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Subclinical myopathy in a child with neutral lipid storage disease and mutations in the *PNPLA2* gene

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ABSTRACT

We report a 14-year-old-boy with markedly elevated serum creatine kinase (CK) levels, in whom massive triglyceride storage was found in peripheral blood leukocytes and in muscle biopsy. Sequencing *PNPLA2*, the gene encoding the adipose triglyceride lipase (ATGL) and responsible for the neutral lipid storage disease with myopathy (NLSDM), we identified two heterozygous mutations, including a previously reported nonsense and a novel missense mutation in the patatin domain of the gene.

Lipid storage myopathy can be clinically silent in childhood and presenting only with hyperCKemia.

1. Introduction

Neutral lipid storage diseases (NLSDs) are multisystem lipid disorders due to a defect either in the adipose triglyceride lipase (ATGL) or in the alpha/beta-hydrolase domain-containing protein 5 (ABHD5) [1,2]. ABHD5 activates ATGL, which catalyzes the first step in the hydrolysis of triacylglycerol to produce free fatty acid and diacylglycerol. Mutations in *PNPLA2*, the gene encoding ATGL, cause NLSD with myopathy (NLSDM, OMIM # 610717) [3], whereas mutations in the CGI-58 gene, coding for ABHD5, cause NLSD with ichthyosis (NLSDI), also termed Chanarin–Dorfman syndrome (CDS, OMIM # 275630) [2,4].

The hallmark of both diseases is the presence of neutral lipid droplets storage in multiple tissues, including skeletal muscle and leukocytes.

CDS/NLSDI is characterized by the presence of ichthyosiform nonbullous erythroderma. Slowly progressive weakness of proximal limb muscles with raised serum muscle enzymes can be detected in about 60% of cases. Hepatomegaly, various ocular symptoms (cataract, nystagmus, and strabismus), hearing loss, mild mental retardation, short stature, microcephaly and intestinal involvement are also described [4,5].

Patients with NLSM start complaining of limb weakness at the beginning of the third decade of life despite lipid accumulation most likely precedes the clinical symptoms [6,7]. Serum CK is always highly elevated (up to 50-fold normal values) and occasionally can be the only sign in childhood as seen in an asymptomatic 18-year-old-girl [8].

The course of the disease is slowly progressive and cardiomyopathy may develop at later stages in half of the patients. No ichthyosis is present and neither the central nor the peripheral nervous systems are involved [1,8].

Here, we report clinical, morphological, magnetic resonance imaging (MRI), and genetic findings of a boy carrying mutations in *PNPLA2* and massive lipid storage in a muscle biopsy without overt muscle symptoms except for persistently markedly elevated serum CK levels.

2. Case report

The propositus is a 14-year-old boy, the first child of healthy non-consanguineous Italian parents.

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He was born at term by normal delivery after an uncomplicated pregnancy. His motor milestones were normal.

At five years of age he was referred to our Center for additional evaluation of elevated levels of serum CK discovered during a routine blood test. Family history was negative for neuromuscular disorders. His younger brother is healthy.

General physical examination was normal and the patient denied the occurrence of myalgia or cramps. Neurologic examination did not reveal any muscle weakness or CNS impairment. Muscle tone, and strength were normal for age and there was no calf hypertrophy or contractures. Serum CK was 1200 U/l (normal values less than 150), while routine laboratory investigations and screening for metabolic disorders were normal. Electromyography of the anterior tibialis muscle showed myogenic signs, while nerve conduction studies were normal. EKG was normal.

