Familial partial lipodystrophy associated with the heterozygous *LMNA* mutation 1445G>A (Arg482Gln) in a Polish family

Ogniskowa rodzinna lipodystrofia związana z heterozygotyczną mutacją w genie LMNA 1445G>A (Arg482Gln) w polskiej rodzinie

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Abstract

Familial partial lipodystrophy (FPLD) belongs to the family of laminopathies – disorders associated with mutation in the lamin A/C gene (*LMNA*). FPLD is characterized by loss of subcutaneous adipose tissue from the limbs, trunk and buttocks, with its concomitant accumulation on the face, neck and intra-abdominal region, and by metabolic disorders.
We present the first Polish family with FPLD confirmed genetically. A 34-year-old woman admitted with myalgia and cushingoid appearance was found to have a round face with double chin, neck bump, and loss of fat on extremities. Diagnostic tests revealed impaired glucose tolerance and increased levels of liver enzymes, and ultrasonography revealed hepatic steatosis. Her 9-year-old daughter presented a similar phenotype, but no fat loss. A genetic test revealed the presence of a heterozygous *LMNA* gene mutation: c.1445G>A, consistent with the "hot spot" for FPLD. Treatment with metformin to improve insulin resistance and address the diabetes proved successful.

Key words: familial partial lipodystrophy (FPLD), *LMNA*, lamin A/C, laminopathies.

Streszczenie

Rodzinna częściowa lipodystrofia (*familial partial lipodystrophy* – FPLD) należy do laminopatii – chorób związanych z mutacjami genu laminy A/C (*LMNA*). Charakteryzuje się utratą podskórnej tkanki tłuszczowej na kończynach, tułowiu i pośladkach z jednoczesnym jej przemieszczeniem na twarz, szyję i do przestrzeni wewnątrzbrzusznej oraz zaburzeniami metabolicznymi. Prezentujemy pierwszą polską rodzinę z genetycznie potwierdzoną FPLD. Trzydziestoczteroletnia chora została przyjęta do szpitala z powodu bólów mięśni oraz cushingoidalnego wyglądu. Klinicznie stwierdzono zaokrąglenie twarzy, podwójny podbródek, nagromadzenie tkanki tłuszczowej na karku oraz zanik tkanki tłuszczowej na kończynach. Badania wykazały nieprawidłową tolerancję glukozy i zwiększoną aktywność enzymów wątrobowych, zaś USG – cechy stłuszczenia wątroby. Dziewięcioletnia córka chorej prezentuje podobieństwo fenotypowe do matki, jednakże obecnie bez zaniku tkanki tłuszczowej. W badaniu genetycznym u chorej i jej córki stwierdzono heterozygotyczną substytucję w genie *LMNA*: c.1445G>A (p.Arg482Gln), która znajduje się w miejscu „hot spot" dla FPLD w 8. eksonie. Chorej podano metforminę w celu leczenia insulinooporności i zaburzeń gospodarki węglowodanowej.

Słowa kluczowe: rodzinna częściowa lipodystrofia, *LMNA*, lamina A/C, laminopatie.

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Introduction

Familial partial lipodystrophy (FPLD), OMIM#151660, is a rare human disorder characterized by partial loss of subcutaneous adipose tissue on the limbs, trunk and buttocks and a concomitant accumulation thereof on the face, neck and intra-abdominal region, this being associated with insulin-resistant diabetes mellitus, hypertriglyceridaemia and hormonal imbalance. The disease onset is usually at puberty, women being more severely affected. Complications of polymetabolic syndrome, such as premature atherosclerosis or acute pancreatitis, may be capable of influencing lifespan [1-5]. FPLD belongs to the group (and accounts for about 8%) of the so-called laminopathies, these being rare inherited human diseases affecting skeletal muscle, peripheral nerves and adipose tissue, or causing multisystemic syndromes, including premature aging [6]. Laminopathies are associated with mutations in the *LMNA* gene that encodes the nuclear envelope proteins lamin A and lamin C. Nuclear lamins are members of the intermediate filament (IF) family of proteins and are present in differentiated cells. Like all IF proteins, they consist of an alpha-helical rod domain flanked by a non-helical N-terminal head and C-terminal tail domains. They form the main component of nuclear lamina, responsible for the maintenance of nuclear structure, for chromatin organization and gene expression and for influencing cell development, differentiation and apoptosis. Apart from FPLD, laminopathies encompass a wide spectrum of different human disorders, affecting skeletal muscles (Emery-Dreifuss dystrophy, EDMD-AD and EDMD-AR; limb-girdle dystrophy 1B, LGMD1B) [7], heart muscle (dilated cardiomyopathy with conduction disturbances, DCM) [8] or peripheral nerves (Charcot-Marie-Tooth 2B, CMT2B) [9], or generating multisystemic diseases bearing features of premature aging, i.e. mandibuloacral dysplasia (MAD) [10], Hutchinson-Gilford progeria (HGPS) [11,12], atypical Werner syndrome and restrictive dermatopathy (RD) [13].

