SHORT REPORT

Clinical and molecular findings in four new patients harbouring the mtDNA 8993T>C mutation

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The 8993T>G (L156R) transversion in the mtDNA ATPase 6 gene is a common cause of maternally inherited Leigh syndrome (MILS; McKusick 516060). *In vitro* studies showed that L156R severely impairs ATP production by altering the proton channel of complex V (DiMauro and Schon 2001). A less common MILS mutation in the same gene (8993T>C; L156P) exhibits a milder phenotype and a remitting–relapsing clinical course, and shows no defective ATP synthesis. Its effects on cellular ATP output are generally unclear. We describe four new 8993T>C patients. In one case, we tested the effects of the mutation on energy production and mitochondrial membrane potential ($\Delta\psi_m$)

Case 1: A 17-year-old girl has had a remitting–relapsing neurodegenerative disease since age 16 months, with worsening during fevers or infectious disease. Neurological examination at age 14 years showed cerebellar signs, increased tendon reflexes in all limbs, and bilateral Babinski sign. CSF lactate was elevated (3.9 mmol/L). Brain MRI was compatible with MILS. Proton magnetic resonance spectroscopy (MRS) showed slight elevation of lactate in the basal ganglia with normal choline-containing compounds (Cho), creatine, phosphocreatine (Cr), and *N*-acetylaspartate (NAA) peaks. Her mother and maternal aunt show a progressive cerebellar ataxia.

Case 2: A 16-year-old boy experienced episodes of loss of consciousness and awkward gait during a febrile illness in childhood with a slow recovery. Blood (8.7 mmol/L) and CSF (4.8 mmol/L) lactate concentrations were elevated. Brain MRI showed basal ganglia involvement.

Case 3: A 21-year-old girl experienced a first episode of lethargy and hypotonia at age 5 months during a fever. Similar episodes reappeared in her first 10 years. At age 11 years, neurological examination showed mental deficiency, severe dysarthria and vertical gaze paralysis. Blood lactate was elevated (4.2 mmol/L).

Brain MRI showed hyperlucencies in the putamen and head of nucleus caudatus. Two older sisters showed peripheral neuropathy with normal MRI and blood lactate.

Case 4: A 16-year-old cousin of case 3 had a subacute episode of leg weakness, ataxia and dysarthria during a fever at age 3 years. She improved but had permanent motor disability. Similar episodes were repeated, always with slow recovery. At age 9 years, she showed elevated blood (3 mmol/L) and CSF (2.8 mmol/L) lactate. Brain MRI was compatible with MILS.

Mutational load in blood was $96.2\% \pm 5.5\%$ in patients but it was lower in 11 maternal relatives (range 0–87%). Individuals with less mutated mtDNAs were either healthy or presented minor neurological symptoms. Only 10% of the wild-type genome was sufficient to prevent basal ganglia involvement and lactic acidosis in family 3, indicating a steep threshold for full-blown disease. MRS in patient 1 showed normal NAA/Cr and Cho/Cr ratios, without a sharp lactate peak in the basal ganglia, findings different from those in a 8993T>G patient. Minimal neuronal loss and breakdown of membrane phospholipids indicate that the effects of the 8993T>C mutation are usually minor but become devastating during attacks triggered by high metabolic demands.

JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) staining showed a normal or increased $\Delta\psi_m$ in > 96% mutant skin fibroblasts from case 3, indicating that cells are more prone to accumulate H^+ , which is not dispersed. The block in complex V seems of no consequence to growth, even under conditions that favour expression of a functional oxidative phosphorylation system. Replication rates were indistinguishable from a control cell line when cells were grown for 5 days in either glucose medium (4.5 g/L) or galactose-medium (5 mmol/L) $\pm$ oligomycin (0.1 ng/ml). ATP synthesis was normal in analysed cells. This differs from data in 8993T>C cybrids (Manfredi et al 1999) but agrees with our model in *E. coli* (Carrozzo et al 2000). The absence of effect on ATP output in the presence of a complex V block suggests an inefficient enzyme, either more rapidly degraded or slipping in the coupling of proton translocation. Proton leaking may account for the observed normal ATP output.

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