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ScienceDirect

Neuromuscular Disorders 29 (2019) 67–69

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Case report

Autophagic vacuolar myopathy caused by a *CLN3* mutation. A case report

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Received 12 July 2018; received in revised form 29 October 2018; accepted 16 November 2018

Abstract

We present a 29-year-old man with visual failure since childhood, muscle weakness, subtle heart muscle hypertrophy, and seizures who was initially considered to be affected by a mitochondrial encephalomyopathy because of the multiple unspecific involvement of brain, muscle and retinal tissues. Only the muscle biopsy findings correctly guided the genetic investigations and the identification of an autophagic vacuolar myopathy due to a homozygous mutation in *CLN3*. We believe that information in autophagic muscle disorders should further alert clinicians to consider *CLN3* in individuals with vacuolar myopathy, especially if they have visual and cardiac involvement.

Keywords: Vacuolar myopathy; Batten disease; *CLN3*; Neuronal ceroid lipofuscinosis; High CPK.

1. Introduction

The autophagic–lysosomal system is critically involved in muscle homeostasis. Lysosomal dysfunction and autophagic pathway defects result in several disorders including muscular dystrophies and metabolic myopathies [1]. Autophagic vacuolar myopathies (AVMs) are heterogeneous muscular disorders characterized by the presence, in the cytoplasm, of autophagic vacuoles with sarcolemmal features and acetylcholinesterase activity on muscle biopsy [2]. Common forms include Danon disease, X-linked myopathy with excessive autophagy, and late-onset Pompe disease, associated with mutations in *LAMP2, VMA2* and *GAA*, respectively [2]. Muscle abnormalities suggestive of AVM have been identified in patients with Batten disease, a common form of neuronal ceroid lipofuscinosis (NCL) [3].

2. Case report

A 29-year-old Italian man presented, since childhood, progressive retinitis pigmentosa and cataracts. As a teen, he developed generalized tonic–clonic seizures and shortly afterwards complained of easy fatigability and progressive neuromotor deterioration. The family history was unremarkable. From the age of 25 years, he has shown progressive cognitive decline and subtle behavioral changes. The latest neurological examination, at age 27, showed festinating gait with bradykinesia and resting tremor, muscular atrophy, and pyramidal tract signs. Brain MRI showed diffuse cerebral cortical-subcortical and mild cerebellar atrophy (Fig. 1). Ophthalmological evaluation revealed optic atrophy, retinitis pigmentosa and cataracts, whereas echocardiography showed initial signs of left ventricular hypertrophy. Metabolic work up showed occasional raise of serum amino acids, and laboratory investigations detected elevated serum CPK levels (2–3 times normal values) on several occasions. The clinical presentation suggested a diagnosis of mitochondrial encephalomyopathy, and an extensive targeted gene panel was analyzed (Mitochip, see supplementary Appendix) but we failed to show gene variants of possible or probable pathogenic significance.

Histological analyses in skeletal muscle showed variation in fiber size, the presence of both atrophic and hypertrophic fibers with increased central nuclei (Fig. 2a) but normal stains for oxidative metabolism. A number of fibers with degenerative features and autophagic vacuoles were also observed

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https://doi.org/10.1016/j.nmd.2018.11.006
0960-8966/

