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Dunnigan-type familial partial lipodystrophy associated with the heterozygous R482W mutation in *LMNA* gene — case study of three women from one family

Związek rodzinnej częściowej lipodystrofii typu Dunnigana z heterozygotyczną mutacją R482W w genie *LMNA* — opis przypadku trzech kobiet pochodzących z jednej rodziny

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Abstract

Lipodystrophies are a heterogeneous group of diseases affecting adipose tissue distribution. Familial partial lipodystrophy of the Dunnigan type (FPLD) is a rare autosomal, dominant disorder caused by missense mutations in lamin A/C (*LMNA*) gene where selective loss of subcutaneous adipose tissue from the limbs and trunk, and accumulation of fat in the neck and face, is usually associated with a variety of metabolic disorders including insulin resistance, diabetes mellitus, dyslipidemia, hepatic steatosis and high blood pressure. In this report we present clinical and molecular features of three Polish women with FLPD phenotype coming from one family (a mother and her two daughters). FPLD was recognised under the circumstances of diabetes treatment, where sequencing of *LMNA* gene revealed heterozygous R482W mutation. In order to be able to recognise monogenic diabetes associated with lipodystrophy, it is important to be very precise in physical examination while diagnosing diabetes and to be aware of the necessity of performing genetic testing. Diabetes-appropriate differential diagnosis is essential for the treatment strategy, anticipation of the disease progression, and determination of the prognosis. It is necessary for an individual mutation carrier to look carefully at the patient's family. **(Endokrynol Pol 2013; 64 (4): 306–310)**

Key words: *diabetes mellitus, familial partial lipodystrophy, laminopathy, LMNA gene*

Streszczenie

Lipodystrofie są heterogenną grupą chorób dotyczących rozmieszczenia tkanki tłuszczowej w organizmie. Rodzinna częściowa lipodystrofia typu Dunnigana (FPLD, *familial partial lipodystrophy of the Dunnigan type*) jest rzadkim autosomalnie dominującym schorzeniem, którego przyczyną jest mutacja braku sensu w genie laminy A/C (gen *LMNA*). W chorobie tej dochodzi do selektywnej utraty podskórnej tkanki tłuszczowej kończyn i tułowia oraz jej kumulacji w okolicy karku i twarzy, co zwykle jest związane z występowaniem różnych schorzeń metabolicznych, w tym insulinooporności, cukrzycy, dyslipidemii, stłuszczenia wątroby i nadciśnienia tętniczego. W przedstawionym raporcie opisujemy kliniczne i molekularne cechy trzech polskich kobiet pochodzących z jednej rodziny charakteryzujących się fenotypem FLPD (matka i jej dwie córki). Obecność FLPD rozpoznano w trakcie leczenia cukrzycy na podstawie sekwencjonowania genu *LMNA* i ujawnienia heterozygotycznej mutacji R482W. W celu rozpoznania cukrzycy monogenowej związanej z lipodystrofią ważne jest przeprowadzenie szczegółowego badania fizykalnego w toku diagnostyki cukrzycy oraz świadomość konieczności diagnostyki genetycznej wątpliwych przypadków. Prawidłowa diagnostyka różnicowa cukrzycy jest niezbędna w celu ustalenia strategii leczenia, przewidywania progresji choroby, określenia rokowania, a także jest potrzebna w celu wzmożenia czujności diagnostycznej w odniesieniu do rodziny osoby będącej nosicielem mutacji. **(Endokrynol Pol 2013; 64 (4): 306–310)**

Słowa kluczowe: *cukrzyca, gen LMNA, laminopatia, rodzinna częściowa lipodystrofia*

Introduction

Lipodystrophies are a rare, heterogeneous group of diseases affecting adipose tissue distribution that can be familial or acquired, generalised or partial. Affected patients are predisposed to metabolic complications such as insulin resistance, diabetes mellitus, hypertriglicerydaemia, hepatic steatosis, acanthosis nigricans and features characteristic for polycystic ovary syndrome namely hirsutism, oligomenorrhoea, and polycystic ovaries [1, 2]. Familial lipodystrophies can broadly be classified into two subtypes. These include autosomal recessive generalised lipodystrophy caused by mutations of either seipin or *AGPAT2* genes and autosomal

