Late onset of neutral lipid storage disease due to a rare *PNPLA2* mutation in a patient with myopathy and cardiomyopathy

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To the Editor: Neutral lipid storage disease with myopathy (NLSDM) is a rare autosomal recessive disorder caused by mutations in the *PNPLA2* gene. The gene encodes adipose triglyceride lipase (ATGL), an enzyme that catalyzes hydrolysis of triglycerides in mammalian adipose tissue and plays key roles in the function of lipid droplets (LDs). The results of many biochemical studies have revealed intracellular localization of ATGL with LDs, but catalytic activity is completely lost in the context of *PNPLA2* mutation.[1] Although NLSDM patients primarily exhibit progressive myopathy, hypertrophic cardiomyopathy (HCM), hepatomegaly, diabetes, and short stature,[2] later onset of the muscle phenotype has been observed in some cases.[2] The most obvious explanation for this muscle weakness is that defects in ATGL activity impair lipolytic breakdown of muscular triacylglycerol (TAG) stores, which alters energy production. Examination of peripheral blood smears always reveals vacuolization of leukocytes with LDs (Jordans' anomaly), which should prompt the search for mutations in the gene encoding ATGL.

Herein, we present a clinical report of a Chinese man with NLSDM presenting with HCM and skeletal myopathy. The study was approved by the Research Ethics Commission of China-Japan Friendship Hospital (No. 2015-ST-4). Written informed consent for publication of clinical details and/or clinical images was obtained from the participant and his relatives.

A 50-year-old man was admitted to our hospital on July 2, 2019, because of progressive weakness of the proximal muscles for 21 years. Ten years previously, he was no longer able to work and could only carry out daily living activities. Seven years prior, he was diagnosed with "ventricular septal thickening, apical HCM" by echocardiography as well as elevated creatine kinase. In the same year, he felt pain in his muscles and joints intermittently. Physical examination indicated proximal muscle strength of both upper limbs at level 2, with level 5 distal muscle strength. The proximal muscle strengths of the left lower limb extremity and distal lower limb were level 4 and level 5^{-}, respectively. Dorsiflexion of the left foot was level 4. The finger-to-nose test and rotation test could not be completed, though the calcaneal tendon test was accurate. No abnormalities were detected by electromyography or somatosensory evoked potential tests. Pathological examination of his left thigh quadriceps muscle revealed a large amount of LDs deposited in the muscle fibers that was accompanied by muscle fiber atrophy and dark cytochrome C oxidase staining in the muscle fibers, consistent with the characteristics of myopathy. Moreover, the fiber type grouping suggested peripheral nerve involvement. None of the pathological changes occurring in muscular dystrophy or inflammatory muscular disease were observed. At that time, no mutation in the *ETFDH* gene was found. In addition, levels of urine organic acids, such as oxalic acid and 3-hydroxypropionic acid, were all increased, suggesting a nutritional disorder. Therefore, treatment was started according to the diagnosis of "lipidosis," including oral administration of vitamin B2 combined with complex vitamin B. However, his symptoms were not relieved after 5 years, and his muscle weakness gradually became serious. Six months ago, he began to need assistance to get up and get dressed. Five days before admission, the family found him in the morning unconscious with cyanosis. Cardiac arrest occurred, and cardiopulmonary resuscitation was applied. After arriving at the Emergency Department, arterial

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blood gas analysis indicated a carbon dioxide partial pressure of >140 mmHg. Endotracheal intubation and norepinephrine were administered, and he regained consciousness two hours later. A computed tomography (CT) scan of the craniocerebrum revealed no abnormalities, but chest CT showed bilateral patchy consolidation of the lower lobes of his lung [Supplementary Figures 1A and 1B, http://links.lww.com/CM9/A949]. The patient visited our hospital for weaning evaluation. He had a history of high blood pressure and was married at the age of 21. His parents were deceased, and his younger brother developed muscle weakness 30 years ago.

On admission to our department, his vital signs were within normal limits. The patient was awake, alert, and fully oriented. Physical examination revealed wet rales in the lower lobes of both lungs, muscle atrophy of the extremities, level 2^+ muscle strength of the upper limbs, level 3 muscle strength of the lower limbs, and weakened tendon reflex of the extremities. Abnormal laboratory values included an alanine aminotransferase level of 51 U/L (normal range, <40 U/L), hypoalbuminemia (albumin level 29.1 g/L; normal range, 35–52 g/L), a creatine kinase isoenzyme level of 6.28 ng/mL (normal range, 0.3–4 ng/ mL), and a troponin I level of 1.0 ng/mL (normal range, <0.04 ng/mL). Bronchoscopy was generally normal [Supplementary Figures 1C–E, http://links.lww.com/CM9/A949], with a small amount of white phlegm in the trachea. Bacterial culture of bronchoalveolar lavage detected *Pseudomonas aeruginosa* (5×10^3 colony-forming units per milliliter), which was sensitive to cephalosporins and aminoglycosides. The patient's electrocardiogram revealed an elevation of the ST segments of the V1 and V2 leads and depression of the ST segments of the V3 and V4 leads [Supplementary Figure 2A, http://links.lww.com/CM9/A949]. Echocardiography showed cardiac apex HCM with pericardial effusion and congenital heart disease with atrial septal defect, and the left ventricular wall was markedly thickened [Supplementary Figures 2B-E, http://links.lww.com/CM9/A949]. Diaphragm ultrasonic examination indicated bilateral diaphragm thinning with left diaphragm paralysis [Supplementary Figures 3A and 3B, http://links.lww.com/CM9/A949]. The structures of both sides of the quadriceps femoris were blurred on ultrasound, with an unclear pennation angle [Supplementary Figures 3C-F, http://links.lww.com/CM9/A949].