We describe here two Polish patients, a mother and daughter, who presented typical symptoms of FPLD, as dependent on the heterozygous *LMNA* mutation Arg482Gln.

Case report

A thirty-four-year-old woman presented to the neuromuscular outpatient clinic complaining of changes in her appearance, i.e. moon-like face, thick neck, slim legs and arms with pronounced muscle profile and myalgia. The symptoms were noticed at first when she was about 20, but she visited her primary care physician only when myalgia occurred; then she was 32. At the time in question she also complained of chronic headaches and depression. While she was suspected of having Cushing syndrome, the hormonal tests did not confirm this diagnosis. On the basis of increased levels of AST, ALT and GGT, hepatopathy was diagnosed, but viral hepatitis was excluded. Although the woman

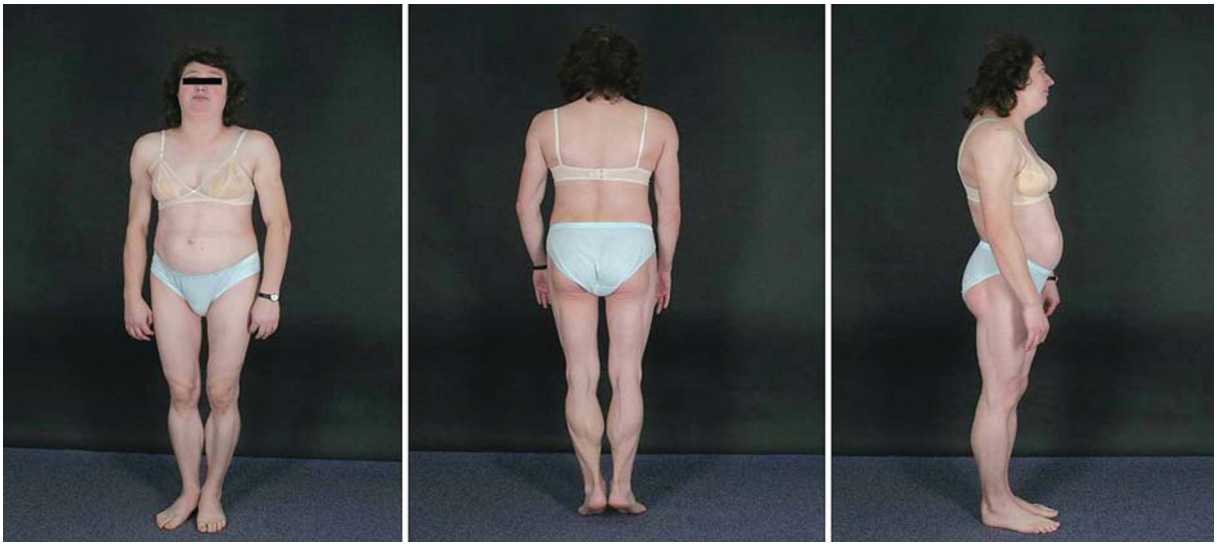

Fig. 1. Patient with familial partial lipodystrophy (FPLD). Note loss of subcutaneous adipose tissue on limbs resulting in prominent muscles, plus concomitant fat accumulation on face and neck

has two children, her first, third and fifth pregnancies miscarried. In addition, she has had irregular menstrual periods since adolescence.

On physical examination we found a round face with double chin and neck bump, and loss of subcutaneous fat on arms and legs, resulting in apparent musculature (Fig. 1). Muscle strength and tendon jerks were normal. No sensory disturbances were detected. Clinical examination of the patient's mother and two children was carried out, the mother proving asymptomatic on neurological examination. The single abnormality in the 8-year-old son was a high palate arch. The 9-year-old daughter presented a similar phenotype to her mother as regards general appearance and posture, though there had been no fat loss at the time of the examination (Fig. 2).

