A Novel Mitochondrial DNA Mutation in COX1 Leads to Strokes, Seizures, and Lactic Acidosis

Authors

E. W. Y. Tam¹, A. Feigenbaum², J. B. L. Addis³, S. Blaser⁴, N. MacKay⁵, M Al-Dosary⁶, R. W. Taylor⁶, C. Ackerley⁵, J. M. Cameron³, B. H. Robinson^{2,3}

Affiliations

Affiliation addresses are listed at the end of the article

Key words cytochrome c oxidase COX stroke seizures mtDNA mutation

Abstract

Cytochrome c oxidase (COX) is the terminal enzyme of the respiratory chain, with subunits originating both from the mitochondrial and nuclear genome. An eleven-year-old female presented initially with a seizure followed two months later with tonic-clonic seizures, weakness and aphasia. MRI of the cerebral hemispheres showed multiple infarcts. Previous history suggested gross and fine motor control deficits with learning difficulties. A muscle biopsy showed a specific decrease of COX staining in all fibres and pleomorphic mitochondria. Respiratory chain studies confirmed an isolated complex IV defect in muscle, whilst fibroblasts showed an initial COX activity below normal which rapidly came up to the normal range on culture. Sequencing of mtDNA revealed an heteroplasmic m.7023G>A mutation in the COX1 gene, with levels of 96% in muscle, 70% in blood and 50% in the initial skin fibroblast culture dropping to 10% in later passages. The mutation was present in a critical region of the COX1 gene, the V374M change being close to the two histidine residues His376 and His378 co-ordinating with the heme a and a₃, and His367 which co-ordinates a magnesium ion. This case highlights that a MELAS-like syndrome can occur with isolated COX deficiency

			Abbreviations v	
	received accepted		CS COX COX1 COX2	citrate synthase cytochrome oxidase cytochrome oxidase subunit 1 cytochrome oxidase subunit 2 cytochrome oxidase subunit 3 histidine
	Bibliography DOI 10.1055/s-0029-1202287 Neuropediatrics 2008; 39: 328–334 © Georg Thieme Verlag KG	5/s-0029-1202287	COX3	
		LRPPRC	leucine rich pentatricopeptide repeat cassette	
	9	Stuttgart - New York ISSN 0174-304X		mitochondrial encephalopathy lactic acidosis and stroke-like episodes
	Correspondence B. H. Robinson Canada Chair in Nutrition and Metabolism		MRI MtDNA SDS/PAGE	magnetic resonance imaging mitochondrial DNA sodium dodecyl sulphate/ polyacrylamide gel electrophoresis
	Research Ins	Programme Head, Metabolism Research Institute Hospital for Sick Children		transfer RNA
	555 University Ave. Toronto M5G 1X8	,	Introduction	
Ontario Canada Tel.: +1/416/813 !		/813 59 89	Defects in	mitochondria result in metabolic dis- t can affect many organs, but most

Tel.: +1/416/813 59 89 Fax: +1/416/813 87 00 bhr@sickkids.ca

metabolic disans, but most commonly result in disease of striated muscle, brain, heart, and other tissues with high energy

demands. Such defects often result from mutation of genes encoding enzymes required for oxidative phosphorylation or gene products required for the synthesis of these enzymes, and the defects can be in either mitochondrial or nuclear DNA. Cytochrome c oxidase (COX) is the terminal enzyme of the respiratory chain, transferring electrons from cytochrome c to molecular oxygen and coupling the transport of protons from the matrix to the cytosol, and is thus essential for the energy breakdown of all substrates. Human COX is a complex macromolecule composed of 13 subunits [28], of which the three largest subunits are encoded by mitochondrial DNA. The remaining subunits are encoded by nuclear DNA.

The majority of known COX defects have been found in nuclear genes implicated in COX assembly, resulting in autosomal recessive disorders that manifest early in infancy, often resulting in fatal outcome. Mutations in these nuclear genes have been described to result in early onset symptoms of cardiomyopathy, hepatic failure, anemia, sensorineural deafness, leukodystrophy, and Leigh syndrome [20]. Fewer mutations have

been reported from the mitochondrial COX subunits (COX1, COX2, and COX3), mostly as case reports of individual families, and their clinical presentations are more varied, including central nervous system, neuromuscular, and systemic disorders. Systemic disorders include acquired idiopathic sideroblastic anemia [12], colorectal and other tumours [7,21], and cerebrohepatic disease [30]. Neuromuscular disorders include recurrent myoglobinuria [13,14], myopathy [9,10,22], and motor neuron disease [5].

