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Adult-onset Leigh syndrome linked to the novel stop codon mutation m.6579G>A in *MT-CO1*

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Abstract

Adult-onset Leigh syndrome is a rare but important manifestation of mitochondrial disease. We report a 17 year old female who presented with subacute encephalopathy, brainstem and extrapyramidal signs, raised CSF lactate, and symmetrical hyperintensities in the basal ganglia on T2-weighted cerebral MRI. The presence of cytochrome *c* oxidase deficient fibres in muscle tissue prompted sequencing of the entire mitochondrial genome which revealed the novel stop codon mutation m.6579G>A; p.Gly226X in *MT-CO1*. Here we present the case and review the clinicopathological and molecular spectrum of previously reported *MT-CO1* truncating mutations.

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1. Introduction

Mitochondrial disorders are a phenotypically diverse group of diseases caused by genetic defects that impair oxidative phosphorylation (OXPHOS). Cytochrome *c* oxidase (COX, complex IV), the terminal component of the mitochondrial electron transport chain (ETC), catalyses the reduction of molecular oxygen to water and helps establish the proton gradient across the inner mitochondrial membrane required for ATP synthesis. The catalytic core of COX is composed of three subunits (COX I-III), encoded by the mitochondrial genome (*MT-CO1-3*), and their prosthetic groups. COX I, II and III are surrounded by 11 nuclear-encoded subunits, many of which are expressed as tissue-specific isoforms, that are suggested to perform an insulating or regulatory role[1]. Assembly of the COX holoenzyme is a complex module-based process that occurs around COX I-III[2].

Over 50 pathogenic mutations, including 14 truncating mutations, are reported in *MT-CO1-3* and are associated with a broad clinical spectrum of disease that includes sideroblastic anaemia, sensorineural deafness, intractable epilepsy, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), and mitochondrial myopathy (MitoMap[3]). Here we report a patient with adult-onset Leigh syndrome in whom the previously unreported stop codon mutation m.6579G>A in *MT-CO1* was detected. We also review the phenotypic spectrum associated with *MT-CO1* truncating mutations and discuss the potential mechanisms that contribute towards their clinical heterogeneity.

2. Patient and Methods

2.1 Clinical case

A 28 year old female presented aged 17 years with a subacute encephalopathy, preceded by a viral gastrointestinal illness, associated with diplopia, dizziness, dysarthria, and hearing loss.

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Cranial nerve examination revealed abnormal eye movements, bilateral lower motor neuron facial weakness, and bulbar dysfunction. There were extrapyramidal and pyramidal signs in the limbs with no evidence of cerebellar or sensory disturbance. She recovered fully after the acute phase of the illness but subsequently developed a transient unilateral facial weakness and persistent fatigue. Past medical history included a possible seizure and episode of confusion following a childhood vaccination. A maternal uncle had epilepsy but there was no other family history of neurological, neuromuscular or multisystem disease (Figure 1A).

Laboratory tests at initial presentation, including blood lactate, ammonia, CPK, ESR, HIV, Lyme serology, copper and caeruloplasmin, ASO titres, amino acids, ANA, ANCA, anti-cardiolipin and anti-basal ganglia antibodies, and urinary organic and amino acids were normal. CSF was acellular, glucose was normal and oligoclonal bands were negative. However, CSF protein and lactate levels were raised (0.45g/L, reference range 0.17-0.36g/L and 3.06mmol/L, reference range 0.8-1.9mmol/L respectively).

Cerebral MRI initially showed high T2 signal intensities in the basal ganglia bilaterally, predominantly affecting the putamen and globus pallidus, without gadolinium enhancement. Repeat cerebral MRI brain 10 weeks after the acute phase of the illness confirmed resolution of the acute changes (Figure 1B and C). An EEG demonstrated a resolving encephalopathy. Nerve conduction studies revealed a mild generalised polyneuropathy with demyelinating features, while EMG was normal.

