Neutral lipid storage disease with myopathy: A 10-year follow-up case report

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Abstract

Mutations in PNPLA2 gene encoding for adipose triglyceride lipase (ATGL), involved in triglyceride degradation, lead to an inborn error of neutral lipid metabolism. The disorder that results in abnormal storage of neutral lipid is known as neutral lipid storage disease with myopathy (NLSDM). We report the follow-up of a 30-year-old woman with NLSDM, asymptomatic until age 23. At the age of 18, a high level of CPK and neutral lipid abnormal accumulation in muscle and skin cells suggested NLSDM diagnosis, afterwards confirmed by *PNPLA2* analysis. After 5 years, she developed weakness in the upper and lower extremities. She was put on a low-fat diet with medium-chain triglycerides (MCT) oil supplementation but, although her CPK level decreased, myopathy continued to progress. At present, she presents severe skeletal myopathy without cardiac involvement. In this patient, no beneficial effects on progressive skeletal muscle weakness were detected after the MCT diet, probably due to complete loss of *PNPLA2* expression.

Key Words: Neutral lipid storage diseases; lipid droplets; myopathy; MCT treatment.

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Errors in fat metabolism can induce lipid accumulation in all tissues. Abnormal lipid storage in skeletal muscle fibers determines the onset of lipid storage myopathies (LSMs), a group of metabolic disorders associated with the loss or decrease of function of different proteins.[1] Adipose triglyceride lipase (ATGL) is the rate-limiting enzyme for the hydrolysis of triglycerides (TAGs) stored into lipid droplets (LDs) (Figure 1a).[2] ATGL is a 504 amino acid-long protein characterized by two main functional regions: a patatin domain (residues I10-L178) and a hydrophobic domain (residues P315-P360). The patatin domain contains the catalytic dyad, S47 and D166, essential for lipase activity, and three LC3-interacting region (LIR) motifs involved in ATGL-LC3 interaction. The hydrophobic domain allows ATGL-LD binding.

ATGL mutations cause the onset of an ultra-rare autosomal recessive LSM form, called neutral lipid storage disease with myopathy (NLSDM; MIM 610717).[3] Until now, 107 NLSDM patients have been described.[4-7] All subjects develop progressive skeletal muscle myopathy, with both proximal and distal involvement. An asymmetric muscle involvement has been observed in almost 50% of patients. Muscle biopsy displays massive lipid storage and muscle atrophy. The other main clinical features are cardiomyopathy (40% of patients), and hepatomegaly with altered hepatic enzymes (20% of patients). Some patients also show diabetes, chronic pancreatitis, and hearing loss.[8-11] In general, there is great variability in NLSDM phenotype, and a correlation between the severity degree of clinical symptoms and mutations identified in ATGL-coding gene, *PNPLA2*, is difficult. To date, 60 different *PNPLA2* mutations have been identified and most of them lead to total loss or severe impairment of ATGL activity (Figure 1b). Nevertheless, some patients carrying severe mutations present late and/or mild progressive myopathy, without cardiac involvement.[3] For this reason, NLSDM pathophysiology is still largely unknown, and, at present, no specific treatment exists.

Here we report the follow-up of an NLSDM patient who followed a restricted diet supplemented with MCT oil in the last four years.

Case report

A 30-year-old woman affected by NLSDM was described for the first time by Akman and colleagues in

