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ARTICLE

Maternally inherited mitochondrial DNA disease in consanguineous families

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Mitochondrial respiratory chain disease represents one of the most common inborn errors of metabolism and is genetically heterogeneous, with biochemical defects arising from mutations in the mitochondrial genome (mtDNA) or the nuclear genome. As such, inheritance of mitochondrial respiratory chain disease can either follow dominant or recessive autosomal (Mendelian) inheritance patterns, the strictly matrilineal inheritance observed with mtDNA point mutations or X-linked inheritance. Parental consanguinity in respiratory chain disease is often assumed to infer an autosomal recessive inheritance pattern, and the analysis of mtDNA may be overlooked in the pursuit of a presumed nuclear genetic defect. We report the histochemical, biochemical and molecular genetic investigations of two patients with suspected mitochondrial disease who, despite being born to consanguineous first-cousin parents, were found to harbour well-characterised pathogenic mtDNA mutations, both of which were maternally transmitted. Our findings highlight that any diagnostic algorithm for the investigation of mitochondrial respiratory chain disease must include a full and complete analysis of the entire coding sequence of the mitochondrial genome in a clinically relevant tissue. An autosomal basis for respiratory chain disease should not be assumed in consanguineous families and that 'maternally inherited consanguineous' mitochondrial disease may thus be going undiagnosed.
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INTRODUCTION

The synthesis of cellular ATP by oxidative phosphorylation (OXPHOS) is coordinated by five multimeric protein complexes – complexes I–IV that form the mitochondrial respiratory chain and ATP synthase (complex V). Mitochondrial disease resulting from defective OXPHOS, represents one of the most common inborn errors of metabolism, with a minimum prevalence of 1 in 10 000.[1] Of the ~85 structural OXPHOS subunits, 13 are encoded by the mitochondria's own genetic material – a 16.6-kb mitochondrial DNA (mtDNA) molecule, which also encodes the necessary rRNA and tRNA machinery for their translation.[2] All other proteins contributing to OXPHOS function are autosomally encoded, therefore defects can occur within nuclear or mitochondrial-encoded genes. Mitochondrial disease is genetically and clinically heterogeneous, and can follow mendelian or X-linked inheritance patterns, or be strictly matrilineal in the case of mtDNA mutations;[3–5] clinical presentations range from isolated organ involvement (deafness, diabetes and cardiomyopathy) to multisystem, syndromic presentations dominated by muscle and CNS involvement.[6,7]

Such heterogeneity can mean that identifying the causative genetic defect is problematic. Given its size, sequencing of the entire mitochondrial genome in clinically affected tissues is often undertaken during the diagnostic work-up unless an autosomal aetiology is suspected, for example, patients born to consanguineous parents or those with affected paternal relatives.[7] Here, we report the histochemical, biochemical and molecular genetic investigations of two patients with suspected mitochondrial disease who, despite being born to consanguineous first-cousin parents, were found to harbour well-characterised mtDNA mutations.

Case reports

All studies were approved and performed under the ethical guidelines issued by each of our Institutions for clinical studies, with written informed consent obtained from each family.

Patient 1

The first child of first-cousin Pakistani parents (Figure 1), this girl was born at term and both microcephalic (<0.4th centile) and of low birth weight (2.32 kg; <0.4th centile). She presented at age 18 months with infrequent generalised seizures. Psychomotor and language delay were noted, but she was able to walk with support and produce polysyllabic babble. An interictal electroencephalogram was normal and seizures resolved after 4 months. Examination was otherwise unremarkable with no family history.

