Spontaneous recovery of a childhood onset mitochondrial myopathy caused by a stop mutation in the mitochondrial cytochrome c oxidase III gene

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In general, the clinical course of patients suffering from different types of mtDNA mediated neurodegenerative disorders progresses with age. The clinical progression of muscle weakness has been reported to correlate with an increase in cytochrome c oxidase (COX) negative fibres or with an increase of mutant mtDNA in skeletal muscle.

In a recent issue we reported on a patient with mitochondrial myopathy with ragged red fibres (RRF), lactic acidosis, exercise intolerance, and delayed growth, with a heteroplasmic G9379A nonsense mutation (W58X) in the mtDNA encoded COIII subunit gene. A follow up examination of the patient showed significant improvement of the neurological symptoms. Here we present the results of detailed clinical, histological, immunohistological, biochemical, and genetic investigations of a repeated muscle biopsy, which all confirm a spontaneous regression of the disease.

PATIENT AND METHODS

Case report

The early clinical history of the patient has been previously reported in detail and is now compared with recent examinations (table 1). In brief, he developed exercise intolerance, generalised muscle weakness with painful muscle cramps, and fatigue at the age of 6 years, and his symptoms were progressing. On examination at 14 years of age, his somatic growth was delayed and he presented generalised mild muscle weakness, muscular hypotonia, and scapular winging. Symptomatic therapy with L-carnitin (2 x 10 ml), sodium hydrogen carbonate, and regular physiotherapy were initiated.

From the age of 16 years the patient’s symptoms improved gradually. At 19 years of age rapid growth was noted, but his weight remained low. A detailed neurological examination was carried out at his present age of 20 years. The patient reported an improvement in his muscle strength and the resolution of exercise intolerance. Episodes of myoglobinuria were never reported.

On examination mild scapular winging was noted. Muscle strength was 5/5 in all muscle groups, except for a very mild weakness in the foot dorsiflexors (mild difficulty in walking on heels). Deep tendon reflexes were mildly decreased on both sides. In addition to his weekly physiotherapy he now performs some sport (cycling) and works full time.

Morphology, immunohistochemistry, and biochemistry of skeletal muscle

At 14 and 20 years of age, open muscle biopsies of the patient were performed. The first biopsy was from the right quadriceps femoris, the second from the right tibialis anterior muscle. Muscle sections of both biopsies were histochemically stained with ATPase at different pH according to standard procedures. A total of 515 fibres (first biopsy, vastus lateralis) and 528 fibres (second biopsy, tibialis anterior) respectively, were differentiated and separately counted for fibre types I and II. Immunohistochecmical stains and activities of respiratory chain (RC) complexes I–IV were determined in skeletal muscle as described.

DNA analysis

The G9379A mutation was originally found by sequencing the entire mitochondrial genome. The mutational load was quantified by PCR and RFLP in total muscle DNA of the patient’s first and second muscle biopsy specimen and in DNA extracted from myoblast cells cultured from the second muscle biopsy as previously described.

RESULTS

Histological regression of the mitochondrial myopathy

When compared with the first muscle biopsy, which showed signs of a chronic myopathy with fibre size variations, 30% RRF, and severe generalised COX deficiency on histochemistry, the second muscle biopsy showed marked improvement. Signs of the myopathic process were less severe (fig 1). The average percentage of RRF remained similar (47%), but the ragged red appearance of the fibres became much less pronounced and the lipid containing vacuoles were also less pronounced.

Key points

- In a recent issue we reported on a patient suffering from mitochondrial myopathy with ragged red fibres, lactic acidosis, exercise intolerance, and delayed growth with a heteroplasmic G9379A nonsense mutation (W58X) in the mtDNA encoded COIII subunit gene.
- A follow up examination of the patient showed remarkable clinical and electrophysiological improvement.
- On a repeated muscle biopsy, signs of histological and immunohistological improvement of the mitochondrial myopathy were found, which was associated with a significant decrease (from 93% to 50%) of the mutational load of G9379A in skeletal muscle.
- Our results demonstrate the variable course of disease caused by mtDNA mutations. A possible positive outcome should be considered when counselling patients with mtDNA disorders.

Abbreviations: CS, citrate synthase; COX, cytochrome c oxidase; RRF, ragged red fibres

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showed a significant recovery of subunits II/III in COX positive fibres. On serial sections, the histochemically COX negative fibres (fig 2A) showed negative staining for COX subunits II/III; however, some of these fibres stained positive for subunits Vab (fig 2B, C). The subsarcolemmal mitochondrial proliferation also became less severe.

