ELECTRONIC LETTER

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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J Med Genet 2004;**41**:e75 (http://www.jmedgenet.com/cgi/content/full/41/6/e75). doi: 10.1136/jmg.2003.015024

In general, the clinical course of patients suffering from different types of mtDNA mediated neurodegenerative disorders progresses with age.[1] The clinical progression of muscle weakness has been reported to correlate with an increase in cytochrome c oxidase (COX) negative fibres[2] or with an increase of mutant mtDNA in skeletal muscle.[3]

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.[4] 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.

Key points

- In a recent issue we reported on a patient suffereing 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 (fro 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.

PATIENT AND METHODS

Case report

The early clinical history of the patient has been previously reported in detail[4] 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 (2×10 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 dorsiflectors (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.[5 6] 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. Immunohistochemical stains[7] and activities of respiratory chain (RC) complexes I−IV were determined in skeletal muscle as described.[8]

DNA analysis

The G9379A mutation was originally found by sequencing the entire mitochondrial genome.[9] 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.[4]

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, 50% RRF, and severe generalised COX deficiency on histochemistry,[4] 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

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

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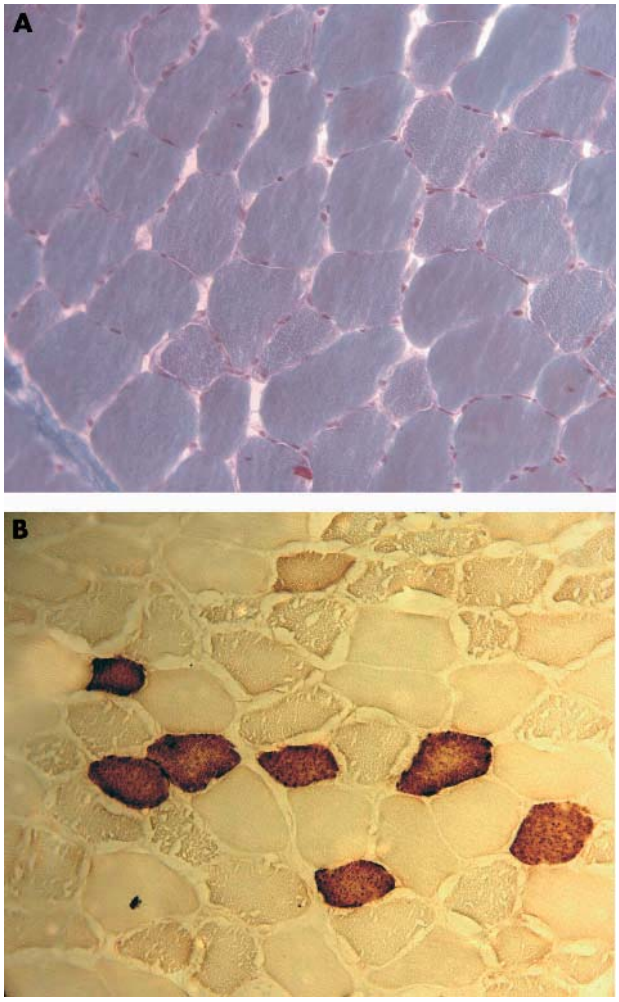

Figure 1 Signs of the myopathic process were less severe in the second muscle biopsy. The average percentage of RRFs was similar (47%) but the ragged red appearance of the fibres became much less pronounced (A). Normal staining for COX was seen in 14% of all fibres in the second biopsy specimen (B). Vacuoles are caused by lipid accumulation.

prevalent (fig 1A). In the second biopsy specimen (fig 1B) 14% of all fibres showed normal staining for COX, a significant change compared with the almost complete absence of COX staining in the first biopsy. In the first biopsy, 48% of all fibres were type I and 52% were type II, compared with 68% and 32% respectively for the second biopsy.

Biochemical measurements of RC enzymes showed increased, but still low activity of COX

Biochemically, there was a 2.5 fold increase in the activity (units) of COX/citrate synthase (CS) in the second biopsy specimen compared with the first, but the activity remained low (table 1). The increased activity of the mitochondrial marker enzyme CS in the first biopsy, probably reflecting compensatory mechanisms, almost normalised in the second biopsy (table 1).

Immunohistochemistry of skeletal muscle points to a recovery of COX II/III subunits

In the first biopsy, immunohistochemistry showed a virtual absence of immunoreactivity of the mtDNA encoded subunits II/III compared with control muscle.[4] Increased immunoreactivity in the subsarcolemmal zone due to the accumulation of mitochondria was observed. The second biopsy 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.[4] 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.[4 10–13] 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.[4] 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.[14] 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*.[15]

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.[5 6] 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

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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 Exercise intolerance and painful muscle cramps; scapular winging	MRC scale 5/5 for both proximal and distal arm and leg muscles, mild difficulty in heelwalking No exercise intolerance, no muscle cramps; mild scapular winging
Somatic growth	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 were 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) CS: 45–105 U/gNCP) Rate of mutant mtDNA	0.03 U/U CS; 256 U/g NCP 93% mutant mtDNA in muscle; 0% in hair follicles; 0% in blood	0.08 U/U 136 U/g NCP 50% mutant mtDNA in muscle; 0% mutant in myoblasts

NR, normal range; MRC, Medical Research Council.

skeletal muscle was shown with time,[3 16] but there are few data known about mtDNA point mutations. In a previous study,[15] one patient with a point mutation in $tRNA^{Leu\ (CUN)}$ 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 concerned 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.