She was readmitted to hospital at 30 months of age with fever, depressed consciousness, stridor, central hypertension and seizures. Further deterioration necessitated mechanical ventilation due to a profound lack of central ventilatory drive. Cranial MRI showed multiple focal areas of abnormal signal consistent with Leigh syndrome (Figure 1). MR angiography was normal. Elevated blood and CSF lactate levels (2.3 mmol/l and 3.4 mmol/l, respectively; normal range 0.7–2.1 mmol/l) prompted muscle and skin biopsies

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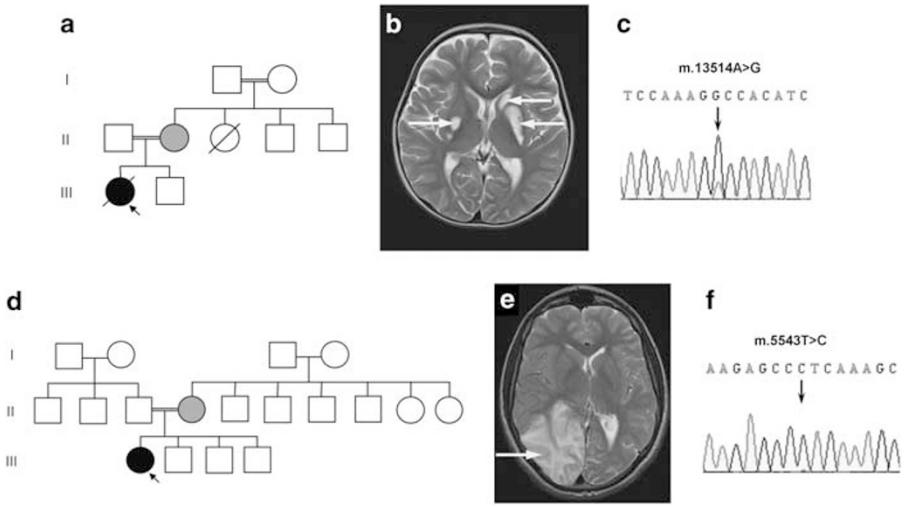


Figure 1 Consanguineous pedigrees, MRI changes and pathogenic mtDNA mutations. (**a**) Patient 1's pedigree with the proband highlighted by arrow; block shading indicates an affected individual, while grey shading represents a known mutation carrier. (**b**) Cranial MRI of Patient 1: T2-weighted axial image demonstrating hyperintensities in both lentiform nuclei (lower arrows) and the head of caudate on the left (upper arrow). (**c**) Sequencing chromatogram showing the pathogenic, heteroplasmic m.13514A>G *MTND5* mutation in the patient's muscle. (**d**) Patient 2's pedigree, with the proband and his clinically unaffected carrier mother is indicated. (**e**) Cranial MRI of Patient 2: T2-weighted axial image reveals hyperintense signal change of the right occipital cortex with mass effect and compression of the occipital horn of the right lateral ventricle (arrow). (**f**) Sequencing chromatogram showing the pathogenic m.5543T>C *MTTW* mutation at very high levels of heteroplasmy in muscle from patient 2.

to investigate a possible metabolic cause. After 3 weeks, and following agreement with the family, supportive treatment was withdrawn; she remained apnoeic and died.

Patient 2

Patient 2 is the eldest of four children born to first-cousin Pakistani parents; she has three healthy younger brothers (Figure 1). Her mother suffered from migraines while taking oral contraceptives, and a maternal aunt has epilepsy. She presented at 13 years of age with a 7-day history of persistent right frontal headache associated with nausea, vomiting and blurred vision. She had experienced a similar episode the previous year and recurrent frontal headaches from the age of 11 years. Examination revealed a left homonymous hemianopia with increased deep tendon reflexes in the left arm and leg, but no weakness. Cranial MRI showed swelling and signal change in the right occipital cortex (Figure 1). CSF and blood lactate concentrations were raised at 3.3 mmol/l and 3.4 mmol/l, respectively. EEG was consistent with an encephalopathy. Although headaches diminished after a few months, the hemianopia persisted.

METHODS

Histological and histochemical analyses of quadriceps muscle biopsy samples were carried out using standard procedures. Respiratory chain complex activities were determined in muscle homogenates as previously described.[8]

Total genomic DNA was extracted from available tissues using standard procedures. Molecular genetic investigations on muscle-derived DNA included long-range and real-time PCR to evaluate large-scale mtDNA rearrangements and mtDNA copy number, followed by direct sequencing of the entire mitochondrial genome as described elsewhere.[9]

Pyrosequencing on the Pyromark Q24 platform permitted quantification of the m.13514A>G and m.5543T>C mutations; PyroMark assay design software v2.0 (Qiagen, Hilden, Germany) was used to design mutation-specific pyrosequencing (PSQ) primer trios as follows: **m.13514A>G assay** - forward: m.13455-13475; biotinylated reverse: m.13539-13560 and pyrosequencing primer: m.13495-13513; **m.5543T>C assay** - biotinylated forward (m.5368-5391), reverse (m.5588-5606) and pyrosequencing primer: m.5547-5563 (Genbank Accesion number: NC_012920). The allele quantification application of Pyromark's proprietary Q24 software was used to calculate mtDNA heteroplasmy levels.