In keeping with the national guidelines on the diagnostic approach to asymptomatic hyperCKemia [9], the patient underwent a muscle biopsy after obtaining parental informed written consent. The biopsy specimen was taken from the quadriceps muscle and processed according to standard techniques for routine histology

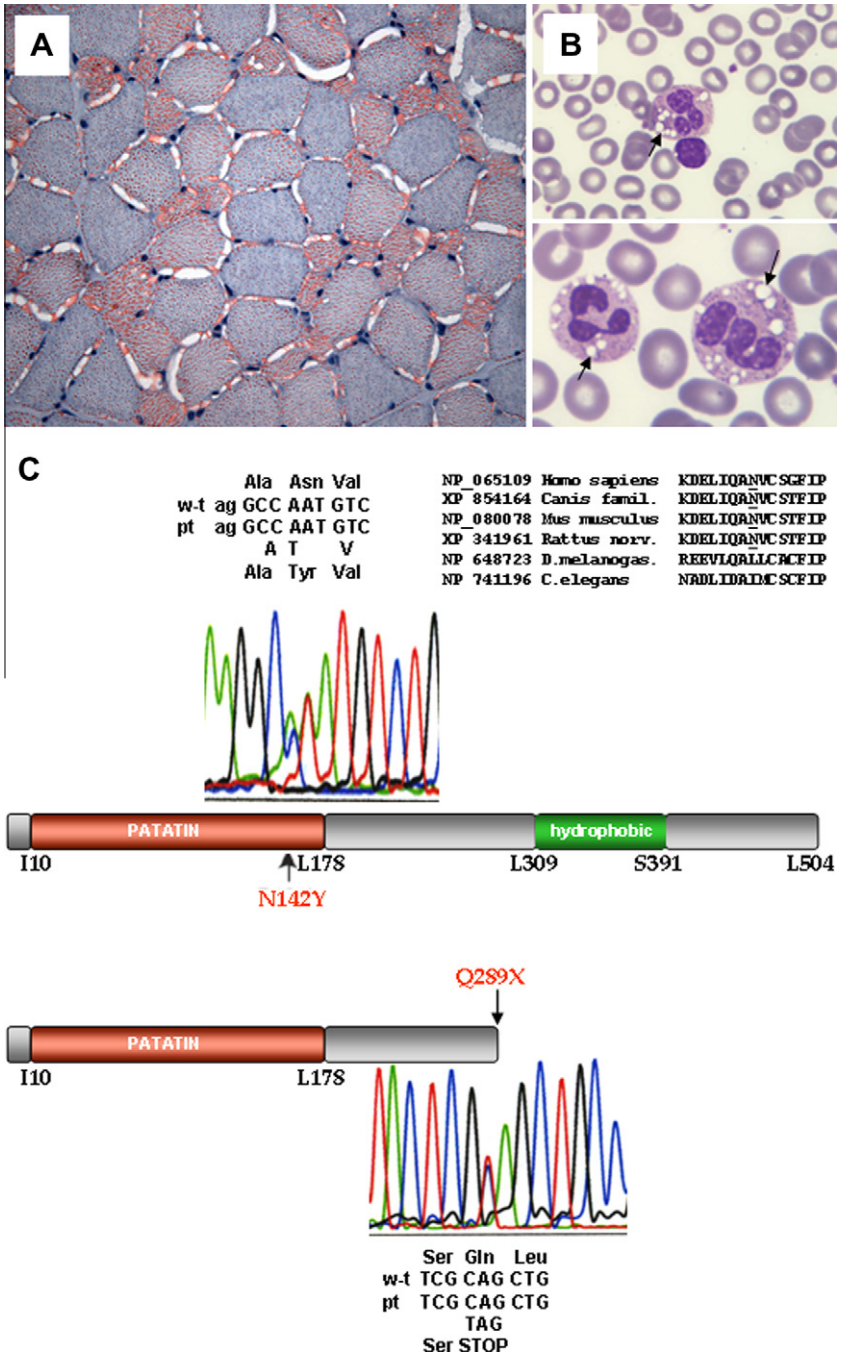

Fig. 1. (A) Oil red O staining for lipids on muscle section highlighted the presence of lipid vacuoles in numerous fibers (orange red dots). (B) Peripheral blood smear showed the presence of lipid droplets in the patient's leukocytes (Jordan anomaly) (arrows). (C) Electropherograms and location of both missense N142Y (upper panel) and nonsense Q289X (lower panel) mutations in the *PNPLA2* gene. For missense N142Y mutation conservation among species is also shown. Protein domains are also schematised as bars of different colors and the patatin and hydrophobic domain are shown with numbers indicating the aminoacid. Diagram is not drawn on scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and histochemistry. We detected multiple neutral lipid vacuoles in both fibers type I and II. Neither necrotic or ragged-red fibers nor rimmed vacuoles were observed. Glycogen was normal (Fig. 1A).

