Dear Author

Please use this PDF proof to check the layout of your article. If you would like any changes to be made to the layout, you can leave instructions in the online proofing interface. First, return to the online proofing interface by clicking "Edit" at the top page, then insert a Comment in the relevant location. Making your changes directly in the online proofing interface is the quickest, easiest way to correct and submit your proof.

Please note that changes made to the article in the online proofing interface will be added to the article before publication, but are not reflected in this PDF proof.

Molecular Syndromology

Novel Insights from Clinical Practice

Mol Syndromol
DOI: 10.1159/000541285

Received: April 9, 2024
Accepted: September 3, 2024
Published online: September ▪▪▪, 2024

A Novel *PNPLA2* Variant in a Female Patient with Neutral Lipid Storage Disease with Myopathy and Hypogonadotropic Hypogonadism

Hüseyin Bahadır Şenol[a] Pelin Teke Kısa[b] Bahar Kulu[b] Hale Ören[c] Nur Arslan[b] Uluç Yiş[a]

[a]Department of Pediatric Neurology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; [b]Department of Pediatric Nutrition and Metabolism, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey; [c]Department of Pediatric Hematology, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey

Established Facts

- Neutral lipid storage disease is characterized by progressive muscle weakness.
- Myopathy and cardiac complications are the main features of this disease. Hearing loss, hepatosteatosis, chronic pancreatitis, diabetes mellitus, short stature, cataracts, and diarrhea are associated findings.
- Muscle magnetic resonance imaging of neutral lipid storage disease with myopathy patients showed more severe involvement of the posterior compartment muscles and fat deposit mainly in the gluteal muscles.
- Neutral lipid accumulation in leucocytes can be seen in peripheral blood smear tests, called Jordans' bodies.

Novel Insights

- Homozygous *PNPLA2* c.496G>C p.Asp166His (NM_020376.4) variant is associated with neutral lipid storage disease with myopathy.
- Hypogonadotropic hypogonadism is observed in neutral lipid storage disease with myopathy.

Keywords
Metabolic disease · Neurodevelopmental disorders · Neutral lipid storage disease with myopathy · Novel mutation · Triacylglycerol · Whole-exome sequencing

Abstract
Background: Neutral lipid storage disease with myopathy (NLSDM) is a rare autosomal recessive disorder characterized by aberrant triacylglycerol metabolism due to mutations in the *patatin-like phospholipase domain-containing 2* (*PNPLA2*) gene. ***Case Presentation:*** This report presents a case study of a 14-year-old female patient exhibiting

karger@karger.com
www.karger.com/msy

© 2024 S. Karger AG, Basel

Correspondence to:
Hüseyin Bahadır Şenol, huseyinbahadirsenol@gmail.com

Karger

symptoms of NLSDM, including recurrent abdominal pain, fatigue, leg pain, and hepatosteatosis. Diagnostic investigations revealed elevated creatinine kinase levels, myopathic findings on electromyography, magnetic resonance imaging findings showing gluteal involvement and Jordans' bodies on peripheral smear. Clinical exome panel showed homozygous of *PNPLA2* c.496G>C p.Asp166His (NM_020376.4) variant. The clinical manifestations, diagnostic challenges, and implications of this novel variant are discussed in the context of current literature. Hypogonadotropic hypogonadism was confirmed in this patient after eliminating possible underlying causes. This was a novel manifestation, and hormone replacement therapy was planned. ***Conclusion:*** This case underscores the significance of genetic testing in elucidating the molecular basis of NLSDM and emphasizes the necessity of comprehensive clinical evaluation for accurate diagnosis and management.
© 2024 S. Karger AG, Basel

Introduction

Neutral lipid storage diseases belong to group of diseases in which triacylglycerol accumulates in tissues due to impaired metabolism [1]. Triacylglycerols are stored in adipose tissue to be used when required for energy [2]. In fasting state, they are metabolized to free fatty acids (FFAs) and provide a source of energy [3, 4]. Enzymatic hydrolysis of intracellular stores of triacylglycerol is mediated by the activation of adipose triglyceride lipase (ATGL), hormone-sensitive lipase, and monoacylglycerol lipase [2, 5, 6]. Various tissues experience an accumulation of neutral lipids due to the disruption of this enzymatic pathway [7]. Depending on the gene involved, lipid storage can lead to two autosomal recessive diseases: neutral lipid storage disease with myopathy (NLSDM) and neutral lipid storage disease with ichthyosis (NLSDI).

