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Diabetes mellitus is one of the heterogeneous phenotypic features of a mitochondrial DNA point mutation within the tRNA$^{Leu(UUR)}$ gene

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A heteroplasmic point mutation (transition A-to-G at nucleotide position 3,243 in the mitochondrial tRNA$^{Leu(UUR)}$ gene) is found in a family suffering from a syndrome with diabetes, deafness and cardiomyopathy as the predominant clinical features.

mtDNA mutation; Diabetes; Deafness; Cardiomyopathy; MELAS

1. INTRODUCTION

Although diabetes has, without doubt, a genetic background, extensive research worldwide has until now been unable to define the genetic lesions and the mode of inheritance of the different forms of the disease. In our attempts to clear up the genetics of diabetes we are confronted with a number of obstacles such as differences and uncertainties in the definition of affected individuals, variabilities in the age of onset and the expression of the disease, its high but different prevalences in different populations, varying rates of concordance in monozygotic twins, etc. [1]. Therefore, the classification of diabetes is less genetic but largely descriptive. Diabetes is currently divided into two idiopathic forms, one insulin-dependent (type 1), one insulin-independent (type 2) and those forms which are associated with several syndromes [1]. Recent observations demonstrating that defects in the mitochondrial DNA (mtDNA) can lead not only to mitochondrial encephalomyopathies [2] but also to diabetes [3–5] have opened a new dimension for the respective genetic research. One of the mtDNA mutations associated with mitochondrial disorders is a A/G transition at nt position 3,243 within the tRNA$^{Leu(UUR)}$ gene which is found in about 90% of patients with MELAS [6–9]. Here we describe a family which carries this mutation but shows different phenotypes of disease expression, including diabetes as the predominant feature.

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Abbreviations: MELAS, mitochondrial encephalo-myopathy, lactic acidosis and stroke-like episodes; mt, mitochondrial; nt, nucleotide.

2. EXPERIMENTAL

2.1. *Patients*

The pedigree is shown in Fig. 1. Both females in the second generation (II_2; II_3) had type 2 diabetes and mild sensorineural hearing loss. The affected male in the third generation (III_1) developed deafness at the age of 22, insulin-dependent diabetes at 26 years, hemiparesis at 41 years. He also suffered from a massive hypertrophic cardiomyopathy and had myocardial infarction at the age of 39. His brother (III_2) had progredient dementia beginning at the age of 10 years. At the age of 20 he developed an insulin-dependent diabetes; at 30 and 32 years he had two episodes with hemianopsia, hemiparesis, motor aphasia, ataxia, frontal headache and two generalized tonic-clonic seizures (grand-mal). During his last years he developed a massive hypertrophic cardiomyopathy. Laboratory investigations revealed elevated lactate levels during rest and mild exercise as well as raised blood sugar values. Ragged red fibers and a mosaic pattern of single cytochrome *c* oxidase negative fibers were found in a muscle biopsy at the age of 32. He died at the age of 42 from cardiac failure. The sister (III_3) showed normal development until the age of 36 years, when she had one single attack of headache and vomiting. During this time mild hearing loss and glucose imbalance treatable with diet was diagnosed. Islet cell antibodies and insulin antibodies were negative. Her son (IV; 1978) is symptomless until now (15 years).

2.2. *Methods*

DNA was extracted from EDTA-blood and other tissues as described [10]. To detect possible deletions, the mtDNA was subjected to digestion with the endonucleases *Bam*HI and *Pvu*II, followed by Southern blotting. For the 3,243 mutation, the relevant region of mtDNA was amplified by PCR using the primer sequences 3,153–3,172 (upstream) and 3,501–3,474 (downstream) according to the Cambridge sequence. The 349 bp product was then digested with *Apa*I. The A/G transition at nt 3,243 creates a new restriction site for *Apa*I leading to two fragments of 255 bp and 94 bp in length. To detect small amounts of mutated mtDNA in the presence of an excess of wild-type mtDNA, we performed allele specific mismatch priming using an upstream primer (nt 3,224–3,243) which carried the mutation (G instead of A) at nt 3,243 and a mismatch (G instead of C) at nt 3,240 on one site and the downstream primer 3,551–3,531 on the other site. The expected fragment was 328 bp long.