Total and free carnitine dosage in blood and in muscle homogenate was normal. Carnitine palmitoyltransferase (CPT) determination and respiratory chain enzyme analysis in muscle homogenate were normal.

In the following years, the patient had variably, but persistently elevated serum CK levels. His clinical picture remained stable and muscle performances were in keeping with his peers.

Routine laboratory investigations, including cholesterol and triglycerides, were again normal.

Peripheral blood smear showed the presence of lipid droplets in the patient's leukocytes (Fig. 1B).

Liver and heart ultrasound were normal, as well as brain MRI spectroscopy. Oral test glucose was normal whilst euglycemic clamp showed a severe hepatic and a mild peripheral insulin resistance.

MRI of skeletal muscle, including whole body coronal T1-weighted, axial T1-weighted sequences in lower girdle muscles, thighs and lower legs, and in-phase and out-of phase scans of the legs, was performed on a 1.5-T MR system (Achieva Intera, Philips Medical System, Eindhoven, Nederland) using standard protocols [7,10].

Genomic DNA was obtained from peripheral lymphocytes using a standard salting-out technique. The entire coding regions and the exon–intron boundaries of the *PNPLA2* gene were PCR-amplified, and directly sequenced using BigDye 3.1 chemistry on a multicolor fluorescence-based DNA analysis system (ABI Prism 3100 Genetic Analyzer; Applied Biosystems, Foster City, CA).

3. Results and discussion

A muscle biopsy performed in an asymptomatic child with elevated levels of serum CK when he was 5-years-old, showed a lipid storage myopathy. Dosage of free and total carnitine, carnitine palmitoyltransferase and respiratory chain enzymes were all normal. At that time, the clinical condition remained undiagnosed. In the following years, the identification of the *PNPLA2* gene [3] prompted us to revaluate the diagnostic approach. A simple peripheral blood smear revealed the presence of lipid accumulations in granulocytes (the so-called Jordans anomaly) leading to a possible diagnosis of NLSM. Moreover, muscle MRI images in our patient were similar to previous report [7] and consistent with a prevalent fatty degeneration of gluteal muscles and posterior thigh and leg muscles with a relative sparing of the anterior compartments, further contributing to define a selective pattern of muscle involvement in NLSM (Fig. 2). However, in our child, minimal changes in anterior compartment of the leg were already detectable in an early and asymptomatic stage of the disease. These findings were highlighted using out-of-phase scans so underlying the importance of this technique in lipid storage myopathies [11]. Expectedly, analysis of *PNPLA2* in the patient's genomic DNA detected two heterozygous mutations, a nonsense C>T mutation at nucleotide 865 (c.865C>T) in exon 7, predicting premature protein truncation at codon 289(p.Q289X) on the paternal allele and a novel missense mutation at nucleotide 424 (c.424A>T, p.N142Y) in exon 4 on the maternal allele (Fig. 1C). The latter variant was scored to be predictably deleterious to protein function in silico (Polyphen2, genetics.bwh.harvard.edu/pph/), and was absent in 200 healthy Italian chromosomes or in large SNP databases (NHLBI Exome Sequencing Project, evs.gs.washington.edu/EVS/).

