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Original article

Cardiometabolic assessment of *lamin A/C* gene mutation carriers: A phenotype–genotype correlation

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ABSTRACT

Aims. – Mutations of the *LMNA* gene encoding lamin A/C induce heterogeneous phenotypes ranging from cardiopathies and myopathies to lipodystrophies. The aim of this study was to compare cardiometabolic complications in patients with heterozygous *LMNA* mutations at the 482nd codon, the 'hotspot' for partial lipodystrophy, with carriers of other, non-R482 *LMNA* mutations.
Methods and results. – This study included 29 patients with R482 *LMNA* mutations, 29 carriers of non-R482 *LMNA* mutation and 19 control subjects. Cardiac and metabolic phenotypes were compared between groups. A family history of either cardiac implantable electronic devices (CIEDs; $P < 0.001$) or sudden death ($P < 0.01$) was more frequent in non-R482 than R482 carriers. The non-R482 carriers also had more abnormalities on electrocardiography and received CIEDs more often than R482 carriers ($P < 0.001$). On cardiac ultrasound, non-R482 patients had greater frequencies of left atrial enlargement ($P < 0.05$) and lower left ventricular ejection fractions ($P < 0.01$) than R482 carriers. In contrast, R482 carriers had lower BMI ($P < 0.05$), leptin ($P < 0.01$) and fat mass ($P < 0.001$), but higher intra-/total abdominal fat-mass ratios ($P < 0.001$) and prevalences of diabetes ($P < 0.01$) and hypertriglyceridaemia ($P < 0.05$) than non-R482 carriers, with a trend towards more coronary artery disease. However, non-R482 carriers had higher intra-/total abdominal fat-mass ratios ($P < 0.02$) and prevalences of diabetes ($P < 0.001$) and hypertriglyceridaemia ($P < 0.05$) than the controls.
Conclusion. – Non-R482 carriers present more frequently with arrhythmias than R482 carriers, who twice as often have diabetes, suggesting that follow-up for laminopathies could be adjusted for genotype. Non-R482 mutations require ultra-specialized cardiac follow-up, and coronary artery disease should not be overlooked. Although overlapping phenotypes are found, *LMNA* mutations essentially lead to tissue-specific diseases, favouring genotype-specific pathophysiological mechanisms.

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Introduction

Lamin A/C gene mutations are associated with heterogeneous phenotypes ranging from cardiomyopathies with or without muscular dystrophies to lipodystrophies—collectively referred to as 'laminopathies' [1,2]. The *LMNA* gene encodes A-type lamins (lamins A/C), members of the intermediate filament protein family that are required for nuclear lamina formation. Recent studies have suggested that *LMNA* mutations affect epigenetic regulation of developmental pathways [3–5]. *Lamin A/C* gene mutations have been described over the entire gene. Nevertheless, the rarity of the disease makes phenotype–genotype correlation difficult. Familial partial lipodystrophy type 2 (FPLD2) is characterized by progressive fat loss in the extremities at puberty, associated with severe insulin resistance, diabetes and hypertriglyceridaemia with muscle hypertrophy [6,7]. FPLD2 is related to heterozygous *LMNA* gene mutations, especially those at the 'hotspot' codon Arg482 (R482) in exon 8.

The severity of *LMNA*-related cardiomyopathies has been demonstrated in several studies showing frequent atrioventricular blocks and ventricular arrhythmias often requiring cardiac implantable electronic devices (CIEDs), as well as severe heart failure, especially in the context of dilated cardiomyopathy [8–23]. Recommendations have already been given concerning the need for implanting defibrillators rather than pacemakers in laminopathies with cardiac involvement [24,25]. However, some of these reports could have been biased by exclusive cardiac recruitment [23]. Indeed, patients with the R482 genotype, whose metabolic phenotype is well described, have rarely been reported to have primary cardiomyopathies, although ischaemic heart disease might be more prevalent [26,27]. Nevertheless, unlike the cardiovascular phenotype, the metabolic phenotype of cardiac laminopathies remains largely unknown.

Thus, the aim of the present study was to compare cardiometabolic complications in patients with *LMNA* mutations at R482, the hotspot for FPLD2, with those in carriers of other *LMNA* mutations.

Patients and methods

Study design

This retrospective longitudinal study was conducted at one university hospital over a 15-year period (from 2000 to 2015). All patients referred to the institution's endocrinology and metabolism department with a diagnosis of laminopathy were included. Patients were classified into two groups according to genotype:

- those with the heterozygous *LMNA* R482 mutation;
- those with other lamin A/C mutations. The cardiometabolic phenotypes in these two groups were then compared, while the metabolic phenotypes in these two groups were further compared with a control group.