NLSDM is an autosomal recessive disorder caused by mutations in the *patatin-like phospholipase domain-containing 2* (*PNPLA2*) gene encoding the ATGL enzyme, resulting in accumulation of lipids in various tissues [8]. Myopathy and cardiac complications are the main features of this disease. Hearing loss, hepatosteatosis, chronic pancreatitis, diabetes mellitus, short stature, cataracts, and diarrhea are associated findings [9, 10].

NLSDI, also known as Chanarin-Dorfman syndrome (MIM: 275630), is caused by mutations in the *abhydrolase domain-containing gene 5* (*ABHD5*). This disease is characterized by skin and liver manifestations. Ichthyosis, liver failure, hearing and vision problems may be observed [7].

Neutral lipid storage diseases are extremely rare. To date, 149 NLSDI and 56 NLSDM patients have been reported. NLSDM is associated with 58 *PNPLA2* variants in ClinVar, 15 of which are single nucleotide variants. The novel *PNPLA2* variant is associated with the NLSDM case with previously unidentified clinical manifestations presented in this report.

Case Presentation

A 14-year-old girl presented with recurrent abdominal pain, fatigue, and leg pain. The patient was born to consanguineous parents at 41st gestational week with birth weight of 3,350 g (25th–50th centiles), length of 48 cm (10th–25th centiles), and occipitofrontal head circumference of 34 cm (25th–50th centiles), and had no postnatal adaptation problems. No regression but mild delay in neuromotor development was reported; head control and sitting without support were achieved at 2 and 7 months, respectively. Canonical babbling was reported to start at the age of 6 months, and there were meaningful words at 12 months. A delay in walking was observed, finally achieved at 19 months.

Three years before admission, patient had undergone appendectomy due to abdominal pain 3 years and had been hospitalized twice due to pancreatitis attack. Exercise intolerance was not significant during the first 2-year period. Elevated creatinine kinase (CK) (985 U/L), which was noticed during hospitalization, was thought to be related to the operation. In the last year before the admission, fatigue and muscle aches started. An increase was described on admission. The patient had a short stature and had not yet experienced menarche. Physical examination revealed the following: a rough round face, truncal obesity, abdominal skin scar, hepatomegaly, and muscle power grade 4/5 in both proximal lower limbs, according to the Medical Research Council (MRC) scale. According to Tanner physical development scale, she was prepubertal girl (stage 1). Her body weight was 47 kg (10th–25th centiles), and height was 144 cm (0–3rd centiles). Other examination findings were unremarkable. CK levels were 846 U/L (reference range 29–168 U/L) at the time of assessment. Aspartate aminotransferase (86 U/L, reference range 0–35 U/L) alanine aminotransferase (106 U/L, reference range 0–35 U/L), triglyceride (TG) (198 mg/dL, reference range 39–120 mg/dL), total cholesterol (155 mg/dL, reference range 49–123 mg/dL) levels were increased. Follicle-stimulating hormone (0.59 mIU/mL, pubertal reference range 2.53–7.04 IU/mL), luteinizing hormone (<0.07 mIU/mL, pubertal reference range 0.7–17.3 mIU/mL), estradiol 33.5 pg/mL, reference range