ACKNOWLEDGEMENTS

The excellent technical assistance of A Zimmermann, S Galuschka, and E Schmidtmeyer and the clinical work of Dr C Schröder and Dr M Sievers are gratefully acknowledged. This work is supported by

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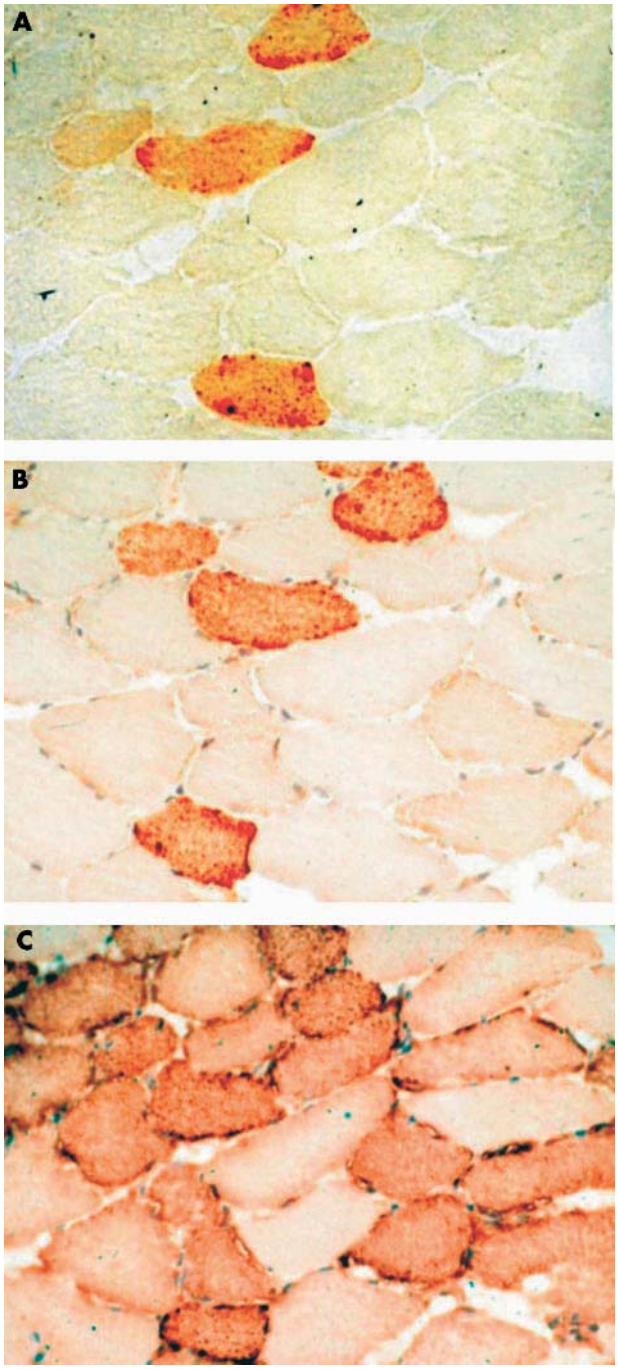

Figure 2 On serial sections of the second muscle biopsy, histochemical stain for COX (A), and immunohistochemistry for COX II/III (B) and Vab (C) subunits were performed. COX staining was positive for 14% of all fibres (A). There was a significant recovery of subunits II/III in COX positive fibres (B). 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).

grants from the Deutsche Forschungsgemeinschaft Ja802/2–1 (M Jaksch) and the Ernst and Berta Grimmke Stiftung (M Jaksch, R Horváth). Human myoblast cultures were obtained from the Muscle Tissue Culture Collection (Friedrich Baur Institute, Munich, Germany), part of the German network on muscular dystrophies (MD-NET, service structure S1, 01GM0302) funded by the BMBF (Bonn, Germany), partner of Eurobiobank (Eurordis; scientific coordinator: Dr C Jaeger, Paris, France), funded by the EC within the Fifth Framework (QLRT-2001–02769).

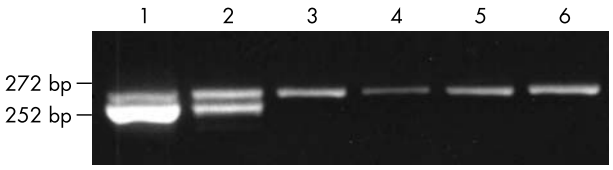

Figure 3 RFLP analysis of the heteroplasmic G9379A point mutation revealed a high rate (93%) in DNA extracted from the first muscle biopsy specimen (lane 1), but a lower rate (50%) in the muscle DNA extracted from the second biopsy (lane 2). The mutation was not present in myoblast cells of the patient 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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Conflict of interest: none declared

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Revised version received 17 November 2003
Accepted for publication 19 November 2003

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