardiac Medication	2.6 (1.4-4.6)	0.002
LVEF Less than 55% (*n* = 85)	3.3 (1.6-6.6)	<0.001
LVESV Indexed to BSA Greater than 36.43 mL/m^2 (*n* = 50)	1.5 (0.8-2.9)	0.19
LVESV Indexed to Height Greater than 40.68 mL/m (*n* = 76)	1.9 (1.0-3.5)	0.04
LVEDV Indexed to BSA Greater than 82.73 mL/m^2 (*n* = 66)	1.1 (0.6-2.1)	0.75

LVEDV Indexed to Height Greater than 88.45 mL/m (n = 79)	1.6 (0.9-2.9)	0.14
LV Mass Indexed to BSA Greater than 46.09 g/m^2 (n = 47)	1.6 (0.8-3.1)	0.15
LV Mass Indexed to Height Greater than 49.81 g/m (n = 96)	1.5 (0.8-2.8)	0.24
Circumferential Strain Amplitude Less than -13.01% (n = 55)	3.7 (1.8-7.4)	<0.001
Longitudinal Strain Amplitude Less than -14.00% (n = 44)	3.7 (1.9-7.1)	<0.001
Radial Strain Amplitude Less than 42.58% (n = 54)	3.3 (1.6-6.6)	<0.001
Minimum Principal Strain Amplitude Less than -24.44% (n = 34)	5.2 (2.7-10.2)	<0.001
Maximum Principal Strain Amplitude Less than 59.31% (n = 48)	3.4 (1.7-6.8)	<0.001
Late Gadolinium Enhancement Presence (n = 71)	1.2 (0.7-2.2)	0.55
Late Gadolinium Enhancement Burden (%)	1.8 (0.9-3.7)	0.10
Extracellular Volume Greater than 28.26% (n = 56)	1.1 (0.4-2.7)	0.86

Assessment of the prognostic ability of patient categorization by parameters obtained by cardiac magnetic resonance for major adverse cardiac events. HR, hazards ratio; CI, confidence interval; LV, left ventricular; EF, ejection fraction; BSA, body surface area; ESV, left ventricular end-systolic volume; EDV, left ventricular end-diastolic volume.

Multivariable risk modelling was performed to assess the independent predictive utility of all CMR parameters following their assessment by univariable cox regression (Table 5.10). Candidate CMR markers were assessed according to optimal binary thresholds, established by ROC analysis. Following adjustment for age, sex, MD type, respiratory disease, and baseline cardiac medication use, the following CMR markers were independently associated with MACE: LVEF < 55% remained independently predictive of MACE (aHR: 3.0; 95% CI: 1.4-6.4; p=0.006) 3D-GCS less than 59.31% (aHR: 3.4; 95% CI: 1.6-7.2; p=0.001), 3DmaxPS less than 59.31% (aHR: 3.3; 95% CI: 1.6-6.8; p=0.002), 3D-GLS less than -14.00% (aHR: 3.4; 95% CI: 1.7-6.9; p<0.001), and 3D-GRS less than 42.58% (aHR: 3.0; 95% CI: 1.4-6.1; p=0.003) (Table 5.11 and

Figure 5.9). Greatest performance was provided by 3DminPS amplitude less than -24.44% (aHR: 5.5; 95% CI: 2.5-11.9, $p<0.001$) with the lowest AIC and highest C-index (Table 5.10). Chamber volumes, LGE presence, LGE burden, and ECV were not independently associated with MACE in the overall cohort. However, LGE presence was associated with MACE in sub-group analysis of hemizygous DMD and BMD patients (Figure 5.6). The predictive value of 3D strain markers over supported by the likelihood ratio test where 3D strain provided incremental value to LVEF for the prediction of MACE across all directions of deformation, including 3DminPS (change in $\chi^2=13.2$, $p<0.001$), 3D-GCS (change in $\chi^2=6.1$, $p=0.01$), 3D-GLS (change in $\chi^2=6.9$, $p=0.009$), 3D-GRS (change in $\chi^2=4.2$, $p=0.04$), and 3DmaxPS (change in $\chi^2=5.6$, $p=0.02$).

Table 5.10 Association Between CMR-Derived Parameters and Major Adverse Cardiac Events.