2.2 Skeletal muscle histochemical, biochemical and molecular genetic studies

Muscle biopsy of the vastus lateralis was performed following informed consent aged 18 years. Standard muscle section staining was undertaken, including COX and modified

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Gomori trichrome. Mitochondrial respiratory chain enzyme activities were measured by spectrophotometric analysis. Blue native polyacrylamide gel electrophoresis (BN-PAGE) and in-gel activity staining of complex IV was completed using muscle homogenate[4-6]. The entire mitochondrial genome, extracted from muscle tissue, was sequenced as previously described[7].

3. Results

Histochemical analysis of muscle tissue revealed several COX deficient fibres (Figure 2A), but no ragged red fibres. There was a relative reduction of complex IV activity compared with complex I and complexes II+III activities (Figure 2B and C). Protein levels of mitochondrial respiratory chain complexes and complex IV in-gel activity in muscle tissue was comparable with controls (Supplementary Figure 1).

Sequencing of the entire mitochondrial genome revealed the novel truncating mitochondrial DNA (mtDNA) mutation m.6579G>A; p.Gly226X predicted to result in a premature stop codon in *MT-CO1*. The mutation was present at 70% mutant load in muscle tissue compared with 40% mutant load in blood, with no detectable mutant mtDNA in maternal blood (Figure 1A). The variant is not reported in Genbank (45,494 full length and 69,730 short control region containing sequences[3], and occurs within a residue which is highly conserved across different species[8]. Residual muscle tissue was unavailable for single fibre mtDNA heteroplasmy studies or measurement of the relative abundance of mtDNA encoded COX subunits.

4. Discussion

We report the novel stop codon mutation in *MT-CO1* causing adult-onset Leigh syndrome. The diagnostic criteria for Leigh syndrome include a progressive neurological disease with

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developmental delay, basal ganglia involvement (clinically and on imaging studies and/or neuropathological examination), and elevated blood or CSF lactate caused by genetically mediated mitochondrial dysfunction[9,10]. Adult-onset Leigh syndrome is a rare but well-recognised presentation of mitochondrial disease, frequently associated with COX deficiency[11] and typically caused by mtDNA mutations[10].

The m.6579G>A; p.Gly226X variant is highly likely to be pathogenic. A similar encephalomyopathic phenotype caused by a truncating *MT-CO1* mutation has previously been described[12]. The m.6579G nucleotide is highly conserved and the premature stop codon introduced by the mutation is predicted to result in loss of more than half of the total polypeptide. The mutation is heteroplasmic, with higher mutant levels in muscle than blood, typical for pathogenic mtDNA mutations, and it segregates with disease with no mutant *MT-CO1* detectable in the unaffected mother's blood. Histochemical and biochemical analyses of muscle tissue were consistent with impaired COX function. Finally, the mutation is absent from control samples.

Seven, primarily *de novo*, truncating mutations in *MT-CO1* are reported (Table 1). The clinical spectrum is varied and includes anaemia, myopathy, rhabdomyolysis, and central neurological or multisystem disease phenotypes. The patient described in this report presented with adult-onset Leigh syndrome. The relatively mild histochemical and biochemical COX deficiency detected in the patient's skeletal muscle tissue is consistent with the late onset presentation and clinically undetectable muscle weakness. A low normal, rather than reduced, complex IV activity in the muscle tissue of patients with pathogenic *MT-CO3* mutations is reported with CNS phenotypes[13], and a more severe enzymatic defect would be expected in brain tissue. Interestingly, the muscle heteroplasmy levels in our patient are similar to previously reported cases. This potentially reflects the influence nuclear genetic

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modifiers have on the phenotypic presentation of pathogenic mtDNA mutations[14]. For instance, cybrid cells harbouring the m.7339A>G mutation in *MT-CO1* still incorporate the truncated COX I protein into the COX sub- and holocomplex, although the presence of the abnormal subunit causes instability of the holoenzyme. COX subunit I interacts with several other factors that determine complex stability and it is proposed that the rapid clearance of unstable complexes by quality control pathways might explain the pathogenesis of *MT-CO1* truncation mutations rather than impaired complex assembly[15]. It is therefore feasible that inter-individual variations in these quality control processes across different tissues contributes towards the clinical heterogeneity of *MT-CO1* truncating mutations. Furthermore, the relative, rather than absolute, reduction in CIV activity in muscle demonstrates the importance of interpreting the results of these assays in their clinical context.