Disorders associated with mitochondrial mutations in COX subunits present with a wide spectrum of symptoms. Seizures have been described, including myoclonic seizures [12], neonatal seizures [27], and epilepsia partialis continua [31]. Specific known syndromes found to be associated with mitochondrial COX subunit mutations including Leigh syndrome and other multisystem disorders of variable ages of presentation [2–4]. Of note, these syndromes can include optic atrophy and visual impairment, sensorineural hearing loss, and cardiomyopathy.

Of the reported cases of mitochondrial COX subunit mutations, two mutations have been reported with a presentation of stroke-like episodes. One child, diagnosed with an m.9957T>C (F251L) mutation in *COX3*, presented with hyperventilation from 18 months of age, followed by stroke-like episodes from the age of 6 years, as well as blindness, hearing loss, spasticity, weakness, and behavioural regression [17]. The other patient, found to have an m.6328T>C (S142F) mutation in *COX1*, presented with exercise intolerance since the age of 16, hypertrophic cardiomyopathy diagnosed at 22, and a stroke-like episode at the age of 33 years [16].

In this manuscript, we describe the clinical, biochemical, and molecular characteristics of a novel m.7023G > A mutation in the *COX1* gene resulting in recurrent strokes and seizures.

Patient and Methods

Case history

The patient is the daughter of Polish parents, born at 42 weeks, after an uneventful pregnancy, by emergency Caesarian section due to failure to progress. There were no neonatal complications. Early developmental milestones were unremarkable, and she learned to walk independently soon after 12 months of age. Concerns were first raised at the age of 6 years, when it was noted that she had trouble keeping up with other children in physical activities. By the age of 9 years, she was noted to have exercise intolerance, being able to run for approximately 2 seconds at a time. She learned to ride a bicycle by the age of 10. Her fatigability also extended to fine motor activities including handwriting. By the age of 11, she was starting to master zippers, but continued to have difficulty with buttons. She continued to require assistance for bathing. She attended a regular classroom, with some modifications for mild developmental delay.

A detailed three-generation review of family history did not reveal any evidence for mitochondrial disorders aside from some learning disabilities reported in both the patient's mother and maternal grandmother. Neither of these individuals reported any exercise intolerance, or strokes. The patient's maternal halfbrother and paternal half-brother were both reported as asymptomatic (**• Fig. 1A**).

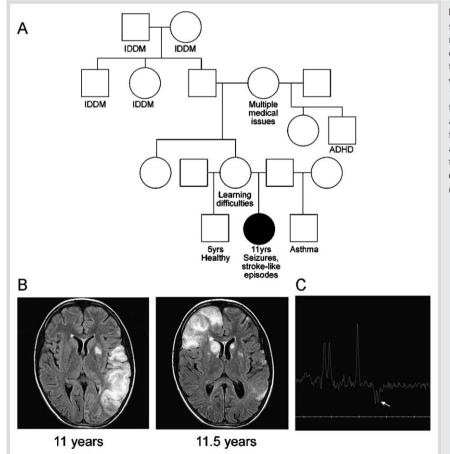


Fig. 1 A Family pedigree, the proband is shown in black. **B** Axial FLAIR image at 11 years reveals focal left basal ganglia signal increase and extensive abnormally increased signal intensity of the left temporal lobe. The cortex and peripheral white matter are involved. Axial FLAIR image at 11.5 years demonstrates near total resolution of the left basal ganglia and temporal lobe signal abnormalities and swelling. There is extensive right frontal swelling and signal increase. New focal areas of signal abnormality are in the heads of the caudate nucleus and in the left frontal cortex. **C** MRS (TE 144) demonstrates a lactate doublet (arrow).

