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Cardiac Phenotype in Familial Partial Lipodystrophy

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ABSTRACT

Objectives: *LMNA* variants have been previously associated with cardiac abnormalities independent of lipodystrophy. We aimed to assess cardiac impact of familial partial lipodystrophy (FPLD) to understand the role of laminopathy in cardiac manifestations.

Study design: Retrospective cohort study.

Methods: Clinical data from 122 patients (age range: 13-77, 101 females) with FPLD were analyzed. Mature human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from a patient with an *LMNA* variant were studied as proof-of-concept for future studies.

Results: Subjects with *LMNA* variants had a higher prevalence of overall cardiac events than others. The likelihood of having an arrhythmia was significantly higher in patients with *LMNA* variants (OR: 3.77, 95% CI: 1.45-9.83). These patients were at higher risk for atrial fibrillation or flutter (OR: 5.78, 95% CI: 1.04-32.16). The time to the first arrhythmia was significantly shorter

in the *LMNA* group, with a higher HR of 3.52 (95% CI: 1.34-9.27). Non-codon 482 *LMNA* variants were more likely to be associated with cardiac events (vs. 482 *LMNA*: OR: 4.74, 95% CI: 1.41-15.98 for arrhythmia; OR: 17.67, 95% CI: 2.45- 127.68 for atrial fibrillation or flutter; OR: 5.71, 95% CI: 1.37- 23.76 for conduction disease). *LMNA* mutant hiPSC-CMs showed a higher frequency of spontaneous activity and shorter action potential duration. Functional syncytia of hiPSC-CMs displayed several rhythm alterations such as early afterdepolarizations, spontaneous quiescence, and spontaneous tachyarrhythmia, as well as significantly slower recovery in chronotropic changes induced by isoproterenol exposure.

Conclusions: Our results highlight the need for vigilant cardiac monitoring in FPLD, especially in patients with *LMNA* variants who have an increased risk of developing cardiac arrhythmias. In addition, hiPSC-CMs can be studied to understand the basic mechanisms for the arrhythmias in patients with lipodystrophy to understand the impact of specific mutations.

Keywords: arrhythmia, atrial fibrillation, conduction disease, lipodystrophy, *LMNA*.

INTRODUCTION

Familial partial lipodystrophy (FPLD) is a heterogeneous rare disease characterized by selective fat loss, mainly affecting the limbs [1]. The most common type, FPLD2 (Dunnigan variety), is attributed to *LMNA* pathogenic variants [2]. The *LMNA* gene produces lamin A and C by alternative splicing of exon 10. *LMNA* pathogenic variants demonstrate remarkable allelic heterogeneity and pleiotropy, and can give rise to more than 16 different diseases (laminopathies) [3]. Mutations in the N terminal region usually lead to a more severe phenotype, because both lamins A and C are affected, unlike C terminal mutations where only lamin A is involved. In general, cardiolaminopathies mostly arise from pathogenic variants in the amino-terminal and central rod domain, 5' to the nuclear localization signal (NLS). Conversely, lipodystrophy mainly originates from C- terminal domain mutations, 3' to the NLS [4]. Variants in the hot-spot (codon 482 in C-terminal domain) make up approximately 80% of all pathogenic variants in known patients with lipodystrophy [1].

LMNA is a well-known genetic cause of dilated cardiomyopathy [5]. A few notable N terminal variants present overlap between lipodystrophy and cardiac disease: in exon 1 (R28W [6,7], R60G [8], and R62G [7] reported with FPLD, atrioventricular (AV) block, and cardiomyopathy), exon 2 (R131L [9] reported with generalized lipodystrophy, cardiomyopathy, and valvular disease), exon 3 (D192V [8] presented with FPLD and cardiomyopathy), exon 6 (R349W [10,11] associated with cardiomyopathy and arrhythmias), and cardiomyopathy resulting in cardiac transplants in T10I

carriers [12]. A recent study reported a higher prevalence of arrhythmia and cardiac disease in non-482 *LMNA* variants (including non-lipodystrophy patients) [13]. A large study of 444 subjects with *LMNA* variants (including 72 with lipodystrophy) reported that male sex, non-missense *LMNA* variants, first degree and higher atrioventricular block, non-sustained ventricular tachycardia, and left ventricular ejection fraction were predictors of life-threatening ventricular tachyarrhythmias [14]. However, the exact degree of cardiac disease among the different subtypes of FPLD (specifically in those with *LMNA* variants versus those that do not harbor *LMNA* variants) still remains unknown. Also, specific evidence documenting the presence of atherosclerotic heart disease in lipodystrophy syndromes is insufficient [15,16].

In this retrospective multi-center cohort study, we aimed to i) characterize the cardiac disease phenotype of FPLD2 and other FPLD types; and ii) define the risk for developing cardiac disease; and iii) explore the association of molecular etiology and cardiac manifestations. Also, spontaneous rhythm and action potential duration of functional syncytia of mature human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from a patient with a pathogenic variant at codon 349 of the *LMNA* gene were assessed in optical mapping experiments using a voltage-sensitive dye to provide the proof of concept that these cells can become important tools in understanding the pathophysiology of cardiac disease and arrhythmias in patients with genetic heart diseases.

METHODS

Description of the cohort and clinical data

One hundred twenty-two patients (median age 49 years, IQR 38-59 years, age range: 13-77; male/female: 21/101) with FPLD who have been followed at the University of Michigan (n = 83) and major academic centers in Turkey (n = 39) with an available cardiac evaluation were included. The diagnosis of lipodystrophy was established based on comprehensive clinical assessment and genetic testing by experienced clinicians at referral centers for this rare disease.

