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Diabetologia

Letters

Clinical relevance of heteroplasmic concentration of mitochondrial A 3243 G mutation in leucocytes

Dear Sir,

A number of studies have found diabetes mellitus to be caused by mitochondrial DNA (mtDNA) mutations which often coexist with deafness or MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episode) [1]. The most common pathogenic mtDNA mutation is an A to G transition at np 3243 in the dihydrouridine loop of mitochondrial transfer RNA leucine $^{(UUR)}$ [2, 3].

Using the dot-blot hybridization method for detection of point-mutations in the $tRNA^{Leu(UUR)}$ region of the mitochondrial gene [4], we identified 23 patients with A 3243 G mutation among 1461 diabetic patients screened. The heteroplasmic concentrations in the leucocyte DNA of these 23 patients were then studied using PCR amplification with [^{32}P]-dCTP, followed by Apa I digestion and agarose gel electrophoresis. A correction for heterodimer formation was made by preparing a standard curve using samples with various amounts of mutated mtDNA as described previously [5]. A negative relation between heteroplasmic concentration in leucocyte DNA and age at onset of diabetes mellitus was observed (Fig. 1 A) ($r = -0.623$), in agreement with a previous report [6]. Taking the age-dependent decline in heteroplasmic concentration as described in another earlier report [7] into consideration, the relation between the onset of diabetes mellitus and the corrected heteroplasmic concentration was not statistically significant ($r = -0.2923$) (data not shown).

In addition, we examined the heteroplasmic concentrations in various tissues obtained from one of the patients at autopsy. This patient, a 65-year-old man, had been treated with 120 mg of gliclazide, for the 8 years preceeding his death, with fair control of blood glucose concentrations (HbA_{1c} value; 7.0–7.5 %). As his mother had been diabetic, he had been screened for mitochondrial gene mutations and found to have an A 3243 G mutation in his leucocytes. He had mild subclinical hearing loss but no neuromuscular symptoms. He had, however, been admitted to hospital because of sudden onset of weakness in his lower extremities and two weeks later, died of respiratory failure due to bacterial pneumonia. Microscopic examination of the pons showed an acute infarction, which was attributed to MELAS and considered to be the cause of his sudden onset of lower extremity weakness. We also studied the percentages of mutated A 3243 G mtDNA in various tissues. The percentage of mutated mtDNA exceeded 50 % in muscle, brain and intestine whereas the percentage was 13.2 % in the pancreas and only 1.4 % in leucocytes (Fig. 1 B). The latter was the lowest percentage among tissues examined. To our knowledge, heteroplasmic concentrations in different tissues have not previously been studied as extensively as in the present report. Our results show clearly that the A 3243 G mutation percentage varies considerably among tissues, with leucocytes being among those having the least heteroplasmy.

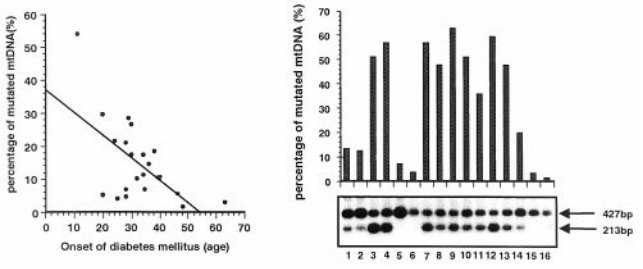

Fig. 1. **A.** Correlation between the heteroplasmic concentration in leucocytes and age at onset of diabetes ($y = 37.022–0.6838\,x$; $r = -0.623$, $p < 0.01$). **B** The heteroplasmic concentrations in pancreas (lane 1), liver (lane 2), left ventricle (lane 3), occipital lobe (lane 4), spleen (lane 5), spinal cord (lane 6), colon (lane 7), skin (lane 8), gastric mucosa (lane 9), cerebellum (lane 10), aorta (lane 11), adrenal gland (lane 12), femoral quadriceps (lane 13), kidney (lane 14), lung (lane 15), leucocytes (lane 16)

Although a negative relation was observed between the heteroplasmic concentration in leucocytes and the age at onset of diabetes, it is virtually impossible to predict the prognosis or seriousness or both of clinical manifestations including diabetes, deafness, and encephalomyopathies in a particular patient. In other words, whether or not the mutated mtDNA is

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present, rather than the percentage of mutated mtDNA in leucocytes, is of major importance.