Patients

A total of 157 patients were referred because of suspected laminopathy by either endocrinologists who suspected lipodystrophic syndromes, or by cardiologists or geneticists because of unexplained cardiac abnormalities or familial screening. After subjecting these patients to careful clinical and biological examinations, and obtaining their written informed consent, their LMNA genes were studied by direct sequencing. Clinical and biological data were collected from patients' medical files. In addition to gender, their age at the time of their first and last cardiometabolic evaluations were recorded for calculation of the average follow-up duration. In addition, 19 healthy subjects matched for age and gender, and recruited from the PHRC-Clin.gov2009-AO-1169-48 trial, served as the control group for metabolic assessment.

Cardiac outcomes

The following cardiac parameters were collected from the patients' medical files:

- family history of CIED or sudden death, considered positive for any patient with at least one first-degree relative with an implanted device or who had died suddenly;
- abnormal electrocardiography (ECG), defined as the presence of at least one of the following during follow-up: atrial fibrillation, atrial flutter, high-grade atrioventricular (AV) block, ventricular ectopy, or complete left bundle branch block;
- abnormal ECG on Holter monitoring, defined as the presence of non-sustained (NSVT) or sustained ventricular tachycardia (VT), high-grade AV block, or atrial flutter or atrial fibrillation;
- use of a CIED, defined as a pacemaker or automatic implantable cardiac defibrillator (ICD) acquired during follow-up (before or after diagnosis of the *LMNA* mutation);
- CIED interrogation, when ventricular rhythm disorders correspond to the presence of at least one NSVT or VT episode retrieved from stored electrograms during scheduled follow-up visits;
- abnormalities on echocardiography, defined by the presence of at least one of the following as per international guidelines for echocardiography measurements: left ventricular ejection fraction (LVEF) $< 50\%$; left atrial enlargement; and/or LV hypertrophy or LV enlargement [28]; patients with non-ischaemic heart disease (cardiomyopathies), defined by LV dilatation and/or altered LVEF, were also noted, and LV diastolic function was assessed by E/A ratio if available;
- smoking status, recorded as either currently active or over the past 3 years;
- screening for ischaemic heart disease, which included myocardial ischaemia screening with an exercise stress test in five patients with R482 and six patients with non-R482 mutations, radionuclide angiography (six R482, one non-R482) or dobutamine stress echocardiography (two R482); and coronary angiography in four patients because of positive non-invasive screening or acute coronary syndrome (two patients);
- screening for atheromatosis by Doppler ultrasound of the carotid and lower-limb arteries.

Metabolic outcomes

The following metabolic parameters were also collected from patients' medical records of the last endocrinological evaluation or, for the control group, from the PHRC-Clin.gov2009-AO-1169-48 database:

- body mass index (BMI), assessment of fasting blood glucose (FBG) and triglycerides, as measured by routine techniques, and fasting C-peptide and leptin using radioimmunoassay [RIA-coat C-peptide (Mallinckrodt France SARL, Paris, France), detection limit: 0.2 ng/mL] and Human Leptin RIA kits (EMD Millipore Corporation, Burlington, MA, USA; normal range in normal-weight subjects: women 7.4 ± 3.7 ng/mL, men 3.8 ± 1.8 ng/mL), respectively;
- diabetes and glucose intolerance by subjecting participants not already being treated for diabetes at inclusion to a 75-g Oral

Glucose Tolerance Test (OGTT), which was interpreted according to American Diabetes Association criteria;
- hypertension, defined as blood pressure > 140/90 mmHg or use of an antihypertensive drug;
- use of lipid-lowering agents (such as statins, fibrates, ezetimib);
- use of antidiabetic treatments (such as metformin or any other antidiabetic drugs, including glucagon-like peptide-1 receptor agonists and insulin);
- body fat-mass percentage, as measured by dual-energy X-ray absorptiometry (DXA; Lunar DPX-IQ, GE Healthcare, Chicago, IL, USA);
- ratio of intra-abdominal/total abdominal fat mass, as calculated by measurement of subcutaneous and visceral fat surface areas from 1-cm reconstructed slices of abdominal L4 magnetic resonance imaging (MRI), which is contraindicated in cases of CIED and was therefore only performed in 22 R482 and 12 non-R482 patients.

