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# Mitochondrial DNA depletion in sporadic inclusion body myositis

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**Abstract**

Sporadic inclusion body myositis (sIBM) is a late onset disorder of unkown aetiology. Mitochondrial changes such as cytochrome oxidase deficient fibres are a well recognised feature and mitochondrial DNA (mtDNA) deletions have also been reported, but not consistently. Since mtDNA deletions are not present in all cases, we investigated whether other types of mtDNA abnormality were responsible for the mitochondrial changes. We studied 9 patients with sIBM. To control for fibre loss or replacement with inflammatory cells, we compared sIBM patients with necrotising myopathy ($n=4$) as well as with healthy controls. Qualitative anlysis for mtDNA deletions and quantitative measurement of mtDNA copy number showed that muscle from patients with sIBM contained on average 67% less mtDNA than healthy controls ($P=0.001$). The level of mtDNA was also significantly depleted in sIBM when compared to necrotising myopathy. No significant difference in copy number was seen in patients with necrotising myopathy compared to controls. Deletions of mtDNA were present in 4 patients with sIBM, but not all. Our findings suggest that mtDNA depletion is a more consistent finding in sIBM, and one that may be implicated in the pathogenesis of the disease.


*Keywords:* Sporadic inclusion body myositis; Mitochondrial DNA depletion; Mitochondrial DNA deletion; Necrotising myopathy.

## 1. Introduction

Sporadic inclusion body myositis (sIBM) is a late-onset muscle disease of unknown aetiology [1]. Patients manifest slowly progressive weakness and atrophy that characteristically affects specific muscle groups[1]. The disorder is considered the most common acquired myopathy in the middle-aged and elderly [2] with estimates of prevelance between 3.3 and 5.1 per 100,000 [2,3]. Although classified with the inflammatory myopathies, immunomodulatory treatments have been disappointing in controlling disease progression. Patients with co-existent rheumatological diseases such as Sjogrens syndrome or systemic lupus erythematosus, or younger patients with accelerated disease course, may respond to immunomodulatory therapy [2], but evidence suggests the benefit is only short-term [4]. The mainstay of treatment remains non-pharmacological and supportive [5].

Muscle biopsy changes in sIBM include lymphocytic infiltrates in non-vacuolated muscle fibres, vacuolar degeneration and the deposition of proteins such as beta amyloid. This, together with the lack of response to immunosuppressive treatment, [6] has raised questions as to whether sIBM is primarily a degenerative disease and not an inflammatory one [7]. Interestingly, several cases of sIBM have also been linked to retroviral infections [1].

Mitochondrial abnormalities in sIBM are well known [8] and include loss of cytochrome c oxidase activity, the presence of ragged-red fibres, accumulation of deleted

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mtDNA [9,10] and abnormal mitophagy [7]. Deletions of mtDNA are reported to accumulate in COX negative fibres [9,11] and patients with the highest numbers of COX negative fibres also appear to have a higher proportion of deletion [10]. It is also reported that there is a greater degree of lymphocytic and macrophage infiltration in sIBM muscle fibres with more respiratory chain dysfunction [11]. MtDNA deletions in sIBM are similar to those seen in normal aging and in autosomally inherited external opthalmoplegia suggesting a similar mechanism [12]. There are also rare reports of mtDNA depletion and dysregulation of Krebs cycle and respiratory chain proteins [13] and variants in nuclear mitochondrial genes such as POLG and RRM2B have been recorded [10].

While there is considerable evidence of mitochondrial dysfunction and especially mtDNA abnormalities in sIBM, mtDNA deletions have not been seen in all patients [8]. Since mtDNA is a multicopy genome, we asked whether the absence of qualitative abnormalities such as deletions simply reflected the presence of other changes in this genome namely the quantitative change of mtDNA depletion.

## 2. Patients and methods

Nine Norwegian patients with clinically and histologically established sIBM were studied. All patients were over 55 years of age at diagnosis and all had symptoms for at least 6 months. None of the patients was on treatment for their sIBM. Diagnositic muscle biopsy was taken from either quadriceps or tibialis anterior, snap frozen in isopentane, cooled in liquid nitrogen and kept at −80 °C until use. Histological and histochemical studies were performed as previously [14]. To control for the effects of inflammation and muscle fibre loss, we also studied four patients of similar age with late onset necrotising myopathy in which the evolution or pattern of weakness or biopsy were suggestive of sIBM. These patients were suspected of having statin induced myopathy. Eight controls, whose biopsy was taken for diagnostic purposes, but who subsequently were found not to have muscle disease, were used for comparison. The study was approved by our local ethical committee (2017/1730).