In conclusion, we report a novel stop mutation in *MT-CO1* causing adult-onset Leigh syndrome. Inter-individual variations in the quality control pathways that regulate mutant protein degradation and/or mitochondrial respiratory chain complex IV stability within different tissues is one potential explanation for the remarkable clinical heterogeneity associated with *MT-CO1* truncating mutations.

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NHS Highly Specialised Commissioners to provide the "Rare Mitochondrial Disorders of Adults and Children" Service.

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Table 1: Summary of clinicopathological, biochemical and molecular characteristics of reported *MT-CO1* truncating mutations

***MT-CO1* truncating mutation**	**Amino acids lost (of 513 total)**	**Age at symptom onset, sex**	**Clinical phenotype**	**Lactate**	**Heteroplasmy (%)**				**Muscle histology**	**Muscle CIV RCEA**	**Functional studies**	**Inherited or *de novo***	**Reference**
					Blood	**Urine**	**Fibs**	**Muscle**					
5920G>A; p.W6X	508	33y, M (EI since childhood)	Rhabdomyolysis	Blood N	0	NA	0	61	COX -ve ++ RRF	↓	SF studies	Absent from mother and sister's blood	[16]
m.6020_6025delCGAGC; p.E40Gfs*4	471	29y, M	MND	Blood ↑	NA	NA	NA	47-68.7	COX -ve ++ RRF	↓	SF studies, WB of COX	Absent from mother and 3	[17]

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											subunits	sibling's muscle	
6579G>A; p.G226X	288	17y, F	Leigh syndrome	Blood N, CSF ↑	40	NA	NA	70	COX -ve	Relative reduction	NA	Absent from mother's blood	Current study
6698delA; p.K265fs271X	243	36y, M	MM, rhabdomyolysis	NA	NA	15	NA	70	COX -ve	↓	WB of COX subunits	Absent from mother's blood	[18]
6708G>A; p.G269X	245	28y, F (EI since childhood)	MM, myalgia, EI, rhabdomyolysis	NA	0	NA	NA	81-89	COX -ve ++ RRF	↓	SF studies, WB of COX subunits	Absent from mother's blood	[19]

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6930G>A; p.G343X	171	3y, F	Cataracts, optic atrophy, SNHL, epilepsy, ataxia, MM	Blood ↑	27	NA	NA	75	COX -ve ++	↓	Confirmed in cybrids	Absent from mother, sister and 4 maternal aunt's blood	[20, 21]
7339A>G; p.K479X	35	NA	Anaemia	NA	NA	NA	NA	NA	NA	NA	Confirmed in cybrids	NA	[15]
7402delC; p.P500Hfs*12	1	18y, F	Encephalopathy, NSCE, EI, MM, SNHL, cataracts,	Blood ULN	7	27	NA	76	COX -ve ++ RRF	↓	SF studies, WB of COX subunits	Present at 2-5% using SF of mother's muscle,	[22]

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			cognitive decline									absent from mother and sister's blood and urine	

Abbreviations: CIV; complex IV, COX -ve; cytochrome *c* oxidase negative fibres, EI; exercise intolerance, F; female, Fibs; fibroblasts, M; male, MM; mitochondrial myopathy, MND; motor neuron disease, N; normal, NA; not available, NSCE; non-convulsive status epilepticus, ref; reference, RCEA; respiratory chain enzyme activity, RRF; ragged red fibres, SF; single fibre PCR, SNHL; sensorineural hearing loss, ULN; upper limit of normal, WB; western blot, Y; years, ++; severe deficiency.

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Figure legends

Figure 1: (A) Family pedigree. Proband is indicated by black symbol and arrow. The m.6579G>A heteroplasmy levels are annotated. (B) Axial T2-weighted cerebral MRI during the acute phase of the patient's illness. Symmetrical hyperintensities in the basal ganglia illustrated by arrows. (C) Repeat cerebral MRI 10 weeks following acute phase of illness confirmed resolution of the original imaging changes.

