Pregnancy and progression of cardiac disease in genetic cardiomyopathies

Thesis of the degree of Philosophiae Doctor (Ph.D.)

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Summary

Genetic cardiomyopathies are hereditary cardiac diseases often diagnosed in young patients and are characterized by live-threatening arrhythmias and heart failure. Among them are arrhythmogenic (AC) and Lamin A/C cardiomyopathy. At genetic diagnosis, penetrance of the disease is often incomplete and factors promoting the disease progression are poorly recognized. Physical exercise is a hemodynamic stress and was previously associated with higher disease penetrance in AC and Lamin A/C cardiomyopathy. Pregnancy is also a hemodynamic stress, therefore with a potential detrimental effect on cardiac disease for patients with a genetic predisposition; however, the role of pregnancy as a factor potentially promoting disease progression in AC and Lamin A/C cardiomyopathy is poorly identified. The results of this thesis contributed to increase our knowledge in the field, showing no significant effect of pregnancy on long term structural and functional disease progression, and no major effect on arrhythmias, in AC and Lamin A/C cardiomyopathy. Additionally, we described the stages of disease progression in Lamin A/C cardiomyopathy, showing a high prevalence of electrical disease at young age, followed by left and right ventricular dysfunction, where the latter was independently associated with adverse prognosis.

Sammendrag

Genetiske kardiomyopatier er arvelige hjertesykdommer hvor diagnose ofte stilles tidlig i voksen alder. De kjennetegnes ved alvorlige hjerterytmeforstyrrelser og hjertesvikt. Blant genetiske kardiomyopatier finnes arytmogen kardiomyopati (AC) og Lamin A/C kardiomyopati. Innenfor en enkelt genetisk diagnose er fenotypen heterogen og faktorer som fremmer sykdommens penetrans og utvikling av hjertesykdom er dårlig kartlagt. Trening er assosiert med høy sykdomspenetrans i AC og Lamin A/C kardiomyopati. I likhet med trening, er graviditet en hemodynamisk stressor, som kan utløse hjertesykdom hos pasienter med genetisk disposisjon. Det er så langt lite undersøkt i hvilken grad graviditet fremmer sykdomsprogresjon hos pasienter med AC og Lamin A/C. Dette forskningsprosjektet bidro til å øke vår kunnskap på feltet, og viste ingen signifikant effekt av graviditet på langsiktig strukturell og funksjonell sykdomsprogresjon, og ingen større effekt på arytmier ved AC og Lamin A/C kardiomyopati. I tillegg beskrev vi stadiene av sykdomsprogresjon i Lamin A/C kardiomyopati, og viste en høy forekomst av elektrisk sykdom i ung alder, etterfulgt av venstre og høyre ventrikkeldysfunksjon, hvor sistnevnte var uavhengig assosiert med ugunstig prognose.

Acknowledgements

The work included in this thesis was started in 2016, when I came to Norway for the first time and worked for nine months as research fellow at Center for Cardiological Innovation, Department of Cardiology, Oslo University Hospital, Rikshospitalet. Later, from August 2019 until September 2022, I came back and continued my research work as PhD student at ProCardio Center for Innovation, Department of Cardiology, University of Oslo, and Institute of Clinical Medicine, Faculty of Medicine, University of Oslo. Funding was provided through the Norwegian Research Council.

Professor Kristina H. Haugaa has been my supervisor since 2016, and it was after meeting with her that I matured my decision to apply for the PhD program. Kristina Haugaa is internationally recognized for her knowledge in genetic cardiomyopathies; she is a brilliant cardiologist and researcher. She is a hard-worker and one of the most productive researchers in Norway. She is a dedicated teacher and a supportive supervisor. Her passion for scientific research is contagious, and she has inspired me all along the way. She is an iconic woman. Thank you, Kristina. You encouraged my ambitions and enthusiasm and shaped my career. I will always be grateful for the opportunity to have worked with you.

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To conclude, thank you to all the patients I met on my path. You are the real inspiration for my work. Thank you.

List of papers

(1) **Number of pregnancies and subsequent phenotype in a cross-sectional cohort of women with arrhythmogenic cardiomyopathy**.

Castrini AI, Lie ØH, Leren IS, Estensen ME, Stokke MK, Klæboe LG, Edvardsen T, Haugaa KH.

Eur Heart J Cardiovasc Imaging. 2019 Feb 1;20(2):192-198.

(2) **Pregnancy and Progression of Cardiomyopathy in Women with LMNA Genotype-Positive.**

Castrini AI, Skjølsvik E, Estensen ME, Almaas VM, Skulstad H, Lyseggen E, Edvardsen T, Lie ØH, Picard KCI, Lakdawala NK, Haugaa KH

J Am Heart Assoc. 2022 Apr 19;11(8):e024960

(3) **Progression of cardiac disease in patients with lamin A/C mutations.**

Skjølsvik ET, Haugen Lie Ø, Chivulescu M, Ribe M, Castrini AI, Broch K, Pripp AH, Edvardsen T, Haugaa KH

Eur Heart J Cardiovasc Imaging. 2022 Mar 22;23(4):543-550

Abbreviations:

AC = arrhythmogenic cardiomyopathy

AF = atrial fibrillation

AV = atrio-ventricular

BSA = body surface area

DCM = dilated cardiomyopathy

EDD = end diastolic diameter

EDV = end diastolic volume

EF = ejection fraction

FAC = fractional area change

GLS = global longitudinal strain

HTx = heart transplantation

ICD = implantable cardioverter defibrillator

LA = left atrial

LMNA = gene encoding for Lamin A/C proteins

LMNA+ = pathogenic and likely pathogenic variants of LMNA

LS = longitudinal strain

LV= left ventricular

LVAD = left ventricular assist device

LVEF = left ventricular ejection fraction

MD = mechanical dispersion

NYHA = New York heart association

PLAX = parasternal long axis

PSAX = parasternal short axis

RV = right ventricular

RVD = right ventricular basal diameter

SAECG = signal averaged electrocardiogram

SCD = sudden cardiac death

SD = standard deviation

TAPSE = tricuspid annual plan excursion

TFC = Task Force Criteria

TR = tricuspid regurgitation

VA = ventricular arrhythmias

VT = ventricular tachycardia.

Introduction

Cardiomyopathies

Cardiomyopathies are a heterogeneous group of heart diseases, characterized by abnormal structure and function of the myocardium in absence of ischemic heart disease or pathological loading condition (4). Cardiomyopathies are progressive in nature and end-stage cardiomyopathies contribute to substantial morbidity and mortality by heart failure. In a recent study, the hospital-based prevalence was 809 per million inhabitants per year, accounting for 51% of all heart transplants, 33% of defibrillator implantations, 38% of mechanical circulatory supports, and 11% of hospitalizations for heart failure (5). The classification of cardiomyopathies is based on the clinical phenotype (6). Increasing knowledge in genetics and wider application of genetic tests have contributed to identifying the existence of an inherited predisposition for cardiomyopathies. Inherited cardiomyopathies are often diagnosed at relatively young age. These diseases rise attention due to the potential severity of the clinical manifestations characterized by malignant arrhythmias and/or heart failure. Sudden cardiac death (SCD) can be the first clinical manifestation and can occur in subjects previously considered healthy or "super healthy", like athletes. Variability of the penetrance and incomplete clinical expression are characteristic of cardiomyopathies (4). Environmental factors are known to have a potential role in influencing gene expression. The knowledge about environmental factors influencing penetrance and disease progression in inherited cardiomyopathy is limited, and this represent an important challenge for clinicians. Among inherited cardiomyopathies are arrhythmogenic cardiomyopathy and Lamin A/C cardiomyopathy, further discussed in the present thesis.

Arrhythmogenic Cardiomyopathy (AC)

AC, also known as arrhythmogenic right ventricular cardiomyopathy, is the inherited cardiomyopathy addressed by paper 1. In AC, the genetic defect is mostly located in genes encoding for desmosomal proteins, resulting in inadequate attachment of neighboring cardiomyocytes and progressive

fibro-fatty replacement of cardiac muscle (7). AC has a predominant autosomal dominant inheritance (8) and is a leading cause of SCD at age ≤ 35 (7). The estimated prevalence is of 1:1000 to 1:5000 (8). AC diagnosis is based on 2010 Task Force Criteria (TFC) (Table 1). TFC include a combination of data from clinical history, cardiac imaging, electrocardiographic examination, and tissue biopsy (9).

The clinical phenotype is characterized by ventricular arrhythmias (VA), where SCD can be the first disease manifestation (10). Structural and functional impairment of the right ventricle is often recognized; however, the penetrance is variable, and the cardiac phenotype can vary from asymptomatic concealed structural abnormalities to advance right ventricle dysfunction, requiring cardiac transplantation (8). Although originally considered predominant of the right ventricle, left ventricular involvement in AC has been increasingly identified (11).

Identification of AC patients at risk for SCD is difficult, and the evidence supporting risk factors for malignant VA and progression of the disease are poorly recognized. High-intensity exercise is the main well-recognized potential trigger for VA and SCD (12-14), and all patients with definite AC diagnosis are recommended to not participate in competitive and/or endurance sports (15). Implantable cardioverter defibrillator (ICD) implantation is the only effective therapy to prevent SCD in this population (15). Considering the young age of these patients and the lifetime risk of ICD-related complications, the decision of ICD implantation needs an accurate evaluation of pros and cons.

Table 1 – Diagnostic criteria for AC. Modified from Marcus et al (9).

Cardiac dysfunction and structural alteration	
Major criteria	Minor criteria
Regional contraction abnormalities [a] AND: • Severe dilatation of RVOT or Severe reduction in RVFAC by echocardiography or • Severe increase of RV volume or Severe reduction of RVEF by CMR	Regional contraction abnormalities [a] AND: • Mild dilatation of RVOT or Mild reduction of RVFAC by echocardiography or • Mild increase of RV volume or Mild reduction of RV EF by CMR
Tissue characterization by biopsy	
Major criteria	Minor criteria
Residual myocytes <60% with fibrous replacement, +/- fatty replacement	Residual myocytes 60% to 75% with fibrous replacement, +/- fatty replacement
Repolarization abnormalities	
Major criteria	Minor criteria
TWI ≥ V3 in precordial leads	• TWI ≥ V2 in precordial leads • TWI in V4, V5, V6 • TWI ≥ V4 n precordial leads with RBBB
Depolarization abnormalities	
Major criteria	Minor criteria
Epsilon wave in right precordial leads	• Late potential by SAECG • TAD ≥ 55 ms
Arrhythmias	
Major criteria	Minor criteria
NSVT or VT with LBBB and superior axis	• NSVT or VT with LBBB, inferior or unknown axis configuration • PVCs>500 per 24 hours
Family history	
Major criteria	Minor criteria
• Definite AC diagnosis by TFC or autopsy in a first degree relative • Pathogenic or likely pathogenic mutation identified by genetic test	• AC in first degree relative without possibility of definite diagnosis • SCD <35 due to suspect AC in first degree relative • Definite AC diagnosis in second degree relative

[a] Regional contraction abnormalities included akinesia, dyskinesia, or aneurism, identified by the corresponding echocardiography or CMR modality. AC = arrhythmogenic cardiomyopathy; CMR = cardiac magnetic resonance imaging; LBBB = left bundle branch block; NSVT = non-sustained ventricular tachycardia; PVCs = premature ventricular contractions; RBBB = right bundle branch block; RV = right ventricular; RVEF = right ventricular ejection fraction; RVFAC = right ventricular fractional area change; RVOT = right ventricular outflow tract; SAECG = signal averaged electrocardiogram; SCD = sudden cardiac death; TAD = terminal activation duration; TD = Terminal activation duration; TFC = Task force criteria; TWI = T wave inversion; VT = ventricular tachycardia.
Definite AC diagnosis: 2 major criteria *or* 1 major and 2 minor *or* 4 minor criteria from different categories.
Borderline AC diagnosis: 1 major criterion and 1 minor criterion *or* 3 minor criteria from different categories.
Possible AC diagnosis: 1 major criterion and 2 minor from different categories.

Lamin A/C cardiomyopathy

Dilated cardiomyopathy (DCM) is defined by the presence of left ventricular dilatation and impaired systolic function without signs of abnormal loading conditions (as arterial hypertension or valve diseases) or severe coronary artery disease (16). About half of the cases of DCM without detectable causes have a genetic substrate (17). Variants of Lamin A/C proteins, encoded by LMNA gene, are found in 4% to 8% of DCM (18), and in 33% of DCM with concomitant electrical conduction disease (19). The LMNA gene is located on chromosome 1 and encodes two proteins of the nuclear envelope, namely Lamin A and C, first described in 1999 in the research for genetic basis of Emery-Dreifuss muscular dystrophy (20). Lamin A/C are nuclear proteins with a critical role in preserving nuclear cytoskeletal integrity (21); however, the pathogenic mechanisms linking variation in Lamin A/C to myocardial dilatation and dysfunction are not known.

Lamin A/C cardiomyopathy is a malignant disease with high frequency of VA, and severe HF with need of heart transplantation (Htx) (22). The disease is mostly inherited as autosomal dominant. Complete age-related penetrance, early onset of atrio-ventricular (AV) block, atrial fibrillation, VA, and progressive DCM characterize the clinical phenotype (22-24). Neuromuscular disorders have been identified in about 20% of patients presenting with cardiac symptoms (25).

Increasing PR interval and AV block (22), together with non-sustained ventricular tachycardia (NSVT), left ventricular ejection fraction (EF) < 45% at diagnosis, male sex and non-missense mutations are known risk factors for malignant VA (25). However, factors influencing disease progression are poorly recognized. The knowledge about morphologic features characterizing Lamin A/C cardiomyopathy and the stages of progression towards end stage heart failure is limited, with important implications in the planning of follow-up programs.

Hemodynamic changes during pregnancy and cardiomyopathies

Pregnancy induces a series of physiological adaptations in the cardiovascular system (Figure 1). One of the earliest adaptations is peripheral vasodilatation, causing a fall in systemic vascular resistance

(26). This triggers a 40% increase in maternal blood volume matched by a comparable increase in cardiac output. The increase in cardiac output results from a rise in stroke volume and, later in pregnancy, from an increase in heart rate (27). From the second trimester, remodeling of the placenta is associated with a further reduction of systemic resistance and the lowering of blood pressure. Myocardial mass increases by 10-20%. During labor, anxiety, pain, and uterine contractions contribute to increase the stroke volume. After delivery, a further increase in cardiac output is observed as consequence of blood-loss, auto-transfusion of blood after labor due to uterus contractions, decompression on the inferior vena cava and resorption of edema (27). Pregnancy is known to be associated with increased risk of arrhythmias (28). Pro-arrhythmic mechanisms during pregnancy presumably include 1) an increase of circulating catecholamine; 2) the chronotropic effect of relaxin; 3) the mechanical effect of atrial stretch; 4) increased ventricular end-diastolic volume, as well as hormonal and emotional changes (29,30).

Cardiovascular adaptations related to pregnancy are mostly well tolerated in healthy females. However, in patients with a predisposition to cardiovascular disease or overt heart disease, pregnancy is associated with increased morbidity and mortality (31), and cardiovascular disease is the leading cause of maternal morbidity worldwide (32,33). Cardiomyopathies are associated with potential severe cardiovascular complications during pregnancy (34). Patients affected by genetic cardiomyopathies have a genetic predisposition to heart disease and are often diagnosed at a relatively early age compared to other cardiovascular diseases. Female patients are often at childbearing age (22,35), therefore, questions regarding the effect of pregnancy on disease progression, the safety, tolerance, and potential risks related to gestation and delivery often arise during clinical consultations with these patients. However, data about pregnancies in genetic cardiomyopathies are limited.

Sports activity has been recognized as a factor increasing disease's penetrance in AC (14,36-38) and patients are advised to avoid competitive and/or endurance sports (15). Hemodynamic adaptations occurring during pregnancy might mimic the mechanical stress induced by exercise in AC patients, therefore one may speculate about a possible negative effect of pregnancy on disease penetrance in AC;

however, data on the effect of one or repeated pregnancies on disease progression in AC are sparse (39-42) which makes it challenging to give advice to this population.

Pregnancy is considered as a potential "second hit" for the development of DCM in patients with genetic predisposition (43). In addition, sport active patients with variants in Lamin A/C gene were described to have worse LV function compared to sedate patients (44); however, the effect of pregnancy as hemodynamic stress, so potentially detrimental, on disease progression has not been previously explored in this population.

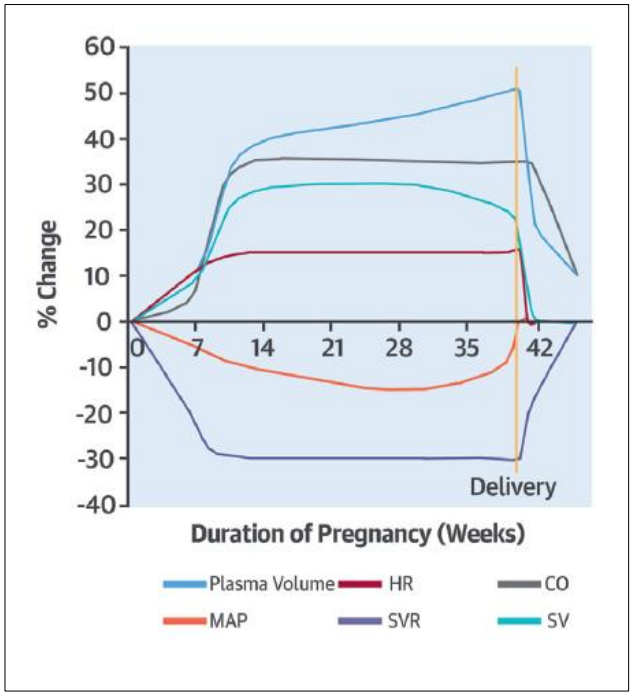


Figure 1 – Cardiovascular adaptation during pregnancy. Modified from Halpern et al.(45) HR = heart rate; CO = cardiac output; MAP = mean arterial pressure; SVR = systemic vascular resistance; SV = stroke volume.

Aims of the thesis

General aims of the thesis

We aimed to explore factors related to the progression of cardiac disease in patients with AC and Lamin A/C cardiomyopathy, including the effect of previous pregnancies on disease penetrance and progression. We aimed to describe the stages of progression of cardiac disease in patients with Lamin A/C cardiomyopathy.

Specific aims

Paper 1

We aimed to explore the effect of one or repeated pregnancies on cardiac phenotype in AC female patients. We hypothesized that pregnancy, as a state of prolonged hemodynamic stress, had a detrimental effect on cardiac disease. Thus, we hypothesized that AC female patients with repeated pregnancies had worse cardiac function and a higher burden of arrhythmias compared to AC females without history of pregnancy.

Paper 2

We aimed to explore the effect of pregnancy on disease progression in women with pathogenic or likely pathogenic variants of LMNA gene (LMNA+). We hypothesized that pregnancy, as a hemodynamic stress, was associated with a worse progression of cardiomyopathy in this population. We hypothesized pregnancy to accelerate the progression of cardiac dysfunction and arrhythmic disease LMNA+ female patients.

Paper 3

We aimed to describe the steps of progression of cardiac disease in LMNA+ patients, considering both structural and functional changes of left ventricle and electrical disease manifestations. We hypothesized that specific markers of disease progression were associated with heart failure outcomes in this population.

Objectives of the thesis

Paper 1

We had the following objectives:

1) To compare the severity of cardiac phenotype in AC female patients grouped by number of pregnancies.
2) To identify associations between number of previous pregnancies and structural and functional disease progression in AC female patients.
3) To explore the effect of number of pregnancies as risk markers of VA.
4) To compare the age at VA between AC female patients grouped by number of pregnancies.
5) To report maternal and fetal outcomes during pregnancy and delivery.

Paper 2

We had the following objectives:

1) To compare the severity of cardiac phenotype, arrhythmias, and heart failure outcomes in LMNA+ female grouped by pregnancy.
2) To explore the structural progression of cardiac disease in LMNA+ women grouped by pregnancy.
3) To explore the age at onset of atrial fibrillation, VA, and heart failure outcomes in nulliparous and women with previous pregnancies.
4) To report maternal and fetal outcomes during pregnancy and in the peripartum period.

Paper 3

We had the following objectives:

1) To describe the clinical presentation of Lamin A/C cardiomyopathy according to age.
2) To compare cardiac structural and functional progression, together with progression of electrical disease, in LMNA+ patients grouped by age.

3) To identify echocardiographic risk markers associated with adverse clinical outcomes in Lamin A/C cardiomyopathy.

Material

Study population

The participants in this thesis were recruited at the Department of Cardiology, Oslo University Hospital, Rikshospitalet, a tertiary referral hospital. Patients were referred for diagnostic evaluation and/or therapeutic approach by local hospitals; additionally, Unit for Cardiac and Cardiovascular Genetics, Department of Medical Genetics, Oslo University Hospital, referred patients to our department for a first cardiac evaluation after diagnosis of a pathogenic or likely pathogenic genetic variant. The frequency of clinical visits during follow-up time was based on clinical indication.

Paper 2 was the result of a collaboration with Brigham and Women's Hospital, Boston, USA, and we included patients followed by Cardiovascular Genetic Program at their center.

Only patients with available genetic test were included in this thesis. We defined probands as the first individuals in a family who were diagnosed with a genetic variant after having sought medical attention for clinical manifestations of the disease.

Paper 1

We performed a single center, cross-sectional cohort study. The study population in *paper 1* included AC female patients followed at Oslo University hospital. At the time of this study, the AC database included 162 participants with probands and family members, of whom 77 were female patients (Figure 2). Several patients were previously included in other AC studies from our research group (38,46,47). All participants were tested for AC related mutations and included if:

- Gene negative with a definite AC diagnosis based on the 2010 TFC (9)

  Or

- Gene positive with pathogenic or likely pathogenic variant regardless of fulfilling definite, borderline, or possible AC diagnosis according to 2010 TFC (9).

Patients with cardiopulmonary comorbidity were excluded.

A sub-cohort of 5 AC female patients followed-up at Rikshospitalet during pregnancy and post-partum period was considered for additional analysis (Figure 2).

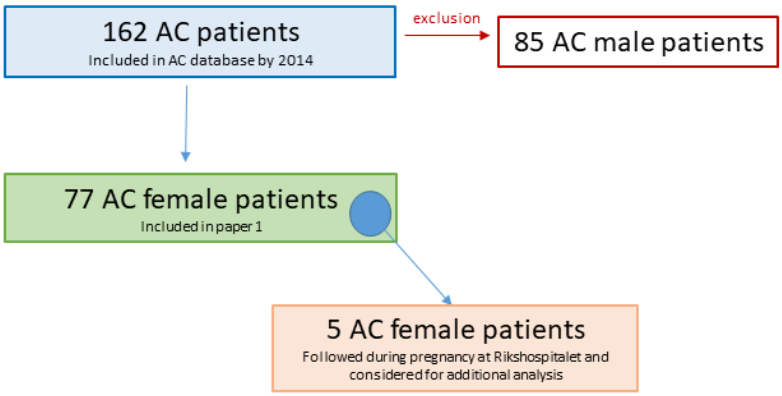


Figure 2 - Inclusion of patients in paper 1.

Paper 2

We performed a multicenter, retrospective, longitudinal study. The study population in paper *2* included LMNA+ female patients followed at Oslo University Hospital, Rikshospitalet, Norway and at Brigham and Women's Hospital, Boston, USA by the end of October 2019 (Figure 3). All included patients carried a pathogenic or likely pathogenic LMNA variant. Data on a selected cohort of six patients followed at our centers during pregnancy and delivery were included in the results.

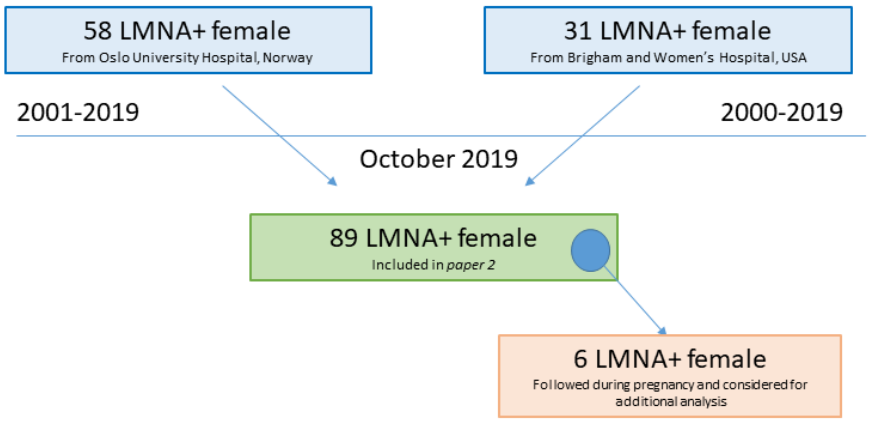


Figure 3 - Inclusion of patients in paper 2.

Paper 3

We performed a single-center, retrospective, longitudinal study. By September 2019, 121 patients were included in the Lamin A/C database at Oslo University Hospital. Female patients from Oslo included *in paper 2* were part of this population. All included patients carried a pathogenic or likely pathogenic LMNA variant. Patients without echocardiographic examination compatible with EchoPAC® GE Healthcare version 2.02 were excluded. After applying this exclusion criteria, 101 patients had echocardiographic exams available for the purpose of the study (Figure 4).

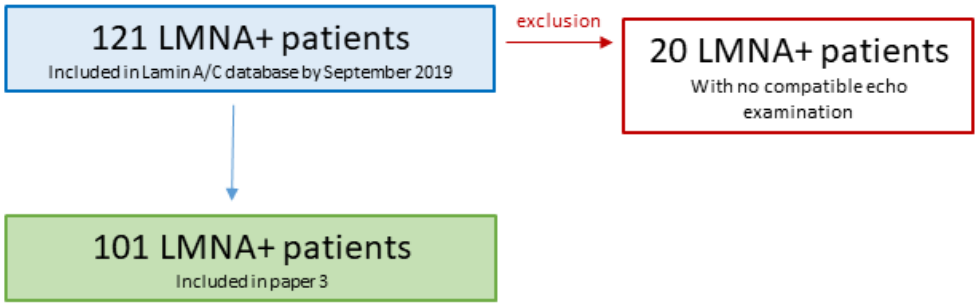


Figure 4 - Inclusion of patients in paper 3.

Methods

Clinical characteristics

Anthropometric measures, physical examination, symptoms, and medical interview

All included patients from Oslo University Hospital, Rikshospitalet, were evaluated at outpatient's clinic, Department of Cardiology, as part of follow-up. Anthropometric data such as weight and height, together with age and sex, were recorded in the electronic patients' journals at each clinical visit. During consultation, patients underwent physical examination and medical interview, which included a detailed anamnestic description of cardiac symptoms and medications. These data were collected from electronic patient's journal. For *paper 2,* Brigham and Women's hospital collected information from the electronic patients' journals and shared data with Oslo after anonymization.

Genetic analysis

Genetic testing was offered as part of evaluation at Oslo University Hospital from 2003 to all patients presenting with anamnestic and clinical criteria suspected of genetic cardiomyopathies (48). Genetic counselling and cascade genetic screening was offered to family members of patients carrying pathogenic/likely pathogenic variants.

All patients included in *paper 1* were tested for AC-related variants and genomic DNA was isolated from peripheral blood. From 2003 at our institute, genetic testing was performed by Sanger sequencing of translated exons with flanking intron sequences of selected genes (48) and included Plakophilin-2 (PKP2), Desmoplakin (DSP), Desmoglein (DSG-2), Desmocollin (DSC-2) and transmembrane protein 43 (TMEM43) (and 29 of the 105 exons of ryanodine receptor-2 RYR2). DNA from patients was stored, so when genetic testing for new genes relevant for cardiomyopathy was available later, genetic testing was most often supplemented.

Probands in *paper 2* and *paper 3*, were genetic tested from 2003 by Sanger sequencing for the following genes: beta-myosis heavy chain (MYH7); cardiac myosin binding protein C (MYBPC3); cardiac muscle isoform of troponin T (TNNT2); cardiac troponin I (TNNI3); myosin light chain 2

(MYL2); myosin light chain 3 (MYL3); Lamin A/C (LMNA). Selected exons of Phospholamban (PLN), BAG3, RBM20, and Titin (TTN) were included. Family members were offered with cascade genetic screening (48). From 2016, patients with a family history of cardiomyopathy and no findings by previous Sanger sequencing were further tested by NGS, a massive parallel sequencing technology (TruSight Cardio Sequencing Kit, Illumina Inc., San Diego, CA, USA) (49). Since 7 November 2018 all genetic testing of probands has been performed by NGS.

The pathogenicity of the genetic variants was evaluated according to the guidelines of American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACG guidelines) (50) by the Unit for Cardiac and Cardiovascular Genetics, Department of Medical Genetics, Oslo University Hospital. In paper 2, for patients included at Brigham and Women's Hospital, LMNA variants were classified locally (Boston) in keeping with the consensus (50).

Pregnancy data

Specific data related to pregnancy and delivery, relevant for the purpose of *paper 1* and *2*, were collected from electronic patients' journals. Additionally, we contacted by phone female patients who experienced pregnancy to collected specific pregnancy related information. In paper 1 and 2, pregnancies ended with birth of a viable or dead fetus were included in the analysis. Spontaneous abortions were not included in the total number of pregnancies. The questionnaire applied for *paper 2* is reported in Appendix 2.

AC severity score

For the purpose of *paper 1*, we introduced AC severity score and calculated the score for each patient, based on the 2010 TFC criteria (9). Two points were assigned to each major criterion and one point to each minor criterion, if not included in the same category of major criteria (1). Numerical quantification of AC criteria in paper 1 was applied to compare the disease's penetrance between the groups of pregnancies.

Outcomes

In *paper 2* and *3*, we included a composite outcome for the purpose of our analysis. In both papers, the outcome included death, left-ventricular assist device (LVAD) and heart transplantation (Htx). However, in paper 2 "death" was defined as all causes of death, while in paper 3 only death for cardiovascular reasons was considered. We retrieved outcomes data from electronic patients' journal.

Electrophysiology

Electrocardiogram (ECG)

Twelve-lead ECG was obtained from all participants included in this thesis at first clinical consultation, and repeated at each clinical consultations during the follow-up, based on clinical indication. ECGs were taken during the same visit as the echocardiographic examination.

In AC patients included in *paper 1*, we recorded the presence of epsilon waves and extent of T-wave inversion (TWI) according to 2010 TFC (Table 1) from ECG registration at last follow-up. In *paper 2* and *3*, we analyzed all available ECGs and recorded PR-interval and QRS-width. For this purpose, we excluded ECGs with ventricular pacing. In paper 3, we defined electrical progression as an increase PR-interval.

