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ORIGINAL ARTICLE

LMNA gene mutation search in Polish patients: new features of the heterozygous Arg482Gln mutation phenotype

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Abstract Mutations of the *LMNA* gene have been shown to cause an autosomal dominant form of insulin resistance with familial partial lipodystrophy (PLD), frequently accompanied by diabetes. *LMNA* mutations are considered to be a rare cause of monogenic diabetes; however, they are probably sometimes misdiagnosed as type 2 diabetes (T2DM). We examined whether skin fold thickness measurements may be an effective screening procedure to select individuals with T2DM for molecular testing of the *LMNA* gene. We also aimed to search for mutations in diabetic patients with evident clinical features of lipodystrophy. Skin fold measurements were performed in 249 not pre-selected T2DM patients. The sum of two trunk skin fold measurements divided by the sum of two peripheral was obtained. Men with a skin fold ratio above 2.5 and women above 1.5 were selected for further molecular analysis of the *LMNA* gene by direct sequencing. We also examined eight patients presenting typical clinical features of lipodystrophy. We selected 16 patients with T2DM on the basis of skin fold measurements. *LMNA* gene sequencing in this group revealed no mutation that could be attributable to diabetic phenotype. However, in the group of subjects with apparent lipodystrophic phenotype, we identified one Arg482Gln mutation. This female, diagnosed with diabetes at the age of 51 years, was characterized by insulin resistance but, unlike previously reported *LMNA* Arg48Gln mutation carriers, she was not overweight. The patient also presented with chronic kidney disease and pulmonary fibrosis that could potentially be a part of the phenotype related to the identified *LMNA* mutation. We did not find the evidence that screening based on skin fold measurements alone could be an efficient approach to select T2DM patients for molecular testing of the *LMNA* gene; the presence of features typical for laminopathy seems to be required for such testing. A clinical picture related to the *LMNA* Arg482Gln mutation may be more diversified than it was previously considered and include low BMI and pulmonary fibrosis.

Keywords Diabetes · Insulin resistance · Laminopathy · *LMNA* gene · Mutation

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Introduction

Diabetes mellitus includes many forms of disease that are all characterized by elevated glucose level [1]. The risk of type 1 and type 2 diabetes is associated with the impact of numerous genes and their interaction with environmental factors [2, 3]. In addition, there are also some rare forms of diabetes inherited as monogenic traits, where a single mutation produces disease phenotype. Altogether, they probably account for not more than a few percent of all diabetic cases [4–6]. The studies of monogenic diabetes, however, offer the chance of gaining profound insights into

the disease mechanisms and, in addition, a proper molecular diagnosis may be of great importance for an individual mutation carrier. Monogenic diabetes represents a wide range of clinical defects from significant β-cell failure [4, 5] to deeply decreased insulin sensitivity [6]. The latter clinical picture has been associated with mutations in *LMNA*, peroxisome proliferator-activated receptor γ, insulin receptor, V-act murine thymoma viral oncogene homolog 2 (AKT2), and some other genes [6].

Genetic testing can currently be applied in clinical practice only in monogenic forms of diabetes, as establishing proper diagnosis may sometimes influence treatment method, help to predict progress of disease in affected individuals, and define prognosis in other family members. Unfortunately, diagnosis based on molecular genetics is usually costly and time-consuming. Molecular testing is usually limited only to patients meeting rigorous clinical criteria. Thus, one can expect that monogenic forms of diabetes are underdiagnosed, as many carriers of "lighter" mutations not producing evident clinical phenotype are not referred for genetic testing. This is why there is a need for cheap and simple screening, tests pre-selecting patients in whom we suspect monogenic diabetes for further molecular testing. For example, we have recently published a paper showing the clinical usefulness of a new biomarker measurement in the screening for monogenic, HNF1α-MODY, diabetes [7].

