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Mitochondrial pathology in inclusion body myositis

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Abstract

Inclusion body myositis (IBM) is usually associated with a large number of cytochrome *c* oxidase (COX)-deficient muscle fibers and acquired mitochondrial DNA (mtDNA) deletions.

We studied the number of COX-deficient fibers and the amount of mtDNA deletions, and if variants in nuclear genes involved in mtDNA maintenance may contribute to the occurrence of mtDNA deletions in IBM muscle.

Twenty-six IBM patients were included. COX-deficient fibers were assayed by morphometry and mtDNA deletions by qPCR. *POLG* was analyzed in all patients by Sanger sequencing and *C10orf2* (Twinkle), *DNA2*, *MGME1*, *OPA1*, *POLG2*, *RRM2B*, *SLC25A4* and *TYMP* in six patients by next generation sequencing.

Patients with many COX-deficient muscle fibers had a significantly higher proportion of mtDNA deletions than patients with few COX-deficient fibers. We found previously unreported variants in *POLG* and *C10orf2* and IBM patients had a significantly higher frequency of an RRM2B variant than controls. *POLG* variants appeared more common in IBM patients with many COX-deficient fibers, but the difference was not statistically significant.

We conclude that COX-deficient fibers in inclusion body myositis are associated with multiple mtDNA deletions. In IBM patients we found novel and also previously reported variants in genes of importance for mtDNA maintenance that warrants further studies.

Keywords: Inclusion body myositis; Mitochondrial DNA; mtDNA; Cytochrome *c* oxidase deficiency

1. Introduction

Sporadic inclusion body myositis (IBM) is an idiopathic inflammatory myopathy that causes progressive muscle weakness and atrophy, predominantly affecting quadriceps and long finger flexors, and dysphagia is common [1–4]. IBM is the most common inflammatory muscle disease in patients over 50 years of age, one study showed a prevalence of 51 cases per million inhabitants in this age group [5]. However, the pathogenesis remains enigmatic, and no therapies have yet proven effective [1,6,7]. Morphological findings in IBM muscle fibers include atrophy, rimmed vacuoles, ragged red fibers, cytochrome *c* oxidase (COX) deficiency and infiltration of mononuclear inflammatory cells. Eventually the muscle is replaced by fat and fibrous connective tissue.

Occasional COX-deficient muscle fibers can be found in normal aging [8], but patients with IBM display a larger amount of such fibers than age-matched controls [9]. A recent study from Rygiel et al. showed that fibers with respiratory deficiency were more prone to be atrophic and therefore of pathogenic importance [10]. COX-deficient fibers are the second most common histopathological finding in IBM patients [11], being 100% sensitive and 73% specific for IBM in inflammatory myopathy without rimmed vacuoles [12] and they are associated with somatic deletions in mtDNA [8–10].

An accelerated aging process in muscle has been discussed as a part of the pathogenesis in IBM [9,13–15]. Variants in genes encoding for proteins associated with mtDNA maintenance such as mitochondrial DNA polymerase gamma (*POLG*) [14] and Twinkle (*C10orf2*) (RJ Wiesner, personal communication) have been shown to induce a mitochondrial ageing phenotype in mice by causing somatic mtDNA mutations.

Mitochondrial DNA polymerase gamma is the only known polymerase to replicate and repair mtDNA, and the catalytical