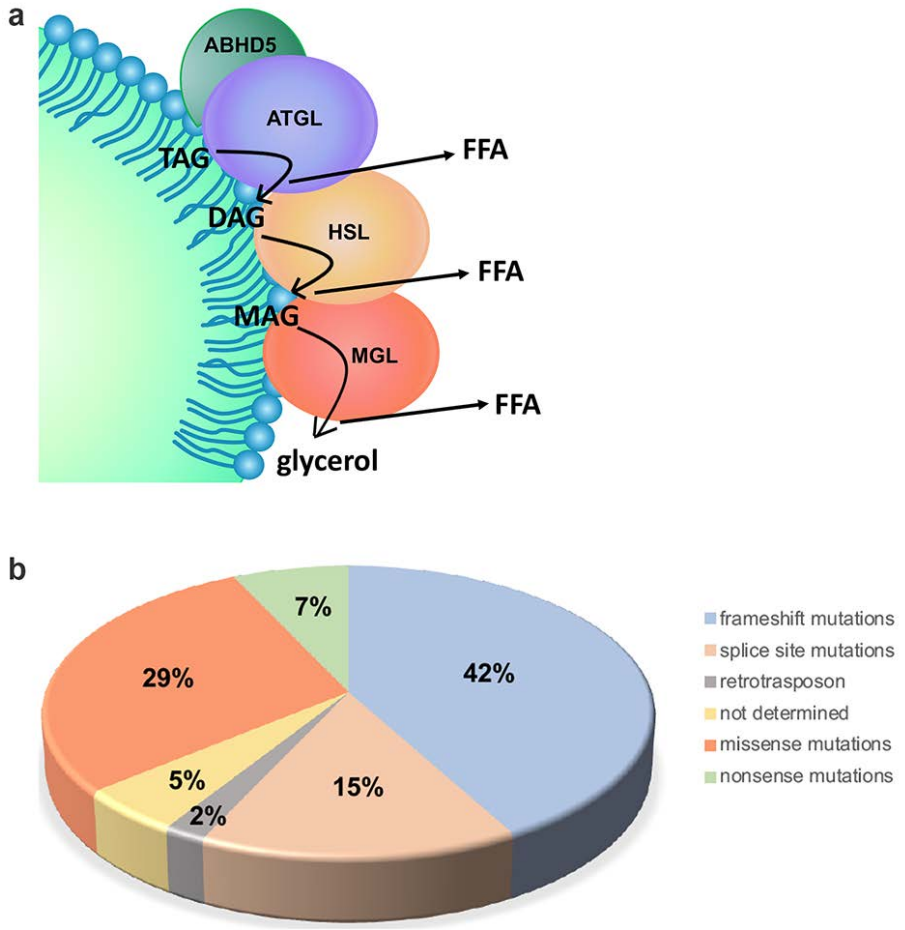

Fig 1. *ATGL pathway and mutations in NLSDM patients. (**a**) Triacylglycerol hydrolysis is composed of a series of interrelated reactions. The primary reaction is performed by the adipose triglyceride lipase (ATGL). This lipase, after activation through the interaction with ABHD5, promotes the release of the first free fatty acid (FFA) from triacylglycerol (TAG), producing diacylglycerol (DAG). In the second step the hormone-sensitive lipase (HSL) leads to DAG conversion into monoacylglycerol (MAG). The lipolytic cascade culminates with the monoglyceride lipase activity (MGL), which induces third FFA and glycerol release. (**b**) Percentage of ATGL different variations found in NLSDM patients. Most of these mutations, such as frameshift and splice site mutations, and retrotransposon insertion, lead to a loss or truncated protein production.*

2010.[12] Since the age of 10, she presented elevated muscle and liver enzymes without myopathy. At the age of 18, the first muscle and skin biopsies revealed abnormal accumulation of intracellular LDs in muscle fibers, in particular in type I fibers, and in skin fibroblasts. These results suggested an NLSDM diagnosis that was confirmed by a genetic investigation. Indeed, sequencing analysis revealed a retrotransposon insertion in exon 3 of *PNPLA2* gene leading to the complete loss of *PNPLA2* mRNA synthesis.[12] Therefore, it could be hypothesized that ATGL protein was completely lacking in the cells of patient. This mutation was not found in 100 healthy subjects.

The patient remained asymptomatic except for hyperCKemia until the age of 23, when she has been experiencing muscle weakness in both arms, shoulders, and hands. These symptoms have then extended to her lower extremities. A calves MRI performed in 2015 showed lipid accumulation.

