

A Late-Onset Case of Neutral Lipid Storage Disease with Myopathy, Dropped Head Syndrome, and Peripheral Nerve Involvement

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Abstract

Neutral lipid storage disease with myopathy (NLSDM) is a rare autosomal recessive disorder of neutral lipid metabolism. Clinical manifestations include progressive skeletal myopathy, cardiomyopathy, and liver dysfunction. Clinical severity is variable and additional symptoms may include diabetes mellitus, chronic pancreatitis, hypothyroidism, neurosensory hearing loss, and short stature. We report a 79-year-old man with progressive proximal arm weakness, lipid storage myopathy, dropped head syndrome, and peripheral nervous system involvement. He harboured a novel homozygous missense mutation, c.570A>C (p.S191R) in the patatin-like phospholipase domain containing 2 (*PNPLA2*) gene, confirming the diagnosis of NLSDM. The S191R mutation causes late-onset NLSDM without cardiac dysfunction. The previously unreported association with dropped head syndrome expands the clinical spectrum of NLSDM.

Keywords: Neutral lipid storage disease; Jordan anomaly; Dropped head syndrome; Lipid metabolism; Adipose Triglyceride lipase; Myopathy; Diabetes mellitus

Introduction

Neutral lipid storage diseases (NLSDs) are inherited metabolic disorders resulting in the accumulation of triglycerides (TG) in various tissues of the body. They are due to defects in the degradation of TG and include two rare autosomal recessive syndromes: the Chanarin-Dorfman syndrome (CDS; MIM 275630), or Neutral Lipid Storage Disease with Ichthyosis (NLSDI) [1], and the Neutral Lipid Storage Disease with Myopathy (NLSDM; MIM 610717) [2]. The presence of characteristic cytoplasmic vacuoles (Jordan's anomalies) in granulocytes is the most common finding in these syndromes [3].

CDS is due to mutations in α/β -hydrolase domain-containing protein 5 (*ABHD5*) and is characterized by non-bullous congenital ichthyosiform erythroderma (NCIE), hepatomegaly, and liver steatosis. Additional inconsistent symptoms include muscle weakness, ataxia, neurosensory hearing loss, sub-capsular cataracts, nystagmus, strabismus, and mental retardation [4-6].

NLSDM is caused by mutations in the *PNPLA2* gene [2], which is located on chromosome 11, contains 10 exons and encodes the enzyme adipose triglyceride lipase (ATGL), a member of the patatin-like phospholipase domain-containing proteins, which catalyzes the first step in the hydrolysis of triacylglycerols. The human ATGL is a 504 amino acid-long protein, containing a patatin domain (amino acids 10 to 178) with catalytic residues S47 and D166 and a hydrophobic lipid-binding domain at positions 315-360 [7,8]. Patients with mutations in *PNPLA2* typically have progressive myopathy and elevated serum CK levels [9-11], often associated with cardiomyopathy, and, less frequently, with hepatomegaly and diabetes mellitus [12,13].

To date, 33 patients with NLSDM have been reported, harboring 25 different *PNPLA2* mutations that include deletions, insertions, missense, nonsense and frameshift variations [2,10,11,14-18].

In this study, we describe the clinical and molecular features of an Italian patient with a novel *PNPLA2* missense mutation in the region between the patatin and hydrophobic domain of the protein.

Materials and Methods

Muscle biopsy

A muscle specimen was obtained under local anesthesia and frozen in liquid nitrogen-cooled isopentane. Cryosection was used for conventional stains and enzyme histochemical analyses, including hematoxylin & eosin (H&E), Oil-Red-O (ORO), and respiratory chain enzymes (NADH-TR, COX, SDH).

Electron microscopy

Electron microscopy was performed with a conventional Transmission Electron Microscope (TEM) on specimens embedded in epoxy resin.

Genomic analysis

Genomic DNA was extracted from peripheral blood and from muscle biopsy using a Puregene DNA Isolation kit (Gentra Systems, Minneapolis), according to the manufacturer's instructions. The

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PNPLA2 coding regions (GeneBank NM_02376) was amplified by PCR using primers and conditions previously described [2].

PCR products were purified with the NucleoSpin Extract II kit (M-Medical) and sequenced on 3730 DNA Analyzers by the BigDye[®] Terminator V1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

Bioinformatic analysis of mutations

The effect of amino acid substitutions on protein function was predicted using ClustalW, SIFT and PolyPhen software. A multiple sequence alignment of mammalian ATGL proteins was used as input for ClustalW. The NCBI reference sequence (NP_065109.1) of the human ATGL protein was used as the input for SIFT and PolyPhen, with default query options.

Informed consent for genetic and histological muscle analysis was obtained from the patient and from control subjects. Moreover, written informed consent from patient was obtained for publication of this Case Report and any accompanying images.