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dominant partial lipodystrophy linked to mutation in the *PPARG* or *LMNA* genes [3–7]. *LMNA* is localised to chromosome 1q21 and encodes for the A-type lamins (lamin A and lamin C) arising by alternative splicing of the LMNA pre-mRNA that are expressed in most differentiated somatic cells [8, 9]. Mutations in the *LMNA* gene cause a variety of monogenic, multisystem disorders called laminopathies that comprise such diseases as skeletal and/or cardiac muscle dystrophies, axonal neuropathy, and premature ageing syndromes as well as familial partial lipodystrophy of Dunnigan type (FPLD; OMIM 151660) [10]. FPLD is one of the partial lipodystrophies, usually inherited, resulting from heterozygous missense mutations in the *LMNA* gene [11]. Approximately 90% of the FLPD-linked LMNA mutations generate aminoacid substitutions within an immunoglobulin-type domain of lamins A and C and it has been suggested that the immunoglobin-type fold in the tail of A-type lamins has a specific function in adipose tissue [12, 13]. The mutations affect the ability of A-type lamins to bind DNA which can potentially change adipocyte differentiation or survival [14]. The great majority of patients have heterozygous substitutions within exon 8 at codon 482 which is suggested as a 'hot spot' for the disease [15]. Patients with FPLD are born with normal fat distribution and after puberty a regional loss of subcutaneous adipose tissue from extremities and trunk followed by progressive, excess fat accumulation on the face and neck, as well as the development of metabolic complications, are observed. Due to accumulation of fat in the neck and upper trunk, FPLD can be misdiagnosed as Cushing's syndrome [2, 16–18].

We present the case of a woman diagnosed with FPLD and her two daughters also affected by the disease.

Case presentation

Case No. 1

A 50 year-old woman with diabetes mellitus recognised at the age of 37 was admitted to the Department of Internal Medicine and Diabetology because of hyperglycaemia. On physical examination during admission she was 154 cm tall, weighed 54 kg and body mass index was 23 kg/m^2. At the time of admission we noted fat accumulation around the face, neck and shoulders accompanied by thin limbs due to atrophy of subcutaneous adipose tissue, acanthosis nigricans in the cervical region and hirsutism. Laboratory tests revealed increased plasma blood glucose levels: fasting ones in the range of 122–130 mg/dL and prandial ones in the range of 164–223 mg/dL as well as HbA1c level of 6.6%. The blood cell count, liver function, creatinine level and uric acid levels were normal. Abnormalities in lipid parameters such as total cholesterol — 6.93 mmol/L and triglycerides — 2.5 mmol/L were discovered, so lipid-lowering therapy (20 mg atorvastatin) was applied. Abdominal ultrasonography revealed slight hepatomegaly with features of hepatic steatosis. Hormonal tests (thyroid hormone and cortisol level) were normal. Blood pressure measurements were optimal and an had been treated with ACE-I and diuretics. An ophthalmological examination revealed signs of hypertensive retinopathy. The insulin dose was escalated up to 56 units per day and metformin treatment was continued in order to obtain optimal blood glucose levels in a 24-hour profile.

She was diagnosed with gestational diabetes mellitus at the age of 36 and insulin therapy was initiated. After pregnancy blood glucose levels became normal, without the necessity for any treatment. Six months later fasting hyperglycaemia was noticed and she was initially treated with metformin and insulin. Her menarche was at the age of 11 years and she had irregular menstrual periods since adolescence. The woman has five children – three daughters and two sons and she has miscarried three other pregnancies.

Due to typical phenotypic traits of lipodystrophy, accompanied by insulin resistance, acanthosis nigricans, lipid abnormalities and a history of miscarriages, she was suspected of FPLD and genetic studies were performed. Her sons presented normal phenotype, but her two daughters aged 21 and 17 presented phenotype similar to the studied woman. All the children were considered to be healthy. The woman's mother suffered from diabetes mellitus type 2 but her phenotype was not known. The woman did not know the medical history of her father. As soon as the outcome of the genetic study was obtained, we verified the misdiagnosed type 2 diabetes mellitus and confirmed the FPLD2 as well as invited the woman's five children to participate in the genetic examination. The woman's sons, and her mother and father did not agree to participate in the study.

The pedigree of the FPLD family with heterozygous R482W mutation in *LMNA* gene is presented at Figure 1.