Diagnostic tests

Laboratory tests revealed increased levels of liver enzymes, i.e. AST – 85 U/L (N: 5-40); ALT – 111 U/L (N: 7-56) and GGTP – 70 U/L (N: 7-50); as well as impaired glucose tolerance: fasting glucose 109 mg%, 120 min – 167 mg%; high levels of insulin: fasting – 61.24 μIU/mL, 120 min – 92.24 μIU/mL (N: 2.1-22); peptide C: fasting – 7.88 ng/mL, 120 min – 10.62 ng/mL (N: 0.48-3.3); and HbA_{1c}: 6.6% (N: 4.5-6.5); and slight dyslipidaemia: total cholesterol: 90 mg/dL (N: 120-200), HDL cholesterol – 32 mg/dL (N: > 40). Serum CK, thyroid hormones and cortisol proved normal, as did EMG and ENG. The patient had two muscle biopsies done: from the brachial biceps (in another hospital) and from the femoral quadriceps. The first biopsy revealed normal muscle morphology, but in the second biopsy there was only adipose tissue in the specimen. Ultrasonography revealed hepatomegaly with hepatic steatosis. Computed tomography scans of the neck and upper mediastinum were normal, and no abnormalities were revealed by cardiological evaluation (ECG, echocardiography). The patient is under the care of a diabetologist and she has been treated successfully with metformin and diet – glucose tolerance is improved. Unfortunately, sometimes the patient is not fully compliant with the treatment schedule, although possible complications of metabolic

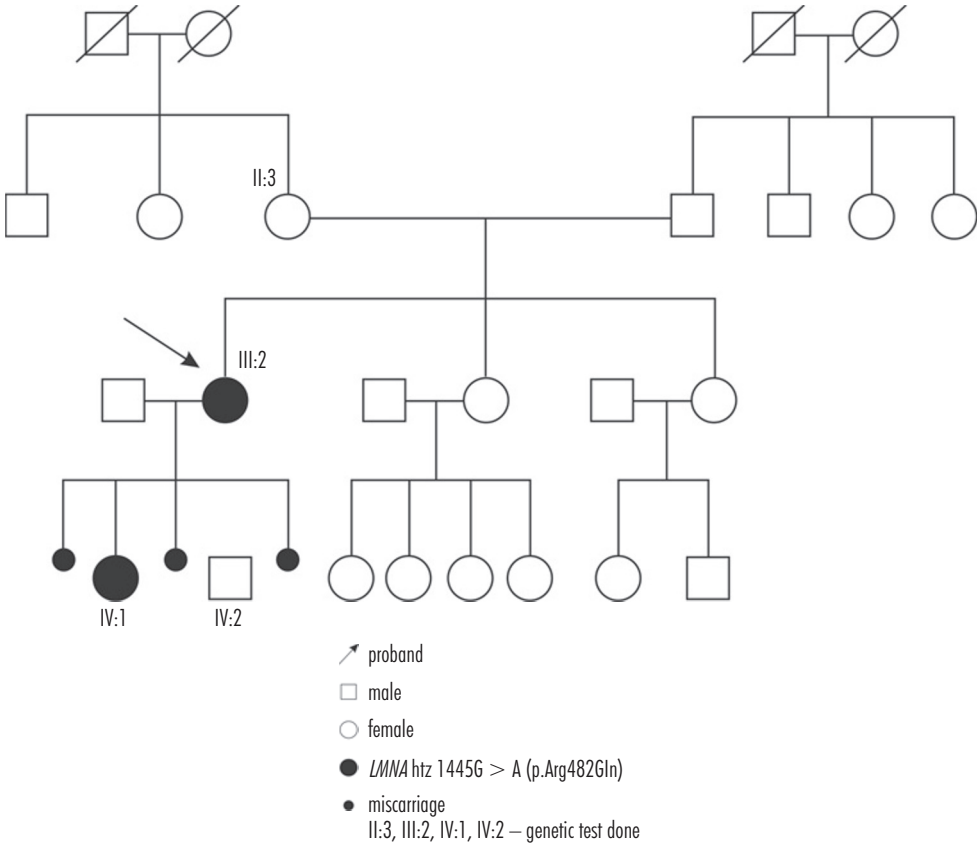

Fig. 2. Pedigree of family affected by familial partial lipodystrophy (FPLD), showing autosomal dominant trait of inheritance

syndrome were discussed with her. The affected child of the patient had glucose and liver enzyme testing done – the results were within the normal range.