Figure 2: (A) Histochemical analysis of muscle tissue with cytochrome *c* oxidase (COX) stain showing COX negative and deficient fibres. (B) Mitochondrial respiratory chain enzyme activity measured using spectrophotometric analysis. Results expressed as ratio to citrate synthase (CS) activity to correct for mitochondrial enrichment of sample. Measurement uncertainty (%) derived from quality control data for each enzyme assayed as part of United Kingdom Accreditation Service (UKAS) accreditation process. COX activity is at the lower level of the reference range and is disproportionately reduced in comparison to the other mitochondrial respiratory chain enzymes. (C) Graphical representation of mitochondrial respiratory chain enzyme activity. Lines indicate reference ranges and black dots represent mitochondrial respiratory chain enzyme activity in patient. Abbreviations: CS; citrate synthase, I; complex I, II+III; complexes II+II, IV; complex IV.

Supplementary Figure 1: (A) Coomassie blue staining showing relative abundance of protein complexes. Levels of complexes are comparable between patient and control. (B) Complex IV in-gel activity stain demonstrating normal complex IV activity in patient. Abbreviations: I; complex I, II; complex II, III; complex III, IV; complex IV.

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- The novel stop codon mutation m.6579G>A; p.Gly226X in *MT-CO1* causes adult-onset Leigh syndrome.

- *MT-CO1* truncating mutations are associated with remarkable clinical heterogeneity.

- The broad phenotypic spectrum potentially relates to inter-individual and tissue-specific variations in the quality control pathways that regulate mutant protein degradation and/or mitochondrial respiratory chain complex IV stability.

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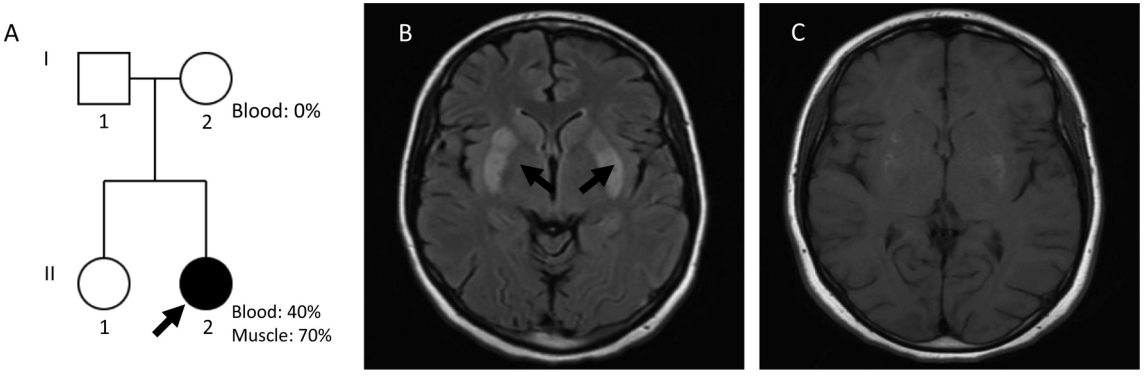
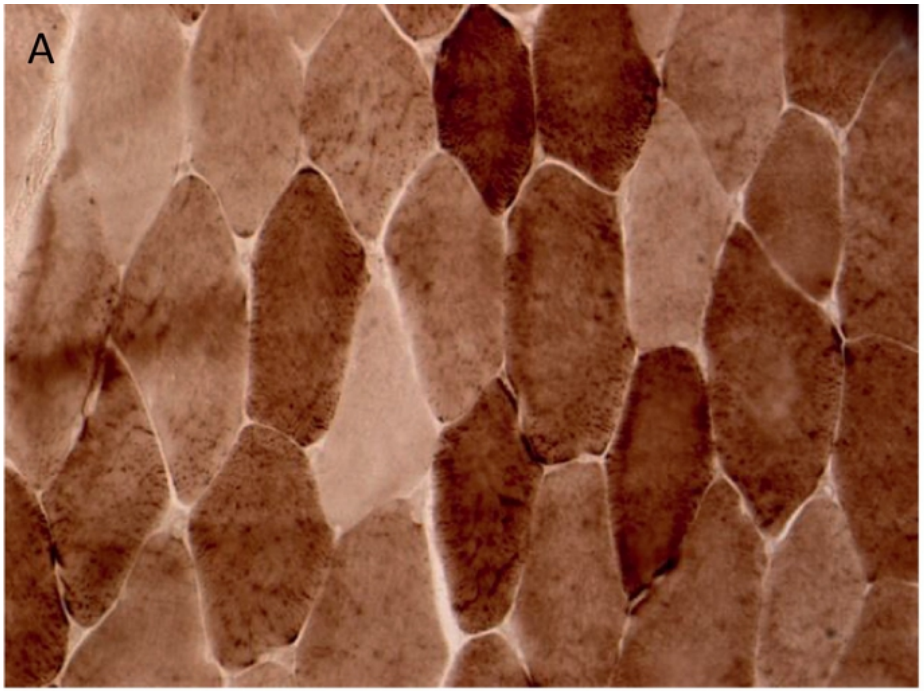


