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Mitochondrial dysfunction in juvenile and adult dermatomyositis.

Degree Project in Medicine

Daniel Lööf

Programme in Medicine

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Supervisors: Anders Oldfors and Christer Thomsen

University of Gothenburg, Institute of Biomedicine.
Department of Pathology and Genetics

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Abstract

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Introduction

Previous studies have shown perifascicular regions in dermatomyositis with mitochondrial abnormalities affecting complex IV also known as cytochrome c oxidase (COX) in the respiratory chain. The aim of this study was to further investigate the mitochondrial dysfunction that is present in dermatomyositis by investigating complex I-V in the respiratory chain within skeletal muscle areas diagnosed with dermatomyositis, which show a manifest COX deficiency.

Method

Muscle biopsy specimens from the muscle biobank at the Sahlgrenska University Hospital in Gothenburg were used. Ten cases (four juvenile and six adults) were selected based on a diagnosis of dermatomyositis and presence of marked deficiency of cytochrome c oxidase in the perifascicular region. Two age matched controls and one disease control were chosen for a total of 13 patients in this study. Sections, with 8µm thickness, were incubated through enzyme histochemistry with cytochrome c oxidase COX (CIV), succinate dehydrogenase SDH (CII) and COX/SDH or through immunohistochemistry with antibodies for the five different complexes (complex I-V) in the respiratory chain.

Results

COX deficiency was profound in the perifascicular regions. Immunohistochemical analysis of complex I and IV subunits showed markedly reduced expression in perifascicular regions corresponding to the same areas showing low COX enzymatic activity.

No deficiency of complex II, III and V were found.

Conclusion

This study has provided new evidence that the mitochondrial dysfunction in dermatomyositis is due to an absence of complex I and IV in the respiratory chain. Further research on the mitochondrial dysfunction of complex I and IV in dermatomyositis may lead to better understanding of the disease and through that create better treatment and management.

Keywords. Mitochondrial dysfunction, dermatomyositis, inflammatory myopathies, Complex I and IV deficiency.

Introduction

Aims and purpose

Idiopathic inflammatory muscle diseases are, besides hereditary muscle diseases, a major cause of muscle weakness and disability in children and adults. The pathophysiology of muscle weakness in idiopathic inflammatory myopathies remains elusive. Knowledge about the mechanism is important for correct treatment and training of patients afflicted by muscle inflammation, myositis. In spite of immunosuppressive treatment many patient suffer from a lifelong disease leading to severe disability and reduced quality of life (1). It has been demonstrated that mitochondrial dysfunction is one important component of disease mechanism leading to muscle weakness. Previous studies have shown perifascicular regions in dermatomyositis with mitochondrial abnormalities affecting complex IV also known as cytochrome c oxidase (COX) in the respiratory chain (2, 3). The aim of the study is to further investigate the mitochondrial dysfunction that is present in dermatomyositis, by investigating the presence of complex I-V in the respiratory chain within skeletal muscle regions that show manifest COX deficiency. Three major questions will be addressed: 1. Is the cytochrome c oxidase (Complex IV in the respiratory chain) deficiency associated with a corresponding lack of the protein that builds up the enzyme? 2. Are other complexes than complex IV affected in dermatomyositis? 3. Are there indications that the mitochondrial disturbance involves mitochondrial DNA or its transcription and translation? The investigation will be made by enzyme histochemistry (EHC) to localize the COX deficient regions and immunohistochemistry (IHC) to examine the presence of the five complexes in the respiratory chain in the same regions.

Background

Inflammatory myopathies

Neuromuscular disorders comprise a group of disorders with muscle weakness as a predominant symptom and they have a prevalence of approximately 1/1000. Many muscle diseases lead to a severe physical handicap of afflicted individuals.

An important group of muscle disease are acquired autoimmune inflammatory myopathies (4). Inflammatory myopathies are rare disorders. The incidence for inflammatory myopathies were in a study estimated at 7.98 cases/million/year with a prevalence of 14.00 cases/100 000 inhabitants (5). The major types of idiopathic inflammatory myopathies are dermatomyositis, polymyositis and inclusion body myositis. In addition, there are other inflammatory myopathies such as immune mediated necrotizing myopathy, myositis associated with autoimmune connective tissue diseases, granulomatous myopathy and focal myositis and other rare conditions.

Inclusion body myositis is characterized by onset after 50 years of age of muscle weakness with a typical distribution affecting long finger flexor and quadriceps muscle. Muscle histopathology is characterized by inflammatory cell infiltration in the endomysium and muscle fibres with so-called rimmed vacuoles (6, 7).

Polymyositis, which is rarer, cause muscle weakness with variable distribution and onset mostly in adulthood. Histopathology looks similar to inclusion body myositis but without rimmed vacuoles (6, 7).

Dermatomyositis is characterized by subacute onset of muscle weakness in children or adults along with a characteristic skin rash. It occurs with equal frequency in children and adults. The disease is associated with a complement mediated intramuscular microangiopathy,

leading to loss of capillaries, muscle ischemia and muscle fibre degeneration (6-8).

In Olmsted County, Minnesota the incidence of dermatomyositis was found in a study to be 9,63 in a million, with a prevalence of 21.42 per 100 000 persons (9).

Muscle histopathology in dermatomyositis shows atrophy and degeneration of muscle fibres, frequently in the outer parts of the muscle fascicles, so-called perifascicular atrophy this is the hallmark of dermatomyositis in histology (fig 2, slide a and c). Biopsy also shows increased inflammation and an increase expression of MHC-I (10). MHC-I or major histocompatibility complex I is a cell surface protein present in all nucleated cells which role is to present intracellular antigens for T cell lymphocytes. The expression of MHC-I in cells can be increased by the immune system through cytokines. When stained, it is usually only seen in blood vessels and connective tissue in healthy muscle tissue (fig 1, MHC-I slide). But when stained in inflammatory myopathies there is appreciable expression on the sarcolemma and internally in several fibres (fig 2, slide b and d)

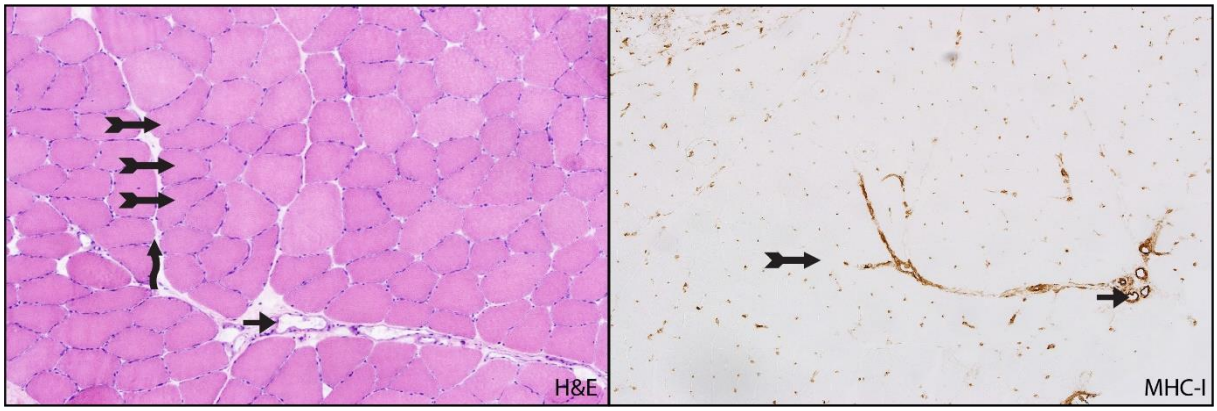

Fig 1. Muscle biopsy from a patient with normal muscle. In the **MHC-I** stain to the right**,** notice the lack of MHC-I expression in the muscle cells which leaves it pale (➤). Whereas capillaries and other vessels and connective tissue express MHC-I and will be stained brown (➔). In the **H&E** stain to the left there are normal perifascicular regions (➥) with no atrophy of the fibres (➤) and no inflammation around blood vessels (➔). This figure was self-produced. Sections presented were provided by professor Anders Oldfors with consent to

use.