Genetic analysis

Once written informed consent had been received, we performed genetic analysis in the proband, her mother and two children. Genomic DNA was extracted from peripheral blood lymphocytes. All 12 exons of *LMNA* and exon-intron boundaries were amplified by PCR, sequenced using the Big Dye Terminator Sequencing Ready Reaction kit (Applied Biosystems), and analysed on an ABI PRISM 373 fluorescent DNA sequencer (Applied Biosystems). Primer sequences and PCR protocols are available upon request.

Direct DNA sequencing revealed a heterozygous missense mutation at codon 482 (c.1445G>A), resulting in arginine-to-glutamine change in the patient (III:2) and her daughter (IV:1). The mutation was not found in the second healthy child (IV:2), or in the patient's mother (II:3) (Fig. 3).

Discussion

As a disorder, lipodystrophy, i.e. a loss of subcutaneous fat, can be acquired (more commonly) or inherited (less commonly), generalized or partial [14]. Although genetically conditioned Dunnigan familial partial lipodystrophy is a rare lipodystrophy, its clinical presentation is well described and the consequences of metabolic complications are relevant as to general health status and lifespan.

Patients with FPLD have normal fat distribution during childhood, abnormalities only occurring in adolescence. This is probably the reason for the absence of lipodystrophy in the patient's 9-year-old daughter, in whom we found the same *LMNA* mutation. Our patient's phenotype, i.e. fat atrophy on the extremities, buttocks and thorax, with accumulation thereof on the face, neck and axillae, and in the pubic region, suggested familial partial lipodystrophy [1]. Acanthosis nigricans, polycystic ovaries and hypertension can also occur in such patients, though our patient did not have them. Our patient's hepatomegaly with hepatic steatosis found on ultrasonography, plus slightly increased ALT and AST, are consistent with observations in FPLD patients described in the literature [15]. The muscle pathology in FPLD can manifest as severe myalgia, and this symptom was present in our patient. Recent studies have shown that FPLD patients may display hypertrophy of type 1 and 2 muscle fibres, as well as non-specific changes in muscles, and myelin swellings at paranodal regions. A recent hypothesis holds that the myopathy and neuropathy in FPLD may be associated with interference of lipodystrophy-associated mutation with Smad signalling [16]. It is not possible to assess skeletal muscle morphology in our patient as the specimen from the femoral quadriceps contained only adipose tissue, although in the previous biopsy from the brachial biceps, done in another hospital, no abnormalities were found on light microscopy. Lipodystrophy may be accompanied by heart pathology, i.e. septum hypertrophy, atheromatosis in coronary vessels and conduction disturbances [17]. Our additional tests showed no heart abnormalities in our patient at the time of examination, though we may not exclude them in future. The abnormal balance as regards female hormones may be responsible for the irregular periods, which are frequently observed in women with Dunnigan lipodystrophy, who may also experience infertility and miscarriages.

Results of diagnostic tests in FPLD reveal insulin resistance, hyperinsulinaemia, hyperglycaemia, hypertriglyceridaemia and low HDL cholesterol. The earliest abnormality is hyperinsulinaemia, which may initially compensate for insulin resistance. Dyslipidaemia also precedes the onset of impaired glucose tolerance and diabetes and it seems to be related to the extent of fat loss [18]. Diabetes arises in the third decade of life, the predisposing factors being higher BMI, higher body fat content and an elevated fasting level of triglycerides [4,5]. At diagnosis, our patient had impaired glucose tolerance with insulin resistance, and slight dyslipidaemia with a decreased HDL cholesterol level. We did not expect serum lipid abnormalities in the patient's daughter, who was 9 years old at the time of examination. Dyslipidaemia appears later, when fat loss is evident, i.e. in adolescence, and then regular control of metabolic parameters is recommended.