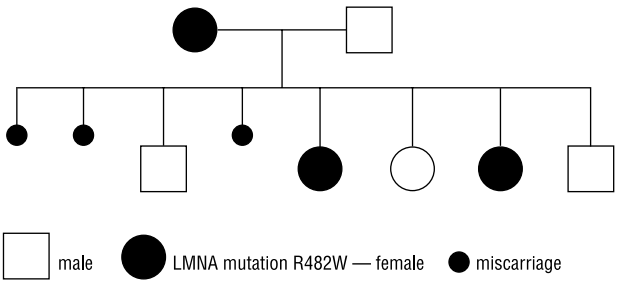

Figure 1. *Pedigree of FPLD family with heterozygous R482W mutation in LMNA gene*

Rycina 1. *Rodowód rodziny z heterozygotyczną mutacją R482W w genie LMNA chorującej na FPLD*

Case No. 2

The 21 year-old daughter had a blood glucose level screening test performed, and because it displayed an abnormal outcome she was admitted to hospital. At the time of admission she was 164 cm tall, weighed 62 kg and body mass index was 23 kg/m^2. She had moderate typical signs of diabetes mellitus such as polyuria and polydipsia. She presented typical phenotype characteristic for lipodystrophy — fat accumulation around the face, neck, shoulders, upper back associated with lean muscular limbs and acanthosis nigricans in the cervical region, but unlike her mother she was free from signs of hirsutism. HbA_{1c} level was 9.4%. Mean fasting plasma blood glucose level was in the range of 152–254 mg/dL and mean prandial plasma blood glucose level in the range of 157–344 mg/dL. Multiple injections of insulin therapy in conjunction with metformin were initiated. The blood cell count, creatinine level and uric acid level were normal but levels of liver enzymes (AST — 69 IU/L and ALT — 113 IU/L) and lipid parameters (triglycerides — 2.14 mmol/L) were elevated. She was advised to implement a low fat diet and control the lipid profile after optimisation of blood glucose levels. Abdominal ultrasonography revealed slight hepatomegaly with features of hepatic steatosis as similarly observed in her mother. Hormonal tests (thyroid hormone and cortisol level) were normal. A performed electrocardiogram did not show any irregularities but her blood pressure was elevated up to 160/90 mm Hg. She required multidrug therapy in order to obtain good blood pressure control (calcium blocker, ACE-I, diuretic). On ophthalmological examination, no signs of diabetic or hypertensive retinopathy were observed. The final insulin dose was escalated up to 90 units per day in order to obtain optimal blood glucose levels in a 24-hour profile. Her past medical history was insignificant. Her first menarche was at the age of 11 and she had regular menstruations.

Genetic analysis was performed and FPLD recognised and the young woman put under the care of a diabetologist. She has been successfully treated with functional intensive insulin therapy, diet and hypertensive therapy.

Case No. 3

The 17 year-old daughter had a blood glucose level screening test performed and, similarly to her sister, as it displayed an abnormal outcome she was admitted to hospital. She had had discrete symptoms of polydipsia and polyuria for a few days preceding admission. At the time of examination, she was 161 cm tall, weighed 62 kg and body mass index was 23 kg/m^2. She presented phenotype characteristic for lipodystrophy — fat accumulation around the face, neck, shoulders, upper back associated with lean muscular limbs, acromegaloid features including enlargement of jaw and moderate acanthosis nigricans on the neck. She, unlike her mother, was free from signs of hirsutism. Performed laboratory measurements revealed mean fasting plasma blood glucose level in the range of 142–166 mg/dL, mean prandial plasma glucose level in the range of 160–180 mg/dL, HbA_{1c} level of 7.4% and a fasting C-peptide level of 2.72 ng/mL (normal range: 0.78–1.89). The girl was started on insulin therapy with the use of multiple injections. The blood cell count and creatinine level were within normal range. Liver parameters were elevated (AST — 87 IU/L and ALT — 129 IU/L) but unlike her mother and sister, lipid profile was in the normal range and abdominal ultrasonography did not reveal any features of hepatomegaly or hepatic steatosis. Polycystic ovaries were observed upon ultrasound examination and a diagnosis of polycystic ovary syndrome was established by the gynaecologist. Hormonal tests (thyroid hormone and cortisol level) were normal. Although there were no abnormalities in a performed electrocardiogram, echocardiography identified concentric left ventricular hypertrophy and 24-h blood pressure monitoring revealed elevated above normal blood pressure values. Therefore antihypertensive therapy with calcium blocker was initiated and good blood pressure control was obtained. No signs of diabetic or hypertensive retinopathy were found on ophthalmological examination. The final insulin dose was escalated up to 70 units per day in order to obtain optimal blood glucose levels in a 24-hour profile. Her past medical history was unremarkable. Pubertal development was normal with menarche at the age of 13 and she had irregular menstruations, similarly to her mother.