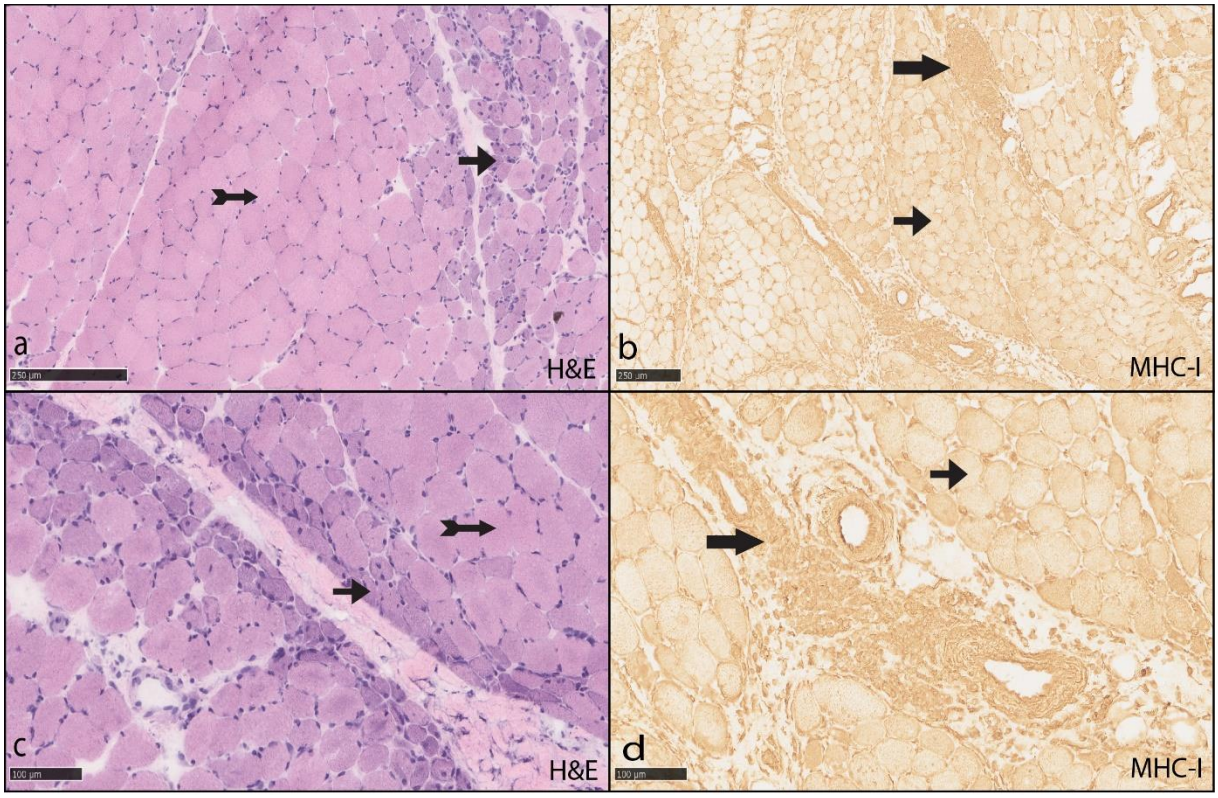

Fig 2. Muscle biopsy from patient with dermatomyositis. Notice in picture **a and c** the atrophic fibres highlighted by ➔ in the perifascicular region where **c** is showing a higher magnification of a perifascicular region. This may be compared to a more normal region (➳). **B and d** demonstrate MHC-I expression in the muscle fibres showing a brown colour (➔), together with an increased number of inflammatory cells around blood vessels (➡) where **d** is showing a higher magnification of the tissue. Scale bar = 250 µm for **a** and **b** (bar shown in both)**.** Scale bar in **C** and **D** = 100 µm (shown in both). This figure was self-produced. Sections were provided by professor Anders Oldfors with consent to use.

Mitochondria and mitochondrial diseases

Mitochondria are cell organelles, which have multiple functions, but most importantly they produce energy in the form of ATP, which is essential for numerous cellular functions. Muscle contraction is an energy demanding process and therefore muscle tissue is depending on well –functioning mitochondria. Disease that are due to primary mitochondrial dysfunction are named mitochondrial disease (11). These disorders frequently affect muscle tissue and cause muscle weakness and wasting and also exercise intolerance. Mitochondrial disease is

caused by dysfunction of the respiratory chain, which is located in the inner mitochondrial membrane. The respiratory chain is composed of five enzyme complexes (complex I-V). All subunits of complex II, also known as succinate dehydrogenase, are encoded by nuclear DNA. Complex I, III, IV (cytochrome c oxidase) and V contains subunits that are encoded from mitochondrial DNA in addition to subunits encoded from nuclear DNA. Therefore, mitochondrial DNA is essential for the function of the respiratory chain. Many mitochondrial diseases are caused by defects in mitochondrial DNA. These defects include point mutations, large scale deletions or lack of mitochondrial DNA. The synthesis of mitochondrial DNA is under nuclear control because many enzymes involved in mitochondrial DNA replication and maintenance are encoded from nuclear DNA. Therefore, mitochondrial DNA defects can be primary or secondary to defects of enzymes encoded from nuclear DNA.

Mitochondrial dysfunction in inflammatory myopathies

It has long been known that there is a mitochondrial dysfunction associated with inflammatory myopathies but the pattern differs between different types of inflammatory myopathies. It has been speculated that these mitochondrial changes are important for the muscular symptoms in inflammatory myopathies, since patients with mitochondrial myopathies show muscle weakness and exercise intolerance.

In inclusion body myositis, which has been best studied with regard to mitochondrial dysfunction, there is a large proportion of muscle fibres that show deficiency of complex IV, cytochrome C oxidase, but not complex II, succinate dehydrogenase, in a mosaic pattern (fig 3, IBM slide). This is due to accumulation of mitochondrial DNA with large-scale deletions

(12). There are multiple different deletions present in each muscle but the deletions are clonally expanded in muscle fibre segments and thereby causing the cytochrome c oxidase deficiency (13).

In dermatomyositis many patients show a more or less pronounced deficiency of cytochrome c oxidase but not succinate dehydrogenase in the perifascicular region (fig 3, DM slide). The explanation remains enigmatic, because mitochondrial DNA deletions or point mutations are not present (3).

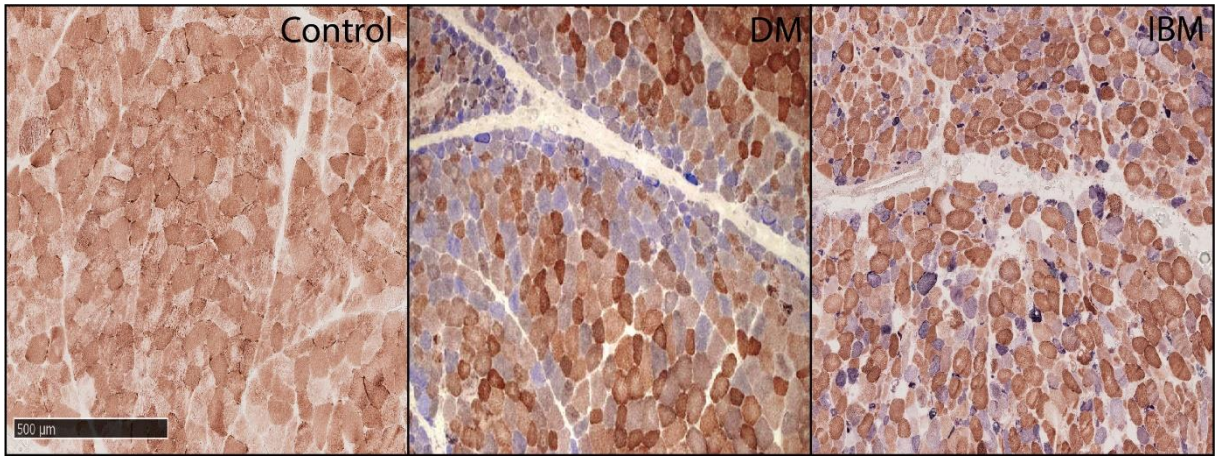

Fig 3. Muscle biopsies from three different patients demonstrating a normal (control), dermatomyositis (DM) and inclusion body myositis (IBM). All incubated for succinate dehydrogenase and cytochrome c oxidase. If there is no enzymatic COX deficiency all fibres will appear brown as seen in the **contro**l. In **DM** note the numerous blue fibres that lack cytochrome c oxidase activity in perifascicular regions, which also show atrophy. In **IBM** (inclusion body myositis) notice the numerous scattered blue fibres that lack cytochrome c oxidase activity. Scale bar = 500 µm for **control, DM** and **IBM** (shown in **control**). This figure was self-produced using patients from the study (Control and IBM). The case representing DM was provided by professor Anders Oldfors with consent to use.