Typical lamin-dependent FPLD is very rare in the general population, estimated to be present with a frequency of 1 : 10 000 000. The majority of patients described in the literature originate from Europe, though this is the first report of genetically confirmed FPLD in the Polish population. Among the more than 320 *LMNA* mutations described in the UMD-LMNA database and confirmed as causal factors in FPLD, nearly 90% affect exon 8 of the *LMNA* gene. The majority of these are in the "hot spot", which includes

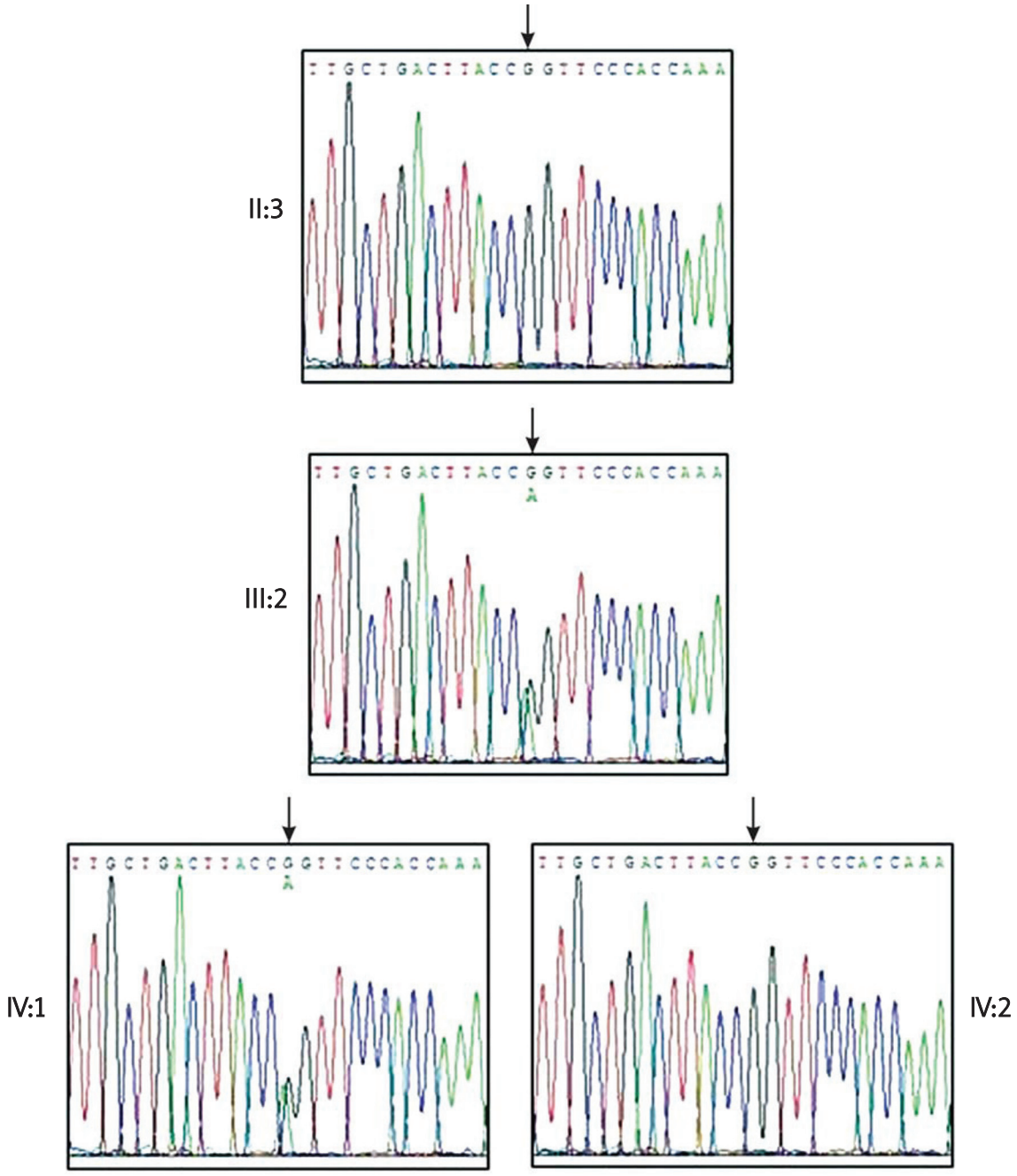

Fig. 3. Sequencing of the direct stand of *LMNA* exon 8 revealing heterozygous point mutation 1445G>A (p.Arg482Gln) in proband (III:2), and the same heterozygous point mutation in the patient's daughter (IV:1)

codon 482. The most frequent substitutions are c.1444C>T (p.Arg492Trp) and c.1445G>A (p.Arg482Gln), accounting for 103 and 75 of 327 described cases, respectively. [19,20] Noting the existence of the *LMNA* "hot spot" for FPLD, we began our genetic screening in the patients with analysis of exon 8 of *LMNA*, in this way confirming the presence of nucleotide substitution at exactly the expected location, i.e. c.1445G>A (p.Arg482Gln). We found no mutations in the remaining *LMNA* exons.