Statistical analysis

Qualitative variables, expressed in absolute numbers and percentages, were compared by Chi^2 or Fisher's exact test for values < 5. For quantitative variables, the Shapiro–Wilk test for normality was performed, with results expressed as medians with interquartile ranges (Q25–Q75), and compared using the Mann–Whitney test. Analyses were carried out with GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA). Any differences with *P*-values < 0.05 were considered significant.

Results

Description of the two groups

In all, 60 patients carried *LMNA* mutations; however, as two were excluded because their medical files were incomplete, 58 patients were ultimately included in this study: 29 patients from eight families carried the heterozygous R482W mutation, and 29 patients from 16 families carried another heterozygous *LMNA* mutation.

The gender ratio between the *LMNA* R482 and non-R482 groups did not differ, although the number of women tended to be higher in the R482 group (22 vs. 16; *P* = 0.09). There were also no differences between the two groups in terms of age at first evaluation [R482: 43 (24–50.5) years vs. non-R482: 39 (28.2–47.5) years; *P* = 0.9] and duration of follow-up [R482: 5 (2–11) years vs. non-R482: 4 (1–9) years; *P* = 0.42]. These patients' genotypes and their main phenotypic features are presented in Table 1, and their diagnostic circumstances are depicted in Fig. 1. The proportion of diabetes patients in the R482 group increased during the follow-up period from 11 to 24 out of 29 patients (from 37.9% to 82.7%), and from 6 to 12 out of 29 patients (from 20.7% to 41.4%) in the non-R482 group, as determined at the last evaluation.

Table 1
Main characteristics of the R482 and non-R482 mutation groups.

	Families (*n*)	Patients (*n*)	ITG or diabetes (*n*)	Personal CIED use (*n*)	Specific features (*n*)	Variant dbSNP ID or reference
R482 group	**8**	**29**	**24**	**1**	**29 FPLD**	
c.1444C>T p.(Arg482Trp)	6	23	19	0	1 severe myopathy	rs57920071
c.1445G>A p.(Arg482Gln)	2	6	5	1		rs11575937
Non-R482 group	**16**	**29**	**12**	**16**		
c.139G>T p.(Asp47Tyr)		1	1	1	1 CGL + progeria	[1,2]
c.310C>G p.(Leu104Val)		1	0	1	1 FPLD	NPR
c.398G>T p.(Arg133Leu)		2	1	1	2 CGL + progeria	rs60864230
c.448A>G p.(Thr150Ala)		4	2	2	1 NIHD	rs58917027
c.467G>A p.(Arg156His)		1	0	0	1 FPLD	rs764475194
c.481G>A p.(Glu161Lys)		4	0	1	1 NIHD	rs28933093
c.694G>C p.(Gly232Arg)		1	1	0	1 CGL + myopathy	rs267607609
c.751C>T p.(Gln251*)		1	0	1	1 NIHD	NPR
c.860del p.(Ala287Valfs*193)		1	1	1	1 FPLD	NPR
c.949G>A (p.Glu317Lys)		3	1	2	1 NIHD	rs56816490
c.1157G>C p.(Arg386Thr)		2	1	1	1 FPLD	rs267607545
c.1173dup p.(Ser392Glnfs*34)		3	1	3	1 NIHD	NPR
c.1238del p.(Gly413Alafs*67)		1	1	1	1 FPLD + myopathy	NPR
c.1315C>T p.(Arg439Cys)		1	0	0	FPLD	rs62636506
c.1357C>T p.(Arg453Trp)		1	1	1	Severe myopathy	rs58932704
c.1930C>T p.(Arg644Cys)		2	1	0	1 FPLD	rs142000963

[1] Caron et al., Cell Death Differ 2007;14(10):1759–67; [2] Todorovic et al., Horm Res 2012;78(Suppl. 1):164.
ITG: glucose intolerance; CIED: cardiac implantable electronic device; dbSNP ID: Single Nucleotide Polymorphism Database identification number; CGL: congenital generalized lipodystrophy; FPLD: familial partial lipodystrophy; NPR: never previously reported; NIHD: non-ischaemic heart disease.