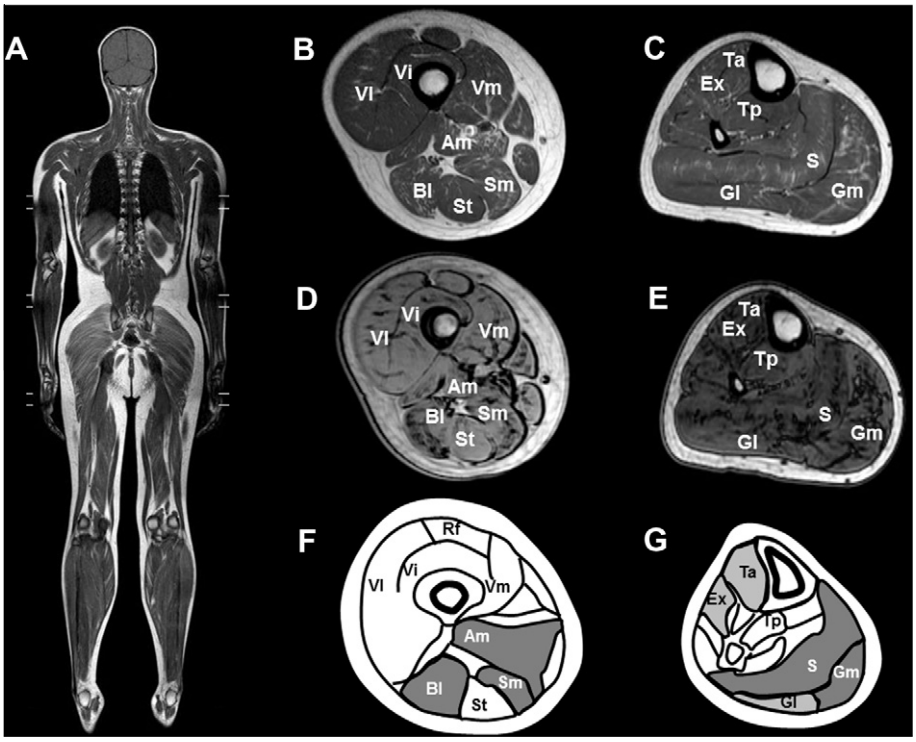

Fig. 2. (A) Coronal T1-weighted slices showed prominent fatty degeneration in gluteal muscles, posterior thigh and posterior leg muscles with sparing of upper girdle muscles. (B) At thigh level, MRI showed predominant involvement of the posterior compartment with marked fatty infiltration of adductor magnus (Am), semimembranous (Sm) and biceps femoris long head (Bl). Quadriceps muscles (Rf, Vl, Vi, Vm) and semitendineous (St) appear to be spared. (C) At lower leg level moderate fatty infiltration of medial gastrocnemius (Gm) and soleus (S) in the posterior compartment was evident with hypotrophy of lateral gastrocnemius (Gl). Mild hyperintense signal changes were present in anterior compartment muscles, tibialis anterior (Ta) and extensor longus digitorum and hallucis (Ex) with sparing of tibialis posterior (Tp). (D and E) Using out-of phase imaging, at thigh and leg level fatty degeneration is reflected by loss of signal intensity in the same muscles. In anterior compartment of the leg, more evident abnormalities in anterior compartment (Ta, Ex), compared to T1 sequences, were present. (F and G) Patterns of muscle involvement in neutral lipid storage disease with myopathy (NLSDM), based on the literature data and our experience. The replacement of muscle tissue by fat is reflected by the dark gray color of the muscle. Initial and/or variable involvement of the muscle is represented by the light gray color. The white color means spared muscles.

To the best of our knowledge, NLSM has been reported in 22 cases [3,7,8,12–19], and 17 mutations have been identified throughout the entire *PNPLA2* gene. On clinical ground, the NLSDM phenotype is characterized by proximal, often asymmetric, muscle weakness in both upper and lower girdle muscles starting in early 30's, a presentation highly resembling limb girdle muscular dystrophies (LGMD). Distal involvement of finger extensors and foot flexors can manifest later in the disease course. In all the patients serum CK is elevated (around 5-fold) and laboratory investigations might reveal hypertriglyceridemia. A few cases develop diabetes mellitus. To date, one case of NLSM has been reported at young age, namely in a 18-year-old-girl that was asymptomatic for myopathy presenting only elevated CK levels [8]. Here we report a NLSDM case detected in childhood without clinically evident myopathy but only hyperCKemia. This finding may further support the notion that hyperCKemia is the early sign of NLSDM in childhood preceding the manifestation of clinical overt myopathy.

PNPLA2 gene encodes for ATGL, a hormone-sensitive lipase that catalyzes the initial step in triglyceride hydrolysis in mammalian adipose tissue. At its N-terminal, ATGL contains a 'patatin domain' common to the acyl-hydrolases while at the C-terminal it displays an hydrophobic region responsible to bind the lipid droplets [3].