The pathogenesis of mitochondrial dysfunction in juvenile and adult onset dermatomyositis

The mitochondrial dysfunction in dermatomyositis is probably due to other factors than mitochondrial DNA deletions or point mutations, since such changes have not been identified.

A working hypothesis is that mitochondrial DNA depletion (reduced mtDNA copy number) causes the cytochrome c oxidase deficiency in spite of normal or increased number of mitochondria in the perifascicular region.

Staining methods

Immunohistochemistry (IHC) is a powerful technique used for visualizing cellular components' topographical localization and presence in tissue samples.
It implies detection of specific epitopes expressed by a single protein-target. This is done by using a "primary antibody" which is capable to bind highly specific epitopes. After the primary antibody has bound the specific epitope and an epitope-antibody complex has been created a "secondary antibody" is added which is capable of binding the primary antibody with high specificity. The secondary antibody is used as a "signal beacon" by that it is coupled to a reporter molecule which will create a coloured precipitate when it reacts with a chemical substrate which is added. This will create a colour at the specific area where our target protein is located giving us the topographical localization of the protein we are searching for in the tissue sample (14)

Enzyme histochemistry combines the biochemical analysis of enzyme activity with information on its topographical localization. The principle of it is to examine enzyme activity by providing the enzyme with a special substrate where, if the enzyme is active, the substrate

will be used and a colour will appear at the location of activity in the tissue sample. For example, in this study succinate dehydrogenase (SDH) and cytochrome c oxidase (COX) are studied.

In a succinate dehydrogenase reaction, if the enzyme is active, the enzyme substrate sodium succinate will be oxidized and the stoichiometric colour indicator tetra-nitrotetrazolium chloride blue (TNBT) will be reduced to black or blue formazan. The formazan will immediately bind to the local proteins and through that provide the location of the enzyme succinate dehydrogenase in the tissue sample (15). The principle behind the enzyme histochemical method for cytochrome c oxidase is that cytochrome c acts like an electron transfer between complex III and IV. Diaminobenzidine (DAB), which is added to the histochemical reaction as an electron donor to cytochrome c and if cytochrome c oxidase (complex IV) is present, DAB will be polymerized and leave a brown reaction product (16).

Material and methods

Muscle biopsy

Muscle biopsy specimens from the muscle biobank at the Sahlgrenska University Hospital in Gothenburg were used. The biobank is comprised of several thousands of muscle biopsies collected since more than 30 years and stored at -80^0C. Ten cases were selected based on a diagnosis of dermatomyositis and presence of marked deficiency of cytochrome c oxidase in the perifascicular region, of which four were juvenile and six were adults. Since characteristics of dermatomyositis are abnormalities in the perifascicular region the

apparently normal regions in the sections were used for comparison along with two age matched controls. Muscle biopsy of a patient with inclusion body myositis (IBM) was also included as a disease control including a total of 13 patients in this study.

Ethics

The use of the biopsies in this study were approved by the patients.

Section preparation

Sections 8µm thick, of fresh frozen muscle biopsy specimens were cut on a cryostat and mounted on glass slides and then stored at -20^{0}C until histochemical preparation. The slides were numbered so they could be stained in a selective order.

Antibodies and histochemical techniques

In this study the following antibodies for subunits in the different complexes in the respiratory chain are to be used. Anti-ATPB antibody (Complex V), Anti-MTCO1 (Complex IV), Anti-UQCRC2 (Complex III), Anti-SDHB (Complex II), Anti-NDUFB8 (Complex I). Anti-VDAC1/Porin antibody (a marker used for porins localised in the mitochondrial membrane and plasma membrane and is used for detecting mitochondria in the cells).
The histochemical techniques used in this study will be cytochrome c oxidase (COX), succinate dehydrogenase (SDH) and a double staining of COX/SDH.
The antibodies used for immunohistochemistry had been previously tested and dilutions calculated for the best results.

Staining protocols

For *immunohistochemistry*, the 8 µm sections was labelled for designation of which antibody

The Autostainer would apply. The sections were then prepped for the Dako Autostainer by following the protocol for "OXPHOS Subunit Immunohistochemistry for Fixed Frozen Sections by MitoSciences". The sections were fixed in 4% formaldehyde at 4^0C for 10 min, rinsed in dH2O washed in Tris-buffered saline-Tween 20 (TBS-T) for 10 min, permeabilized in a graded methanol series (70% 10 min, 95% 10 min, 100% 20 min, 95% 10 min, 70% 10 min), washed in TBS-T for 5 min and then further processed in a Dako Autostainer using the Dako EnVision™ FLEX High pH kit, the Autostainer was loaded with the specific primary antibody and dilutions seen in table 1. The 8 µm sections were then put in the Autostainer and incubated with the antibodies during a 2,5 hours procedure. The sections were then removed from the Autostainer and dehydrated in a graded alcohol series (70% 2min, 95% 2min, 100% 2min) ending with 4 min in xylene and then mounted in synthetic resin.

Table 1

Antibodies used	Subunit for	dilutions	Subunits coded by
Anti-NDUFB8 ab110242 abcam	CI (NADH dehydrogenase)	1:100	Nuclear DNA
Anti-SDHB ab14714 abcam	CII (succinate dehydrogenase)	1:500	Nuclear DNA
Anti-UQCRC2 ab14745 abcam	CIII (cytochrome c reductase)	1:4000	Mitochondrial DNA
Anti-MTCO1 ab14705 abcam	CIV (cytochrome c oxidase)	1:2000	Mitochondrial DNA
Anti-ATPB ab14730 abcam	CV (F1F0 ATP synthase)	1:500	Mitochondrial DNA
Anti-VDAC1 ab14734 abcam	Mitochondrial porin marker	1:2000	Nuclear DNA

For *enzyme histochemistry*, 8µm sections were stained for cytochrome c oxidase (COX), succinate dehydrogenase (SDH) and combined COX/SDH.

For cytochrome c oxidase the sections were removed from the freezer and thawed in room temperature for 15min. It was then incubated with a premixed *COX-incubation solution* containing DAB, disodium phosphate, sodium dihydrogen phosphate, cytochrome c, catalase and sucrose for 60 min in a light protected box. Sections were then washed in desterilized water before being dehydrated in a graded alcohol series (70% 2min, 95% 2min, 100% 2min) ending with 4 min in xylene and then mounted in synthetic resin. They were then left to dry and later scanned.

Sections that were to be stained for COX/SDH were not dehydrated and incubated with SDH following the SDH protocol bellow.

For SDH staining, the sections taken from freezer were thawed in room temperature for 15min and then incubated with a premixed *SDH-incubation solution*, containing sodium succinate, sodium dihydrogen phosphate, disodium phosphate and nitro tetrazolium blue chloride for 60 min at 37^0C in an oven. Sections were then washed thoroughly with desterilized water and mounted in aqueous mountant. Then left to dry and later scanned.

Software usage

After staining, the slides were processed and imaged in a Hamamatsu scanner with 40x magnification. Thereafter organized through usage of the program *NDP.view 2* from Hamamatsu.

Figures were then created by use of the program *Adobe Illustrator*.

Results

Both controls show normal enzymatic activity (fig 4 and 5, slides a-c) which can be seen clearly by SDH staining blue, COX-SDH dark brown and COX light brown.
In immunohistochemistry the controls show normal presence of Complex I-V subunits (fig 4 and 5, slides d-h) shown by the brown staining and a normal presence of mitochondria (fig 4 and 5, slide i).

