Atypical Generalized Lipoatrophy and Severe Insulin Resistance due to a Heterozygous LMNA p.T10I Mutation

clinical case report

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ABSTRACT

Lipodystrophies are a group of heterogeneous disorders characterized by the loss of adipose tissue and metabolic complications. The main familial forms of lipodystrophy are Congenital Generalized Lipodystrophy and Familial Partial Lipodystrophy (FPLD). FPLD may result from mutations in the *LMNA* gene. Besides FPLD, mutations in *LMNA* have been shown to be responsible for other inherited diseases called laminopathies. Here we describe the case of a 15-year-old girl who was referred to our service due to diabetes mellitus and severe hypertriglyceridemia. Physical examination revealed generalized loss of subcutaneous fat, confirmed by DEXA (total body fat 8.6%). As the patient presented with pubertal-onset of generalized lipodystrophy and insulin resistance, molecular analysis of the *LMNA* gene was performed. We identified a heterozygous substitution in exon 1 (c.29C>T) predicting a p.T10I mutation. In summary, we describe an atypical phenotype of lipodistrophy associated with a *de novo* appearance of the p.T10I mutation in *LMNA* gene. **(Arq Bras Endocrinol Metab 2008; 52/8:1252-1256)**

Keywords: Lamin A/C; Lipodystrophy; Insulin resistance; *LMNA* gene

RESUMO

Lipoatrofia Generalizada Atípica e Resistência Insulínica Grave Devido à Mutação p.T10I em Heterozigose no Gene *LMNA*.

As lipodistrofias são um grupo heterogêneo de doenças caracterizadas por perda de tecido adiposo e complicações metabólicas. As formas hereditárias mais importantes de lipodistrofias são: lipodistrofia congênita generalizada e lipodistrofia parcial familiar (LDPF). LDPF resulta de mutações no gene *LMNA* que codificam as lâminas tipo A. Além da LDPF, mutações no gene *LMNA* são responsáveis por outras doenças hereditárias, denominadas laminopatias. Descrevemos o caso de uma paciente de 15 anos de idade encaminhada por diabetes melito e hipertrigliceridemia grave. Ao exame físico, apresentava perda generalizada de gordura subcutânea que foi confirmada por DEXA (gordura corporal total 8,6%). Como a paciente apresentava perda de gordura de início na puberdade e resistência insulínica, foi realizada análise molecular do gene *LMNA*. Identificamos uma substituição em heterozigose no éxon 1 (c.29C>T), resultando na mutação p.T10I. Em sumário, um caso de fenótipo atípico de lipodistrofia generalizada devido à mutação *de novo* p.T10I no gene *LMNA* é descrito. **(Arq Bras Endocrinol Metab 2008; 52/8:1252-1256)**

Descritores: Lâminas A/C; Lipodistrofia; Resistência insulínica; Gene *LMNA*

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Lipodystrophies are a group of clinically heterogeneous disorders characterized by the loss of adipose tissue. Metabolic complications such as insulin resistance, impaired glucose tolerance, dyslipidemia and hepatic steatosis are generally present in affected patients and their severity is determined by the extent of fat loss (1,2). Lipodystrophies are classified according to their origin as familial (or genetic) and acquired types; and according to their clinical pattern of fat loss as generalized or partial.

The main familial forms of lipodystrophy are Congenital Generalized Lipodystrophy (CGL) or Berardinelli-Seip Syndrome (OMIM 269700) and Familial Partial Lipodystrophy (FPLD), Dunnigan variety (OMIM 51660). CGL is a rare autosomal recessive disorder characterized by a nearly complete absence of adipose tissue since birth or early infancy. Familial Partial Lipodystrophy, Dunnigan variety is an autosomal dominant disease characterized by gradual loss of adipose tissue from the extremities and trunk starting at the puberty, and subsequent fat accumulation on the face and neck. Two distinct genes were found to be responsible for the majority of CGL cases: the gene encoding the enzyme 1-acyl-glycerol phosphate acyltransferase 2 (*AGPAT2*) and the *BSCL2* gene which encodes a protein called seipin of unknown function (3,4). FPDL may result from heterozygous mutations in the *LMNA* gene (5,6). This gene is located on chromosome 1q 21-22 and contains 12 exons. *LMNA* gene, through alternative splice, encodes lamin A and lamin C (7). Lamins are structural components of nuclear lamina and belong to the intermediate filament family of proteins. Besides FPLD, mutations in *LMNA* have been shown to be responsible for other inherited diseases such as Emery-Dreifuss muscular dystrophy type 2, dilated cardiomyopathy and conduction system disease, Hutchinson-Gilford Progeria Syndrome, Charcot-Marie-Tooth disorder and others (8-11). The term laminopathies is used to collectively call these diseases. Furthermore, *LMNA* mutations were found in atypical progeroid syndromes and in a pubertal-onset generalized lipodystrophy (12-14).

