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Review

Centronuclear myopathies: A widening concept

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ABSTRACT

Centronuclear myopathies (CNM) are a group of congenital myopathies classically defined by the presence of an abnormally high number of muscle fibres with nuclei organised in rows in the central part of the fibre. Over recent years there have been important advances in the knowledge of the genetic bases of the three main forms of CNM: the X-linked recessive form or myotubular myopathy (XLMTM) with severe neonatal phenotype, caused by mutations in the *MTM1* gene; the classical autosomal dominant forms with mild, moderate or severe phenotypes caused by mutations in the *DNM2* gene; and an autosomal recessive form presenting severe and moderate phenotypes caused by mutations in the *BIN1* gene.

Although at present the histopathological distinction between these described forms of CNM seems well established, these three genes do not explain all the cases of CNM and there still exist an important number of genetically unresolved cases with prominent myonuclei internalisation and centralisation. This mini-review lays emphasis on the particular histopathological abnormalities associated with specific gene mutations, the high significance of establishing a distinction between nuclear centralisation (i.e. the presence of one nucleus at the geometric centre of the fibre) and nuclear internalisation (i.e. one or more nuclei anywhere inside the sarcoplasm) for CNM categorisation, and demonstrates how additional structural alterations within muscle fibres are a useful criterion for suggesting or discarding *DNM2*-, *BIN1*- or *MTM1*-related CNM.

1. Introduction

The centronuclear myopathies (CNM) emerged early in the group of congenital myopathies (CM), with the initial reports by Spiro et al. [1] and by Sher et al. [2,3] of "myotubular" myopathies. The identifying characteristic was indeed an abnormally high number of muscle nuclei organised in long rows at the centre of the fibres, which initially suggested some similarities with the myotubes normally present during the development of the muscle fibre. This analogy was however severely criticised, in particular by Banker [4], since nobody could prove that the muscle was arrested in its development at the myotubular stage in this CM; therefore the term of "centronuclear" myopathies was preferred.

The clinical presentations were highly heterogeneous and the initial classification of these myopathies was mainly based on the age of onset of the disorder and the severity of clinical symptoms; thus, rapidly, a number of severe cases were detected in the neonatal period, in male newborns with an X-linked inheritance, and for that category the morphological similarities with the myotubes were more pronounced. So, the term "myotubular" was used again to designate just that neonatal form, as opposed to the childhood or late-onset centronuclear myopathies (see for review Fardeau [5], Fardeau and Tome [6] and North [7]). Afterwards, the first breakthrough came with the refined locus mapping of the gene locus at Xq27-q28 of the "myotubular myopathy" thanks to the collective work of several teams under the European Neuromuscular Centre (ENMC) [8]. This made it possible to identify the mutations in the *MTM1* gene, encoding for the 3′-phosphoinositides phosphatase myotubularin 1 (*MTM1*), in that "myotubular" myopathy [9].

For the other forms, dominant and recessive pedigrees were presented. An initial precise analysis of the clinical, muscle histopathology and genetic features in the group of 29 patients from 12 families studied in our laboratory allowed us to identify different individual subgroups in the autosomal centronuclear myopathies [10]. Amongst these patients, a clinical homogeneous group of autosomal dominant myopathy was determined, which enabled us to find mutations in the dynamin-2 gene (*DNM2*), encoding for a large GTPase implicated in endocytosis and membrane trafficking [11]. Two years later, in an autosomal recessive form of centronuclear myopathy with severe or moderate phenotype, mutations were found in a gene encoding for amphiphysin 2 (*BIN1*) [12].

By pursuing the study of centronuclear myopathies in patients in whom mutations in genes *DNM2* and *BIN1* were excluded, we identified, mainly on clinical and morphological

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features, different subgroups. In one of these subgroups, mutations in an already identified gene were found, but with a different clinico-pathological pattern; noticeably, mutations in the *MTM1* gene were identified, in both male and female, in adult and adolescent patients whose biopsies harboured unusual "necklace" fibres [13]. Furthermore, a heterozygous *RYR1*-mutation was found in a single patient with an initial diagnosis of CNM and core-like lesions in the muscle biopsy [14].

At present, about 70% of all our CNM cases are genetically identified but there still exist a number of unresolved cases with an initial diagnosis of centronuclear myopathy.

Prospective suggestions will be indicated in the conclusion of this review.

2. CNM with genetic identified bases

2.1. Autosomal dominant – DNM2-related centronuclear myopathy

DNM2-related CNM account about for 50% of CNM cases. Three clinical presentations of CNM related to *DNM2* have so far been described: the autosomal dominant (AD), clinically corresponding to mild or late-childhood and adult onset forms [11], sporadic cases that correspond with the early-severe onset clinical form [15], and an intermediate form [16].