Signal-average ECG

Signal-average ECG (SAECG) (MAC 5000, GE medical system, Milwaukee, WI, USA) was obtained at the first clinical consultation in AC patients without bundle brunch block and data were included in *paper 1.* The aim was to identify late potential as part of 2010 TFC. SAECG was considered pathological in presence of one or more of the following criteria: filtered QRS duration ≥114 ms, high-frequency low-amplitude (<40 microV) duration (HFLA) >38 ms and root-mean-*square* voltage during the last 40 ms (RMS) <20 microV (9).

ECG Holter registration

Twenty-four hours ECG Holter monitoring (Schiller Medilog ® AR and AR+) was obtained at the time of the first clinical control and during follow-up, according to clinical indication. Arrhythmic events were analyzed and reported in patients' journals.

For paper 1, ECG Holter registrations at AC diagnosis were analyzed and VA were recorded. In paper 2 and 3, all available ECG Holter registrations taken during follow-up were included. (Please see the section on arrhythmias definitions).

Exercise test ECG

Data from available exercise test ECGs, performed according to clinical indications, were included. The test was performed on a cycle ergometer, starting at a workload of 50 Watts, and increasing by 25 Watts every second minute until exhaustion or sustained ventricular tachycardia (VT). Twelve-lead ECG was registered at the beginning and monitored continuously, to detect arrhythmias induced by exercise.

Arrhythmias' definition

For *paper 1*, we defined VA as aborted cardiac arrest, sustained VT (> 100 beats per minute > 30 seconds or terminated earlier due to hemodynamic instability)/ventricular fibrillation or appropriate therapy from an ICD (anti tachycardia pacing or shock therapy). In *paper 2*, the definition of sustained VA was comparable. However, we defined sustained VT from heart rate ≥ 120 beats per minute lasting > 30 seconds, as in previously published study in Lamin A/C (22,25). VA was documented on 12 lead ECG, ECG Holter monitoring, exercise ECG and ICD records.

In *paper 2* and *3*, we recorded atrial fibrillation (AF), defined as in the current guidelines (51). AF and AV block I-III were detected by ECG, and ECG Holter monitoring. AV block I was defined as PR ≥ 220 ms. For the purpose of our analysis, we included AF detected by implanted cardiac electronic device.

Cardiac imaging

Echocardiography

Echocardiographic imaging has a 70-years long history of continued innovation. In modern cardiology, echocardiography is widely applied and essential in diagnostic investigation, risk

stratification and prognosis estimation (52). 2D transthoracic echocardiography was the main imaging method applied in this thesis.

AC and LMNA+ patients included in our studies underwent echocardiographic examinations at first consultation and repeatedly during follow-up (Vivid 7, 9, E9 or E95 scanners, GE Healthcare, Horten, Norway), as part of clinical evaluation. Analyses were performed offline (EchoPAC, GE, Healthcare, Horten, Norway). Either echo-technicians or doctors at the echocardiographic laboratory, outpatient clinic or cardiologic department performed image acquisition.

In *paper 1*, a cardiologist in training, blinded for clinical data, analyzed the last available echocardiographic examination of our cohort of AC female patients. For the purpose of this study, we collected measurements on left ventricular (LV) and right ventricular (RV) dimensions and systolic function. From apical views, we measured LV ejection fraction (EF) by modified Simpson's biplane method (53). We measured RV outflow tract in parasternal long (PLAX) and short axis (PSAX). RV-focused 4-chambers apical view was analyzed to measure RV basal diameter (RVD) and RV fractional area change (FAC). We performed RV and LV strain analysis by 2D speckle tracking at a frame rate > 50 m/s (54). From apical views, the software automatically tracked RV and LV endocardial borders throughout the cardiac cycle and manual corrections were performed if necessary**.** RV-focused apical view and all three standard apical views were applied for this purpose. Images with poor quality tracking were excluded. For the left ventricle, peak negative longitudinal strain was obtained from 16 LV segment and expressed as average to GLS. For the right ventricle, RV free wall longitudinal strain (LS) was calculated on three free wall segments in *paper 1* and on six wall segments in *paper 3* (55). RV mechanical dispersion (MD) was calculated as the standard deviation to the peak negative longitudinal strain for six RV segments, as more robust for calculation of standard deviation (SD) (55).

For the purpose of *papers 2 and 3*, we collected all available echocardiography examinations of LMNA+ patients taken during the follow-up period and data were analyzed by four echocardiographers blinded to clinical data. The same echocardiographer analyzed all available examinations from the same patient, to minimize variability in the measurements. In *paper 2*, echocardiographic data from patients

followed at Brigham and Women's hospital, USA, were included. Examinations obtained during hospitalization for acute heart failure, on circulatory assist device and after transplantation were excluded from our analysis.

In *paper 2*, we assessed LV dilatation by end –diastolic diameter (EDD), consistent with previous studies on LMNA+ population (22,25,56). LV EDD was measured in PLAX in accordance with guidelines (53). Both *paper 2* and *3* included LV EF (53) and LV GLS (54), measured as described above.

In *paper 3,* we assessed LV dilatation by end diastolic volume (EDV), obtained by the modified Simpson method. Left atrial volume was measured by the area length biplane method, and indexed for body surface area to obtain the left atrial volume index (53). We measured RVD in right-focused apical 4-chambers view. Additionally, we analyzed RV systolic function by tricuspid annular plane systolic excursion (TAPSE)(53) and RV LS from 6 segments (57). Right atrium size was measured as right atrial area in the right-focused apical 4-chambers view (58). Mitral and tricuspid valve regurgitations were quantified by qualitative and semi-quantitative methods (59).

Cardiac magnetic resonance (CMR)

CMR is characterized by a superior spatial resolution compared to echocardiography. However, due to being more resource demanding and often less available, it is often used complementary to echocardiography. Available data from CMR, were included in *paper 1.* AC patients underwent CMR based on clinical indications, often on the first clinical control. CMRs were performed in a 1.5 Tesla unit (Magnotom Sonata, Vision Plus or Avanto Siemens, Erlangen, Germany) using a phased array body coil. As previously described (13), RV and LV volumes and EF were calculated by radiologist at Oslo University Hospital.

Statistical analysis

The statistical analysis was performed on SPSS (21.0 SPSS Inc, Chicago, IL, USA) for *paper 1*, and on STATA (16.1, StataCorp LLC, Texas, USA) for *paper 2* and *3*. Data were presented as mean ± SD or standard error (SE), median with interquartile range (IQR) or frequencies with percentage (%).

Continuous variables were compared by Student's t-test, one-way analysis of variance (ANOVA) with Bonferroni correction and Kruskal-Wallis test, as appropriate. Categorical variables were compared by chi-squared test or Fischer exact test. Two side p-value <0.05 was considered significant.

Paper 1

To investigate the effect of number of pregnancies, age, body surface area (BSA) and AC severity score on RV and LV dimension and function, we performed multiple linear and multivariable logistic regression analysis. To avoid collinearity, we excluded AC score from the analysis when a parameter included in 2010 TFC was the outcome variable. Cox regression analysis was performed to access risk markers of age at VA, adjusted for numbers of pregnancies. We constructed Kaplan Meier curves and performed log rank test to assess cumulative lifetime arrhythmias-free survival.

Paper 2

For paper 2 and 3, we recorded longitudinal nested data to increase the statistical strength of our analysis within each participant. In paper 2, generalized estimating equation was applied to assess the odds of impeding AF, AV block, sustained VA, ICD, or cardiac resynchronization therapy (CRT) defibrillator implantation, EF ≤ 45%, and of the composite outcome. The covariates in our multivariable analysis included pregnancy, age at last follow-up, missense mutation and probands status. Linear mixed model analysis with random intercept and an exchangeable covariance structure was applied to evaluate the progression of LV EF, LV GLS and LV EDD in nulliparous and female with previous history of pregnancy. To assess the effect of pregnancy on structural and functional progression of cardiomyopathy, an interaction term between the time-varying covariate number of pregnancies and age at examination was introduced. To compare the cumulative hazard risk of AF, sustained VA, and composite outcome in nulliparous and women with history of pregnancy, we generated Kaplan-Meier curves and performed long-rank test. The results of survival analysis were adjusted with a Cox regression multivariable analysis, including EF at baseline for the outcome sustained VA and composite outcome. For the outcome AF, we included in addition age at baseline and probands status.

Paper 3

For the purpose of *paper 3*, we divided patients according to age at inclusion into tertiles where the first tertile was called "younger", the second "middle" and the third "older". Linear mixed model analysis with random intercept and an exchangeable covariance structure was applied to compare disease progression at different age tertiles. We compared disease progression at different age tertiles by an interaction term between age group and time since inclusion, and the middle age tertile was used as the reference group for comparison. Individuals younger than 18 years old were excluded from the analysis to avoid confounders related to physiologic growth. Markers of adverse outcome were tested in a mix model logistic regression with random intercept and exchangeable covariance structure. Further, we adjusted the results with a multivariate mixed model logistic regression including sex, comorbidities, age, LV-systolic function, New York Heart Association (NYHA) class, as time varying co-variables. Expected PR-interval as a function of age were constructed by applying a multivariable fractional polynomial linear regression. Standard error was used as robust estimator. Kaplan-Meier survival curves were introduced to illustrate age at first episode of AF.

Ethics

All participants included at Oslo University Hospital provided written informed consent. All projects complied with the Declaration of Helsinki and were approved by the Regional Medical Ethics Committee of South-Eastern Norway. Approval documents are the following:

Study 1: REK 17.01.2008/S-07469a

Study 2: 2020/95617 REK sør-øst D.

Study 3: REK 17.01.2008/S-07469a

Brigham and Women's hospital waived consent for retrospective data in compliance with all applicable federal and state regulations, and the requirements of the Partners Human Research Committee at Massachusetts hospitals.

Summary of results

Paper 1

Clinical characteristics

We collected data of 77 Caucasian AC female patients (age 47±16 years), including 43 (56%) probands and 34 (44%) AC mutation positive female relatives. Both groups included patients with definite, borderline, or possible diagnosis as reported in Figure 5. A detailed description of major and minor criteria for AC patients grouped by number of pregnancies is reported in Appendix A1.

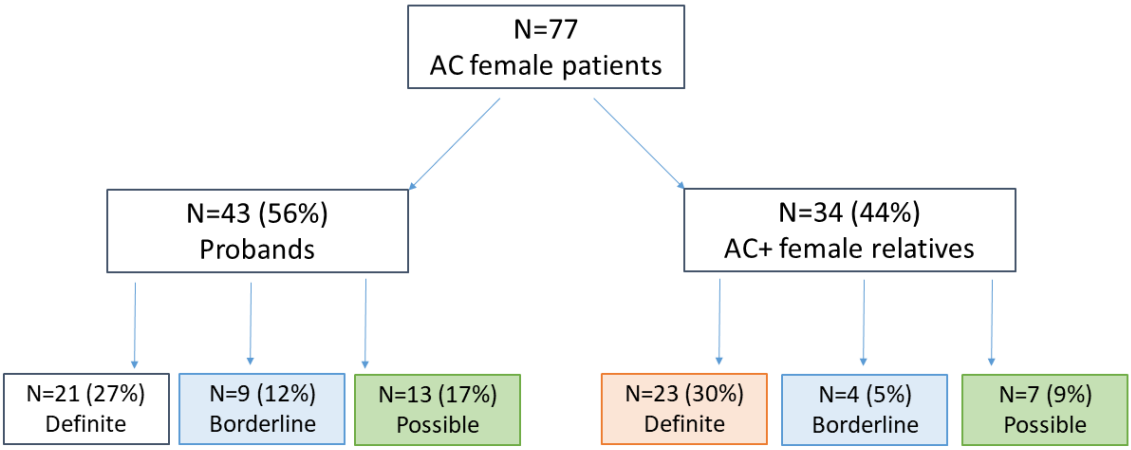


Figure 5 - Schematic representation of number of AC female patients in our population grouped by definite, borderline, and possible AC diagnosis according to 2010 TFC. N= frequencies (%).

Sixty-one patients carried an AC pathogenic variant, where PKP2 (66%) was the gene most often affected. For the purpose of our study, we grouped AC patients according to the number of previous pregnancies (Table 2) and analyzed data at last clinical follow-up. Most of the women had experienced pregnancy before start of clinical follow-up and average time from last pregnancy was of 19±14 years.

Age at examination showed a parallel increase with number of pregnancies, as expected (Table 2). Comparing women with and without history of pregnancy, we did not observe significant differences in the number of probands, burden of symptoms and penetrance of AC disease, electrocardiographic findings, arrhythmic burden, and ICD treatments (Table 2). No significant difference in the number of heart transplantations was identified between the two groups (data not shown).

Imaging findings

In the whole population of AC female patients, analyses of echocardiographic data showed dilated RVOT PLAX (19.5±4.2 mm/m2) and RVD at the upper limit of the reference value (41.0±8.0 mm). RV function was impaired when measured by RV FAC (37±10%), while LV EF (56±6%) and LV GLS (-19.6±2.8%) suggested a preserved LV function. Comparing AC female patients with and without history of pregnancy, we did not observe differences in echocardiographic and CMR findings (Table 2). A trend toward increasing RVOT PSAX with multiple pregnancies was noted (Table 2). However, multiple linear regression analysis showed that number of pregnancies was not significantly associated with increase in RVOT diameter when adjusted for age and BSA (β-value 0.87, 95% CI -0.76 to 2.5, P=0.29). Moreover, we introduced RVOT PSAX ≥ 32 mm as outcome variable in a multivariable logistic regression, and the results confirmed no association between number of pregnancies and RVOT dilatation after adjustment (Table 3). No other measurement of RV or LV structure or function were associated with number of pregnancies (Table 2 and 3). Interestingly, AC severity score was independently associated to RV dilatation by RVD and reduced LV EF (Table 3).

Number of pregnancies and arrhythmic events

Women with history of VA had a higher AC severity score, more often pathological SAECG, TWI, and sign of RV affection, with increased RVD, RV akinesia by CMR and sign of impaired RV free wall LS (Table 4). However, after adjustment for number of pregnancies, only AC severity score, together with RV diameter, regional akinesia and strain were markers of VA. Number of pregnancies was not associated with VA in univariable analysis, not when adjusted for the severity of AC disease (Table 4). AC female patients without history of pregnancy experienced VA earlier than patients with one pregnancy (Figure 6); however, no differences were observed between the other groups of pregnancies (Figure 6).

Table 2 – Clinical characteristics and cardiac imaging parameters in AC women grouped by number of pregnancies at last follow-up

	0 pregnancies (n=19)	1 pregnancy (n=16)	2 pregnancies (n=30)	≥3 pregnancies (n=12)	*p-value from F test*	>0 pregnancies (n=58)	*p-value 0 vs >0 pregnancies*
BSA (m^2)	1.7±0.2	1.8±0.2	1.8±0.2	2.9±4.3	0.25	2.1±2.2	0.47
Age at examination (years)	36±18	48±16*	51±13*	55±13*	**<0.01**	52±13	**<0.01**
Age at arrhythmic event (years)	35±17	48±16*	48±13*	54±13*	**0.02**	49±14	**<0.01**
Probands (n, (%))	9 (47)	5 (31)	17 (57)	4 (33)	0.33	25 (43)	0.50
AC severity score	4.3±2.8	3.4±1.6	4.7±2.8	4.3±2.3	0.53	4.2±2.4	0.83
Definite AC by TFC (n, (%))	11 (58)	6 (38)	19 (63)	8 (66)	0.36	33 (57)	0.58
Syncope (n, (%))	8 (42)	2 (13)	11 (37)	2 (17)	0.17	15 (26)	0.16
VA (n, (%))	9 (47)	4 (25)	15 (50)	3 (25)	0.25	22 (38)	0.34
ICD (n, (%))	6 (32)	2 (13)	11 (37)	4 (33)	0.38	16 (28)	0.50
ICD discharge (n, (%))	1 (5)	0 (0)	6 (20)	3 (25)	0.36	10 (17)	0.16
ATP (n, (%))	0 (0)	1 (6)	5 (17)	2 (17)	0.97	8 (14)	0.05
TWI major criteria (n, (%))	7 (37)	5 (31)	10 (33)	4 (33)	0.99	19 (33)	0.74
Epsilon waves (n, (%))	1 (5)	0 (0)	4 (13)	0 (0)	0.37	4 (7)	0.64
SAEG pathological (n, (%))	4 (21)	6 (38)	10 (33)	2 (17)	0.54	18 (31)	0.27
Echocardiographic findings							
RVOT PLAX (mm/m^2)	19±5	18±2	20±4	21±6	0.23	20±4	0.82
RVOT PSAX (mm/m^2)	19±5	17±3	19±3	22±6†	0.06	19±4	0.96
RVD (mm)	41±8	39±4	43±10	42±9	0.62	42±8	0.98
RVFAC (%)	39±9	37±7	34±12	39±11	0.37	36±10	0.27
RV free wall LS (%)	-23.6±6.1	-26.1±6.0	-21.9±5.8	-24.0±9.0	0.25	-21.2±4.8	0.82
RVMD (ms)	41±22	32±21	53±23	40±21	0.07	44±28	0.60
LVEF (%)	56±7	56±6	54±7	58±7	0.42	55±6	0.88
LVGLS (%)	-19.3±2.8	-18.9±2.8	-19.9±2.9	-20.4±1.9	0.50	-19.7±2.7	0.56
CMR findings	n=17	n=10	n=25	n=8		n=43	
CMR EDV RV (ml)	199±71	138±29	192±63	216±127	0.26	184±75	0.64
CMR EDV LV (ml)	146±24	135±22	149±35	137±36	0.74	144±32	0.87
CMR EF RV (%)	46±12	56±4	47±12	43±18	0.23	48±12	0.64
CMR EF LV (%)	60±6	63±6	56±7	56±15	0.28	57±10	0.50

Data presented as mean±SD unless otherwise stated. P from ANOVA F-test with post hoc Bonferroni correction, chi-square, or Fisher exact test as appropriate. * Post hoc *p*<0.05 versus 0 pregnancy. † Post hoc *p*<0.05 versus 1 pregnancy. AC = Arrhythmogenic cardiomyopathy; ATP = anti-tachycardia therapy; BSA = body surface area; EDV = end-diastolic volume; EF = ejection fraction; ICD = implantable cardioverter defibrillator; LS = longitudinal strain; LV = left ventricular; LVEF = left ventricular ejection fraction; LVGLS = left ventricular global longitudinal strain; MR= magnetic resonance; PLAX = parasternal long axis view; PSAX = parasternal short axis view; RV = right ventricular; RVD = right ventricular basal diameter; RVFAC = right ventricular fractional area change; RVMD = right ventricular mechanical dispersion; RVOT = right ventricular outflow tract; SAECG = signal averaged ECG; TFC = Task Force Criteria; TWI = T-wave inversion, VA = ventricular arrhythmias. Modified from Castrini AI et al (1)

Table 3 – Markers of pathological cardiac structure or function by echocardiography in 77 AC female patients

	Univariable analysis		Multivariable analysis	
	OR (95%CI)	*p-value*	OR (95%CI)	*p-value*
Markers of LVEF<54% (n=25)				
Age (years)	1.04 (1.01-1.07)	0.03	1.02 (0.98-1.05)	0.42
Number of pregnancies (n)	1.76 (1.08-2.87)	0.24	1.67 (0.96-2.92)	0.71
AC severity score	1.37 (1.07-1.75)	0.01	1.35 (1.05-1.74)	**0.02**
Markers of LVGLS>-18 (n=20)				
Age (years)	1.00 (0.97-1.03)	0.98	1.00 (0.97-1.04)	0.89
Number of pregnancies (n)	0.79 (0.48-1.31)	0.37	0.77 (0.44-1.34)	0.36
AC severity score	1.1 (0.93-1.38)	0.23	1.13 (0.92-1.39)	0.25
Markers of RVOT PSAX≥32 mm (n=45)				
Age (years)	1.04 (1.00-1.01)	0.03	1.02 (0.98-1.05)	0.40
Number of pregnancies (n)	1.76 (1.08-2.87)	0.02	1.56 (0.91-2.66)	0.11
BSA (m^2)	18.3 (0.99-340.58)	0.05	12.58 (0.54-292.44)	0.12
Markers of RVD>41 mm (n=25)				
Age (years)	1.01 (0.98-1.05)	0.40	0.99 (0.95-1.04)	0.86
Number of pregnancies (n)	0.95 (0.59-1.50)	0.81	0.89 (0.48-1.64)	0.70
AC severity score	1.91 (1.41-2.58)	<0.01	1.93 (1.41-2.64)	**<0.01**
Markers of RVFAC≤40% (n=41)				
Age (years)	1.02 (0.99-1.05)	0.16	1.02 (0.98-1.05)	0.27
Number of pregnancies (n)	0.99 (0.64-1.55)	0.98	0.85 (0.51-1.42)	0.53
BSA (m^2)	5.23 (0.38-72.34)	0.22	3.61 (0.24-54.39)	0.35

Univariable and multivariable logistic regression, retaining all potential confounders in multivariable analyses. AC = Arrhythmogenic cardiomyopathy; BSA = body surface area; LVEF = left ventricular ejection fraction; LVGLS = left ventricular global longitudinal strain; PSAX = parasternal short axis view; RVD = right ventricular basal diameter; RVFAC = right ventricular fractional area change; RVOT = right ventricular outflow tract. *Modified from Castrini AI et al (1)*

Table 4: Comparisons of AC female patients without and with history of VA, and markers for age at VA adjusted for number of pregnancies

	AC Without Ventricular Arrhythmias (n=46)	AC With Ventricular Arrhythmias (n=31)	*p-value*	Risk markers of Ventricular Arrhythmias Multivariable HR (95%CI)	*Multivariable p-value*
Age (years)	46±18	49±12	0.45		
AC severity score	3.1±1.7	6.0±2.5	<0.001	1.31 (1.15-1.50)*	**<0.001**
Number of pregnancies (n)	1.5±1.1	1.4±1.1	0.69	0.72 (0.51-1.03)†	0.08
SAECG pathological (n, (%))	7 (15)	15 (48)	<0.001	0.76 (0.53-1.09)*	0.14
TWI major criteria (n, (%))	7 (15)	19 (61)	<0.01	0.74 (0.52-1.06)*	0.10
Epsilon waves (n, (%))	3 (7)	19 (61)	0.99		
RVOT PSAX (mm)	32±6	36±6	0.14		
RVD (mm)	38±4	46±10	<0.001	1.09 (1.05-1.14)*	**<0.001**
RVFAC (%)	40±9	33±11	0.01	0.98 (0.95-1.01)*	0.22
RV free wall LS (%)	-22.3±4.5	-19.6±4.0	<0.001	1.09 (1.03-1.17)*	**0.006**
LVEF (%)	56±7	55±5	0.46		
LVGLS (%)	-19.7±2.7	-19.6±3.0	0.90		
CMR EDV RV (ml)	168±70	213±71	0.07		
CMR EDV LV (ml)	138±30	153±30	0.14		
CMR EF RV (%)	50±12	45±12	0.15		
CMR EF LV (%)	58±8	58±10	0.96		
Regional akinesia RV (n, (%))	7 (15)	18 (58)	<0.01	3.01 (1.38-6.60)*	**0.006**

Data presented as mean±SD or n (%). P-value from Student's t-test or chi-square as appropriate. Multivariable Cox regression of age-adjusted incident VA. *Adjusted for number of pregnancies. †Adjusted for AC severity score. AC = Arrhythmogenic cardiomyopathy; EDV = end-diastolic volume; EF = ejection fraction; MR= magnetic resonance; LS = longitudinal strain; LV = left ventricular; LVEF = left ventricular ejection fraction; LVGLS = left ventricular global longitudinal strain; PSAX = parasternal short axis view; RMS= root-mean-square voltage; RV = right ventricular; RVD = right ventricular basal diameter; RVFAC = right ventricular fractional area change; RVOT = right ventricular outflow tract; SAECG = signal averaged electrocardiogram; TWI = T-wave inversion. Modified from Castrini AI et al. (1)

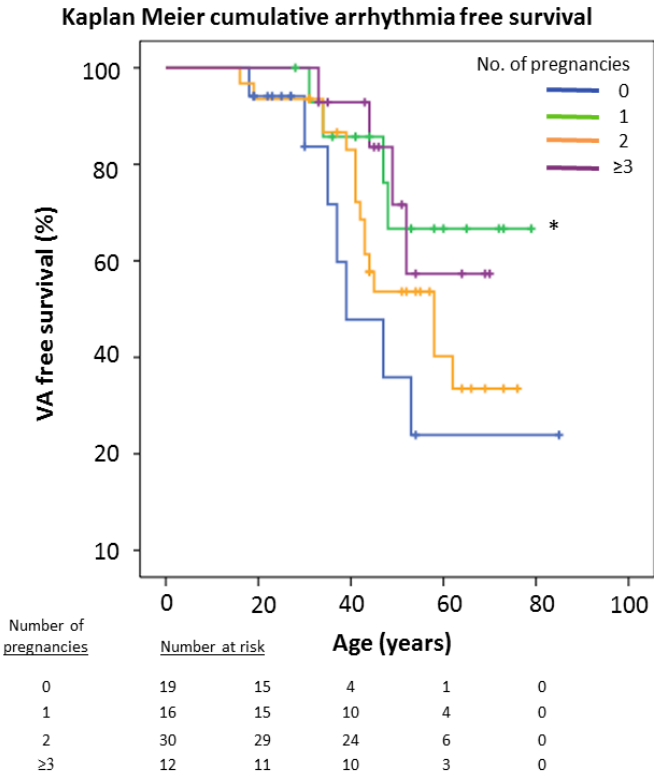


Figure 6 - Kaplan Meier curves of 77 AC female patients and their mutation positive female relatives. Women with 0 pregnancies had VA at younger ages compared to women with 1 pregnancy (*log rank = 0.04). No significant differences were observed in arrhythmic events and prognosis between 1, 2, and ≥3 pregnancies. VA = ventricular arrhythmias*. *Modified from Castrini AI et al (1).*

Gestation and delivery

Forty-seven (81%) patients replied to our questionnaire. Age at first pregnancy was 29±6 years among 58 AC female patients with previous history of pregnancy. Few participants (10%) were aware of AC disease or AC gene predisposition at time of first pregnancy, and 8% were on beta-blocker medication during pregnancy. Twelve patients (21%) reported symptoms during pregnancy. Six (10%) patients reported worsening symptoms during pregnancy, including palpitation and dizziness; however, none reported serious cardiac events.

Most of deliveries were vaginal and no cardiac complication during birth was reported. Two cases of spontaneous abortion occurring before week 12 were noted. Both patients experienced other pregnancies, respectively 2 and 3, reported as uncomplicated and concluded by vaginal delivery.

Selected cohort of AC female patients followed during pregnancy and delivery

A selected cohort of 5 AC genotype positive female patients (age at first pregnancy 34±3 years) were followed with serial echocardiographic examinations during a total of 6 pregnancies at our department (1 patient experienced 2 pregnancies and 4 had 1 pregnancy). No arrhythmias were reported during pregnancy, delivery, and post-partum. Echocardiographic findings showed no significant changes in RV and LV function and structure, comparing before and within 6 months after pregnancy (Table 5).

Table 5: Serial investigation in 5 AC genotype positive female patients followed during pregnancy

Echocardiographic parameters	Before pregnancy	Within 6 months post-partum	*p-value*
RVOT PSAX (mm)	33±6	32±7	0.52
RVFAC (%)	46±7	41±7	0.08
RV free wall LS (%)	-24.3±3.1	-21.9±2.3	0.55
LVEF (%)	54±2	54±2	0.36
LVGLS (%)	-19.5±2.7	-17.9±4.6	0.44

Data presented as mean±SD. P-value from Student's t-test. AC = Arrhythmogenic cardiomyopathy; LS = longitudinal strain; LVEF = left ventricular ejection fraction; LVGLS = left ventricular global longitudinal strain; PSAX = parasternal short axis view; RV = right ventricular; RVFAC = right ventricular fractional area change; RVOT = right ventricular outflow tract. (Modified from Castrini AI et al (1).

Paper 2 – Results

Clinical characteristics

We collected data from 89 LMNA+ female patients (58 from Oslo University Hospital, 31 from Brigham and Women's Hospital, Boston, USA). Mean age at baseline was 41 ±16 years, 28% were probands and 27% carried a missense mutation (Table 6, Appendix A3). We analyzed 452 echocardiographic examinations, with a median of 4 (IQR 17-32) examinations per patient and 5 (IQR 3-9) years follow-up time.

At the first clinical consultation, 24% of patients had a NYHA class between II-IV, 21% had AV block grade I-III, 44% had AF, 11% experienced VA, and 11% had already undergone ICD/CRT-D implantation (Table 6). Echocardiographic findings showed mild LV impairment, although 21% had LV EF <45% (Table 6).

At the last clinical examination, we grouped our cohort according to the number of previous pregnancies (Table 7). Among nulliparous, 23 (79%) were in childbearing age (15-49 years) at last follow-up and 13 (45%) were aged ≤25 years. Most of participants experienced pregnancy before clinical debut of cardiac symptoms or diagnosis of LMNA+ variant.

Lamin A/C cardiomyopathy and electrical disease in patients grouped by pregnancy

At the last clinical follow-up, women with previous pregnancy were older than nulliparous and age showed a parallel increase with number of pregnancies (Table 7). Women with previous pregnancy had a higher prevalence of AV block I-III and AF compared with nulliparous (Table 7). However, the number of pregnancies was not associated with AV block I-III (OR, 1.63; 95% CI 0.65-4.07; P=0.30), nor with AF (OR 1.17, 95% CI, 0.68-2.03; P=0.56) when adjusted for age, proband status and missense mutation (Table 8). No difference in age at onset of AF was observed between nulliparous and patients with previous pregnancy (long rank 0.73), and time to AF was not significantly different between the two group after adjustment for age and EF at baseline, and proband status (Figure 7).

Pregnancy was not a marker of sustained VA when corrected for age, probands status and missense mutation (Table 8). Age at onset of VA was similar in the two groups (Log rank p=0.87), and

time to sustained VA was not significantly different between the groups after adjustment for EF at baseline (Figure 7).