Figure 1

A



B

Respiratory chain complex	Activity corrected for CS (measurement uncertainty/%)	Reference range
NADH ubiquinone reductase (I)	0.148 (9.71)	0.104-0.268
Succinate-cytochrome c reductase (II+III)	0.121 (12.77)	0.040-0.204
Cytochrome *c* oxidase (IV)	0.016 (7.14)	0.014-0.034

C

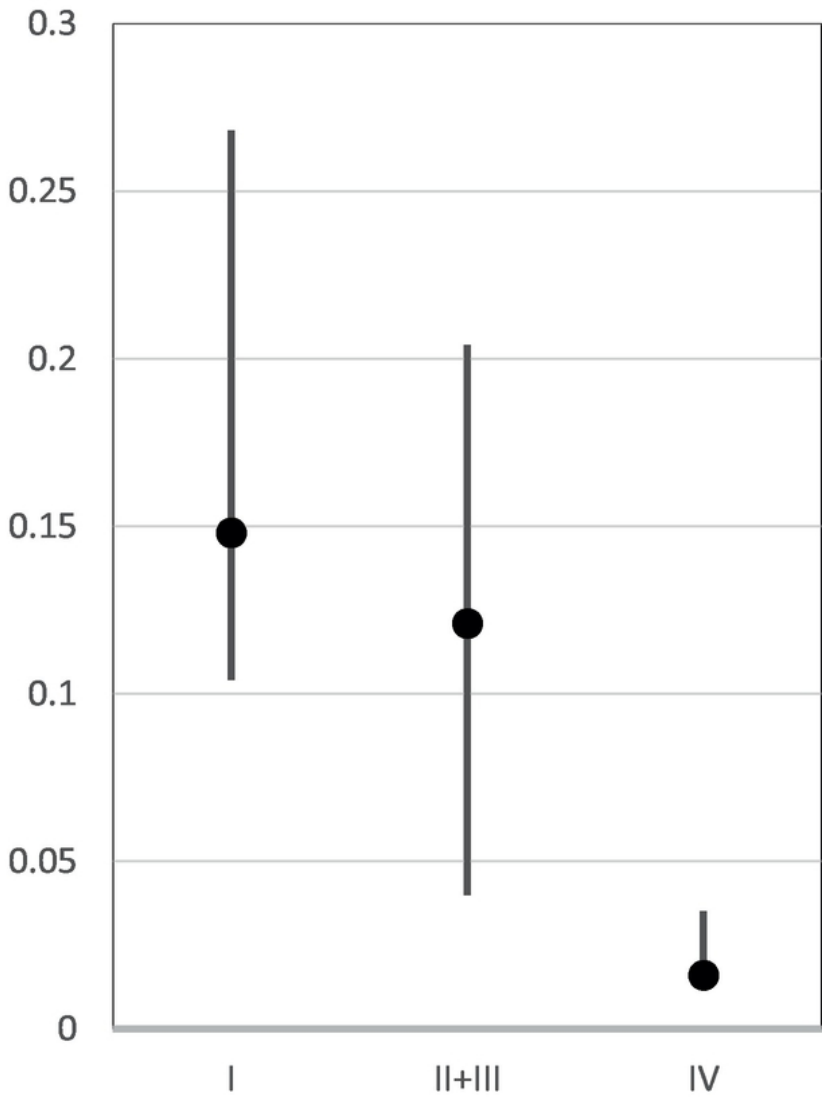


Figure 2