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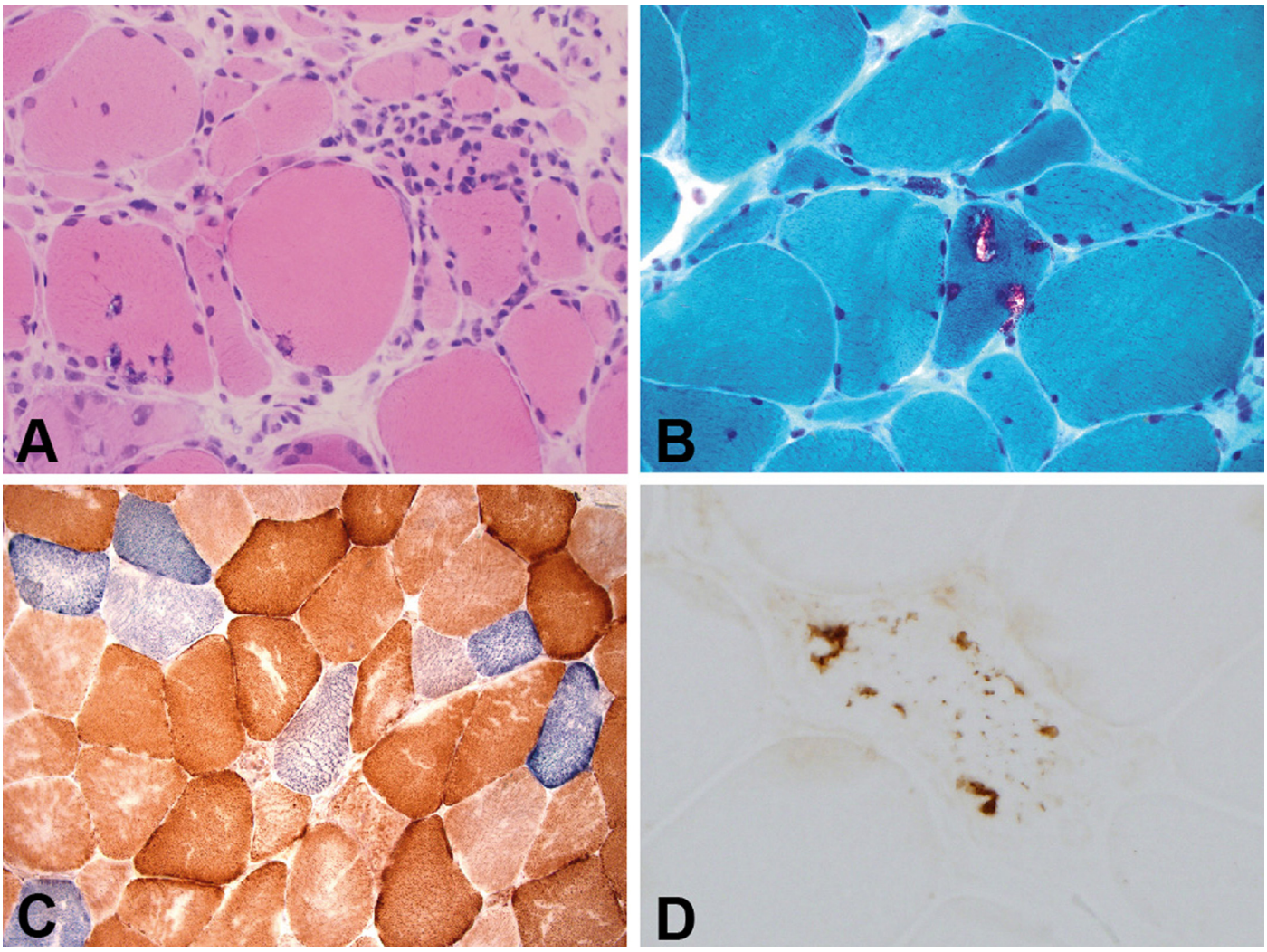

Fig. 1. Morphological features of IBM muscle. A. Variation in muscle fiber size, inflammatory cells and rimmed vacuoles in IBM muscle (Hematoxylin and eosin). B. Rimmed vacuoles in a muscle fiber (Gomori trichrome). C. Double staining with COX and succinate dehydrogenase (SDH) identifies COX-deficient muscle fibers (blue). D. p62-positive inclusions aggregated in a muscle fiber.

part is encoded by *POLG*. Many pathogenic *POLG* variants are characterized by multiple mtDNA deletions and disruption of mitochondrial function in post-mitotic tissues. *POLG* contains a CAG trinucleotide repeat, which normally consists of 10 or 11 repeats, encoding a poly-Q tract [16,17]. It has been shown that abnormal length of the *POLG* CAG repeat is associated with Parkinson's disease in Sweden and Finland [16,18], and a meta-analysis has confirmed an association between non-10Q-alleles and Parkinson's disease [17].

Variants in additional nuclear DNA (nDNA) genes, which regulate transcription, replication and maintenance of mtDNA, can cause accumulation of somatic mtDNA deletions leading to human disease. They include *C10orf2* (Twinkle), *DNA2* [19], *MGME1* (earlier *C20orf72*) [20], *POLG2* and *OPA1* [21]. An imbalance in the mitochondrial deoxyribonucleotide (dNTP) pools, which may be caused by variants in *TYMP*, *RRM2B* [22] and *SLC25A4* (*ANT1*) [23] can also result in mtDNA deletions and disease [22,24].

In this study, we wished to examine if IBM is associated with sequence variants in these genes. We also aimed to confirm the association between COX-deficient muscle fibers and mtDNA deletions.

2. Material and methods

2.1. Patients and morphology

From a register of approximately 150 patients diagnosed with IBM at Sahlgrenska University Hospital in Gothenburg, Sweden, fresh frozen muscle specimens were examined regarding the amount of COX-deficient muscle fibers as previously described [25]. The patients were included in the study if they had typical clinical symptoms and morphology for IBM including inflammation, rimmed vacuoles and positive staining for p62/Sequestosome1, and either very few or very numerous COX-deficient fibers (Fig. 1). Exclusion criteria were lack of material for further analysis and large artifacts in the specimen. In total, 26 patients were included in the study and divided into two groups, consisting of 15 patients (5.81–23.5% COX-deficient fibers) and 11 patients (0.48–2.65% COX-deficient fibers), respectively (Table 1). All included patients