All ten cases of dermatomyositis showed profound COX deficiency in the perifascicular regions (fig. 6, 7, 8 and 9, slides **a** and **c**). Immunohistochemical analysis of complex I and IV subunits showed markedly reduced expression in perifascicular regions corresponding to the same regions showing low COX enzymatic activity (fig. 6, 7, 8 and 9, slides **d** and **g**).
No deficiency of complex II, III and V were found. The absence of activity was not due to loss of mitochondria as shown by the mitochondrial marker VDAC1, these results are illustrated clearly through high magnification in (Fig. 7 and 9). These results were seen in all patients with COX deficiency.

The results seen in fig 6,7,8 and 9 can be compared to the normal expression of enzyme activity and protein presence in the two control cases (fig 4 and 5). Where we can see that there is no perifascicular atrophy and no corresponding deficiency of enzyme activity or proteins.

The disease control of inclusion body myositis (fig 10) is showing a mosaic pattern of COX deficient fibres (fig 10, slide a and c). This is seen as pale fibres in slide c and blue fibres in slide a. In immunohistochemistry we can see a mosaic pattern showing decreased expression of subunits for complex I and V (fig 10, slide d and g) clearly seen in the pale fibres lacking expression of the proteins. These fibres correspond with the same fibres showing COX deficiency. There is no decrease in expression of complex II, III and V subunits (fig 10, slide e, f, h).

Muscle pathology

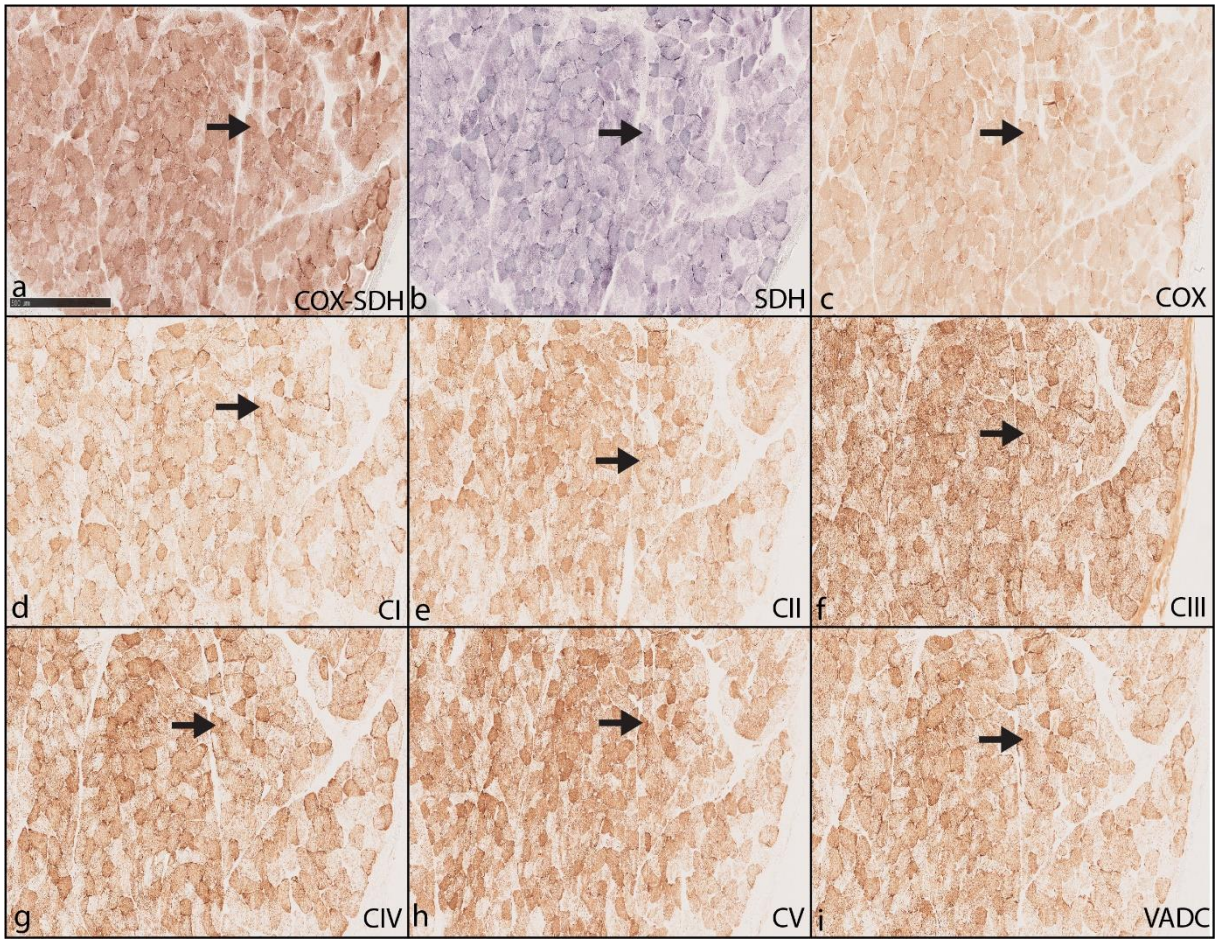

Fig 4. Control biopsy from adult patient showing healthy muscle. (**a-i**) are serial sections showing a view of a perifascicular region. (**a-c**) are stained through enzyme histochemistry

(enzyme activity) and (**d-j**) through immunohistochemistry (proteins). Notice the fibres highlighted by ➜ in section **a-c** showing normal expression of both enzymatic activity and the normal presence of complex I-V in **d-i.** This expression of both protein presence and enzymatic activity is seen throughout the entire sections. Scale bar = 500 µm for **a-i** (shown in **a**)

