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CLINICAL CASE SEMINAR:
MELAS syndrome, diabetes and thyroid disease: the role of mitochondrial oxidative stress

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Short title
Oxidative stress in MELAS, diabetes and thyroid disease

Key words
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Abstract
The mitochondrial DNA mutation MTTL1 A3243G causes MELAS syndrome (Mitochondrial Encephalomyopathy, Lactic Acidosis, Stroke-like episodes). The A3243G mutation is also associated with variable endocrinopathies. We describe 2 case histories of patients with the A3243G mutation. The first patient presented with diabetes and uncontrolled Graves' thyrotoxicosis in association with a stroke-like episode, seizures and cortical blindness. The second patient presented initially with primary hypothyroidism and vitiligo, and later with glucose intolerance and hypoadrenalism. She went on to develop the classical features of MELAS syndrome. These cases illustrate the range of endocrine abnormalities associated with this mitochondrial DNA mutation. MELAS syndrome should be considered in patients presenting with disparate endocrine conditions in association with neurological problems such as stroke-like episodes, myoclonic epilepsy, sensorineural deafness and cortical blindness. The development of thyrotoxicosis or the introduction of thyroid hormone replacement may precipitate a 'bioenergetic crisis' by imposing cellular oxidative stress.

Case descriptions

Case 1 A 26-year-old woman presented with a "stroke-like episode" resulting in left-sided neglect. She had a background of diabetes mellitus diagnosed at the age of 22, which was treated with insulin. A right parietal-occipital infarct was identified on CT scanning of the brain. Blood tests at this time showed evidence of subclinical thyrotoxicosis with free T4 and T3 within normal ranges at 15.1 pmol/l (normal range 9.0-26.0) and 3.7 pmol/l (normal range 2.5-5.7) respectively, but with undetectable TSH at <0.05 mU/l (normal range 0.3-4.2).

Her neurological condition subsequently deteriorated over the course of two months. She developed cortical blindness, seizures and global dysphasia. Further investigations showed overt thyrotoxicosis with free T4 27.2 pmol/l, free T3 7.3 pmol/l, TSH <0.05 mU/l. TSH receptor antibodies were weakly positive at 2.5 U/ml (normal range 0-1.5), consistent with a diagnosis of Graves' disease. A T2-weighted MRI scan of the brain showed hyperintensity in the right parietal-occipital region. Serum and CSF lactate were elevated (4.4 and 4.3 mmol/l respectively, NR <1.8). Mitochondrial DNA analysis revealed an MTTL1 A3243G mutation with heteroplasmy of 85% in skeletal muscle, 63% in urinary epithelial cells and 33% in blood. Muscle biopsy revealed the characteristic changes of MELAS syndrome (Figure 2). She was commenced on coenzyme Q10 300 mg tds. Euthyroidism was restored and maintained with carbimazole 10 mg daily, and later radioiodine treatment. With this treatment, her limb weakness, cortical blindness and left-sided neglect have all improved over 4 years of follow-up.

Case 2 A 23-year-old woman was referred for evaluation of low body weight and hypothyroidism. She was diagnosed with hypothyroidism at age 12 and had been treated with levothyroxine, but had discontinued treatment for 6 months prior to assessment. Examination revealed an under-nourished woman with normal sexual development, widespread patches of vitiligo on the hands, a weight of 28.4 kg, height 1.42 m and BMI of 14.1 kg/m^2. Thyroid function tests were consistent with primary hypothyroidism with undetectable free T4 of <3.9 pmol/l and TSH of 375 mU/l. Thyroid peroxidase antibodies were within normal limits at 12 IU/ml (normal <50).