She presented to the hospital at the age of 10 with five generalized tonic-clonic seizures, with post-ictal right arm and leg weakness. Despite rapid resolution of deficits, neuroimaging abnormalities were seen. On MRI, diffusion restriction was seen in the left basal ganglia, right splenium of the corpus callosum, and right occipital ribbon, corresponding to infarcts (• Fig. 1B). MR spectroscopy in the left basal ganglia showed an elevated lactate peak (• Fig. 1C). To investigate for possible vasculitis or thromboembolic phenomena, cerebral angiogram and echocardiogram were recorded but found to be normal. Ophthalmological examination was unremarkable. Cerebrospinal fluid testing showed an elevated lactate level at 3.3 mmol/L, although serum lactate was within normal limits. Metabolic investigations including serum amino acids, urine organic acids, and carnitine levels were within normal range. The hospital stay was complicated by frequent headaches. She was discharged home on acetylsalicylic acid 80 mg daily for stroke prevention and phenytoin for seizure prophylaxis.

Two months after initial presentation, she returned again to the hospital with right focal tonic-clonic seizures and expressive aphasia. A repeat MRI study at 11 years revealed a changing pattern, now with focal left basal ganglia signal increase and extensive abnormally increased signal intensity of the left temporal lobe. The cortex and peripheral white matter were also involved. MRI at 11.5 years demonstrated near total resolution of the left basal ganglia and temporal lobe signal abnormalities and swelling. There was extensive right frontal swelling and signal increase. New focal areas of signal abnormality were identified in the heads of the caudate nucleus and in the left frontal cortex (**o Fig. 1B**). MR spectroscopy again confirmed a lactate doublet (**o Fig. 1C**).

With a growing concern for a mitochondrial disorder and persistent headaches, acetylsalicylic acid was discontinued, and a trial of L-arginine, 3g per hour, and dexamethasone was given. Clobazam was added to aid in seizure control. Due to a residual mild right-sided motor and sensory deficit, as well as word-finding difficulty, she was transferred to a rehabilitiation hospital. Further readmissions occurred in the ensuing months for seizure management, without evidence of recurrent infarcts. Furthermore, she was also started on coenzyme Q10 100 mg bid.

A diagnosis of a mitochondrial disorder was made on muscle biopsy, sampled from the vastus lateralis. Isolated muscle fibres with conspicuous subsarcolemmal accumulations of mitochondria were seen on modified Gomori trichrome staining, whilst repeated histocytochemical studies showed significant, widespread reduction in cytochrome oxidase activity. Electron microscopy revealed pleomorphic and often large mitochondria.

Cell cultures and biochemical assays

Fibroblast cell lines were established from the patient with informed consent for diagnostic purposes. Blood and buccal cell samples for DNA analysis were obtained with informed consent from family members. Biochemical assays of NADH:ubiquinone oxidoreductase (complex I), rotenone-sensitive NADH-cytochrome c reductase (complexes I and III), succinate cytochrome c reductase (complexes II and III), cytochrome oxidase, COX (complex IV) and citrate synthase (CS) were performed as described [24].

Total DNA was extracted from blood, buccal cells, fibroblasts or muscle tissue [19]. MtDNA was amplified by PCR as described previously for all tRNA species, for subunits ND1-6 and ND4L and ATP6 and ATP8, and COX1, 2 and 3 as described previously [18, 19]. PCR products were sequenced by fluorescent di-deoxy sequencing (ACGT Corp, Toronto, CANADA). This was followed by automated sequencing of the mitochondrial genome to screen for other novel pathogenic mutations. The entire mitochondrial genome was sequenced in a series of overlapping fragments using M13-tagged oligodeoxynucleotide primers to facilitate direct sequencing of the PCR amplified products and direcly compared to the revised Cambridge reference sequence for human mtDNA [1,29]. Screening for common MELAS mitochondrial DNA mutations at 3243 and 3271, as well as Leigh syndrome mutation at 13513, were negative. Biochemical testing showed reduced cytochrome oxidase activity, and a 7023G > A mutation was found in the mitochondrial COX subunit 1.

Determination of heteroplasmy for 7023G>A

A segement of mtDNA from bp 6900 to 7063 was amplified using the primers: forward 5'-AAATGATCTGCTGCAGTGCT-3' and reverse:5'-AATACAGCTCCTATTGATAGGACATAGTGGAAGTGGGATA-3'. The reverse primer has a mismatched T instead of G at position 7026 that creates a recognition site for *Bful* which then digests the wild-type PCR product at the consensus sequence site GTATCC(N)₆. This recognition site is not affected by the polymorphism T or C at position 7034. Levels of heteroplasmy were determined by area measurements of ethidium bromide stained DNA bands using ImageJ [23].