DNA extraction was performed with a QIamp DNA Mini Kit DNA (Qiagen) according to the manufacturer's protocol. We performed long PCR using primers that produce ~8 kb product, as previously described [15]. Relative quantification of mtDNA copy-number and major arc deletions was performed by qPCR as described previously using a triplex reaction to detect mtDNA genes ND1, ND4 and the single copy, nuclear gene *APP*. Relative mtDNA copy number and deletion fraction were calculated from the ratio of ND1/APP and ND4/ND1 respectivelly [15].

A Kruskall-Wallis test was used to compare the relative mtDNA copy number (ND1/APP ratio) between sIBM, necrotising myopathy and control groups. All data is illustrated as (mean ± SEM). Individual Mann–Whitney tests were performed to compare relative mtDNA copy number between each pair. Prism 7.0 was utilised for statistical analysis and to create graphs (GraphPad Software, La Jolla California USA). Error bars in graphs represent SEM. To assess whether demographics (age, gender) or muscle atrophy had a significant effect on mtDNA copy number measurements, we performed linear multiple regression in SPSS (v25, IBM). In all cases, $P < 0.05$ was considered statistically significant.

## 3. Results

All sIBM patients had asymmetric finger flexor weakness, quadriceps femoris weakness, wrist and hip flexor weakness. Table 1 shows the histological features of the nine patients with sIBM and four patients with necrotising myopathy. Characteristic histopathological features of sIBM were present: rimmed vacuoles were present in eight out of nine patient biopsies with sIBM. Eight of nine patients also showed considerable inflammation while one patient only showed infiltration of a few scattered immune cells. The presence of CD8+ve T cells was the predominant inflammatory cell population. Four of nine sIBM patients had large numbers of COX-negative fibres, but as can be seen in the table, there is no correlation between the number of COX negative fibres and disease duration.

Long PCR showed evidence of multiple mtDNA deletions in four of the nine sIBM patients (data not shown), although this was not confirmed on qPCR, which showed a normal ND4/ND1 ratio for all samples. No deletions were seen in controls or patients with necrotising myopathy.

MtDNA quantification (Fig. 1) showed significant mtDNA depletion in all patients with sIBM with on average 67% lower mtDNA copy number compared with healthy controls ($P = 0.001$). Patients with necrotising myopathy showed a trend toward lower mtDNA copy number, but this was not statistically significant ($P = 0.23$) (Fig. 1).

Linear regression analysis showed no significant association between the degree of mtDNA depletion and patient gender ($P = 0.1$), age ($P = 0.2$) or severity of muscle atrophy ($P = 0.2$).

## 4. Discussion

Our results confirm significant mtDNA abnormalities in sIBM, however, in contrast to earlier studies [9], we found the major defect to be a decrease in mtDNA copy number (i.e. depletion). While long PCR suggested the presence of mtDNA deletions in four of nine patients, these were not detected by the ND4/ND1 qPCR assay suggesting they were either at very low levels (< 10%), or not localized in the major arc. Since diseased muscle can contain fewer muscle fibres and significant numbers of other cell types such as inflammatory cells that have lower mtDNA content, we used necrotising myopathy to control for these factors. Muscle from patients with necrotising myopathy showed a trend for slightly lower levels of mtDNA compared to controls, but this was not statistically significant. These results suggest that changes in cellular composition were not a major factor in our analyses. Further, we looked at the correlation

Table 1
Histological features of sIBM and necrotising myopathy muscle biopsies.