While genetic defects resulting in profound impairment of insulin secretion have been extensively studied over last decade, diabetes arising from genetically determined insulin resistance seems to be, to some degree, neglected. Insulin resistance can be defined as the inability of insulin to produce its usual biological effects at circulating concentrations that are effective in normal subjects [8]. Significant percentage of monogenic cases of genetically determined insulin resistance is due to mutations in the *LMNA* gene encoding Lamin A/C [6]. Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin [9]. *LMNA* mutations produce a very heterogeneous phenotype that may include not only insulin resistance with diabetes, but also muscular dystrophy, neuropathy, cardiac conduction defect, and many others [10]. As Lamin acts as an inhibitor of adipocyte differentiation, *LMNA* gene mutations result in a redistribution of adipose tissue and the phenotype of generalized or partial lipodystrophy (PLD) [10]. Some patients with *LMNA* gene mutations and a less evident phenotype are probably misdiagnosed as type 2 diabetes (T2DM) subjects. The prevalence of *LMNA* gene mutations among patients of diabetic clinics is unknown. An interesting idea has recently been proposed for a potential screening test to select individuals for an *LMNA* gene mutation search that could be based on skin fold measurements [11].

The aim of our study was to test whether skin fold thickness measurements may be an effective screening procedure to select individuals with T2DM from the Polish population for molecular testing of the *LMNA* gene. We also aimed to search for mutations in diabetic patients with evident clinical features of lipodystrophy.

Results

In the group of patients with a diagnosis of T2DM, the median trunk/peripheral skin fold ratio was 2.1 (range 1.1–3.1) and 1.3 (range 0.6–2.0) for males and females, respectively; there was not any difference between the subgroups of subjects on diet and different pharmacological treatments. On the basis of skin fold measurements, we selected 16 patients, 9 females and 7 males, with the highest trunk/peripheral skin fold ratio, 2.8 (range 2.5–3.1) and 1.8 (range 1.6–2.0) for males and females, respectively. Their mean age was 54.9 (range 46–61 years) and diabetes duration was 6.6 (range 3–11 years), while their mean BMI being 26.1 kg/m^2. Among them, there were 9 subjects treated with oral hypoglycemic agents and 7 with insulin. The clinical characteristics of both study groups are provided in Table 1.

LMNA gene sequencing in patients selected from subjects with a T2DM diagnosis revealed no mutations that could be attributable to diabetes. Polymorphisms found in this group (NCBI GeneBank reference numbers: rs4641, rs505058) were previously excluded as variants with a significant effect on diabetes risk [12, 13]. The allele frequencies of identified polymorphisms (T/C: 25%/75% and C/T: 3.12%/96.88% for SNPs rs4641 and 505058, respectively) were similar to previously reported frequencies from other European populations [12, 13]. No new polymorphisms were found.

The only diabetes-causative mutation was found in a 53 year old female patient from the group of 8 subjects with typical PLD. The patient was heterozygous for the G → A substitution at codon 482, which predicted the replacement of arginine (CGG) to glutamine (CAG) (Fig. 1). The *LMNA* Arg482Gln mutation was previously reported [14–18]. Of interest, she was also heterozygous for a polymorphism in intron 6 of the *LMNA* gene (NCBI refSNP ID: rs 534807) and for the silent D446D (GAT → GAC) mutation in exon 7 (NCBI refSNP ID: rs505058). The patient was diagnosed with diabetes at the age of 51 years. She presented with PLD with a profound loss of subcutaneous fat from the upper and lower limbs, however, without neck or face fat accumulation (Fig. 2). Her skin fold trunk/peripheral ratio was 3.2. The clinical

Table 1 Characteristics of patients examined for *LMNA* mutations

Characteristics	Patients selected on the basis of skin fold measurements	Patients selected on the basis of the presence of clinical features of PLD
Number	16	8
Sex (females/males)	9/7	5/3
Age at examination (years, ± SD)	54.9 ± 7.4	44.1 ± 8.6
Duration of diabetes (years, range)	6.6; 3–11	4.9; 2–11
Diabetes treatment (diet/oral agents/insulin)	0/10/6	1/4/3
BMI (kg/m^2)	26.1	24.9
Average skin fold ratio (females/males)	1.9/2.7	2.1/3.2
Antihypertensive therapy (%)	100%	100%
1–2 agents	93.75%	12.5%
More than 2 agents	6.25%	87.5%
Lipid-lowering therapy (%)	93.75%	100%
Statin	93.75%	37.5%
Fibrate	0%	12.5%
Statin + Fibrate	0%	50%
Heterozygous *LMNA* mutations	0	1