Clinical Report

A 79-year-old man had a 15-year history of progressive muscle weakness in the arms. He was born to healthy, non-consanguineous Italian parents. He suffered from dropped head since the age of 66 (Figure 1).

When the patient was 70 years old, he complained of pain in the cervical region and received radiation therapy to the upper limbs. He also complained of weakness in the arms accompanied by increased levels of serum CK (330 U/L; normal <170). Blood lactate and pyruvate levels were normal. Electrocardiography (ECG) showed nonspecific alterations and electromyography (EMG) showed a chronic diffuse neurogenic pattern. A needle muscle biopsy revealed lipid storage myopathy (Figure 2B). A study of plasma carnitine and acyl-carnitines was normal. Biochemical analysis of respiratory chain enzymes in muscle was normal. Nerve biopsy showed axonal neuropathy. Brain Magnetic Resonance Imaging (MRI) showed chronic vascular abnormalities in the white matter. Spinal MRI showed protrusion of disk C5-C6. Cervical Doppler analysis showed kinking of both carotid arteries. He had bilateral neurosensory hearing loss. Genetic studies for Charcot-Marie-Tooth and Facioscapulohumeral Muscular Dystrophy genes yielded negative results.

At age 75, he complained of increased weakness and was unable

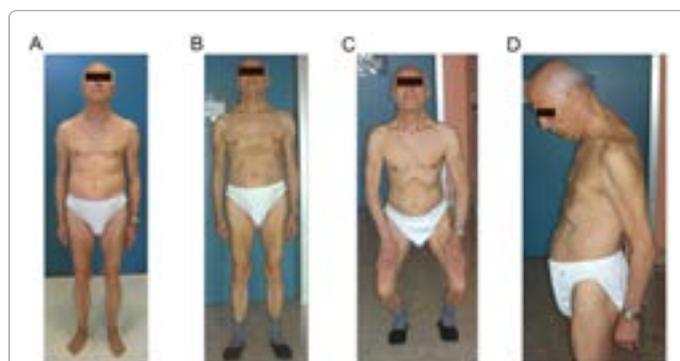


Figure 1: Clinical phenotype of NLSMD patient. A) In 2005 the patient shows atrophy of pectoral muscles; B) In 2007: advanced atrophy of legs; C) Patient's squatting; D) The patient developed the dropped head syndrome with anterocollis and kyphosis.