Western blotting

Mitochondria were prepared from muscle and resolved through a 4–12% Bis-Tris NUPAGE gel (Invitrogen). The NUPAGE gel was electroblotted onto nitrocellulose membrane and blocked with 5% skim milk/TBST. The membrane was then probed with antirabbit LRPPRC, holo-COX or citrate synthase antibody.

Immunogold labelling

Muscle from patient and normal control were harvested and fixed in 4% paraformaldehyde, 0.1. M phosphate buffer (pH 7.4) containing 0.1% glutaraldehyde, for a minimum of 4h. The tissues were then washed several times with phosphate buffer. They were then stored at 4°C in PBS containing 20 mM sodium azide until further processing. Prior to cryoultramicrotomy, the cells were infused overnight in 2.3 M sucrose. The tissues were then frozen in liquid nitrogen on aluminum pins and sections cut on a diamond knife in a cryoultramicrotome (Ultracut R, Leica Canada, Willowdale, ON). Sections were transferred to formvar nickel coated grids in a loop of molten sucrose. The grids were then blocked in PBS containing 0.5% BSA and 0.15% glycine. After several rinses in PBS/BSA the sections were incubated in COX1 antibody diluted 1:10 in PBS for 1 hour. Following several rinses in PBS/BSA the specimens were then incubated with 10 nm gold goat anti-rabbit IgG particles (Amersham, Oakville, ON) for an additional hour, rinsed in PBS and distilled water and stabilized in a thin layer of methyl cellulose containing 0.2% uranyl acetate. The grids were then examined in a transmission electron microscope (JEOL JEM 1200, JEOL USA Peabody, MA) and images captured using a CCD camera (AMT corp. Danvers, MA).

Results

Biochemical testing of the muscle biopsy from the patient showed robust activities for complex I, complex I + III, complex

V and citrate synthase (• Fig. 2). Complex IV however showed a level which was below the normal range of activities. This value was below the normal range and relative to citrate synthase was considerably below the level (0.058) cf. > 0.21 for controls. Initial activities of complex IV were also decreased in skin fibroblast cultures, just below the bottom end of the normal range. These activities rapidly normalized on subculture to values within the control range. Thawing out fresh early generation cells gave reproducible activities below the normal range.

Sequencing of the individual COX genes from mtDNA derived from muscle revealed normal sequences for COX2 and COX3 but showed the presence of a novel, heteroplasmic m.7023G>A mutation in COX1. This mutation appeared to be present at a lower level in mtDNA isolated from fibroblasts (• Fig. 3A). Whole genome sequencing of mtDNA revealed no further unreported nucleotide changes other than a synonymous m.7034C>T mutation. Since the m.7023G>A mutation produced an amino acid substitution (Val374Met) in the critical heme-binding transmembrane helices of the COX1 protein, it was thought likely to be responsible for the pathology seen in this patient. Furthermore, the m.7023G>A mutation has not been identified in more than 300 sequences obtained in our own laboratory, is not represented on publicly available databases [11,25] and affects an evolutionary conserved amino acid. The mutation was not identified in DNA prepared from blood or buccal cells from

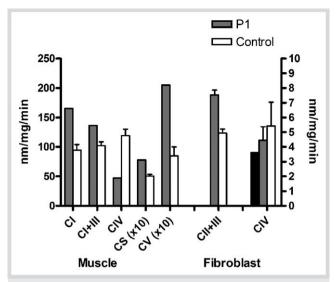


Fig. 2 Respiratory chain enzyme activities are shown for muscle and fibroblasts. CI: NADH:ubiquinone oxidoreductase, CI + III: rotenonesensitive NADH-cytochrome c reductase (complexes I and III), COX: cytochrome oxidase (complex IV), CV: ATP synthase (complex V), CS: citrate synthase, CII + III: succinate cytochrome c reductase. Patient enzyme rates are shown in grey, with the initial CIV rate from low passage fibroblasts shown in black. Control rates are shown in white.

