Pancreatic exocrine dysfunction associated with mitochondrial tRNA^{Leu(UUR)} mutation

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Abstract

We report on pancreatic exocrine dysfunction in families that have the mitochondrial tRNA^{Leu(UUR)} gene mutation. These families exhibited maternally inherited diabetes mellitus (DM) and an A to G substitution at nt 3243 of the mitochondrial tRNA^{Leu(UUR)} gene (A3243G mutation). Pancreatic necropsy samples from one proband showed accumulation of degenerated mitochondria in pancreatic acinar cells. Pancreatic exocrine dysfunction was recognised by a functional pancreatic study. This study indicates that exocrine pancreatic dysfunction may be associated with the A3243G mutation.

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Many mitochondrial DNA (mtDNA) mutations are associated with various diseases.¹ One of the most common mutations involves an A to G substitution at nt 3243 of the tRNA^{Leu(UUR)} gene (A3243G mutation).² Recent studies have shown that there is a close association between the A3243G mutation and maternally inherited DM.³ Multiorgan involvement has also been suggested.⁴ However, pancreatic exocrine dysfunction has not been investigated, although it has been reported in patients with mtDNA deletion.^{5,6} Here, we report on pancreatic exocrine dysfunction in families with the A3243G mutation.

Clinical characteristics of the family members are as follows (fig 1).

Family A. The proband (A-II), a 39 year old man, was diagnosed as having DM at the age of 29 years. He developed stroke-like episodes and was diagnosed with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) at the age of 31 years. He continued to experience stroke-like episodes. In the later stages of the disease, he had chronic diarrhoea. He died at the age of 39. A necropsy was performed six hours after death.

Family B. The proband (B-I) is a 52 year old woman with a history of DM from the age of 50 years. The proband's son (B-II), aged 24 years, has had Wolff-Parkinson-White syndrome since the age of 5 years, a slight decrease in hearing threshold from the age of 20, and developed impaired glucose tolerance at the age of 24. The proband's mother, aged 83 years, has had DM since the age of 73 and sensory hearing disturbance from the age of 80.

Family C. The proband (C-I) is a 55 year old woman with a history of DM from the age of 53 years. Her son (C-II), aged 25, developed stroke-like episodes and haemianopia at the age of 21 and was diagnosed as having MELAS. He was further diagnosed as having DM at the age of 23. The clinical symptoms and molecular genetic analysis of A-II and B-II have been described in previous reports.^{7,8}

As a test of exocrine pancreatic function, we performed a bentiromide test using benzoyl-tyrosyl-p-aminobenzoic acid (Bz-Ty-PABA, PFD oral, Eisai Co Ltd)⁹ on the probands and their sons in families B and C, as they were the only ones who agreed to the test. The test was performed according to the manufacturer's instructions. Results were expressed as percentage recovery of PABA in urine (normal range=81.9±8.5% in non-diabetic subjects and 77±12.6% in diabetic patients).^{10,11} We could not perform the test on the proband in family A (A-II) because of his poor condition.

Genomic DNA extraction, PCR amplification, restriction enzyme analysis, sequencing, and densitometric analysis were performed as previously described.⁸

The pathological findings of the pancreas of patient A-II at necropsy are as follows. The pancreas showed no acinar atrophy or fibrosis. In the pancreatic acini, degenerative acinar cells with pycnotic nuclei were found to be scattered or clustered. As determined by electron microscopy, these cells had lost most of the rough endoplasmic reticulum, Golgi apparatus, and secretory granules, and were occupied by closely congregated vacuolar mitochondria (fig 2). No dilatation of the ducts or liposis was present. The number of islets was markedly decreased.

Molecular genetic analysis of the five patients showed that they had an A3243G mutation to different degrees. Densitometric analysis showed the percentage of mutant mtDNA in the white blood cells and the necropsy samples ranged from 6% to 63% (fig 1).

The five patients showed normal serum amylase and lipase levels. The bentiromide test indicated that three of the four patients showed a decreased percentage recovery of PABA, which in patients B-I, B-II, C-I, and C-II was 68%, 19%, 50%, and 40%, respectively.

The present study showed pancreatic exocrine dysfunction in families with the A3243G mutation. Although DM is commonly associated with the A3243G mutation, exocrine pancreatic dysfunction is not a recognised feature. However, it is worth noting that a significant

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Figure 1 Identification and quantitation of the A3243G mutation of the mitochondrial tRNA^{Leu(UUR)} gene in white blood cells (B) of all the patients and of the quadriceps muscle (Q) and pancreas (P) of necropsy samples from patient A-II. The percentage of mutant mtDNA and the percentage recovery of PABA are shown. M: size marker.

Figure 2 Electron micrograph of the degenerative acinar cells. Loss of rough endoplasmic reticulum and secretory granules and marked accumulation of vacuolar mitochondria in the cytoplasm can be seen. Note that the adjacent cell containing zymogen granules also shows enlargement of mitochondria (arrow).

degree of exocrine pancreatic dysfunction is a feature of Pearson syndrome (McKusick No 26056), a disease associated with deletions of mtDNA.^{5,6}

The electron microscopic appearance at necropsy of the pancreas of patient A-II is characteristic. Accumulation of vacuolar mitochondria seen in degenerative acinar cells is analogous to that seen in skeletal and cardiac muscle cells^{12,13} as well as smooth muscle and endothelial cells of the cerebral vessels¹⁴ of patients with mitochondrial encephalomyopathy. These findings led us to investigate pancreatic exocrine function.

The percentage recovery of PABA was variable between patients regardless of age. The clinical phenotype of mitochondrial disease depends on the level of heteroplasmy in each organ, organ threshold, and age.^{15,16} In our patients, the percentage of mutant mtDNA in the exocrine pancreas may be different.

Pancreatic exocrine dysfunction was recognised only by the bentiromide test, which is non-invasive and has a significant correlation with the results of the pancreozymin secretin test.⁹ However, the bentiromide test showed relatively little impairment. These data, together with the minimal changes in pancreatic cell morphology, indicate that the pancreatic exocrine dysfunction in these patients is subtle. One patient (A-II) showed chronic diarrhoea in the later stages of the disease. This might have resulted from steatorrhoea, although this was not documented clinically.

In conclusion, we observed variable clinical symptoms in the patients and our results indicate that exocrine pancreatic dysfunction may be associated with the A3243G mutation.

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