When following subjects with mitochondrial A 3243 G mutations, we must keep in mind that many tissues other than leucocytes are likely to have high amounts of mutated mtDNA. This mutated mtDNA can eventually lead to serious disorders in tissues such as the brain and muscle, which appear normal early in the disease course.

Yours sincerely.

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Autoantibodies to human tissue transgutaminase identify silent coeliac disease in Type I diabetes

Dear Sir,

Coeliac disease is a concomitant condition in a appreciable number of patients with Type I (insulin dependent) diabetes mellitus. This could be explained by a similar genetic background associated with HLA DQ2 and DQ8 genotypes. Coeliac disease is triggered by the ingestion of gliadin, a component of wheat gluten, and is characterised by mucosal inflammation, crypt hyperplasia and villous atrophy. Coeliac disease specific IgA antibodies to endomysium have been described in 1.0–7.8 % of non-selected patients with Type I diabetes. This emphasises the importance of screening programmes to identify subjects with oligosymptomatic or silent coeliac disease [1, 2]. Recently, endomysium antibodies were shown to target the enzyme tissue transglutaminase [3, 4]. In this study we investigate the value of this new marker in a large cohort of patients with Type I diabetes to identify subjects with coexisting latent or silent coeliac disease.

Autoantibodies to human recombinant tissue transglutaminase were measured in 305 consecutive patients with newly diagnosed Type I diabetes (age 1 month–71 years, mean age 19.0 ± 14.5 years, 118 females, 187 males). None of them had a history of coeliac disease. Screening for tissue transglutaminase antibodies was done by combined IgG/IgA radioligand assay in a 96-well format using human recombinant [^{35}S]-methionine labelled tissue transglutaminase as described previously [5]. Receiver-operating characteristic analysis showed a diagnostic sensitivity of 95.6 % (n = 45 untreated patients with Coeliac disease and a specificity of 99.5 % (n = 574 healthy control subjects). Intra-assay and inter-assay coefficients of variation of this test were 10.4 % and 13.9 %, respectively. Where results were positive, sera were tested for IgA isotype and IgG isotype specific tissue transglutaminase antibodies [5].

Table 1. Immunological and histological findings in study groups

Subjects	Number	Antibodies to TG		Abnormal biopsies[a]
		IgA	IgG	
Type I diabetes	305	11 (3.6 %)	12 (3.9 %)	5/5 (100 %)
Autoimmune Thyroid disease	100	0	0	
Healthy control subjects	100	0	0	

TG = tissue transglutaminase

[a] a small bowel biopsy specimen was available five TG antibody positive subjects

In the combined assay tissue transglutaminase antibodies were observed in 12 subjects of whom 11 (3.6 %) and 12 (3.9 %) had IgA-tissue transglutaminase antibodies and IgG-tissue transglutaminase antibodies, respectively (Table 1). All of the IgA-antibody positive patients were also found positive for antibodies to endomysium studied by an indirect immunofluorescence test. Patients with tissue transglutaminase antibodies were significantly younger (age 8.3 ± 4.6 years) than antibody negative diabetic subjects (18.6 ± 14.4 years) ($p < 0.05$). All subjects with tissue transglutaminase antibodies were clinically undetected; eight were asymptomatic and four suffered from non-specific symptoms (weight loss n = 3; recurrent abdominal pain n = 1; growth retardation n = 1; diarrhoea n = 1). The patient with positive IgG-tissue transglutaminase but negative IgA-tissue transglutaminase and endomysium antibodies suffered from IgA deficiency. Thus, in this subject only IgG-tissue transglutaminase antibodies indicate silent co-

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