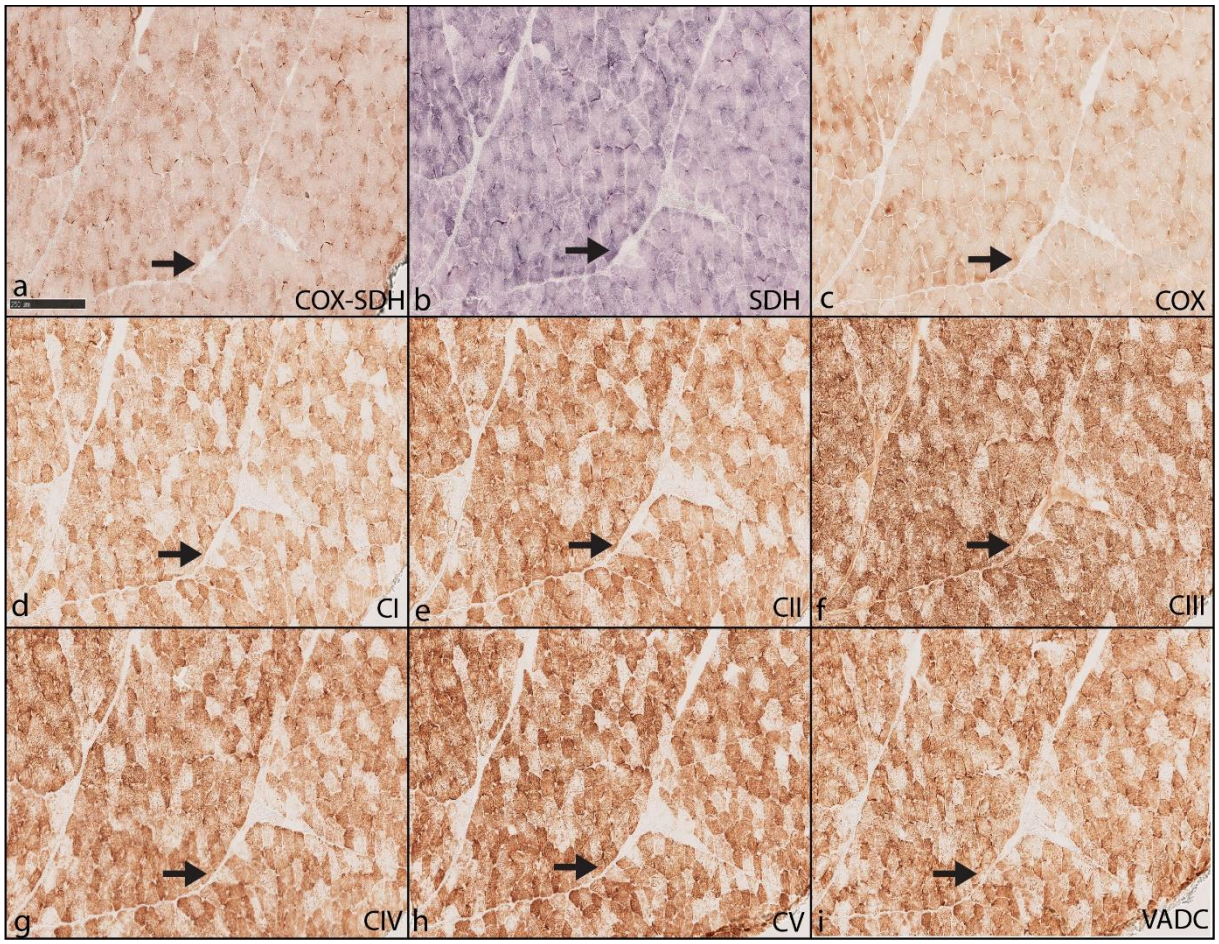

Fig 5. Control biopsy from juvenile patient showing healthy muscle. (**a-i**) are serial sections showing a view of a perifascicular region. (**a-c**) are stained through enzyme histochemistry (enzyme activity) and (**d-j**) through immunohistochemistry (proteins). Notice the fibres highlighted by ➜ in section **a-c** showing normal expression of enzymatic activity and normal presence of complex I-V in **d-i.** This normal expression of protein and enzymatic activity is seen throughout the entire sections. Scale bar = 250 µm for **a-i** (shown in **a**)

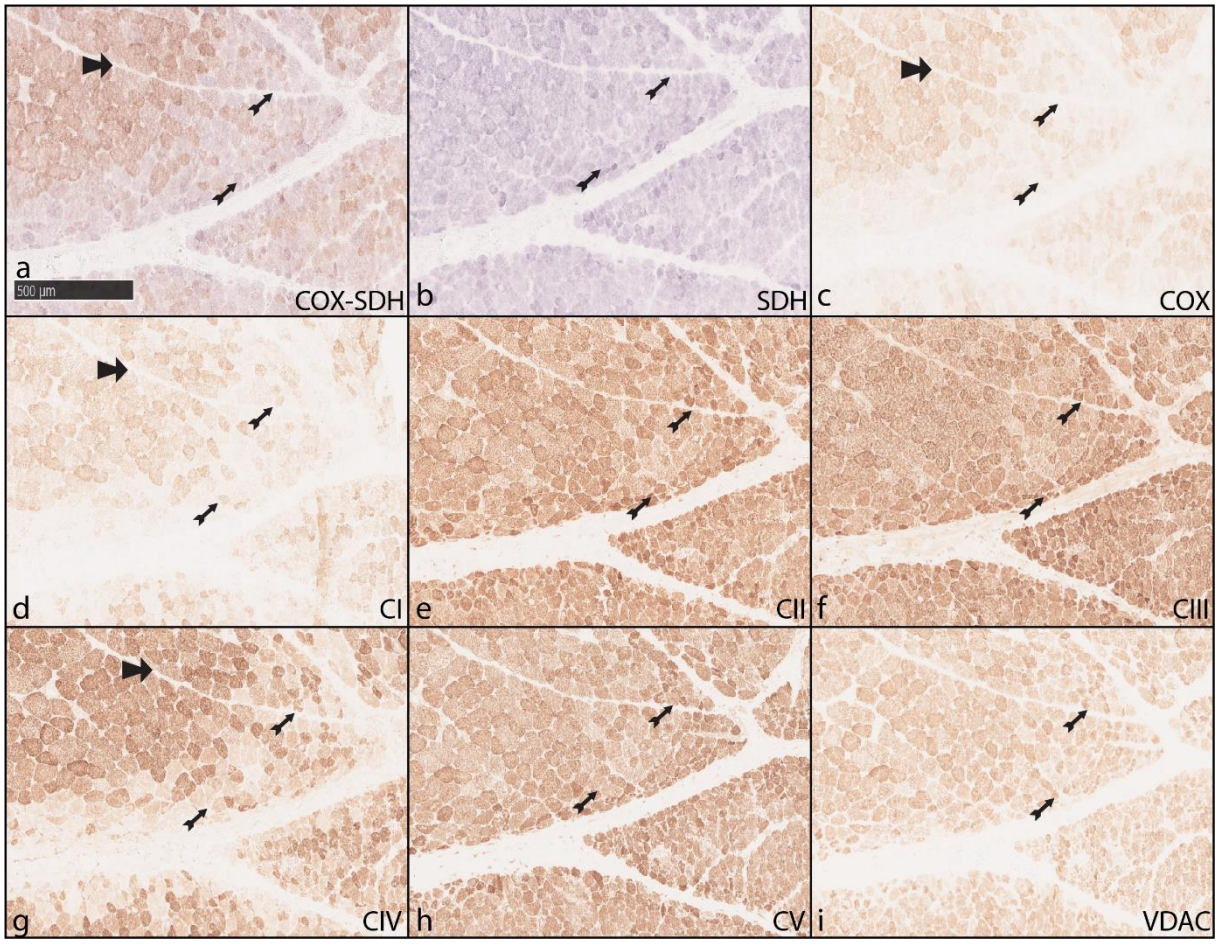

Fig 6. Muscle biopsy from patient with juvenile dermatomyositis.
(**a-i**) are serial sections showing a perifascicular region. (**a-c**) are stained with enzyme histochemistry (enzyme activity) and (**d-j**) with immunohistochemistry (proteins). Note the blue fibres in **b** and the pale fibres in **a** showing low COX enzymatic activity (➤) compared to normal (⏩). In **d and g** note the pale fibres showing low expression of complex I and IV highlighted by ➤ compared to healthy area shown by ⏩. Scale bar = 500 µm for **a-i** (shown in **a**)