Here, we report the case of a patient with generalized lipodystrophy harboring the heterozygous p.T10I mutation in *LMNA* gene.

SUBJECTS AND METHODS

We studied a 15-year old girl who was referred to our hospital due to diabetes mellitus and hypertriglyceridemia. The patient and her parents were informed about the aims of the study and gave their written consent.

Molecular analysis of LMNA gene

DNA was extracted from peripheral blood leukocytes using a commercial kit (Puregene DNA Isolation Kit, Gentra System, Minneapolis, MN, USA). Exons 1-12 and the intron-exon boundaries of *LMNA* gene were amplified by PCR. The PCR products were directly sequenced with the use of Big Dye Terminator Cycle Sequencing Reaction Kit version 3.1 and analyzed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, CA, USA).

Analysis of total and segmental body fat

Dual-energy-x-ray absorptiometry (DEXA) was used to evaluate whole-body and regional fat with the Hologic QDR-4500A equipment.

Biochemical measurements

Plasma glucose was determined by the glucose-oxidase method. Cholesterol contents of lipoproteins fractions and triglycerides were measured enzymatically. Plasma adiponectin concentrations were measured by radioimmunoassay (Linco Research, St Charles, MI, USA).

RESULTS

Case Report

In 2001, a 15-year-old girl was referred to our hospital due to diabetes mellitus and hypertriglyceridemia. Both conditions were diagnosed at age of 14 yr during investigation for delayed puberty. She was using NPH Insulin 14U/day, Metformin 1 g/day and Bezafibrate 400 mg/day. Physical examination revealed generalized loss of subcutaneous fat, including palmar aspects of hand and plantar aspects of feet. Prominent superficial veins and xantomas were seen on limbs (Figure 1). The patient referred that her subcutaneous fat gradually disappeared from the age of 11. Acanthosis nigricans was present on axilla and neck. Her weight was 33.9 kg, height 1.51 m and BMI 15 kg/m^2. Blood pressure was 150x100 mmHg. She had pubic hair at Tanner stage 4,

however no breast development (Tanner stage 1). The liver was palpable at 6 cm below the right costal margin and spleen at 1 cm below the left costal margin. She presented thinning of scalp hair and sparse grey hair; no other progeroid characteristics were noted.

Laboratory measurements showed fasting plasma glucose of 143 mg/dl, HbA1C of 6.5%, triglycerides of 877 mg/dL, total cholesterol of 170 mg/dL, HDL-cholesterol of 20 mg/dL. Liver function tests showed alanine aminotransferase of 42 U/L (normal <31 U/L), aspartate aminotransferase of 36 U/L (normal <32 U/L), alkaline phosphatase of 562 U/L (normal <460 U/L) and γ glutamyltranspeptidase of 56 U/L (normal <32 U/L). Plasma adiponectin concentration of 0.6 ng/mL (reference range: 5-30 ng/mL). Blood electrolytes, renal function and thyroid hormone level were normal.