	Null Model		**LVEF** (< 55%; *n* = 85*)		**3D-GCS** (<-13.01%; *n* = 55*)		**3D-GLS** (<-14.00%; *n* = 44*)		**3D-GRS** (<42.58%; *n* = 54*)		**3DminPS** (<-24.44%; *n* = 34*)		**3DmaxPS** (<59.31%; *n* = 48*)	
	aHR (95% CI)	*P* value	aHR (95% CI)	*P* value	aHR (95% CI)	*P* value	aHR (95% CI)	*P* value	aHR (95% CI)	*P* value	aHR (95% CI)	*P* value	aHR (95% CI)	*P* value
CMR Parameter	-	-	3.0 (1.4-6.4)	0.006	3.4 (1.6-7.2)	0.001	3.4 (1.7-6.9)	<0.001	3.0 (1.4-6.1)	0.003	5.5 (2.5-11.9)	<0.001	3.3 (1.6-6.8)	0.002
Age (5y)	1.1 (1.0-1.2)	0.17	1.1 (1.0-1.2)	0.13	1.0 (1.0-1.2)	0.21	1.1 (0.9-1.2)	0.37	1.1 (0.9-1.2)	0.28	1.0 (0.9-1.2)	0.87	1.1 (1.0-1.3)	0.21
Sex	1.4 (0.7-2.9)	0.37	1.2 (0.6-2.4)	0.71	1.2 (0.5-2.8)	0.69	1.1 (0.5-2.6)	0.87	1.3 (0.5-3.1)	0.56	1.0 (0.4-2.3)	0.93	1.1 (0.5-2.6)	0.79
MD Group														
Dys (ref)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LGMD	1.4 (0.6-3.2)	0.41	1.8 (0.8-4.0)	0.16	1.5 (0.7-3.7)	0.33	1.6 (0.7-3.8)	0.28	1.3 (0.6-3.2)	0.52	2.1 (0.9-5.1)	0.09	1.2 (0.5-3.0)	0.62
DM1	1.6 (0.7-3.6)	0.31	1.7 (0.7-4.0)	0.21	1.2 (0.5-3.2)	0.70	1.4 (0.6-3.6)	0.48	1.3 (0.5-3.4)	0.65	2.2 (0.8-5.9)	0.10	1.2 (0.5-3.3)	0.68
Respiratory Disease	0.9 (0.5-1.7)	0.75	1.0 (0.5-1.8)	0.94	0.7 (0.4-1.6)	0.48	0.7 (0.3-1.4)	0.30	0.6 (0.3-1.3)	0.22	0.7 (0.3-1.4)	0.27	0.6 (0.3-1.3)	0.23
Baseline Cardiac Medication	2.6 (1.4-5.1)	0.004	2.0 (1.0-3.8)	0.04	2.1 (1.0-4.3)	0.05	2.2 (1.1-4.4)	0.04	2.1 (1.0-4.4)	0.05	1.9 (0.9-4.1)	0.08	2.1 (1.0-4.5)	0.05
AIC	142.4		166.4		140.2		139.2		142.1		127.2		135.3	
C-index	0.71 (0.64-0.79)		0.75 (0.68-0.82)		0.75 (0.67-0.84)		0.75 (0.66-0.83)		0.73 (0.65-0.82)		0.84 (0.78-0.91)		0.72 (0.62-0.82)	

*n indicates number of patients affected by reduced CMR parameter. LVEF, left ventricular ejection fraction; 3D-GCS, Circumferential strain amplitude; 3D-GLS, Longitudinal strain amplitude; 3D-GRS, Radial strain amplitude; 3DminPS, Minimum principal strain amplitude; 3DmaxPS, Maximum principal strain amplitude; aHR, adjusted hazard ratio; CI, confidence interval; Dys, Dystrophinopathies; LGMD, limb-girdle muscular dystrophy; DM1, type 1 myotonic dystrophy; AIC, Akaike's information criterion; C-index; Concordance index.