In this study, we specifically inquired the impact of genotype, BMI, and glucose control on the occurrence of cardiac events. Data were collected on demographics, past medical history, genotype, body mass index (BMI), laboratory assessments (specifically lipid and glucose control parameters such as HbA1c, triglyceride levels, liver function tests, etc.), medications, comorbidities, cardiac manifestations, and cardiac disease dates. Cardiac exam was done as

part of standard medical care. Typically, patients are seen at least once a year and cardiac exam is part of their visits. Further tests are ordered based on clinical symptoms and findings in the system review, and physical exam. Baseline ECG and ECHO are typically obtained at least when patients are evaluated for the first time and studies repeated based on findings and follow up symptoms/clinical events. ECGs and echocardiograms were reviewed by an experienced electrophysiology subspecialist (*H.O*) and cardiac imaging subspecialist *(N.B*), respectively. Both experts were blinded to any clinical data.

The primary end-point was the first record of a cardiac event. Events included arrhythmias, conduction disease, cardiomyopathy, ischemic cardiac disease, and any other cardiac disease. Arrhythmias included sinus arrhythmia, premature ventricular contractions (PVC), premature atrial contractions (PAC), supraventricular tachycardia (SVT), atrial fibrillation or flutter, and ventricular tachycardia or fibrillation. Conduction abnormality included atrioventricular block and intraventricular conduction delay.

Proof-of-concept hiPSC studies

Dermal fibroblasts were isolated from a skin biopsy of a patient with *LMNA* variant R349W. Briefly, the skin sample was minced into ≈1mm^3 fragments under sterile conditions and plated on a six-well plate with 1 ml of fibroblast media (DMEM: F12, 10% FBS, 1%NEAA, 200μM of L-Glutamine) for 24 hours. Fibroblast outgrowth was monitored, and media was changed every other day until fibroblasts reached confluence and were passed with trypsin for expansion and reprogramming into hiPSC with a commercially available non-integrational vector (Cytotunes 2.0, Thermo, USA). Control fibroblasts (BJ foreskin fibroblasts; ATCC® CRL-2522™) were also reprogrammed as described before [17]. In general, we prefer to perform comparisons between genetically-related (parents and/or normal siblings) and genetically-unrelated control cell lines; nevertheless, the patient that donated the disease-specific fibroblasts did not have close relatives alive that could be recruited to provide genetically related control samples and the feasible alternative was to perform the comparison with hiPSC generated from a commercially available normal fibroblast line.

New hiPSC lines were expanded with StemMACS iPS-Brew XF on matrigel for the production of hiPSC-derived cardiomyocytes (hiPSC-CMs). Cardiac directed differentiation was performed with a high-efficiency small molecule protocol [18]. Cardiomyocytes were prepared as high-purity functional syncytia of mature hiPSC-CMs as described previously [19,20]. After cardiac directed differentiation, the cells were submitted to Magnetic Assisted Cell Sorting (MACS) negative selection with PSC-derived cardiomyocyte isolation kit (Miltenyi Biotec). Briefly, cells

were dissociated with trypsin and the enzyme was neutralized with medium containing high serum (20% FBS). Cells were pelleted down by centrifugation and washed with HBSS prior to incubation with non-cardiomyocyte depletion antibody cocktail (1:5 dilution) for 10 minutes. Primary antibody cocktail was removed by dilution followed by centrifugation for cells separation. After removal of supernatant, the cell pellets were resuspended and incubated with magnetic bead-conjugated secondary antibodies (1:5 dilution) included in the kit. Volume of each tube was adjusted to 2 mL with MACS separation buffer (Miltenyi) and cell suspensions were individually applied to magnetic separation columns. Flow through separation buffer containing cardiomyocytes was centrifuged prior to discard of supernatant. Cardiomyocytes were resuspended in plating medium and cardiomyocyte concentration was adjusted to 1.10^6 cardiomyocytes/mL. Purified cardiomyocytes were plated as functional syncytia on Matrigel coated PDMS to induce maturation of cardiomyocytes. Optical mapping of voltage changes was used to investigate cardiac electrophysiology. Action potential duration at 80% of repolarization during spontaneous depolarization and paced depolarization frequency were calculated by using the Scroll software. These parameters were also assessed after treatment of the cells with 100nM isoproterenol [20]. Isoproterenol recovery rate was defined as the difference in APD80% immediately after and 5 minutes after isoproterenol treatment divided by APD80% before drug treatment.

Statistical methods

The nature of data distribution was assessed with the Kolmogorov–Smirnov test. Fisher's exact test or Mann-Whitney U test was used as appropriate to compare groups. Unadjusted and adjusted odds ratios with 95% confidence interval (CI) were calculated for arrhythmia, atrial fibrillation or flutter, and conduction disease using a logistic regression model. Models were adjusted for age, body mass index (BMI, kg/m^2), and comorbidities (such as chronic obstructive pulmonary disease (COPD), thyroid disease, chronic kidney disease, amyloidosis, renal tubular acidosis, sleep apnea, and lung cancer). For the subgroup analysis (non-482 and 482 *LMNA*), models were only adjusted for age as a continuous variable due to the limited sample size and number of events. Data were then analyzed with a time to event analysis approach where the reference date was the year of lipodystrophy diagnosis. Survival time was calculated from the reference date to endpoint date or end of study date (2019) or death. Kaplan Meier curves were plotted for conduction disease, and arrhythmia for the entire cohort, the log-rank test was used to compare between *LMNA* and non-*LMNA* groups. Two groups of patients were excluded from the study: patients who developed an event before lipodystrophy diagnosis, and those

diagnosed with lipodystrophy before 2004 (due to limited data accessibility). A cox proportional hazards model was used to drive hazard ratios with 95% CI of arrhythmia and conduction disease. When the proportionality assumption was met, the model was tested with time-dependent covariates. Provided $p < 0.05$, multivariable models were conducted to adjust for age at the cardiac exam (continuous variable) and comorbidity. Analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC), SPSS v.20, and Prism version 7 (GraphPad Software Inc., San Diego, CA). A p value < 0.05 was considered statistically significant.