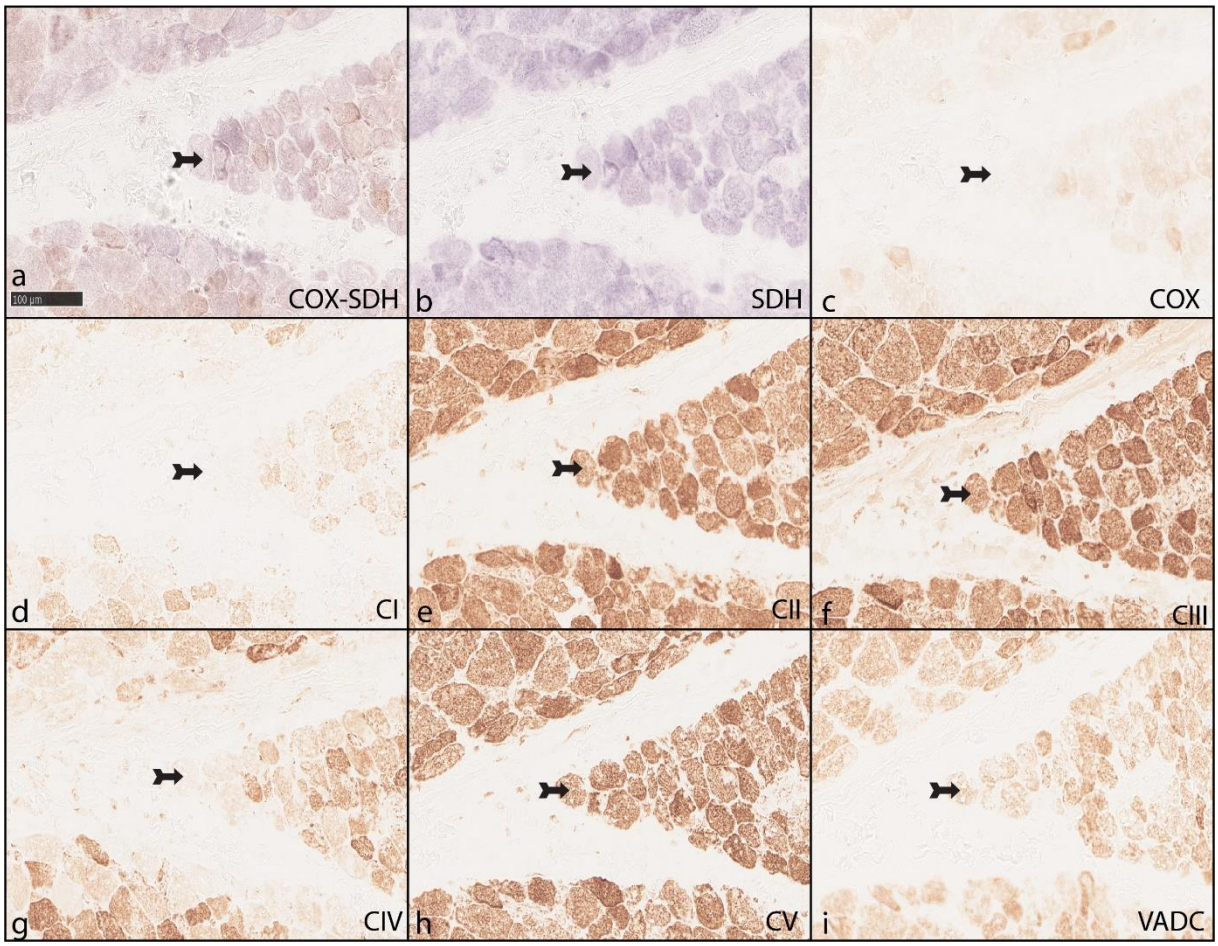

Fig 7. Muscle biopsy from a patient with juvenile dermatomyositis.
(**a-i**) are serial sections showing a higher magnification compared to Fig 4. To study single muscle fibres. (**a-c**) are stained with enzyme-histochemistry (enzyme activity) and (**d-j**) are stained with immunohistochemistry (proteins).
Notice the fibres highlighted by ➳. In **a, b and c** we can see a lack of COX enzymatic activity but no deficiency of SDH enzymatic activity. **D and g** demonstrate a markedly reduced expression of complex I and IV compared to the other complexes shown in **e, f and h.** Mitochondrial expression are shown by **i.** Scale bar = 100 µm for **a-i** (shown in **a**)

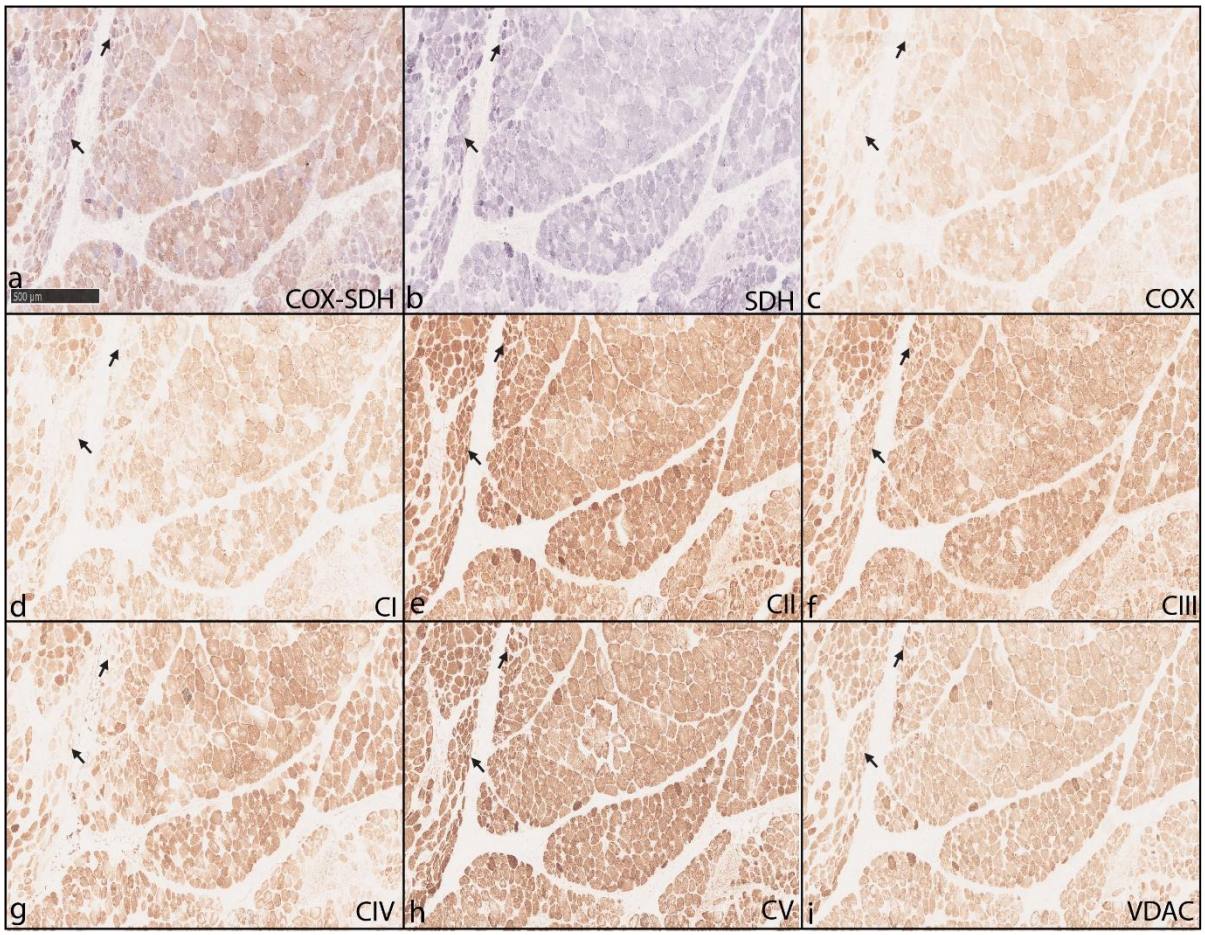

Fig 8. Deltoideus muscle biopsy from patient with adult dermatomyositis. (**a-i**) are serial sections showing a view of a perifascicular region. (**a-c**) are stained with enzyme histochemistry (enzyme activity) and (**d-j**) with immunohistochemistry (proteins). Note the small atrophic fibres in the perifascicular area highlighted by ➜ and the lack of COX enzymatic activity shown by **a and b** in corresponding areas**. D and g** are showing reduced expression of Complex I and IV (➜). Mitochondrial expression is shown by **i.** Scale bar = 500 µm for **a-i** (shown in **a**)