She was re-commenced on levothyroxine and this was titrated to achieve euthyroid levels with a maintenance dose of 75 micrograms daily. With this, she started complaining of frontal headaches, poor appetite, nausea, vomiting, and and suffered a further loss of weight. A short Synacthen test was performed and this revealed a subnormal cortisol response (baseline 0900h cortisol 292 nmol/l, peak cortisol 485 nmol/l – normal peak response >550). Anti-adrenal and tissue transglutaminase antibodies were both negative. A 75 g oral glucose tolerance test showed a fasting plasma glucose of 4.2 mmol/l, and 2-hour glucose of 8.7 mmol/l. In summary, investigations revealed a combination of primary hypothyroidism with vitiligo, a subnormal cortisol response to Synacthen, and glucose intolerance.

She was commenced on hydrocortisone replacement, 20 mg daily in divided doses. Despite this treatment, her weight loss continued to a nadir of 24 kg. She developed tonic-clonic and myoclonic seizures, cortical blindness, and a deterioration in consciousness. An MRI scan revealed evidence of bilateral T2 hyperintensity of the cortex and subcortex in the right temporo-parietal and the left temporo-occipital lobes (Figure 3). Her serum lactate levels were elevated at 6.70 mmol/l (normal range 0.6-2.4). A muscle biopsy revealed the characteristic changes of MELAS syndrome. Mitochondrial DNA sequencing revealed the MTTL1 A3243G mutation in blood with heteroplasmy of 43%. She was commenced on coenzyme Q10 200 mg bd and intensive nutritional supplementation, with nightly enteral feeding using liquid feed via a percutaneous gastrostomy. She has had no further seizures and has gained 13 kg in weight over 9 months of follow-up.

Discussion

We describe two cases presenting with endocrine manifestations of the A3243G mutation in the mitochondrial gene MTTL1, the

mitochondrial tRNA for leucine (UUR). This gene is encoded in mitochondrial DNA (mtDNA – Figure 1). In an epidemiologic survey of a Finnish population of 245,101 individuals, the frequency of the A3243G mutation was estimated to be at least 16.3/100,000 (1).

Mutations in mtDNA are maternally inherited as the zygote inherits mitochondria exclusively from the oocyte (mitochondria from sperm are ubiquitinated and degraded). The zygote will contain a mixture of wild-type and mutant mtDNA: this is called 'heteroplasmy'. Mitochondria are distributed stochastically between daughter cells during mitosis. Moreover, mitochondria actively divide, and can fuse with other mitochondria, leading to the mixture of mtDNA molecules within each mitochondrion (2). Lastly, for unknown reasons, A3243G mutant mtDNA appears to have a replicative advantage over wild-type mtDNA (3). Thus, heteroplasmy may vary widely between different tissues in patients carrying the A3243G mutation, with the proportion of mutant mtDNA varying from 3-98% (4).

The A3243G mutation of MTTL1 is manifest in four main syndromes: (1) classical MELAS syndrome, as illustrated in the current cases; (2) maternally-inherited hereditary sensorineural deafness, diabetes mellitus, ataxia and deafness, but without stroke-like episodes; (3) progressive external ophthalmoplegia; and (4) limb weakness and exercise intolerance (5). The highly variable clinical presentation, age at presentation and progression may be due to differences in the degree of heteroplasmy between tissues, due to variations in the threshold levels of mutant mtDNA required to cause cellular or organ malfunction, and perhaps due to the influence of the nuclear genome (2).

Disease pathogenesis resulting from the A3243G mutation is thought to be caused by defects in mitochondrial function. The mutated mitochondrial tRNA Leu(UUR) lacks a critical taurinomethyluridine modification to the third nucleotide of the tRNA's anticodon (the so-called 'wobble' position), weakening its ability to bind the UUG codon. The mutated tRNA also has a shortened half-life (6, 7). This causes a translational defect of the ND6 component in the respiratory chain complex I, which is also encoded in mtDNA ((8) and Figure 1). As a result, cells with a higher load of mutant mtDNA are not able to respire normally (9). Skeletal muscle, cardiac muscle, and neurons appear to be most vulnerable, with mitochondrial dysfunction occurring with mutant mtDNA loads of 80-90% (10, 11). Furthermore, mitochondrial dysfunction causes the generation of reactive oxygen species, which overwhelms the normal mechanisms for detoxifying these, so-called 'mitochondrial oxidative stress' (12). Reactive oxygen species cause damage to the mitochondrial membrane and DNA, and the release and activation of pro-apoptotic factors, triggering the activation of the caspase cascade and apoptotic cell death (13).