Table 6: Clinical characteristics and imaging parameters of 89 LMNA+ women at baseline and last follow-up

Clinical characteristics	Baseline (n=89)	Last follow-up (n=89)
Age at first pregnancy (years ± SD)	-	27±5
Age (years ± SD)	41±16	46±16
NYHA class II-IV (n, (%))	21 (24)	32 (36)
Atrioventricular block I-III (n, (%))	19 (21)	24 (27)
• AV block I	11 (12)	9 (10)
• AV block II	2 (2)	5 (6)
• AV block III	6 (7)	10 (11)
Atrial fibrillation (n, (%))	39 (44)	52 (58)
Sustained VA (n, (%))	10 (11)	22 (25)
Medications and device therapy		
Beta-blockers (n, (%))	22 (25)	48 (54)
ACE inhibitors/ARBs (n, (%))	15 (17)	31 (35)
MRAs (n, (%))	8 (9)	16 (18)
AAs (n, (%))	8 (9)	2 (2)
ICD/CRT-D (n, (%))	10 (11)	51 (57)
Echocardiographic findings		
LV EF (%)	53±11	50±13
LV EF≤ 45% (n, (%))	19 (21)	17 (19)
LV End-diastolic diameter (mm)	51±6	51±7
LV GLS (%)	-18±4	-16±4

Data are presented as n (%) or means ± standard deviation. Prevalence of arrhythmias and of treatments (medical and device therapy) is reported. LV GLS refers to a subgroup of 58 patients with available strain measurements. Abbreviations: AAs= Anti-arrhythmic medications (sotalol, amiodarone, verapamil, flecainide and dronedarone); ACEi=Angiotensin converting enzyme inhibitors; ARBs=Angiotensin receptor blockers; CRT-D=cardiac resynchronization therapy defibrillator; ICD=implantable cardioverter-defibrillator; LV EDD= left ventricular end-diastolic diameter; LV EF=left ventricular ejection fraction; LV GLS=left ventricular global longitudinal strain; MRAs=Mineralocorticoid-receptor antagonist; NYHA=New York heart association; TIA=transient ischemic attack; VA=ventricular arrhythmias. *Modified from Castrini AI et al.(2)*

Table 7: Clinical parameters and outcomes of 89 LMNA+ women grouped by previous pregnancies at last follow-up

	0 previous pregnancy (n=29)	1 previous pregnancy (n=13)	2 previous pregnancies (n=31)	≥3 previous pregnancies (n=16)	*p value*	≥1 previous Pregnancies (n=60)	*p value 0 vs* ≥1 *pregnancy*
Clinical characteristics							
Age at last follow-up (years)	33±17	44±9	53±9*	57±12*	**<0.001**	52±11	**<0.001**
Follow-up time (years)	4 [2-7]	4 [3-8]	6 [3-10]	5 [4-12]	0.83	5 [3-10]	0.13
Proband status (n, (%))	5 (17)	3 (23)	9 (29)	8 (50)	0.16	20 (33)	0.20
Missense mutation (n, (%))	6 (21)	3 (23)	8 (26)	6 (38)	0.68	17 (28)	0.60
NYHA class II-IV (n, (%))	11 (38)	2 (15)	13 (42)	6 (38)	0.30	21 (35)	0.53
Atrioventricular block I-III (n, (%))	4 (14)	4 (31)	13 (42)	6 (38)	**0.004**	23 (38)	**<0.001**
Atrial fibrillation (n, (%))	9 (31)	6 (46)	25 (81)	12 (75)	**0.001**	43 (72)	**0.001**
Sustained VA (n, (%))	4 (14)	3 (23)	9 (29)	6 (38)	0.36	18 (30)	0.11
Echocardiographic examination							
LV EF (%)	53±14	53±12	48±13	45±12	0.25	48±12	0.18
LV EF≤ 45% (n, (%))	2 (7)	3 (23)	6 (19)	6 (38)	0.11	15 (25)	0.08
Delta EF (%))	-3±11	-7±8	-3±8	-4±10	0.82	-4±10	0.73
LV GLS (%)	-16±4	-16±5	-15±4	-16±3	0.68	-15±4	0.42
LV End-diastolic diameter (mm)	50±7	50±4	53±7	50±6	0.34	51±6	0.31
Medications and device therapy							
Beta-blockers (n, (%))	12 (41)	7 (54)	19 (61)	10 (63)	0.56	36 (60)	0.12
ACEi/ARBs (n, (%))	5 (17)	3 (23)	15 (48)	8 (50)	**0.04**	26 (43)	**0.02**
MRAs (n, (%))	3 (10)	0 (0)	7 (23)	6 (38)	**0.04**	13 (22)	0.17
AAs (n, (%))	0 (0)	0 (0)	2 (6)	0 (0)	0.63	2 (3)	1.00
ICD/CRT-D (n, (%))	9 (31)	7 (54)	25 (80)	10 (63)	**0.003**	44 (73)	**0.001**
Outcomes							
Death (n, (%))	2 (7)	1 (8)	3 (10)	2 (13)	0.95	6 (10)	0.52
Heart transplantation (n, (%))	4 (14)	1 (8)	3 (10)	4 (25)	0.50	8 (13)	0.57
LVAD (n, (%))	1 (3)	0 (0)	1 (3)	1 (6)	1.00	2 (2)	0.69
Death/LVAD/HTx, (n, (%))	6 (21)	2 (15)	5 (16)	6 (38)	0.39	13 (22)	0.56

Data are presented as *n* (%), means ± standard deviation or median [interquartile range]. Prevalence of arrhythmias, treatments (medical and device therapy) and outcome is reported. P-value from ANOVA F-test with Bonferroni correction, Fisher exact test and Kruskal-Wallis test. Abbreviations: AAs= Anti-arrhythmic medications (sotalol, amiodarone, verapamil, flecainide and dronedarone); ACEi=Angiotensin converting enzyme inhibitors; ARBs=Angiotensin receptor blockers; CRT-D=cardiac resynchronization therapy defibrillator; Delta EF=difference between EF baseline and EF last follow-up; HTx=heart transplantation; ICD=implantable cardioverter-defibrillator; LVAD=Left ventricular assistance device; LV EDD= left ventricular end-diastolic diameter; LV EF=left ventricular ejection fraction; LV GLS=left ventricular global longitudinal strain; MRAs=Mineralocorticoid-receptor antagonist; NYHA=New York heart association; TIA=transient ischemic attack; VA=ventricular arrhythmias. * Post hoc p<0.05 versus 0 pregnancy. *Modified from Castrini AI et al (2).*

LV functional-structural disease progression and outcome

No difference in echocardiographic parameters were observed between pregnancy groups (Table 7) and pregnancy was not associated with reduced LV EF ≤ 45% (Table 8). Annual progression rate of LV EF, LV EDD, and LV GLS in nulliparous and female patients with previous pregnancy was similar (Table 9). The outcome death/LVAD and HTx occurred in 19 (21%) patients, without differences across pregnancy groups.

Table 8: Multivariable analysis of repeated observations in 89 LMNA+ women assessing predictive effects of known prognostic factors and number of previous pregnancies

Primary outcome and markers of disease progression	Prognostic factors	Odds ratio	95% CI	*P value*
Death/LVAD/HTx, n=452 (100%)	Age	1.05	0.99 to 1.11	**0.09**
	Pregnancy	0.67	0.35 to 1.30	0.24
	Proband status	13.7	3.67 to 50.8	**<0.01**
	Missense mutation	1.00	0.25 to 4.1	0.96
Atrioventricular block, n=279 (62%)	Age	1.05	0.98 to 1.11	**0.16**
	Pregnancy	1.63	0.65 to 4.07	0.30
	Proband status	13.5	0.93 to 195.4	**0.05**
	Missense mutation	0.61	0.14 to 2.69	0.51
Atrial fibrillation, n=447 (98%)	Age	1.06	1.02 to 1.11	**<0.01**
	Pregnancy	1.17	0.68 to 2.03	0.56
	Proband status	7.59	1.55 to 37.5	**0.01**
	Missense mutation	0.56	0.16 to 1.92	0.36
ICD/CRT-D, n=434 (96%)	Age	1.09	1.04 to 1.15	**<0.01**
	Pregnancy	0.70	0.36 to 1.38	0.31
	Proband status	11.2	2.13 to 58.8	**<0.01**
	Missense mutation	0.48	0.14 to 1.69	0.26
Sustained VA, n=447 (98%)	Age	1.02	0.98 to 1.06	0.32
	Pregnancy	1.13	0.68 to 1.86	0.64
	Proband status	2.25	0.77 to 6.57	0.14
	Missense mutation	1.30	0.44 to 3.84	0.63
LV EF≤ 45%, n=452 (100%)	Age	1.06	1.02 to 1.11	**<0.01**
	Pregnancy	1.04	0.66 to 1.63	0.88
	Proband status	7.17	2.26 to 22.8	**<0.01**
	Missense mutation	1.00	0.28 to 3.52	0.99

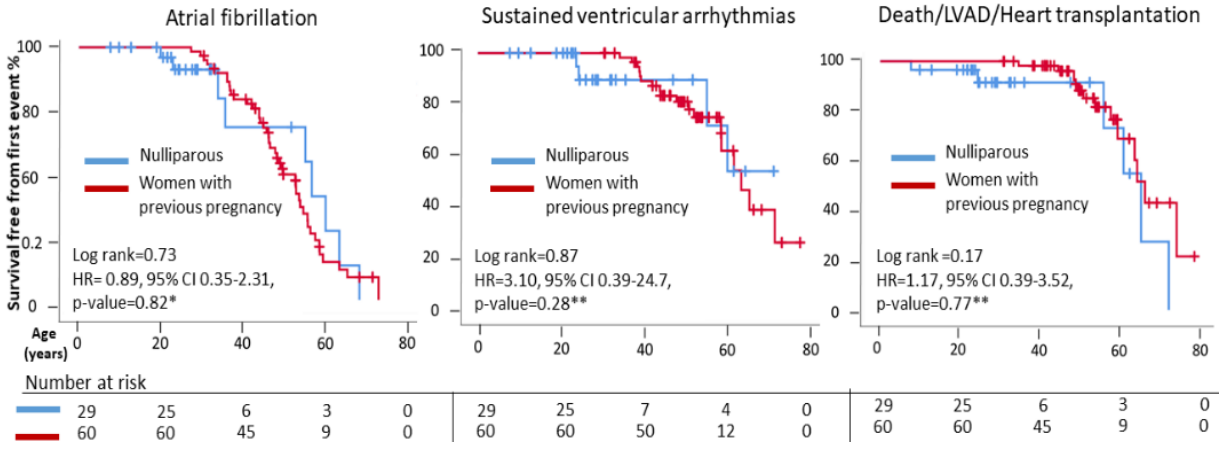
Generalized estimating equation (GEE) with repeated observations; n=number of examinations (percent) with available data. Random effects by individuals, logit link, binomial family, and independent covariance structure. n=number of examinations (percent) with available data; CRT-D=cardiac resynchronization therapy defibrillator; ICD=implantable cardioverter-defibrillator; LVAD=Left ventricular assistance device; LV EF=left ventricular ejection fraction; HTx=heart transplantation; VA=ventricular arrhythmias. *Modified from Castrini AI et al.*

Table 9: Annual structural progression by echocardiography in LMNA+ women grouped pregnancy

	At baseline (n=89)	Progression rate 1 year (SE)	Last follow-up (n=89)	*P value*
LV EF (%), n=415 (92%)	53±11	-0.4 (0.0)	50±13	<0.001
Nulliparous	55±13	-0.3 (0.1)	53±14	0.003
Women with previous pregnancy	53±10	-0.5 (0.1)	49±12	<0.001
P for interaction		***0.37***		
LV EDD (mm), n=416 (92%)	50±6	0.1 (0.0)	51±6	<0.001
Nulliparous	49±7	0.2 (0.1)	50±7	<0.001
Women with previous pregnancy	51±6	0.1 (0.0)	51±6	0.02
P for interaction		***0.09***		
LV GLS (%), n=230 (51%)	-17±4	0.1 (0.0)	-16±6	0.03
Nulliparous	-17±5	0.0 (0.1)	-16±4	0.53
Women with previous pregnancy	-17±3	0.1 (0.0)	-15±4	0.01
P for interaction		***0.35***		

Values at baseline and last follow-up are presented as mean±SD; n=number of examinations (percent) with available data. Yearly progression rate with standard errors, *p value* for progression and interaction are calculated by linear mixed models' statistics with exchangeable covariance structure and random individual intercept. Abbreviations: LV EDD = left ventricular end-diastolic diameter; LV EF = left ventricular ejection fraction; LV GLS = left ventricular global longitudinal strain.

Figure 7 - Survival free from arrhythmias and primary outcome



Survival free from incident atrial fibrillation (panel A), sustained ventricular arrhythmias (panel B) and death, need for LVAD or heart transplantation (panel C) did not differ between women with previous pregnancy (red line) and nulliparous (blue line) women. HR: Hazard ratio from Cox models regression, exploring time to AF, sustained VA and death/LVAD/heart transplantation. *Adjusted for pregnancy, age, and EF at baseline, and probands status. ** adjusted for pregnancy and EF at baseline. *From Castrini AI et al. (2)*

Number of pregnancies did not increase the odds for the combined outcome death/LVAD/HTx (Table 8). We observed no differences in the age at primary outcome between LMNA+ nulliparous and with history of pregnancy (log rank 0.17), and time to primary outcome was not different between the groups after adjustment for EF at baseline (Figure 7).

Table 10: Maternal clinical characteristics and obstetric/fetal outcomes

Maternal adverse cardiac event (MACE)	**Total number of pregnancies (n=109)**
Maternal mortality	0 (0)
Heart failure	0 (0)
Atrial fibrillation	2 (2)
PVCs	3 (3)
nsVA	2 (2)
Sustained VA	2 (2)
Thrombo-embolic complications	0 (0)
AAs	5 (5)
Symptoms	
Palpitations	9 (8)
Dyspnea	4 (4)
Syncope	3 (3)
Obstetric outcomes	
Vaginal deliveries	94 (86)
Caesarean section	15 (14)
Emergency CS for cardiac reason	0 (0)
Pre-eclampsia	2 (2)
Spontaneous abortions > 12 weeks	4 (4)
Bleeding	1 (1)
Adverse fetal outcomes	
Fetal or neonatal death < 1 week	3 (3)
Low birth weight (<2500 g)	2 (2)
Preterm birth (< 37 weeks)	6 (6)

Data are presented as *n* (%). Data on AF, nsVA and sustain VA refers to incident arrhythmias. Abbreviations: AAs = Anti-arrhythmic therapy (Metoprolol, Bisoprolol, Sotalol); CS = Caesarean section; nsVA = non-sustained ventricular arrhythmias; PVCs = Premature ventricular contractions; VA = ventricular arrhythmias. *Modified from Castrini et al (2).*

Pregnancy and peripartum outcomes

Age at first pregnancy was 27±5 years in our cohort. Most of patients reported well-tolerated pregnancy and uncomplicated delivery (Table 10). Vaginal delivery was predominant (Table 10). No caesarian section due to cardiac reason was noted. Four spontaneous abortions in the early second trimester were reported; however, none was reported associated to cardiac complications. Patients who experienced abort reported previous or subsequent successful births after uncomplicated pregnancies. Three stillbirths were reported, none where the situation could suggest cardiac reason.

Selected cohort of LMNA+ patients followed during pregnancy

Six LMNA+ female patients (age 31±3, 67% probands) were followed during pregnancy and peripartum period at our centers. Fifty percent of them developed sustained VA during pregnancy, effectively treated with betablocker. One patient was implanted with ICD. All experienced uncomplicated delivery.

Paper 3 - Results

Baseline characteristics

We included 101 LMNA+ patients (50% female, 39% probands) with mean age at inclusion of 44 years [IQR 29-54], from 33 different families. Mean BSA at inclusion was 1.9±0.3. Patients were divided in tertiles based on age at inclusion, and we defined a young tertile (age < 32 years), a middle (age 32-49 years), and an older tertile (age >49). Ninety-four patients had follow-up visits with a mean follow-up time of 4.4 [2.5-6.6] years.

Follow-up time was shorter in the older tertile. Patients in this group had more often sign of electrical cardiac disease, medication, and comorbidities, as expected (Table 11).

Structural and functional disease progression

We collected 576 echocardiographic examinations taken during follow-up time. In the total population, LV and RV function deteriorated, while both atria and right ventricle dilated. LV volume did not change significantly (Figure 8, Table 12).

Structural progression in the young tertile was characterized by dilatation of left atrial size, with left atrial volume index reaching values beyond normal references (Table 12), without sign of impairment of RV and LV function. We observed a tendency to increased LV EDV in the young tertile, however not statistically significant (Table 12).

From the middle tertile, the LV function had a significant worsening. Interestingly, we did not observe a concomitant dilatation of left ventricle by EDV. In addition, RV showed a substantial dilatation with a slow worsening of RV systolic function (Table 12, Figure 8).

The older tertile showed a similar deterioration in LV function as observed in the middle tertile. RVD increased in older tertile as in middle; however, RV function showed a faster deterioration in the older group (Table 12, Figure 8).

Left and right atria dimension increased with similar rates in middle and older tertile (Table 12). Mitral and tricuspid regurgitation showed progression in all groups, more evident in the middle and older

tertiles. We observed no significant differences in progression when comparing probands and family members (data do not show).

Table 11. Baseline characteristics of 101 lamin A/C genotype patients

Clinical data	Total	Younger tertile	Middle tertile	Older tertile	p-value
Included:	n=101	n=34	n=34	n=33	
Follow-up time, years	4.4(2.5 - 6.6)	6.1(3.4-9.6)	2.6(1.7-4.6)	2.5(2.5-3.0)	**<0.01**
Clinical visits, n	5(3-9)	6(4-10)	6(4-9)	4(3-7)	0.11
Age at inclusion, years	44(29-54)	22(15-29)	44(38-47)	57(55-60)	**<0.001**
Female sex, n(%)	50(50)	17(50)	19(56)	14(42)	0.57
Proband, n(%)	39(39)	3(9)	15(44)	21(64)	**<0.001**
Families	33	18	20	15	NA
NYHA I, n(%)	70(69)	30(88)	27(79)	13(41)	**<0.001**
NYHA II, n(%)	15(15)	2(6)	4(12)	9(28)	**0.045**
NYHA III, n(%)	12(13)	1(3)	3(9)	8(25)	0.03
NYHA IV, n(%)	3(3)	1(3)	0(0)	2(6)	0.31
Electrophysiological					
AV-block I, n(%)	14(14)	3(10)	7(23)	4(14)	0.39
AV-block II, n(%)	3(3)	0(0)	1(3)	2(7)	0.32
AV-block III, n(%)	27 (27)	0(0)	9(30)	18(62)	**<0.001**
Atrial fibrillation, n(%)	45 (45)	4(12)	16 (47)	25 (78)	**<0.001**
Pacemaker, n(%)	15(15)	0(0)	7(20)	8(24)	**<0.01**
ICD, n(%)	2(2)	0(0)	0(0)	2(6)	0.10
CRT-D, n(%)	9(9)	0(0)	0(0)	9(28)	**<0.001**
Medication					
ACE inhibitor, n(%)	19(19)	0(0)	5(15)	14(43)	**<0.001**
Beta blocker, n(%)	23(23)	1(3)	8(24)	14(44)	**<0.001**
MCRA, n(%)	6(6)	0(0)	1(3)	5(16)	**0.01**
Comorbidities					
Hypertension, n(%)	3(3)	0(0)	0(0)	3(9)	0.32
Coronary artery disease, n(%)	3(3)	0(0)	0(0)	3(9)	0.03
Stroke, n(%)	5(5)	1(3)	0(0)	4(13)	0.04
COPD, n(%)	1(1)	0(0)	1(3)	0(0)	0.65
Diabetes, n(%)	5(5)	1(3)	1(3)	3(9)	0.44

Values are mean ± standard deviation, median (Inter-quartile range [IQR]), or frequency (%). P-values by one-way ANOVA, Kruskal Wallis test, or Fisher's exact test as appropriate. Abbreviations: ACE inhibitor; Angiotensin converting enzyme inhibitor, AV-block; Atrioventricular block, BMI; Body mass index, COPD; Chronic obstructive pulmonary disease, CRT-D; Cardiac resynchronization therapy defibrillator, DCM; Dilated cardiomyopathy, MCRA; Mineral corticoid receptor antagonist, NSVT; Non sustained ventricular tachycardia, NYHA; New York Heart Association functional class, VA; Ventricular arrhythmia. *From Skjølsvik at al*. (3)

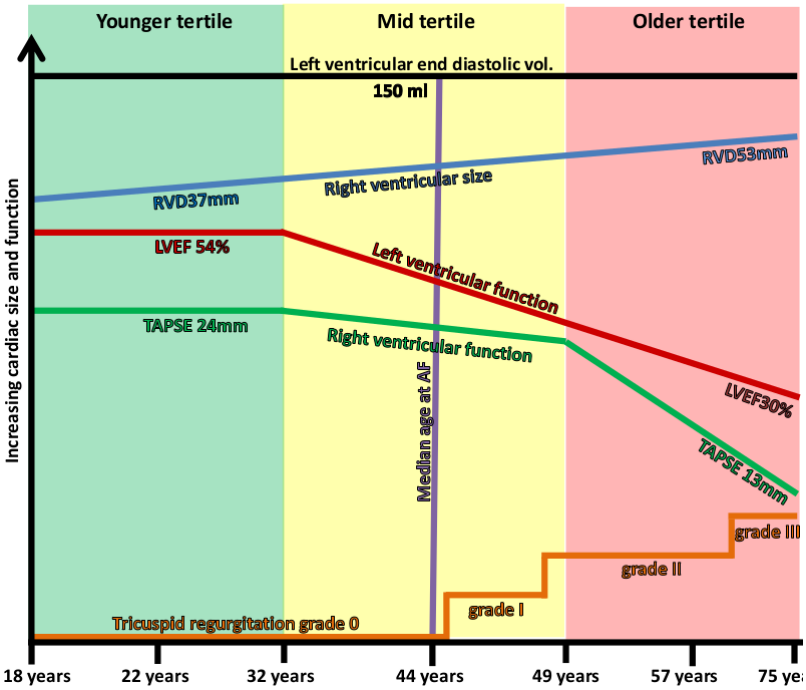


Figure 8- Schematic figure showing progression of Lamin A/C cardiomyopathy with increasing age. The X-axis displays by color the age of patients in the younger (green), middle (yellow) and older (red) tertiles. Cardiac function worsened and the right ventricle dilated with increasing age, while left ventricular volume did not increase. Y axis displays increasing cardiac size and function. Abbreviations: LVEF; Left ventricular ejection fraction, RVD; Right ventricular basal linear Dimension, TAPSE; Tricuspid annular plane systolic excursion. *Skjølsvik et al.* (3)

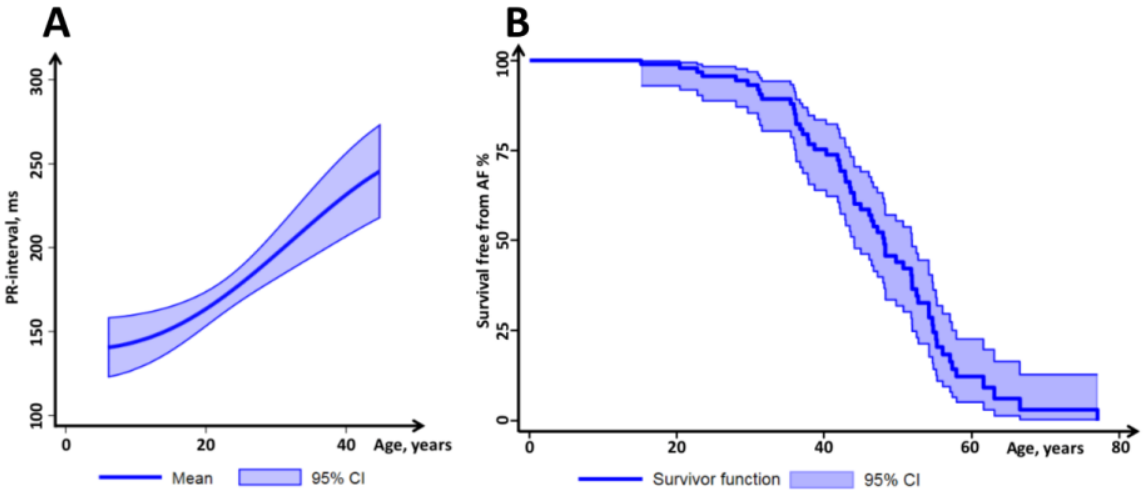


Figure 9 - A: Mathematical derived figure showing the expected PR-interval according to age.
B: Kaplan-Meier survival curve displaying survival free from atrial fibrillation (AF) according to age. Data are based on 576 echocardiographic exams and 258 electrocardiograms obtained during 4.4 (IQR: 2.5 - 6.6) years of follow-up. Blue line represents average or survival function, light blue shaded area represents 95% confidence interval. *Modified from: Skjølsvik et al.*(3)

Electrical disease

ECG data were recorded from 258 ECGs taken during follow-up. We observed a significant progression of PR interval in the young tertile, although the increase did not reach pathological value. A further acceleration of PR-interval progression was observed in the middle and older tertiles, showing in addition a higher incidence of AF (Figure 9). This suggested that electrical progression was faster from the middle tertile.

Structural progression and adverse outcome

In our population of LMNA+ patients, the outcome cardiac death/Htx/LVAD occurred in 18 patients during the follow-up period (n=3 (10%) young tertile; n= 3 (10%) middle tertile; n= 12 (44%) older tertile, p= 0.001). Increasing cardiac chamber size, worsening of LV and RV systolic function, increasing in severity of AV valve regurgitation, proband status and evolution of heart failure symptoms were all associated with increased odds for adverse outcome (Table 13). However, after adjustment for sex and age, comorbidities, LV EF, and NYHA class, only RV and LV dilatation, RV function by RV LS and increased severity of AV-regurgitation remained markers for adverse outcome. Interestingly, we observed that both proband status and LV EF disappear as marker for adverse outcome (Table 13).

Table 12. Structural and functional progression in 101 lamin A/C genotype patients by 576 echocardiographic assessments and 258 electrocardiograms

Variable	At inclusion, n=101	Progression rate / 1year (SE)	At last follow-up, n=94	p progression
LVEF, %	50 ± 12	-0.5 (0.1)	47 ± 13	<0.001
Younger (229/34)	54 ± 10	-0.2 (0.1)	53 ± 12	0.13
p for interaction		<0.01		
Middle (219/35)	51 ± 11	-0.8 (0.2)	49 ± 10	<0.001
p for interaction		0.60		
Older (128/32)	43 ± 13	-1.2 (0.3)	38 ± 12	<0.001
LVGLS, %	-15.5 ± 4.7	0.2 (0.1)	-14.5 ± 4.5	0.001
Younger (229/34)	-17.4 ± 4.2	0.02 (0.1)	-16.9 ± 4.1	0.70
p for interaction		<0.01		
Middle (219/35)	-16.5 ± 4.3	0.3 (0.1)	-14.2 ± 3.3	<0.001
p for interaction		0.94		
Older (128/32)	-13.0 ± 4.4	0.3 (0.2)	-11.8 ± 4.4	0.051
LVEDV, ml	136 ± 45	0.2 (0.3)	138 ± 43	0.60
Younger (229/34)	120 ± 23	0.2 (0.5)	131 ± 35	0.68
p for interaction		0.72		
Middle aged (219/35)	149 ± 51	-0.1 (0.5)	138 ± 41	0.90
p for interaction		0.01		
Older (128/32)	145 ± 47	-2.5 (0.7)	146 ± 54	<0.001
LAVI, ml/m2	45 ± 22	1.4 (0.2)	50 ± 25	<0.001
Younger (229/34)	30 ± 5	1.0 (0.2)	36 ± 10	<0.001
p for interaction		0.08		
Middle (219/35)	42 ± 15	1.7 (0.3)	51 ± 21	<0.001
p for interaction		0.17		
Older (128/32)	61 ± 28	2.4 (0.5)	69 ± 29	<0.001
MR, Grade 0-3	0.7 ± 0.8	0.04 (0.01)	1.0 ± 0.8	<0.001
Younger (229/34)	0.3 ± 0.6	0.02 (0.01)	0.5 ± 0.6	<0.01
p for interaction		<0.01		
Middle (219/35)	0.6 ± 0.7	0.07 (0.01)	1.0 ± 0.8	<0.001
p for interaction		0. 51		
Older (128/32)	1.1 ± 0.7	0.05 (0.02)	1.4 ± 0.8	0.03
RVD, mm	41 ± 7	0.2 (0.06)	43 ± 9	<0.001
Younger (229/34)	37 ± 6	0.1 (0.1)	39 ± 5	0.18
p for interaction		0.21		
Middle (219/35)	42 ± 6	0.3 (0.09)	44 ± 6	0.001
p for interaction		0.26		
Older (128/32)	45 ± 8	0.2 (0.1)	48 ± 11	0.15
TAPSE, mm	23 ± 6	-0.3 (0.06)	21 ± 6	<0.001
Younger (229/34)	24 ± 5	-0.03 (0.8)	23 ± 5	0.70
p for interaction		<0.01		
Middle (219/35)	24 ± 7	-0.4 (0.1)	23 ± 6	<0.001

p for interaction		<0.01		
Older (128/32)	21 ± 5	-0.9 (0.1)	16 ± 5	<0.001
RVLS, %	-19.7 ± 5.8	0.3 (0.1)	-17.8 ±5.9	<0.001
Younger (229/34)	-21.5 ± 5.2	0.1 (0.1)	-21.4 ± 4.1	0.40
P for interaction		0.03		
Middle (219/35)	-20.8 ± 5.5	0.4 (0.1)	-17.6 ± 4.7	<0.01
P for interaction		0.27		
Older (128/32)	-17.1 ± 6.3	0.6 (0.1)	-13.5 ± 5.9	<0.001
RA area, cm2	21 ± 8	0.5 (0.06)	24 ± 9	<0.001
Younger (229/34)	16 ± 5	0.1 (0.09)	18 ± 7	0.13
p for interaction		<0.001		
Middle (219/35)	20 ± 4	0.6 (0.1)	24 ± 6	<0.001
p for interaction		<0.001		
Older (128/32)	26 ± 9	1.3 (0.2)	31 ± 10	<0.001
TR, Grade 0-3	1.0 ± 0.8	0.06 (0.01)	1.4 ± 0.9	<0.001
Younger (229/34)	0.7 ± 0.7	0.03 (0.01)	0.9 ± 0.6	0.001
p for interaction		0.01		
Middle (219/35)	1.1 ± 0.5	0.06 (0.01)	1.5 ± 0.8	<0.001
p for interaction		0.01		
Older (128/32)	1.4 ± 0.9	0.12 (0.02)	2.0 ± 0.8	<0.001
Electrical parameters				
PR-interval, ms	204±73	4 (0.5)	211 ± 69	<0.001
Younger (167/34)	177 ± 54	3 (0.5)	185 ± 58	<0.001
p for interaction		<0.001		
Middle (62/35)	224 ± 83	11 (1.7)	248 ± 74	<0.001
p for interaction		0.42		
Older (29/32)	257 ± 76*	9 (2.3)	245 ± 43**	<0.001
QRS-width, ms	95 ± 21	0.4 (0.3)	96 ± 21	0.10
Younger (167/34)	89 ± 11	0.04 (0.2)	90 ± 16	0.85
p for interaction		<0.01		
Middle (62/35)	100 ±25	2 (0.7)	99 ± 17	<0.01
p for interaction		0.59		
Older (29/32)	106 ± 26	3 (1.7)	108 ± 39	0.10

Observed values at inclusion and last follow-up are mean ± standard deviation. Yearly progression rates with standard errors are calculated by linear mixed models' statistics with exchangeable covariance structure and random intercept, middle aged patients are reference category calculating p for interaction. Patients <18 years of age were excluded in evaluation of cardiac chamber size to avoid confounding by physiological growth. n, imaging, or ECG examinations/patients are reported in parentheses. * Average of 5 observations, **average of 5 observations. *Abbreviations:* LAVI indicates; Left Atrial Volume Index, LVEDV; Left Ventricular End Diastolic Volume, LVEF; Left Ventricular Ejection Fraction, LVGLS; Global Longitudinal Strain MR; Mitral Regurgitation, RA area; Right Atrial area, RVD; Right Ventricular basal linear Dimension, RVLS; Right Ventricular Longitudinal Strain, TR; Tricuspid Regurgitation, TAPSE; Tricuspid Annular Plane Systolic Excursion. *Modified from: Skjølsvik et al. (3)*

Table 13: Odds for adverse cardiac outcome (Htx or LVAD, or death from heart disease) in 101 lamin A/C genotype patients in univariate and multivariate mixed model logistic regression

	Univariate			Multivariate Adjusted for age, sex, LVEF, NYHA functional class and comorbidities		
	OR	CI	p	OR	CI	p
Age per year	1.3	1.21-1.42	**<0.001**	NA	NA	NA
Female	0.4	0.06-2.80	0.36	NA	NA	NA
Proband	160	2.2-11925	**0.02**	3.5	0.6-21	0.17
NYHA I-IV	30	11-84	**<0.001**	NA	NA	NA
LVEF per 5 %	2.5	1.9-3.3	**<0.001**	NA	NA	NA
LVGLS per %	1.5	1.3-1.7	**<0.001**	NA	NA	NA
LVEDV per 10 ml	1.5	1.2-1.9	**0.001**	1.2	1.006-1.4	**0.04**
LAVI per $5cm^3/m^2$	1.6	1.3-2.0	**<0.001**	1.0	0.8-1.1	0.65
MR per 0-3	20	6.1-69	**<0.001**	2.4	1.004-6.0	0.049
RVLS per %	1.7	4.7-42	**<0.001**	1.3	1.03-1.65	**0.03**
RVD per 3 mm	3.0	1.4-2.1	**<0.001**	1.5	1.1-2.1	**0.01**
RA area per $5cm^2$	4.6	2.5-8.8	**<0.001**	1.3	0.8-2.0	0.28
TR per 0-3	158	11.3-2182	**<0.001**	5.0	1.64-14.8	**<0.01**
TAPSE per 3 mm	3.1	2.1-4.6	**<0.001**	1.4	0.96-2.0	0.08

P values by mixed models logistic regression analyses. Comorbidities included hypertension, coronary artery disease, peripheral arterial disease, cerebrovascular disease, and diabetes mellitus. Parameters LAVI, LVEDV LVEF, RA area, RVD, and TAPSE are all continuous scale parameters. Abbreviations: LAVI; Left atrial volume index, LVEDV; Left ventricular end diastolic volume, LVEF; Left ventricular ejection fraction, LVGLS; Left ventricular global longitudinal strain, MR; Mitral regurgitation, NYHA; New York Heart Association functional class, OR; odds ratio, RA; Right atrium, RVD; Right ventricular basal linear dimension, RVLS; Right ventricular longitudinal strain, TAPSE; Tricuspid annular plane systolic excursion, TR; Tricuspid regurgitation. *Modified from: Skjølsvik et al. (3)*

Discussion

Main findings

Paper 1 and *2* showed that pregnancy did not seem to be associated with long-term disease penetrance in AC, nor with disease progression in Lamin A/C cardiomyopathy. A history of pregnancy was not a marker of arrhythmias, was not associated with worsening of cardiac function or structure, nor with adverse outcome on long-term follow-up. Pregnancies and deliveries were mostly well tolerated and uncomplicated. *Paper 3* provided a detailed description of the stages characterizing cardiac disease in Lamin A/C cardiomyopathy, suggesting a predominant electric disease in young LMNA+ patients, followed by structural and functional cardiac disease later in life, with a significant prognostic role of RV dysfunction.