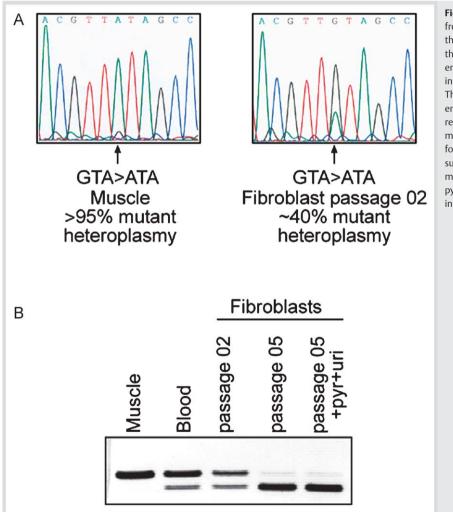


Fig. 3 A Sequencing chromatogram for patient from muscle and low passage fibroblast showing the varying levels of mutant heteroplasmy of the m.7023G > A mutation in *COX1*. **B** Restriction enzyme digestion of a 163-bp segment of mtDNA in the *COX1* gene covering the site of the mutation. The wild-type sequence was cut by the restriction endonuclease *Bful*, whereas the mutant sequence remained uncut. Mutant heteroplasmy was high in muscle (96 %), blood (70 %) and fibroblasts (60 %), followed by a drop to 10% levels after only two subcultures. Supplementation of the tissue culture medium with high levels of glucose, additional pyruvate and uridine did not prevent the decrease in mutant heteroplasmy in fibroblasts.

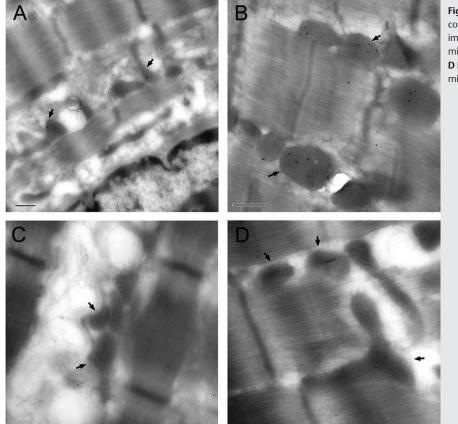


Fig. 4 Immunogold labelling of patient and control muscle. A and B Control muscle showing immunogold labeling of COX1 specifically in the mitochondria (arrows). Bar equals 500 nm. C and D Patient muscle showing no COX1 present in the mitochondria (arrows). Bar equals 500 nm.

the mother or half-brother, neither of whom had symptoms in common with the proband, suggesting it is *de novo*.

PCR primers were designed to amplify a 163-bp segment of mtDNA in the COX1 gene covering the site of the mutation. The wild-type sequence was cut by the restriction endonuclease Bful, whereas the mutant sequence remained uncut. When this digest was performed with DNA amplified from various tissues, it was found that the mutant heteroplasmy was high in muscle (96%), buccal cells (84%) (not shown) and blood (70%) with an initial heteroplasmy of 60% in fibroblasts followed by a drop to 10% levels after only two subcultures (**Fig. 3B**). This is an important finding that could easily have been missed, had not serial measurements been made. Supplementation of the tissue culture medium with high levels of glucose, additional pyruvate and uridine, a medium in which ρ^{o} (cells depleted of mtDNA) cells will grow well, did not prevent the precipitous decrease in mutant heteroplasmy in fibroblasts. The restriction digest reflected the proportion of mutants seen on the original sequencing of mtDNA.

Muscle samples were examined by immunogold electron microscopy for the presence of the COX1 subunit protein. This showed that compared to control muscle the COX1 subunit was almost completely absent from the patient muscle (• Fig. 4).

Mitochondria were prepared from muscle and subjected to SDS/ PAGE electrophoresis followed by immunoblotting for citrate synthase, LRPPRC and for the subunits of cytochrome oxidase (• Fig. 5). Since the same amount of protein was loaded in each lane, it is obvious that the citrate synthase and LRPPRC levels are increased in the patient compared to controls, while the COX subunit levels are uniformly decreased to <10% of normal values. This may be part of a co-ordinated response of mitochondrial respiratory chain enzyme expansion in the light of

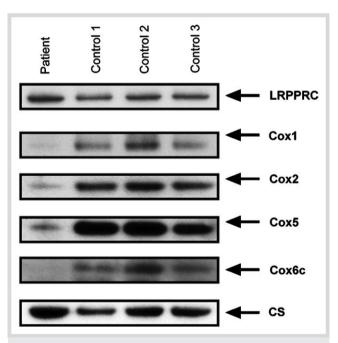


Fig. 5 Immunoblot of patient and control COX subunit, LRPPRC and citrate synthase protein levels prepared from fibroblast mitochondria.

cytochrome oxidase deficiency and decreased ATP production in muscle.