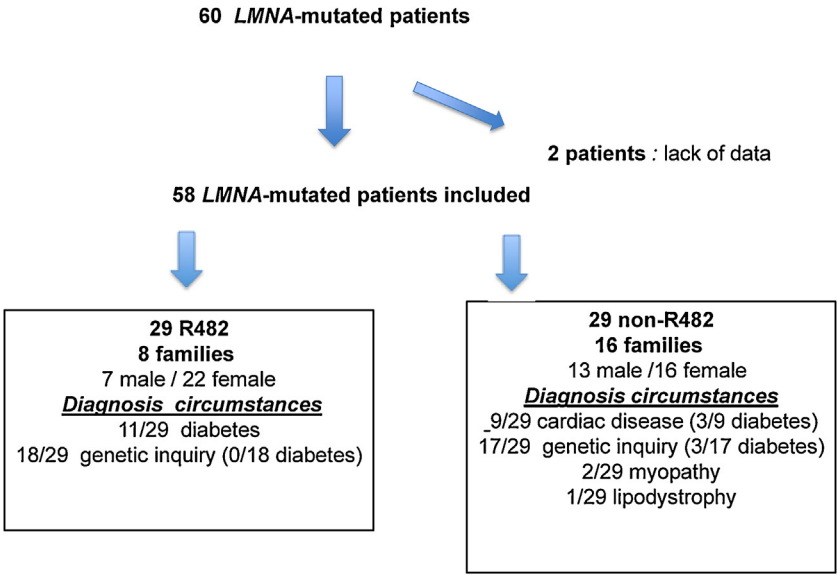

Fig. 1. Flow chart of patient recruitment for the study.

Cardiovascular phenotype

The frequency of a family history of sudden death ($P < 0.01$) or CIED use ($P < 0.001$) was significantly lower in the R482 vs. non-R482 mutation groups, as was the frequency of abnormal ECG ($P < 0.001$) (Table 2, Fig. 2A). The frequency of abnormal Holter monitoring also differed significantly between the two groups, with a greater frequency of abnormalities, especially NSVT, in the non-R482 mutation group compared with the R482 group ($P < 0.01$).

In addition, the frequency of implantable CIEDs was significantly lower in R482 vs. non-R482 mutation patients ($P < 0.001$). More specifically, the only patient in the R482 group (R482Q mutation) with a CIED, a pacemaker implanted at age 46, died at age 60 after several years of dialysis. In the non-R482 group, there were 16 CIEDs, including eight pacemakers, seven of which were

Table 2
Cardiac phenotype: comparison of R482 and non-R482 mutation groups.

	R482 (n = 29)	Non-R482 (n = 29)	P
Arrhythmia disorders			
Familial history of sudden cardiac death, n/N (%)	3/29 (10.3%)	13/29 (44.8%)	< 0.01
Familial history of CIED, n/N (%)	1/29 (3.4%)	16/29 (55.2%)	< 0.001
Abnormal ECG n^1/N (%)	2/29 (6.9%)	15/29 (51.7%)	< 0.001
Abnormal Holter ECG, n/n^1 (%)	0/16 (0%)	9/26 (35.6%)	< 0.01
CIED, n/N (%)	1/29 (3.4%)	16/29 (55.2%)	< 0.001
Type of CIED	1 PM	1 PM, 15 Defib	
VT episodes	0 recorded	7/16	
Abnormal/performed echocardiography, n/n^2 (%)	3/19 (15.8%)	10/26 (38.5%)	0.18
Atrial enlargement, n/n^2 (%)	1/19 (5.3%)	9/26 (34.6%)	< 0.05
LV ejection fraction, median (%) [IQR]	67.5 [61.2–70]	56.5 [45–62.7]	< 0.01
LV hypertrophy, n/n^2 (%)	2/19 (10.5%)	2/26 (7.7%)	1
LV diastolic diameter, median (mm) [IQR]	50 [43–54]	51 [44–54]	0.76
Number of E/A < 1/N E/A measured	4/7 (57%)	0/9 (0%)	–
Number of E/A > 2/N E/A measured	0/7 (0%)	3/9 (33%)	–
Cardiac ischaemic disorders			
Smokers, n/N (%)	4/29 (13.8%)	8/29 (27.6%)	0.33
Screening test for myocardial ischaemia, n3/N (%)	13/29 (45%)	7/29 (24%)	0.09
Abnormal test, n/n^3 (%)	5/13 (38%)	0/7 (0%)	0.11
Coronarography, n^4/N (%)	4/29 (14%)	2/29 (7%)	0.67
Significant coronary stenosis, n/n^4	4/4 (2 ACS)	0/2	0.06
Angioplasty/stenting, n/N (%)	2/29 (7%)	0/29 (0%)	0.46
Death during follow-up, n/N (%)	3/29 (10.3%) 1 Parkinson's disease 1 myopathy 1 dialysis + PM	3/29 (10.3%) 2 cardiac failure 1 dialysis + PM	1
Peripheral atheromatosis			
Lower-limb vascular Doppler US, n^5/N (%)	15/29 (52%)	11/29 (38%)	0.29
Atheromatosis, n/n^5 (%)	6/15 (40%)	5/11 (45%)	0.78
Carotid Doppler US, n^6/N (%)	15/29 (52%)	11/29 (38%)	0.29
Atheromatosis, n/n^6 (%)	4/15 (26.6%)	5/11 (45%)	0.42