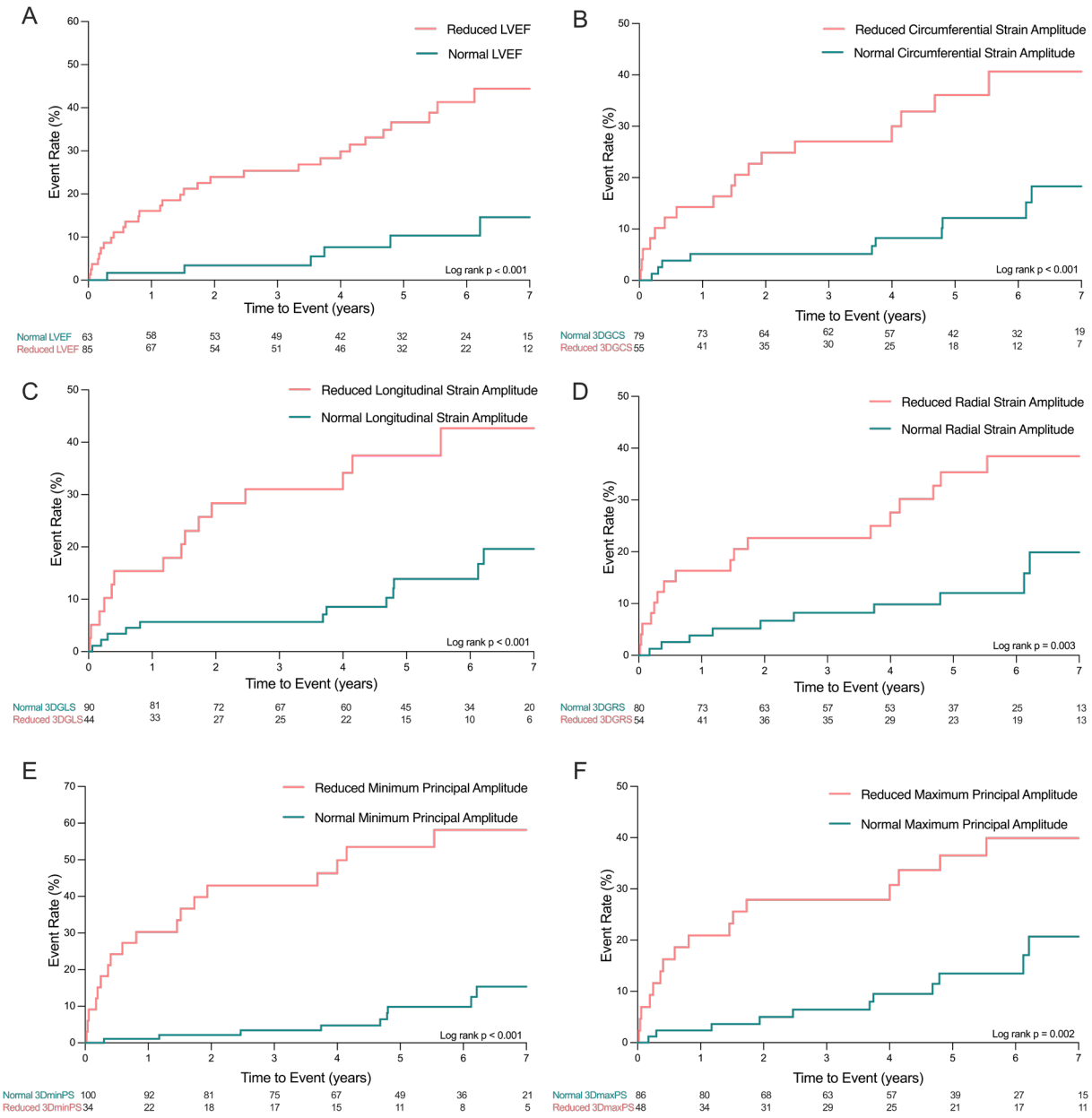

Figure 5.9 Kaplan-Meier analysis of major adverse cardiac events from baseline over time for risk stratification based on CMR-derived parameters in muscular dystrophy patients.

LVEF, Left ventricular ejection fraction; 3D-GCS, Circumferential strain amplitude; 3D-GLS, Longitudinal strain amplitude; 3D-GRS, Radial strain amplitude; 3DminPS, Minimum principal strain amplitude; 3DmaxPS, Maximum principal strain amplitude.

5.4 Discussion

In this study we examined the distribution and predictive utility of CMR-derived markers for the prediction of MACE in patients with MD. Supported by the largest adult study cohort examined to date by CMR, we identified clinically relevant phenotypic differences between MD subtypes, showing a prominent replacement fibrosis with LV dysfunction phenotype in dystrophinopathy patients, a milder but similar phenotype in LGMD, and reduced cardiac mass with minimal fibrosis phenotype in DM1. Of all investigated CMR markers, 3D myocardial strain offered the strongest capacity to predict cardiovascular outcomes in our cohort of MD patients. In particular, the geometry-independent strain marker of 3DminPS amplitude identified patients at a 5.5-fold elevated risk of MACE independent of baseline characteristics, showing superior predictive performance over LVEF. Similarly, the composite model of 3DminPS and LVEF had significant additive value over LVEF alone for risk stratification.