Paper 1

Pregnancy and functional/structural impairment in AC

Our study was the first relatively large casuistic analyzing the effect of repeated pregnancies on cardiac dimensions and systolic function in AC female patients. We observed no significant long-term effect of pregnancy on the structure and function of right and left ventricles. Although we observed a tendency of increased in RVOT PSAX with repeated pregnancies, number of pregnancies was not associated with enlarged RVOT in the multivariable analysis. A larger independent analysis from a Scandinavian registry, including our cohort, confirmed these findings (60). In our selected cohort of women followed during pregnancy, no statistically significant differences in RV and LV parameters before pregnancy and in peripartum period were observed. This is in line with other reports in AC patients (40,61,62). Previous studies reported worsening of heart failure during pregnancy in AC patients (42); however, patients had known major RV structural abnormalities and LV dysfunction before the start of pregnancy (42).

Pregnancy and ventricular arrhythmias in AC

Number of pregnancies was not different in patients with or without history of VA, and in the multivariable analysis pregnancy was not a marker of VA. Interestingly, VA occurred at an earlier age in nulliparous. Younger age at VA in nulliparous was noted in another AC cohort (60). This could indicate a possible confounder as women with severe arrhythmias at young age might decide to avoid pregnancy.

During pregnancy, rare cases of arrhythmias were reported in our casuistic, and no episodes of sustained VA were noted. Other cohorts described sustained VA occurring during pregnancy in AC female population; in those, a higher percent of patients with definite AC diagnosis and symptoms before pregnancy were noted, and this could explain a more pronounce arrhythmic tendency (61) (42,60); however, in all these reports, most pregnancies were still reported uncomplicated and free from VA (40,42,61).

Paper 2

Pregnancy and progression of cardiomyopathy in LMNA+ female patients

In our large cohort of LMNA+ female patients, pregnancy was not associated with long-term progression of cardiac dilatation or systolic dysfunction. Annual progression of structural and functional LV parameters by echocardiography was not different in nulliparous and women with previous pregnancy. The combined outcome death, LVAD implantation and Htx showed no differences in the two groups. At age ≤32, LMNA+ patients showed mainly sign of electrical cardiac disease (3). Most of participants in our cohort experienced pregnancy at this age, and this could explain our findings. One previous case report on pregnancy characteristic in a cohort of LMNA+ patients showed no major cardiac events and no worsening in cardiac condition (63), in line with our results.

Pregnancy, electrical disease, and maternal/fetal outcomes

Prevalence of atrial fibrillation and AV conduction disease seemed to increase with the number of pregnancies. However, this association was not confirmed after adjustment for age. Lamin A/C

cardiomyopathy was shown to have a strong age-related penetrance, and our results were in line with these findings (22). ICD/CRT-D implantation was not associated with pregnancy in multivariable analysis. A relative higher prevalence of ICD/CRT-D was observed in our population compared to prevalence of VA. In our centers, LMNA+ patients are implanted with ICD/CRT-D when pacemaker indications are fulfilled, as previously suggested (64,65). This explained the high number of defibrillators in our population.

During pregnancy, a tendency to develop of VA was observed in our cohort of patients followed during pregnancy. This was a small and selected cohort; nevertheless, a tendency to develop arrhythmic disease during pregnancy cannot be excluded in LMNA+ population. No life threatening arrhythmias were reported in previous casuistics (63).

In our cohort, most deliveries were vaginal with low incidence of fetal and obstetrical complications. Three stillbirths were reported, and in two cases the causes of death were not completely clarified. We did not identify clues suggesting a causative effect of Lamin A/C disease in these reports; however, in women with lipodystrophy related to Lamin A/C defect, the burden of obstetrical and fetal complication was reported higher than in general population and we cannot excluded with certainty a possible relation with underlying LMNA+ variant (66).

Do AC and LMNA+ patients tolerate pregnancy?

We cannot give conclusive answers based on the results of *paper 1 and 2*. However, these studies provided some important insight. From a long-term perspective, pregnancy seemed to not resemble the effect of exercise on AC and LMNA+ patients, as we hypothesized. There are different possible reasons for this. First, exercise and pregnancy are different kinds of hemodynamic stress. In fact, cardiac work increases in both, although, while exercise is characterized by peripheral vascular constriction, during pregnancy peripheral vascular dilation is predominant (34). In pregnancy, conversely to regular sport exercise, the gradual developing of hemodynamic load might limit the effect on arrhythmias and/or on cardiac function/structure. Further, high intensity exercise was showed to have the main premise for

adverse outcome in AC (12). However, pregnancy could be compared to a prolonged low-intensity exercise, therefore probably not associated with adverse outcome as the previous. Histopathological studies suggested a role of inflammation in AC pathogenesis since lymphocyte infiltration was observed (67). However, pregnancy is characterized by physiological immunosuppression (68) that might protect the myocardium from external factors and stress. Hormonal adaptation occurring during pregnancy might also play a protective mechanism in AC and LMNA+ female patients (27). In addition, a previous study showed a later onset of cardiac phenotype in female mice homozygous for a missense variant of LMNA gene (69).

From a short-term perspective, the available data showed low risk related to pregnancy for the majority of AC and LMNA+ patients. Genetic cardiomyopathies showed in general an age-dependent and often complete penetrance. Thus, pregnant women might not be "old enough". The main maternal risk during pregnancy seemed to be the development of arrhythmic disease, including VA (2,42,61,62), while structural and functional disease manifestations were rarely reported during gestation. In LMNA+ population, childbearing period corresponds to the electrical phase of the disease (3), and this could explain the tendency to arrhythmic manifestations. Similarly, AC gene positive female showed more often electrical disease at first clinical control (70), occurring when in early adulthood.

History of previous arrhythmias might suggest a predisposition to electric disorders during gestation. Probands, patients with definite diagnosis and patients symptomatic during pre-pregnancy period for arrhythmias, might have higher risk of arrhythmias occurring during pregnancy in AC (42). However, sustained VA were reported also in patients without previous arrhythmic history, and even in women with a previously uneventful gestation (42,61). Thus, the risk of arrhythmias during pregnancy should be considered in all AC female patients. Discontinuation of medical therapy during pregnancy might play an important role and predispose to arrhythmic episodes.

According to our findings, most of studies showed uncomplicated vaginal delivery in AC and LMNA+ patients, with lower rate of obstetric complications. Low birth weight was reported as possible fetal outcome associated to antiarrhythmic treatment with beta-blocker (71). Poor long-term prognosis in

AC children was previously described (61). Although not included in the present analysis, these results are in line with a recent study from our group showing the high incidence of severe events in pediatric AC population (72).

Paper 3

Our paper described the stages of disease progression in Lamin A/C cardiomyopathy. We showed progression of electric disease from young age, while structural and functional modifications of left ventricle occurred from third decade of age. Left ventricle function deteriorated without compensatory LV dilatation. RV remodeling accelerated from the third decade together with tricuspid valve regurgitation, and both were independent markers of adverse cardiac outcome.

Early atrial affection and hypokinetic non- dilated cardiomyopathy

Analysis of structural and functional disease progression showed no significant change in LV chamber in the young tertile. On the other hand, the left atrium reached pathological dilatation at this age. The mechanism behind left atrium dilatation in the youngest patient is not clear. Some patients had already developed AF; however, whether AF represent a potential cause or a consequence of left atrial dilatation remains to be explored. A recent publication showed high prevalence of intrinsic LA myopathy in LMNA+ population defined by AF and impaired LA strain, even in absence of LV dysfunction and LA dilation (73). The left atrium is characterized by thin wall thickness and this was described as a possible reason for earlier atrial affection in Lamin A/C cardiomyopathy (73).

From middle to older tertiles, a progressive deterioration of LV and RV systolic function occurred. However, we observed no LV dilatation. Dilatation of left ventricle is a well-described compensatory mechanism occurring in the initial stages of heart failure (74). Reasons for this maladaptive mechanism in Lamin A/C cardiomyopathy are poorly identified; however, we may speculate that progressive myocardial fibrosis and stiffening of the left ventricle can make it less prone to dilatation (75). Failure in compensatory LV dilatation, together with RV dysfunction, dilatation and increased

tricuspid regurgitation, may describe the reasons for further decline of cardiac output, occurring earlier compared to other familiar DCM. These could probably partially explain the highly malignant course of the disease. Our observation of no significant LV dilatation is in line with previous studies and contribute to categorized Lamin A/C cardiomyopathy as hypokinetic non-dilated cardiomyopathy (76).

A slight reduction in LV volume was observed in the older tertile. This may reflect the selection of the "healthiest" LMNA+ patients, survived to this age. Another mechanism explaining this finding could be related to the higher prevalence of severe tricuspid regurgitation observed at this age. Due to massive volume regurgitation, RV shape becomes more spherical and this, combined with limited pericardial capacity and ventricular interdependence, could induce a leftward septal shifting during diastole. This phenomenon may lead to systematic errors of LV volumes by echocardiography in the old group and explain our results (77).

Right ventricle affection in Lamin A/C cardiomyopathy

The prevalence of right ventricular dysfunction in DCM varied in previous reports, ranging from 20 to 65% (78). In our cohort of LMNA+ patients, mean RV diameter, and RV function measured by TAPSE and RV LS, were normal at inclusion in the young and middle tertile. RV function by strain analysis was impaired at baseline from the fifth decade of age, and a faster progression was observed in this group. On other the hand, left ventricle systolic dysfunction by LV GLS was present from baseline in all groups of age. These results showed that in our cohort, RV and LV deterioration did not seem to occur at the same time. This might suggest that in this population the deterioration of right ventricle is probably a consequence of LV impairment and postcapillary pulmonary hypertension acting on the thin RV wall, combined with chronic volume overload due to tricuspid regurgitation. Thus, in Lamin A/C cardiomyopathy, RV impairment is probably a consequence of LV dysfunction more than the results of a primary cardiomyopathic process.

A previous report on a cohort of DCM patients with Titin genetic variants showed a prevalence of 20% of RV dysfunction at diagnosis, with a significant improvement of RV function during follow-up

(79). On the contrary, we found a significant deterioration of RV function during follow-up despite optimized medical therapy. This confirmed findings from previous studies showing a significant improve in cardiac function in Titin DCM patients after optimized medical therapy compared to Lamin A/C related DCM (80), suggesting the heterogeneity in the pathophysiological mechanism behind DCM and highlighting the need for further research in the field to tailor medical therapy according to genetic diagnosis.

Progression of RV dilatation, together with dysfunction and worsening in tricuspid regurgitation, were associated with adverse outcome in our cohort, and this association was independent from LV dysfunction and heart failure symptoms. Our findings are in line with previous reports emphasizing the prognostic role of tricuspid regurgitation and RV systolic dysfunction in heart failure (81,82).

The observation of a non-dilating LV combined with massive myocardial fibrosis (75), bi-atrial enlargement and failing RV could indicate an important role of restrictive physiology in Lamin A/C cardiomyopathy.

Clinical implications and future perspectives

Management of patients with cardiomyopathy planning a pregnancy

Our studies are reassuring for female patients with predisposition to AC and Lamin A/C cardiomyopathy, suggesting the unlikeliness of major effect of pregnancy on disease penetrance and progression on long-term follow-up. Considering that most of female patients tolerated pregnancies and deliveries, we found no holding point to prevent a priori these patients from gestation. However, data from our small cohorts of patients followed during pregnancy, together with results from previous literature, showed that arrhythmic events and worsening in heart failure could occur in these populations. This remarks the need for pre-pregnancy counseling with a tailored follow-up strategy during gestation and peripartum period.

The evaluation of cardiovascular maternal risk and indication to follow-up during pregnancy should be based on previous clinical manifestations, according to the latest ESC guidelines (34). No clear indication to follow-up during pregnancy is reported for women with a genetic predisposition as in AC or LMNA+ patients, without overt arrhythmical or structural clinical disease. However, based on the available data, I believe that women with a genetic predisposition to cardiomyopathy may benefit from pre-pregnancy counselling and clinical evaluation. A history of previous pregnancies, previously masked symptoms, and family history should be collected by detailed anamnesis, together with clinical assessment including ECG, 24 hours Holter ECG and echocardiographic examination. Based on the results of this evaluation, a dedicated pregnancy and peripartum follow-up could be planned. Moreover, these patients may take advantage of being informed on possible “alarm” symptoms and discussing the possible need for medications during pregnancy and eventual fetal/maternal consequences. It seems reasonable to centralize patients in a highly experienced center where a multidisciplinary team, including genetic counselors, cardiologists, obstetricians, midwifes and neonatologists, is available.

In a previous report on AC patients followed during pregnancy, all patients were advice against breast-feeding (40). Beta-blockers were the only reported medical treatment in this cohort during

pregnancy. Unfortunately, we did not collect data on breast-feedings in our population, so we cannot conclude about the safeness of breast-feeding. However, considering the amount of evidence on the benefits of breast-feeding for both mothers and babies, the choice to prevent AC mothers from this practice should be deeply evaluated (83). If the potential effect on electrolytes deprivation and subsequent arrhythmic risk in AC population could be a reason to prevent breast-feeding, this should be considered further in large cohort studies, while no clear evidence supports this decision nowadays. On the other hand, higher vagal control was shown in breast-feeding mothers (84) and this could potentially play an important effect against arrhythmic risk in these patients.

Tailored follow-up and treatment in LMNA+ patients

The results of our study confirmed previous evidence and increased our understanding about the mode of development of cardiomyopathy in LMNA+ patients. Thus, it may help to improve clinical follow-up and therapeutic strategies for these population.

Observation of the early onset of electrical disease, with increasing PR interval and early onset of AF in some patients, suggests the need for ECG and ambulatory ECG monitor from young age. Previous studies showed that VA might occur before manifest DCM in LMNA+ patients (4,64) and increased PR-interval was a risk marker for ventricular events. Therefore, focus on detection of potential life-threatening arrhythmic events should start from young age. We observed an early onset of LA dilatation, and a recent study identified LA myopathy in patients without evidence of ventricular impairment (73). These observations, together with previous evidence on higher thrombotic risk in LMNA+ patients (85), could suggest starting earlier with anti-coagulation therapy in case of AF in this population. However, further studies are needed to conclude on the benefit of this choice.

Structural and functional deterioration together with AV valve regurgitations progressed from the third decade of life, and RV deterioration was an important marker of end stage cardiomyopathy and adverse outcome. Thus, an echocardiographic assessment should be included in the routine clinical

follow-up of these patients and signs of RV impairment should alert the clinician, identifying patients at a potentially high risk of adverse outcome.

LVAD therapy showed excellent results on mortality in advanced stages of heart failure, both as end stage therapy and bridge to recovery (86,87), and represents an important therapeutic option in patients with advanced heart failure. However, the combination of non-dilated LV, progressive RV dilatation and dysfunction with large tricuspid regurgitation that we described, may suggest that this population is less suitable for LVAD. In fact, low LV end-diastolic diameter was shown to be an independent predictor of mortality after primary LVAD implantation (88). LVAD therapy was associated with increased risk in patients with moderate to severe RV dysfunction, with enlarged RV, and low RV longitudinal strain (89). Furthermore, LVAD therapy did not improve already established RV-failure (90). Additional studies should evaluate the effect of LVAD therapy in LMNA+ patients to identify eventual indication for earlier timing of LVAD in this population.

Progression of cardiac disease in inherited cardiomyopathies: what are we missing?

The findings of this thesis suggested the presence of a pattern in the pathophysiology and phenotypical presentations of AC and Lamin A/C cardiomyopathy, wherein physiological triggers such as pregnancy do not seem to play an influential role. Except for the role of high intensity exercise, we are yet to identify factors that promote penetrance and progression of the disease in most patients with inherited cardiomyopathies. Consequently, from a clinical point of view, we are currently unable to provide our patients carrying a genetic variant with adequate guidance on how to avoid disease progression. These observations underscore the pressing need for further investigations to identify such factors that can either promote or retard the development of clinical manifestations in these populations.

Limitations

General limitations

In *paper 1 and 3* we performed single-center studies, and the external validity of our results is undetermined. On the other hand, in *paper 2* we included patients from two different large referral centers and variability related to local clinical practice could be introduced. Although relatively large, our AC- and LMNA+ cohort are small in epidemiological terms, and this has implications in relation to statistical power and robustness of statistical analysis.

Both in paper 1 and 2, data on pregnancy/post-partum and delivery were collected retrospectively and most of patients experienced pregnancy years before. This predisposed to recall bias. Additionally obstetric and fetal outcomes were mostly self-reported, exposing to potential report bias. Most of participants in these studies were young at time of pregnancy and in relatively early phase of the disease, then relatively healthy at time of pregnancy.

ECG Holter registration and exercise ECG were performed by clinical indication, identifying potential selection bias.

Echocardiography was the main imaging modality of this thesis. The method is widely available, however variability within and between operators and observers is a known limitation. To compensate for this, in *paper 1* all measurements were taken by the same sonographer. In *paper 2 and 3*, all measurements taken at OUS Rikshospitalet were made by experienced echocardiographers, and we assured that all measurements clustered within a patient were performed by the same sonographer to avoid systematic measurement bias. In *paper 2*, we included echocardiographic data from Brigham and Women Hospital, USA, and potential variability related to sonographer, echocardiographic protocol and echo machine cannot be excluded.

CMR data, reported in paper 1, might be affected by a selection bias. In fact, CMR was performed based on clinical indication. On the other hand, this technique is less susceptible to observed variability.

Specific limitations

Paper 1

This was a cross-sectional study. However, pregnancy characteristics and outcome were collected retrospectively with inherent limitation. Our AC population had a high prevalence of PKP2 mutations, and our findings may not be applicable to other AC populations. We observed that women without history of pregnancy experienced arrhythmias at young age. This represents an important potential bias difficult to control. Survival bias cannot be excluded in older groups.

Paper 2

The retrospective design of this study suggests potential recall bias. The multicenter design introduced potential bias related to local clinical practice. A substantial number of echocardiographic data on LV EDV were missing from Brigham and Women Hospital (Boston, USA), and this parameter could not be considered when analyzing LV dilatation. We cannot exclude that LMNA+ patients with more serious family history or disease penetrance could have decided to avoid pregnancy.

Paper 3

Subtle signs of structural progression might be difficult to detect and to distinguish from variability of echocardiographic measurements. Lack of diastolic data is a limitation in this study. In fact, restrictive physiology with secondary pulmonary hypertension could explain RV dysfunction as an important marker for outcome. Further study should clarify the role of diastolic dysfunction in this population. The three tertiles are not homogenous and other potential confounders, such as medications and comorbidities, could have influenced the echocardiographic progression we reported. The older tertile could include more probands, although no difference of disease progression in probands and family members was previously shown. We focused on heart failure endpoints. However, Lamin A/C cardiomyopathy is highly arrhythmogenic and association between structural disease and VA should be included in further studies.

Conclusions

General conclusions

The results of this thesis increased our knowledge about effect of pregnancy on cardiac disease in AC and LMNA+ female patients, and on progression of cardiac disease in patients with Lamin A/C cardiomyopathy. A history of pregnancy was not associated with increase penetrance or adverse progression of the cardiac disease in our cohort of patients with AC and LMNA+. In our LMNA+ population, we identified a pattern in the progression of cardiomyopathy, with electrical disease preceding functional and structural disease. All the three studies had important limitations. Our observations should be validated by prospective analysis in separate AC and LMNA+ cohorts.

Specific conclusions

Paper 1

On long-term follow-up, number of pregnancies was not associated with adverse cardiac phenotype in our cohort of AC female patients. The number of pregnancies was not associated with a worse structural or functional cardiac function and was not a risk marker of VA. Age at VA was not different between AC patients grouped by number of previous pregnancies. Gestation and delivery were mostly well tolerated and uncomplicated.

Paper 2

In our cohort of female LMNA+ patients, pregnancy was not associated with worse cardiac phenotype, nor with arrhythmias or adverse heart failure outcomes. The progression of structural cardiac disease was not different in women with or without previous history of pregnancy. Age at onset of atrial fibrillation, VA and heart failure outcomes was not different in nulliparous and female with history of pregnancy. Gestation, delivery and peripartum were mostly well tolerated and uncomplicated, with low prevalence of maternal and fetal complications. However, we could not exclude that during pregnancy arrhythmias might be triggered in selected patients.

Paper 3

In our cohort, the clinical presentation of Lamin A/C cardiomyopathy started at young age and was mainly characterized by electrical manifestations, including AF and AV block, together with atrial dilatation. Structural and function progression of cardiac disease was slow at young age. With increasing age, we observed a progressive deterioration of left-ventricle function, and a subsequent rapid deterioration of RV function, which was an independent marker of adverse outcome. Limited compensatory LV dilatation was observed, suggesting different pathogenic mechanisms from other forms of DCM.

Appendix

Appendix 1– TFC major and minor criteria in AC female patients grouped by number of pregnancies

Number of pregnancies / TFC	0 (n=19)	1 (n=16)	2 (n=30)	≥3 (n=12)	*p-value**	>0 (n=58)	*p-value 0 vs >0*
AC severity score	4.3±2.8	3.4±1.6	4.7±2.8	4.3±2.3	0.53	2.1±2.2	0.47
Global or regional dysfunction							
Major TFC criteria (n, (%))	5 (26)	2 (13)	16 (53)	4 (33)	0.38	22 (38)	0.41
Minor TFC criteria (n, (%))	1 (5)	1 (6)	0 (0)	0 (0)	0.37	1 (2)	0.44
Tissue characterization							
Major TFC criteria (n, (%))	1 (5)	0 (0)	0 (0)	0 (0)	0.61	0 (0)	0.25
Minor TFC criteria (n, (%))	1 (5)	0 (0)	0 (0)	0 (0)	0.61	0 (0)	0.25
Repolarization abnormalities							
Major TFC criteria (n, (%))	8 (42)	5 (31)	12 (40)	3 (25)	0.79	20 (35)	0.59
Minor TFC criteria (n, (%))	0 (0)	1 (6)	1 (3)	2 (17)	0.21	4 (7)	0.57
Depolarization abnormalities							
Major TFC criteria (n, (%))	4 (21)	1 (6)	4 (13)	2 (17)	0.71	7 (12)	0.50
Minor TFC criteria (n, (%))	2 (11)	6 (38)	7 (23)	1 (8)	0.19	14 (24)	0.33
Arrhythmias							
Major TFC criteria (n, (%))	3 (16)	0 (0)	3 (10)	3 (25)	0.18	6 (10)	0.68
Minor TFC criteria (n, (%))	7 (37)	7 (44)	14 (47)	3 (25)	0.64	24 (41)	0.79
Family history							
Major TFC criteria (n, (%))	15 (79)	13 (81)	25 (83)	10 (83)	0.98	48 (83)	0.74
Minor TFC criteria (n, (%))	0 (0)	0 (0)	0 (0)	1 (8)	0.16	1 (2)	1.0

Data are presented as n (%) and mean±SD. Modified from Castrini AI et al. *p-value from F test.

Appendix 2 - Pregnancy and peripartum questionnaire applied in *paper 2*

Lamin A/C mutasjon og svangerskap - Spørreskjema

Kryss av for korrekt svar. Du kan avgi flere svar når det er passende.

1. Hvor mange svangerskap har du hatt?
   - ☐ 0
   - ☐ 1
   - ☐ 2
   - ☐ 3
   - ☐ >3

2. Har du hatt spontanabort før uke 12 (innen først trimester?) Hvor mange?
   - ☐ 0
   - ☐ 1
   - ☐ 2
   - ☐ 3
   - ☐ >3

3. Hvor mange barn har du?
   - ☐ 0
   - ☐ 1
   - ☐ 2
   - ☐ 3
   - ☐ ……

4. Hva er dato(ene) for din/din fødsel/fødsler?
   - 1. fødsel ……
   - 2. fødsel ……
   - 3. fødsel ……
   - 4. fødsel ……
   - ….. fødsel ……

5. Hadde du vaginal fødsel eller keisersnitt?
   - 1. fødsel:
     Vaginal/keisersnitt
   - 2. fødsel:
     Vaginal/keisersnitt
   - 3. fødsel:
     Vaginal/keisersnitt
   - 4. fødsel:
     Vaginal/keisersnitt
   - ….

6. Når har du fått vite om at du har mutasjon for Lamin genet?
   - ☐ Før første svangerskap?
   - ☐ Før andre svangerskap?
   - ☐ Før tredje svangerskap?
   - ☐ Før fjerde svangerskap?
   - ☐ Før femte svangerskap?

7. Hadde du noe ubehag/symptomer før ditt første svangerskap?
   - ☐ Nei
   - ☐ Hjertebank
   - ☐ Tung pust
   - ☐ Smerte i brystet
   - ☐ Svimmelhet
   - ☐ Besvimelse

8. Hadde du noe ubehag/symptomer:
   - Gjennom ditt først svangerskap?
     - ☐ Nei
     - ☐ Hjertebank
     - ☐ Tung pust
     - ☐ Smerte i brystet
     - ☐ Svimmelhet
     - ☐ Besvimelse
   - Gjennom ditt andre svangerskap?
     - ☐ Nei
     - ☐ Hjertebank
     - ☐ Tung pust
     - ☐ Smerte i brystet

- Svimmelhet
- Besvimelse

- Gjennom ditt tredje svangerskap?
  - Nei
  - Hjertebank
  - Tung pust
  - Smerte i brystet
  - Svimmelhet
  - Besvimelse

- Gjennom ditt fjerde svangerskap?
  - Nei
  - Hjertebank
  - Tung pust
  - Smerte i brystet
  - Svimmelhet
  - Besvimelse

- Gjennom ditt … svangerskap?
  - Nei
  - Hjertebank
  - Tung pust
  - Smerte i brystet
  - Svimmelhet
  - Besvimelse

9. Gjennom ditt svangerskap var dine symptomer…
   - redusert
   - uendret
   - forverret

   … i forhold til før svangerskapet?

10. Hadde du noen symptomer/ubehag <u>etter</u> ditt siste svangerskap?
    - Nei
    - Hjertebank
    - Tung pust
    - Smerte i brystet
    - Svimmelhet
    - Besvimelse

11. Ble du <u>innlagt</u> på sykehus de seks første månedene etter fødsel på grunn av en eller flere av følgende?
    - Nei
    - Ja, på grunn av hjerte bank
    - Ja, på grunn av tung pust
    - Ja, på grunn av smerte i brystet
    - Ja, på grunn av svimmelhet
    - Ja, på grunn av besvimelse
    - Ja, på grunn av hjertesvikt
    - Ja på grunn av arytmier
      - Atrieflimmer
      - Ventrikulær arytmi
      - Langsom rytme
    - Annet?