n: number of cases; N: number of investigated patients; $^{1, 2, 3, 4, 5, 6}$ refer to the number of cases performed in each category.
CIED: cardiac implantable electronic device; ECG: electrocardiography; PM: pacemaker; Defib: defibrillator; VT: ventricular tachycardia; LV: left ventricular; IQR: interquartile range (Q25–Q75); ACS: acute coronary syndrome; US: ultrasound.

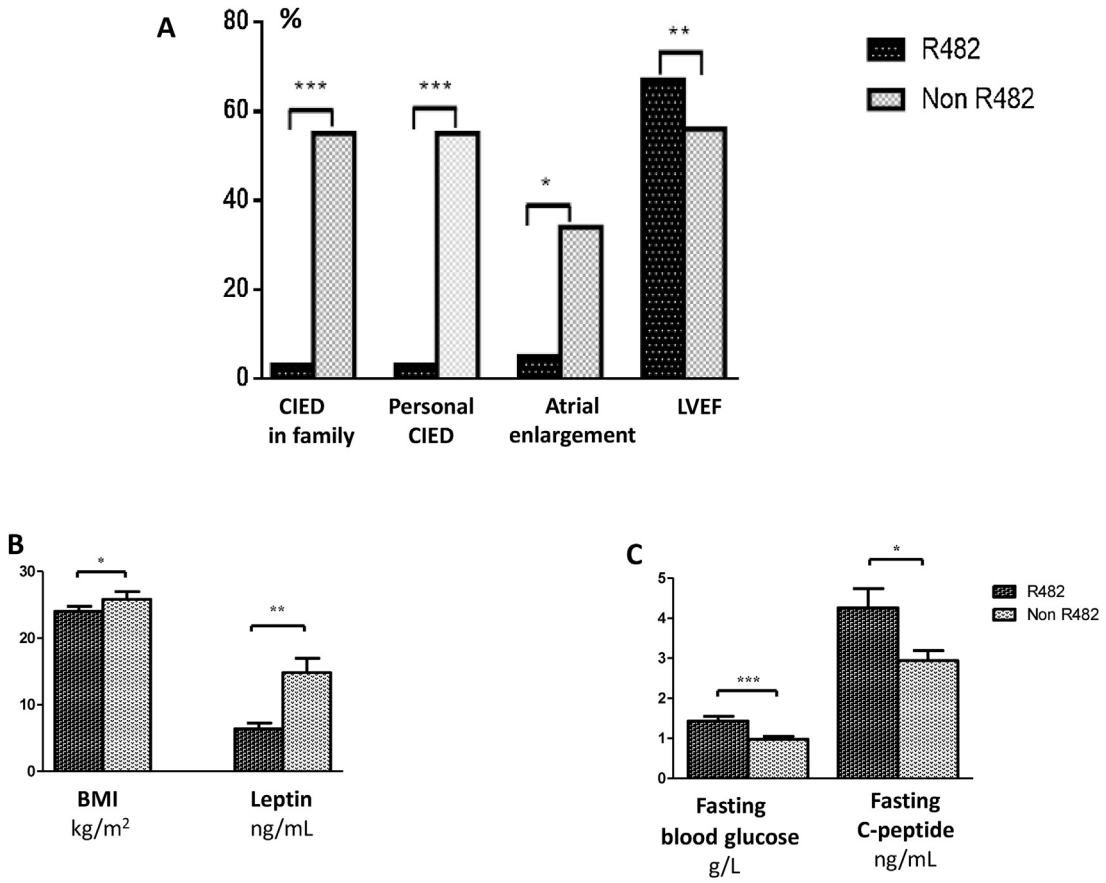

Fig. 2. Main differences between R482 and non-R482 groups in cardiac and metabolic outcomes. CIED: cardiac implantable electronic device; LVEF: left ventricular ejection fraction; BMI: body mass index; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

later upgraded to defibrillators when their indication for cardiac laminopathies was standardized [23], and eight defibrillators as first-line devices. The only remaining non-R482 patient with a pacemaker, implanted at age 33, developed end-stage renal disease (ESRD) and died suddenly during dialysis at age 50, just before genetic results were obtained. Seven VT episodes were recorded by CIEDs in seven different non-R482 patients.