The A3243G mutation is associated with endocrine disease (reviewed in {Stark, 2007 #148}). The most common endocrine abnormality is diabetes mellitus and glucose intolerance, as in the cases described here. Typically, diabetes develops at an early age (mean age of onset 38), but there is an age-related increase in penetrance such that approximately 85% of carriers of the A3243G mutation develop diabetes or impaired glucose tolerance by the age of 70 (14). Affected individuals are lean, require insulin treatment, but, typically, do not present with ketoacidosis (15). The pathogenesis of mitochondrial diabetes has been partially characterized and is thought to involve a primary beta-cell defect in insulin secretion and synthesis (16). Part of the problem is due to impaired coupling of glucose sensing to insulin secretion: defective mitochondrial function leads to impaired generation of ATP from glucose and therefore a lower ATP/ADP ratio. There is therefore a reduced depolarization of the beta-cell membrane in response to glucose, reduced calcium intake and reduced insulin exocytosis (17). This parallels the situation in MODY2, in which glucokinase mutations impair the generation of glucose-6-phosphate from glucose and cause a non-progressive defect in glucose-insulin secretion coupling. Mitochondrial dysfunction also causes oxidative stress within beta-cells, triggering their aging and apoptosis. Mitochondrial diabetes is

therefore characterized by a progressive decline in beta-cell function over time, explaining the age-related penetrance (14, 18).

Scattered case reports describe other endocrinopathies associated with mitochondrial encephalomyopathies. MELAS syndrome can be associated with GH deficiency (19); deficiencies in TSH, gonadotrophins and GH (20); and deficiencies in GH and gonadotrophins (21). The deficiencies may therefore be isolated or multiple in nature. Three case reports also describe hypoparathyroidism in association with MELAS syndrome (22, 23) and MELAS combined with Kearns-Sayre syndrome (progressive ptosis, ophthlamoplegia, myopathy and pigmentary retinopathy) (24).

MELAS syndrome has also been associated with thyroid disease in case reports. These include a case of a woman presenting with features of Kearns-Sayre syndrome and polyglandular autoimmune syndrome type 2 manifesting as primary hypoadrenalism, Type 1 diabetes mellitus, Hashimoto's thyroiditis and primary ovarian failure. The A3243G mutation in this case was found in association with a large deletion of mitochondrial DNA (25). Hyperthyroidism in association with MELAS syndrome has also been described in two cases (26, 27).

Thyroid disease itself can influence the development of MELAS syndrome. It has long been known that thyroid hormone increases mitochondrial oxygen consumption, oxidative phosphorylation, the transcription of mitochondrial RNA and the expression of mitochondrial proteins, and the biogenesis of mitochondria (reviewed in (28)). These effects are mediated indirectly via nuclear thyroid receptors and directly via mitochondrial thyroid receptors (29, 30). Thyrotoxicosis increases mitochondrial oxidative stress and the generation of reactive oxygen species, a phenomenon which underlies the pathophysiology of thyroid myopathy, and the deleterious effects of thyrotoxicosis on cardiac tissue and on the liver (31). Therefore, the development of thyrotoxicosis in patient 1 may have been instrumental in precipitating her neurological crisis, by increasing oxidative stress and lowering the threshold of heteroplasmy required to trigger apoptosis. We also speculate that the deterioration seen in patient 2 might in a similar fashion have been triggered by the re-introduction of levothyroxine treatment for her primary hypothyroidism.

