A new mtDNA mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS)

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In 3 of 40 MELAS patients, a new common mutation, a T-to-C transition at nucleotide position 3271 in the mitochondrial tRNA Leu(UUR) gene was recognized and was very near to the most common mutation site at 3243. With a simple detection method using polymerase chain reaction with a mismatch primer, none of 46 patients with other mitochondrial diseases and 50 controls had this mutation.

MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) is a clinical entity of mitochondrial diseases [1]. Recently, a transition mutation in the mitochondrial tRNA Leu(UUR) gene was found in 26 of 31 MELAS patients [2] and by a comparative analysis on the affected and normal cultured muscle cell lines from a patient [3]. We identified another 9 MELAS patients and found that 32 of the 40 patients (80%) had this mutation, but the remaining 8 patients did not. We, therefore, tried to detect other molecular abnormalities specific to MELAS. Sequence analysis was performed in all mitochondrial tRNA regions as described previously [2], and we found T-to-C transition mutation at nucleotide position 3271 in three unrelated patients (Fig. 1).

To develop a simple test to detect this mutation, we synthesized a modified PCR primer:

5'- (3301)TAAGAAGAGGAA'I'FGAACCTCTGACC'ITAA(3272)-3',

with a G-to-T mismatch at 3275 and a T-to-C at 3276 (*). If there was a transition mutation at 3271, the PCR products (170 bp) with this mismatch reverse primer and the forward primer:

5'-(3130)AGGACAAGAGAAATTGACCTGACCATTA(3372)-3',

could be cleaved into two fragments (140 and 30 bp) by endonuclease AflII because a new recognition site emerged:

5'-(3267)CTTAAG(3272)-3'.

This test revealed that three patients had mutant DNA in heteroplasmic fashion (Fig. 2). However, 32 MELAS patients with the most common mutation at 3243, 6 MERRF (myoclonus epilepsy associated with ragged-red fibers) with the mutation at 8344, 32 and 8 CPEO (chronic progressive external ophthalmoplegia) with and without large-scale deletions, respectively, and 50 normal controls from different racial groups did not have this mutation.

The nucleotides in the anticodon stem are not invariant in contrast to the most common mutation in the dihydrouridine loop during evolution [4-12]. However, the preserved nucleotides forming the anticodon stem are complementary each other, e.g., nucleotides T at 3271 and A at 3261 in normal human individuals (Figs. 3 and 4). As the tRNA with the most common mutation at 3243 in MELAS patients is deprived of the

Fig. 1. Nucleotide sequences around the mutation in the mitochondrial tRNA Leu(UUR) gene. Patient 1 had the mutation at nucleotide position 3243 and Patient 2 at 3271.

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hydrogen bond to T-residue in the amino-acid acceptor arm [2], the new transition should have a similar adverse effect on the structure and function of tRNA

Fig. 2. Amplified DNA was obtained after 30 cycles of 94 °C for 30 s, 45 °C for 30 s and 72 °C for 2 min. New bands (arrowheads) after digestion by AflII in addition to the initial PCR products (arrow) were electrophoretically detected in 3 of 8 MELAS patients without the 3243 mutation (lanes 2, 7 and 8).

Fig. 3. Interspecies similarity of mitochondrial tRNA

Fig. 4. A cloverleaf-like schema of human mitochondrial tRNA

References