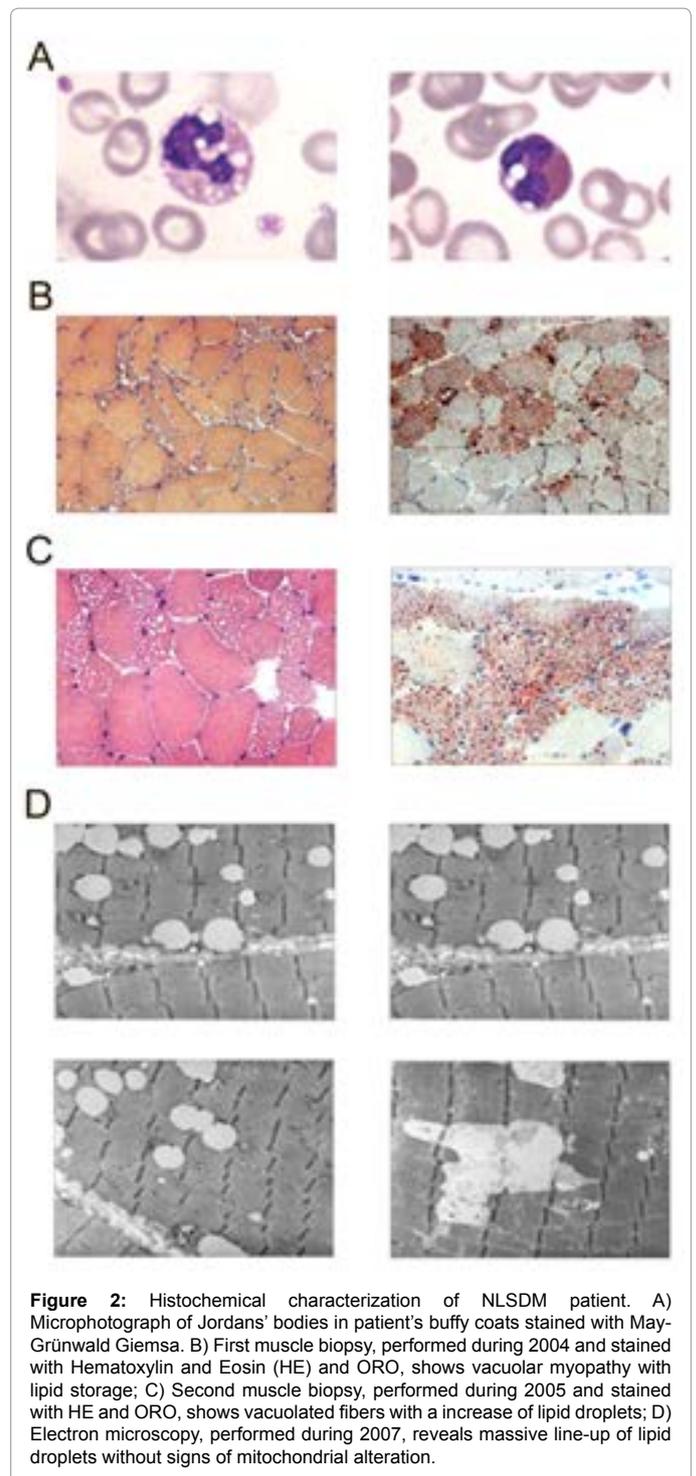


Figure 2: Histochemical characterization of NLSMD patient. A) Microphotograph of Jordans' bodies in patient's buffy coats stained with May-Grünwald Giemsa. B) First muscle biopsy, performed during 2004 and stained with Hematoxylin and Eosin (HE) and ORO, shows vacuolar myopathy with lipid storage; C) Second muscle biopsy, performed during 2005 and stained with HE and ORO, shows vacuolated fibers with an increase of lipid droplets; D) Electron microscopy, performed during 2007, reveals massive line-up of lipid droplets without signs of mitochondrial alteration.

to lift his arms over the head. He had anterocollis and kyphosis but was still able to hop on each foot, although he had bilateral pes cavus. Strength was 4/5 in biceps and 4/5 in finger extensors and he had atrophy of the pectoral muscles. A second muscle biopsy showed vacuolated fibers with a profusion of lipid droplets and lobulated fibers (Figure 2C). Serum CK was 300 U/L and the EMG showed a myogenic pattern. Jordans' anomalies were detected in buffy coats of patient stained with May-Grünwald Giemsa (Figure 2A).

At the age of 77, he developed diabetes mellitus. ECG showed occasional ventricular ectopic beats. A third muscle biopsy of the vastus lateralis showed subsarcolemmal mitochondrial aggregates and increased lipid droplets in 30% of fibers. The acid phosphatase reaction was increased in atrophic fibers. Electron microscopy confirmed the excessive accumulation of lipid droplets (Figure 2D).

Sequencing of the *PNPLA2* gene confirmed the suspected diagnosis of NLSDM by revealing a novel homozygous missense mutation in exon 5, the c.570A>C (p.S191R) (Figure 3), located in the sequence connecting the patatin and the hydrophobic domains of the ATGL protein. This mutation was not observed in >200 alleles from control subjects and was submitted to GenBank (accession number BankIt1658178 Seq KF601369). Molecular analysis was performed on DNA from muscle and peripheral blood and repeated twice.

RT-PCR analysis of *PNPLA2* cDNA from muscle of patient does not reveal aberrant mRNA, demonstrating that the c.570A>C mutation maintains wild-type mRNA splicing (Supplementary information).

Discussion

To the best of our knowledge, this is the oldest patient with a diagnosis of NLSDM. He also had symptoms never before associated with NLSDM, including late-onset progressive proximal weakness of the upper limbs, atrophy of pectoral muscles, and dropped head syndrome (DHS). DHS is a relatively rare condition with a broad differential diagnosis. It has been described in patients with disorders of the central nervous system (amyotrophic lateral sclerosis, Parkinson

disease, multiple system atrophy, cervical dystonia), neuromuscular diseases (myasthenia gravis), inflammatory and metabolic myopathies, as well as other conditions (malignancy, postsurgical) [19]. To date, DHS has never been associated with NLSDM. Although NLSDM is a rare disease, it has a characteristic pattern of muscle involvement, which includes a relatively early onset (3rd decade), progressive and often asymmetric weakness of both upper and lower limbs, and severe involvement of the deltoid. In addition, cardiomyopathy is frequent. However, the severity of symptoms varies considerably among patients [10,11,13,17]. Our patient adds to the variability of the clinical presentation, with his very late onset upper and lower limb myopathy, anterocollis, and severe kyphosis (Figure 1). In particular, he complains of increased weakness in upper limbs and of pain in the cervical region. The first symptom can be due to the genetic disease with the contribution of cervical protrusion, while the second one is to be attributed mainly to C5-C6 protrusion. Moreover, although C5-C6 protrusion alone is not enough to cause the dropped head syndrome -since neck is mainly supported by muscles innervated by cervical nerves C1-C3- we cannot exclude a partial involvement.

In addition, the involvement of central or peripheral nervous system has not been described in NLSDM, whereas mental retardation is seen in approximately 10% of NLSDI cases [12,18]. In our patient, although the vascular changes in the MRI of the brain can be attributed to his advanced age, the axonal neuropathy revealed by the nerve biopsy is likely due to the genetic disease.