Discussion

V

The condition coined by the acronym MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) is strongly associated with various defects in the mtDNA sequence, particularly in tRNA sequences, the m. 3243G>A mutation being the most common [8]. MELAS has also been associated with mitochondrial complex I subunit mutations, particularly ND5, of which m.13513G>A is the most frequently occurring [6,26] and also ND1 [15].

In this instance, the patient presented at 11 years of age with no obvious family history of strokes, but with four instances of type I diabetes in the grand-paternal section of the pedigree. The mother has had some learning difficulties in school.

The findings of a specific cytochrome oxidase deficiency in muscle, combined with increases in other complexes relative to citrate synthase activity suggested a COX defect possibly in isolation. Since such apparent COX defects are often caused by mutations in tRNA genes, it is important to note that there was mitochondrial proliferation in muscle and a general decrease in cytochrome oxidase staining, as opposed to an assortment of COX-ve and COX+ve fibres usually seen in tRNA defects of mtDNA. Whole mitochondrial genome sequencing confirmed that there were in fact no tRNA mutations, but rather a single, heteroplasmic missense mutation in the COX1 subunit. This m.7023G>A mutation was present at high mutant heteroplasmy in muscle. The heteroplasmy levels seen in cultured skin fibroblasts were informative, in that the initial high mutant value associated with COX deficiency decreased rapidly into a much lower level of heteroplasmy and normal COX activity within 2 passages. This combined with the muscle data strongly suggested that the m.7023G>A mutation was pathogenic. It also emphasizes the importance of making determinations of enzyme activity and heteroplasmy on early passage skin fibroblasts. The mother and half-brother who showed no symptoms in common with the propositus had no visible levels of the mutation in their buccal cells, suggesting that this mutation occurred de novo.

As seen in the crystal structure of bovine cytochrome C oxidase, valine 374 is a catalytic residue which is found in helix 17 of subunit I which is within the close packed interior of the complex. Helix 17 is important in that not only does it contain valine 374, which interacts with heme A, but also includes histidine 376, phenylalanine 377 and histidine 378. Histidines 376 and 378 are the distal ligands which coordinate to the iron of heme A3 and heme A, respectively (Fig. 6). Phenylalanine 377 forms a ligand interaction with the porphyrin of heme A. The backbone carboxy-oxygen of valine 373 forms strong hydrogen bonds with the backbone nitrogens of both histidine 376 and phenylalanine 377. The mutation of valine to the rather larger methionine residue would greatly interfere with the close helical packing of subunit I which is likely required for the correct positioning of the distal histidines within helix 17 to coordinate correctly with their proper heme irons. As well, this mutation may also negate the valine 374 interaction with heme A and carboxy oxygen hydrogen bond with the amide backbone of histidine 378. Modelling of this mutation was performed with the 3D coordinates of the bovine structure. We could not identify any rotamer of the mutated residue 374 to methionine which did not cause either steric interference or greatly interfere with existing hydrogen bonds.

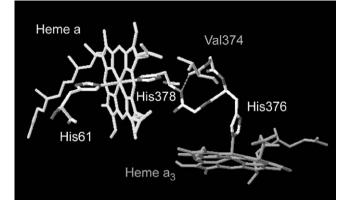


Fig. 6 Protein model showing COX1 from the crystal structure of *B. taurus* cytochrome c oxidase demonstrating how valine 374 mutated to methionine will likely affect the tertiary formation of COX1 and its ability to interact normally with heme a, by causing steric interference or interfering with existing hydrogen bonds. The three catalytic histidine residues are shown, as well as valine 374 which interact with both heme a and a_3 .

Thus, the modelling suggests that this mutation would interfere with tertiary formation of COX1 and result in failure of this misformed subunit to incorporate heme and further hinder quaternary complex formation.