At 26 years, she was put on a restrictive low-fat diet (15 gr of natural fat per day) supplemented with 30 gr of MCT oil per day. After beginning MCT diet, CPK lowered (from 2640 U/l to 1424 U/l). Nevertheless, muscle weakness did not improve. GSGC test performed in 2020 confirmed progressive myopathy: Walking 10 meters = 10 seconds, score 2 (for mild waddling); Climbing stairs = 13 steps up = 8.10 seconds, 13 step down = 6.40 seconds, score 1 (does not need assistance); Raising from seated floor position with no hands = 2.76 seconds, score 4 (left hand on left thigh); Getting up from chair = 0.61 seconds (< less than 1 second), score 1 (normal). A new MRI at the age

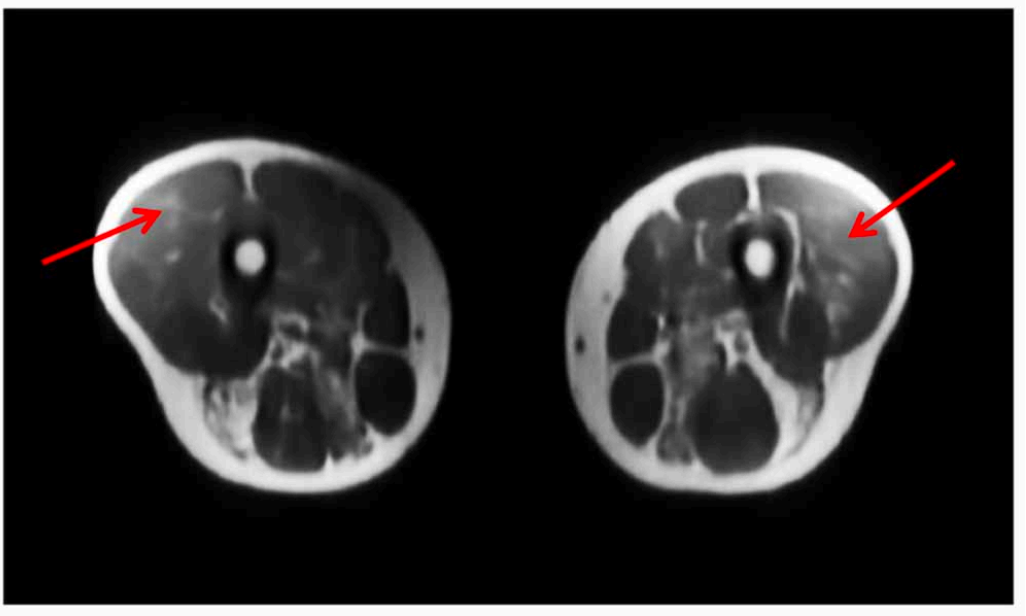

Fig 2. *MRI evaluation in lower limbs. After MCT oil treatment, muscle MRI highlighted advanced fat and connective tissue substitution (arrows) in both lower extremities*

of 27 displayed muscle atrophy as well as tissue substitution in the following muscles: bilateral in tensor fascia-lateral, bilateral in gluteus muscles, in vastus lateralis of the thigh and partially in sartorius, bilaterally in femoral biceps, and bilaterally in semimembranosus (Figure 2). Moreover, fat tissue replacement of deltoid and biceps muscles in the right upper extremity was also observed. In 2021 she has had an increased muscle weakness in both upper and lower extremities. She had trouble walking up the stairs and walking long distances, lifting her arms above her head, and grabbing moderate to heavy objects (i.e. lifting a glass to her mouth with one hand). No heart involvement was referred for cardiac MRI.

Discussion

Defects of neutral lipids metabolism can be caused by ATGL deficiency resulting in excessive storage of TAGs in cytoplasmic LDs and determining NLSDM onset. To date, it is difficult to correlate the clinical phenotype severity with genotype. Most of patients presented ATGL mutations that cause total or dramatic decrease of lipase function. In general, these subjects show an early progressive skeletal muscle myopathy with often a cardiac involvement (40% of patients). Patients carrying ATGL variants which partially maintain enzymatic activity usually develop a slowly progressive myopathy, without hearth defects.