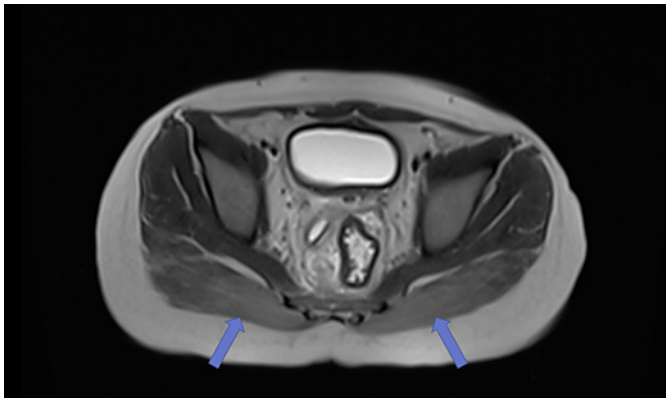


Fig. 1. Hyperintense areas consistent with involvement of bilateral gluteus maximus muscles on T2 sequence abdominal MRI cross-section.

19.5–214 pg/mL) levels were prepubertal. Thyroid function tests were within normal range. Results of nerve conduction studies were within normal limits, and needle electromyography showed myopathic findings.

Two weeks after the examination, patient presented with fever, vomiting, abdominal pain, and seizure. Her amylase levels were 64 U/L (reference range 28–100 U/L), and lipase levels were elevated to 117 U/L (reference range 0–38 U/L). Magnetic resonance imaging (MRI) of the abdomen showed hepatomegaly due to hepatosteatosis, inflammation in the pancreas, and a hypointense solid lesion measuring 5 × 4.5 × 5 centimeters. Endoscopic ultrasound-guided pancreatic biopsy showed no histopathologic finding consistent with chronic pancreatitis. Cross-sectional images of abdomen MRI also revealed bilateral involvement of the gluteus maximus muscles (shown in Fig. 1).

Vacuolations of leucocytes (Jordans' bodies) were observed in examination of peripheral blood smear (shown in Fig. 2). Brain MRI and electroencephalography yielded normal results, as did electrocardiography and echocardiography. Ophthalmological evaluation was normal, and results of hearing test were within its normal range. In pubertal evaluation, results of hormone tests performed were compatible with hypogonadotropic hypogonadotropism (HH).

The highest value of CK was 1,054 U/L and the lowest value was 503 U/L. The highest value was observed during pancreatitis, when there was an increase in tissue destruction. The patient was discharged after supportive treatment. The exercise intolerance persisted, although the weakness regressed. The only treatment she received was physical therapy. Routine follow-ups were continued.

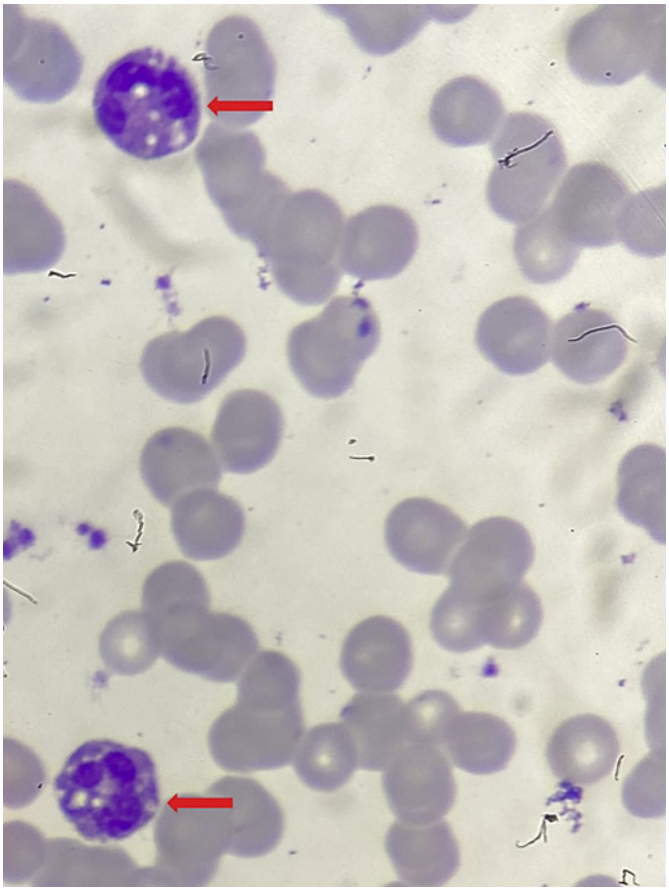


Fig. 2. Peripheral blood smear revealing leucocytes with fat deposits (Wright stain. ×1,000).