We observed both geometry-dependent and -independent 3D markers of myocardial deformation to be uniformly reduced compared to healthy controls and were predictive of future. Following adjustment, 3D-minPS delivered optimal performance for the prediction of outcomes, a marker describing the maximal amplitude of local tissue shortening when measured along its innate direction of deformation.[135-137] Superior performance for this marker relative to geometry-dependent strain estimates is postulated to reflect more accurate estimation of local myofibril contractile health through elimination of off-axis errors.[138] Several studies have previously examined the role of myocardial strain in patients with MD.[140-144] Of particular relevance, a recent study Azzue, *et al.,* also applied a 3D strain analysis technique to study conventional, geometry-dependent measures in 111 patients with a broad range of neuromuscular diseases inclusive of, but not limited to MD.[144] Similar to our study they observed both 3D-GCS and 3D-GRS are associated

with the presence of replacement fibrosis. Incremental to this publication, our study provided a greater focus on MD, assessed geometry-independent 3D strain markers, and followed patients for MACE to determine the predictive value of these markers. LVEF in neuromuscular disease patients is valuable for risk stratification and associated with plasma markers for cardiac remodeling.[139] Recognizing LVEF is a well-established prognostic marker in patients with MD[139, 145, 146], our study provided objective evidence that myocardial strain delivers incremental prognostic value to LVEF in this vulnerable patient population.

We observed important differences in cardiac remodeling among the different types of MD in our cohort. Cardiac phenotypes in the dystrophinopathies cohort were distinguishable from LGMD and DM1 cohorts, given larger LV volumes, greater prevalence and burden of LGE, and greater reduction in LVEF with corresponding low 3D-GRS amplitudes. The heterozygote population in dystrophinopathies are frequently overlooked or dismissed as a milder version of dystrophinopathies; in contrast to this belief, our results demonstrate the heterozygotes were at risk of experiencing MACE and exhibit impaired systolic function, reduced geometry-dependent and 3DminPS amplitudes, and significant burden of fibrosis consistent with the dystrophinopathies phenotype.[54] The LGMD cohort experienced similar features, including elevated volumes, reduced systolic function and high burden of fibrosis, to a milder extent. However, these cohorts lacked objective evidence of eccentric hypertrophy; in particular, DM1 patients had a unique phenotype with lower LV mass compared to healthy controls, reduced atrial and ventricular chamber volumes. Type 1 myotonic dystrophy is a multisystemic disorder caused by a CTG repeat expansion within the 3'-UTR of the myotonic dystrophy protein kinase (*DMPK*) gene. Interestingly, the primary defect associated with this genetic variant is impaired myogenesis[147-149], suggesting that impaired cardiac growth is primarily responsible for the reduced LV mass and

volume. Moreover, physical impairment was less abundant in the DM1 cohort and therefore a sedentary lifestyle with limited exercise ability is not likely leading to cardiac atrophy. While the cardiomyopathy in patients with MD is reminiscent of a dilated cardiomyopathy phenotype[117, 150, 151], our data shows relatively mild chamber dilation, small left atrial volumes, and minimal LV hypertrophy. This contrasts with other cardiomyopathy states, whereby adverse and progressive eccentric or concentric hypertrophy is commonly observed during disease progression.[152, 153]

Assessment of replacement fibrosis by LGE and diffuse fibrosis by T1-mapping showed a high prevalence of the former among patients with dystrophinopathies. The pattern and distribution of these findings were as described by prior reports with subepicardial or transmural replacement fibrosis of the inferolateral segments.[154, 155] In addition, myocardial fibrosis was associated with decreased function in affected regions. In contrast, fibrosis was less prevalent in DM1 and LGMD patients and when present was localized to the lateral wall subepicardial regions. Even though LGE presence has prognostic utility in other cardiomyopathies and is associated with adverse remodeling in MD patients, our investigation showed that LGE was not a significant predictor of MACE except in the dystrophinopathies cohort.[156-158] Furthermore, early-onset of myocardial fibrosis in pediatric DMD patients is associated with a corresponding reduction in systolic function and increase in risk of mortality; however, the associated risk of mortality declines with age.[145] Meanwhile, the link between myocardial fibrosis and heart disease in DM1 patients is unclear. While Chmielewski *et al*. report LGE presence is an independent predictor of atrial fibrillation and flutter events, Petri *et al*. demonstrated LGE presence is not associated with ECG or Holter monitor abnormalities.[159, 160] Considering the low burden of fibrosis and lack of predictive value of MACE in our cohort, myocardial fibrosis is not likely to be the primary driver of heart disease experienced by DM1 patients. Diffuse fibrosis has been previously observed in patients with MD using native