In this report we described a patient with a severe *PNPLA2* mutation causing the total lack of gene expression. This genotype correlates with an early muscle weakness onset which slowly progressed over the years, but it does not determine cardiac disfunction. Nine patients with severe *PNPLA2* mutations have previously been described.[2,9,13-16] Five of them are males who develop both skeletal muscle myopathy and cardiomyopathy. In particular, Pasanisi and coll. reported the case of a man who developed skeletal myopathy at the age of 20 (similar to our patient) and cardiac dysfunction after 4 years.[15] The other patients are females presenting variable age at onset and severity of clinical manifestations. Chen and coll. reported a 40-year-old woman who presented muscle weakness since the age of 35, without cardiomyopathy. Her brother developed general muscle weakness later, at the age of 45, and after 10 years he manifested dilated cardiomyopathy.[9] The second is a Chinese female who presented progressive muscle weakness at the age of 45. In the following 3 years, skeletal myopathy worsened, but there was not cardiac involvement.[13] In 2017 Missaglia et al. described a 54-year-old woman showing progressive skeletal myopathy since the age of 39. A mild left ventricular diastolic dysfunction was detected at 53 of age, but after 12 months heart function appeared normal.[16] Finally, Tavian et al. described an Italian woman presenting progressive skeletal myopathy with onset at the age of 18. After 30 years, she required a pacemaker implantation. Her brother presented muscle weakness since the age of 30 and died at 54 years probably of cardiac disfunction.[2] These data suggest a phenotypic heterogeneity in NLSDM which is not explained by genotype but can depend on other factors. Some authors suggest the protective role of the

estrogens on cardiac function.[2,7] There could be other factors capable of modulating the severity of clinical phenotype, such as the diet or exercise. It was reported that the exercise can have an anti-inflammatory effect which protects against cardiovascular diseases.[17] Long-term physical activity improves the metabolism and works as a natural, strong anti-inflammatory factor because muscles can produce different anti-inflammatory molecules, called myokines, during exercise. Myokines secreted by skeletal muscles can modulate the anti-inflammatory mechanism in other organs. A moderate increase of one of these myokines, interleukin-6 (IL-6), can activate macrophages, maintaining glucose homeostasis, and can inhibit the production of pro-inflammatory TNF-α. These actions improve and maintain cardiac function. Our patient was a ballet dancer who practiced regular exercise for several hours every week for years. This could partially explain the preservation of heart condition.

Differences in environmental factors (diet and different lifestyle) as well as gender, can easily be seen to underlie a proportion of inter-familial manifestations. However, intra-familial variability, especially in siblings, cannot be so readily accounted for these types of mechanisms.[11] Therefore, although it is known that the defect of "LD-TAG-lipolysis" mediated by ATGL is primarily implicated in the pathogenesis of the disease, the reported data suggest that the clinical manifestations of NLSDM could be influenced also by "modifier genes".

Our patient followed a restricted diet supplemented by MCT oil for several years without beneficial effects on myopathy progression. MCT oil is a highly concentrated source of medium-chain triglycerides which plays a key role in increasing mitochondrial fatty acid oxidation (FAO) in some disorders.[18] In skeletal muscle mitochondrial FAO is activated and maintained by PPARα.[19] It has been demonstrated in mice model that ATGL provides ligands for PPARα activity and, in case of ATGL depletion, there is a decrease in PPARα function.[19] It could be hypothesized that in our NLSDM patient there is a negative loop in which ATGL is not produced, PPARα is negatively regulated and FAO is not maintained, even in the case of MCT supplementation. To date, no positive effect has been described in NLSDM patients treated with MCT. On the contrary, some beneficial effects of special diet (poor in long chain fatty acids and enriched with medium chain fatty acids) have been reported on children affected by NLSDI (Neutral Lipid Storage Disease with Ichthyosis), which is due to ABHD5 mutations. However, the molecular mechanisms explaining the different effect of MCT are completely unknown.

We reported the follow-up of an NLSDM patient with a progressive skeletal muscle myopathy, without cardiac involvement. From the genetic point of view, she carried a dramatic *PNPLA2* mutation, causing the total loss of gene expression. This genotype correlates with the early onset of muscle damage. On the contrary, the preservation of heart condition is probably related to intensive previous activity performed by the patient for many times. The special diet followed by the patient did not improve muscle function.