The patient underwent cardiological and neurological consultations, which suggested myocardial biopsy to confirm the reason for his unexplained myocardial injury as well as genetic testing to determine the type of lipid-deposition disease. We performed whole-exome sequence capture using peripheral blood DNA samples from the patient after obtaining signed informed consent for genetic analysis. Histochemical characterization of myocardial biopsy of the right ventricular endocardium by hematoxylin-eosin staining showed vacuolar degeneration of cardiomyocytes [Supplementary Figure 4A, http://links.lww.com/CM9/A949]. Masson staining showed mild hyperplasia of the mesenchymal fibrous tissue [Supplementary Figure 4B, http://links.lww.com/CM9/A949]. Electron microscopy confirmed excessive accumulation of LDs, and the abundance of interstitial collagen fibers was increased, without signs of mitochondrial alteration or accumulation of glycogen [Supplementary Figures 4C and 4D, http://links.lww.com/CM9/A949]. Sanger sequencing was carried out to validate the *PNPLA2* (NM_020376: c.187+1G>A) homozygous variant identified by whole-exome sequencing, and the genetic analysis indicated that the patient carries a novel homozygous mutation, c.187 +1G>A, in *PNPLA2* [Supplementary Figure 5A, http://links.lww.com/CM9/A949]. Screening of his family showed his son and daughter to be heterozygous for this *PNPLA2* gene mutation [Supplementary Figures 5B and 5C, http://links.lww.com/CM9/A949, Supplementary Figure 6, http://links.lww.com/CM9/A949].

Weaning, which was evaluated by a spontaneous breathing trial (SBT), failed (the oxygen saturation dropped to 80%, and he had obvious dyspnea after 5 min of SBT). Finally, tracheotomy was performed. Cefoperazone sulbactam was given to treat hospital-acquired pneumonia, and indwelling gastric tubes were inserted to support enteral nutrition. The patient was prescribed long-term oral administration of butylphthalide, 1-carnitine, vitamin B2, and vitamin B complex after discharge on July 22, 2019.

NLSDM patients have been genetically reported worldwide,[3] and cardiac dysfunction has been observed in many cases. Moreover, some studies have demonstrated that sex may influence the phenotypic clinical expression of NLSDM, and despite presenting with HCM, females as well as males may die of heart failure.[3] Additionally, more than 90% of NLSDM patients with cardiac involvement carry harmful *PNPLA2* mutations resulting in no production or function of the ATGL protein. Deficiency of ATGL results in accumulation of re-esterified triglycerides in cardiomyocytes, leading to HCM and heart failure.[4] In contrast, patients with missense mutations that partially preserve lipase activity generally do not have cardiac involvement.[5] Accordingly, these patients should undergo long-term follow-up of heart function. As hepatomegaly, diabetes, and pancreatitis have all been reported in such patients, blood biochemical examination should also be performed in long-term follow-up.

There is no specific therapy for NLSDM at present; although oral carnitine has been used with some improvement, these findings have not been confirmed.[6] As reported in glycogen-storage disease, enzyme replacement therapy may constitute an optional treatment.[5] Peroxisome proliferator-activated receptor (PPAR)-α agonists are applied to reduce TAGs in serum and mobilize lipids from LDs.[7] Indeed, there have been recent advances in using PPARγ agonist treatment (i.e., bezafibrate), benefitting fibroblasts in carnitine palmitoyltransferase II deficiency. In a human study, bezafibrate treatment in NLSDM individuals resulted in a significant reduction in cardiac and muscle fat.[8,9] In our clinical observation, a defibrillator is necessary in cases of severe arrhythmogenic cardiopathy. Furthermore, physical exercise and muscle rehabilitation play important roles in activation of lipid metabolism. Overall, the identification and analysis of *PNPLA2*

mutations is particularly important for the development of more efficient and targeted therapies.

To the best of our knowledge, reports of NLSDM in Chinese individuals are very rare, and its onset typically occurs in adults. We will attempt to sequence the genes of all family members of the patient to determine whether there is common lineage separation. In addition, physicians should pay more attention to muscular dystrophies as a differential diagnosis for difficult weaning caused by skeletal muscle weakness with the presence of cardiomyopathies.

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Conflicts of interest

None.

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