RESULTS

Genotypic and Clinical Characteristics

The main characteristics of the study population are reported in Supplemental Table 1. The majority of patients first underwent hot spot screening for codon 482 of the *LMNA* gene followed by targeted sequencing of genes of interest, and whole-exome sequencing (WES) if the previous studies were negative. Seventy-one patients (58%) had pathogenic variants in known lipodystrophy genes (Supplemental Table 2). *LMNA* pathogenic variants were found in 60 patients. Eight patients had pathogenic or likely pathogenic variants in the *PPARG* gene. Also, pathogenic variants were confirmed in the *POLD1* (2 patients), and *MFN2* (1 patient) genes. WES identified variants of unknown significance (VUS) in novel genes in 15 patients. We were not able to identify any pathogenic variant or VUS in 19 patients. The genetic characterization was incomplete in 17 patients with no causative genetic mutation for lipodystrophy on targeted clinical testing (none of them underwent WES). Of these 17 patients, 9 underwent sequencing of *LMNA* and *PPARG* genes, 7 were sequenced only for *LMNA* codons 10 and 482 and one patient had clinical lipodystrophy panel testing from the University of Chicago Genetics Laboratory that includes 13 genes associated with lipodystrophy.

Cardiac Phenotype

Table 1 shows the cardiac characteristics of our study population. The median (IQR) age at the cardiac exam was 46 years (34-55). Among 122 patients with FPLD, 95 (78%) were diagnosed with cardiac disease in their lifetime. Of those, 30 (25%) were diagnosed with ischemic heart disease, 45 (37%) with an arrhythmia, 20 (16%) with conduction disease, 24 (20%) with prolonged QT interval, 9 (7%) with axis deviation, and 13 (11%) with cardiomyopathy (CMP).

Of those with ischemic heart disease, 15 (12%) patients had balloon angioplasty, 16 (13%) had a cardiac stent, 16 (13%) had myocardial infarction, and 6 (5%) had a history of CABG. Of those with arrhythmia, 12 (10%) had atrial fibrillation or flutter, 10 (8%) PVCs, 7 (6%) PAC/SVPC, and 21 (17%) sinus arrhythmia. For those with CMP diagnosis, 7 (6%) patients had dilated CMP, 3 (3%) hypertrophic, 2 (2%) ischemic, and 1 (1%) postpartum CMP. Clinically, 18 (15%) patients developed congestive heart failure in their lifetime. Among the 90 patients who had an echocardiogram (74%), 29 (32%) had left ventricular hypertrophy, 21 (23%) had diastolic dysfunction, and 27 (30%) had valvular heart disease.

There were only 4 observed deaths caused by cardiac events during the study observation period. Sudden cardiac death occurred in a 41-year-old female with *LMNA* pathogenic variant R349W. The cause of death was myocardial infarction in a 72-year-old male with *LMNA* R482W variant and heart failure in 2 patients (a 58-year-old female with the *LMNA* pathogenic variant R62G and a 77-year-old male with *LMNA* pathogenic variant R482Q). Dual-chamber cardiac defibrillators were implanted in 5 patients (4 with non-482 *LMNA* pathogenic variants, and in another patient from the non-*LMNA* group).

Impact of glycemic control and BMI on cardiac disease presentation

Overall, ischemic heart disease was more prevalent among patients with HbA1c > 7.5% versus those with lower HbA1c [23 subjects (34.8%) vs. 6 subjects (10.9%); p = 0.003; one subject excluded as no HbA1c level was available at the time of cardiac evaluation]. Also, percutaneous coronary interventions [13 subjects (19.7%) vs. 3 subjects (5.5%), p = 0.030] were more commonly performed in the group with higher HbA1c. No significant association was found between BMI (adjusted for age) and cardiac events.

Presence of a pathogenic variant in LMNA gene versus other causes

The study cohort was stratified by genotype into two groups, those harboring pathogenic variants in *LMNA* (*LMNA* group, n = 60) vs. those that do not (non-*LMNA group*, n = 55) groups (Table 2). Seven patients were excluded from this analysis because they did not have complete gene sequencing for *LMNA* (they were only tested for 482 hotspot). Patients in the non-*LMNA* group had a significantly higher median BMI, glucose, HbA1c, triglycerides, total cholesterol, and non-HDL cholesterol levels than patients in the *LMNA* group. Their unfavorable metabolic profile was also reflected in medication use as patients in the non-*LMNA* group required significantly higher doses of insulin, more frequent use of concentrated insulin, and high dose statins (Supplemental Table 3). Leptin levels were lower in the *LMNA* group.

Although patients with *LMNA* variants had lower BMI, and better metabolic control than patients in the non-*LMNA* group, both groups had similar ratios of ischemic heart disease, myocardial infarction, or stroke events. On the other hand, patients with pathological *LMNA* variants had a higher prevalence of arrhythmia, specifically atrial fibrillation or flutter (Table 2). The odds ratio for arrhythmia and atrial fibrillation or flutter were higher in patients with *LMNA* pathogenic variants (Table 3). After adjusting for age at the cardiac exam, BMI, and comorbidities, the odds of arrhythmia and atrial fibrillation or flutter were still significantly higher among patients with *LMNA* variants. The median time to first recorded arrhythmic event from the diagnosis of lipodystrophy was significantly shorter for patients with *LMNA* variants compared to the non-*LMNA* group (Figure 1). The hazard rate of arrhythmia was 3.52 times (95% CI: 1.34 - 9.27) higher among patients with *LMNA* variants compared to those with no *LMNA* variant.