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12. Hadde du noen komplikasjoner gjennom en eller flere av dine fødsler eller rett etter?
    - Nei
    - Hjerte bank
    - Tung pust
    - Smerte i brystet
    - Svimmelhet
    - Besvimelse
    - Hjertesvikt
    - Arytmier
      - Atrieflimmer
      - Ventrikulær arytmi
      - Langsom rytme
    - Annet?……
      ……
      ……

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13. Var det komplikasjoner for barnet gjennom svangerskapet eller ved fødsel?
    - Første barn:
        - ☐ Ingen komplikasjoner
        - ☐ Dødfødsel
        - ☐ Prematur fødsel
        - ☐ Lav vekt for svangerskapsalder
        - ☐ Infeksjon
        - ☐ Behov for intensiv overvåkning
    - Andre barn:
        - ☐ Ingen komplikasjoner
        - ☐ Dødfødsel
        - ☐ Prematur fødsel
        - ☐ Lav vekt for svangerskapsalder
        - ☐ Infeksjon
        - ☐ Behov for intensiv overvåkning
    - Tredje barn:
        - ☐ Ingen komplikasjoner
        - ☐ Dødfødsel
        - ☐ Prematur fødsel
        - ☐ Lav vekt for svangerskapsalder
        - ☐ Infeksjon
        - ☐ Behov for intensiv overvåkning
    - Fjerde barn:
        - ☐ Ingen komplikasjoner
        - ☐ Dødfødsel
        - ☐ Prematur fødsel
        - ☐ Lav vekt for svangerskapsalder
        - ☐ Infeksjon
        - ☐ Behov for intensiv overvåkning
    - …….. svangerskap:
        - ☐ Ingen komplikasjoner
        - ☐ Dødfødsel
        - ☐ Prematur fødsel
        - ☐ Lav vekt for svangerskapsalder
        - ☐ Infeksjon
        - ☐ Behov for intensiv overvåkning

14. Bruker du nå medisiner for hjertet?
    - JA/NEI
    - Husker du navn på medisin?
      ……
      ……
    - Husker du når har du begynt å bruke det (dd.mm.åååå):
      ……
      ……

15. Har du brukt hjertemedisiner før et eller flere svangerskap?
    - JA/NEI
    - Husker du navn på medisin?
      ……
      ……
    - Husker du når har du begynt å bruke det (dd.mm.åååå):
      ……
      ……

16. Har du begynt å bruke medisiner gjennom et eller flere svangerskap?
    JA/NEI
    - Første svangerskap?

      JA/NEI
      - Husker du navn på medisin?
      - Husker du når har du begynt å

bruke det (dd.mm.åååå):
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- Husker du navn på medisin?
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- Ca. når har du sluttet å bruke disse medisinene?
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- Andre svangerskap?

JA/NEI
- Husker du navn på medisin?
- Husker du når har du begynt å bruke det (dd.mm.åååå):
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Kommentarer:
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- Tredje svangerskap?

JA/NEI
- Husker du navn på medisin?
- Husker du når har du begynt å bruke det (dd.mm.åååå):
……
……

- Fjerde svangerskap?

JA/NEI
- Husker du navn på medisin?
- Husker du når har du begynt å bruke det (dd.mm.åååå):
……
……

17. Har du sluttet å bruke medisiner <u>gjennom en eller flere svangerskap</u>?

JA/NEI

Appendix 3 - Pathogenic and likely pathogenic gene variants found in our cohort of 89 LMNA+ female patients

Variant (HGVS)	Variant (protein)	Number of patients (%)	Type of mutation
c.43C>T	p.Q15X	2 (2)	Nonsense
c.73C>T	p.R25C	2 (2)	Missense
c.215G>T	p.R72L	1 (1)	Missense
c.234G>T	p.K78N	1 (1)	Missense
c.305T>C	p.L102P	1 (1)	Missense
c.427T>C	p.S143P	1 (1)	Missense
c.481G>A	p.E61K	1 (1)	Missense
c.585C>G	p.N195K	1 (1)	Missense
c.608A>G	p.E203G	4 (5)	Missense
c.629T>G	p.I210S	1 (1)	*not know
c.642delG	p.E214DfsX266a	6 (7)	Frameshift
c.673C>T	p.R225Ter	1 (1)	Nonsense
c.725C>T	p.A242V	1 (1)	*not know
c.863C>G	p.A288G	2 (2)	Missense
c.868G>A	p.E290K	1 (1)	Missense
c.886_887insA	p.R296QfsX35	16 (18)	Frameshift
c.961C>T	p.R321X	26 (29)	Nonsense
c.992G>A	p.R331Q	3 (3)	Missense
c.1003C>T	p.R335W	2 (2)	Missense
c.1129C>T	p.R377C	3 (3)	Missense
c.1189delC	p.Arg397Alafs*83	2 (2)	Frameshift
c.1146C>T	p.G382G	2 (2)	*not know
c.1215_1218delCTCA	p.Ser406Profs*73	1 (1)	Frameshift
c.1300_1307del	p.A434X	1 (1)	Nonsense
c.1541G>A	p.W514Ter	1 (1)	Nonsense
c.1609-1G>A	*not known	2 (2)	Splice site
c.1621C>T	p.R541C	1 (1)	Missense
c.(?_1)_(356_?)del	p.? (deletion exon 1)	2 (2)	*not know
c.?	p.? (deletion exons 10-12)	1 (1)	*not know

Values are frequency (%). HGVS= Human Genome Variation Society. Modified from Castrini AI et al.(2)

Appendix 4 – Pathogenic and likely pathogenic gene variants found in our cohort of 101 LMNA+ patients.

Variant (HGVS)	Variant (protein)	Number of patients (%)	Type of mutation
c.961C>T	p.R321X	32 (32)	Nonsense
c.886-887insA	p.R296QfsX35a*	27 (27)	Frameshift/Nonsense
c.1063C>T	p.Q355X	4 (4)	Nonsense
c.1129C>T	p.R377c	2 (2)	Missense
c.642delG	p.E214DfsX266*	14 (14)	Frameshift
c.1381-1 G>A	"_"*,†	2 (2)	Splice site
c.427T>C	p.S143P	1 (1)	Missense
c.868G>A	p.E290K	1 (1)	Missense
c.43C>T	p.Q15X	2 (2)	Nonsense
c.730G>A	A244T*	1 (1)	Missense
c.992G>A	p.R331Q	8 (8)	Missense
c.1003C>T	p.R335W	4 (4)	Missense
c.305T>C	p.L102P	1 (1)	Missense
c.1016C>A	p.A339E*	1 (1)	Missense
c.568C>T	p.R190W*	1 (1)	Missense

Values are frequency (%). *Novel mutation, †Mutations in an acceptor site may cause more than one mutant transcript with different effects at the protein level. HGVS= Human Genome Variation Society. Modified from Skjølsvik ET et al.(3)

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Errataliste

Abbreviations for type of corrections: Cor - corrected

Page	Line/ table	Section	Original text	Type of correction	Corrected text
3	1	Table of content	Arrhythmia's definition	Cor	Definition of arrhythmias
5	4	Summary	..disease's penetrance..	Cor	..penetrance of the disease..
5	9-12	Summary	..disease's progression..	Cor	..disease progression..
13	4	Introduction	..of myocardium..	Cor	..of the myocardium..
13	10	Introduction	..test have contribute to identify..	Cor	..tests have contributed to identifying..
14	12	Introduction	..disease's progression	Cor	..progression of the disease..
15	Table 1	Caption	Define	Cor	Definite
16	26	Introduction	..adaptation is the peripheral vasodilation, causing fall..	Cor	..adaptations is peripheral vasodilation, causing a fall…
17	2	Introduction	Increase in cardiac output is the results from..	Cor	The increase in cardiac output results from..
17	3	Introduction	..increase in..remodeling of placenta..with further..	Cor	..an increase in..remodeling of the placenta..with a further..
17	4	Introduction	..and lowering..of 10..	Cor	..and the lowering..by 10
17	7	Introduction	..on inferior ..	Cor	..on the inferior..
17	9	Introduction	..includes 1) increase	Cor	..include 1) an increase
17	10	Introduction	2) chronotropic..3) mechanical..	Cor	2) the chronotropic…3) the mechanical…
17	11	Introduction	..and hormonal..	Cor	..as well as hormonal..
17	12	Introduction	..heathy	Cor	..healthy
17	13	Introduction	..with predisposition	Cor	..with a predisposition
17	17	Introduction	..at relatively..	Cor	..at a relatively..
17	23	Introduction	..are advice..	Cor	..are advised..
18	1	Introduction	..about effect..	Cor	..on the effect..
18	2	Introduction	..and makes it.. to give advices..	Cor	..which makes it…to give advice..
19	3	Aims	..to progression..	Cor	..to the progression..
19	4	Aims	..on disease's penetrance	Cor	..on disease penetrance..
19	12	Aims	..and higher burden..	Cor	..and a higher burden..
22	6-7	Material	…referred patients to first cardiac evaluation to our department..	Cor	..referred patients to our department for a first cardiac evaluation..
22	21	Material	..define..	Cor	..definite..
25	5-6	Methods	..data as weight, height…	Cor	..data such as weight and height..
25	6	Methods	..patient's journals..	Cor	..patients' journals..
25	8	Methods	..a detail anamnestic..	Cor	..a detailed anamnestic..
25	10	Methods	..from electronic patient`s journal	Cor	..from the electronic patients`journals..

26	5-6	Methods	From 7 November…was performed..	Cor	Since 7 November….has been performed..
26	9	Methods	..Unit for..	Cor	..the Unit for..
26	14	Methods	..patient's journals..		..patients' journals..
26	16	Methods	..or death fetus..	Cor	..or dead fetus..
27	9	Methods	..ECG were taken at same..	Cor	ECGs were taken during the same..
27	10	Methods	..recorded presence..	Cor	..recorded the presence..
27	17	Methods	..was obtain at first..	Cor	…was obtained at the first…
27	23-24	Methods	..was taken at first clinical..	Cor	…was obtained at the time of the first..
27	25	Methods	…patients' journal.	Cor	..patients' journals.
28	3	Methods	..the section about..	Cor	..the section on…
28	5	Methods	…test ECG…to clinical indication..	Cor	..test ECGs..to clinical indications…
28	6	Methods	..on cycle ergometer	Cor	..on a cycle ergometer
28	7	Methods	..increasing of 25 Watts	Cor	..increasing by 25 Watts
28	14	Methods	..per minut..	Cor	..per minute..
28	15	Methods	..as in a previous..	Cor	..as in a previously..
28	23	Methods	..of continue innovation..	Cor	..of continued innovation..
29	23	Methods	..paper..	Cor	…papers..
29	24	Methods	..taken during follow-up..	Cor	…taken during the follow-up..
30	2	Methods	..exams taken..	Cor	..examinations obtained..
30	8	Methods	..by modified..	Cor	..by the modified..
30	9	Methods	..by area length	Cor	..by the area length
30	10	Methods	..to obtain left atrial..	Cor	..to obtain the left atrial..
30	19	Methods	..on clinical indication, often at first clinical control.	Cor	..on clinical indications, often on the first clinical control.
31	2	Methods	, as appropriated.	Cor	, as appropriate.
31	7	Methods	..AC score form..	Cor	..AC score from..
31	20	Methods	..between time-varying..	Cor	..between the time-varying..
32	6	Methods	.. and middle age..	cor	..and the middle age..
32	8	Methods	..confounder..Marker..	Cor	..confounders..Markers..
32	12	Methods	..constructed applying..	Cor	..constructed by applying..
33	6	Summary of results	..with defined..	Cor	..with definite..
33	6	Summary of results	Detailed description..	Cor	A detailed description..
33	14	Summary of results	Most of women experienced..	Cor	Most of the women had experienced..
33	18	Summary of results	..in number of probands..	Cor	..in the number of probands..
34	10	Summary of results	, we introduce..	Cor	,we introduced..
34	16	Summary of results	..VA had higher..	Cor	..VA had a higher..
34	19	Summary of results	..marker of VA.	Cor	..markers of VA.
39	4	Summary of results	..1 patients..	Cor	..1 patient..

40	8	Summary of results	At firsts clinical..	Cor	At the first clinical..
40	9	Summary of results	..underwent..	Cor	..undergone..
40	17	Summary of results	At last clinical…	Cor	At the last clinical..
40	17	Summary of results	..older then..	Cor	..older than..
40	19-20	Summary of results	However, number of pregnancy..	Cor	However, the number of pregnancies..
40	21-24	Summary of results	..probands status..	Cor	..proband status
47	18	Summary of results	From middle tertile, LV function ..	Cor	From the middle tertile, the LV function..
47	25	Summary of results	..in middle..	Cor	..in the middle..
48	1	Summary of results	..tertile.	Cor	..tertiles.
50	3	Summary of results	..in young tertile..	Cor	..in the young tertile..
50	4	Summary of results	..and older tertile,..	Cor	..and older tertiles,..
50	6	Summary of results	..middle tertile.	Cor	..the middle tertile.
50	11-15	Summary of results	probands status	Cor	proband status
50	12-14	Summary of results	increase	Cor	increased
55	3	Discussion	Interesting, VA occurred at earlier..	Cor	Interestingly, VA occurred at an earlier..
55	8	Discussion	..with define AC..	Cor	..with definite AC
55	10	Discussion	..in all this reports..	Cor	..in all these reports..
55	17	Discussion	The combine..	Cor	The combined..
56	1	Discussion	..was showed..	Cor	..was shown..
56	5	Discussion	..indication is fulfilled..	Cor	..indications are fulfilled..
56	7	Discussion	..to development..	Cor	..to develop..
56	10	Discussion	..previous casuistic.	Cor	..previous casuistics.
56	11	Discussion	.,most of deliveries..	Cor	, most deliveries..
56	12	Discussion	..the reasons of death..	Cor	..the causes of death..
56	13	Discussion	..did not identified..	Cor	..did not identify
56	19	Discussion	On long-term…	Cor	From a long-term...
56	21-22	Discussion	In fact in both cardiac work increases..	Cor	In fact, cardiac work increases in both..
57	7	Discussion	..later-onset..	Cor	..later onset..
57	7	Discussion	..muse homozygote for missense variant..	Cor	..mice homozygous for a missense variant..
57	9	Discussion	On the short-term..	Cor	From a short-term..
57	21	Discussion	..with previous..	Cor	..with a previously..
58	10	Discussion	..markers adverse..	Cor	..markers of adverse..
58	13	Discussion	Contrary, left atrium..	Cor	On the other hand, the left atrium..
58	14	Discussion	..at the youngest..	Cor	..in the youngest..

58	14-15	Discussion	..developed already AF; however, if AF represent..	Cor	..had already developed AF; however, whether AF represents…
58	18	Discussion	Left atrium..	Cor	The left atrium…
58	22	Discussion	Reasons of..	Cor	Reasons for ..
59	24	Discussion	Previous reports on DCM cohort with Titin…reported..	Cor	A previous report on a cohort of DCM patients with Titin…showed..
60	1	Discussion	Contrary..	Cor	On the contrary..
60	5	Discussion	..in field..	Cor	..in the field..
60	7	Discussion	..RV dilation..	Cor	..RV dilatation..
61	16	Clinical implications	..previous masked..	Cor	..previously masked..
61	17	Clinical implications	..,family history	Cor	..,and family history, ..
61	20	Clinical implications	..advantage from being..	Cor	..advantage of being..
61	20	Clinical implications	..from discuss possible need of medications..and possible fetal…	Cor	..and discussing the possible need for medications…and eventual fetal..
61	22	Clinical implications	..to a high experience center…team, including genetic counselors..	Cor	..in a highly experienced center…team of genetic counselors..
61	25	Clinical implications	Beta-blocker was the only reported treatment..	Cor	Beta-blockers were the only reported medical treatment..
62	2	Clinical implications	..amount of evidences on benefits..	Cor	..amount of evidence on the benefits..
62	7	Clinical implications	..was showed..	Cor	..was shown..
62	13	Clinical implications	The observation on early onset..	Cor	Observation of the early onset..
63	1-2	Clinical implications	…patients at potential high risk for..	Cor	..patients at a potentially high risk of…
63	3	Clinical implications	..stage..	Cor	..stages..
63	5	Clinical implications	..advance..	Cor	..advanced..
63	6	Clinical implications	..regurgitation we described..	Cor	..regurgitation that we described..
63	7	Clinical implications	..was showed..	Cor	..was shown..
63	19	Clinical implications	..on a clinical..	Cor	..from a clinical..
64	6	Limitations	..epidemiological regards, and this has implications in terms of..	Cor	..epidemiological terms, and this has implications in relation to..
64	24	Limitations	..this technic..	Cor	..this technique..
65	4	Limitations	..may not be applicate..population	Cor	..may not be applicable..populations
65	11	Limitations	..to analyzed…	Cor	..when analyzing..
65	18	Limitations	..as medication..	Cor	.. such as medications..
66	13	Conclusions	Number of pregnancies was not associated with worse…	Cor	The number of pregnancies was not associated with a worse..

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Paper 1

Number of pregnancies and subsequent phenotype in a cross-sectional cohort of women with arrhythmogenic

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Number of pregnancies and subsequent phenotype in a cross-sectional cohort of women with arrhythmogenic cardiomyopathy

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Aims We aimed to assess the relation between number of pregnancies and cardiac structure, function, and arrhythmic events in women with arrhythmogenic cardiomyopathy (AC).

Methods and results We included female AC patients in a cross-sectional study. Number of pregnancies and pregnancy related symptoms were recorded. Ventricular arrhythmias were defined as aborted cardiac arrest, sustained ventricular tachycardia, or appropriate implantable cardioverter-defibrillator therapy. Right and left ventricular dimensions and function, including strain analyses, were assessed by echocardiography and magnetic resonance imaging. We created a new AC severity score to grade the severity of AC disease. We included 77 women (age 47 ± 16, 43 probands and 34 AC mutation positive female relatives), 19 ± 14 years after last pregnancy. Median number of pregnancies was 2 (0–4); 19 had no previous pregnancies, 16 had 1 pregnancy, 30 had 2, and 12 had ≥3 pregnancies. Presence of a definite AC diagnosis ($P = 0.36$), severity of AC disease ($P = 0.53$), and arrhythmic events ($P = 0.25$) did not differ between groups of pregnancies. Number of pregnancies was related to increased right ventricular outflow tract diameter in single variable analyses [odds ratio (OR) 1.76, 95% confidence interval (CI) 1.08–2.87; $P = 0.02$], but not when adjusted for body surface area and age (OR 1.56, 95% CI 0.91–2.66; $P = 0.11$). The number of pregnancies was not associated with any other measures of cardiac structure and function.

Conclusion Higher number of pregnancies did not seem to relate to a worse phenotype in women with AC.

Keywords Arrhythmogenic right ventricular cardiomyopathy • Pregnancy • Right ventricle • Cardiomyopathy

Introduction

Arrhythmogenic cardiomyopathy (AC) is an inheritable, chronic, and progressive cardiomyopathy, and one of the leading causes of sudden cardiac death in young individuals.[1,2] The most common forms of AC follow autosomal dominant inheritance patterns and affect cardiac desmosomes leading to progressive loss of cardiomyocytes, followed by fibrofatty replacement.[1,3] The cardiac phenotype presents as right ventricular (RV) dilatation, wall thinning, aneurysms and impaired function, with increased occurrence of life-threatening ventricular arrhythmias (VA). Pathology is not restricted to the right ventricle as left ventricular involvement is commonly recognized.[1,2]

Recent reports have indicated that occurrence of VA and disease progression in AC is worsened by vigorous exercise.[1,4–7] Pregnancy could be regarded as a comparable state of prolonged haemodynamic stress with increased wall stress due to volume expansion, rise in stroke volume and heart rate, in addition to sympathetic stimulation and hormonal changes.[8,9] Thus, pregnancy might affect disease progression in AC. As the disease is frequently diagnosed in women

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of childbearing age,[10] data regarding the effect of pregnancy on disease progression is needed to improve patient advice.[11] However, such data are sparse with only two published studies on the effect of pregnancy in 6 and 26 women with AC,[12,13] respectively, in addition to case reports.[14,15] We aimed to investigate the relation between parity and cardiac structure, function, arrhythmias, and clinical course in female AC patients.

Methods

Study population

In this cross-sectional study, consecutive female AC patients were recruited from the Unit for genetic cardiac diseases, Oslo University Hospital, Rikshospitalet, Norway. The AC diagnosis was based on the 2010 Task Force Criteria (TFC).[16] Patients with cardiopulmonary comorbidity were excluded. All included women were genetically tested for AC-related mutations. Medical history, including the number of pregnancies and miscarriages was collected from patient interview and medical records. Women with previous pregnancies were additionally contacted by telephone to collect specific pregnancy related information, including age at pregnancies, awareness of AC diagnosis in pregnancy, AC related symptoms and use of medications before or during pregnancy, and vaginal delivery or caesarian section. An echocardiographic study was performed in all patients.

All participants gave written informed consent. The study complied with the Declaration of Helsinki and was approved by the Regional Committees for Medical Research Ethics.

Arrhythmogenic cardiomyopathy patients followed during pregnancy and delivery

A subgroup of AC mutation positive female relatives was followed during pregnancy and delivery at our hospital. In this subgroup, echocardiography was performed before pregnancy and within 6 months post-partum. Findings during pregnancy and after delivery in these women were reported in detail.

Arrhythmogenic cardiomyopathy severity score

To evaluate disease severity in each patient, we introduced an AC disease severity score yielding one point for each minor criterion and two points for each major criterion according to the 2010 TFC. If a patient fulfilled a major criterion, points for minor criteria in the same category were not included. Therefore, a maximum score across 6 diagnostic categories was 12, and patients with definite AC had ≥ 4 points (2 major, or 1 major and 2 minor, or 4 minor criteria).

Electrocardiography and arrhythmias

A 12-lead electrocardiogram was obtained in all participants at the same visit as the echocardiographic examination. Signal averaged electrocardiograms (SAECGs) were recorded and analysed as previously reported.[17] Ambulatory 24 h Holter registrations at AC diagnosis were analysed when available. Ventricular arrhythmias were defined as arrhythmic events, and included aborted cardiac arrest, sustained ventricular tachycardia/ventricular fibrillation or appropriate therapy from an implantable cardioverter-defibrillator (ICD). Appropriate ICD therapy was defined as anti-tachycardia pacing (ATP) or ICD shock due to correct recognition of ventricular tachycardia or ventricular fibrillation. Non-arrhythmic death or heart transplantation was not considered an arrhythmic event.

Echocardiographic study

2D echocardiographic studies were performed in all patients on Vivid 7 or 9 scanners with off-line data analyses (EchoPAC version 112, GE Healthcare, Horten, Norway). From 2D echocardiography, we assessed proximal right ventricular outflow tract (RVOT) diameter in the parasternal short- and long-axis views. We assessed RV basal diameter and right ventricular fractional area change (RVFAC) from the RV focused four-chamber view.[18] Regional RV akinesia, dyskinesia, or aneurisms were detected in parasternal long- or short-axis view and RV focused four-chamber view. Left ventricular (LV) ejection fraction was calculated by the modified Simpson's biplane method.[19] Longitudinal strain was assessed by speckle tracking technique[20] at frame rates >50/s. Left ventricular global longitudinal strain (LVGLS) was defined as the average of peak negative longitudinal from a 16 segments LV model.[20] Right ventricular free wall longitudinal strain was defined as an average peak negative longitudinal strain from three RV segments.[21,22] Right ventricular mechanical dispersion was calculated as the standard deviation of time from the onset of the Q/R wave in the electrocardiogram to the peak negative longitudinal strain from six RV segments.[22] All measurements were performed by an independent observer blinded to patients' previous pregnancies.

Cardiac magnetic resonance

Cardiac magnetic resonance was performed in subset of patients. The RV free wall was imaged by axial and sagittal breath-hold cine sequences. Parameters from the TFC 2010[16] together with LV volumes and function were analysed as previously described in Ref.[17]

Genetic analyses

Genetic testing was performed as part of the diagnostic work-up in women with suspected AC as described previously,[23] including genes encoding plakophilin-2 (*PKP2*), desmoplakin (*DSP*), desmoglein-2 (*DSG2*), and desmocollin-2 (*DSC2*), as well as 29 of the 105 exons of the gene encoding the ryanodine receptor-2. Cascade genetic screening was performed in family members of AC probands with confirmed pathogenic mutations.

Statistical analyses

Data were presented as mean ± standard deviation, median with range, or frequencies with percentages, as appropriate. Comparisons of continuous data were performed by the unpaired Student's *t*-test (SPSS 21.0, SPSS Inc., Chicago, IL, USA) or by the analysis of variance (ANOVA) *F*-test and the Bonferroni *post hoc* correction when >2 groups were compared. Categorical variables were compared by the χ^2 test or Fisher's exact test as appropriate. Bivariate correlations were assessed to test for linear trends. Multivariable logistic and multiple linear regression models were performed to investigate the impact of number of pregnancies, age, body surface area (BSA), and AC severity score on measures of RV and LV dimensions and function. To avoid collinearity, the AC severity score was not added to models with the dependent variable from the TFC. Kaplan–Meier curves were constructed and log-rank test was performed to assess cumulative lifetime arrhythmia-free survival. Cox regression analyses were performed to assess risk markers of age at VA, adjusted for number of pregnancies. *P*-values were two sided and values ≤ 0.05 were considered statistically significant.

Table 1 Clinical characteristics and cardiac imaging parameters in AC women grouped by number of pregnancies

	0 pregnancies ($n = 19$)	1 pregnancy ($n = 16$)	2 pregnancies ($n = 30$)	≥3 pregnancies ($n = 12$)	*P*-value from *F*-test	>0 pregnancies ($n = 58$)	*P*-value 0 pregnancies vs. >0 pregnancies
BSA (m^2)	1.7 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	2.9 ± 4.3	0.25	2.1 ± 2.2	0.47
Age at examination (years)	36 ± 18	48 ± 16[a]	51 ± 13[a]	55 ± 13[a]	<0.01	52 ± 13	<0.01
Age at arrhythmic event (years)	35 ± 17	48 ± 16[a]	48 ± 13[a]	54 ± 13[a]	0.02	49 ± 14	<0.01
Probands	9 (47)	5 (31)	17 (57)	4 (33)	0.33	25 (43)	0.50
AC severity score	4.3 ± 2.8	3.4 ± 1.6	4.7 ± 2.8	4.3 ± 2.3	0.53	4.2 ± 2.4	0.83
Definite AC by TFC	11 (58)	6 (38)	19 (63)	8 (66)	0.36	33 (57)	0.58
Syncope	8 (42)	2 (13)	11 (37)	2 (17)	0.17	15 (26)	0.16
VA	9 (47)	4 (25)	15 (50)	3 (25)	0.25	22 (38)	0.34
ICD	6 (32)	2 (13)	11 (37)	4 (33)	0.38	16 (28)	0.50
ICD discharge	1 (5)	0 (0)	6 (20)	3 (25)	0.36	10 (17)	0.16
ATP	0 (0)	1 (6)	5 (17)	2 (17)	0.97	8 (14)	0.05
TWI major criteria	7 (37)	5 (31)	10 (33)	4 (33)	0.99	19 (33)	0.74
Epsilon waves	1 (5)	0 (0)	4 (13)	0 (0)	0.37	4 (7)	0.64
SAEG pathological	4 (21)	6 (38)	10 (33)	2 (17)	0.54	18 (31)	0.27
Echocardiographic findings							
RVOT PLAX (mm/m^2)	19 ± 5	18 ± 2	20 ± 4	21 ± 6	0.23	20 ± 4	0.82
RVOT PSAX (mm/m^2)	19 ± 5	17 ± 3	19 ± 3	22 ± 6[b]	0.06	19 ± 4	0.96
RVD (mm)	41 ± 8	39 ± 4	43 ± 10	42 ± 9	0.62	42 ± 8	0.98
RVFAC (%)	39 ± 9	37 ± 7	34 ± 12	39 ± 11	0.37	36 ± 10	0.27
RV free wall LS (%)	-23.6 ± 6.1	-26.1 ± 6.0	-21.9 ± 5.8	-24.0 ± 9.0	0.25	-21.2 ± 4.8	0.82
RVMD (ms)	41 ± 22	32 ± 21	53 ± 23	40 ± 21	0.07	44 ± 28	0.60
LVEF (%)	56 ± 7	56 ± 6	54 ± 7	58 ± 7	0.42	55 ± 6	0.88
LVGLS (%)	-19.3 ± 2.8	-18.9 ± 2.8	-19.9 ± 2.9	-20.4 ± 1.9	0.50	-19.7 ± 2.7	0.56
CMR findings	$n = 17$	$n = 10$	$n = 25$	$n = 8$		$n = 43$	
CMR EDV RV (mL)	199 ± 71	138 ± 29	192 ± 63	216 ± 127	0.26	184 ± 75	0.64
CMR EDV LV (mL)	146 ± 24	135 ± 22	149 ± 35	137 ± 36	0.74	144 ± 32	0.87
CMR EF RV (%)	46 ± 12	56 ± 4	47 ± 12	43 ± 18	0.23	48 ± 12	0.64
CMR EF LV (%)	60 ± 6	63 ± 6	56 ± 7	56 ± 15	0.28	57 ± 10	0.50

Data are represented as *n* (%) and mean ± SD unless otherwise stated.
P-value from ANOVA *F*-test with the *post hoc* Bonferroni correction, χ^2 test, or Fisher's exact test as appropriate.
AC, arrhythmogenic cardiomyopathy; ATP, anti-tachycardia therapy; BSA, body surface area; CMR, cardiac magnetic resonance; EDV, end-diastolic volume; EF, ejection fraction; ICD, implantable cardioverter-defibrillator; LS, longitudinal strain; LV, left ventricular; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; MR, magnetic resonance; PLAX, parasternal long-axis view; PSAX, parasternal short-axis view; RV, right ventricular; RVD, right ventricular basal diameter; RVFAC, right ventricular fractional area change; RVMD, right ventricular mechanical dispersion; RVOT, right ventricular outflow tract; SAECG, signal average ECG; TFC, Task Force Criteria; TWI, T-wave inversion; VA, ventricular arrhythmias.
[a]*Post hoc* $P < 0.05$ vs. 0 pregnancy.
[b]*Post hoc* $P < 0.05$ vs. 1 pregnancy.

Results

Clinical and electrocardiogram characteristics

We included 77 caucasian women (age 47 ± 16 years) with AC; 43 (56%) were probands [21 (27%) with a definite AC diagnosis, 9 (12%) with a borderline diagnosis, and 13 (17%) with a possible diagnosis] and 34 (44%) were AC mutation positive female relatives [23 (30%) with definite diagnosis, 4 (5%) with borderline AC, and 7 (9%) with possible AC diagnosis]. A table with the description of major and minor criteria of AC women grouped for number of pregnancies is reported (see Supplementary data online, *Table S1*). Pathogenic mutations were found in 61 of which the majorities were found in *PKP2* gene (51 *PKP2*, 4 *DSP*, 5 *DSG2*, and 1 *DSC2*). Among the 77 AC women, 23 (30%) had a history of syncope, 31 (40%) had documented VA, and 23 (30%) had an ICD. Twenty-four hours Holter registrations were available in 26 patients (*Table 1*). Twelve had >500 premature ventricular contractions (10 definite, 1 borderline, and 1 possible diagnosis). Two AC women died: 1 (2%) from non-AC related death; 1 (2%) with primary preventive ICD died for unknown reasons.