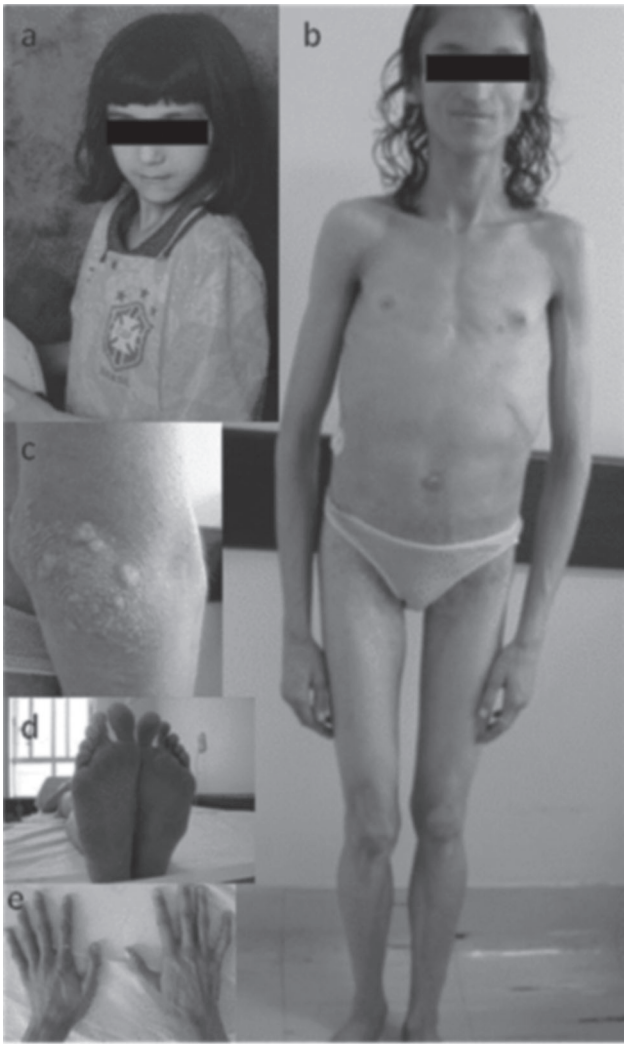

Figure 1. Photography of the patient at approximately 8 years of age showing the normal appearance (A) and at current age showing generalized lipoatrophy, sparse body hair, protuberant abdomen, and no breast development (B). C, detail of xantomas in the elbow. D,E, close-up of her feet and hands. Note loss of fat and the presence of callosities in her feet.

Abdominal ultrasonography showed hepatosplenomegaly and liver steatosis. Doppler echocardiography showed small atrial septum defect, *ostium secundum* type (4 mm), without any other abnormality. Dual energy x-ray absorptiometry was performed to evaluate whole-body and regional fat. The percentage of total body fat was 8.6%, corresponding to 2,924 g (reference range: 30.3 %± 1.5), legs 6.3% (reference range: 33.1%±1.5), arms 8.5% (reference range: 30.2%±1.8), trunk 7.8% (reference range: 29.0%±1.6). Therefore, a generalized loss of body fat was present. The reference values were reported by Mazess and cols. (15).

During the follow-up period a worsening on her metabolic state was observed coincident with a decrease in body fat (percentage of total body fat in 2004: 4.0%). The most recent evaluation showed an HbA1C of 13.5% despite high doses of insulin (1200 U/day) and triglycerides of 9,838 mg/dL despite the use of statin, fibrate and nicotinic acid.

Molecular analysis of LMNA gene

As the patient presented with pubertal-onset of generalized lipodystrophy and insulin resistance, molecular analysis of the *LMNA* gene was performed. We identified a heterozygous substitution in exon 1 (c.29C> T) predicting the substitution of threonine, a polar hydrophilic amino acid, to isoleucine, a non polar hydrophobic amino acid, at codon 10 (p.T10I mutation) in the N-terminal domain of the protein (Figure 2). The amino acid threonine at this position in *LMNA* gene is hi-

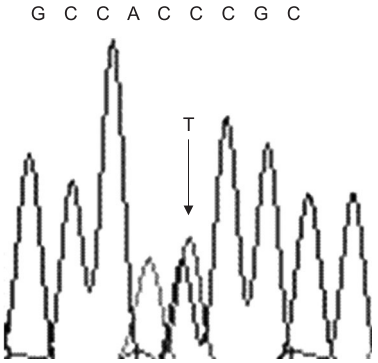

Figure 2. Sequencing electropherogram showing the heterozygous C→ T substitution at nucleotide 29 (exon 1)

Table 1. Amino acid sequence alignment for lamin A/C from various species. The residue affected by the mutation described (*LMNA* T10I) is shown in bold.