The lack of overt cardiac involvement is also notable, especially

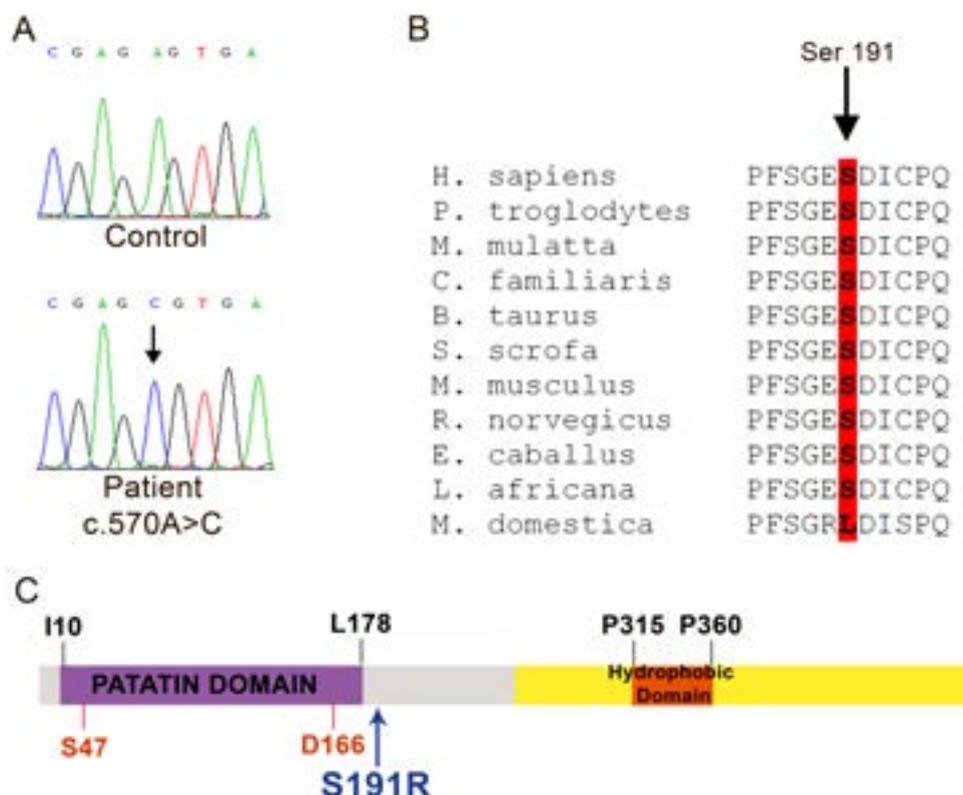


Figure 3: Identification of *PNPLA2* mutation. A) Electropherogram of *PNPLA2* exon 5 shows the c.570A>C (p.S191R) mutation; B) The amino acid sequence alignment of ATGL in 11 mammals (H. sapiens, P. troglodytes, M. mulatta, C. familiaris, B. taurus, S. scrofa, M. musculus, R. norvegicus, E. caballus, L. africana and M. domestica) reveals complete conservation of serine 191; C) Schematic representation of ATGL protein shows the localization of p.S191R mutation in the region between patatin and hydrophobic domain.

considering the age of our patient. Cardiac involvement in NLSDM is still an open issue. A recent review of all reported cases showed that only about 60% of NLSDM patients develop cardiomyopathy, which is usually identified at a young age (<40 yrs) [13]. The severity of the cardiomyopathy varies markedly in different patients. It was lethal or it required cardiac transplantation in young patients, whereas older NLSDM patients had less severe cardiac involvement. The different severity of both skeletal and cardiac myopathy may well be due to different ATGL mutations. In our patient, molecular analysis of *PNPLA2* documented a homozygous c.570A>C mutation, resulting in the substitution of a serine (a polar amino acid) at position 191 with an arginine (a positively charged amino acid). This amino acid substitution is located near to the patatin domain within a highly conserved region among mammals (Figure 3B). Although the mutation is outside the catalytic site, it may perturb the ATGL conformation and affect its lipase function. According to bio-informatic prediction tools, the p.S191R mutation is considered “probably damaging” by Poliphen-2, while it is defined “tolerated” by Sift. Functional characterization of missense ATGL mutations has shown that those mutations that completely abrogate lipase function cause more severe phenotypes than mutations that only partially impair enzymatic activity [2,11,20]. It has also been shown that a small amount of correctly localized lipase activity is sufficient to preserve cardiac function in NLSDM [11]. The molecular and clinical data in our patient are in agreement with these findings. It is likely that in his case the mutant ATGL protein retains partial lipase activity, which, however, cannot prevent slowly progressive accumulation of neutral lipids in skeletal muscle and in peripheral nerves, as pointed out by nerve biopsy.

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