"Hot spot" versus other pathogenic LMNA variants

LMNA group was then subdivided according to the most frequent hotspot mutation site, exon 8 codon 482. Table 4 shows the characteristics of both groups. There was no difference in age, sex, BMI, presence of diabetes, hypertension, hepatic steatosis, and pancreatitis, lipid profiles, and HbA1c levels; however, patients with non-482 *LMNA* variants were more likely to have myocardial infarction, arrhythmia, atrial fibrillation/flutter, axis deviation, cardiomyopathy, and congestive heart failure. Among patients with *LMNA* pathogenic variants, those with non-482 codon variants had higher odds of arrhythmia, atrial fibrillation or flutter, and conduction abnormality. The higher risk persisted after adjusting for age at the cardiac exam (Table 3). Patients with non-482 *LMNA* pathogenic variants also had higher odds of arrhythmia and atrial fibrillation or flutter compared to the rest of the cohort (data not shown).

We also evaluated how patients with the hot spot variants compared to those with the non-*LMNA* genotypes. Premature ventricular contractions were more commonly observed in patients with codon 482 *LMNA* variants than in the group with no *LMNA* pathogenic variants (Supplemental Table 4). Numerically, patients with codon 482 *LMNA* variants had arrhythmia and atrial fibrillation/flutter at a higher rate, but these differences were not statistically significant. Additionally, BMI and leptin levels were lower in patients with codon 482 *LMNA* variants than those with no *LMNA* variants.

Clinical characteristics of the LMNA R349W variant

We had the opportunity to study the hiPSCs of a patient harboring a pathogenic variant (R349W) in the *LMNA* gene. The cell samples were obtained from this case when she was 39. This patient had multiple comorbidities including diabetes, hyperlipidemia, hypertension, hyperandrogenism, and NASH. She presented with a unique form of lipodystrophy at age 36, previously completely undiagnosed, with profound fat loss from her extremities, neck and face, and anterior trunk, but with increased fat deposition in the back and specifically with an exaggerated buffalo hump. She had acrolysis and underdeveloped breasts, but high circulating testosterone levels without aberrant signs of hirsutism. She developed progressive proteinuria while she was followed. She had history of infertility and had one pregnancy that resulted in fetal loss at 35 weeks. The patient's cardiac history included stent implantation for coronary artery disease, myocardial infarction (non-ST-elevation MI), cardiomyopathy, and conduction abnormality all of which were uncovered after we initiated cardiac workup for the observed variant. The patient had sudden cardiac death at age 41, 2 years after we obtained her skin biopsy.

We also had the opportunity to observe additional patients harboring the same mutation in an unrelated pedigree from Turkey and a completely unrelated male case originally from England. This latter patient with *LMNA* R349W from England had diabetes, hyperlipidemia, and hepatic steatosis. He was diagnosed with supraventricular tachycardia when he was 28 years old. He also had aortic stenosis. Other pertinent clinical features included anxiety, partial alopecia, and proteinuria.

The Turkish *LMNA* R349W pedigree had previously been reported [10]. Data from three living members of this pedigree are included in this study. In contrast to codon 482 *LMNA* variants, these patients had fat loss affecting the face and neck, and limb lipoatrophy was prominent distally. Paroxysmal atrial fibrillation/flutter was detected in the proband (42-year-old female) who later developed an episode of stroke. She had additional clinical features such as hearing loss, micrognathia, scoliosis, partial alopecia, skin atrophy, and proteinuria. Her son and daughter were also studied. Among these younger generation members, the older brother developed episodes of atrial tachycardia. There was further cardiac history in the family. Although not included in this analysis, her father's medical records indicate that he died at age 44 after being admitted with coronary artery disease and cardiac arrhythmias to the emergency room. Her brother died at age 33 due to heart failure. His medical records indicate that he had atrial fibrillation, severe mitral and tricuspid valve regurgitation, and pulmonary hypertension.

Cellular Electrophysiologic Characteristics in the cells harboring LMNA R349W variant

Functional syncytia of mature hiPSC-CMs from a patient with *LMNA* R349W variant were prepared for the study of action potential and rate of spontaneous activation.

Affected patient's cardiomyocytes had a higher frequency of spontaneous depolarizations (Figure 2A), and shorter uncorrected APD80% (action potential duration at 80% repolarization) at 1 Hz compared to control cardiomyocytes (Figure 2B). Action potential duration was adjusted with Fridericia correction formula because of differences in spontaneous depolarization rhythm and showed that corrected APD80% is shorter in the patient's cardiomyocytes than normal cardiomyocytes (Figure 2C). Furthermore, cardiomyocytes from the patient with *LMNA* R349W variant also demonstrated several rhythm alterations such as early afterdepolarizations, spontaneous quiescence, and spontaneous tachyarrhythmia (Supplemental data; movie 1); none of those were observed in the control cell lines (Figure 2D). Cardiomyocytes differentiated from hiPSC-CMs carrying the R349W variant presented heterogeneous rhythm of spontaneous repolarization varying from normal rhythm to Torsade de Pointes-like activation and quiescence. Therefore, we have obtained several recordings per functional mature syncytia to be able to document rhythm abnormalities in these cells (6 syncytia and 65 optical mapping recordings). We also have obtained multiple optical mapping recordings from control cardiomyocytes (6 syncytia and 17 optical mapping recordings). *LMNA* R349W cardiomyocytes had preserved chronotropic response to isoproterenol, although chronotropic changes were greater in control than affected cardiomyocytes. Additionally, the recovery rate was significantly lower in affected than in control cardiomyocytes (Figure 2E), which indicates disrupted beta-adrenergic response.