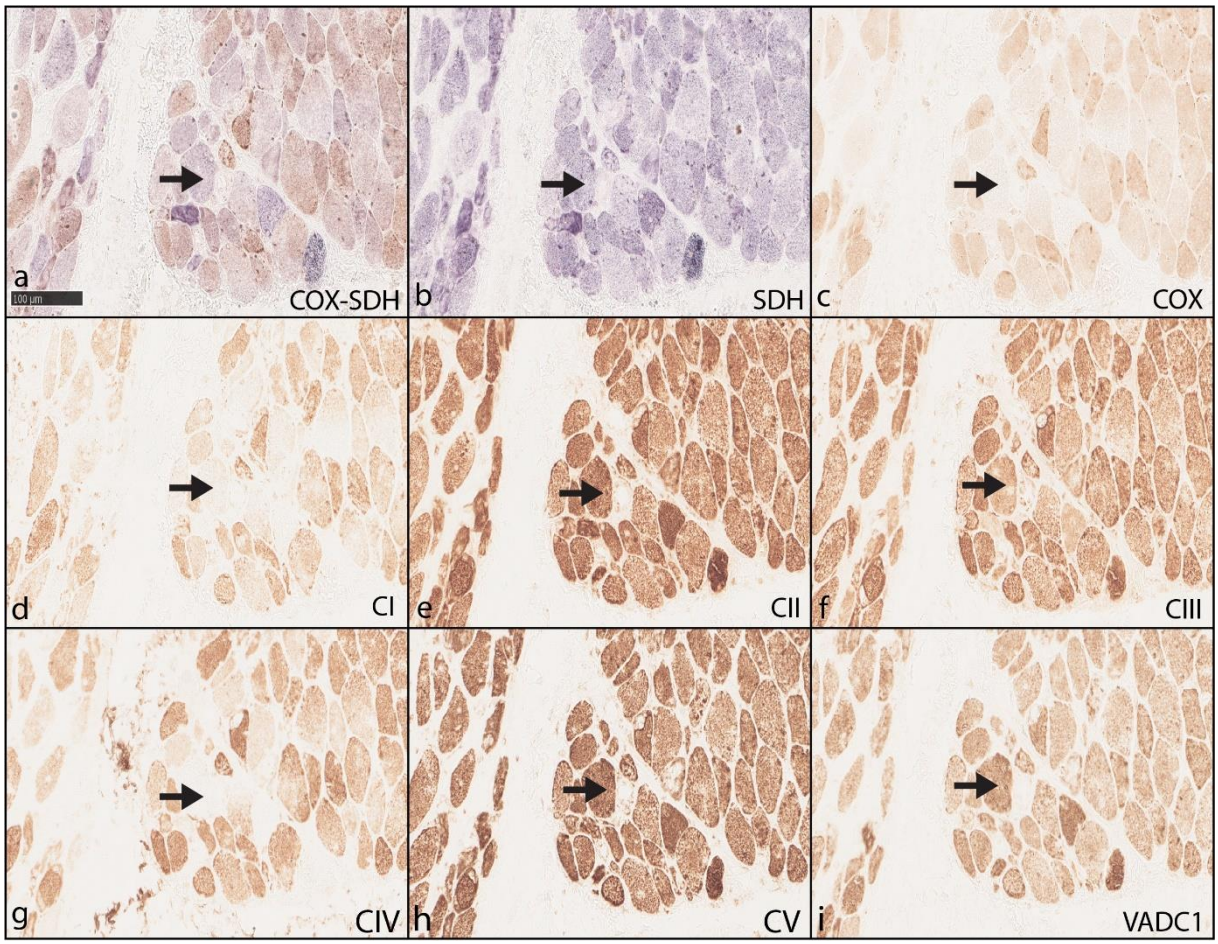

Fig 9. Deltoideus muscle biopsy from patient with adult dermatomyositis. **(a-i)** are serial sections showing a higher magnification compared to Fig 6. To study single muscle fibres. (**a-c**) are stained through enzyme-histochemistry (enzyme activity) and (**d-j**) are stained through immunohistochemistry (proteins). Notice the fibres highlighted by ➔. In **a, b and c** there is a lack of COX enzymatic activity but no deficiency of SDH enzymatic activity. **D** and **g** demonstrate a markedly reduced expression of complex I and IV compared to the other complexes shown in **e, f and h.** Mitochondrial expression are shown by **i.** Scale bar = 100 µm for **a-i** (shown in **a**).

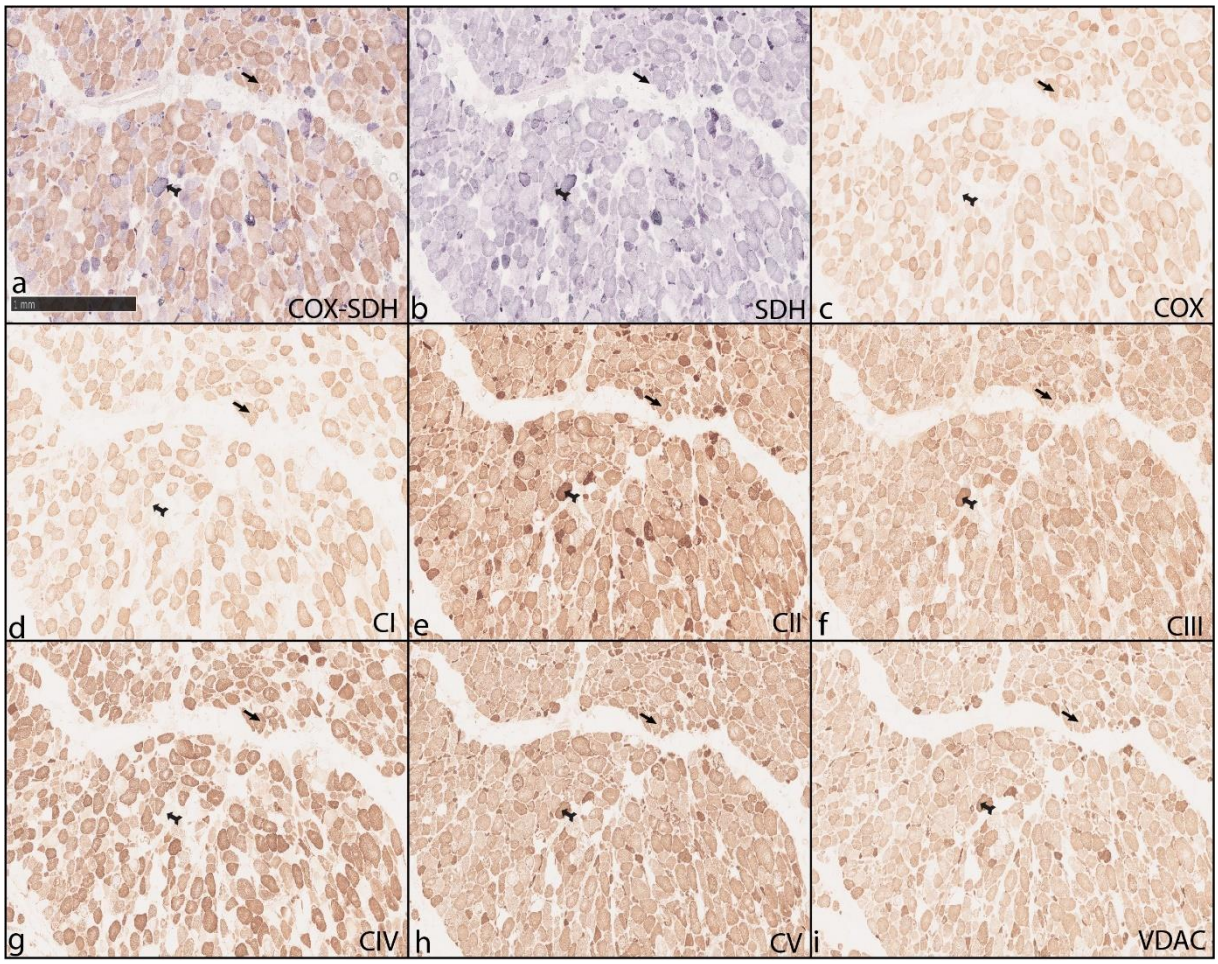

Fig 10. Disease control muscle biopsy from a patient with inclusion body myositis. (**a-i**) are serial sections. (**a-c**) are stained with enzymehistochemistry (enzyme activity) and (**d-j**) with immunohistochemistry (proteins). Note the scattered fibres showing low COX enzymatic activity in **b and c.** In **d and g,** we see a reduced expression of complex I and IV corresponding to the same fibres that show low COX seen in **b and c.** Scale bar = 1mm for **a-i** (shown in **a**).

Discussion

Dermatomyositis is characterized by muscle weakness and inflammation in the muscle. The pathogenesis of the sometimes profound muscle weakness is not fully understood, since there is usually no major loss of muscle bulk as compared to muscular dystrophies that are characterized by severe muscle wasting with replacement of the muscle by fat and fibrous tissue. Muscle fibre function and contractility may be affected in various ways, one being a metabolic disturbance as in metabolic myopathies affecting the energy metabolism and ATP synthesis. A mitochondrial disturbance in dermatomyositis is indicated by a deficiency of cytochrome c oxidase, which is part of the mitochondrial respiratory chain, that can be seen especially in the perifascicular regions.

The goal of this study was to investigate the mitochondrial dysfunction in dermatomyositis, in order to increase the understanding of the disease mechanisms. Three major questions were addressed: 1. Is the cytochrome c oxidase (Complex IV in the respiratory chain) deficiency associated with a corresponding lack of the protein that builds up the enzyme? 2. Are other complexes than complex IV affected in dermatomyositis? 3. Are there indications that the mitochondrial disturbance involves mitochondrial DNA or its transcription and translation?