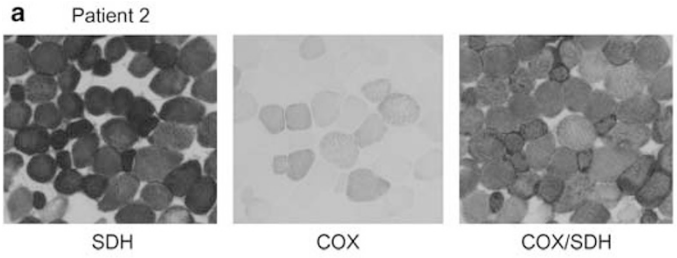
RESULTS

Patient 1

Muscle histology and histochemical analyses were normal, although assessment of respiratory chain complex activities in muscle and fibroblasts demonstrated severe, isolated complex I deficiency (Figure 2). Having excluded mtDNA depletion, we sequenced the entire mitochondrial genome, identifying a previously reported pathogenic mutation (m.13514A>G, p.D393G) within *MTND5*[10,11] (Figure 1) at levels of 69 and 55% heteroplasmy in muscle and fibroblasts, respectively. Analysis of maternal samples clearly identified the m.13514A>G mutation in blood-, buccal- and uroepithelium-derived DNA samples at 5, 57 and 8% heteroplasmy, respectively, confirming maternal transmission.

Patient 2

Histochemical analysis of a muscle biopsy sample revealed ~80% COX-deficient fibres, a significant proportion of which showed evidence of subsarcolemmal mitochondrial accumulation, typical of 'ragged-red' changes (Figure 2). Respiratory chain analyses revealed severe biochemical deficiencies of complexes I and IV. Following exclusion of a mtDNA copy-number abnormality and large-scale mtDNA rearrangements, whole mitochondrial genome sequencing uncovered a previously reported pathogenic mutation, m.5543T>C (Figure 1) within the *MTTW* gene encoding the mitochondrial tryptophan tRNA.[12] Pyrosequencing confirmed very high levels of mutated mtDNA in muscle and uroepithelium (99% and 92%, respectively), with lower levels in blood (42%) and buccal epithelium (55%). Analysis of maternal samples confirmed maternal transmission of the m.5443T>C mutation, being present at low levels of heteroplasmy in blood-, urine- and buccal-derived DNA samples (8, 29 and 14% mutation load, respectively).



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	MUSCLE			FIBROBLASTS	
	Patient 1	Patient 2	Controls (*n*=25)	Patient 1	Controls (*n*=8)
Complex I/CS	0.044	0.007	0.104 ± 0.036	0.055	0.197 ± 0.034
Complex II/CS	0.188	0.082	0.145 ± 0.047	0.410	0.219 ± 0.067
Complex III/CS	0.999	0.506	0.554 ± 0.345	0.525	0.646 ± 0.192
Complex IV/CS	1.020	0.070	1.124 ± 0.511	2.671	1.083 ± 0.186
Complex I:II	0.234	0.085	range 0.52-0.95	0.135	range 0.58-0.90

Figure 2 Histochemical and biochemical assessment of mitochondrial respiratory chain function in affected patients. (**a**) Histochemical analyses of patient 2's serial muscle biopsy sections identifying numerous ragged-red fibres exhibiting marked mitochondrial accumulation (succinate dehydrogenase (SDH) reaction) and a mosaic pattern of cytochrome *c* oxidase (COX) deficiency (COX and sequential COX/SDH reactions). (**b**) Biochemical assessment of respiratory chain complex activities revealed an isolated complex I deficiency in both skeletal muscle and fibroblasts in patient 1, and combined deficiencies of both complex I and IV activities in muscle from patient 2. Enzyme activities are expressed as nmol NADH oxidized per min per unit citrate synthase (CS) for complex I, nmol 2,6-dichlorophenol-indophenol reduced per min per unit citrate synthase for complex II (succinate:ubiquinone-1 reductase) and the apparent first-order rate constant per sec per unit citrate synthase for complexes III and IV ($\times 10^3$). Control values are shown as mean ± SD.