(non-contrast) T1 mapping compared to healthy controls.[161, 162] In contrast to prior investigations, our ECV-based assessment of diffuse fibrosis did not reveal elevated values in our MD cohorts relative to healthy controls. The management of comorbidities (hypertension, diabetes, and dyslipidemia), LV systolic dysfunction and respiratory disease can minimize the risk of heart damage in MD patients.[163-165] Indeed, MRA and ACEi are well known to reverse myocardial fibrosis and the prevention of nocturnal hypoxemia (respiratory therapy) and comorbidities can minimize myocardial ischemia.[166, 167]

Study limitations

We recognize certain limitations associated with our study. While multi-center, and the largest adult MD cohort to date evaluated by CMR, our cohort size remains modest and reflects the challenge of conducting CMR-based studies in patients with MD. Ongoing recruiting of patients to our cohort will increase size for enhanced validation in future studies, including permitting sub-group analyses. Our multi-center design also recognizes differences in imaging hardware. This prevented our capacity to assess the predictive utility of native T1 values, given inherent reliance on field strength and pulse sequence.

5.5 Conclusions

Muscular dystrophy represents a collection of rare genetic diseases, each of which associated with a distinct cardiomyopathy phenotype that can be identified by comprehensive CMR imaging. We showed contractile dysfunction (with replacement fibrosis) among dystrophinopathies, intermediate expression of a similar phenotype in LGMD, and reduced cardiac growth without replacement fibrosis phenotype in DM1. Furthermore, of all studied CMR-based markers, 3DminPS delivered the strongest prognostic value that can be used independently or as a composite to LVEF to identify patients at high risk of future adverse outcomes.

Chapter 6. Discussion

6.1 Discussion and Impact of Research

Current cardiac monitoring modalities, including electrocardiography, echocardiography, and cardiac magnetic resonance (CMR) imaging, can capture a comprehensive view of cardiac manifestation. These clinical tools provide practical and impactful utility for risk stratification and prognostication. Our investigation sought to leverage traditional tools for cardiac assessment to elucidate the phenotypic manifestation and pathology in rare diseases and identify which cardiac features are predictive of poor outcomes. Progressive and chronic diseases like Fabry disease (FD) and muscular dystrophies (MD) are at substantial risk of major adverse cardiac events (MACE), ultimately adding to the burden on healthcare facilities. Through this thesis, we discuss the practical and important value of incorporating the strategic use of these techniques to facilitate therapeutic decision-making and prevent disease progression.

Our study established markers of disease progression, such as left ventricular hypertrophy (LVH) and myocardial fibrosis and inflammation, as predictors of MACE in FD. The beginning stages of the development of heart disease in FD are characterized by the accumulation of lyso-GB3 in several cell types without the presence of LVH and reduced native T1 levels.[168] The lowering of T1 mapping is associated with fat accumulation during the accumulation stage and early signature of FD pathology.[93] In heterozygous women, there is partially preserved α-galactosidase A activity that translates to mild/absent lipid accumulation in myocytes with correspondingly low T1 values and lower prevalence of hypertrophy.[169] Many heterozygous females tend to appear mild or asymptomatic but may manifest with the classic phenotype of FD and are still vulnerable to life-threatening arrhythmias.[169]

The advanced stages of cardiac involvement in FD are characterized by concentric ventricular hypertrophy, inflammation, and myocardial fibrosis.[87] In our investigation, we characterized the clinical profile of the advanced stages of FD identified with elevated LVM and LGE using CMR imaging. Our investigation shows: (1) LVH is more prevalent in male patients then female patients, (2) CMR parameters, LGE and LVH, are predictors of MACE that lead to poor quality of life, and (3) comorbidities, including dyslipidemia and hypertension, are additional predictors of MACE that are important targets for therapeutic intervention (Figure 6.1).

Improvements in diagnostic techniques and strategies targeting unexplained hypertrophic patients have enabled earlier initiation of Fabry-specific therapies in patients' disease courses.[9, 77] Enzyme replacement therapy has been demonstrated to attenuate or even revert the progression of hypertrophy; in contrast, myocardial fibrosis is unmodifiable.[65] Myocardial fibrosis is a significant risk factor that has grown in this population, particularly in female heterozygotes, for which LGE prevalence is equivalent in both sexes. In addition, the presence of comorbidities and FD pathogenesis can exacerbate the progression of each other through interference of endothelial dysfunction, oxidative stress, and activation of inflammatory pathways.[88, 170, 171] Thus, lipid profile and blood pressure monitoring and early intervention with statin therapy can prevent further complications and improve patient outcomes. Employing a clinical management strategy with frequent monitoring of markers of cardiac remodelling, early intervention with Fabry-specific therapies and strict management of comorbidities may attenuate disease progression, reduce risk of MACE, and improve quality of life (Figure 6.1).