There was no difference in frequency of cardiac ultrasound abnormalities between the two patient groups ($P = 0.18$). Nevertheless, the frequency of left atrial enlargement was significantly lower in patients with R482 vs. non-R482 mutations ($P < 0.05$), and median LVEF was significantly higher in patients with R482 vs. non-R482 mutations ($P < 0.01$), but with no difference in LV hypertrophy ($P = 1.00$) or LV diastolic diameter ($P = 0.76$). Five patients, all in the non-R482 group, had a cardiac phenotype of non-ischaemic heart disease. Of the remaining population, 16 (seven R482 and nine non-R482 carriers) had normal systolic function and E/A measurements. In the R482 group, 4/7 had impaired relaxation (E/A < 1) vs. none in the non-R482 group whereas, in the non-R482 group, 3/9 had restrictive filling patterns (E/A > 2) vs. none in the R482 group. The rate of deaths during the follow-up period was similar in both groups ($P = 1.00$), but was more often related to cardiac causes in the non-R482 group. ESRD related to long-term severely insulin-resistant diabetes worsened the prognosis in two cases.

There was no difference in the number of smokers ($P = 0.33$) between the two groups. Also, no patient in the non-R482 group was tested positive for cardiac ischaemia or significant coronary stenosis, which contrasted with positive diagnoses in 38% and 14% of patients, respectively, with R482 mutations. However, screening tests for cardiac ischaemia tended to be performed more often ($P = 0.09$) in patients with R482 mutations, who more often had diabetes ($P < 0.01$) than in the non-R482 patients. Comparison of peripheral atherosclerotic features showed no differences between the two groups (lower limb: $P = 0.78$; carotid atheromatosis: $P = 0.42$).

Metabolic phenotype

BMI ($P < 0.05$), blood leptin ($P < 0.01$) and high-density lipoprotein (HDL) cholesterol levels ($P < 0.05$) and fat-mass percentages ($P < 0.001$) were all significantly lower in patients with R482 vs. non-R482 mutations (Table 3, Fig. 2B and C). In contrast, median fasting C-peptide ($P < 0.05$) and glucose (< 0.001) levels, intra-abdominal/total abdominal fat-mass ratio ($P < 0.001$), and frequencies of diabetes or glucose intolerance ($P < 0.01$), metformin treatment ($P < 0.01$) and hypertriglyceridaemia ($P < 0.05$) were all significantly higher in patients with R482 vs. non-R482 mutations. There were no differences between the two groups in rates of hypertension ($P = 0.11$) or in median triglyceride ($P = 0.15$), total cholesterol ($P = 0.73$) and low-density lipoprotein (LDL) cholesterol ($P = 0.82$) levels, or use of lipid-lowering ($P = 0.10$) or antidiabetic treatments other than metformin ($P = 0.24$), including insulin ($P = 0.14$).

Comparison of the two *LMNA* mutation groups to an age- and gender-matched healthy control group revealed that BMI ($P < 0.02$), intra-/total abdominal fat-mass ratio ($P < 0.05$; Fig. S1; see supplementary materials associated with this article online), leptin levels ($P < 0.01$), frequency of hypertriglyceridaemia ($P < 0.05$) and diabetes or glucose intolerance ($P < 0.001$) were significantly higher, whereas HDL levels ($P < 0.02$) were significantly lower, in the non-R482 group compared with the

Table 3
Metabolic phenotype: R482 and non-R482 mutation groups compared with age- and gender-matched healthy controls.