**DNA analysis reveals a significant decrease in the mutational load of G9379A**

G9379A creates a stop codon (W58X) in COIII and has been identified as the causative mutation. Electrophoresis revealed a high rate (93%) of the heteroplasmic G9379A point mutation in DNA extracted from the first muscle biopsy specimen, but a lower rate in the muscle DNA extracted from the second biopsy (50%). The mutation was not present in myoblast cells of the patient cultured from the second biopsy (fig 3).

**DISCUSSION**

We have described a novel heteroplasmic mtDNA stop mutation (W58X) in COIII in a patient with mitochondrial myopathy. Including this patient, five cases are now reported with different pathogenic mutations of the mitochondrial encoded COIII subunit gene. Despite significant decline in COX activity in skeletal muscle (10–20% residual activity), both clinical outcome and muscle involvement of COX III deficient patients were relatively mild. In three of the reported five patients (including ours) the mutation was found almost exclusively in skeletal muscle.

On recent examinations, our patient presented with an almost complete clinical recovery and with a significant histological improvement (table 1). In support of this, a marked decrease of the mutational load was observed in the second biopsy. The G9379A mutation showed a high rate (93%) in the first muscle biopsy and was absent in blood DNA and hair follicles. In the second biopsy, the mutational load was 50% and as with blood DNA and hair follicles, the mutation is absent in myoblasts. Myoblasts, also known as satellite cells, determine the regenerative capacity of skeletal muscle after injury, surgery, or neuromuscular diseases. The fact that the myoblasts of our patient do not harbour the mutation would support the idea that they fuse into already existing muscle fibres and decrease the mutant/wild type ratio during regeneration as has been suggested by Shoubridge et al.

We are aware of the fact that differences in the mutational load may occur between different muscles and even different biopsy specimens of the same muscle in the same patient. Notably, the different composition of fibre types among the two biopsied muscles may have some effect on mutational load, if the mutation were not evenly shared by type I and type II fibres. The tibialis anterior of the patient contains more type I fibres (68%) compared with the vastus lateralis (48%), which concurs with previous reports on a relative predominance of type I fibres in human tibialis anterior. However, this is unlikely to explain the drop in the mutational load (93% in the first biopsy v 50% in the second biopsy). Even if type II fibres carried only mutated mtDNA molecules (100% mutation) in both biopsies, the calculated mutational load for the second biopsy with the larger contribution of type I fibres would not be less than 89%.

In further support of the genetic improvement, additional analyses in our patient showed clear improvement in clinical, electrophysiological, histological, immunohistological, and biochemical parameters, as summarised in table 1.

Changes in the mutational load in skeletal muscle of patients with different types of mtDNA mediated disorders have also been reported by other groups. For single and multiple mtDNA deletions an accumulation of mutant in...
skeletal muscle was shown with time,⁴ ¹⁴ but there are few data known about mtDNA point mutations. In a previous study,¹⁵ one patient with a point mutation in tRNALeu (CUN) presented a remarkable increase (33.4%) in the ratio of the wild type genome after 11 days of concentrated exercise (from 11.8% to 48.4%). Physiological, biochemical, and genetic testing of patients carrying different mtDNA mutations in skeletal muscle were previously performed before and after 14 weeks of aerobic conditioning training.¹⁷ ¹⁸ There was a biochemical improvement observed in the activity of respiratory chain complexes and in oxygen utilisation; however, the proportion of wild type mtDNA was unchanged or slightly decreased, suggesting a trend toward preferential proliferation of mutant genomes. These results imply a training induced mitochondrial proliferation that might result in changes of the ratio of mutant to wild type mtDNA. Another example of a re-examination of skeletal muscle concern a patient with the recently reported 15 bp microdeletion in COIII. The deletion was detected with a high rate of heteroplasmy in skeletal muscle (92%) and a very low rate in blood (0.7%), and except for episodes of recurrent myoglobinuria the muscle strength of the patient was normal.¹¹ This patient was included in the studies of Taivassalo et al.,¹⁷ ¹⁸ and a second biopsy was performed 5 years later. Like our results, there was a significant difference in the mutational load compared with the original publication (from 92% to 36%). However the mutational load in repeated muscle biopsies of other patients of Taivassalo et al.¹⁷ ¹⁸ carrying different types of mtDNA mutations showed minor differences only. As we do not have enough follow up data about other cases, we cannot predict how often spontaneous recovery occurs in patients with different types of mtDNA mutations. In both our patient and the patient carrying the 15 bp microdeletion and showing decrease in the mutational load, the mutation was located to COIII. Whether or not this phenomenon is characteristic for mutations in COIII needs to be confirmed in a larger number of cases.