DISCUSSION

This multicenter study reveals a high prevalence of cardiac events in patients with FPLD that highlights the need for vigilant cardiac monitoring in FPLD, especially in patients with FPLD2 who exhibited a disproportionately higher risk of developing cardiac arrhythmias such as atrial fibrillation/atrial flutter. A study of hiPSC-CMs from a patient with *LMNA* pathological variant R349W showed further evidence regarding arrhythmogenic potential of the underlying genetic defect as evidenced by a higher frequency of autonomous activity, shorter action potential duration, slower recovery from chronotropic changes, and several rhythm alterations such as early after-depolarizations, spontaneous quiescence, and spontaneous tachyarrhythmia. Although patients with *LMNA* variants had lower BMI, and better metabolic control than non-*LMNA* patients, both groups had similar ratios of ischemic heart disease, myocardial infarction, or stroke events. Several factors may have contributed to high prevalence of metabolic

disturbances in the patients with no *LMNA* variants, which include, but are not limited to, diet, lifestyle, and higher BMI [21]. We want to note that a similar degree of ischemic disease in the group with *LMNA* variants highlights the predisposition of this group despite being in better metabolic state. Overall cardiac events, were more common in the *LMNA* group with a higher prevalence of arrhythmia, specifically atrial fibrillation or flutter, emphasizing the difference in cardiac phenotypes between the groups.

In addition to higher odds of arrhythmia and atrial fibrillation or flutter, the median time from lipodystrophy diagnosis to arrhythmia was significantly shorter for patients with *LMNA* variants. The association of the *LMNA* gene with cardiomyopathy and arrhythmia has been previously demonstrated in several studies [22,23]. It is known that these patients may require ICD as a result of high risk of high degree atrioventricular block and ventricular arrhythmias. On the other hand, cardiac manifestations of *LMNA* variants causing the lipodystrophy phenotype had been only occasionally reported [8,15,24-26], and not systematically studied except in a few clinical case series from France [13,27]. The rarity of disorders caused by the *LMNA* variants makes it difficult to phenotypically classify all variants scattered over the entire *LMNA* gene. Phenotypic heterogeneity is high among carriers of *LMNA* variants ranging from lipodystrophy to neuromuscular and cardiac disorders, and overlaps are sometimes observed [1]. The severity of cardiac phenotype can be different even in patients presenting with primary cardiac disease. For instance, Hoorntje et al. [28] reported that the *LMNA* p.R331G variant was associated with milder clinical events than other *LMNA* variants causing *LMNA*-related cardiac disease. One explanation for the rare reporting of cardiac events in patients with *LMNA* variants causing lipodystrophy might be the presence of relatively milder cardiac disease. Nevertheless, as shown in our study, an increased risk for specific cardiac events exists in patients with *LMNA* variants compared to other etiologies of FPLD; thus, rigorous evaluation and follow-up are required. Among these cardiac events, atrial fibrillation is a well-known cardiac disease to be associated with increased morbidity and mortality, in part due to the risk of thromboembolic disease, but further long-term studies are needed to confirm whether *LMNA* variants increase the risk of clinical outcomes due to atrial fibrillation in patients with FPLD.

It is still not clear how aberrant *LMNA* transcript leads to alterations in cardiac phenotype. Myocardial fibrosis has been previously proposed to be a responsible mechanism for both arrhythmogenesis and cardiomyopathy [29]. On the other hand, crucial cellular processes such as structural integrity of the nucleus, the structural integrity of the cell (via interactions between nuclear lamina, cytoskeleton, and extracellular matrix) and the stiffness of the cell, regulation of

gene expression, and cellular signaling pathways are known to be affected by pathogenic *LMNA* variants [30].

The protocol for differentiation of hiPSCs into cardiomyocytes generates a heterogeneous population of cardiac cells consisting of atrial-, nodal- and ventricular-like cardiomyocytes, and each individual syncytium has a natural pacemaker [31]. We found that this heterogeneous population of cardiomyocytes carrying a non-482 *LMNA* pathogenic variant causative of a lipodystrophy phenotype presented a wide array of arrhythmias, including EADs, quiescence, and tachyarrhythmias. Tachyarrhythmias and quiescence may indicate defective generation of pacemaker activity by nodal-like cells which are governed by the potassium inward funny current (I_f) and transient and long-acting calcium currents (I_{Ca}, T and I_{Ca}, L) [32]. Additionally, with respect to the disturbances in pacemaker activity that may be calcium-mediated, we have observed that studied cardiomyocytes have blunted recovery after an isoproterenol challenge indicating calcium overload or at least delay in the reestablishment of intracellular calcium concentration. Abnormal intracellular calcium concentration and shortened APD have been shown experimentally to induce cardiomyocyte early afterdepolarizations [33], and the presence of EADs in these cardiomyocytes may result from defective intracellular calcium handling as well. The nature of the disturbance in the calcium handling system in affected cardiomyocytes is yet to be determined and future studies should focus on the activity of SERCA2a, phospholamban and other proteins regulating calcium relocation into different intracellular compartments.