| Pt. ID | Sex | Age at biopsy | Symptom duration | Biopsy Muscle | Inflammation | Inflammatory cells present | | | | Rimmed vacuoles | COX neg. fibres | MHC1 up-regulation | E.M. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | CD4 | CD8 | CD68 | CD45 | | | | |
| sIBM | | | | | | | | | | | | | |
| **1** | M | 57 | 6 | Q | Yes | | | | + | Yes | 2 | Yes | |
| **2** | F | 76 | 8 | Q | Yes | (+) | + | (+) | + | Yes | 12 | | |
| **3** | F | 74 | 8 | TA | Yes | (+) | + | (+) | + | Yes | 1 | Yes | Vacuoles with myelin figures |
| **4** | M | 61 | 4 | Q | Yes | | | | | Yes | 15 | | |
| **5** | M | 72 | 5 | TA | Yes | + | + | + | + | Yes | 20 | Yes | Vacuoles with myelin figures |
| **6** | M | 67 | 8 | TA | Yes | (+) | + | (+) | + | Yes | 33 | Yes | Vacuoles with myelin figures, cores |
| **7** | M | 78 | 12 | Q | Yes | (+) | (+) | + | + | Yes | 0 | Yes | Vacuoles with myelin figures, cores |
| **8** | F | 69 | 12 | Q | Yes | (+) | + | + | + | No | 12 | Yes | Core like |
| **9** | M | 71 | 3 | TA | Little | | | | (+) | Yes | 0 | Yes | |
| **Necrotising myopathy** | | | | | | | | | | | | | |
| **10** | M | 64 | 3 | Q | Yes | (+) | + | (+) | + | No | 0 | Yes | |
| **11** | F | 67 | 1 | Q | No | | | | | No | | No | |
| **12** | M | 62 | 1 | Q | Yes | – | + | + | + | No | 0 | Yes | No changes |
| **13** | F | 84 | 0.5 | Q | Some | – | (+) | (+) | (+) | No | 2 | Yes | Myelin figures |

(+) = few scattered cells. + = substantial infiltration of immune cells. Q = quadriceps. TA = tibialis anterior. MHC1 = major histocompatibility locus 1. COX negative fibres – represent numbers in 5 high power fields (×400).

between mtDNA depletion and age, degree of muscle atrophy and gender. While our sample size is small, we found no association between these parameters and the degree of depletion.

Deletions of mtDNA of sIBM patients were first identified in muscle using in-situ hybridisation [9]; this was later confirmed using PCR based techniques [16]. Single fibre analysis [11] showed that 55% of COX deficient fibres harboured deletions and low levels were also present in respiratory competent cells from the same patients. In a subsequent study [17], the same authors showed that individual fibres contained a clonally expanded species of a mtDNA rearrangement, and in some cases more than one type of rearrangement (deletion and duplication). PCR-based techniques do not allow accurate quantification, and many of these rearranged mtDNA molecules are present at low levels raising questions as to their biological significance. More importantly, we have recently shown that the presence of DAB interferes with mtDNA amplification introducing a significant bias in the estimation of mitochondrial DNA copy number and deletion levels between DAB-positive and -negative fibers [18].

The absence of mtDNA deletions in sIBM has been noted previously [8]. This study used Southern blotting to quantify mtDNA and while this technique may be less sensitive than PCR, it does detect deletions that are present at clinically significant levels. Depletion of skeletal muscle mtDNA in sIBM has also been reported in one earlier study [13]. These authors described a mean loss of 36% mtDNA compared with normal controls, however, no disease control group was included. Further, only 57% of the patients had mtDNA deletions, although in these, depletion appeared more severe. Interestingly, this study also suggested lower mtDNA copy number in blood cells. In comparison, our study showed a significant mtDNA depletion in skeletal muscle with an average loss of around 67% compared to control. We also showed that the presence of mtDNA deletion was less than 50%.

While we cannot exclude the presence of low levels of mtDNA deletions in individual fibres, particularly COX deficient fibres, our findings suggest they are not necessarily the hallmark of sIBM. Sporadic IBM is a late onset disease, raising the possibility that the presence of mtDNA abnormalities is an ageing phenomenon and not a disease related change. Age appears more strongly associated with mtDNA deletions [19] than depletion, and in other tissues such as the substantia nigra, increasing levels of mtDNA deletions appear to induce a compensatory increase in mtDNA copy number in non-diseased individuals [18]. An age-related increase in both mtDNA deletions and depletion has been identified in paraspinal muscles [20]. In this study, the number of COX deficient fibres increased with age and single fibre studies showed that these could either contain clonally expanded mtDNA deletions or depletion. The possibility that our findings are simply age-related cannot, therefore, be excluded.