Genetic analysis was performed and FPLD recognised; the girl is under the care of a diabetologist and has been successfully treated with intensive insulin therapy, metformin and hypertensive therapy.

Genetic analysis

Genetic screening and analysis of the exon 8 in the *LMNA* gene were performed at the Childrens' Hospital and Centre for Medical Genetics and Molecular Medicine, Haukeland University Hospital in Bergen in Norway. Genomic DNA from each patient was isolated from peripheral blood lymphocytes using a commercial kit. All coding exons of *LMNA* with flanking intron sequences from the patients were amplified with PCR. DNA sequence information for the *LMNA* gene was obtained from GenBank (accession no. NM_170708.3 and NM_170707.3 www.ncbi.nlm.nih.gov). For sequencing, all exons, exon–intron boundaries, and 900 bp from the 5′ and 3′ untranslated region (UTR)/promoter region of

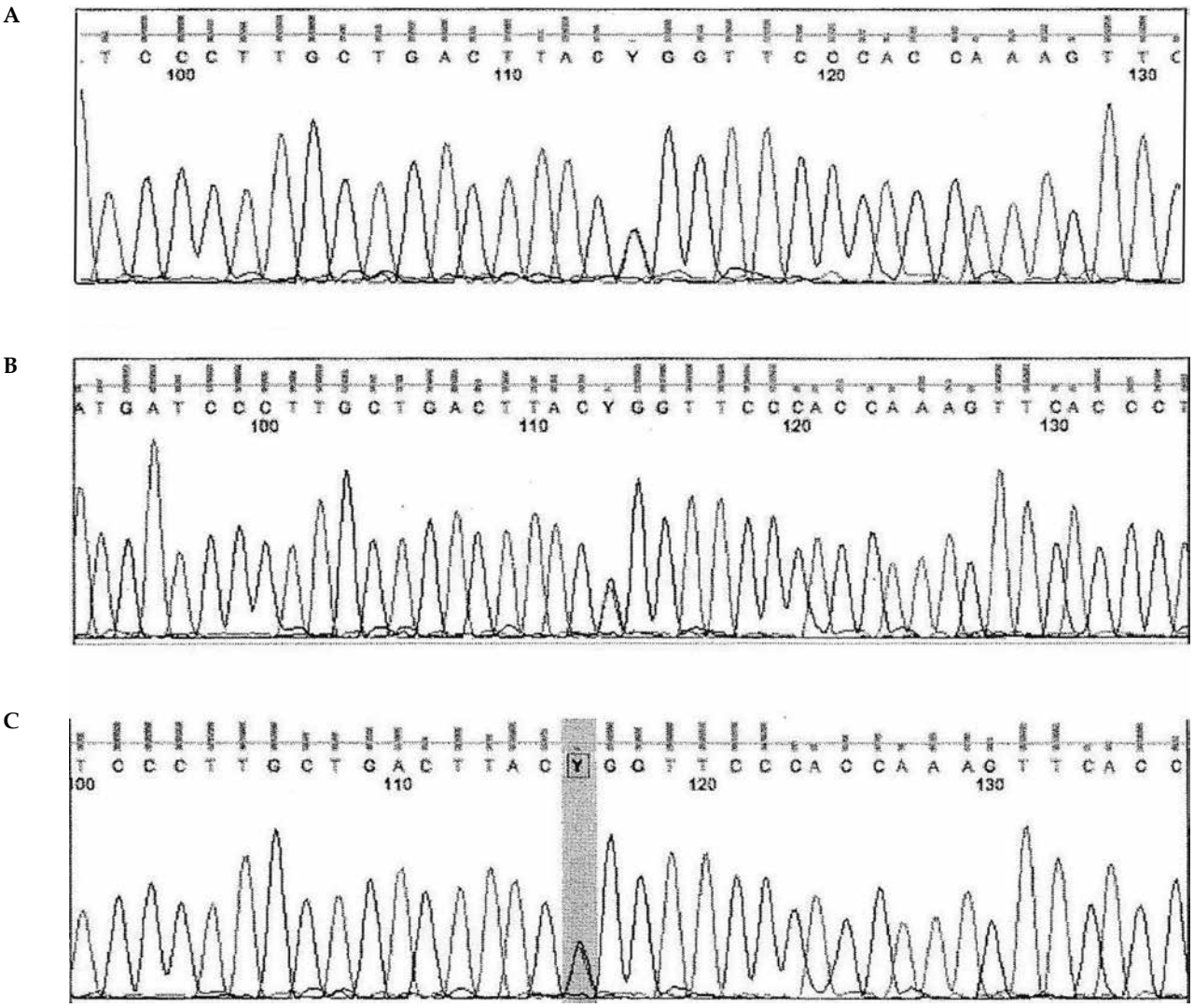

Figure 2. *Chromatograms from DNA sequencing of affected female family members revealing heterozygous substitution c.1444C > T within exon 8 of LMNA gene.* **A.** *50 year-old woman (mother).* **B.** *21 year-old woman (daughter).* **C.** *17 year-old girl (daughter)*

Rycina 2. *Chromatogramy sekwencjonowania DNA chorych członków rodziny ujawniające heterozygotyczną substytucję c.1444 C > T w obrębie exonu 8 genu LMNA.* **A.** *50-letnia kobieta (matka).* **B.** *21-letnia kobieta (córka).* **C.** *17-letnia dziewczynka (córka)*

LMNA were amplified using PCR with sequence-specific primers and analysed by terminator cycle sequencing using Big Dye v3.0 chemistry on an ABI PRISM 310 capillary DNA sequencer (Applied Biosystems, Foster City, CA, USA). All sequence data was compared to RefSeq NM_006005 using Sequencher Software 4.7 (Gene Codes, Ann Arbor, MI, USA). Direct DNA sequencing revealed a heterozygous missense mutation at codon 482 (c. 1444C > T; R482W) located in exon 8 of the *LMNA* gene in all three female patients' blood samples. On the basis of revealed missense mutation W482R, the diagnosis of familial partial lipodystrophy of the Dunnigan type (FPLD) was established. Chromatograms from DNA sequencing of the affected female family members revealing the above mentioned mutation are presented in Figure 2.

Discussion

We report a case of LMNA mutation R482W (c.1444C > T) in exon 8, in the heterozygous state, causing FPLD associated with a variety of metabolic complications. This mutation was identified among three women in one Polish family which has confirmed the diagnosis of FPLD suspected on the basis of phenotype typical for the disease [19]. Even though all three women carried the same mutation of LMNA gene and had diabetes mellitus, the type and severity of concomitant complications were differently pronounced. Insulin resistance was of various degrees, manifested by a large amount of insulin necessary to obtain good metabolic control of diabetes, the most highlighted in the 21 year-old daughter and the least in the mother. Two of the studied