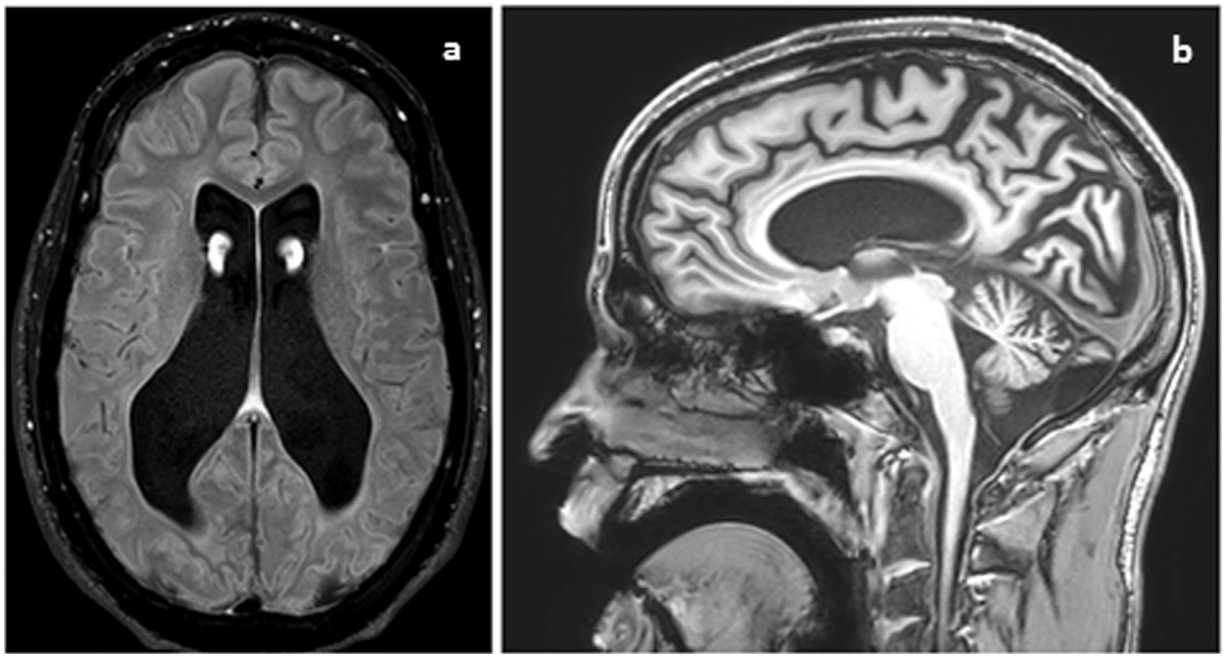

Fig. 1. Brain MRI in autophagic vacuolar myopathy.
Brain MRI in the proband when he was 27 years old. Axial FLAIR (**a**) and sagittal T1-weighted (**b**) images showing diffuse cerebral cortical-subcortical and mild cerebellar atrophy.

(Fig. 2b,c). Spectrophotometric investigations of respiratory chain enzyme complexes in muscle homogenate were also normal. As expected (1), the autophagic vacuoles showed a strong reaction for lysosomal acid phosphatase activity (Fig. 2d), whereas immunofluorescence with dystrophin antibodies revealed positive binding at vacuolar membrane level (data not shown).

Taken together, the patient's clinical features and muscle biopsy findings suggested, as an alternative diagnosis, AVM occurring in NCL. The *CLN3* gene was duly analyzed and the "common" homozygous deletion of 1.0 Kb detected. Both parents and two healthy siblings carried the heterozygous deletion. Informed consent was obtained from all individual participants included in the study.

3. Discussion

Whilst cardiac symptoms and cardiac muscle hypertrophy are frequent (even as late, life-threatening manifestations), skeletal muscle involvement is rarely reported in Batten disease. Nonetheless, muscle weakness, high CPK levels and features of AVM have been described [5–7]. Supplementary Table 1 compares the present case with the clinical features reported in previous *CLN3* patients presenting AVM. Visual symptoms were the onset manifestations in these patients, followed by epilepsy and progressive neuromotor deterioration. The muscle weakness, affecting both proximal and distal muscles, seems to be moderate, correlating well with the finding of slightly increased serum CPK levels (range 220–600 IU/l), however, it is associated with severe muscular atrophy. Brain MRI in all the patients showed atrophy of cerebral and cerebellar regions associated with cognitive decline of varying severity. Histological studies in muscle failed to distinguish AVMs associated with *CLN3* mutations from other AVMs, with all the forms showing a strong reaction for lysosomal acid phosphatase activity in the cytoplasm of all fibers. Invariably, the vacuoles displayed immunoreactivity for the sarcolemmal protein dystrophin [5–7].This further case of AVM associated with a mutation in *CLN3* was initially misdiagnosed as a mitochondrial disorder because of the multiple unspecific involvement of brain, muscle and retinal tissues. Two tips are worth remarking. First, only the muscle biopsy findings correctly guided the genetic investigations stressing the importance of this diagnostic procedure even now in the era of rapid gene testing. When clinical features appear to suggest multi-tissue involvement–as in our case–, a correct approach would endorse muscle histology first, leaving gene panel or even analysis of the exome for later analysis to refine the diagnosis. Second, our results further corroborate the important role of *CLN3* protein in the pathomechanisms of vacuolar myopathy. Although the exact physiological function of this lysosomal/endosomal transmembrane protein remains unknown [1,3–4], a recent report of successful pharmacological modulation of the consequences of *CLN3* mutation in mice [8] might also offer new treatment perspectives and opportunities in AVMs and other muscular disorders [1]. In our opinion, *CLN3* should be always considered in the diagnostic workup of individuals with AVM, especially if they have visual and cardiac involvement.