We found, by applying immunohistochemistry, that in dermatomyositis there is a markedly reduced expression of NDUFB8 (subunit of complex I) and MTCO1 (subunit of complex IV) in perifascicular regions corresponding to the same regions and cells that show low COX enzymatic activity. There was no deficiency of succinate dehydrogenase (SDH, complex II) activity and this was in accordance with the normal expression of the complex II subunit SDHB. There was neither any deficiency of the complex III subunit UQCRC2 or complex V

subunit ATPB. From these results we conclude that the enzymatic deficiency is due to lack of enzyme protein. If an enzyme histochemical method to study complex I (NADH-dehydrogenase) had been available it would probably had shown NADH dehydrogenase deficiency in the perifascicular regions, since there was a profounddepletion of NDUFB8 protein indicating deficiency of the complete complex I, since NDUFB8 is essential for the assembly of complex I (17).

By applying immunohistochemical and enzymehistochemical techniques we could show that there is deficiency of complex I and IV in the respiratory chain with a perifascicular distribution. The results do not provide information on why the complex I and IV were deficient but are in accordance with previous studies demonstrating complex IV deficiency in dermatomyositis (2, 3). Further research is needed to identify the underlying mechanisms. In inclusion body myositis, there is also lack of complex I and IV but the deficient fibres are distributed completely different from in dermatomyositis. In inclusion body myositis the deficient fibres are distributed as scattered isolated cytochrome c oxidase deficient fibres and no regional accentuation. The molecular basis for deficiency of complex I and IV in muscle fibers in inclusion body myositis has been demonstrated to be due to accumulation of mtDNA with multiple different deletions in muscle fiber segments but with mainly one type of deletion in each single fiber indicating a clonal expansion in single fiber segments (12, 13). This situation is similar to what is seen in patients with polymerase gamma mutations that cause multiple mtDNA deletions and mitochondrial myopathy with mainly complex I and IV deficiency (18). However, in inclusion body myositis there is no such explanation for the occurrence of mtDNA deletions.

The two deficient enzyme complexes (I and IV) are both encoded by nuclear and mitochondrial DNA. Among the complexes not affected (II, III and V), we know that complex II is entirely coded by nuclear genes, whereas complex III and V are encoded by both nuclear and mitochondrial DNA. Since only complex I and IV were absent a question that arose were if there exist a break in the chain from transcription to translation of the mitochondrial complexes I and IV affecting either the nuclear encoded subunits or the mitochondrial. Deficiency of complex I and IV is typically found in disorders caused by defects in mitochondrial DNA (mtDNA) or the transcription or translation of mtDNA. In inclusion body myositis the mosaic deficiency of cytochrome c oxidase is caused by clonal expansion of mtDNA with multiple deletions, but so far mtDNA deletions have not been identified in dermatomyositis (3). Further studies may unravel the pathogenesis leading to reduced expression of complex I and IV in dermatomyositis.

The clinical significance of these findings remains to be evaluated. In primary mitochondrial myopathies clinical signs and symptoms include muscle weakness and fatigue at exercise similar to what is seen in dermatomyositis. It may be speculated that the mitochondrial dysfunction significantly adds to the muscle functional deficiency in many cases of dermatomyositis. Ways to reverse the mitochondrial dysfunction would then be a treatment strategy. However better understanding of the pathogenesis of the mitochondria deficiency is needed to develop such therapies.

Further investigation

A working hypothesis is that mitochondrial DNA depletion (reduced mtDNA copy number) causes the deficiency of complex I and IV in spite of normal or increased number of

mitochondria in the peri-fascicular region. Further studies on this subject should be focused on investigating mtDNA and mtRNA to further examine the underlying reason for the absence of complex I and IV, and explore a possible break in the chain from replication to transcription and translation of mtRNA. MtDNA could be investigated by examining the mtDNA through quantitative PCR analysis as seen in (19), together with long-range PCR analysis examining possible large-scale mtDNA deletions. To study the specific regions with respiratory chain deficiency a possibility would be to dissect these regions by laser microdissection and compare with regions showing normal activity. MtRNA could be examined through in-situ hybridization where mtRNA levels could be studies in regions that show cytochrome c oxidase deficiency by using mtRNA probes.

Methodological considerations

The methods used in this study were enzyme histochemistry to examine enzyme activity of complex II and IV and immunohistochemistry to examine the presence of complex I-V of the respiratory chain. An advantage of using histochemical techniques is that they can identify focal alterations where biochemical studies may be unable to disclose deficiency in the homogenate, but they are less quantitative. Biochemical methods would have implied isolation of mitochondria and then oximetric analysis of their oxygen consumption when adding different substrates or spectrophotometric analysis of the different enzymes. Such studies could possibly be added to get a more quantitative evaluation of the overall mitochondrial function in dermatomyositis.

Conclusion

This study has provided new evidence on the mitochondrial dysfunction in dermatomyositis in that it is limited to an absence of complex I and IV in the respiratory chain. Further research on the mitochondrial dysfunction of complex I and IV in dermatomyositis may lead to better understanding of the disease and through that create better treatment and management.

Populärvetenskaplig sammanfattning

Mitokondriell påverkan i barn och vuxna med dermatomyosit

Dermatomyosit tillhör gruppen inflammatoriska muskelsjukdomar. Den förkommer hos både barn och vuxna och drabbar ungefär 20 svenskar per år. Orsaken till sjukdomen är okänd men den anses vara en autoimmun sjukdom, vilket innebär att immunförsvaret är felaktigt riktat mot kroppens egna vävnader. Symtomen från dermatomyosit är en gradvis försämring av muskelstyrka och funktion. Diagnosen ställs utifrån symtom och laboratorieundersökningar av muskler och blod. Behandlingar syftar till att dämpa immunsystemets aktivitet och återställa muskelfunktion. En del vuxna kan så småningom avsluta behandlingen, medan andra måste fortsätta med den under många år och ibland livet ut.

Att djupare förstå den bakomliggande orsaken till hur en sjukdom uppkommer och förklaring till de symtom som medföljer är viktigt för att kunna ge korrekt behandling och träning av drabbade patienter. Det ger även möjlighet till upptäckt av nya behandlingsstrategier.

Orsaken till muskelsvagheten i dermatomyosit är inte helt förstådd. Man vet dock att det finns en påverkan på cellernas mitokondrier. Mitokondrier finns inuti våra celler och fungerar som cellernas kraftverk. När de inte fungerar kan många olika symtom uppkomma, exempelvis svaghet i musklerna.

I mitokondrierna omvandlas energin från maten vi äter till kroppens egna form av energi i form av adenosintrifosfat (ATP) med hjälp av komplicerade system av olika enzymer (en sorts proteiner). Bildningen av ATP sker i den så kallade mitokondriella andningskedjan eller med andra ord elektrontransportkedjan. Denna kedja är uppbyggd av fem olika enzymkomplex vars namn och ordning i kedjan är följande.

- komplex I (1); NADH-ubiquinon-oxidoreduktas
- komplex II (2); succinat-dehydrogenas
- komplex III (3); ubiquinon-cytokrom-c-oxidoreduktas
- komplex IV (4); cytokrom-c-oxidas
- komplex V (5); ATP-syntetas.

Tidigare studier har sett att inom visa områden av sjuka muskler hos patienter med dermatomyosit så saknas aktivitet från komplex IV. Vårt syfte med denna studie är att undersöka orsaken till varför detta enzym inte fungerar och se om det finns flera enzymer som inte fungerar. För att undersöka påverkan av de fem enzymkomplexen i patienter med dermatomyosit använda vi oss av specifika färgningsmetoder. Där man på mikroskopisk nivå kan färga för både närvaro av specifika proteiner och aktivitet för olika enzymer.

De två tekniker vi använde var *enzymhistokemi* där man mäter enzymaktivitet i muskelvävnad och *immunhistokemi* där man undersöker närvaron av specifika proteiner i en muskelvävnad.

Genom dessa tekniker kunde vi undersöka närvaron av alla fem komplex i elektrontransportkedjan och enzymaktivitet hos komplex II och IV.

I denna studie såg vi att komplex I och komplex IV uppvisade tydliga brister i muskelvävnaden, medans komplex II, III och V var normala. Denna studie använde sig av tekniker vars begränsning låg i att de endast kunde se om det var en brist men inte varför. Vidare forskning behövs för att finna den bakomliggande orsaken till denna brist

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