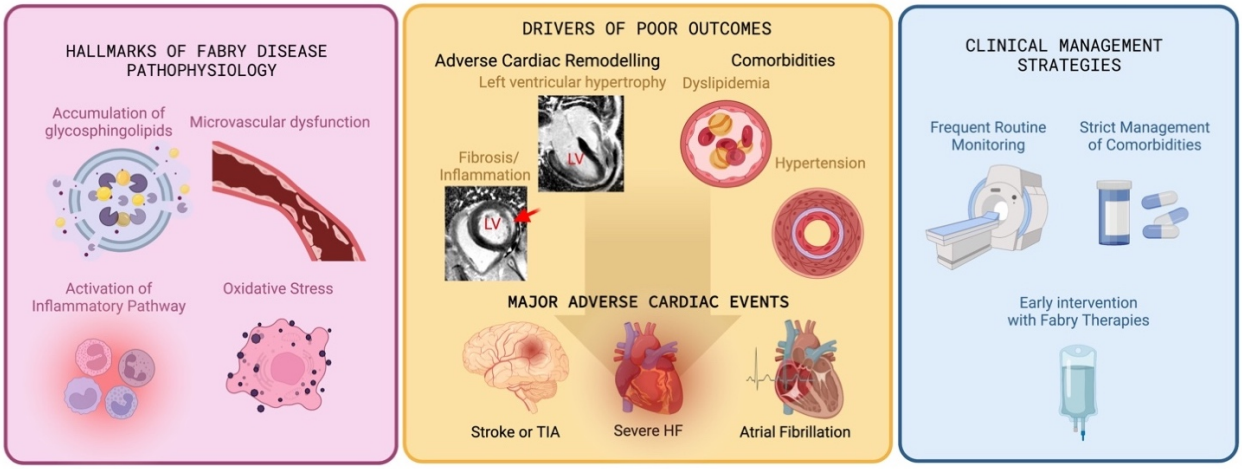

Figure 6.1 The combined pathological effects of the hallmarks of Fabry disease pathology lead to changes in heart morphology and function that can be detected by cardiac magnetic resonance imaging. Presence of markers of cardiac remodelling and comorbidities predict major adverse cardiac events. Frequent monitoring and management of risk factors and early intervention with Fabry-specific therapies attenuate disease progression and prevent poor outcomes.

Patients with muscular dystrophies commonly develop heart disease and are at substantial risk of poor outcomes. Adverse cardiac remodeling in this population is commonly associated with dilated cardiomyopathy (DCM); however, phenotyping the types of MD can identify key features of MD-driven heart disease beyond the DCM phenotype. Our investigations sought to leverage traditional cardiac monitoring tools to explore phenotypic characterization that can be used to guide the care of patients with MD. Variability in cardiac manifestations poses a challenge to the clinical management of EDMD patients. Conduction system disease and dilated atria are the two most prevalent cardiac manifestations in patients with EDMD. Early detection of conduction disease with Holter monitoring and ECG for bradycardia, prolonged PR interval, reduced P wave amplitude, nodal dysfunction, and ventricular arrhythmias is crucial. Underlying conduction disease indicate pacemaker insertion at the first sign of bradycardia or nodal dysfunction. As such,

all four cases of EDMD required cardiac device intervention between late teens to early thirties. Regular monitoring with device interrogation, ECG, and TTE allows for close monitoring of disease progression is recommended. Overall, these patients exhibited preserved systolic function despite eccentric ventricular hypertrophy or mild chamber dilation. As seen with Patient 3, serial tracking of blood pressure and TTE parameters allow for efficient and adaptable therapeutic decision-making that reverses potentially adverse cardiac remodelling. Consistent monitoring and managing of cardiac risk factors in a multidisciplinary setting allow for a patient-centred approach to managing this rare disease.

Understanding the complexities of managing heart disease in patients with rare diseases can improve outcomes, quality of life, management practices, and therapeutic decision-making. Close monitoring and early intervention are necessary to prevent adverse cardiac events. Ongoing communication and collaboration between healthcare providers, patients, and their families are critical in optimizing care and reducing the burden of cardiac disease in these populations. Incorporating a multidisciplinary care approach involving cardiologists, neurologists, respiratory therapists, and dieticians can help provide comprehensive care to individuals with EDMD and ensure appropriate cardiac monitoring and interventions are in place to prevent adverse cardiac events.[172]

Cardiac magnetic resonance imaging is a valuable tool for phenotypic characterization and prognostication in patients with MD. By detecting and characterizing structural, kinetic, and tissue composition features through conventional cardiac monitoring modalities, we hope to improve our understanding of these diseases and enhance our ability to manage and treat patients. Our study assessed three major types of MD using CMR imaging to explore differential adverse myocardial remodeling compared to a healthy control cohort and its influence on MACE (Figure 6.2).