Organism	Protein		Sequence	
Homo sapiens	Lamin A/C	1	METPSQRRA**T**RSGAQASSTPLSPTRI	26
Macaca mulatta	Lamin A/C	1	METPSQRRA**T**RSGAQASSTPLSPTRI	26
Equus caballus	Lamin A/C	1	METPSQRRA**T**RSGAQASSTPLSPTRI	26
Mus musculus	Lamin A	1	METPSQRRA**T**RSGAQASSTPLSPTRI	26
Rattus norvegicus	Lamin-A	1	METPSQRRA**T**RSGAQASSTPLSPTRI	26
Xenopus tropicalis	Lamin A/C		METPGQKRA**T**RSTHTPLSPTRITRLQ26	26

ghly conserved across different species (Table 1), suggesting an important role in protein function. We did not observe this mutation in both of her parents, indicating that it is a *de novo* mutation.

DISCUSSION

Here we report the case of a patient with pubertal-onset generalized lipoatrophy, severe insulin resistance and hepatic steatosis harboring the p.T10I mutation in *LMNA* gene. The switch from polar hydrophilic threonine to non polar hydrophobic isoleucine in the lamin A/C N-terminal head may have functional consequences. Accordingly, Csoka et al showed in cultured fibroblasts that the p.T10I mutation causes severe consequences for nuclear morphology such as lobulations of the nuclear membrane, and some of these lobulations contained no chromatin, indicating that the lamina had detached from the chromatin (14).

Laminopathies are characterized by a complex genotype/phenotype relationship and *LMNA* mutations and redistribution of adipose tissue are more frequently observed in Familial Partial Lipodystrophy (Dunnigan variety) (6,16,17). Also, lipodystrophy and metabolic complications associated with insulin resistance have been reported in Mandibuloacral Dysplasia (18), and some affected patients were found to carry homozygous missense mutations in *LMNA* gene (19-21). The clinical phenotype of the patient reported herewith does not resemble any of these two conditions.

Recently, Caux et al described a male patient harboring the heterozygous R133L *LMNA* mutation that has some features similar to those found in our patient: pubertal-onset generalized lipodystrophy, insulin-resistant diabetes, hypertriglyceridemia and liver steatosis (13). However, hypertrophic cardiomyopathy and disemined leucomelanodermic papules also described by Caux et al were not observed in our patient.

The p.T10I mutation was previously described by Csoka et al in a patient who was originally diagnosed with atypical progeria, but was later reclassified as Seip Syndrome based on generalized lipoatrophy, hyperglycemia and hypertriglyceridemia (14). However, no further information is provided such as the age of onset of these abnormalities, making difficult the comparison with this patient. Metabolic alterations such as insulin resistance, hypertriglyceridemia and liver steatosis are characteristics of lipodystrophies. Recent studies describe new phenotypes of metabolic laminopathies, even in the absence of obvious clinical lipoatrophy (22) or associated with premature ageing process (14).

Mutations associated with FPLD are frequently clustered in the *LMNA* region encoding the carboxi-terminal domain (2). The present observation illustrates a generalized lipoatrophy with a very severe insulin resistance due to a mutation in *LMNA* region encoding the N-terminal head. Previous reports of generalized lipoatrophy as a manifestation of laminopathy were also associated with mutations which affect the amino-terminal head domain or the α-helical rod domain of the protein (13,14,23). These observations pointed out that the heterogeneity of lipodystrophies as a manifestation of laminopathy.

In summary, here we describe an atypical phenotype of lipodystrophy associated with a *de novo* appearance of the p.T10I mutation in *LMNA* gene. The identification of mutation in this gene implies in a careful clinical and laboratory evaluation and follow-up of the affected patient. Also, family members should be screened for an early detection and adequate treatment.