Nineteen (24%) patients had no previous pregnancies, 16 (21%) had one pregnancy, 30 (39%) had 2 pregnancies, and 12 (16%) had ≥3 pregnancies (*Table 1*). As expected, age at examination was significantly lower in women without previous pregnancies (*Table 1*). In those with previous pregnancies, time since last pregnancy was 19 ± 14 years. All patients were in sinus rhythm at inclusion. There were no differences between women without previous pregnancies and women with previous pregnancies regarding ratio of probands, definite AC diagnosis,

Table 2 Markers of pathological cardiac structure or function by echocardiography in 77 AC women

	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Markers of LVEF <54% (*n* = 25)				
Age (years)	1.04 (1.01–1.07)	0.03	1.02 (0.98–1.05)	0.42
Number of pregnancies (*n*)	1.76 (1.08–2.87)	0.24	1.67 (0.96–2.92)	0.71
AC severity score	1.37 (1.07–1.75)	0.01	1.35 (1.05–1.74)	0.02
Markers of LVGLS >-18 (*n* = 20)				
Age (years)	1.00 (0.97–1.03)	0.98	1.00 (0.97–1.04)	0.89
Number of pregnancies (*n*)	0.79 (0.48–1.31)	0.37	0.77 (0.44–1.34)	0.36
AC severity score	1.1 (0.93–1.38)	0.23	1.13 (0.92–1.39)	0.25
Markers of RVOT PSAX ≥32 mm (*n* = 45)				
Age (years)	1.04 (1.00–1.01)	0.03	1.02 (0.98–1.05)	0.40
Number of pregnancies (*n*)	1.76 (1.08–2.87)	0.02	1.56 (0.91–2.66)	0.11
BSA (m^2)	18.3 (0.99–340.58)	0.05	12.58 (0.54–292.44)	0.12
Markers of RVD >41 mm (*n* = 25)				
Age (years)	1.01 (0.98–1.05)	0.40	0.99 (0.95–1.04)	0.86
Number of pregnancies (*n*)	0.95 (0.59–1.50)	0.81	0.89 (0.48–1.64)	0.70
AC severity score	1.91 (1.41–2.58)	<0.01	1.93 (1.41–2.64)	<0.01
Markers of RVFAC ≤40% (*n* = 41)				
Age (years)	1.02 (0.99–1.05)	0.16	1.02 (0.98–1.05)	0.27
Number of pregnancies (*n*)	0.99 (0.64–1.55)	0.98	0.85 (0.51–1.42)	0.53
BSA (m^2)	5.23 (0.38–72.34)	0.22	3.61 (0.24–54.39)	0.35

Univariable and multivariable logistic regression, retaining all potential confounders in multivariable analyses.
AC, arrhythmogenic cardiomyopathy; BSA, body surface area; CI, confidence interval; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; PSAX, parasternal short axis view; RVD, right ventricular basal diameter; RVFAC, right ventricular fractional area change; RVOT, right ventricular outflow tract.

presence of AC symptoms, AC severity score, arrhythmic events, ICD implantations, ICD shock, ATP, or heart transplantations, indicating similar disease burden in all groups (*Table 1*). Two (5%) probands patients with definite diagnosis needed heart transplantation. One woman (3%) was transplanted twice, 17 and 20 years after the first and second pregnancy, respectively; one woman (5%) had no previous pregnancies. Electrocardiogram and SAECG parameters did not show significant differences with increased number of pregnancy (*Table 1*).

Pregnancy characteristics

Among the 58 AC women with previous pregnancies, age at first pregnancy was 29 ± 6 years. Six (10%) women knew they had AC or AC-associated mutations at the time of their first pregnancy. Five (8%) patients were on beta-blocker medication during pregnancy, four on metoprolol, and one on sotalol. Symptoms during pregnancy were reported by 12 (21%) patients (6 with palpitations, 3 symptomatic ventricular extrasystoles, 1 syncope, 1 atrial fibrillation, and 1 dizziness due to low blood pressure). Six (10%) patients reported that their symptoms had worsened during pregnancy, including palpitations and dizziness, but none reported an increase in serious events. Among a total of 88 deliveries, 83 (94%) were vaginal and 6 (6%) were by caesarian sections. The majority of deliveries [*n* = 85, (97%)], were reported as uncomplicated. The 3 (3%) complicated deliveries included 2 (2%) cases of breech birth and 1 (1%) case of caesarian section due to preeclampsia. No delivery was complicated by AC disease or symptoms. A deep venous thrombosis occurred in 1 (1%) patient post-partum. Two (3%) women reported miscarriages: 1 (2%) with definite and 1 (5%) with possible AC diagnosis, but both miscarriages occurred early in pregnancy and were therefore not included in number of pregnancies. Both women experienced other pregnancies, 2 and 3, respectively, with uncomplicated pregnancies and vaginal deliveries.

Imaging findings and comparisons based on number of pregnancies

For the total population of patients with AC, RVOT parasternal long-axis was increased (19.5 ± 4.2 mm/m^2) and RV basal diameter was at the upper limit of the reference values (41 ± 8 mm). Right ventricular function was reduced when measured by RVFAC (37 ± 10%). Left ventricular function was preserved based on measurements of LV ejection fraction (56 ± 6%) and LVGLS (-19.6 ± 2.8%).

Separate analyses of women with AC who had not been pregnant compared to women with AC who had ≥1 pregnancy did not show any differences in echocardiographic measurements (*Table 1*).

By dividing into four groups according to number of pregnancies, we observed a linear trend towards increased RVOT with multiple pregnancies (linear trend $P = 0.02$ and ANOVA *F*-test, $P = 0.05$) (*Table 1*). However, in multiple linear regression, the number of pregnancies was not a marker of increased RVOT diameter, adjusted for age and BSA [beta value 0.87, 95% confidence interval (CI) -0.76 to 2.5; $P = 0.29$]. No other measurements of RV or LV structure or function were associated with the number of pregnancies (*Tables 1* and *2*). As expected, AC severity score was an independent marker

Table 3 Comparisons of female AC patients without and with history of ventricular arrhythmias, and markers for age at ventricular arrhythmias adjusted for number of pregnancies

	AC without ventricular arrhythmias (*n* = 46)	AC with ventricular arrhythmias (*n* = 31)	*P*-value	Risk markers of ventricular arrhythmias multivariable HR (95% CI)	Multivariable *P*-value
Age (years)	46 ± 18	49 ± 12	0.45		
AC severity score	3.1 ± 1.7	6.0 ± 2.5	<0.001	1.31 (1.15–1.50)[a]	<0.001
Number of pregnancies (*n*)	1.5 ± 1.1	1.4 ± 1.1	0.69	0.72 (0.51–1.03)[b]	0.08
SAECG pathological	7 (15)	15 (48)	<0.001	0.76 (0.53–1.09)[a]	0.14
TWI major criteria	7 (15)	19 (61)	<0.01	0.74 (0.52–1.06)[a]	0.10
Epsilon waves	3 (7)	19 (61)	0.99		
RVOT PSAX (mm)	32 ± 6	36 ± 6	0.14		
RVD (mm)	38 ± 4	46 ± 10	<0.001	1.09 (1.05–1.14)[a]	<0.001
RVFAC (%)	40 ± 9	33 ± 11	0.01	0.98 (0.95–1.01)[a]	0.22
RV free wall LS (%)	-22.3 ± 4.5	-19.6 ± 4.0	<0.001	1.09 (1.03–1.17)[a]	0.006
LVEF (%)	56 ± 7	55 ± 5	0.46		
LVGLS (%)	-19.7 ± 2.7	-19.6 ± 3.0	0.90		
CMR EDV RV (mL)	168 ± 70	213 ± 71	0.07		
CMR EDV LV (mL)	138 ± 30	153 ± 30	0.14		
CMR EF RV (%)	50 ± 12	45 ± 12	0.15		
CMR EF LV (%)	58 ± 8	58 ± 10	0.96		
Regional akinesia RV	7 (15)	18 (58)	<0.01	3.01 (1.38–6.60)[a]	0.006

Data are represented as mean ± SD or *n* (%).
P-value from the Student's *t*-test or χ^2 test as appropriate.
Multivariable Cox regression of age-adjusted incident VA.
AC, arrhythmogenic cardiomyopathy; CI, confidence interval; CMR, cardiac magnetic resonance; EDV, end-diastolic volume; EF, ejection fraction; HR, hazard ratio; MR, magnetic resonance; LS, longitudinal strain; LV, left ventricular; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; PSAX, parasternal short-axis view; RMS, root-mean-square voltage; RV, right ventricular; RVD, right ventricular basal diameter; RVFAC, right ventricular fractional area change; RVOT, right ventricular outflow tract; SAECG, signal averaged electrocardiogram; TWI, T-wave inversion.
[a]Adjusted for number of pregnancies.
[b]Adjusted for AC severity score.

of reduced LV ejection fraction (<54%) [odds ratio (OR) 1.35, 95% CI 1.05–1.74; $P = 0.02$] and of dilated RV basal diameter (>41 mm) (OR 1.93, 95% CI 1.41–2.64; $P < 0.01$).

Relation between pregnancies, arrhythmic events, and cardiac transplantation

As expected, AC women with a history of arrhythmic events had more frequently a pathological SAECG, regional akinesia, increased RV dimensions, as well as reduced RVFAC and RV free wall longitudinal strain compared to AC women without arrhythmic events (*Table 3*). Furthermore, AC severity score was a marker of VA as expected ($P < 0.001$) (*Table 3*). The number of pregnancies was not associated with VA in univariable analyses, nor when adjusted for severity of AC disease (*Table 3*). The Kaplan–Meier curves showed earlier onset of VA in those with 0 pregnancies compared with those with 1 pregnancy ($P = 0.04$) (*Figure 1*). No differences in onset of VA were observed between the other groups of pregnancies.

Two (3%) patients needed cardiac transplantation: One 27-year-old woman without previous pregnancy had severely affected right ventricle and was transplanted due to recurrent and refractory VA. One 50-year-old woman with two previous pregnancies was transplanted due to severe RV heart failure and arrhythmic storms.

Patients followed during pregnancy and delivery

Five AC mutation positive female relatives (4 plakophilin-2, 1 desmoglein-2) were followed with serial echocardiographic examinations during a total of six pregnancies at our department (1 patient had 2 pregnancies, and 4 had 1 pregnancy). Age at AC genetic diagnosis was 31 ± 3 years and age at first pregnancy was 34 ± 3 years. Arrhythmogenic cardiomyopathy severity score was 2.5 ± 1.0. No arrhythmias were reported during pregnancy, delivery or postpartum. Importantly, there were no indications of changes in RVOT diameter, RVFAC, RV free wall longitudinal strain, LVGLS, or LV ejection fraction within 6 months after pregnancy (*Table 4*).

Intra- and inter-observer variability for RV and LV strain and for RV mechanical dispersion was previously reported.[17,23]

Discussion

This is the first larger study exploring the effect of previous pregnancies on outcome in women with AC. The number of previous

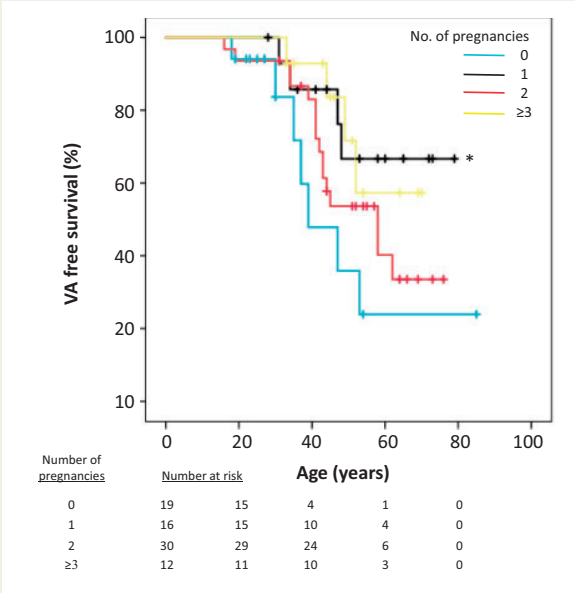

Figure 1 The Kaplan–Meier analyses of 77 female AC patients and their mutation positive female relatives. Women with 0 pregnancies had ventricular arrhythmias at younger ages compared with women with 1 pregnancy (*log rank = 0.04). No significant differences were observed in arrhythmic events and prognosis between 1, 2, and ≥3 pregnancies. AC, arrhythmogenic cardiomyopathy; VA, ventricular arrhythmias.

pregnancies in AC women did not seem to aggravate RV and LV structure or function, or increase the occurrence of VA in long-term follow up. Furthermore, the majority of the women reported their pregnancies as uncomplicated with no increase of serious adverse events during pregnancy.

Cardiac effects of pregnancy

In our AC population, RVOT diameter tended to increase with the number of previous pregnancies. However, number of previous pregnancies was not a marker of dilated RVOT when adjusted for age and BSA. Other markers of RV and LV structure and function did not show any relation to the number of pregnancies, even when adjusted for age and AC severity score. Although a small number, no changes were observed in RV diameters and function during pregnancy in the five AC women followed during pregnancy and delivery at our hospital. Overall, our findings indicated that pregnancy did not affect cardiac structure or function in AC, supporting previous case series and reports of well tolerated pregnancies in this patient group.[12,13,15] Our results indicated that the long-term effect of pregnancy is well tolerated and safe in women with AC. Nevertheless, we suggest that these patients should be referred to a high experience centre for structured follow-up from early pregnancy until after delivery. A recent large study on pregnancy in hypertrophic cardiomyopathy indicated overall good survival, but cardiovascular complications during pregnancy were not uncommon. The results highlight that pregnancy alters haemodynamic and arrhythmic risk and requires appropriate follow up and management.[24]

Table 4 Serial investigations in five AC mutation positive female relatives before pregnancy and within 6 months post-partum

	Before pregnancy	Within 6 months post-partum	*P*-value
RVOT PSAX (mm)	33 ± 6	32 ± 7	0.52
RVFAC (%)	46 ± 7	41 ± 7	0.08
RV free wall LS (%)	-24.3 ± 3.1	-21.9 ± 2.3	0.55
LVEF (%)	54 ± 2	54 ± 2	0.36
LVGLS (%)	-19.5 ± 2.7	-17.9 ± 4.6	0.44

Data represented as mean ± SD.
P-value from the Student's *t*-test.
AC, arrhythmogenic cardiomyopathy; LS, longitudinal strain; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; PSAX, parasternal short-axis view; RV, right ventricular; RVFAC, right ventricular fractional area change; RVOT, right ventricular outflow tract.

The rationale for this study was the recently reported negative effects of repeated haemodynamic stress due to athletic activity on cardiac function and arrhythmias in AC.[4,5] Pregnancy shares some physiological aspects with exercise and represents several months of continuous haemodynamic stress. The normal physiological changes in the cardiovascular system during pregnancy includes an increase in maternal blood volume by 40%, resulting in a 30–50% increase in cardiac output[8] achieved by a rise in stroke volume and heart rate.[8,9] Based on our results, the effects of haemodynamic stress during pregnancy seem to differ from the effects of exercise. Several explanations are possible; previous reports have reported doses of harmful athletic activity to range from 1 to 4 h activity per week for one to several years.[4,5,25] Pregnancy, however, is limited to a 9 months period and the largest increase in cardiac stress is on lower intensity and spans an even shorter time period than 9 months. Furthermore, during pregnancy there is a decrease in vascular resistance by approximately 30%, causing peripheral vasodilatation, unlike exercise where there is an exercise induced increase in afterload. Other possible speculations may include hormonal and adaptive mechanisms involved during pregnancy, which may protect the myocardium.[26,27]

Arrhythmic events and outcome

Arrhythmic events were equally prevalent in women without and with previous pregnancies, and multivariable analyses showed no influence of pregnancies on VA outcome. As expected, AC severity score was highly predictive for the occurrence of VA and echocardiographic and SAECG were markers of VA. Arrhythmogenic cardiomyopathy women without previous pregnancies had experienced arrhythmic events at younger age compared with those with previous pregnancies as also shown in the Kaplan–Meier plot. This could indicate a possible confounding factor as women with severe arrhythmias at young age might have decided not to become pregnant and reflect more severe disease in the end. However, less than half of patients without pregnancy had experienced arrhythmic events, indicating limited confounding effect. Furthermore, no differences were found in age at arrhythmic events between those with 1, 2, and >3 pregnancies, and proband status and AC severity score were not different between the groups based on number of pregnancies.

Limitations

This study had a cross-sectional design, while data on symptoms during pregnancy were collected retrospectively with the inherent limitations. We cannot exclude survival bias and disease is underestimated in the older groups. We included AC mutation positive family members with early disease, and our population was therefore relatively healthy. We cannot exclude the possibility that women with symptomatic AC avoided pregnancy on the basis of diagnosis, a potential bias that is difficult to explore and control. Although being the largest study on this topic, the numbers of events were limited and larger studies are needed to confirm our findings.

Conclusions

Higher number of pregnancies was not associated with worse outcome in women with AC or in mutation positive female relatives. Serious cardiac symptoms did not worsen during pregnancy and number of pregnancies was not associated with arrhythmic events.

Supplementary data

Supplementary data are available at *European Heart Journal - Cardiovascular Imaging* online.

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Conflict of interest: none declared.

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Table S1: TFC major and minor criteria in AC women grouped by number of pregnancies

	0 pregnancies (n=19)	1 pregnancy (n=16)	2 pregnancies (n=30)	≥3 pregnancies (n=12)	*p-value from F test*	>0 pregnancies (n=58)	*p-value 0 pregnancies vs >0 pregnancies*
AC severity score	4.3±2.8	3.4±1.6	4.7±2.8	4.3±2.3	0.53	2.1±2.2	0.47
Global or regional dysfunction							
Major TFC criteria (n, (%))	5 (26)	2 (13)	16 (53)	4 (33)	0.38	22 (38)	0.41
Minor TFC criteria (n, (%))	1 (5)	1 (6)	0 (0)	0 (0)	0.37	1 (2)	0.44
Tissue characterization							
Major TFC criteria (n, (%))	1 (5)	0 (0)	0 (0)	0 (0)	0.61	0 (0)	0.25
Minor TFC criteria (n, (%))	1 (5)	0 (0)	0 (0)	0 (0)	0.61	0 (0)	0.25
Repolarization abnormalities							
Major TFC criteria (n, (%))	8 (42)	5 (31)	12 (40)	3 (25)	0.79	20 (35)	0.59
Minor TFC criteria (n, (%))	0 (0)	1 (6)	1 (3)	2 (17)	0.21	4 (7)	0.57
Depolarization abnormalities							
Major TFC criteria (n, (%))	4 (21)	1 (6)	4 (13)	2 (17)	0.71	7 (12)	0.50
Minor TFC criteria (n, (%))	2 (11)	6 (38)	7 (23)	1 (8)	0.19	14 (24)	0.33

Arrhythmias							
Major TFC criteria (n, (%))	3 (16)	0 (0)	3 (10)	3 (25)	0.18	6 (10)	0.68
Minor TFC criteria (n, (%))	7 (37)	7 (44)	14 (47)	3 (25)	0.64	24 (41)	0.79
Family history							
Major TFC criteria (n, (%))	15 (79)	13 (81)	25 (83)	10 (83)	0.98	48 (83)	0.74
Minor TFC criteria (n, (%))	0 (0)	0 (0)	0 (0)	1 (8)	0.16	1 (2)	1.0

P from chi-square or Fisher exact test as appropriate. AC = Arrhythmogenic cardiomyopathy; TFC = Task Force Criteria.

Paper 2

Pregnancy and Progression of Cardiomyopathy in Women With LMNA Genotype-Positive

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Journal of the American Heart Association

ORIGINAL RESEARCH

Pregnancy and Progression of Cardiomyopathy in Women With LMNA Genotype-Positive

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BACKGROUND: We aimed to assess the association between number of pregnancies and long-term progression of cardiac dysfunction, arrhythmias, and event-free survival in women with pathogenic or likely pathogenic variants of gene encoding for Lamin A/C proteins (LMNA+).

METHODS AND RESULTS: We retrospectively included consecutive women with LMNA+ and recorded pregnancy data. We collected echocardiographic data, occurrence of atrial fibrillation, atrioventricular block, sustained ventricular arrhythmias, and implantation of cardiac electronic devices (implantable cardioverter defibrillator/cardiac resynchronization therapy defibrillator). We analyzed retrospectively complications during pregnancy and the peripartum period.

We included 89 women with LMNA+ (28% probands, age 41±16 years), of which 60 had experienced pregnancy. Follow-up time was 5 [interquartile range, 3–9] years. We analyzed 452 repeated echocardiographic examinations. Number of pregnancies was not associated with increased long-term risk of atrial fibrillation, atrioventricular block, sustained ventricular arrhythmias, or implantable cardioverter defibrillator/cardiac resynchronization therapy defibrillator implantation. Women with previous pregnancy and nulliparous women had a similar annual deterioration of left ventricular ejection fraction (–0.5/year versus –0.3/year, P=0.37) and similar increase of left ventricular end-diastolic diameter (0.1/year versus 0.2/year, P=0.09). Number of pregnancies did not decrease survival free from death, left ventricular assist device, or need for cardiac transplantation. Arrhythmias occurred during 9% of pregnancies. No increase in maternal and fetal complications was observed.

CONCLUSIONS: In our cohort of women with LMNA+, pregnancy did not seem associated with long-term adverse disease progression or event-free survival. Likewise, women with LMNA+ generally well-tolerated pregnancy, with a small proportion of patients experiencing arrhythmias.

Key Words: arrhythmias ■ cardiomyopathy ■ Lamin A/C ■ LMNA ■ outcome ■ pregnancy

Variants in gene encoding for Lamin A/C proteins (LMNA), are an important genetic cause of dilated cardiomyopathy (DCM).[1] In familial DCM, LMNA variants are found in 4% to 8% of the cases,[2] and in up to 33% in DCM with concomitant electrical conduction disease.[3] The penetrance of LMNA variants is age-dependent and approaches 100% by middle age with variable clinical expression, including early onset of atrioventricular block, atrial fibrillation (AF), and DCM.[4] The disease course is malignant with high rates of ventricular arrhythmias (VA) and sudden cardiac death, stroke, and progression to end stage heart

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CLINICAL PERSPECTIVE

What Is New?

- In women with LMNA+, number of previous pregnancies was not associated with long-term worsening of electrical disease and occurrence of sustained ventricular arrhythmias, and did not accelerate cardiac dilatation.
- Number of deaths, left ventricular assistance device implantations, and heart transplantations did not differ significantly between nulliparous women and women with previous pregnancy in our cohort.
- Pregnancy was mostly well tolerated with a low number of maternal and fetal complications, but risk of arrhythmias during pregnancy cannot be excluded.

What Are the Clinical Implications?

- Women with LMNA+, without overt electric/structural cardiomyopathy, should not be suggested to refrain pregnancy.
- Pre-pregnancy counseling in high experience cardiomyopathy centers, to assess disease status and pregnancy-related risks, should be considered in these patients.
- During pregnancy, because of lack of systematic data on triggering of arrhythmic events, it can be reasonable to use ambulatory ECG monitoring in women with known LMNA+, independently of current phenotype.

Nonstandard Abbreviations and Acronyms

DCM	dilated cardiomyopathy
HTx	heart transplantation
LMNA	gene encoding for Lamin A/C proteins
LMNA+	pathogenic or likely pathogenic variants of LMNA
VA	ventricular arrhythmias

failure, with frequent need of left ventricular assistance device (LVAD) and heart transplantation (HTx).[5]

Recent reports have suggested a negative effect of both competitive[6] and non-competitive[7] sport on prognosis of patients with LMNA variants. Pregnancy can be regarded as a comparable state of prolonged exercise because of the hemodynamic stress related to increase in circulating blood volume, rise in stroke volume and heart rate, in addition to sympathetic stimulation and hormonal changes.[8,9] Thus, pregnancy might be associated with increased cardiac complications and number of pregnancies might affect long-term disease progression in LMNA cardiomyopathy. Cardiac disease in these patients often develops in early adulthood, but the tolerance and effect of pregnancy on disease progression have not been explored, with only few case reports available.[10] We aimed to investigate the association between pregnancy and long-term progression of cardiac dysfunction, arrhythmias and, survival outcomes in women with pathogenic or likely pathogenic variant of LMNA (LMNA+). Furthermore, we wanted to explore fetal and maternal adverse events, during pregnancy and peripartum period.

METHODS

Data Availability

The authors do not have the authority to share the data reported in the present article, because of the sensitive nature of the data collected for this study. The Approval of the Regional Committee for Medical Research Ethics limits sharing data with researchers inside or outside Norway for purposes of reproducing the results or replicating the procedures. The data can be made available to any additional research after formal application to the Regional Committee for Medical Research Ethics and explicit consent given from every study subject.

Independent Data Access and Analysis

Authors had full access to all the data in the study and take responsibility for its integrity and the data analysis.

Study Population

We conducted a multicenter, retrospective, longitudinal study and included consecutive women with LMNA+ from the Unit for Genetic Cardiac diseases, Oslo University Hospital, Rikshospitalet, Norway, and from the cardiovascular genetics program of Brigham and Women's Hospital, Boston, USA. LMNA variants were classified locally (Oslo/Boston), in conjunction with reference laboratories and in keeping with consensus.[11] Only patients with pathogenic or likely pathogenic LMNA variants were included. We defined proband as the first individual in a family, without known history of LMNA+ related disease, who sought medical attention attributable to clinical manifestation of disease and underwent genetic testing. We defined baseline as time of first available echocardiographic examination, and last follow-up as last available echocardiographic examination before October 2019 or before implantation of LVAD or HTx. We recorded symptoms of heart failure at each visit and reported as New York Heart Association functional class. We collected medical history, including the number of previous pregnancies/births and spontaneous abortions

from medical records. Pregnancy ended with birth of a viable or death fetus were included in our analysis, and women with LMNA+ who experienced pregnancy with these characteristics were defined *women with previous pregnancy*. We defined *nulliparous* women with LMNA+ who never carried a pregnancy to birth of a viable or death fetus. Spontaneous abortions were not included in the total number of pregnancies. We recorded heart failure and anti-arrhythmic medical therapy. We additionally contacted patients by telephone to collect specific pregnancy-related information, including age at pregnancies, awareness of being LMNA+ at time of pregnancy, presence of symptoms before/during pregnancy, and use of medications before/during pregnancy. We recorded type of delivery and obstetric complications. In addition, we reported a detailed description on the subgroup of women with LMNA+ followed during pregnancy and peripartum period at our hospitals.

All patients from Oslo gave inform consent. Brigham and Women's Hospital waived consent for retrospective data. The study complied with the Declaration of Helsinki and was approved by the Regional Committees for Medical Research Ethics.

Electrocardiography, Conduction Disease, and Arrhythmias

Arrhythmias were recorded during clinical visits from 12-lead resting ECG, exercise ECG, ambulatory ECG monitoring, and interrogation of implantable cardiac electronic devices. We obtained and analyzed 12-lead ECG in all participants at the time of echocardiographic examinations. All patients underwent at least one ECG Holter and exercise ECG. We recorded atrioventricular block I-III, AF and VA. Sustained VA was defined as aborted cardiac arrest or ventricular tachycardia with a frequency ≥120/min lasting >30 seconds or appropriate implantable cardioverter defibrillator (ICD) therapy.[5]

Echocardiography

All completed transthoracic echocardiographic examinations between inclusion and last clinical follow-up were analyzed. We excluded echocardiographic exams performed after LVAD implantation and/or HTx, and examinations done during infusion of cardiac inotropes. Data about left ventricular (LV) end-diastolic diameter and LV ejection fraction (EF) were collected.[12] We considered LV ejection fraction (EF)≤45% as threshold for significant LV systolic dysfunction.[13] A subset of patients were investigated with LV strain. LV global longitudinal strain was derived from speckle tracking analyses on 2D gray scale image loops with >50 frames per second from the 3 apical views and expressed as the average peak negative strain in a 16 segment LV model.[14] All measurements were performed masked to clinical outcome.

Statistical Analysis

We performed statistical analyses using Stata SE 16.1 (StataCorp LLC, Texas, USA). Values were expressed as mean with standard deviations, median with interquartile range (IQR), and frequencies with percentages. At last follow-up continuous variables were compared by unpaired Student *t*-test, Kruskal–Wallis test, or by ANOVA *F* test with Bonferroni correction, when more than 2 groups were compared. Categorical variables were compared by Fisher exact test. The composite of all-cause mortality, LVAD implantation, or HTx constituted the primary outcome (death/LVAD/HTx). To assess the effect of pregnancies, we divided the study population in subgroups according to the number of pregnancies and we compared nulliparous women and women with previous pregnancy at last follow-up.

We used a generalized estimating equation with individual level random effects, binomial family, and independent working correlation within our data. We recorded longitudinal nested data to increase the statistical strength of our analysis within participant. We applied generalized estimating equation to assess the odds of impending AF, atrioventricular block, sustained VA, ICD, or cardiac resynchronization therapy (CRT) defibrillator implantation, EF ≤45%, and of primary outcome in up to 452 examinations (=100% of observations), or less if data were not available. We aimed to adjust our analyses for statistically and clinically relevant covariates, and to interpret the results in the context of relatively few events. Therefore, we have kept the following 4 covariates in our multivariate analyses; (1) pregnancy, as main dependent variable; (2) age at last follow-up as a statistically and clinically important confounder, in addition to (3) missense mutations, and (4) probands status. The 2 latter were not statistically significant parameters in univariate analyses but were considered clinically important for prognosis in Lamin disease.

Key echocardiographic parameters from all the examinations taken during the study period were entered into a linear mixed model regression analyses with random individual intercept and exchangeable covariance structure. LV structural and functional deterioration in nulliparous women and women with previous pregnancy was assessed by interaction term between the time varying covariates number of pregnancies and age at examination.

Kaplan–Meier curves were generated, and we performed log-rank test to compare cumulative hazard risk of AF, sustained VA, and primary outcome between nulliparous women and women with previous pregnancy. The results of survival analysis were adjusted with a Cox regression multivariable analysis exploring

time to AF, to sustained ventricular arrhythmias and to death/LVAD/HTx. We included EF at baseline (for the outcomes sustained VA and primary outcome) in combination with age at baseline, and probands status (for the outcome AF). These covariates were considered clinically important. We used proportional hazard test to check deviation from proportionality and results confirmed the fitness of our models.

P values were 2-sided, and values <0.05 were considered significant.