If the changes in mtDNA are not simply age-related, what is the underlying mechanism and why do some patients have deletions while others have depletion and others have both? The combination of multiple deletions and depletion is

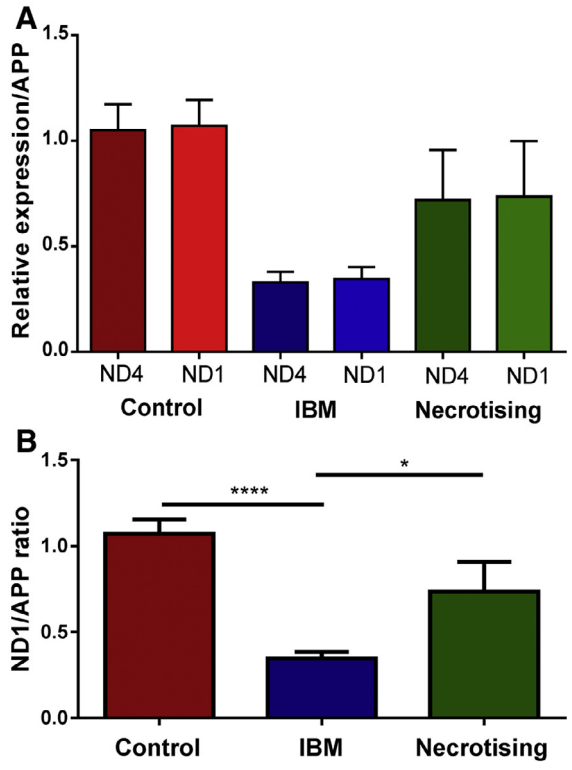


Fig. 1. mtDNA analysis in skeletal muscle using relative qPCR quantification. (A) mtDNA deletion assessement by comparison of *ND4* (commonly deleted) and *ND1* (rarely deleted) amplification against the nuclear gene *APP*. There is no significant difference in the ND4/ND1 ratio between sIBM, controls or necrotizing myopathy patients. Results are compared by Kruskall–Wallis test ($P = 0.785$). Error bars show SEM. (B) Relative quantification of mtDNA (*ND1*) against the nuclear gene *APP*. Patients with sIBM show significant mtDNA depletion compared to controls ($P = 0.001$). Patients with necrotizing myopathy show no significant difference from controls ($P = 0.23$) or sIBM ($P = 0.14$). Mean ND1/APP ratio ± SEM for sIBM: 0.35 ± 0.06; necrotising myopathy: 0.74 ± 0.26; healthy controls: 1.07 ± 0.12. Results are compared by Kruskall–Wallis test. Error bars show SEM.

similar to that found in the presence of mutations in genes involved in mtDNA homeostasis. For example, mutations in the catalytic subunit of polymerase gamma can cause both mtDNA deletion and depletion and this varies from tissue to tissue [21]. Oldfors and colleagues [16] looked in sIBM patients for mutations in *POLG* as wells as *SLC25A4* (ANT1) and *TWNK* (Twinkle, C10orf2), but found none. In a follow up study this group examined a larger panel of genes including - *C10orf2* (Twinkle), *DNA2, MGME1, OPA1, POLG2, RRM2B, SLC25A4* (*ANT1*) and *TYMP* [10]. They found several variants in *POLG* which are likely polymorphisms and no association with 10/22 CAG repeats. Variants in *RRM2B* and *TWNK* were also identified, but the suggestion that sIBM may be secondary to abnormalities in genes involved in mtDNA homeostasis has yet to be verified.

Sporadic IBM is a common disorder with a high prevalence in Norway (Dobloug 2015). Currently, the aetiology remains unknown, but growing evidence supports a mechanistic role for mitochondria in disease pathogenesis. Whatever the proximal event, mtDNA deletion/depletion will itself adversely affect energetic cells such as muscle and even if these changes are not the primary cause of sIBM, they are highly likely to play a role in disease development. The presence of mtDNA depletion in white blood cells [13] is a potential biomarker that, in the light of our studies, should perhaps be investigated further.

## 5. Conclusion

We have demonstrated the presence of significant mtDNA depletion in sIBM, with varying degrees of mtDNA deletions. This is a novel finding. The absence of mtDNA depletion in necrotising myopathy samples suggests that this finding is specific for sIBM. However, we do not imply that mtDNA damage is an aetiological factor of sIBM, but may accumulate as the disease progresses. Clearly, the role of mtDNA damage in the pathogenesis of sIBM remains to be elucidated and should be further investigated.

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