Two pieces of evidence confirm such a mechanism. Firstly the immunogold localization of COX1 in skeletal muscle of the patient shows a distinct lack of labelling in the mitochondria of the patient. Secondly, the Western blotting of mitochondria from the muscle of the patient showed that there was not only low titre of COX1 but also of COX2, COX4 and COX6c subunits. This points to a lack of assembly of the cytochrome oxidase complex, possibly brought about because COX1 is unable to adopt the correct conformation in the membrane to permit assembly. COX1 appears to be the first subunit inserted into the membrane.

The unusual presentation of this patient with seizures and stroke-like episodes is reminiscent of the case of Lucioli et al. (2006) [16]. This patient had a 14-year history of exercise intolerance and muscular weakness before having a stroke-like episode at the age of 33. This individual has an m.6327C>T mutation, which was homoplasmic. The S142F mutation in COX1 was reproduced in *Paracoccus* and found to produce a complex IV lacking the COX3 subunit but retaining some residual (50%) activity. A patient with an m.9957T>C mutation also showed hemiparesis at 7 years of age.

In conclusion, the investigation of this family serves to illustrate the ever-expanding biochemical and genetic diversity of mtDNA disease and the importance of performing diagnostic testing on early stage cultured cells, when investigating mtDNA mutations in fibroblasts.

Acknowledgements

This work was supported by the Canadian Institutes for Health Research and the MitoMarch for Kirkland. RWT is funded by the Wellcome Trust and Department of Health (UK). Affiliations

- Divison of Neurology, Hospital for Sick Children and the University of Toronto, Toronto, Canada
- ² Division of Clinical Genetics, Department of Paediatrics, Hospital for Sick Children and the University of Toronto, Toronto, Canada
- ³ Programme in Genetics and Genome Biology, Research Institute, Hospital for Sick Children, Department of Biochemistry, University of Toronto, Toronto, Canada
- ⁴Department of Diagnostic Imaging, Hospital for Sick Children, Toronto, Canada
- ⁵Department of Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Canada
- ⁶ Mitochondrial Research Group, School of Neurology, Neurobiology and Psychiatry, University of Newcastle upon Tyne, Newcatsle upon Tyne, U.K.

References

- 1 Andrews RM, Kubacka I, Chinnery PF et al. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 1999; 23: 147
- 2 Bruno C, Martinuzzi A, Tang Y et al. A stop-codon mutation in the human mtDNA cytochrome c oxidase I gene disrupts the functional structure of complex IV. Am J Hum Genet 1999; 65: 611–620
- 3 *Campos Y, Garcia-Redondo A, Fernandez-Moreno MA et al.* Early-onset multisystem mitochondrial disorder caused by a nonsense mutation in the mitochondrial DNA cytochrome C oxidase II gene. Ann Neurol 2001; 50: 409–413
- 4 *Clark KM*, *Taylor RW*, *Johnson MA et al*. An mtDNA mutation in the initiation codon of the cytochrome C oxidase subunit II gene results in lower levels of the protein and a mitochondrial encephalomyopathy. Am J Hum Genet 1999; 64: 1330–1339
- 5 *Comi GP, Bordoni A, Salani S et al.* Cytochrome c oxidase subunit I microdeletion in a patient with motor neuron disease. Ann Neurol 1998; 43: 110–116
- 6 Corona P, Antozzi C, Carrara F et al. A novel mtDNA mutation in the ND5 subunit of complex I in two MELAS patients. Ann Neurol 2001; 49: 106–110
- 7 Gallardo ME, Moreno-Loshuertos R, Lopez C et al. m.6267G>A: a recurrent mutation in the human mitochondrial DNA that reduces cytochrome c oxidase activity and is associated with tumors. Hum Mutat 2006; 27: 575–582
- 8 *Goto Y, Nonaka I, Horai S.* A new mtDNA mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS). Biochim Biophys Acta 1991; 1097: 238–240
- 9 *Hanna MG*, *Nelson IP*, *Rahman S et al*. Cytochrome c oxidase deficiency associated with the first stop-codon point mutation in human mtDNA. Am J Hum Genet 1998; 63: 29–36
- 10 Horvath R, Scharfe C, Hoeltzenbein M et al. Childhood onset mitochondrial myopathy and lactic acidosis caused by a stop mutation in the mitochondrial cytochrome c oxidase III gene. J Med Genet 2002; 39: 812–816
- 11 Ingman M, Gyllensten U. mtDB: Human Mitochondrial Genome Database, a resource for population genetics and medical sciences. Nucleic Acids Res 2006; 34: D749–751
- 12 Jaksch M, Hofmann S, Kleinle S et al. A systematic mutation screen of 10 nuclear and 25 mitochondrial candidate genes in 21 patients with cytochrome c oxidase (COX) deficiency shows tRNA(Ser)(UCN) mutations in a subgroup with syndromal encephalopathy. J Med Genet 1998; 35: 895–900