RESULTS

Clinical Characteristics

Eighty-nine women with LMNA+ (28% probands) were included (58 from Oslo University Hospital and 31 from Brigham and Women's Hospital), 24 (27%) of them with missense mutations. Age at baseline was age 41±16 years (Table 1). A list of included LMNA variants is provided on Table S1. Patients were followed from November 2001 to October 2019 with median follow-up time of 5 (IQR, 3–9) years, without differences between the pregnancy groups (Table 2). Most women with previous pregnancy, experienced pregnancy before clinical debut of cardiac symptoms or diagnosis of an LMNA variant. Clinical follow-up started in median 14 (IQR, 10–22) years after last pregnancy. The total time from first pregnancy to the last follow-up was median 22 (IQR, 17–32) years. We analyzed 452 available echocardiographic examinations, with a median of 4 (IQR, 2–8) examinations per patient.

At baseline, 19 (21%) patients had atrioventricular block, 39 (44%) AF, 10 (11%) patients had experienced sustained VA, and 10 (11%) had ICD/CRT-defibrillator (Table 1). Twenty-one (24%) patients were in New York Heart Association class II–IV, and 10% had history of stroke or transient ischemic attack. Most of patients had LV diameter and systolic function, by LV EF and global longitudinal strain, preserved at baseline, although 21% of patients had LV EF≤45 (Table 1).

At last follow-up, 29 (33%) women were nulliparous and 60 (67%) were women with previous pregnancy, including 13 (22%) with 1 pregnancy, 31 (51%) with 2 pregnancies, and 16 (27%) with ≥3 or more pregnancies (Table 2). Among nulliparous women, 23 (79%) were still in childbearing age (15–49 years) at last follow-up, and 13 (45%) women were aged ≤25 years.

Electrical, Structural Disease Progression, and Outcome in Patients Grouped by Pregnancy

At last follow-up, women with previous pregnancy were older then nulliparous and, as expected, age had a parallel increase with number of pregnancies (Table 2).

Table 1. Clinical Characteristics and Imaging Parameters of 89 Women With LMNA+ at Baseline and Last Follow-Up

Clinical characteristics	Baseline (n=89)	Last follow-up (n=89)
Age at first pregnancy (y±SD)	…	27±5
Age (y±SD)	41±16	46±16
NYHA class II–IV (n, (%))	21 (24)	32 (36)
Atrioventricular block I–III (n, (%))	19 (21)	24 (27)
Atrioventricular block I	11 (12)	9 (10)
Atrioventricular block II	2 (2)	5 (6)
Atrioventricular block III	6 (7)	10 (11)
Atrial fibrillation (n, (%))	39 (44)	52 (58)
Sustained VA (n, (%))	10 (11)	22 (25)
Medications and device therapy		
Beta-blockers (n, (%))	22 (25)	48 (54)
ACE inhibitors/ARBs (n, (%))	15 (17)	31 (35)
MRAs (n, (%))	8 (9)	16 (18)
AAs (n, (%))	8 (9)	2 (2)
ICD/CRT-D (n, (%))	10 (11)	51 (57)
Echocardiographic		
LV EF, %	53±11	50±13
LV EF≤45% (n, (%))	19 (21)	17 (19)
LV EDD, mm	51±6	51±7
LV GLS, %	−18±4	−16±4

Data are presented as n (%) or means±SD. Prevalence of arrhythmias and of treatments (medical and device therapy) is reported. LV global longitudinal strain refers to a subgroup of 58 patients with available strain measurements. AAs indicates anti-arrhythmic medications (sotalol, amiodarone, verapamil, flecainide, and dronedarone); ACE, angiotensin-converting enzyme; ARBs, angiotensin receptor blockers; CRT-D, cardiac resynchronization therapy defibrillator; ICD, implantable cardioverter-defibrillator; LV EDD, left ventricular end-diastolic diameter; LV EF, left ventricular ejection fraction; LV GLS, left ventricular global longitudinal strain; MRAs, mineralocorticoid-receptor antagonist; NYHA, New York Heart Association; TIA, transient ischemic attack; and VA, ventricular arrhythmias.

During follow-up, 5 patients developed atrioventricular block, 3 patients developed atrioventricular block II, and 4 atrioventricular block III (Table 1). Thirteen new cases of AF occurred. At last follow-up, in total atrioventricular block was present in 24 (27%) patients and AF in 52 (58%), with higher prevalence in women with previous pregnancy compared with nulliparous women (Table 2). However, number of pregnancies was not associated with atrioventricular block (OR, 1.63; 95% CI, 0.65–4.07; *P*=0.30), nor with AF (OR, 1.17; 95% CI, 0.68–2.03; *P*=0.56), when adjusted for age, proband status, and missense mutation (Table 3). There was no difference in the age at onset of AF in nulliparous women and women with previous pregnancy (log rank, *P*=0.73), and time to AF was not significantly different between the groups after adjustment for age and EF at baseline, and probands status (Figure 1). Twelve patients developed VA during follow-up with an incidence of 13% and VA prevalence was of 22 (25%) patients at

Table 2. Clinical Parameters and Outcomes of 89 Women With LMNA+ Grouped by Previous Pregnancies at Last Follow-Up

	0 previous pregnancy (n=29)	1 previous pregnancy (n=13)	2 previous pregnancies (n=31)	≥3 previous pregnancies (n=16)	*P* value	≥1 previous Pregnancies (n=60)	*P* value 0 vs ≥1 pregnancy
Clinical characteristics							
Age at last follow-up, y	33±17	44±9	53±9*	57±12*	<0.001	52±11	<0.001
Follow-up time, y	4 [2–7]	4 [3–8]	6 [3–10]	5 [4–12]	0.83	5 [3–10]	0.13
Proband status (n, (%))	5 (17)	3 (23)	9 (29)	8 (50)	0.16	20 (33)	0.20
Missense mutation (n, (%))	6 (21)	3 (23)	8 (26)	6 (38)	0.68	17 (28)	0.60
NYHA class II–IV (n, (%))	11 (38)	2 (15)	13 (42)	6 (38)	0.30	21 (35)	0.53
Atrioventricular block I–III (n, (%))	4 (14)	4 (31)	13 (42)	6 (38)	0.004	23 (38)	<0.001
Atrial fibrillation (n, (%))	9 (31)	6 (46)	25 (81)	12 (75)	0.001	43 (72)	0.001
Sustained VA (n, (%))	4 (14)	3 (23)	9 (29)	6 (38)	0.36	18 (30)	0.11
Echocardiographic examination							
LV EF, %	53±14	53±12	48±13	45±12	0.25	48±12	0.18
LV EF≤45% (n, (%))	2 (7)	3 (23)	6 (19)	6 (38)	0.11	15 (25)	0.08
Delta EF (%)	–3±11	–7±8	–3±8	–4±10	0.82	–4±10	0.73
LV GLS, %	–16±4	–16±5	–15±4	–16±3	0.68	–15±4	0.42
LV End-diastolic diameter, mm	50±7	50±4	53±7	50±6	0.34	51±6	0.31
Medications and device therapy							
Beta-blockers (n, (%))	12 (41)	7 (54)	19 (61)	10 (63)	0.56	36 (60)	0.12
ACE inhibitors/ARBs (n, (%))	5 (17)	3 (23)	15 (48)	8 (50)	0.04	26 (43)	0.02
MRAs (n, (%))	3 (10)	0 (0)	7 (23)	6 (38)	0.04	13 (22)	0.17
AAs (n, (%))	0 (0)	0 (0)	2 (6)	0 (0)	0.63	2 (3)	1.00
ICD/CRT-D (n, (%))	9 (31)	7 (54)	25 (80)	10 (63)	0.003	44 (73)	0.001
Outcomes							
Death (n, (%))	2 (7)	1 (8)	3 (10)	2 (13)	0.95	6 (10)	0.52
Heart transplantation (n, (%))	4 (14)	1 (8)	3 (10)	4 (25)	0.50	8 (13)	0.57
LVAD (n, (%))	1 (3)	0 (0)	1 (3)	1 (6)	1.00	2 (2)	0.69
Death/LVAD/HTx, (n, (%))	6 (21)	2 (15)	5 (16)	6 (38)	0.39	13 (22)	0.56

Data are presented as n (%), means±SD or median [interquartile range]. Prevalence of arrhythmias, treatments (medical and device therapy), and outcome is reported. *P* value from ANOVA F-test with Bonferroni correction, Fisher exact test, and Kruskal–Wallis test. AAs indicates anti-arrhythmic medications (sotalol, amiodarone, verapamil, flecainide and dronedarone); ACE, angiotensin-converting enzyme; ARBs, angiotensin receptor blockers; CRT-D, cardiac resynchronization therapy defibrillator; Delta EF, difference between ejection fraction baseline and ejection fraction last follow-up; HTx, heart transplantation; ICD, implantable cardioverter-defibrillator; LVAD, Left ventricular assistance device; LV EDD, left ventricular end-diastolic diameter; LV EF, left ventricular ejection fraction; LV GLS, left ventricular global longitudinal strain; MRAs, mineralocorticoid-receptor antagonist; NYHA, New York heart association; TIA, transient ischemic attack; and VA, ventricular arrhythmias.

*Post hoc *P*<0.05 versus 0 pregnancy

last follow-up, with similar rates in nulliparous women and women with previous pregnancy (Table 2), and with no effect of number of pregnancies (Table 3). Age at onset of sustained VA was similar between the 2 groups (log rank *P*=0.87), and time to VA was not significantly different between the groups after adjustments for EF at baseline (Figure 1).

LV systolic function was mildly impaired at last follow-up (Table 1) along with normal LV end-diastolic diameter. We found no difference in echocardiographic parameters between pregnancy groups (Table 2). Pregnancy did not increase the odds for LV EF ≤45% (Table 3). Nulliparous women and women with previous pregnancy had similar annual progression of

Table 3. Multivariable Analysis of Repeated Observations in 89 Women With LMNA+ Assessing Predictive Effects of Known Prognostic Factors and Number of Previous Pregnancies

Primary outcome and markers of disease progression	Prognostic factors	Odds ratio	95% CI	*P* value
Death/LVAD/HTx, n=452 (100%)	Age, y	1.05	0.99–1.11	0.09
	Pregnancy	0.67	0.35–1.30	0.24
	Proband status	13.7	3.67–50.8	<0.01
	Missense mutation	1.00	0.25–4.1	0.96
Atrioventricular block, n=279 (62%)	Age, y	1.05	0.98–1.11	0.16
	Pregnancy	1.63	0.65–4.07	0.30
	Proband status	13.5	0.93–195.4	0.05
	Missense mutation	0.61	0.14–2.69	0.51
Atrial fibrillation, n=447 (98%)	Age, y	1.06	1.02–1.11	<0.01
	Pregnancy	1.17	0.68–2.03	0.56
	Proband status	7.59	1.55–37.5	0.01
	Missense mutation	0.56	0.16–1.92	0.36
ICD/CRT-D, n=434 (96%)	Age, y	1.09	1.04–1.15	<0.01
	Pregnancy	0.70	0.36–1.38	0.31
	Proband status	11.2	2.13–58.8	<0.01
	Missense mutation	0.48	0.14–1.69	0.26
Sustained VA, n=447 (98%)	Age, y	1.02	0.98–1.06	0.32
	Pregnancy	1.13	0.68–1.86	0.64
	Proband status	2.25	0.77–6.57	0.14
	Missense mutation	1.30	0.44–3.84	0.63
LV EF≤ 45%, n=452 (100%)	Age, y	1.06	1.02–1.11	<0.01
	Pregnancy	1.04	0.66–1.63	0.88
	Proband status	7.17	2.26–22.8	<0.01
	Missense mutation	1.00	0.28–3.52	0.99

Generalized estimating equation with repeated observations; n=number of examinations (percent) with available data. Random effects by individuals, logit link, binomial family, and independent covariance structure. CRT-D indicates cardiac resynchronization therapy defibrillator; HTx, heart transplantation; ICD, implantable cardioverter-defibrillator; LV EF, left ventricular ejection fraction; LVAD, Left ventricular assistance device; n, number of examinations (percent) with available data; and VA, ventricular arrhythmias.

structural and functional LV disease, measured by LV EF, end-diastolic diameter, and global longitudinal strain (Table 4).

Women with previous pregnancy had higher prevalence of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers medication, mineralocorticoid-receptor antagonist, and ICD/CRT-defibrillator device therapy (Table 2), while these differences were not present in age-adjusted analyses.

The primary outcome death/LVAD/HTx occurred in 19 (21%) patients, without differences across pregnancy groups. Number of pregnancies did not increase the odds for the primary outcome (OR, 0.67; 95% CI, 0.35–1.30; *P*=0.24). There was no difference in the age at occurrence of the primary outcome between nulliparous women and women with previous pregnancy (log rank 0.17), and time to primary outcome was not different between the groups after adjustments for EF at baseline (Figure 1).

Pregnancy and Peripartum Outcomes

Among the 60 women with previous pregnancies, mean age at first pregnancy was 27±5 years. A total of 125 pregnancies were reported, and we had available details of pregnancy and peripartum outcomes in 109 (87%) of them. Most of the women reported well-tolerated pregnancies and uncomplicated deliveries. Palpitations, dyspnea, and syncope were reported in 9 (8%), in 4 (4%), and in 3 (3%) pregnancies, respectively. Arrhythmias were detected in 9 (9%) pregnancies (Table 5). Two (2%) patients experienced sustained VA and anti-arrhythmic therapy was started or modified, and 1 patient received an ICD during pregnancy. Four patients were on medications before pregnancy, and 5 (5%) were on anti-arrhythmic medications during pregnancy (Table 5).

Vaginal delivery was predominant (Table 5). Because of obstetric reasons, 14% of women had caesarean sections, none for cardiac reasons. In total, 4 spontaneous abortions occurred in the early second trimester, but

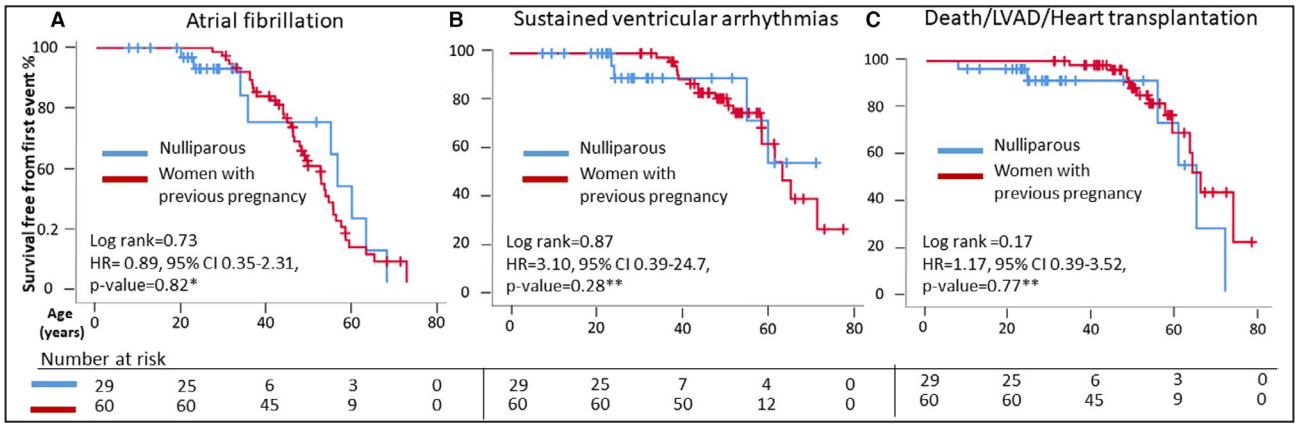

Figure 1. Survival free from arrhythmias and primary outcome.
Survival free from incident atrial fibrillation (**A**), sustained ventricular arrhythmias (**B**) and death, need for left ventricular assistance device or heart transplantation (**C**) did not differ between women with previous pregnancy (red line) and nulliparous (blue line) women. HR indicates hazard ratio from Cox models regression, exploring time to atrial fibrillation, sustained ventricular arrhythmias, and death/left ventricular assistance device/heart transplantation; and LVAD, left ventricular assistance device. *Adjusted for pregnancy, age, and ejection fraction at baseline, and probands status. **Adjusted for pregnancy and ejection fraction at baseline.

Table 4. Annual Structural Progression by Repeated Echocardiographic Assessments in Women With LMNA+ Grouped by Previous Pregnancy

	At baseline (n=89)	Progression rate 1 year (SE)	Last follow-up (n=89)	*P* value
LV EF (%), n=415 (92%)	53±11	−0.4 (0.0)	50±13	<0.001
Nulliparous	55±13	−0.3 (0.1)	53±14	0.003
Women with previous pregnancy	53±10	−0.5 (0.1)	49±12	<0.001
P for interaction		0.37		
LV EDD (mm), n=416 (92%)	50±6	0.1 (0.0)	51±6	<0.001
Nulliparous	49±7	0.2 (0.1)	50±7	<0.001
Women with previous pregnancy	51±6	0.1 (0.0)	51±6	0.02
P for interaction		0.09		
LV GLS (%), n=230 (51%)	−17±4	0.1 (0.0)	−16±6	0.03
Nulliparous	−17±5	0.0 (0.1)	−16±4	0.53
Women with previous pregnancy	−17±3	0.1 (0.0)	−15±4	0.01
P for interaction		0.35		

Values at baseline and last follow-up are presented as mean±SD; n=number of examinations (percent) with available data. Yearly progression rate with standard errors, *P* value for progression and interaction are calculated by linear mixed model statistics with exchangeable covariance structure and random individual intercept. LV EDD indicates left ventricular end-diastolic diameter; LV EF, left ventricular ejection fraction; and LV GLS, left ventricular global longitudinal strain.

without associated cardiac symptoms/complications. All women who experienced spontaneous abortion reported previous or subsequent successful births with uncomplicated pregnancies. No arrhythmic events or heart failure were described during peripartum period.

Three stillbirths were reported, none attributable to cardiac cases (1 umbilical cord strangulation, 1 of unknown etiology in a twin pregnancy, and 1 of unknown etiology in a single pregnancy through in vitro fertilization with sperm donation). None of these 3 women were aware of LMNA+ diagnosis, nor received cardiac medical therapy at the time of stillbirth, and all reported prior or subsequent uncomplicated pregnancy.

Patients With LMNA+ Prospectively Followed During Pregnancy and Peripartum Period

Of all 89 patients, we prospectively followed 6 women with LMNA+ (aged 31±3 years) during pregnancy and peripartum period at our hospitals (5 in Oslo, 1 in Boston). Four patients were probands, and 1 of them

Table 5. Maternal Clinical Characteristics During Pregnancy and Peripartum Period and Obstetric and Fetal Outcomes

Maternal adverse cardiac event	(n=109)
Maternal mortality	0 (0)
Heart failure	0 (0)
Atrial fibrillation	2 (2)
PVCs	3 (3)
nsVA	2 (2)
Sustained VA	2 (2)
Thrombo-embolic complications	0 (0)
AAs	5 (5)
Symptoms	
Palpitations	9 (8)
Dyspnea	4 (4)
Syncope	3 (3)
Adverse obstetric outcomes	
Vaginal deliveries	94 (86)
Caesarean section	15 (14)
Emergency CS for cardiac reason	0 (0)
Pre-eclampsia	2 (2)
Spontaneous abortions >12 wk	4 (4)
Bleeding	1 (1)
Adverse fetal outcomes	
Fetal or neonatal death<1 wk	3 (3)
Low birth weight (<2500 g)	2 (2)
Preterm birth (<37 wk)	6 (6

Data are presented as *n* (%). Data on AF, nsVA and sustain VA refers to incident arrhythmias. AAs indicates anti-arrhythmic therapy (Metoprolol, Bisoprolol, Sotalol); CS, Caesarean section; nsVA, non-sustained ventricular arrhythmias; PVCs, premature ventricular contractions; and VA, ventricular arrhythmias.

was diagnosed with LMNA+ attributable to symptoms occurring during pregnancy.

Patients #1 and #2, with unknown genetic status, were referred because of palpitations during pregnancy. Sustained VA was detected in both cases. Patient #1, with a previous asymptomatic pregnancy, was effectively treated with Sotalol. She had spontaneous pre-term birth at week 31, without maternal complications. Patient #2 had syncope while on beta-blocker and was implanted with ICD during her second trimester. She had an uncomplicated delivery. Patient #3, proband and with 3 previous asymptomatic pregnancies, had palpitations attributable to premature ventricular complexes during her second pregnancy. She was efficiently treated with beta-blocker without further complications. Patient #4, proband, developed non-sustained VA during pregnancy, effectively treated with beta-blockers without further complication. Patients #5 and #6 were referred because of proband status and were free of cardiac symptoms. Both experienced uncomplicated pregnancies and deliveries.

All patients underwent at least 1 echocardiographic examination during pregnancy and all had normal LV end-diastolic diameter and LV EF.

DISCUSSION

To our knowledge, this is the largest study exploring the association between previous pregnancies, long-term development of cardiomyopathy, and outcomes in patients with LMNA+. Number of pregnancies was not associated with long-term worsening of electrical disease and occurrence of sustained VA and was not associated with worse primary outcome. Furthermore, pregnancy did not accelerate the progression of cardiac dysfunction in our cohort. The majority of women retrospectively reported uncomplicated pregnancies without increase in serious obstetric or fetal adverse events. A few selected patients followed during pregnancy experienced increased arrhythmic symptoms.

Effect of Pregnancy on Progression of Electrical Disease

The prevalence of AF and atrioventricular block was higher in women with previous pregnancy and increased with number of pregnancies. However, LMNA disease is strongly age-related and there was no association between number of pregnancies and AF or atrioventricular block when adjusted for age. Likewise, pregnancy did not affect prevalence of sustained VA, nor age at onset VA. We interpret these results as reassuring for long-term outcome in women with LMNA+. Our results support that pregnancy in LMNA+ is not comparable with exercise on arrhythmic risk. These results are similar to reports in arrhythmogenic cardiomyopathy with no effect of pregnancy on arrhythmic outcome.[15,16]

In our cohort, ICD/CRT-defibrillator implantation was higher in mothers, but not when adjusted for age. In our centers, we implant ICD/CRT when the pacemaker indication is fulfilled because of atrioventricular block, as recommended,[17] and this explains the high number of these devices in our study population. Overall, our finding suggested that 1 or several pregnancies in patients with LMNA+ did not accelerate electrical disease.

Effect of Pregnancy on Development of DCM

At the last follow-up, reduced LV function reflected the development of cardiomyopathy in our cohort, but reduced LV function did not relate to pregnancy or to numbers of pregnancies. Pregnancy was not associated with worse long-term progression of the structural

heart disease. This is in contrast to reports on harmful effects of physical exercise in LMNA+.[7] Possible explanations for this difference are hemodynamic adaptive mechanisms occurring in pregnancy, including systemic reduction in vascular resistance,[18] in contrast with prevalent increase in systemic resistance related to physical exercise.[19] Previous case reports showed no change in cardiac function in patients with LMNA+ during and after pregnancy.[10] Our study supports these case reports by a larger multi-center study.

Other studies reported data about pregnancy in women with overt DCM, without information on genotype.[20,21] Our patients were mostly pregnant in a pre-symptomatic phase, or during the "electrical phase" of LMNA cardiomyopathy, which may explain the non-eventful pregnancy reported in our cohort.

Effect of Pregnancy on Primary Outcome

A high proportion of patients died, received LVAD, or were heart transplanted in our cohort, in line with previous results.[5] Pregnancy did not increase odds for the primary outcome death/LVAD/HTx, which is reassuring for women with LMNA+ in childbearing age. Most of the patients in our cohort experienced pregnancy before LMNA+ genetic diagnosis and underwent the last follow-up years after last pregnancy. Furthermore, the total time from first pregnancy to last follow-up was median 22 years. Therefore, we believe to have covered a reasonable long-term follow-up.

Pregnancy Tolerance

It is well known that arrhythmic symptoms can increase during pregnancy.[22] In our small cohort of 6 patients followed prospectively, arrhythmias increased/occurred in 4 patients, and 2 of them had first time symptoms and sustained VA during pregnancy. Although a small and selected group, a tendency to triggered arrhythmias during pregnancy cannot be excluded.

In our population, pregnancy and delivery mostly occurred at a pre-symptomatic age. However, pregnancy in older women with LMNA+ with more advanced disease may be less tolerated.

Maternal and fetal complications were low, in line with results of previous case reports.[10] We reported 3 stillbirths, which is a higher number compared with the general population. However, we found no evident causative relationship with LMNA cardiomyopathy as described above.

Limitations

This was a retrospective cohort study with inherent limitations. The multicenter design allowed a higher number of included patients, but it could have introduced variability related to different clinical practice. Obstetric and fetal outcomes were mostly self-reported, which may lead to report- and recall bias, especially in patients who experienced pregnancy many years before the start of clinical follow-up. Most patients were in a relatively early phase of the disease when pregnant, so our population was therefore relatively healthy at time of pregnancy. Most nulliparous women in our cohort were young women and most of them still in childbearing age at last follow-up. Nevertheless, we cannot exclude that more seriously affected individuals, or individuals who have experienced a malignant family history, chose not to carry offspring, even if they were unaware of their genotype.

CONCLUSIONS

Pregnancies did not seem to be associated with worse electrical or structural cardiac disease, nor to worse event-free survival in women with LMNA+. Pregnancies and deliveries were globally well tolerated and uncomplicated, but a tendency of triggering arrhythmias during pregnancy could not be excluded in selected patients.

ARTICLE INFORMATION

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Supplemental Material

Table S1

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SUPPLEMENTAL MATERIAL

Table S1: Pathogenic and likely pathogenic gene variants found in our cohort of LMNA+ women

Variant (HGVS)	Variant (protein)	Number of patients (%)	Type of mutation
c.43C>T	p.Q15X	2 (2)	Nonsense
c.73C>T	p.R25C	2 (2)	Missense
c.215G>T	p.R72L	1 (1)	Missense
c.234G>T	p.K78N	1 (1)	Missense
c.305T>C	p.L102P	1 (1)	Missense
c.427T>C	p.S143P	1 (1)	Missense
c.481G>A	p.E61K	1 (1)	Missense
c.585C>G	p.N195K	1 (1)	Missense
c.608A>G	p.E203G	4 (5)	Missense
c.629T>G	p.I210S	1 (1)	*not know
c.642delG	p.E214DfsX266a	6 (7)	Frameshift
c.673C>T	p.R225Ter	1 (1)	Nonsense
c.725C>T	p.A242V	1 (1)	*not know
c.863C>G	p.A288G	2 (2)	Missense
c.868G>A	p.E290K	1 (1)	Missense
c.886_887insA	p.R296QfsX35	16 (18)	Frameshift
c.961C>T	p.R321X	26 (29)	Nonsense
c.992G>A	p.R331Q	3 (3)	Missense
c.1003C>T	p.R335W	2 (2)	Missense
c.1129C>T	p.R377C	3 (3)	Missense
c.1189delC	p.Arg397Alafs*83	2 (2)	Frameshift
c.1146C>T	p.G382G	2 (2)	*not know
c.1215_1218delCTCA	p.Ser406Profs*73	1 (1)	Frameshift
c.1300_1307del	p.A434X	1 (1)	Nonsense
c.1541G>A	p.W514Ter	1 (1)	Nonsense
c.1609-1G>A	*not known	2 (2)	Splice site
c.1621C>T	p.R541C	1 (1)	Missense
c.(?_1)_(356_?)del	p.? (deletion exon 1)	2 (2)	*not know
c.?	p.? (deletion exons 10-12)	1 (1)	*not know

HGVS=Human Genome Variation Society

SUPPLEMENTAL MATERIAL

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Paper 3

Progression of cardiac disease in patients with lamin A/C mutations

Eystein T Skjølsvik, Øyvind Haugen Lie, Monica Chivulescu, Margareth Ribe, Anna Isotta Castrini, Kaspar Broch, Are Hugo Pripp, Thor Edvardsen, Kristina H Haugaa

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ESC European Society of Cardiology

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Progression of cardiac disease in patients with lamin A/C mutations

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Aims We aimed to study the progression of cardiac dysfunction in patients with lamin A/C mutations and explore markers of adverse cardiac outcome.

Methods and results We followed consecutive lamin A/C genotype-positive patients divided into tertiles according to age. Patients underwent repeated clinical examinations, electrocardiograms (ECGs), and echocardiograms. We followed left ventricular (LV) and right ventricular (RV) size and function, and the severity atrioventricular-valve regurgitations. Outcome was death, LVAD implant, or cardiac transplantation. We included 101 patients [age 44 (29–54) years, 39% probands, 50% female]. We analysed 576 echocardiograms and 258 ECGs during a follow-up of 4.9 (interquartile range 2.5–8.2) years. The PR-interval increased at young age from 204 ± 73 to 212 ± 69 ms ($P < 0.001$), LV ejection fraction (LVEF) declined from middle age from 50 ± 12% to 47 ± 13% ($P < 0.001$), while LV volumes remained unchanged. RV function and tricuspid regurgitation worsened from middle age with accelerating rates. Progression of RV dysfunction [odds ratio (OR) 1.3, 95% confidence interval (CI) (1.03–1.65), $P = 0.03$] and tricuspid regurgitation [OR 4.9, 95% CI (1.64–14.9), $P = 0.004$] were associated with outcome when adjusted for age, sex, comorbidities, LVEF, and New York Heart Association functional class.

Conclusion In patients with lamin A/C genotype, electrical disease started at young age. From middle age, LV function deteriorated progressively, while LV size remained unchanged. Worsening of RV function and tricuspid regurgitation accelerated in older age and were associated with outcome. Our systematic map on cardiac deterioration may help optimal monitoring and prognostication in lamin A/C disease.

Keywords heart failure • echocardiography • genetic disease

Introduction

Mutations in the lamin A/C gene account for 5–8% of cases of familial dilated cardiomyopathy (DCM).[1–3] Lamin A/C cardiomyopathy has autosomal dominant inheritance and complete age-related disease-penetrance.[3,4] The disease is highly malignant with high risk of sudden cardiac death and end-stage heart failure.[3,5]

Lamin A/C cardiomyopathy commonly presents with atrial fibrillation (AF) and progresses with atrioventricular (AV) block and ventricular arrhythmias (VA). Heart failure occurs later, and often progresses to end-stage heart failure.[3] Systematic reports on the cardiac disease progression in lamin A/C cardiomyopathy are lacking, although there have been suspected less left ventricular (LV) dilatation than in DCM of other aetiologies.[6] In addition to medical treatment,

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cardiac resynchronization therapy, and circulatory assist devices are established in heart failure therapy.[7] The success of device therapy is partly dependent on cardiac structure and function, including LV size, right ventricular (RV) function, and AV valve competence.[8–12] The timing of intervention and follow-up of these patients may benefit from knowledge about the clinical course and cardiac structural and functional progression of lamin A/C cardiomyopathy.

We aimed to describe the individual cardiac electrical, structural, and functional progression in lamin A/C genotype-positive patients. We wanted to evaluate if disease progression were prognostic markers of heart failure outcome.