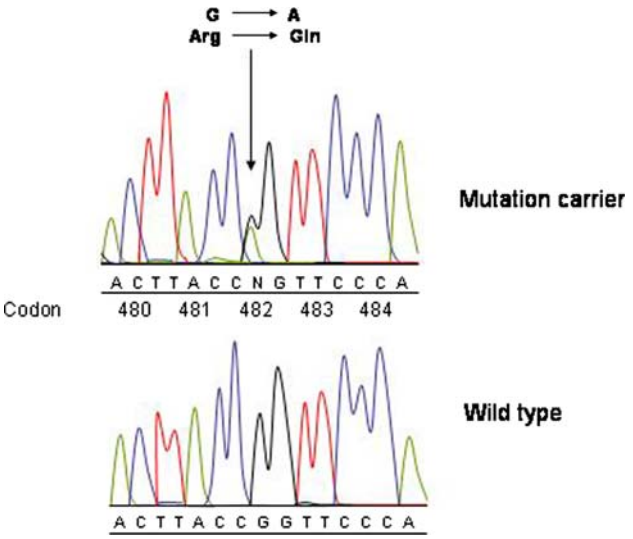

Fig. 1 Partial sequence of exon 8 of *LMNA* gene of a carrier of the Arg482Gln mutation is presented. The *arrow* indicates a G → A substitution at codon 482. Below, the sequence of the non-carrier is shown

picture also included combined hyperlipidemia, fatty liver disease, coronary heart disease (with myocardial infarction at the age of 39 years), bilateral partial cataract, and chronic kidney disease (creatinine level 124 μmol/l, creatinine clearance 35.1 ml/min) with an albumin excretion rate within normal limit. On the ophthalmological examination, the patient was free from any signs of diabetic retinopathy. Interestingly, she was also diagnosed with pulmonary fibrosis at age 48 years. This diagnosis was based on transthoracal biopsy that showed the processes of intensive fibrosis. The patient was suspected of sarcoidosis, however, this diagnosis has never been definitely confirmed as typical mediastinal lymphadenopathy was lacking and bronchoalveolar lavage (BAL) fluid did not show typical features.

There was no sign of hirsutism or history of menstrual irregularities before menopause occurred at the age of 48 years. The patient gave birth to two healthy children. Her BMI at diagnosis was 20.7 kg/m^2, much lower than in previously described *LMNA* Arg482Gln mutation carriers [14–18]. She was 150 cm in height, the shortest among all 5 siblings as the height of her 2 brothers and 2 sisters varied between 162 and 180 cm.

At the time of examination, she was on insulin therapy, with a daily dose of 67 units (1.3 units/kg), C-peptide level 6.4 ng/ml. In addition, she was receiving metformin, although its dose was reduced to 500 mg/day due to decreased glomerular filtration rate. On this treatment, the patient's HbA_{1c} was 6.9%. Insulin sensitivity of this *LMNA* Arg482Gln mutation carrier was assessed by hyperinsulinemic euglycemic clamp technique as described by us elsewhere [19, 20]. The clamp examination, with a constant insulin infusion rate of 3 mU/kg/min, proved insulin resistance, as *M* value was 3.3 mg/kg/min. The patient was not on any insulin-sensitizing agents directly before and during the test. In spite of aggressive lipid-lowering therapy including 40 mg of atorvastatin, 267 mg of micronized fenofibrate, and 2 g of omega-3 fatty acids, the patient did not to reach the goals of lipid control for subjects with diabetes and history of a myocardial infarction [21]. Her total cholesterol level was 4.21 mmol/l, LDL-cholesterol 2.61 mmol/l, HDL-cholesterol 0.74 mmol/l, and triglyceride level was 1.89 mmol/l.