	R482 (n = 29)	Non-R482 (n = 29)	P (R482 vs. non-R482)	Controls (n = 19)	P (controls vs. R482 and/or non-R482)
Body mass index, kg/m^2	24 [22–27]	27 [22–29]	< **0.05**	22 [21–24]	< **0.02** vs. non-R482
Diabetes or glucose intolerance, n/N (%)	24/29 (82.7%)	12/29 (41.4%)	< **0.01**	0/19 (0%)	**0.001** vs. R482 and non-R482
Hypertension, n/N (%)	17/29 (58.6%)	11/29 (37.9%)	0.11	0/19 (0%)	–
HDL cholesterol, mg/dL	0.4 [0.3–0.4]	0.48 [0.4–0.5]	< **0.05**	0.63 [0.45–0.74]	< **0.02** vs. R482 and non-R482
Triglyceride > 150 mg/dL, n/N (%)	16/29 (55.2%)	8/29 (27.6%)	< **0.05**	0/19	< **0.05** vs. R482 and non-R482
Metformin, n/N (%)	17/29 (58.6%)	7/29 (24%)	< **0.01**	0/19 (0%)	–
Lipid-lowering treatment, n/N (%)	14/29 (48.3%)	8/29 (27.6%)	0.10	0/19 (0%)	–
Fat mass, %	20 [17.7–22.8]	29.7 [18.7–38.1]	< **0.001**	22 [20–30]	< **0.01** vs. R482, 0.07 vs. non-R482
Intra-/total abdominal fat-mass ratio	0.59 [0.47–0.67]	0.36 [0.22–0.45]	< **0.001**	0.20 [0.11–0.30]	< **0.001** vs. R482, < **0.02** vs. non-R482
Leptin, ng/mL	5.2 [2.8–8.0]	15.9 [5.2–22.3]	< **0.01**	4.6 [4.1–10.7]	< **0.01** vs. non-R482

Data are expressed as medians [Q25–Q75] unless otherwise indicated.
HDL: high-density lipoprotein. Bold characters are present on significant comparisons.

controls. Similarly, intra-/total abdominal fat-mass ratio ($P < 0.001$; Fig. S1) and frequency of hypertriglyceridemia ($P < 0.05$) and diabetes or glucose intolerance ($P < 0.001$) were significantly higher, but HDL level ($P < 0.02$) and fat-mass percentage significantly lower, in the R482 vs. the control group.

Discussion

This large-scale, single-centre, *LMNA* mutation cohort comparing cardiometabolic complications in patients with R482 mutations with carriers of other *LMNA* mutations showed more arrhythmias in the non-R482 than R482 group, which tended to have a greater frequency of coronary artery disease. On the other hand, the rate of diabetes in the non-R482 group reached 40%, and was twofold higher in the R482 group. Each group also showed higher intra-/total abdominal fat-mass ratios, a hallmark of lipodystrophy, than the control group.

Recognized only since 1999, laminopathies are rare diseases with multiple phenotypes, some of which overlap [1]. Yet, despite their rarity, nearly 60 patients from 24 families were identified at our centre alone, with a greater number of families with non-R482 than R482 mutations (16 vs. 8 patients, respectively). As the northern region of France, where our patients were from, has four million inhabitants, the prevalence of patients with *LMNA* mutations and *LMNA*-related FPLD2 in this area is estimated to be 14.5 and 7.25 cases/million people, respectively, which is twice the prevalence reported in a recent study [29]. Moreover, our results do not reflect any founder-effect bias, as 16 different mutations were identified in the non-R482 group, two of the eight R482 families were of Portuguese descent, and two of the remaining six families had two different types of mutations (R482W and R482Q). Indeed, laminopathies are most likely underrecognized, given their variable phenotype [1,2,7], although this phenotype may perhaps be better recognized in this region, known for its high levels of obesity and diabetes, due to an anticipation phenomenon [30].

One limitation of our study is that it was retrospective. All investigations were not performed in every patient, but were adjusted according to clinical situation and guidelines for follow-up of type 2 diabetes. For this reason, the number of patients who underwent each investigation has been systematically mentioned. On the other hand, the size of our cohort was relatively large for such a rare disease, and all evaluations were performed at the same centre using the same methods, thereby strengthening all data comparisons.