Methods

The authors do not have the authority to share the data used in the analyses described in this article. The approval of the Regional Committee for Medical Research Ethics limits sharing of data with researchers outside of Norway for purposes of reproducing the results or replicating the procedures. The data can only be made available to any additional researchers if a formal request is filled with the Regional Committee of Research Ethics and explicit consent is given from every study subject.

Study population

We conducted a single-centre, longitudinal cohort study. The patients were recruited from the Unit for Genetic Cardiac Diseases, Department of Cardiology, Oslo University Hospital Rikshospitalet, Oslo, Norway. We included consecutive lamin A/C genotype-positive patients with at least one clinical visit including an echocardiogram and followed them at our institution. Follow-up were performed according to clinical protocol. We defined probands as the first member of a family who sought medical attention due to cardiac or neuromuscular disease caused by a lamin A/C mutation. Family members were identified by cascade genetic screening. Inclusion was the first clinical visit at our centre. We stratified the patients by age into tertiles. The age tertiles were defined as younger, middle, and older.

We recorded demographical data, clinical characteristics including New York Heart Association (NYHA) functional class I–IV and comorbidities at each visit. Comorbidities were defined as having at least one of the following: hypertension, coronary artery disease, chronic obstructive pulmonary disease, peripheral arterial disease, cerebrovascular disease, and diabetes mellitus.[13]

Outcome

Outcome was defined as cardiac death, heart transplantation, or LV assist device (LVAD) placement. Last follow-up was the last visit before September 2019.

The study complied with the Declaration of Helsinki. The research protocol was approved by the regional committee for medical research ethics (REK no. 17.01.2008/S-0746a). All patients gave written informed consent.

Structural and functional progression by echocardiography

We analysed repeated echocardiograms and excluded echocardiograms on intravenous inotropes, on treatment with circulatory assist devices, and post-heart transplantation.

Echocardiograms were evaluated offline (Echo Pac® GE Healthcare version 2.02), by three expert echocardiographers (E.T.S., M.C., and M.R.), blinded to clinical data. The same echocardiographer analysed all repeated exams within one patient. We measured LV end-diastolic volume (LVEDV), end-systolic volume (LVESV), and LV ejection fraction (LVEF) by Simpson's biplane method.[14] LV global longitudinal strain (LVGLS) was assessed in a 16 segments model.[15] Left atrial (LA) volume was calculated by the area length method indexed for body surface area as LA volume index (LAVI).[14] RV size was measured as RV basal linear dimension (RVD).[14] We assessed RV systolic function by tricuspid annular plane systolic excursion (TAPSE) and by 6 segments RV longitudinal strain (RVLS).[14,16] Right atrial (RA) size was measured as RA area.[14] We quantified mitral- and tricuspid valve regurgitation as mild, moderate, and severe.[17]

Electrical parameters and progression

We examined all available electrocardiograms (ECG) during follow-up and recorded heart rate, PR-interval, and occurrence of AV-block grade I–III. We excluded ECGs with ventricular pacing. Electrical progression was defined as an increase in PR-interval. Occurrence of AF was noted from ECG, exercise ECG, Holter monitorings, and implantable cardiac electronic devices. We noted time and indication of implanted cardiac electronic devices.

Statistics

Continuous variables were presented as mean ± (SD) or (SE), or as median with interquartile range (IQR) and were compared by one-way ANOVA or Kruskal–Wallis test as appropriate. Categorical data were presented as numbers (percentages) and compared by Fisher's exact test.

Repeated echocardiographic and electrical parameters were analysed in a linear mixed model regression with random intercept. The effect of time on disease progression was compared across age tertiles by an interaction term with the middle tertile serving as the reference group. For evaluating cardiac chamber size progression, we excluded patients <age 18 to eliminate bias by physiologic growth. We compared the disease progression of probands vs. family members separately in each age tertile.

We used uni- and multivariate mixed model logistic regression with random intercept to test whether disease progression were markers for outcome. Univariate markers associated with outcome ($P < 0.05$) were included in a multivariate model adjusted for sex, and the evolution of age, comorbidities, LVEF, and NYHA class as time varying covariates.

We used a multivariable fractional polynomial linear regression model to fit an age curve for the expected PR-interval. Kaplan–Meier survival curves illustrated age at first episode of AF (STATA version 16.1, StataCorp LLC, TX, USA). Two-sided *P*-values <0.05 were considered significant.

Results

Clinical characteristic

We included 101 lamin A/C genotype-positive patients [age 44 (IQR: 29–54) years, 39% probands, 50% female, 33 different families] with median follow-up 4.9 (IQR: 2.5–8.2) years (*Table 1*). Genetic testing revealed 15 different pathological lamin A/C mutations (Supplementary data online, *Table S1*). At inclusion, age tertiles were distributed as younger tertile <32 years, middle tertile 32–49 years, and older tertile >49 years (*Figure 1*). Patients in the older tertile were more often probands and had more severe cardiac disease as expected (*Table 1*).

Table 1 Baseline characteristics of 101 lamin A/C genotype patients

Clinical data	Total, *n* = 101	Younger tertile, *n* = 34	Middle tertile, *n* = 34	Older tertile, *n* = 33	*P*
Follow-up time (years)	4.4 (2.5–6.6)	6.1 (3.4–9.6)	2.6 (1.7–4.6)	2.5 (2.5–3.0)	<0.01
Clinical visits, *n*	5 (3–9)	6 (4–10)	6 (4–9)	4 (3–7)	0.11
Age at inclusion (years)	44 (29–54)	22 (15–29)	44 (38–47)	57 (55–60)	<0.001
Female sex, *n* (%)	50 (50)	17 (50)	19 (56)	14 (42)	0.57
Proband, *n* (%)	39 (39)	3 (9)	15 (44)	21 (64)	<0.001
Families	33	18	20	15	NA
NYHA I, *n* (%)	70 (69)	30 (88)	27 (79)	13 (41)	<0.001
NYHA II, *n* (%)	15 (15)	2 (6)	4 (12)	9 (28)	0.045
NYHA III, *n* (%)	12 (13)	1 (3)	3 (9)	8 (25)	0.03
NYHA IV, *n* (%)	3 (3)	1 (3)	0 (0)	2 (6)	0.31
Electrophysiological					
AV-block I, *n* (%)	14 (14)	3 (10)	7 (23)	4 (14)	0.39
AV-block II, *n* (%)	3 (3)	0 (0)	1 (3)	2 (7)	0.32
AV-block III, *n* (%)	27 (27)	0 (0)	9 (30)	18 (62)	<0.001
Atrial fibrillation, *n* (%)	45 (45)	4 (12)	16 (47)	25 (78)	<0.001
Pacemaker, *n* (%)	15 (15)	0 (0)	7 (20)	8 (24)	<0.01
ICD, *n* (%)	2 (2)	0 (0)	0 (0)	2 (6)	0.10
CRT-D, *n* (%)	9 (9)	0 (0)	0 (0)	9 (28)	<0.001
Medication					
ACE inhibitor, *n* (%)	19 (19)	0 (0)	5 (15)	14 (43)	<0.001
β-Blocker, *n* (%)	23 (23)	1 (3)	8 (24)	14 (44)	<0.001
MCRA, *n* (%)	6 (6)	0 (0)	1 (3)	5 (16)	0.01
Comorbidities					
Hypertension, *n* (%)	3 (3)	0 (0)	0 (0)	3 (9)	0.32
Coronary artery disease, *n* (%)	3 (3)	0 (0)	0 (0)	3 (9)	0.03
Stroke, *n* (%)	5 (5)	1 (3)	0 (0)	4 (13)	0.04
COPD, *n* (%)	1 (1)	0 (0)	1 (3)	0 (0)	0.65
Diabetes, *n* (%)	5 (5)	1 (3)	1 (3)	3 (9)	0.44

Values are mean ± standard deviation, median (IQR), or frequency (%). *P*-values by one-way ANOVA, Kruskal–Wallis test, or Fisher's exact test as appropriate.
ACE inhibitor; angiotensin converting enzyme inhibitor, AV-block; atrioventricular block, BMI; body mass index, COPD; chronic obstructive pulmonary disease, CRT-D; cardiac resynchronization therapy defibrillator, DCM; dilated cardiomyopathy, MCRA; mineral corticoid receptor antagonist, NSVT; non-sustained ventricular tachycardia, NYHA; New York Heart Association functional class, VA; ventricular arrhythmia.

Structural and functional disease progression in lamin A/C

We obtained 576 echocardiographic exams during follow-up. LV systolic function deteriorated during follow-up without compensatory LV dilatation (*Table 2* and *Figure 1*).

RV systolic function deteriorated along with a progressive RV dilatation (*Table 2* and *Figure 1*). LA and RA size increased along with increased mitral- and tricuspid regurgitation (*Table 2*).

Comparison of LV and RV structural and functional progression in different age-tertiles

LV systolic function remained unchanged in the younger tertile, but deteriorated in the middle and older age tertiles with an average LVEF reduction of 1% per year (*Table 2* and *Figure 1*). LV volume did not change (*Table 2* and *Figure 1*).

LA size increased with similar rates in all tertiles and pathological LAVI values were reached in the younger tertile (*Table 2*). Mitral regurgitation progressed in all age groups with faster progression in the middle and older tertiles (*Table 2*).

RV and RA size and RV function remained unchanged in the younger tertile, and worsened from the middle tertile. The deterioriation of TAPSE and RA dilatation accelerated with increasing age (*Table 2* and *Figure 1*). Tricuspid regurgitation progressed in all age groups with accelerated rates with increasing age (*Table 2*).

We observed no clear differences in cardiac disease progression in probands compared with family members (data not shown).

Electrical disease

We analysed in all 258 ECGs (*Table 2*). The PR-interval increased during follow-up in all age groups with faster progression rates in the middle and older tertile (*Table 2* and *Figure 2a*).

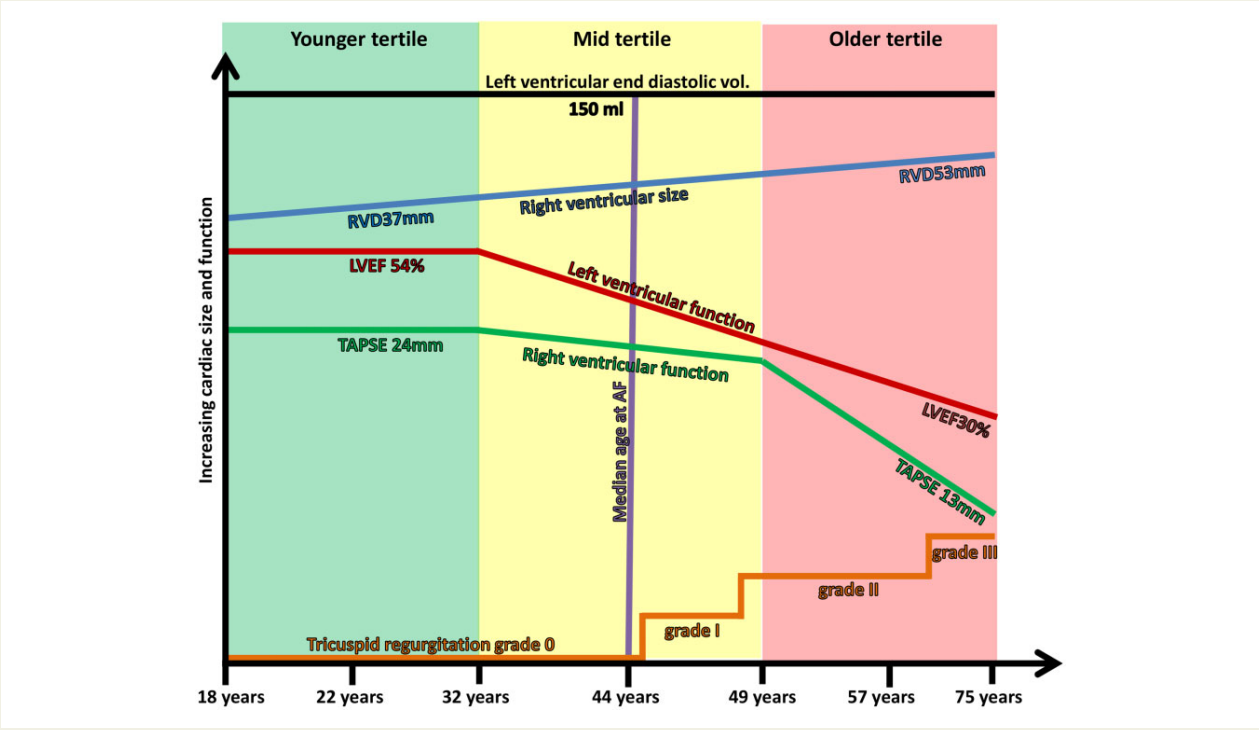

Figure 1 Schematic figure showing structural and functional progression of lamin A/C cardiomyopathy with increasing age. Age tertiles are colour coded on the *x*-axis by younger (green), middle (yellow), and older (red) . LVEF; left ventricular ejection fraction, RVD; right ventricular basal linear dimension, TAPSE; tricuspid annular plane systolic excursion.

The frequency of AF increased from 44% at inclusion to 62% during follow-up and occurred at median age 44 (range 15–77) years (*Figures 1 and 2b*).

Frequency of implantable cardiac electronic devices increased during follow-up from $n = 26$ (26%) (*Table 1*) to $n = 52$ (56%) (pacemakers: $n = 2$, ICDs: $n = 20$, and CRT-D: $n = 30$).

Association between structural progression and outcome

Eighteen patients had adverse cardiac outcome during follow-up [younger tertile $n = 3$ (10%), middle tertile $n = 3$ (10%), and older tertile $n = 12$ (44%), $P = 0.001$].

Worsening of LV and RV function, increasing ventricular and atrial sizes, worsening of AV-valvular regurgitations, NYHA functional class, increasing age, and proband status were all associated with increased odds for outcome in univariate analysis (*Table 3*). RV and LV dilatation, RV dysfunction by RVLS, and increase in AV-regurgitations remained significant markers for outcome when adjusted for sex, evolution of age, LVEF, comorbidities, and NYHA class (*Table 3*).

Discussion

We describe the progressive and dynamic cardiac deterioration in patients with lamin A/C genotypes. At young age electrical progression was most predominant, followed by decline in LV systolic function from middle age. LV systolic function deteriorated without compensatory LV dilatation, indicating an important structural difference in patients with lamin A/C compared with DCM of other aetiologies. RV dysfunction progressed at accelerating rates during the middle and older tertiles and was together with RV dilatation, and AV-valvular regurgitations associated with adverse cardiac outcome independent of the traditional selection criteria for heart transplantation or LVAD implantation. We provide hereby a comprehensive map of the expected cardiac deterioration in a cohort of patients with lamin A/C genotypes. Our findings implicate a closer monitoring of RV parameters in advanced disease as markers of adverse outcome and may be valuable in planning of LVAD and heart transplantation in lamin A/C patients.

Structural and functional disease progression in lamin A/C

LV function declined in patients in the middle and older tertiles with no obvious compensatory LV dilatation. These findings suggest different LV progression in lamin A/C cardiomyopathy compared with typical DCM and support the suggestion of lamin A/C cardiomyopathy as a hypokinetic non-DCM.[6,18] In heart failure, compensatory LV dilatation restore stroke volume.[19] A decline in LV systolic function without compensatory LV dilatation as in lamin A/C, may lead to a steeper decline in stroke volumes and earlier activation of neuro-hormonal systems known to stimulate heart failure progression[20] and may explain the rapid clinical deterioration seen in clinical practice.

Table 2 Cardiac structural and functional progression in 101 patients with lamin A/C mutation by 576 echocardiographic assessments and 258 ECG

Variable	Observed values at inclusion, *n* = 101	Observed values at last follow-up, *n* = 94	Progression rate/1 year (SE)	*P* for progression
LVEF (%)	50 ± 12	47 ± 13	-0.5 (0.1)	<0.001
Younger tertile	54 ± 10	53 ± 12	-0.2 (0.1)	0.13
P for interaction			<0.01	
Middle tertile	51 ± 11	49 ± 10	-0.8 (0.2)	<0.001
P for interaction			0.60	
Older tertile	43 ± 13	38 ± 12	-1.0 (0.3)	<0.001
LVGLS (%)	-15.5 ± 4.7	-14.5 ± 4.5	0.2 (0.1)	<0.01
Younger tertile	-17.4 ± 4.2	-16.9 ± 4.1	0.02 (0.1)	0.70
P for interaction			<0.01	
Middle tertile	-16.5 ± 4.3	-14.2 ± 3.3	0.3 (0.1)	<0.001
P for interaction			0.94	
Older tertile	-13.0 ± 4.4	-11.8 ± 4.4	0.3 (0.2)	0.051
LVEDV (mL)	136 ± 45	138 ± 43	0.2 (0.3)	0.60
Younger tertile	120 ± 23	131 ± 35	0.2 (0.5)	0.68
P for interaction			0.72	
Middle tertile	149 ± 51	138 ± 41	-0.1 (0.5)	0.90
P for interaction			0.01	
Older tertile	145 ± 47	146 ± 54	-2.5 (0.7)	<0.001
LAVI (mL/m^2)	45 ± 22	50 ± 25	1.4 (0.2)	<0.001
Younger tertile	30 ± 5	36 ± 10	1.0 (0.2)	<0.001
P for interaction			0.08	
Middle tertile	42 ± 15	51 ± 21	1.7 (0.3)	<0.001
P for interaction			0.17	
Older	61 ± 28	69 ± 29	2.4 (0.5)	<0.001
MR, Grades 0–3	0.7 ± 0.8	1.0 ± 0.8	0.04 (0.01)	<0.001
Younger tertile	0.3 ± 0.6	0.5 ± 0.6	0.02 (0.01)	<0.01
P for interaction			<0.01	
Middle tertile	0.6 ± 0.7	1.0 ± 0.8	0.07 (0.01)	<0.001
P for interaction			0. 51	
Older tertile	1.1 ± 0.7	1.4 ± 0.80	0.05 (0.02)	0.03
RVD (mm)	41 ± 7	43 ± 9	0.2 (0.06)	<0.001
Younger tertile	37 ± 6	39 ± 5	0.1 (0.1)	0.18
P for interaction			0.21	
Middle tertile	42 ± 6	44 ± 6	0.3 (0.09)	0.001
P for interaction			0.48	
Older tertile	45 ± 8	48 ± 11	0.2 (0.1)	0.15
TAPSE (mm)	23 ± 6	21 ± 6	-0.3 (0.06)	<0.001
Younger tertile	24 ± 5	23 ± 5	-0.03 (0.8)	0.70
P for interaction			<0.01	
Middle tertile	24 ± 7	23 ± 6	-0.4 (0.1)	<0.001
P for interaction			<0.01	
Older tertile	21 ± 5	16 ± 5	-0.9 (0.1)	<0.001
RVLS (%)	-19.7 ± 5.8	-17.8 ± 5.9	0.3 (0.1)	<0.001
Younger tertile	-21.5 ± 5	-21.4 ± 4.1	0.1 (0.1)	0.40
P for interaction			0.03	
Middle tertile	-20.8 ± 5.0	-17.6 ± 4.7	0.4 (0.1)	<0.01
P for interaction			0.27	
Older tertile	-17.1 ± 6.3	-13.5 ± 5.9	0.6 (0.1)	<0.001
RA area (cm^2)	21 ± 8	24 ± 9	0.5 (0.06)	<0.001
Younger tertile	16 ± 5	18 ± 7	0.1 (0.09)	0.13

Continued

Table 2 *Continued*

Variable	Observed values at inclusion, *n* = 101	Observed values at last follow-up, *n* = 94	Progression rate/1 year (SE)	*P* for progression
P for interaction			<0.001	
Middle tertile	20 ± 4	24 ± 6	0.6 (0.1)	<0.001
P for interaction			<0.001	
Older tertile	26 ± 9	31 ± 10	1.3 (0.2)	<0.001
TR, Grades 0–3	1.0 ± 0.8	1.4 ± 0.9	0.06 (0.01)	<0.001
Younger tertile	0.7 ± 0.7	0.9 ± 0.6	0.03 (0.01)	0.001
P for interaction			0.047	
Middle tertile	1.1 ± 0.5	1.5 ± 0.8	0.06 (0.01)	<0.001
P for interaction			0.01	
Older tertile	1.4 ± 0.9	2.0 ± 0.8	0.1 (0.02)	<0.001
Electrical parameters				
PR-interval (ms)	204 ± 73	211 ± 69	4 (0.5)	<0.001
Younger tertile	177 ± 54	185 ± 58	3 (0.5)	<0.001
P for interaction			<0.001	
Middle tertile	224 ± 83	248 ± 74	11 (1.7)	<0.001
P for interaction			0.42	
Older tertile	[a]	[a]	9 (2.3)	<0.001

Observed data expressed as mean ± SD. Progression rate expressed as mean (SE). *P* values for yearly progression rate and for time-interaction in linear mixed models statistics.
[a]Data not shown due to <6 observations.
LAVI; left atrial volume index, LVEDV; left ventricular end-diastolic volume, LVEF; left ventricular ejection fraction, LVGLS; global longitudinal strain, MR; mitral regurgitation, RA area; right atrial area, RVD; right ventricular basal linear dimension, RVLS; right ventricular longitudinal strain, TR; tricuspid regurgitation, TAPSE; tricuspid annular plane systolic excursion.

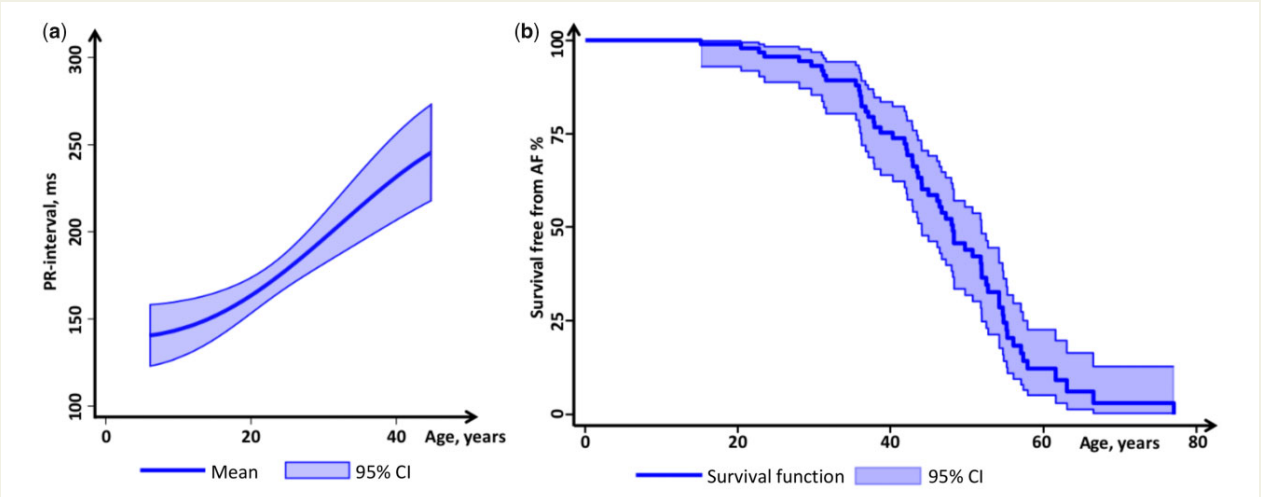

Figure 2 PR interval and survival from atrial fibrillation, according to age in 101 lamin A/C genotype-positive patients. (a) Mathematical-derived curve showing the expected PR-interval (*y*-axis) according to age (*x*-axis). (b) Kaplan–Meier survival curve displaying survival free from AF according to age.

LA size reached pathological values already in the younger tertile. Whether the high prevalence of AF explains the increase in atrial size or vice versa the atrial cardiomyopathy induce AF is unknown.

Mitral regurgitation worsened in all ages. The progressive regurgitation despite unchanged LV volumes may seem contradictory, but may be explained by the combination of progressive LV dysfunction, dyssynchrony, and mitral annular dilatation even without LV dilation.[21]

We found an accelerating deterioration of RV function and increase in tricuspid regurgitation in the middle and older tertiles. RV

Table 3 Odds for adverse cardiac outcome (cardiac transplantation or LVAD, or death from heart disease) in 101 lamin A/C genotype patients in univariate and multivariate mixed model logistic regression

	Univariate			Multivariate Adjusted for age, sex, LVEF, NYHA functional class, and comorbidities		
	OR	CI	*P*	OR	CI	*P*
Age per year	1.3	1.21–1.42	<0.001	NA	NA	NA
Female	0.4	0.06–2.80	0.36	NA	NA	NA
Proband	160	2.2–11 925	0.02	3.5	0.6–21	0.17
NYHA I–IV	30	11–84	<0.001	NA	NA	NA
LVEF per 5%	2.5	1.9–3.3	<0.001	NA	NA	NA
LVGLS per %	1.5	1.3–1.7	<0.001	NA	NA	NA
LVEDV per 10 mL	1.5	1.2–1.9	0.001	1.2	1.006–1.4	0.04
LAVI per 5 cm^3/m^2	1.6	1.3–2.0	<0.001	1.0	0.8–1.1	0.65
MR per 0–3	20	6.1–69	<0.001	2.4	1.004–6.0	0.049
RVLS per %	1.7	4.7–42	<0.001	1.3	1.03–1.65	0.03
RVD per 3 mm	3.0	1.4–2.1	<0.001	1.5	1.1–2.1	0.01
RA area per 5 cm^2	4.6	2.5–8.8	<0.001	1.3	0.8–2.0	0.28
TR per 0–3	158	11.3–2182	<0.001	5.0	1.64–14.8	<0.01
TAPSE per 3 mm	3.1	2.1–4.6	<0.001	1.4	0.96–2.0	0.08

P values by mixed models logistic regression analyses. Comorbidities included hypertension, coronary artery disease, peripheral arterial disease, cerebrovascular disease, and diabetes mellitus.
Parameters LAVI, LVEDV LVEF, RA area, RVD, and TAPSE are all continuous scale parameters.
LAVI; left atrial volume index, LVEDV; left ventricular end-diastolic volume, LVEF; left ventricular ejection fraction, LVGLS; left ventricular global longitudinal strain, MR; mitral regurgitation, NYHA; New York Heart Association functional class, OR; odds ratio, RA; right atrium, RVD; right ventricular basal linear dimension, RVLS; right ventricular longitudinal strain, TAPSE; tricuspid annular plane systolic excursion, TR; tricuspid regurgitation.

disease progression may be explained by progressive increase in RV afterload due to elevated LV filling pressures, and at later stages, chronic RV volume overload due to severe tricuspid regurgitation.[22] Chronic AF leading to atrial and annular dilatation[23] may worsen tricuspid regurgitation and transvalvular pacing leads can further increase the unfavourable situation.[24]

Together, the progressively failing LV without compensatory LV dilatation, accompanied by RV disease and worsening of tricuspid regurgitation, all contribute to low systemic stroke volumes. These factors may contribute to the high incidence of end-stage heart failure in patients with lamin A/C genotypes.[3]

Electrical progression in lamin A/C disease

The PR-interval increased across all age tertiles with increased progression rate from the middle tertile resulting in the frequent need of cardiac pacing.[3] Studies have shown early replacement fibrosis in the AV junction in lamin A/C cardiomyopathy[1] supported by findings of septal late gadolinium enhancement and septal dysfunction.[25] Conduction delays leading to LV dyssynchrony and an inadequate chronotropic response may further reduce cardiac output.

Clinical implications

During the younger tertile, we observed an increasing risk of AF accompanied by increasing LA volumes, while other structural features were stable. Management of young lamin A/C patients should therefore focus on Holter monitoring to detect asymptomatic AF which may warrant anticoagulation therapy.[26] Furthermore, it is important to detect AV-block and VA leading to therapeutic measures including device therapy.

From around 40 years of age, we observed increasing cardiac LV and RV structural and functional deterioration, accompanied by increased severity of AV valve regurgitations. RV deterioration was a strong marker for adverse cardiac outcome independent of other typical markers indicating need of LVAD or heart transplant. In addition to management of electrical disease, management of adult lamin A/C patients should focus on assessment of both LV and RV size and function.

LV dilatation was uncommon in lamin A/C cardiomyopathy and the clinician should be aware that an end-stage phenotype can occur without LV dilatation. Nevertheless, occurrence of LV dilatation should be regarded as sign of poor prognosis. The lack of severe LV dilatation in lamin A/C cardiomyopathy may have additional clinical relevance. Smaller hearts may respond better to cardiac resynchronization therapy.[12] Furthermore, smaller LV size and progressive RV dysfunction and tricuspid regurgitation may imply non-optimal effect of LVAD therapy.[8,9,11,27] Future studies should evaluate the effect and optimal timing of LVAD treatment in lamin A/C patients.

Limitations

This was a single-centre longitudinal cohort study conducted in a tertiary reference centre with inherent limitations. Subtle structural progression may overlap with echocardiographic measurement variability and may be difficult to identify clinically. The number of

outcomes was limited and potential outcome markers from his study should be validated in a separate cohort.

We did not include CMR examinations in this study due to the high frequency of cardiac devices at older ages and thereby missing data.

Conclusion

We provided a comprehensive map of cardiac disease progression in patients with lamin A/C genotypes. Cardiac disease progressed slowly at younger age, mainly with electrical disease including AV block and further AF. With increasing age, LV function gradually deteriorated with a subsequent rapid RV deterioration to end-stage heart failure. We observed limited compensatory LV dilatation, suggesting disease mechanisms different from other DCM. These findings may have impact on prognostication, clinical management, follow-up, and timing of interventions in patients with lamin A/C disease.

Supplementary data

Supplementary data are available at *European Heart Journal - Cardiovascular Imaging* online.

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1 “Supplemental material”

Supplemental table S1. Distribution of lamin A/C genotypes in 101 lamin A/C mutation positive patients followed for 4.9 years.

c DNA	Amino Acid	n (%)	Mutation type
c.961C>T	p.R321X	32 (32)	Nonsense
c.886-887insA	p.R296QfsX35a*	27 (27)	Frameshift/Nonsense
c.1063C>T	p.Q355X	4 (4)	Nonsense
c.1129C>T	p.R377c	2 (2)	Missense
c.642delG	p.E214DfsX266*	14 (14)	Frameshift
c.1381-1 G>A	"_"*,†	2 (2)	Splice site
c.427T>C	p.S143P	1 (1)	Missense
c.868G>A	p.E290K	1 (1)	Missense
c.43C>T	p.Q15X	2 (2)	Nonsense
c.730G>A	A244T*	1 (1)	Missense
c.992G>A	p.R331Q	8 (8)	Missense
c.1003C>T	p.R335W	4 (4)	Missense
c.305T>C	p.L102P	1 (1)	Missense
c.1016C>A	p.A339E*	1 (1)	Missense
c.568C>T	p.R190W*	1 (1)	Missense

Values are frequency (%)

c DNA indicates complimentary DNA

*Novel mutation, †Mutations in an acceptor site may cause more than one mutant transcript with different effects at the protein level.