Similar to our observation, Kwapich et al. [13] recently reported more frequent sudden death and more frequent use of cardiac implantable electronic devices in non-482 than codon 482 *LMNA* pathogenic variant carriers. Also, non-482 *LMNA* pathogenic variant carriers had more abnormalities on electrocardiography, had greater frequencies of left atrial enlargement, and lower left ventricular ejection fractions than codon 482 pathogenic variant carriers. Although it remains unknown why patients with non-482 *LMNA* pathogenic variants were more likely to present with cardiac manifestations, overlapping progeroid characteristics (such as stiffness of the extracellular matrix or intracellular architecture abnormalities) might contribute to increased cardiac risk in these patients [1,10,34].

Our study has several limitations. First, the retrospective nature of the study makes it liable to unmeasured potential confounding factors. Although extensive efforts have been put into data collection, data were obtained from records, and availability was limited to what already exists. Second, only patients who had an available ECG in the records were included. This might distort the result if more ECGs were clinically indicated in one group compared to another though ECG can be considered a routine test in all patients with lipodystrophy. Sample

heterogeneity was another limiting factor. Patients were included from two countries. It is quite possible that patient characteristics might differ between the two countries. For example, Michigan patients had higher BMI, which could be due to the difference in diet in the US compared to the diet in Turkey. There were country-specific differences in treatment algorithms of patients with lipodystrophy and hyperlipidemia. Smoking is an important contributor to cardiac risk. The Turkish data set did not include any smoking history, so we could not account for smoking in this retrospective study. More than 90 percent of the Michigan patients are not current smokers and only a small percentage of the patients had substantial smoking history (more than 15 pack-years that would impact cardiac events). There were also differences in genetic testing algorithms. Besides, no formal family screening algorithm was performed in any of the centers. Nevertheless, lipodystrophy is a rare disease, and achieving the case numbers of this report was only possible with international collaboration. Finally, the hiPSC-CM study lacks an isogenic control to rule out factors related to genetic background which may have contributed to the arrhythmic phenotype. Another issue is that the study remains descriptive rather than mechanistic. We did not conduct a study of potential structural alterations in the hiPSC-CMs, including altered trafficking of ion channels which might have contributed to the differences between affected and control cells in the electrophysiological phenotype. Our studies in the induced cardiomyocytes are intended as preliminary and for proof of concept. Our data imply that these cells can be utilized to fully characterize the pathophysiology of arrhythmias and also for screening the best cardiac therapeutics in the future

In conclusion, our results highlight a high prevalence of cardiac events in patients with FPLD. Patients with FPLD2 caused by *LMNA* variants are at a higher risk for developing cardiac arrhythmias, especially atrial fibrillation or flutter. Patients with non-482 *LMNA* pathogenic variants are at further risk. For this reason, early diagnosis and providing adequate screening as well as appropriate interventions for cardiac and metabolic abnormalities is crucial to reduce the occurrence of cardiovascular events in this population. The association of the *LMNA* gene and arrhythmogenic potential is further supported by data from hiPSC-CMs that may help us better understand the mechanisms of the cardiac phenotype associated with the underlying genetic abnormalities and create precision therapy opportunities in the future.

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Conflict of interest

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Disclosures

The authors report following conflicts: BA has attended Scientific Advisory Board Meetings organized by Aegerion Pharmaceuticals (now Amryt Pharma) and Regeneron Pharmaceuticals, and has received honoraria as a speaker from AstraZeneca, Lilly, MSD, Novartis, Novo Nordisk, Boehringer-Ingelheim, Servier, and Sanofi-Aventis. BA has taken consulting fees from Amryt Pharma and has received writing support from Aegerion Pharmaceuticals (past) and Amryt Pharma (current) in unrelated manuscripts. EAO reports the following conflicts: Grant support: Aegerion Pharmaceuticals (now Amryt Pharma), Ionis Pharmaceuticals, Akcea Therapeutics, Gemphire Therapeutics, GI Dynamics (current), AstraZeneca (past two years). Consultant or Advisor: AstraZeneca, Akcea Therapeutics, Ionis Pharmaceuticals, Thera Therapeutics, and BMS (Past), Regeneron, Aegerion (now Amryt Pharma). Drug support: Aegerion Pharmaceuticals (now Amryt Pharma), Rhythm Pharmaceuticals, Regenereon (all current) and Akcea Therapeutics (past), other support: (specifically writing support in unrelated manuscripts) Aegerion Pharmaceuticals (now Amryt Pharma). AMR reports the following conflict: consultant for CARTOX (current).

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Tables

Table 1. Cardiovascular Manifestations

	Total (n = 122)	Michigan (n = 83)	Turkey (n = 39)	*P* value
Age when cardiac exam, y	46 (34-55)	46 (35-57)	44 (30-53)	0.284
Sex, Female	101 (83)	68 (82)	33 (85)	0.802
BMI, kg/m2	27.2 (22.6-32.4)	30 (24.1-34.4)	24.6 (20.6-26.2)	< 0.001
LMNA variant	60 (49)	33 (40)	27 (69)	*0.011
Any cardiac issue	95 (78)	69 (83)	26 (67)	0.060
Ischemic heart disease	30 (25)	19 (23)	11 (28)	0.653
Heart catheterization	35 (29)	23 (28)	12 (31)	0.830
Balloon angioplasty	15 (12)	7 (8)	8 (21)	0.077
Cardiac stent	16 (13)	8 (10)	8 (21)	0.148
Myocardial infarction	16 (13)	13 (16)	3 (8)	0.383
CABG	6 (5)	5 (6)	1 (3)	0.663
Stroke	11 (9)	9 (11)	2 (5)	0.500
PR interval, ms	156 (142-168)	158 (146-168)	153 (136-180)	0.234
QRS duration, ms	88 (82-98)	88 (82-98)	88 (80-102)	0.761
QTC, ms	442 (421-462)	445 (430-468)	429 (400-455)	0.001
Arrhythmia	45 (37)	36 (43)	9 (23)	0.044
Atrial fib/flutter	12 (10)	6 (7)	6 (15)	0.196
PVC	10 (8)	10 (12)	0	0.030
PAC/SVPC	7 (6)	5 (6)	2 (5)	1.000
Sinus arrhythmia	21 (17)	18 (22)	3(8)	0.072
Conduction abnormality	20 (16)	17 (20)	3 (8)	0.114
Prolonged QT	24 (20)	19 (23)	5 (13)	0.229
Axis deviation	9 (7)	6 (7)	3 (8)	1.000
Cardiomyopathy	13 (11)	9 (11)	4 (10)	1.000
Congestive heart failure	18 (15)	15 (18)	3 (8)	0.175
LV hypertrophy†	29 (32)	21 (40)	8 (21)	0.068
Diastolic dysfunction†	21 (23)	14 (27)	7 (18)	0.451
Valvular heart disease†	27 (30)	13 (25)	14 (36)	0.354
LVEF†, %	60 (60-65)	63 (57-65)	60 (60-65)	0.369