Among genetic tests performed, chromosome analysis and chromosomal microarray did not lead to a conclusive diagnosis. Clinical exome panel showed homozygous of *PNPLA2* c.496G>C p.Asp166His (NM_020376.4) variant. The *PNPLA2* variant in this case was not identified in the Human Gene Mutation Database and ClinVar mutation databases, and it was classified as likely pathogenic based on the American College of Medical Genetics and Genomics criteria (PP3, PM5, PM2). The patatin domain encoded by *PNPLA2* has an important role in the catalytic process of ATGL, and the region containing two amino acid residues provides this (Ser47 and Asp166). The variant in this patient is associated with a change in amino acid 166, which impairs the function of the patatin domain and causes lipid deposits in various tissues. The patient was diagnosed with NLSDM based on clinical, muscle MRI and peripheral blood smear findings.

Table 1. Clinical phenotypes of pediatric patients

Patient	Age of onset	First symptom	Skeletal muscle involvement	Cardiac involvement	Hepatic involvement	Additional findings	Reference
1	12	Abdominal pain	Myopathy	N	HM, HS	SS, pancreatitis, HH	In this report
2	3	EI	Myopathy	N	N	N	Fu et al. [11] (2023)
3	3	EI	Myopathy	Arrhythmia	N	HL	Fu et al. [11] (2023)
4	9	Asymptomatic	Myopathy	Arrhythmia	N	SS	Fu et al. [11] (2023)
5	5	Asymptomatic	Myopathy	N	N	N	Fiorillo et al. [16] (2013)
6	1	WD	Myopathy	N	N	HL, SI	Perrin et al. [17] (2013)
7	1	Hypotonia	Myopathy	N	HM	N	Avila-Smirnow et al. [18] (2021)

EI, exercise intolerance; WD, walking disturbances; SS, short stature; HL, hearing loss; HH, hypogonadotropic hypogonadism; SI, skin involvement, HM, hepatomegaly; HS, hepatosteatosis; N, none.

Discussion

NLSDM has a variable clinical presentation and is characterized by progressive muscle weakness [10]. This disease, which is quite rare, is found less frequently in childhood [11].

Although there are patients who are asymptomatic and diagnosed only with elevated CK, the first symptoms are usually gait disturbance, exercise tolerance, and delay in developmental milestones [11, 12]. Symptoms occurring at different stages of development have been reported ranging from postnatal hypotonia after birth to gait disturbances at age 11 [11].

The most common involvement is observed in the skeletal muscle group and is less frequency in the pediatric age group than in adults [11]. Muscle weakness may show both proximal and distal involvement [10]. Proximal muscle groups are affected more frequently than distal muscle groups, and asymmetric involvement has been reported, more often in the right extremities [12]. The patient presented in this report had similar symptoms such as delayed walking in developmental stages and difficulty in walking at 2 years of age. Muscle involvement was symmetrical.

Cardiac involvement is reported almost as often as skeletal musculoskeletal involvement in both age groups [9, 11]. Cardiac involvement was not observed in the patient presented in this report.

One of the common involvement in the adult group is hepatic involvement [9]. This is also less common in the pediatric age group [11]. In the case presented, hepatic involvement is present even though it is less common in the pediatric age group.

One result of increased TGs in various tissues is pancreatitis [13]. This case had three episodes of acute pancreatitis. However, it did not progress to chronic pancreatitis. Radiologic findings were incompatible with chronic pancreatitis [14]. Likewise, hepatic involvement, the development of pancreatitis is rare in the pediatric population [11].