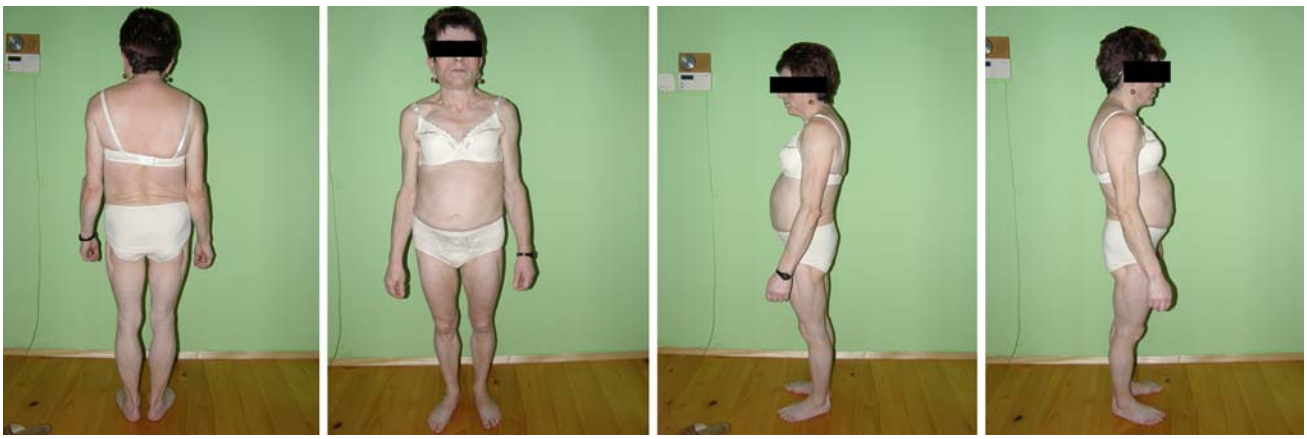

Fig. 2 Anterior, left lateral, right lateral, and posterior views of the *LMNA* Arg482Gln mutation carrier showing loss of subcutaneous fat over the extremities giving muscular appearance. There are no signs of neck or face fat accumulation with increased trunk fat deposits

Interestingly, the probant was the only family member with clinical features of PLD. Sequencing of the *LMNA* gene performed in 7 additional family members (a mother, 4 siblings, a son, and a daughter) did not identify any other mutation carrier. The patient's father died at the age of 65 years due to cerebral hemorrhage. He was free from diabetes and presented no features of PLD, thus, it is unlikely that he was carrier of *LMNA* gene mutation. Therefore, the mutation could have arisen de novo in the identified subject.

Discussion

In this study, we tested a screening procedure based on skin fold measurements to select from T2DM patients the subjects for *LMNA* gene mutation screening [11]. This procedure was suggested by interesting preliminary results of a British study (not published so far as a final report) showing that skin fold ratio for all PLD subjects, those with molecular diagnosis of LMNA mutation and with unknown genetic results, was above the 95% confidence interval of body mass index normalized healthy subjects [11]. The suggested test, however, has not been examined so far in clinical settings and thus its sensitivity, specificity, positive and negative predictive values have not been tested. In the current study, we evaluated the suggested test in a medium sized group of T2DM patients using an arbitrary cut-off point of skin fold ratio above 2.5 and 1.5 for men and women, respectively. This cut-off point was established based on the British study, which showed that for the majority of patients with clinical features of PLD, with or without the molecular diagnosis of laminopathy, the skin fold ratios laid above values corresponding to 2.5 for men and 1.5 for women [11]. We were not able to find any *LMNA* gene mutation in 16 subjects selected for molecular testing based on skin fold measurements. There are several reasons for this negative outcome. First, our sample size might be too small. In addition, the cut-off point might be too relaxed, making the test very nonspecific. Moreover, the frequency of *LMNA* is unknown, thus, in case of its likely rarity any screening test for laminopathy in an unselected population could have very low positive predictive value making it clinically inefficient. Further studies in larger cohorts and using more rigorous cut-off points are necessary to definitively establish whether skin fold measurements may play a role in the process of selecting patients for *LMNA* genetic testing.

The important differences between the British study [11] and ours should be emphasized. The current research should not be considered a simple replication study. They tried to select *LMNA* gene mutation carriers from healthy individuals, while the aim of the current research was different as we tried to screen for such individuals in T2DM patients. The subjects for screening in the British study were selected not only from diabetic clinics, but also from the other patient groups. It is well known, however, that most of the patients with *LMNA* are diabetics [22].