Three morphological features are characteristic of *DNM2*-related CNM and constantly observed: (a) significant nuclear centralisation and internalisation, (b) typical aspects of radiating sarcoplasmic strands (RSS), (c) type 1 muscle fibre predominance and small size. In *DNM2*-related cases, nuclear centralisation is consistently higher than nuclear internalisation (Fig. 1); the percentage of fibres with centralised nuclei is always larger than those with internalised nuclei. With myofibrillar ATPase reactions the centre of fibres with centralised nuclei shows a non-reactive area; these zones correspond to nuclear or perinuclear areas. In transverse sections which do not pass through a nucleus, the central area of the fibre reacts strongly with PAS, phosphorylase and oxidative stains. NADH-TR-, SDH-, COX-reacted sections clearly show numerous fibres with sarcoplasmic strands radiating from the central nucleus (RSS fibres) conferring a spoke-like appearance on the fibre (Fig. 1). In general, the RRS occur throughout the length of the muscle fibre. Noticeably, the complete histopathological triad of features described above, was, until now, exclusively and constantly found in *DNM2*-associated CNM. However, in young *DNM2*-related patients (i.e. below 5 years), this characteristic triad of features could be absent or quantitatively less frequent [15]. An increase in endomysial connective tissue and fibro-adipose replacement may be present in *DNM2*-related CNM [15]. However, none of the other typical CM lesions were ever observed in patients with *DNM2*-related myopathy such as cores, inclusion bodies, vacuoles, rods, etc. In addition, neither necrosis nor regeneration was seen.

The electron microscopy findings in muscle correlate with the histoenzymological ones. The centralised nuclei are normal. The radial distribution of the intermyofibrillar sarcoplasmic strand is easily observed in numerous fibres. The central internuclear spaces are occupied by mitochondria, sarcoplasmic reticulum, golgi complex and glycogen particles; these organelles and materials are also observed between the myofibrils (Fig. 1). Usually, the diameter of the myofibrils progressively decreases from the periphery to the central zone of the fibre, giving rise to the radiating appearance [5,17].

2.2. Autosomal recessive – BIN1-related centronuclear myopathy

Only a few patients have been described with recessive *BIN1*-related myopathy [12,18]. Clinically these patients were defined as intermediate between the XLMTM (X-linked myotubular myopathy) severe neonatal form and the autosomal dominant CNM forms [12].

The muscle biopsies show an almost homogeneous population of rounded hypotrophic type I fibres, with central nuclei in the large majority of them (Fig. 2). It is common to observe several nuclei in the central part of the fibre in the same transversal section, which sometimes looks like small packages of nuclei. In *BIN1*-related CNM, nuclear centralisation is always higher than nuclear internalisation. Uniformity of type 1, or type 1 fibre predominance, appears constant. Staining for NADH-TR activity reveals fibres showing a clear central zone with a dark border, which corresponds to the nucleus (Fig. 2); these perinuclear and internuclear zones contain sometimes PAS-positive material. Typical RSS fibres are rarely observed [18]. An increase in connective tissue and fibro-adipose replacement is consistently present, but necrosis and regeneration are never seen. No other abnormal features are found in these biopsies.

Ultrastructural studies reveal that the central area of the fibres is occupied by one or more nuclei surrounded by an amorphous material containing mitochondria, many membranous profiles and glycogen particles (Fig. 2). Some fibres have a particular myofibrillar organisation showing an abnormal gradient of myofibril diameter which decreases from the periphery to the centre of the muscle fibre.

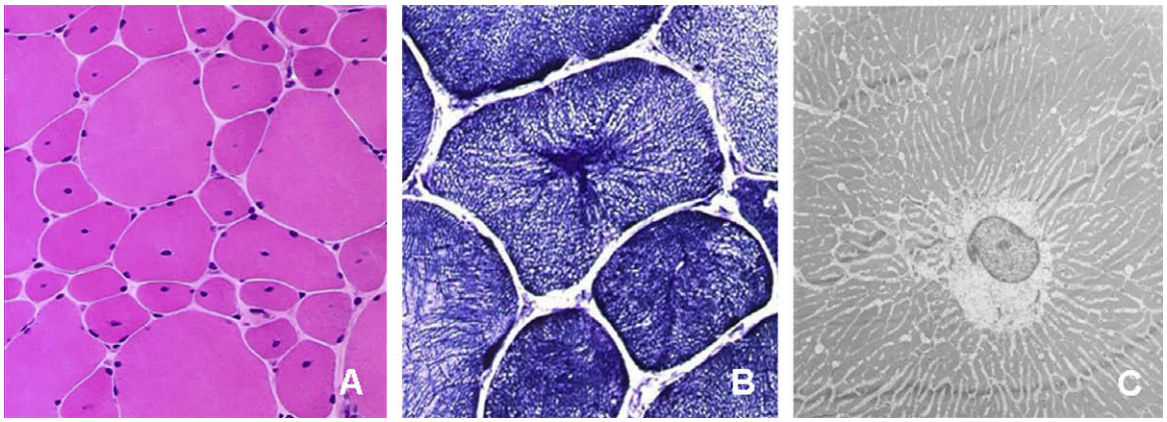

Fig. 1. *DNM2*-related CNM: Transverse muscle sections (A and B) show centralised nuclei especially in small fibres (A; HE). Typical aspect of radiating sarcoplasmic strands is seen on NADH-TR (B). Electron micrograph of transverse section shows the nucleus in the centre of the fibre and the radial distribution of sarcoplasmic strands (C).