Values are median (interquartile range) or n (%). CABG indicates coronary artery bypass graft surgery; PVC, premature ventricular complex; PAC, premature atrial complex; SVPC, supraventricular premature complex; LVEF, left ventricular ejection fraction. †Echocardiogram is available in 91 patients (52 Michigan and 39 Turkey). *Seven patients with no complete gene sequencing for *LMNA* are excluded.

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Table 3. Cardiac Events by *LMNA* Pathogenic Variant

	Odds Ratio (95%CI); *p* value	***Odds Ratio (95%CI); *P* value**
LMNA variant compared to non-*LMNA*		
Arrhythmia	2.93 (1.29-6.64); 0.010	3.77 (1.45-9.83); 0.007
Atrial fibrillation/ Atrial flutter	5.30 (1.11-25.39); 0.037	5.78 (1.04-32.16); 0.045
Conduction abnormality	1.90 (0.70-5.17); 0.211	2.20 (0.71-6.85); 0.173
*Adjusted for age at cardiac exam, BMI, comorbidities. Sex distribution and diabetes were not included in the model since >85% are diabetics and >80% are females. In addition, sex distribution was not different between *LMNA* and non-*LMNA* group, and the sample size did not allow for extra parameters in the model. Seven patients with no complete gene sequencing for *LMNA* are excluded.		
	Odds Ratio (95%CI); *p* value	**†Odds Ratio (95%CI); *P* value**
Non-482 *LMNA variant compared to 482*		
Arrhythmia	3.37 (1.12-10.08); 0.030	4.74 (1.41-15.98); 0.012
Atrial fibrillation/ Atrial flutter	5.44 (1.24- 23.95); 0.025	17.67 (2.45-127.68); 0.004
Conduction abnormality	3.77 (1.05-13.57); 0.042	5.71 (1.37–23.76); 0.017

†Adjusted only for age at cardiac exam due to limited sample size. Seven patients with no complete gene sequencing for *LMNA* are excluded.

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Table 4. Clinical Characteristics and Codon 482 Variants of the *LMNA* Gene

	Non-482 variant (n = 22)	**482 variant (n = 38)**	***P* value**
Cardiac exam age, y	42 (31-49)	47 (33-57)	0.094
Sex, Female	17 (77)	31 (82)	0.744
BMI, kg/m2	22.9 (19.6-27.3)	24.8 (21.9-28.3)	0.123

Table 2. Clinical Characteristics and the *LMNA* Variant

	LMNA **(n = 60)**	**Non-*LMNA*** **(n = 55)**	***P* value**
Cardiac exam age, y	46 (33-54)	51 (36-57)	0.259
Sex, Female	48 (80)	46 (84)	0.638
BMI, kg/m2	24.7 (21.7-27.6)	31.5 (25.9-35.6)	<0.001
Diabetes Mellitus	50 (83)	52 (95)	0.078
Hypertension	42 (70)	36 (66)	0.691
Pancreatitis	12 (20)	20 (36)	0.062
Glucose, mg/dL	123 (93-181)	165 (128-239)	0.006
HbA1c, %	7.0 (6.0- 8.6)	8.3 (7.1-9.2)	0.009
Triglycerides, mg/dL	279 (174-485)	342 (246-896)	0.038
Total cholesterol, mg/dL	193 (160-228)	226 (174-293)	0.007
LDL cholesterol, mg/dL	93 (64-129)	106 (65-145)	0.367
HDL cholesterol, mg/dL	38 (31-44)	33 (29-42)	0.138
Non-HDL cholesterol, mg/dL	149 (116-192)	187 (138-254)	0.003
Leptin†, ng/mL	3.2 (1.5-7.78)	12 (5.29-18.50)	<0.001
Ischemic heart disease	14 (23)	16 (29)	0.528
Stroke	4 (7)	7 (13)	0.348
Arrhythmia	27 (45)	12 (22)	0.011
Atrial fib/flutter	10 (17)	2 (4)	0.031
PVC	7 (12)	1 (2)	0.063
PAC/SVPC	5 (8)	2 (4)	0.442
Sinus arrhythmia	10 (17)	7 (13)	0.607
Conduction abnormality	13 (22)	7 (13)	0.229
Axis deviation	6 (10)	3 (6)	0.494
Prolonged QT	13 (22)	10 (18)	0.816
Cardiomyopathy	10 (17)	3 (6)	0.078
Congestive heart failure	11 (18)	7 (13)	0.451
LV hypertrophy‡	13 (27)	16 (40)	0.256
Diastolic dysfunction‡	9 (18)	12 (30)	0.219
Valvular heart disease‡	18 (37)	9 (23)	0.170

Values are median (interquartile range) or n (%). BMI indicates body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; HbA1c, hemoglobin A1c; PVC, premature ventricular complex; PAC, premature atrial complex; SVPC, supraventricular premature complex. †Leptin levels are before metreleptin treatment

Diabetes Mellitus	19 (86)	31 (82)	0.732

closest to cardiac evaluation. ‡Echocardiogram is available in 88 patients (48 *LMNA* and 40 non-*LMNA*). Seven patients with no complete gene sequencing for *LMNA* are excluded.