- 13 *Karadimas CL, Greenstein P, Sue CM et al.* Recurrent myoglobinuria due to a nonsense mutation in the COX I gene of mitochondrial DNA. Neurology 2000; 55: 644–649
- 14 *Keightley JA*, *Hoffbuhr KC*, *Burton MD et al*. A microdeletion in cytochrome c oxidase (COX) subunit III associated with COX deficiency and recurrent myoglobinuria. Nat Genet 1996; 12: 410–416
- 15 Kirby DM, MacFarland R, Ohtake A et al. Mutations of the mitochondrial ND1 gene as a cause of MELAS. J Med Genet 2004; 41: 784–789
- 16 *Lucioli S, Hoffmeier K, Carrozzo R et al.* Introducing a novel human mtDNA mutation into the Paracoccus denitrificans COX I gene explains functional deficits in a patient. Neurogenetics 2006; 7: 51–57
- 17 Manfredi G, Schon EA, Moraes CT et al. A new mutation associated with MELAS is located in a mitochondrial DNA polypeptide-coding gene. Neuromuscul Disord 1995; 5: 391–398
- 18 Merante F, Tein I, Benson L et al. Maternally inherited hypertrophic cardiomyopathy due to a novel T-to-C transition at nucleotide 9997 in the mitochondrial tRNA(glycine) gene. Am J Hum Genet 1994; 55: 437–446
- 19 *Merante F, Myint T, Tein I et al.* An additional mitochondrial tRNA(Ile) point mutation (A-to-G at nucleotide 4295) causing hypertrophic cardiomyopathy. Hum Mutat 1996; 8: 216–222
- 20 Pecina P, Houstkova H, Hansikova H et al. Genetic defects of cytochrome c oxidase assembly. Physiol Res 2004; 53 (Suppl 1): S213–S223
- 21 Polyak K, Li Y, Zhu H et al. Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 1998; 20: 291–293
- 22 Rahman S, Taanman JW, Cooper JM et al. A missense mutation of cytochrome oxidase subunit II causes defective assembly and myopathy. Am J Hum Genet 1999; 65: 1030–1039
- 23 Rasband WS, Image J. In: U. S. National Institutes of Health, Bethesda, Maryland, USA 1997–2007
- 24 *Robinson BH, Glerum DM, Chow W et al.* The use of skin fibroblast cultures in the detection of respiratory chain defects in patients with lacticacidemia. Pediatr Res 1990; 28: 549–555
- 25 *Ruiz-Pesini E, Lott MT, Procaccio V et al.* An enhanced MITOMAP with a global mtDNA mutational phylogeny. Nucleic Acids Res 2007; 35: D823–D828
- 26 Santorelli FM, Tanji K, Kulikova R et al. Identification of a novel mutation in the mtDNA ND5 gene associated with MELAS. Biochemical and biophysical research communications 1997; 238: 326–328
- 27 Seneca S, Abramowicz M, Lissens W et al. A mitochondrial DNA microdeletion in a newborn girl with transient lactic acidosis. J Inherit Metab Dis 1996; 19: 115–118
- 28 Taanman JW. Human cytochrome c oxidase: structure, function, and deficiency. J Bioenerg Biomembr 1997; 29: 151–163
- 29 *Taylor RW*, *Taylor GA*, *Durham SE et al*. The determination of complete human mitochondrial DNA sequences in single cells: implications for the study of somatic mitochondrial DNA point mutations. Nucleic Acids Res 2001; 29: E74
- 30 *Uusimaa J, Finnila S, Vainionpaa L et al.* A mutation in mitochondrial DNA-encoded cytochrome c oxidase II gene in a child with Alpers-Huttenlocher-like disease. Pediatrics 2003; 111: e262–268
- 31 Varlamov DA, Kudin AP, Vielhaber S et al. Metabolic consequences of a novel missense mutation of the mtDNA CO I gene. Hum Mol Genet 2002; 11: 1797–1805