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REFERENCES

1. Garg A. Acquired and Inherited Lipodystrophies. New Engl J Med. 2004;350(18):1220-34.
2. Agarwal AK, Garg A. Genetic basis of lipodystrophies and management of metabolic complications. Annu Rev Med. 2006;57:297-311.
3. Agarwal AK, Arioglu E, de Almeida S, Akkoc N, Taylor SI, Bowcock AM, et al. AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. Nat Genet. 2002;31(1):21-3.
4. Magre J, Delépine M, Khallouf E, Gedde-Dahl Jr T, Van Maldergem L, Sobel E, et al. Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. Nat Genet. 2001; 28(4):365-70.
5. Peters JM, Barnes R, Bennett L, Gitomer WM, Bowcock AM, Garg A. Localization for the gene for familial partial lipodystrophy (Dunnigan variety) to chromosome 1q21-22. Nat Genet. 1998;18(3):292-5.
6. Cao H, Hegele RA. Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy. Hum Mol Genet. 2000;9(1):109-12.
7. Lin F, Worman HJ. Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. J Biol Chem. 1993;268(22):16321-6.
8. Di Barletta MR, Ricci E, Galluzzi G, Tonali P, Mora M, Morandi L, et al. Different mutations in the LMNA gene cause autosomal dominant and autosomal recessive Emery-Dreifuss muscular dystrophy. Am J Hum Genet. 2000;66(4):1407-12.
9. Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, et al. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. N Engl J Med. 1999;341(23):1715-24.
10. Cao H, Hegele RA. LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). J Hum Genet. 2003;48(5):271-4.
11. De Sandre-Giovannoli A, Chaouch M, Kozlov S, Vallat JM, Tazir M, Kassouri N, 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(3):726-36.
12. Chen L, Lee L, Kudlow BA, Dos Santos HG, Sletvold O, Shafeghati Y, et al. LMNA mutations in atypical Werner's syndrome. Lancet. 2003;362(9382):440-5.
13. Caux F, Dubosclard E, Lascols O, Buendia B, Chazouillères O, Cohen A, et al. A New Clinical Condition Linked to a Novel Mutation in Lamins A and C with Generalized Lipoatrophy, Insulin-Resistant Diabetes, Disseminated Leukomelanodermic Papules, Liver Steatosis, and Cardiomyopathy J Clin Endocrinol Metab.2003; 88(3):1006-13.
14. Csoka AB, Cao H, Sammak PJ, Constantinescu D, Schatten GP, Hegele RA. Novel lamin A/C gene (LMNA) mutations in atypical progeroid syndromes. J Med Genet. 2004;41(4):304-8.
15. Mazess RB, Barden HS, Bisek JP, Hanson J. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. Am J Clin Nutr. 1990;51(6):1106-12.
16. Shackleton S, Lloyd DJ, Jackson SN, Evans R, Niermeijer MF, Singh BM, et al. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. Nat Genet. 2000;24(2):153-6.
17. Speckman RA, Garg A, Du F, Bennett L, Veile R, Arioglu E, 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(4):1192-8.
18. Cutler DL, Kaufmann S, Freidenberg GR. Insulin-resistant diabetes mellitus and hypermetabolism in mandibuloacral dysplasia: a newly recognized form of partial lipodystrophy. J Clin Endocrinol Metab. 1991;73(5):1056-61.
19. Novelli G, Muchir A, Sangiuolo F, Helbling-Leclerc A, D'Apice MR, Massart C, et al. Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. Am J Hum Genet. 2002;71(2):426-31.
20. Garg A, Cogulu O, Ozkinay F, Onay H, Agarwal AK. A novel homozygous Ala529Val LMNA mutation in Turkish patients with mandibuloacral dysplasia. J Clin Endocrinol Metab. 2005;90(9):5259-64.
21. Simha V, Agarwal AK, Oral EA, Fryns JP, Garg A. Genetic and phenotypic heterogeneity in patients with mandibuloacral dysplasia-associated lipodystrophy. J Clin Endocrinol Metab. 2003;88(6):2821-4.
22. Decaudain A, Vantyghem MC, Guerci B, Hécart AC, Auclair M, Narbonne YRH, et al. New Metabolic Phenotypes in Laminopathies: LMNA Mutations in Patients with Severe Metabolic Syndrome. J Clin Endocrinol Metab. 2007;92(12):4835-44
23. Jacob KN, Baptista F, Santos HG, Oshima J, Agarwal AK, Garg A. Phenotypic heterogeneity in body fat distribution in patients with atypical Werner´s Syndrome due to heterozygous Arg133Leu laminin A/C mutation. J Clin Endocrinol Metab. 2005;90(12):6699-06.

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