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Functional characterisation of mitochondrial $tRNA^{Tyr}$ mutation (5877G→A) associated with familial chronic progressive external ophthalmoplegia

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EDITOR—Chronic progressive external ophthalmoplegia (CPEO) is a common clinical manifestation of mitochondrial cytopathies characterised by ophthalmoplegia and ptosis.[1] Approximately two-thirds of CPEO patients harbour a large, heteroplasmic, mitochondrial DNA (mtDNA) deletion.[2] Some other CPEO patients carry a point mutation in the mitochondrial tRNA genes. Twelve point mutations in six mitochondrial tRNA genes have been reported to date in association with CPEO (Mitomap at http://www.gen.emory.edu/mitomap.html). Among the 12 mutations, 5703C→T in the $tRNA^{Asn}$ gene has been functionally characterised.[3] Here we report functional analysis of 5877G→A in $tRNA^{Tyr}$ identified in a patient with CPEO[4] using ρ^0 cells that lack mtDNA.

A 45 year old woman had moderate degrees of ptosis, external ophthalmoplegia, and proximal muscle weakness from the age of 28. She had no sensorineural hearing loss, ataxia, pigmentary retinopathy, hypogonadism, or mental retardation. She had episodic diarrhoea of unknown aetiology. An ECG showed atrioventricular conduction block, while EEG, brain CT, and brain MRI showed no abnormalities. An exercise loading test of 15 watts for 15 minutes on a bicycle ergometer[5] raised her serum lactate from 6.9 mg/dl to 24.0 mg/dl (normal, less than 18.0 mg/dl), and her serum pyruvate from 0.6 mg/dl to 1.4 mg/dl (normal, less than 1.3 mg/dl), thereby increasing the lactate to pyruvate ratio from 10.7 to 17.1 (normal, less than 13.8). A biopsy specimen obtained from the biceps brachii showed 4.0% ragged red fibres and 0.7% cytochrome *c* oxidase negative fibres.

Mutation analysis of muscle mtDNA was briefly described previously (patient 2 in Ozawa *et al*[4]). Determination of the entire mtDNA sequence showed 34 nucleotide changes; 33 were homoplasmic and were observed in 274 controls with variable frequencies. A 5877G→A transition in the $tRNA^{Tyr}$ gene was heteroplasmic and unique to the patient. The ratios of mutant to wild type mtDNA were 73% in skeletal muscle and 0.7% in blood (fig 1B). The 5877G→A mutation is located in the DHU loop of the $tRNA^{Tyr}$ gene (fig 1A). The 5877G base pairs with 5905C in the variable loop (circle in fig 1A) to form the L shaped tertiary structure of the tRNA