Short stature, one of the positive findings in our case, has been previously reported in pediatric cases [11]. Notably, hypogonadotropic hypogonadism caused by *PNPLA2* gene mutations is unprecedented. In obese individuals, increased fatty acids entering the liver raise insulin levels, leading to more intense fat accumulation in adipose tissue and higher levels of inflammatory markers. These conditions are known to suppress the hypothalamic-pituitary-adrenal axis, resulting in reduced levels of follicle-stimulating hormone and luteinizing hormone. In this case, the influx of FFAs to the liver and insufficient release from adipose tissue inhibited this axis, causing hypogonadotropic hypogonadism [15]. In this case, it is conceivable that the heightened influx of FFAs into the liver, combined with potentially insufficient liberation from adipose tissue, might have interfered with hypothalamic-pituitary-adrenal axis, potentially contributing to HH.

Multiple system involvement and varying frequency of involvement have led to many clinical phenotypes. The clinical phenotypes of pediatric patients reported to date are presented in Table 1.

Peripheral blood smear findings may also be used to support the diagnosis, although they may not be clinically significant. In fact, neutral lipid accumulation in leucocytes can be seen in peripheral blood smear tests. Indeed, the first description of NLSDM was made by Jordan by showing fat deposits in leucocytes [19]. In this case, Jordans' bodies were observed and supported the diagnosis.

In addition to clinical and laboratory parameters, muscle MRI can be used to support the diagnosis. Muscle MRI of NLSDM patients showed more severe involvement of the posterior compartment muscles and fat deposits mainly in the gluteal muscles [12]. For this patient, similarly, MRI revealed findings in the gluteal muscles.

Conclusions

A female patient with HH is described as a new manifestation in this report. Additionally, the disease in this case is attributed to a novel *PNPLA2* variant. NLSDM is an exceptionally rare disorder that currently has no known treatment. Describing new cases remains crucial for a better understanding of the disease's clinical picture.

Acknowledgment

We want to thank to family of our patient.

Statement of Ethics

Samples from the patient and her parents were obtained in accordance with the Helsinki Declaration. The paper is exempt from the Ethical Committee approval. Ethical approval was not required for this study in accordance with local/national guidelines. Written informed consent was obtained from the patient's parents for genetic testing and from the parent/legal guardian of the patient for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was not supported by any sponsor or funder.

Author Contributions

Hüseyin Bahadır Şenol: design of the work, acquisition, analysis, and interpretation of data for the work, drafting the work, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Pelin Teke Kısa: substantial contributions to the conception and design of the work, reviewing it critically for important intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Bahar Kulu: analysis of data for the work, drafting the work, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Hale Ören, Nur Arslan, and Uluç Yiş: analysis of data for the work, reviewing it critically for important intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Availability Statement

The authors confirm that all relevant data are included in the article. Further inquiries can be directed to the corresponding author.

References

1 Garibaldi M, Tasca G, Diaz-Manera J, Ottaviani P, Laschena F, Pantoli D, et al. Muscle MRI in neutral lipid storage disease (NLSD). J Neurol. 2017;264(7):1334–42. https://doi.org/10.1007/s00415-017-8498-8

2 Zechner R, Zimmermann R, Eichmann TO, Kohlwein SD, Haemmerle G, Lass A, et al. Fat signals--lipases and lipolysis in lipid metabolism and signaling. Cell Metab. 2012;15(3):279–91. https://doi.org/10.1016/j.cmet.2011.12.018

3 Kerner J, Hoppel C. Fatty acid import into mitochondria. Biochim Biophys Acta. 2000;1486(1):1–17. https://doi.org/10.1016/s1388-1981(00)00044-5

4 Eaton S. Control of mitochondrial beta-oxidation flux. Prog Lipid Res. 2002;41(3):197–239. https://doi.org/10.1016/s0163-7827(01)00024-8

5 Vaughan M, Berger JE, Steinberg D. Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue. J Biol Chem. 1964;239(2):401–9. https://doi.org/10.1016/s0021-9258(18)51692-6