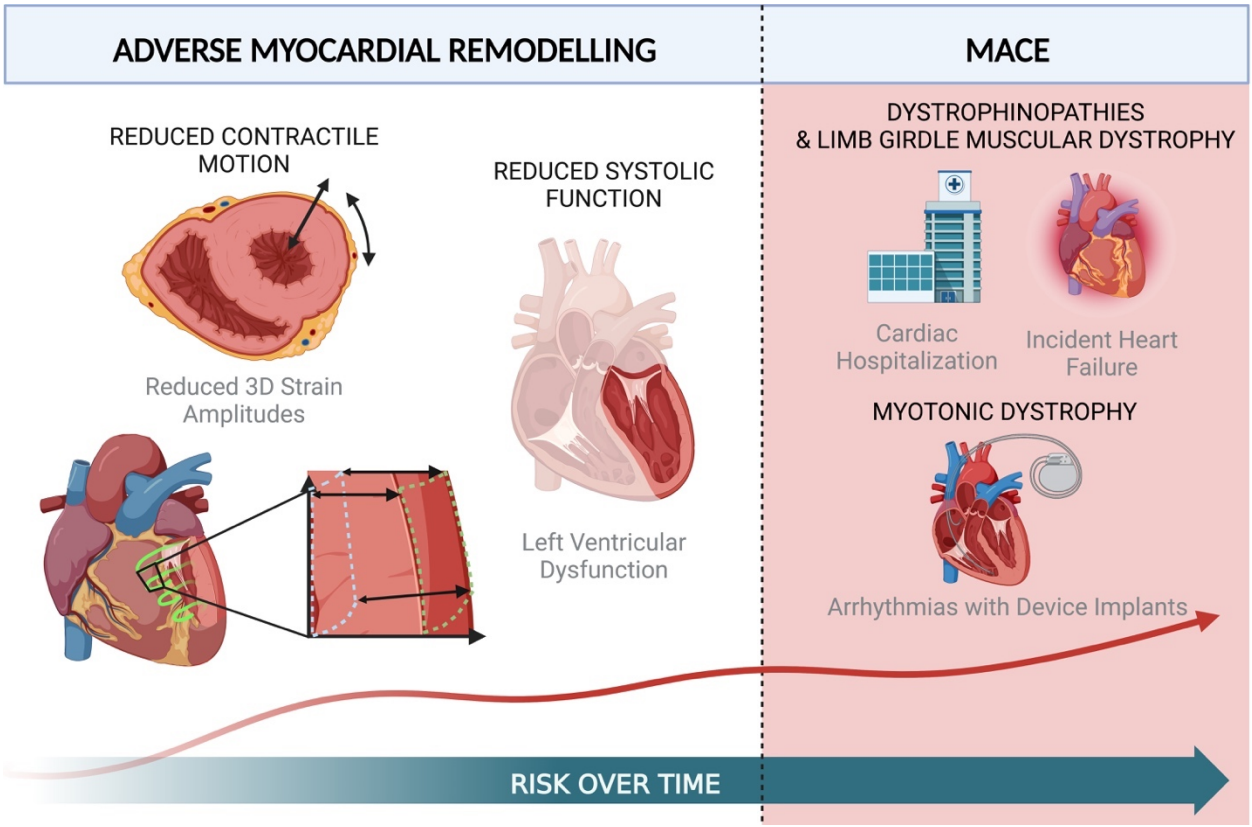

Figure 6.2 In muscular dystrophy patients, markers of adverse cardiac remodelling, reduced 3D strain amplitudes and left ventricular function are indicative of increased risk of major adverse cardiac events (MACE) over time.