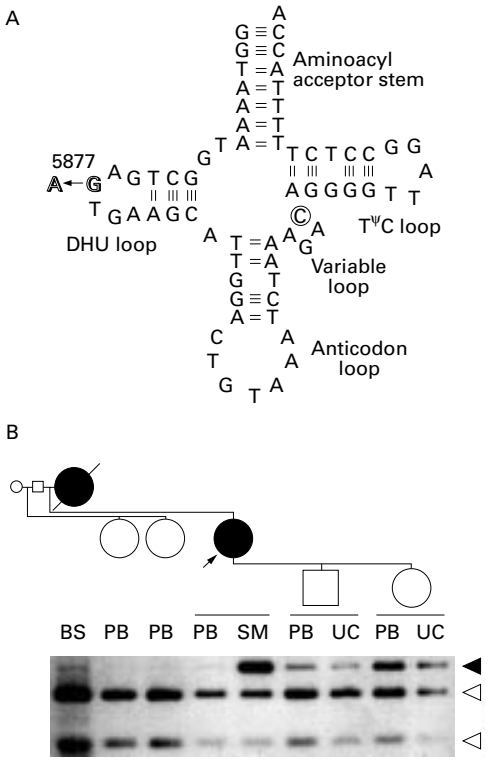

Figure 1 (A) Secondary structure of tRNA^{Tyr} and 5877G→A mutation. A circled C nucleotide in the variable loop (5905C) indicates a residue that base pairs with 5877G to make the L shaped tertiary structure.[6] Short lines in the stems indicate hydrogen bonds linking the paired nucleotides. (B) DdeI restriction analysis of 5877G→A in family members. PCR amplified mtDNA fragments from nucleotides 5681 to 5960 were digested with DdeI, which only cleaves at the wild type 5877G. Closed arrowhead points to a mutant 261 bp fragment. Open arrowheads indicate wild type 178 and 83 bp fragments. A mutant fragment in the patient's blood is scarcely visible in the figure. Arrow indicates the patient. Closed symbols show affected family members. BS, blood spot in the hospital archives from deceased mother, PB, peripheral blood, SM, skeletal muscle, UC, archived umbilical cords of patient's children which had been saved as mementos of birth.

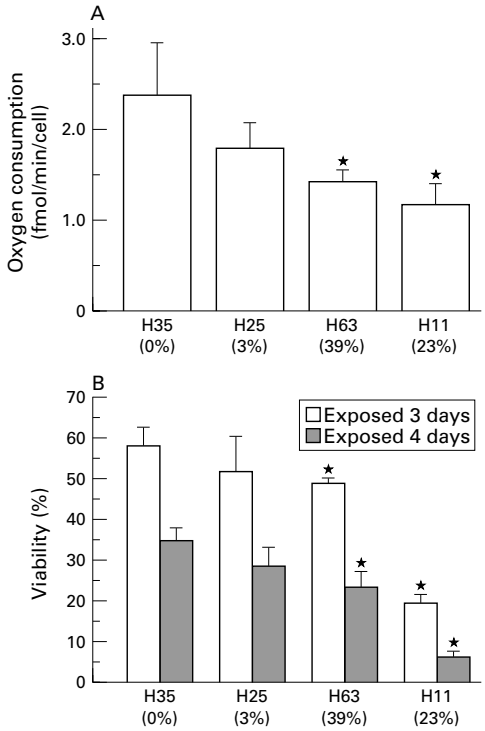

*Figure 2 (A) Oxygen consumption rate and (B) cell viability rate of transmitochondrial cybrid cell lines after exposure to 95% oxygen. Clones H35, H25, H63, and H11 carry 0, 3, 39, and 23%, respectively, of mutant mtDNA carrying 5877G→A. *p<0.05 compared to wild type H35.*

molecule.[6] The 5877G is conserved in 29 of 43 species or in 25 of 28 vertebrates (alignment not shown). The 5877G→A mutation has not been reported in other patients with mitochondrial cytopathies (Mitomap).

Restriction enzyme analysis of blood and umbilical cord DNA of family members showed that the deceased mother, who had ptosis, carried 5877G→A and that two asymptomatic children (a 21 year old son and 19 year old daughter) harbour 5877G→A in peripheral blood and umbilical cord (fig 1B). The patient developed ptosis at the age of 28 and the children may be too young to show any symptoms. Alternatively, the ratio of mutant mtDNA in muscle may be less in the children than in the patient. The children had no increase in resting serum lactate. Two asymptomatic paternal half sisters (aged 60 and 59 years) do not carry 5877G→A (fig 1B). Photographs showed that the maternal grandmother also had ptosis, but her DNA was not available.

To characterise the functional consequences of 5877G→A, we transformed ρ^0 cells with patient's skin fibroblasts as described elsewhere.[7] We obtained four distinct cybrid clones, H35, H25, H63, and H11, harbouring 0, 3, 39, and 23% of mutant mtDNA, respectively. We measured the oxygen consumption as an indicator for the mitochondrial respiratory chain functions, using a Clark type oxygen electrode. The H35 clone showed the highest oxygen consumption and the H11 clone the lowest (fig 2A). We next measured the viability of cybrids under 95% oxygen on days 3 and 4 by staining cells with 0.15% trypan blue, because we assumed that mutant mtDNA increases the leakage of reactive oxygen species, which in turn exerts cytotoxic effects leading to cell death.[8] Even normal mitochondria convert approximately 1-5% of oxygen into the reactive oxygen species, which results in degeneration of proteins, lipids, and nucleic acids.[9] Similar to the oxygen consumption analysis, H35 showed the highest viability rate and H11 the lowest (fig 2B). Although H63 harboured a higher ratio of mutant mtDNA than H11, H63 showed higher oxygen consumption and higher viability rate than H11. This may be because of the difference in the intracellular distribution of mutant mtDNA or in the total number of transformed mtDNA.