In conclusion, as the pathophysiology of NLSDM is largely unknown, further studies are needed to clarify molecular basis of this disease and identify a specific treatment for patients. Moreover, it would be important to collect information regarding patient lifestyle to clarify hypothetical/beneficial influences on clinical phenotype.

List of acronyms

ATGL: adipose triglyceride lipase
FAO: fatty acid oxidation
GSGC test: Gait, Stair, Gowers' Maneuver, Chair test
LDs: lipid droplets
LIR: LC3-interacting region
LSMs: lipid storage myopathies
MCT: medium-chain triglycerides
NLSDM: neutral lipid storage disease with myopathy
TAGs: triglycerides

Contributions of Authors

SM wrote and edited the manuscript; DT critically revised the manuscript; CA conceived the study, supervised it and performed the clinical characterization of patients. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare they have no financial, personal, or other conflicts of interest.

Ethical Publication Statements

We confirm that we have read the journal's position on ethical issues involved in publication and affirm that this report is consistent with those guidelines.

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References

1. Angelini C, Pennisi E, Missaglia S, Tavian D. Metabolic lipid muscle disorders: biomarkers and treatment. Ther Adv Neurol Disord 2019 Apr;12:1756286419843359.doi: 10.1177/1756286419843359. eCollection 2019.
2. Tavian D, Maggi L, Mora M, Morandi L, Bragato C, Missaglia S. A novel PNPLA2 mutation causing total loss of RNA and protein expression in two NLSDM siblings with early onset but slowly progressive severe myopathy. Genes Dis. 2019 Jul;8(1):73-8. doi: 10.1016/j.gendis.2019.07.006. eCollection 2021 Jan.
3. Missaglia S, Coleman RA, Mordente A, Tavian D. Neutral lipid storage diseases as cellular model to study lipid droplet function. Cells. 2019 Feb;8(2),E187. doi: 10.3390/cells8020187.
4. Higashi M, Hirano K, Kobayashi K, Yoshihiko I, Issiki A, Otsuka T, Suzuki C, Nakamura H, Nagasaka H, Miyata T, Miyamoto Y, Kobayashi K, Naito H, Toda T. Distinct cardiac phenotype between two homozygotes born in a village with accumulation of a genetic deficiency of adipose triglyceride lipase. Int J Cardiol. 2015 Aug;192:30-2.doi: 10.1016/j.ijcard.2015.05.004. Epub 2015 May 6.
5. Reilich P, Horvath R, Krause S, Schramm N, Turnbull DM, Trenell M, Holligsworth KG, Gorman GS, Hans VH, Reimann J, MacMillan A, Turner L, Schollen A, Witte G, Czermin B, Holinski-Feder E, Walter MC, Schoser B, Lochmuller H. The phenotypic spectrum of neutral lipid storage myopathy due to mutations in the PNPLA2 gene. J Neurol. 2011 Nov;258(11):1987-97.doi: 10.1007/s00415-011-6055-4. Epub 2011 May 5.
6. Pennisi EM, Arca M, Bertini E, Bruno C, Cassandrini D, D'Amico A, Garibaldi M, Gragnani F, Maggi L, Massa R, Missaglia S, Morandi L, Tasca E, Tavian D, Toscano A, Angelini C, Italian NLSD Group. Neutral Lipid Storage Diseases: clinical/genetic features and natural history in a large cohort of Italian patients. Orphanet J Rare Dis. 2017 May;12(1):90.doi: 10.1186/s13023-017-0646-9.
7. Zhang W, Wen B, Lu J, Zhao Y, Hong D, Zhao Z, Zhang C, Luo Y, Qi X, Zhang Y, Song X, Zhao Y, Zhao C, Hu J, Yang H, Wang Z, Yan C, Yuan Y. Neutral lipid storage disease with myopathy in China: a large multicentric cohort study. Orphanet J Rare Dis. 2019 Oct;14(1):234.doi: 10.1186/s13023-019-1209-z.