women had slight hepatomegaly with hepatic steatosis in ultrasonography examination, of which one was acompanied by elevated levels of liver enzymes and one woman had elevated liver enzymes with normal liver ultrasound examination. Apart from glucose and lipid abnormalities, we diagnosed hypertension in all the female patients and observed concentric hypertrophy of the left ventricle in one of them in performed echocardiography. Unfortunately the other two described women had no echocardiography examination. All the women presented acanthosis nigricans but only the mother had hirsutism. Two of them presented menstrual irregularities and one of them polycystic ovaries syndrome. Additionally, the mother had a history of multiple miscarriages.

All the mentioned abnormalities are consistent with observations in FPLD patients in accordance with previous reports from literature [20, 21]. On the other hand, none of the patients was overweight, while most of the patients carrying the W482R mutation described to date have had BMI above the normal range [22, 23]. Although the molecular basis and precise pathophysiology of FPLD is still not fully known, early diagnosis of Dunnigan-type familial partial lipodystrophy caused by mutation in *LMNA* gene should be performed when characteristic FPLD phenotype with concomitant one or more metabolic abnormalities are observed. This implies that all family members of the studied women should be carefully diagnosed to allow appropriate medical care and clinical treatment if necessary. Unfortunately, in the case of the presented patients, other members of the family, namely two sons and the mother's parents, could not be examined.

All three women visit our Outpatient Clinic regularly, so it will be interesting to observe whether there will be any problems with conception and miscarriages among the two daughters in the future, a problem observed in the past medical history of the mother. It ought to be mentioned that monogenic forms of diabetes account only for a few percent of the disease cases and are not easy to recognise [24]. Diabetes associated with FLPD may be misdiagnosed with type 2 diabetes, but proper diagnosis influences future treatment decisions and prognosis, and may help early recognition of affected members of the patient's family.

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