Concerning the cardiac phenotype, there was a marked difference between the R482 and non-R482 groups in familial history of CIED use or sudden cardiac death, arguing in favour of a better cardiac prognosis for R482 than for other *LMNA* mutations. This point, which has never been emphasized before, was easy to explore during clinical assessment of the patients' medical histories and was confirmed by cardiac investigations. Indeed, the frequencies of abnormal ECG and left atrial dilatation were significantly lower, and LVEF significantly higher, in patients with R482 vs. non-R482 mutations. The latter patients were also more likely to have CIEDs and five cases of non-ischaemic heart disease were found in this group. In contrast, patients with R482 mutations tended to have more frequent positive non-invasive tests for ischaemia or documented severe coronary artery disease. However, diastolic function was altered in some patients in both groups, but with different patterns: there was restrictive filling in non-R482 carriers, but impaired relaxation in R482 carriers, a possible consequence of the high incidence of diabetes in the latter. Also, the large number of carriers in the R482 families suggests that the prognosis for the R482W group in terms of mortality may be better. Finally, although gender did not significantly differ, there tended to be more men in the non-R482 group, which might reflect a bias, as male gender has been reported to be a risk factor for sudden cardiac death in laminopathies [31]. However, this link was possibly related to the fact that male gender is more often associated with non-R482 mutations, including non-missense mutations, which are also known to be associated with higher risk of severe cardiac disorders [31]. Nevertheless, no definitive conclusions concerning coronary artery disease can be drawn, as there was also a trend for more frequent screening in the R482 group, most likely because those patients more often also had diabetes.

From a metabolic perspective, the prevalence of diabetes and hypertriglyceridaemia was higher in R482 than in non-R482 carriers. The R482 carriers also had lower BMIs, and lower levels of leptin and fat mass, but higher MRI-assessed visceral fat levels, than non-R482 carriers, which is in accordance with the lipodystrophic phenotype of R482 carriers. Interestingly, the intra-/total abdominal fat-mass ratio, a hallmark of lipodystrophy [32], was significantly higher in the non-R482 vs. control group, thereby arguing for a specific lipodystrophy fat distribution even in patients with non-R482 mutations. It is also noteworthy that the frequency of diabetes reached 40% in the non-R482 group, but often required OGTT to make the diagnosis. Diabetes is known to promote atherosclerosis and findings in vitro favour a direct proatherogenic effect of the *LMNA* R482W mutation in endothelial cells, which is consistent with the trend towards a greater frequency of coronary heart disease in the R482 group [26,27]. However, the diagnosis of lipodystrophy is easier to

make, as the metabolic syndrome (MetS) is more severe in women than in men [33–35]. This gender difference in disease phenotype might be modulating the expression of coronary artery disease, thereby worsening the cardiovascular prognosis for women which, before menopause, is usually considered better than that for men. Also, our results support more systematic screening for MetS, especially by OGTT, in the non-R482 group, and for silent myocardial ischaemia in both groups of mutations, especially in cases with diabetes.

Finally, comparisons between R482 and non-R482 carriers have revealed different cardiometabolic phenotypes with different types of risk, a point that has never been emphasized before. Patients with R482 mutations have a FPLD2 phenotype, which may sometimes be associated with other features, such as myopathy [7]. In this group, diabetes is present in 80% of cases, yet the cardiac phenotype seems less severe than in non-R482 cases, and is mostly the result of insulin resistance and atherosclerosis. These findings are concordant with the low frequency of arrhythmia events reported in patients with R482 mutations in the literature in contrast to the non-negligible frequency of coronary artery disease [26,27]. Otherwise, patients with non-R482 mutations often have a severe cardiac phenotype, such as an initial conduction or rhythm disorder with a risk of sudden cardiac death, and diabetes is present in 40% of these patients. Thus, such patients should be screened for ventricular arrhythmias and dilated cardiomyopathy by Holter monitoring and echocardiography, with consideration of electrophysiological studies, CIED use or cardiac transplantation where appropriate. In any case, the presence of ventricular arrhythmias, especially when associated with diabetes or a familial history of CIED use, should prompt *LMNA* genetic testing and subsequent ICD implantation.

The reason for the different expressions of the disease in our two patient groups remains unexplained. Limited peripheral adipose tissue storage capacity has recently been emphasized in the pathogenesis of human insulin resistance [36]. Frequent and/or early atherosclerotic complications could be mediated through oxidative stress and mitochondrial dysfunction. Recent studies suggest that *LMNA* mutations affect epigenetic regulation of developmental pathways, and might alter myogenesis and adipogenesis processes in a genotype-specific manner [3–5]. These mechanisms, however, have yet to be explored.

Conclusion

These results suggest that cardiac follow-up of laminopathies might be adjusted according to genotype, with more aggressive screening for arrhythmias in non-R482 patients. Also, it is important to consider laminopathies as a differential diagnosis when faced with an unexplained rhythm disorder, especially if there is a familial history of CIED use, sudden cardiac death and/or a personal history of diabetes. In addition, coronary artery disease should not be overlooked, especially when diabetes is present.

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Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.diabet.2018.09.006.

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