We would therefore suggest that patients with MELAS syndrome should be monitored for the development of hyperthyroidism and aggressively treated, as this may precipitate a 'bioenergetic crisis' (32). The introduction of levothyroxine in the treatment of hypothyroidism should likewise be carefully introduced. To protect against the development of oxidative stress, a combination of antioxidants, such as coenzyme Q10 and vitamins C, E and K (33), plus beta-blockade may be useful. Carvedilol may be an especially promising agent in this context, as it has alpha and beta-blocker properties, and as it has been shown to protect against mitochondrial oxidative stress *in vitro* and *in vivo* (34, 35).

Although the association of MELAS with diabetes mellitus is well-known, its association with thyroid, parathyroid, and neuroendocrine disease is less well-known. The cases described here underscore the point that this diagnosis should be considered in patients presenting insidiously with disparate endocrine conditions, in association with stroke-like episodes, myoclonic epilepsy and cortical blindness.

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Figures

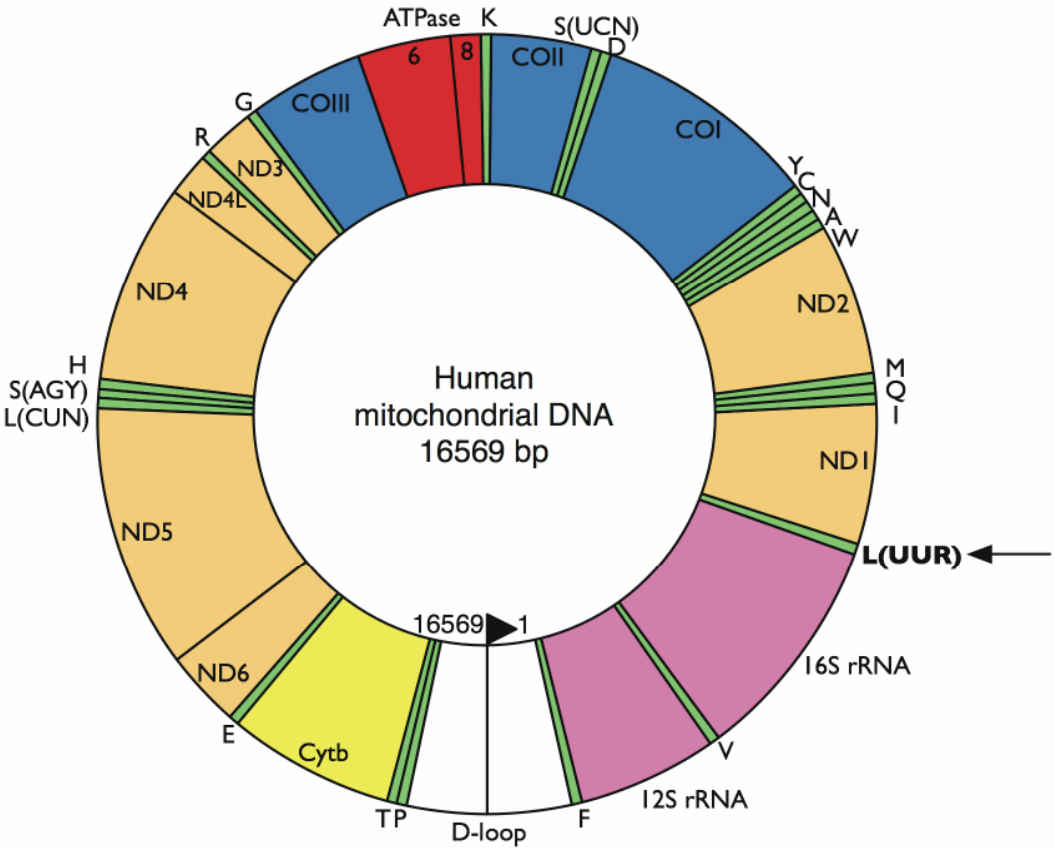

Figure 1: Schematic representing human mitochondrial DNA and genes encoded therein. Uncolored segment indicates the position of the D-loop, the origin of replication for the heavy strand of mitochondrial DNA. Green segments represent mitochondrial tRNA genes, labeled with the