8. Fischer J, Lefèvre C, Morava E, Mussini JM, Laforet P, Negre-Salvayre A, Lathrop M, Salvayre R. The gene encoding adipose triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy. Nat Genet. 2007 Jan;39(1):28-30.doi: 10.1038/ng1951. Epub 2006 Dec 24.
9. Chen J, Hong D, Wang Z, Yuan Y. A novel PNPLA2 mutation causes neutral lipid storage disease with myopathy (NLSDM) presenting muscular dystrophic features with lipid storage and rimmed vacuoles. Clin Neuropathol. 2010 Nov-Dec;29(6):351-6.doi: 10.5414/npp29351.
10. Tavian D, Missaglia S, Redaelli C, Pennisi EM, Invernici G, Wessalowski R, Maiwald R, Arca M, Coleman RA. Contribution of novel ATGL missense mutations to the clinical phenotype of NLSD-M: a strikingly low amount of lipase activity may preserve cardiac function. Hum Mol Genet. 2012 Dec;21(24):5318-28.doi: 10.1093/hmg/dds388. Epub 2012 Sep 17.
11. Missaglia S, Tasca E, Angelini C, Moro L, Tavian D. Novel missense mutations in PNPLA2 causing late onset and clinical heterogeneity of neutral lipid storage disease with myopathy in three siblings. Mol Genet Metab. 2015 Jun-Jul;115(2-3):110-7.doi: 10.1016/j.ymgme.2015.05.001. Epub 2015 May 2.
12. Akman HO, Davidzon G, Tanji K, Macdermott EJ, Larsen L, Davidson MM, Haller RG, Szczepaniak LS, Lehman TJA, Hirano M, DiMauro S. Neutral lipid storage disease with subclinical myopathy due to a retrotransposal insertion in the PNPLA2 gene. Neuromuscul Disord. 2010 Jun;20(6):397-402.doi: 10.1016/j.nmd.2010.04.004. Epub 2010 May 14.
13. Lin P, Li W, Wen B, Zhao Y, Fenster DS, Wang Y, Gong Y, Yan C. Novel PNPLA2 gene mutations in Chinese Han patients causing neutral lipid storage disease with myopathy. J Hum Genet. 2012 Oct;57(10):679-81.doi: 10.1038/jhg.2012.84. Epub 2012 Jul 26.
14. Xu C, Zhao Y, Liu J, Zhang W, Wang Z, Yuan Y. Muscle MRI in neutral lipid storage disease with myopathy carrying mutationc 187fl1G>A. Musc Nerve. 2015 Jun;51(6):922-7. doi: 10.1002/ mus.24507. Epub 2015 Apr 24.
15. Pasanisi MB, Missaglia S, Cassandrini D, Salerno F, Farina S, Andreini D, Agostoni P, Morandi L, Mora M, Tavian D. Severe cardiomyopathy in a young patient with complete deficiency of adipose triglyceride lipase due to a novel mutation in PNPLA2 gene. Int J Cardiol. 2016 Mar;207:165-7.doi: 10.1016/j.ijcard.2016.01.137. Epub 2016 Jan 9.
16. Missaglia S, Maggi L, Mora M, Gibertini S, Blasevich F, Agostoni P, Moro L, Cassandrini D, Santorelli FM, Gerevini S, Tavian D. Late onset of neutral lipid storage disease due to novel PNPLA2 mutations causing a total loss of lipase activity in a patient with myopathy and slight cardiac involvement. Neuromuscul Disord. 2017

May;27(5):481-6 .doi: 10.1016/j.nmd.2017.01.011. Epub 2017 Jan 17.
17. Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. Eur J Clin Invest. 2017 Aug;47(8):600-11.doi: 10.1111/eci.12781. Epub 2017 Jul 19.
18. Merritt JL 2nd, Norris M, Kanungo S. Fatty acid oxidation disorders. Ann Transl Med. 2018 Dec;6(24):473. doi: 10.21037/atm.2018.10.57.
19. Biswas D, Ghosh M, Kumar S, Chakrabarti P. PPARα-ATGL pathway improves muscle mitochondrial metabolism: implication in aging. FASEB J. 2016 Nov;30(11):3822-34. doi: 10.1096/fj.201600571RR. Epub 2016 Aug 2.

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