Hypertension	15 (68)	27 (71)	1.000
Pancreatitis	6 (27)	6 (16)	0.327
Glucose, mg/dL	114 (93-171)	142 (101-184)	0.193
HbA1c, %	6.8 (5.8-8.1)	7.7 (6.0-8.7)	0.337
Triglycerides, mg/dL	190 (126-410)	323 (196-485)	0.079
Total cholesterol, mg/dL	202 (159-268)	193 (160-226)	0.544
LDL cholesterol, mg/dL	96 (75-130)	93 (63-125)	0.520
HDL cholesterol, mg/dL	39 (35-45)	36 (30-44)	0.167
Non-HDL cholesterol, mg/dL	149 (116-207)	149 (116-187)	0.713
Leptin†, ng/mL	4.2 (1.5 -8.5)	3.1 (1.4-7.4)	0.773
Ischemic heart disease	6 (27)	8 (21)	0.753
Myocardial infarction	5 (23)	1 (3)	0.021
Stroke	2 (9)	2 (5)	0.619
Arrhythmia	14 (64)	13 (34)	0.034
Atrial fib/flutter	7 (32)	3 (8)	0.029
PVC	2 (9)	5 (13)	1.000
PAC/SVPC	3 (14)	2 (5)	0.346
Sinus arrhythmia	4 (18)	6 (16)	1.000
Conduction abnormality	8 (36)	5 (13)	0.052
Axis deviation	5 (23)	1 (3)	0.021
Prolonged QT	6 (27)	7 (18)	0.520
Cardiomyopathy	8 (36)	2 (5)	0.003
Congestive heart failure	8 (36)	3 (8)	0.012
LV hypertrophy‡	3 (17)	10 (32)	0.282
Diastolic dysfunction‡	5 (28)	4 (13)	0.400
Valvular heart disease‡	10 (56)	10 (33)	0.441

Values are median (interquartile range) or n (%). †Leptin levels are before metreleptin treatment closest to cardiac evaluation. BMI indicates body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; HbA1c, hemoglobin A1c; PVC, premature ventricular complex; PAC, premature atrial complex; SVPC, supraventricular premature complex. ‡Echocardiogram is available in 48 patients (18 with non-codon 482 variant and 30 patients with codon 482 variant).

FIGURE LEGENDS

Figure 1: Kaplan-Meier survival curve showing arrhythmia for *LMNA* and *non-LMNA patients*.

Figure 2: Functional syncytia of mature human induced pluripotent stem cell-derived cardiomyocytes from a patient carrying a variant (*LMNA* R349W) causative of familial partial lipodystrophy (FPLD) were submitted to optical mapping for assessment of membrane voltage changes.

(A) *LMNA* mutant cardiomyocytes had a higher frequency of spontaneous depolarization in relation to control cell line (MCH) ($p < 0.001$).

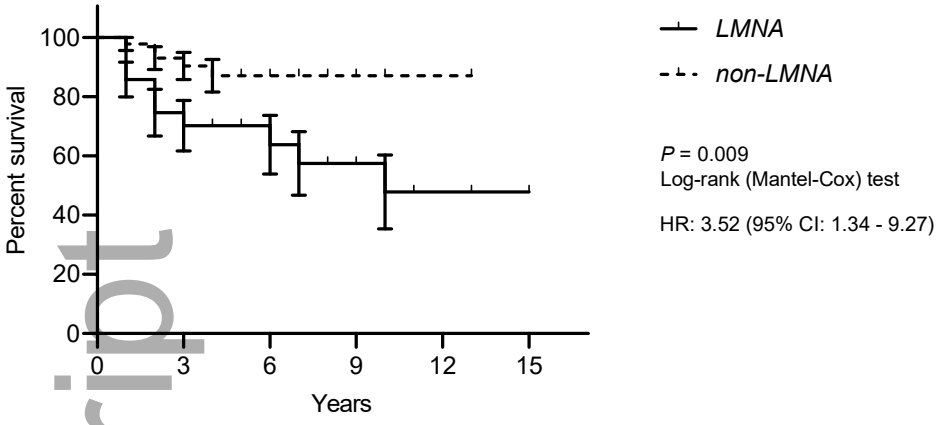
(B) APD80% of repolarization was shorter in cells carrying the *LMNA* variant.

(C) Duration of spontaneous action potential duration at 80% of repolarization was adjusted to the frequency of spontaneous depolarization with Fridericia correction and showed that *LMNA* mutant cardiomyocytes presented shorter corrected APD80% compared to control cardiomyocytes ($p < 0.001$).

(D) Additionally, *LMNA* mutant cardiomyocytes presented several rhythm alterations (red arrows) such as early afterdepolarizations, spontaneous quiescence and spontaneous tachyarrhythmia; none of those were observed in the control cell lines.

(E) Finally, both control and *LMNA* mutant cardiomyocytes showed a positive chronotropic response to isoproterenol. Nevertheless, isoproterenol recovery rate was significantly lower in the *LMNA* mutant cardiomyocytes.

Figure 1: Kaplan-Meier survival curve showing arrhythmia for *LMNA* and non-*LMNA* patients.



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