While speckle tracking echocardiography can be used for strain-based evaluations, its performance can be hindered due to limited acoustic windows as a result of scoliosis, obesity, and lung disease[128, 173], which are highly prevalent in patients with MD.[119] CMR is the preferred method for myocardial strain analysis as a high-resolution, accurate and reproducible alternative to echocardiography. The application of CMR in the MD population can be complicated due to extensive musculoskeletal involvement leading to difficulty in positioning and lying still, and presence of incompatible devices with CMR steel rods or non-invasive ventilators.[130, 174, 175] Widespread use of titanium alloy steel rod implants, which are CMR compatible, have become common practice.[176] As well, enhancements in CMR technology have improved the accessibility

for advanced patients by real-time and motion corrected techniques.[109, 177] As demonstrated in our study, strain analysis is a valuable tool for risk stratification of MD patients, thus we recommend incorporating CMR imaging as part of routine clinical care in patients with MD.[178, 179]

The dystrophinopathies and limb-girdle MD (LGMD) exhibit impaired systolic ventricular function and mild to normal dilated left ventricle. There was a high prevalence and burden of myocardial fibrosis in dystrophinopathies and a moderate presence/burden in LGMD. In contrast, type 1 myotonic dystrophy (DM1) patients exhibit low prevalence and burden of myocardial fibrosis, reduced LV mass, and impaired systolic function. Reduced LVEF was common among patients with left bundle branch block. In addition to markers of replacement and diffuse myocardial fibrosis, we studied 3-dimensional myocardial deformation analysis (3D-MDA) and reported reduced levels of both geometry-dependent and geometry-independent across the different types of MD.

The next stage of our study sought to assess the prognostic value of these distinguishing features. Clinical outcomes over a median of 5.2-years documented 80 MACE. Even though replacement myocardial fibrosis is a common manifestation associated with the MD phenotype, particularly in dystrophinopathy patients, fibrosis was not an independent predictor for MACE in MD patients. However, our additional analysis on dystrophinopathy hemizygotes determined that myocardial fibrosis, excluding subepicardial regions, was an independent predictor. In addition, anterolateral and inferoseptal regions of replacement and diffuse fibrosis had a low to moderate positive correlation to regions of reduced contractile function in the left ventricle. Therefore, fibrosis may not be a contributing factor to regional contractile dysfunction.

The well-established prognostic marker, LVEF, was an important predictor of MACE in these patients, as such is an important clinical factor for risk stratification (Figure 6.2). In addition,

reduced global strain amplitudes were also revealed to be strong predictors of MACE comparable to LVEF. Reduced 3D minimum principal strain amplitude was the strongest independent predictor of MACE, identifying patients at a 5-fold elevated risk of adverse outcomes and was superior to LVEF. Furthermore, 3D strain analysis has been demonstrated to have incremental value to LVEF for risk assessment. By disseminating important CMR parameters for phenotypic characterization and prognostication, we can prioritize monitoring these parameters, guide therapeutic decision-making by identifying at-risk patients, and establish guidelines for the clinical management of MD patients. Ultimately, the impact of heart disease on disease progression, patient longevity and quality of life can be improved for patients with rare genetic diseases.

6.2 Future Directions

This paper outlines future research directions for expanding the understanding of CMR parameters in characterizing FD and MD patients. We discuss current therapeutic management strategies and the utility of novel CMR techniques in predicting poor outcomes. Our future research will address the utility of CMR parameters to guide therapeutic decision-making and illustrate the potential benefits of integrating cardiac biomarkers in clinical practice. In the rapidly advancing field of FD and MD management, CMR imaging is emerging as an essential diagnostic and prognostic tool. We can improve our understanding of FD phenotypic characterization and pathology by assessing a broader range of CMR parameters, such as 3D-MDA and T1/T2 mapping. We can also investigate how CMR-based markers of remodelling correlate with circulating cardiac biomarkers and the additive prognostic value of biomarkers to CMR imaging. This can provide insight into underlying molecular features associated with overt cardiac dysfunction and assess the utility of integrating various therapeutic management strategies. In addition, we can also validate our findings on the utility of CMR markers and other crucial markers

by recapitulating these studies in larger cohorts. There are inherent limitations with studying rare diseases, such as small sample sizes, variable follow-up durations, and heterogeneous phenotypes. We will develop strategies to overcome these challenges and enhance the reliability of our research findings.

One of the primary goals of our future research is to determine the effectiveness of CMR-based parameters in guiding therapeutic decision-making for MD and FD patients. We will assess whether introducing or uptitrating dosages of the classic triad of therapeutic interventions (ACEi, MRA, and BB) can improve left ventricular ejection fraction (LVEF) and strain amplitudes in MD patients. Our research will explore the potential of serial assessments of CMR parameters in capturing the disease-modifying effects of ACE inhibitors, MRA, and beta-blockers. These assessments may provide a more comprehensive understanding of FD and MD patients' cardiac conditions and incorporate the role of pharmacotherapeutics in modifying disease progression and improving outcomes.

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