A previously characterised 5703C→T mutation at the anticodon stem of tRNA^{Asn} destabilises the tRNA secondary or tertiary structure and decreases steady state levels of tRNA^{Asn}.[3] The present 5877G→A mutation in tRNA^{Tyr} alters an essential residue to form the L shaped tertiary structure of tRNA.[6] Similarly, seven out of the 11 other CPEO associated point mutations (Mitomap) are at the tRNA stems

and probably destabilise the tRNA structure. However, four other mutations are at the tRNA loops and their effects on the tRNA structure remain unknown.

The 5877G→A mutation exerts deleterious effects even at 23% of mutant mtDNA, whereas more than 90% of mutant mtDNA is required to show pathogenicity of 3243A→G in tRNA Leu(UUR),[10] which is primarily associated with MELAS.[11] Hao and Moraes[3] proposed that null or near null mtDNA mutations are associated with muscle pathologies, as exemplified in 5703C→T in $tRNA^{Asn}$ and in mtDNA deletions. On the other hand, less severe mtDNA mutations are associated with central nervous system symptoms, as in the MELAS and MERRF associated mutations.[10] That low levels of mutant mtDNA decrease oxygen consumption and compromise cell viability under 95% oxygen in the current studies further supports their hypothesis on phenotype-genotype correlation of mtDNA mutations.

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Mutation detection in long QT syndrome: a comprehensive set of primers and PCR conditions

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EDITOR—Long QT syndrome (LQTS) is an inherited disorder which produces arrhythmia and sudden death. The only presymptomatic indication of the disorder is an extended QT interval, in excess of 460 ms.[1] However, those with LQTS do not always show this prolongation of QT. There are dominantly and recessively inherited forms of the disease, Romano-Ward syndrome and Jervell Lange-Nielsen syndrome, respectively, the latter also exhibiting severe sensorineural deafness.[2–4] About half the familial cases of LQT are known to be caused by mutations in five ion channel or channel associated genes, with over 90% being accounted for by *KCNQ1* and *HERG*, both of which code for potassium channels.[5] The sodium channel gene *SCN5A* is responsible for about 8% of cases with a known gene mutation, while *KCNE1* and *KCNE2*, which code for proteins that associate with *KCNQ1* and *HERG* respectively, are mutated in 1-2%.[5] Mutations in *KCNQ1* can produce both dominant and recessive forms of the disease, depending on the nature of the mutation.[6–12] *HERG* and *SCN5A* mutations are dominant, while those in *KCNE1* and *KCNE2* are recessive.[13–16] There are, however, exceptions to these rules.[17 18]

Mutations have been identified throughout the genes,[19 20] although analysis of both *HERG* and *SCN5A* has tended to be concentrated on the pore regions owing to the substantial number of exons in both genes. The initial publications on *KCNQ1* (formerly *KVLQT1*) also analysed the pore and surrounding regions, although several groups have now produced primers that cover the entire gene.[19 21] The mutation analysis has been by PCR followed by SSCP, and although laborious, this is still the most commonly used method. Investigation of all the genes has not been possible, mainly because the exact sequence of the PCR fragments amplified by the existing primer sets was not available for SCN5A[22] and the published primers for *SCN5A, HERG*, and *KCNQ1* amplified fragments outside the optimum size for SSCP or proved difficult to use.[21–23] Larsen *et al*[24] have reported a robust analysis of *KCNQ1* and *HERG*, but this uses an automated capillary SSCP analysis on an ABI 310 with an in house cooling system, which is not available in most laboratories, and *SCN5A* was not included in this report. To allow extensive investigation of populations potentially at high risk for mutations in genes known to cause LQTS, we have put together primer sets for all five genes, using