The patient described presents typical symptoms of familial partial lipodystrophy. Impaired glucose tolerance and insulin resistance are being treated in line with recommendations. Detection of the *LMNA* heterozygous mutation c.1445G>A in the patient's daughter allows for earlier diagnosis and for treatment of the metabolic syndrome while the sufferer is still in early adulthood.

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Disclosure

Authors report no conflict of interest.

References

1. Garg A., Peshock R.M., Fleckenstein J.L. Adipose tissue distribution pattern in patients with familial partial lipodystrophy (Dunnigan variety). *J Clin Endocrinol Metab* 1999; 84: 170-174.
2. Cao H., Hegele R.A. Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy. *Hum Mol Genet* 2000; 9: 109-112.
3. Garg A. Gender differences in the prevalence of metabolic complications in familial partial lipodystrophy (Dunnigan variety). *J Clin Endocrinol Metab* 2000; 85: 1776-1782.
4. Hegele R.A. Premature atherosclerosis associated with monogenic insulin resistance. *Circulation* 2001; 103: 2225-2229.
5. Haque W.A., Oral E.A., Dietz K., et al. Risk factors for diabetes in familial partial lipodystrophy, Dunnigan variety. *Diabetes Care* 2003; 26: 1350-1355.
6. Worman H.J., Bonne G. "Laminopathies": a wide spectrum of human diseases. *Exp Cell Res* 2007; 313: 2121-2133.
7. Bonne G., Di Barletta M.R., Varnous S., et al. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet* 1999; 21: 285-288.
8. Fatkin D., MacRae C., Sasaki T., et al. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *New Eng J Med* 1999; 341: 1715-1724.
9. De Sandre-Giovannoli A., Chaouch M., Kozlov S., et al. Homozygous defects in LMNA, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot-Marie-Tooth disorder type 2) and mouse. *Am J Hum Genet* 2002; 70: 726-736.
10. Novelli G., Muchir A., Sangiuolo F., et al. Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. *Am J Hum Genet* 2002; 71: 426-431.
11. De Sandre-Giovannoli A., Bernard R., Cau P., et al. Lamin a truncation in Hutchinson-Gilford progeria. *Science* 2003; 300: 2055.
12. Eriksson M., Brown W.T., Gordon L.B., et al. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 2003; 423: 293-298.
13. Navarro C.L., De Sandre-Giovannoli A., Bernard R., et al. Lamin A and ZMPSTE24 (FACE-1) defect cause nuclear disorganization and identify restrictive dermatopathy as a lethal neonatal laminopathy. *Hum Mol Gen* 2004; 13: 2493-2503.
14. Garg A. Acquired and inherited lipodystrophies. *NEJM* 2004; 350: 1220-1234.
15. Lüdtke A., Genschel J., Brabant G., et al. Hepatic steatosis in Dunnigan-type familial partial lipodystrophy. *Am J Gastroenterol* 2005; 100: 2218-2224.
16. Spuler S., Kalbhenn T., Zabojszcza J., et al. Muscle and nerve pathology in Dunnigan familial partial lipodystrophy. *Neurology* 2007; 68: 677-683.
17. Vantyghem M.C., Pigny P., Maurage C.A., et al. Patients with familial partial lipodystrophy of the Dunnigan type due to a LMNA R482W mutation show muscular and cardiac abnormalities. *J Clin Endocrinol Metab* 2004; 89: 5337-5346.
18. Simha V., Garg A. Lipodystrophy: lessons in lipid and energy metabolism. *Curr Opin Lipidol* 2006; 17: 162-169.
19. Speckman R.A., Garg A., Du F., et al. Mutational and haplotype analyses of families with familial partial lipodystrophy (Dunnigan variety) reveal recurrent missense mutations in the globular C-terminal domain of lamin A/C. *Am J Hum Genet* 2000; 66: 1192-1198.
20. Shackleton S., Lloyd D.J., Jackson S.N., et al. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. *Nat Genet* 2000; 24: 153-156.