single-letter IUPAC codes for each amino acid carried by the tRNA (brackets contain the codon bound by the tRNA). Pink segments represent the mitochondrial ribosomal RNA genes. Orange segments represent the components of respiratory chain complex I (ND 1-6: NADH dehydrogenase). Blue segments represent the components of respiratory chain complex IV (CO I-III: cytochrome c oxidase). Red segments represent the components of respiratory chain complex V (ATPase 6,8: ATP synthase). Yellow segment represents the component of respiratory chain complex III (Cytb: cytochrome b). The position of MTTL1, encoding the mitochondrial tRNA for Leu(UUR) is indicated by the arrow.

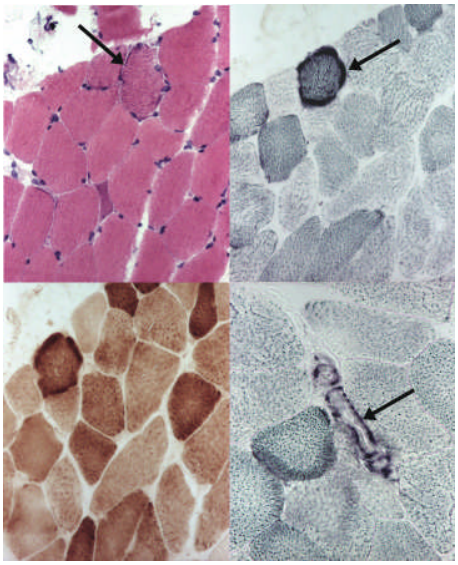

Figure 2: Skeletal muscle biopsy of patient 1 shows abnormal muscle fibers with haematoxylin and eosin stain (arrow, top left panel) and "ragged blue fibers" with a histochemical stain for succinate dehydrogenase (SDH), which stains mitochondria, thus demonstrating mitochondrial proliferation (arrow, top right panel). Cytochrome c oxidase (COX) staining shows marked heterogeneity (bottom left panel). An additional feature is overabundance of mitochondria in smooth muscle and endothelial cells of intramuscular blood vessels on SDH staining (arrow, bottom right panel).

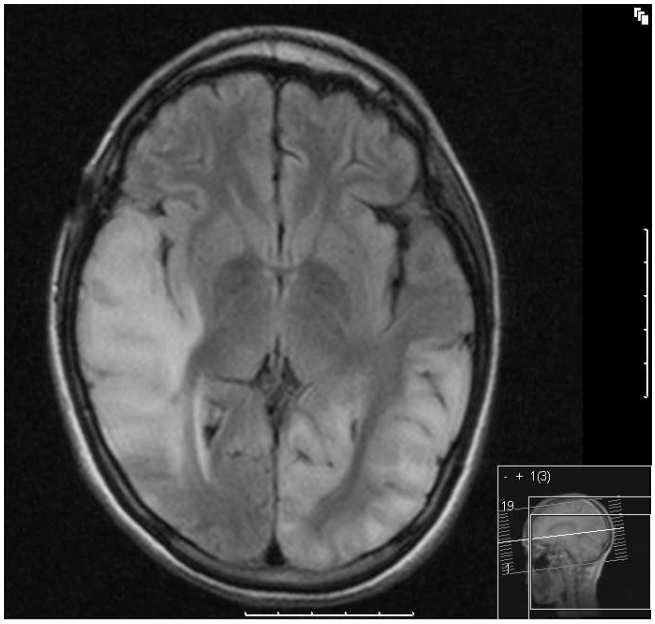

Figure 3: MRI scan of patient 2, showing bilateral T2 hyperintensity of the cortex and subcortex in the right temporo-parietal and the left temporo-occipital lobes.