6 Lass A, Zimmermann R, Oberer M, Zechner R. Lipolysis - a highly regulated multienzyme complex mediates the catabolism of cellular fat stores. Prog Lipid Res. 2011;50(1):14–27. https://doi.org/10.1016/j.plipres.2010.10.004

7 Missaglia S, Coleman RA, Mordente A, Tavian D. Neutral lipid storage diseases as cellular model to study lipid droplet function. Cells. 2019;8(2):187. https://doi.org/10.3390/cells8020187
8 Fischer J, Lefèvre C, Morava E, Mussini JM, Laforêt P, Negre-Salvayre A, et al. The gene encoding adipose triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy. Nat Genet. 2007;39(1):28–30. https://doi.org/10.1038/ng1951
9 Pennisi EM, Arca M, Bertini E, Bruno C, Cassandrini D, D'amico A, et al. Neutral Lipid Storage Diseases: clinical/genetic features and natural history in a large cohort of Italian patients. Orphanet J Rare Dis. 2017;12(1):90. https://doi.org/10.1186/s13023-017-0646-9.
10 Zhang W, Wen B, Lu J, Zhao Y, Hong D, Zhao Z, et al. Neutral lipid storage disease with myopathy in China: a large multicentric cohort study. Orphanet J Rare Dis. 2019;14(1):234. https://doi.org/10.1186/s13023-019-1209-z
11 Fu X, Yang X, Wang X, Jia B, Ma W, Xiong H, et al. HyperCKemia: an early sign of childhood-onset neutral lipid storage disease with myopathy. Neuromuscul Disord. 2023;33(9):81–9. https://doi.org/10.1016/j.nmd.2023.07.007
12 Tan J, Yang H, Fan J, Fan Y, Xiao F. Patients with neutral lipid storage disease with myopathy (NLSDM) in Southwestern China. Clin Neurol Neurosurg. 2018;168:102–7. https://doi.org/10.1016/j.clineuro.2018.03.001
13 Yang AL, McNabb-Baltar J. Hypertriglyceridemia and acute pancreatitis. Pancreatology. 2020;20(5):795–800. https://doi.org/10.1016/j.pan.2020.06.005
14 Freeman AJ, Maqbool A, Bellin MD, Goldschneider KR, Grover AS, Hartzell C, et al. Medical management of chronic pancreatitis in children: a position paper by the north American society for pediatric gastroenterology, hepatology, and nutrition pancreas committee. J Pediatr Gastroenterol Nutr. 2021;72(2):324–40. https://doi.org/10.1097/MPG.0000000000003001
15 Eng PC, Phylactou M, Qayum A, Woods C, Lee H, Aziz S, et al. Obesity-related hypogonadism in women. Endocr Rev. 2024;45(2):171–89. https://doi.org/10.1210/endrev/bnad027
16 Fiorillo C, Brisca G, Cassandrini D, Scapolan S, Astrea G, Valle M, et al. Subclinical myopathy in a child with neutral lipid storage disease and mutations in the PNPLA2 gene. Biochem Biophys Res Commun. 2013;430(1):241–4. https://doi.org/10.1016/j.bbrc.2012.10.127
17 Perrin L, Féasson L, Furby A, Laforêt P, Petit FM, Gautheron V, et al. PNPLA2 mutation: a paediatric case with early onset but indolent course. Neuromuscul Disord. 2013;23(12):986–91. https://doi.org/10.1016/j.nmd.2013.08.008
18 Avila-Smirnow D, Durán-Saavedra G, Ovalle-Besa P, Gejman-Enríquez RJND. Early onset neutral lipid storage disease with myopathy presenting as congenital hypotonia and hepatomegaly. Neuromuscul Disord. 2021;31(1):52–5. https://doi.org/10.1016/j.nmd.2020.11.007
19 Jordans GH. The familial occurrence of fat containing vacuoles in the leukocytes diagnosed in two brothers suffering from dystrophia musculorum progressiva (ERB.). Acta Med Scand. 1953;145(6):419–23. https://doi.org/10.1111/j.0954-6820.1953.tb07038.x