The improvement of the biochemical COX activity and the histological staining of skeletal muscle for COX partly resembles another disease entity, benign infantile COX deficiency syndrome. This rare syndrome is characterised by severe muscular hypotonia, generalised weakness, and lactic acidosis in early infantile period, and if the patient survives this stage, complete or almost complete recovery occurs within 1–2 years.¹⁹ ²⁰ The mode of inheritance and the mechanism of the reversibility are still unknown.²¹ The presence of mitochondrial abnormalities and RRF in this syndrome has been repeatedly confirmed,¹⁹ ²⁰ ²² suggesting a role of mtDNA mutations in the pathomechanism; however, no mutation in the mtDNA has been found so far in these patients.

What might account for the improvement of the muscular symptoms in our patient with a stop mutation in COIII? Functional studies on cybrids containing the 15 bp microdeletion in COIII have shown that only homoplasmic mutants fail to assemble the COX holoenzyme and even 3% wild type COIII can have a marked positive effect on the functioning of the respiratory chain.²³ The decrease in the mutational load of W58X in our patient might be responsible for the clinical improvement, and even the small increase in COX activity might result in a relatively good muscle energy supply. MtDNA wild type containing myoblasts may fuse into existing muscle fibres over time and positively influence the rate of mutant in skeletal muscle. This mechanism may explain the change of the mutational load and indirectly the positive clinical outcome in our patient.

In summary, a remarkable clinical and histological improvement of mitochondrial myopathy together with a decrease in the mutational load was observed in a patient carrying a stop mutation in the mitochondrially encoded COXIII subunit gene. Our results demonstrate again the variable course of diseases caused by mtDNA mutations and a possible positive outcome should be considered in counseling patients with mtDNA mediated disorders.

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| Table 1 Summary of the clinical, histological, biochemical and genetic data of the patient at 14 and 20 years of age |
| Age (years) | 14 | 20 |
| Clinical presentation | MRC scale 4/5 for both proximal arm and leg muscles and for foot dorsiflexors | MRC scale 5/5 for both proximal and distal arm and leg muscles, mild difficulty in heelwalking |
| Exercise intolerance and painful muscle cramps; scapular winging | No exercise intolerance, no muscle cramps; mild scapular winging |
| Height. 3rd–10th percentile; weight: below 3rd percentile | Height: 50th percentile; weight: 5th percentile |
| Serum lactate (NR 0.5–2.2 mmol/l) | Highly elevated: up to 12.8 mmol/l; abnormal increase after exercise | Mildly elevated: 2.9 mmol/l; normal increase after exercise |
| Electromyography | Myopathic changes, polyphasic, low amplitude action potentials in deltoid and quadriceps muscles | Normal in deltoid and quadriceps, but very mild increase in spontaneous activity in the right tibialis anterior muscle |
| Muscle histology | Chronic myopathy; 50% ragged red fibres; severe lipid accumulation; severe COX defect in all fibres | Mild myopathic changes; 47% ragged red fibres; milder lipid accumulation; 14% COX positive fibres |
| Immunohistochemistry | Virtual absence of COX II/III but normal staining for COX IV in all fibres | COX positive fibres where positive for COX II/III, IV, and Vab, but some of the COX negative fibres were also positive for COX IV and Vab |
| COX (NR 0.90–4.70) | 0.03 U/U CS; 256 U/g NCP | 0.08 U/U 136 U/g NCP |
| C5: 45–105 U/gNCP | 256 U/g NCP | 136 U/g NCP |
| Rate of mutant mtDNA | 93% mutant mtDNA in muscle; 0% in hair follicles; 0% in blood | 50% mutant mtDNA in muscle; 0% mutant in myoblasts |

NR, normal range; MRC, Medical Research Council.
On serial sections of the second muscle biopsy, histochemical stain for COX (A), and immunohistochemistry for COX II/III (B) and Vab (C) subunits were performed. COX staining was positive for 14% of all fibres (A). There was a significant recovery of subunits II/III in COX positive fibres (B). The histochemically COX negative fibres showed negative staining for COX subunits II/III, however, some of these fibres stained positive for subunits Vab (fig 2B, C).

Figure 2

Figure 3

RFLP analysis of the heteroplasmic G9379A point mutation revealed a high rate (93%) in DNA extracted from the first muscle biopsy specimen (lane 1), but a lower rate (50%) in the muscle DNA extracted from the second biopsy (lane 2). The mutation was not present in myoblast cells of the patient cultured from the second biopsy (lane 3), or in hair follicles (lane 4) and blood DNA of the patient (lane 5) and his mother (lane 6).

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