Most of the reported mutations of *PNPLA2* gene are nonsense and affect the C-terminal domain of the gene (residues 252–504) and particularly the hydrophobic region (lipid binding domain residues 309–391). Interestingly, our patient carried a novel missense mutation in the patatin-like phospholipase domain (PLPD) (residues 10–179), and a nonsense in the C-terminus. The latter has been previously reported in homozygosis in an Algerian family [Fisher 2007] in association with typical late-onset lipid storage myopathy and classical distribution of muscular weaknesses, In contrast, only two mutations [8,12] have been identified in the PLPD domain which affects the lipase activity, Given the limited penetrance observed in our patient, and in the absence of more functional tests, we can only speculate that the new c.424A>T/p.N142Y alone or in combination with other yet unknown factors – including the patient's genetic background – could protect his muscle from being weak and atrophic notwithstanding the accumulation of lipids, possibly increasing the function of the ATGL protein coded from the non-truncated allele.

Although no effective therapy is currently available for NLSM, encouraging results have recently been showed in patients' cells upon treatment with a beta-adrenergic agent, thought to activate alternative pathway of triglyceride metabolism [7].

In conclusion, we have genetically characterized a 14-year-old boy with hyperCKemia and a subclinical myopathy due to neutral lipid storage.

Search for lipid droplets in peripheral leukocytes and the *PNPL2* gene analysis should be added to the diagnostic work-up of patients with hyperCKemia [20].