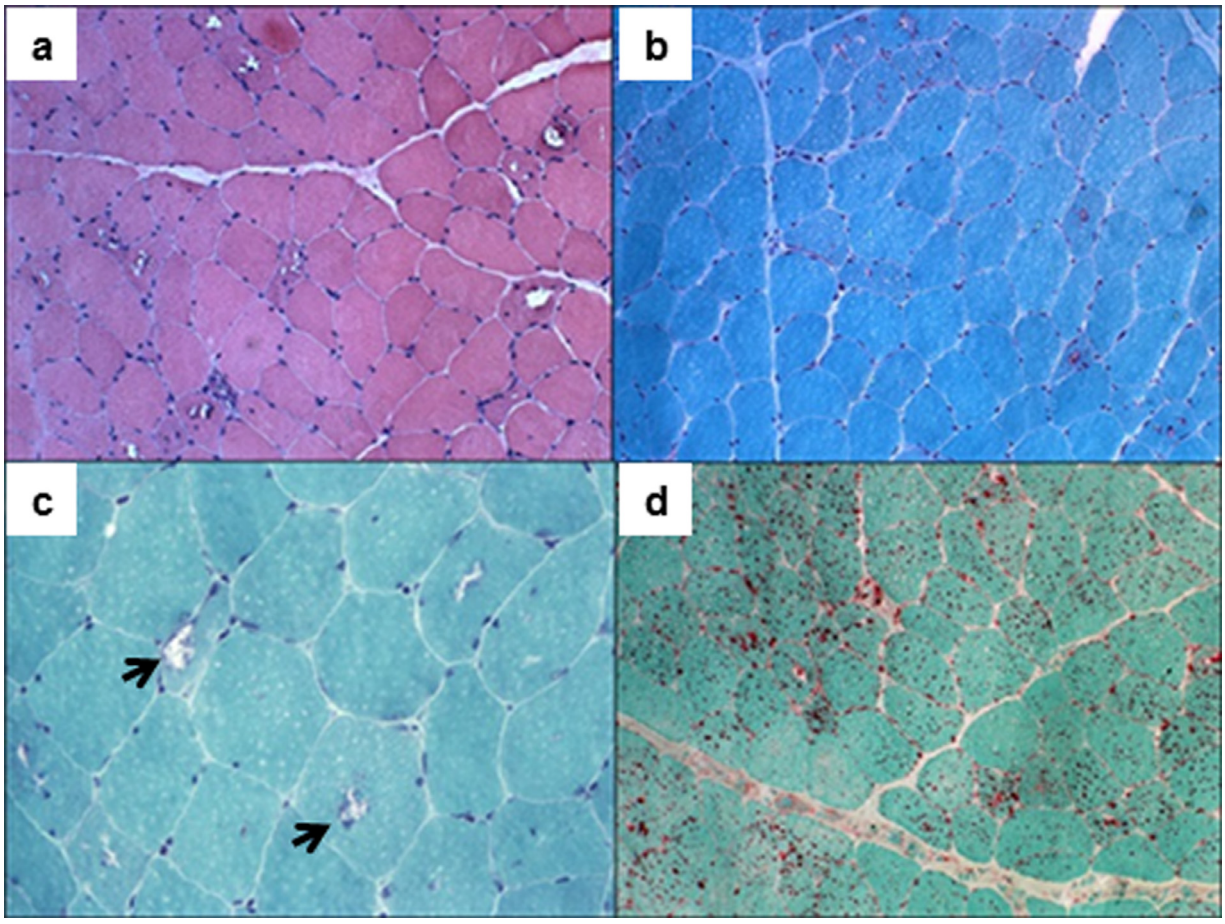

Fig. 2. Muscle histology in a CLN3 patient.
Representative light microscopy images of muscle biopsy (**a**) Hematoxylin and eosin staining demonstrating mild variation in fiber size and the presence of vacuoles. (**b–c**) Gomori trichrome staining shows, in numerous muscle fibers, multiple vacuoles or microvacuoles, some of them with fuchsia rims. Arrows highlight the presence of vacuoles. (**d**) Lysosomal acid phosphatase activity was increased in the cytoplasm of all fibers and present in the vacuoles.

Acknowledgments

The authors thank Catherine J. Wrenn who provided expert editorial assistance. This work was partially funded by grants from Telethon Foundation (GUP11001 to FMS) and Regione Toscana FAS SALUTE 2014 (CUP 4042.16092014.066000060 to FMS).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi: 10.1016/j.nmd.2018.11.006.

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