DISCUSSION

We studied two patients with suspected mitochondrial disease – both born to consanguineous, first-cousin parents – identifying maternally transmitted, mtDNA mutations in each case: m. 13514A>G within *MTND5* in Patient 1 and m.5543T>C within *MTTW* in Patient 2. Quantitative assessment of these mutations in maternal DNA samples confirmed that mutations were not sporadic, having been maternally transmitted in both cases. Both asymptomatic mothers harboured their child's mutation in all available samples, albeit below the threshold required to cause a biochemical defect.

Isolated complex I deficiency (Patient 1) represents the most frequent biochemical abnormality in paediatric presentations of mitochondrial disease.[9,13] Complex I (NADH:ubiquinone oxidoreductase) is the largest component of the respiratory chain with 45 structural subunits, seven of which are mtDNA encoded; the remaining subunits and assembly factors are nuclear encoded. An estimated 25–30% of patients with isolated complex I deficiency harbour a mitochondrial DNA point mutation;[14] nuclear defects must therefore account for the remaining patients. In a consanguineous family, the scope for offspring harbouring autozygous mutations is clearly increased; to date, there have been no reports linking pathogenic mtDNA mutations with isolated complex I deficiency in consanguineous families, while autosomal recessive mutations within nuclear-encoded structural genes or assembly factors are frequently reported.[9,15] When isolated complex I deficiency is identified in non-consanguineous families, mutations of the matrilineal mitochondrial genome are also considered and mtDNA sequencing is performed routinely.

The m.5543T>C mtDNA mutation identified in Patient 2 lies within the gene encoding mitochondrial $tRNA^{Trp}$; mt-tRNA gene mutations are associated with diverse clinical presentations and are often heteroplasmic. Phenotypic variation in mitochondrial disease is thought to be related, at least partly, to the proportion of mutated mtDNA present in different tissues, each with their own disease threshold. The previous report of the m.5443T>C mutation was apparently *de novo*, with no evidence of the mutation in maternal blood; however, it is well documented that heteroplasmy levels of many mt-tRNA gene mutations decrease with age in rapidly dividing tissues.[12] As such, the absence in maternal lymphocytes may be attributable to negative selection, while the mutations may have been detectable in other tissues with lower selective pressures, such as uroepithelial cells. This highlights a potential pitfall in screening familial samples for mt-tRNA mutations and the importance of selecting clinically relevant tissues for investigation.[16,17] In our patient, the age of onset,[14] in tandem with combined respiratory chain deficiencies and marked histological abnormalities, was highly suggestive of an mtDNA rather than nuclear genetic abnormality.

Identifying the genetic basis of mitochondrial disease is essential for accurate genetic counselling and calculating the recurrence risks.[18] Although mtDNA mutations can arise sporadically,[19] we demonstrate that in both our families, the causative mtDNA mutation has been maternally transmitted. Parental consanguinity is often assumed to infer an autosomal recessive aetiology, which means that mtDNA analysis may be overlooked in the pursuit of a presumed autosomal defect. Although next-generation sequencing and autozygosity mapping represent increasingly cost-effective tools for investigating consanguineous mitochondrial disease,[5,20–23] our findings support a thorough investigation of mtDNA in all patients. Consequently, we strongly advocate that any diagnostic algorithm for investigating mitochondrial disease should include analysis of the entire mitochondrial genome in a clinically relevant tissue. Furthermore, we show that an autosomal basis for respiratory chain disease should not be assumed in consanguineous families and that 'maternally inherited consanguineous' mitochondrial disease may be undiagnosed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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