Our study could perhaps be qualified for the fact that most of the patients were on pharmacotherapy. It is known that some drugs, for example, insulin and sulfonylureas, predispose T2DM patients to weight gain [23] and may increase adiposity. There is no evidence, however, that such treatment affects the skin fold ratio that we examined in this study. The only exceptions are probably thiazolidinediones, as a case report was published describing the increase of the fat tissue on the limbs of an *LMNA* gene mutation carrier [24]. None of our patients was on medication from this group. In addition, there was not any difference between the skin fold ratio in T2DM patients in various treatment groups.

We also report the heterozygous Arg482Gln *LMNA* mutation carrier characterized by some new, previously not reported clinical features. First, she had low BMI, while most of other described Arg482Gln mutation carriers were overweight [15–18]. This may mean that the phenotype resulting from the *LMNA* Arg482Gln mutation includes some patients with BMI at the lower edge of normal limit. It should be mentioned that cases of low BMI for the other *LMNA* mutation, Arg482Trp, affecting the same codon, were reported [25]. We cannot exclude that the phenotype was modified by the presence of the silent D446D mutation that was found to be associated with the development of T2DM in a recently performed metaanalysis [13]. There is no scientific evidence that may suggest a role of the other *LMNA* variant (SNP rs 534807), also found in our patient. On the other hand, her phenotype may reflect an effect of unidentified environmental or genetic factors modifying BMI of *LMNA* Arg482Gln carriers. Our case confirms the recent description that the diminished renal function or even renal failure may be a part of the clinical picture related to *LMNA* Arg482Gln mutation [18].

Pulmonary fibrosis was not earlier reported as a feature associated with any mutation in the examined gene. We should, however, take into account that the existence of this phenomenon with other typical laminopathy characteristics was a coincidence.

A relatively low insulin dose sufficient to keep satisfactory metabolic control in the presented patient is probably partially a result of high preserved β-cell output as measured with C-peptide level. Alternatively, elevated C-peptide level in this case could be a result of its decreased renal and, possibly, muscular metabolism. The alternative explanation for relative low insulin requirement may be only moderately decreased insulin sensitivity. The level of insulin resistance reported previously in *LMNA* Arg482Gln mutation carriers was highly variable, with some patients with PLD due to Arg482Gln mutation free from diabetes or with the disease developing later in life [14–17].

In summary, we did not find evidence that a screening procedure based on skin fold measurements alone could be an efficient approach in a clinical setting to select T2DM patients for molecular testing of the *LMNA* gene; the presence of features typical for laminopathy seems to be required for such testing. A clinical picture related to the *LMNA* Arg482Gln mutation may be more diversified than it was previously considered and include low BMI and pulmonary fibrosis.

Materials and methods

Skin fold measurements were performed in a group of 249 consecutive, not pre-selected T2DM patients of Outpatient Clinic of the Department of Metabolic Diseases, Jagiellonian University, Medical College, Krakow, Poland (143 men and 106 women), using a Lange caliper (Cambridge Scientific Industries, Cambridge, USA). Patients' mean age was 61.7 years (range 44–68 years), mean diabetes duration was 7.9 years (range 3–16 years). There were 81 (33%) patients on metformin, 24 (9%) on sulphonylurea, and 61 (25%) were using both drugs. In addition, 83 patients (33% of the study group) were on insulin either as a monotherapy or as a part of combined model with oral agents. Measurements were performed according to previously described procedure [26] and with the same instruments throughout the study by two trained nurses. The following skinfolds were measured: subscapular (SS), suprailiacal (SI), biceps (BI), triceps (TRI). The sum of two trunk skin fold measurements (SS + SI) divided by the sum of two peripheral (TRI + BI) skin folds was obtained. Men with a skin fold ratio above 2.5 and women above 1.5 were selected for further molecular analysis of the *LMNA* gene by direct sequencing.

We also examined 8 patients with typical features of PLD referred to Department of Metabolic Diseases. The inclusion criteria were diabetes, PLD (based on clinical estimation and defined as a loss of subcutaneous fat from the limbs giving a muscular appearance) and the presence of at least one of the following: (a) lipid abnormalities; (b) fatty liver disease; (c) hypertension; (d) coronary artery disease diagnosed under the age of 50 years. The molecular testing for the *LMNA* mutation was performed by direct sequencing of the promoter and all exons of LMNA with sets of primers published before [27]. The sequencing was performed with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster, USA) and analyzed with ABI PRISM Sequencing Analysis 3.7 software.

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