References

[1] C. Bruno, S. Dimauro, Lipid storage myopathies, Curr. Opin. Neurol. 21 (2008) 601–606.
[2] M. Schweiger, A. Lass, R. Zimmermann, T.O. Eichmann, R. Zechner, Neutral lipid storage disease: genetic disorders caused by mutations in adipose triglyceride lipase/PNPLA2 or CGI-58/ABHD5, Am. J. Physiol. Endocrinol. Metab. 297 (2009) E289–E296.
[3] J. Fischer, C. Lefèvre, E. Morava, J.M. Mussini, P. Laforêt, A. Negre-Salvayre, M. Lathrop, R. Salvayre, The gene encoding adipose triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy, Nat. Genet. 39 (2007) 28–30.
[4] C. Lefevre, F. Jobard, F. Caux, B. Bouadjar, A. Karaduman, R. Heilig, H. Lakhdar, A. Wollenberg, J.L. Verret, J. Weissenbach, M. Ozgüc, M. Lathrop, J.F. Prud'homme, J. Fischer, Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin–Dorfman syndrome, Am. J. Hum. Genet. 69 (2001) 1002–1012.
[5] C. Bruno, E. Bertini, M. Di Rocco, D. Cassandrini, G. Ruffa, T. De Toni, M. Seri, M. Spada, G. Li Volti, A. D'Amico, F. Trucco, M. Arca, C. Casali, C. Angelini, S. Dimauro, C. Minetti, Clinical and genetic characterization of Chanarin–Dorfman syndrome, Biochem. Biophys. Res. Commun. 369 (2008) 1125–1128.
[6] P. Laforêt, C. Vianey-Saban, Disorders of muscle lipid metabolism: diagnostic and therapeutic challenges, Neuromuscul. Disord. 20 (2010) 693–700.
[7] P. Reilich, R. Horvath, S. Krause, N. Schramm, D.M. Turnbull, M. Trenell, K.G. Hollingsworth, G.S. Gorman, V.H. Hans, J. Reimann, A. MacMillan, L. Turner, A. Schollen, G. Witte, B. Czermin, E. Holinski-Feder, M.C. Walter, B. Schoser, H. Lochmüller, The phenotypic spectrum of neutral lipid storage myopathy due to mutations in the PNPLA2 gene, J. Neurol. 258 (2011) 1987–1997.
[8] H.O. Akman, G. Davidzon, K. Tanji, E.J. Macdermott, L. Larsen, M.M. Davidson, R.G. Haller, L.S. Szczepaniak, T.J. Lehman, M. Hirano, S. DiMauro, Neutral lipid storage disease with subclinical myopathy due to a retrotransposal insertion in the PNPLA2 gene, Neuromuscul. Disord. 20 (2010) 397–402.
[9] L. Morandi, C. Angelini, A. Prelle, A. Pini, B. Grassi, G. Bernardi, L. Politano, C. Bruno, D. De Grandis, P. Cudia, A. Citterio A, High plasma creatine kinase: review of the literature and proposal for a diagnostic algorithm, Neurol. Sci. 27 (2006) 303–311.
[10] E. Mercuri, A. Pichiecchio, S. Counsell, J. Allsop, C. Cini, H. Jungbluth, C. Uggetti, G. Bydder, A short protocol for muscle MRI in children with muscular dystrophies, Eur. J. Paediatr. Neurol. 6 (2002) 305–307.
[11] M. Gaeta, F. Minutoli, A. Toscano, A. Celona, O. Musumeci, S. Racchiusa, S. Mazziotti, Opposed-phase MR imaging of lipid storage myopathy in a case of Chanarin–Dorfman disease, Skeletal Radiol. 37 (2008) 1053–1057.
[12] M. Akiyama, K. Sakai, M. Ogawa, J.R. McMillan, D. Sawamura, H. Shimizu, Novel duplication mutation in the patatin domain of adipose triglyceride lipase (PNPLA2) in neutral lipid storage disease with severe myopathy, Muscle Nerve 36 (2007) 856–859.
[13] K. Kobayashi, T. Inoguchi, Y. Maeda, N. Nakashima, A. Kuwano, E. Eto, N. Ueno, S. Sasaki, F. Sawada, M. Fujii, Y. Matoba, S. Sumiyoshi, H. Kawate, R. Takayanagi, The lack of the C-terminal domain of adipose triglyceride lipase causes neutral lipid storage disease through impaired interactions with lipid droplets, J. Clin. Endocrinol. Metab. 93 (2008) 2877–2884.
[14] K. Hirano, Y. Ikeda, N. Zaima, Y. Sakata, G. Matsumiya, Triglyceride deposit cardiomyovasculopathy, N. Engl. J. Med. 359 (2008) 2396–2398.
[15] F. Campagna, L. Nanni, F. Quagliarini, E. Pennisi, C. Michailidis, F. Pierelli, C. Bruno, C. Casali, S. DiMauro, M. Arca, Novel mutations in the adipose trigliceride lipase gene causing neutral lipid storage disease with myopathy, Biochem. Biophys. Res. Commun. 377 (2008) 843–846.
[16] A. Ohkuma, S. Noguchi, H. Sugie, M.C. Malicdan, T. Fukuda, K. Shimazu, L.C. López, M. Hirano, Y.K. Hayashi, I. Nonaka, I. Nishino, Clinical and genetic analysis of lipid storage myopathies, Muscle Nerve 39 (2009) 333–342.
[17] J. Chen, D. Hong, Z. Wang, Y. Yuan, A novel PNPLA2 mutation causes neutral lipid storage disease with myopathy (NLSDM) presenting muscular dystrophic features with lipid storage and rimmed vacuoles, Clin. Neuropathol. 29 (2010) 351–356.
[18] P. Lin, W. Li, B. Wen, Y. Zhao, D.S. Fenster, Y. Wang, Y. Gong, C. Yan, Novel PNPLA2 gene mutations in Chinese Han patients causing neutral lipid storage disease with myopathy, J. Hum. Genet. 84 (2012), http://dx.doi.org/10.1038/jhg.2012.84.
[19] D.B. Ash, D. Papadimitriou, A.P. Hays, S. DiMauro, M. Hirano M, A novel mutation in PNPLA2 leading to neutral lipid storage disease with myopathy, Arch. Neurol. 69 (2012) 1190–1192.
[20] T. Kyriakides, C. Angelini, J. Schaefer, S. Sacconi, G. Siciliano, J.J. Vilchez, D. Hilton-Jones, European federation of neurological societies, EFNS guidelines on the diagnostic approach to pauci- or asymptomatic hyperCKemia, Eur. J. Neurol. 17 (2010) 767–773.