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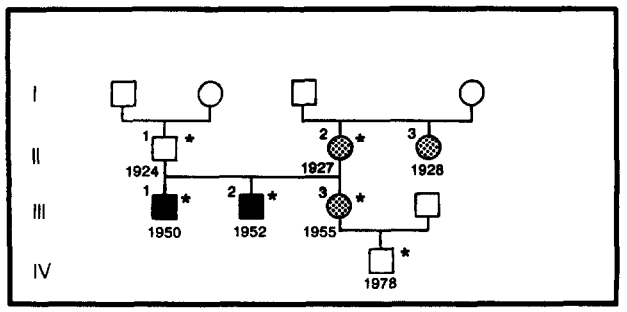

Fig. 1. Pedigree. Fully filled symbols = diabetes mellitus, deafness, cardiomyopathy and various neurological symptoms; dotted symbols = diabetes and deafness without other symptoms.

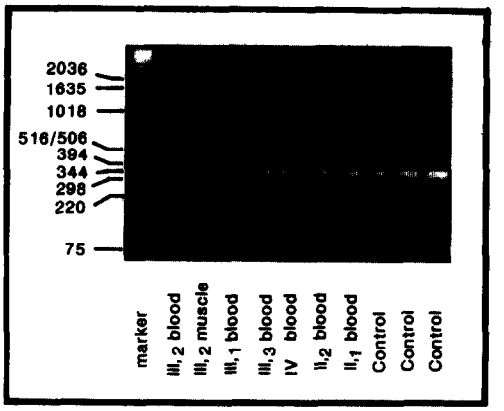

Fig. 2. PCR amplified mtDNA fragments after cleavage with *ApaI* visualized on ethidium bromide agarose gel. In the presence of the A/G transition at 3,243 *ApaI* cuts the 349 bp fragment into two smaller fragments 255 and 94 bp in length. Numbers represent pedigree members in Fig. 1.

3. RESULTS

Restriction analysis and Southern blotting failed to detect any deletion of native mtDNA extracted from blood. PCR amplification and digestion by *ApaI* yielded the point mutation at nt 3,243 in all samples from the family members of the third and fourth generation in a heteroplasmic form (Fig. 2). In the blood samples from both parents in the second generation, however, the respective PCR products were neither digested by *ApaI* (Fig. 2) nor by *Hae*III (data not shown). The use of the allele specific mismatch primer produced the expected fragments in the blood samples from the subjects III_1, III_3, IV, and a very faint, hardly visible band in that from the mother (II_2), while in the father's sample (II_1) no amplified fragment was detectable (Fig. 3).

4. DISCUSSION

The question of whether the mtDNA is involved in the pathogenesis of diabetes was recently discussed in detail [11]. In the meantime several publications have confirmed the hypothetical assumption that some forms of diabetes are associated with or even caused by defects of the mtDNA [4–6]. Our observation emphasizes the relation between diabetes and a specific A/G transition mutation at nt 3,243 within the $tRNA^{Leu(UUR)}$ gene [4,5] which has most commonly been observed in MELAS patients [6–9]. Although all family members along the matrilineage carry the 3,243 mutation their clinical features are different. Diabetes in its insulin-dependent and in its insulin-independent forms as well as hearing loss are the predominant clinical features in all family members with the exception of the young healthy boy in the last generation. MELAS typical symptoms such as strokes, seizures, hemiparesis, dementia, muscle weakness as well as severe cardiomyopathy are found only in the two brothers in the third generation and not in the others. Since the 3,243 mutation occurs in a highly conserved portion of the $tRNA^{Leu(UUR)}$ gene and has, so far, not been observed in healthy subjects [2,6–9], it is likely to cause the disease. Its phenotypic variability seems to be due to differential expression of the mutant genotype in different tissues with different thresholds. Measurements in blood cells might not reflect the situation in the affected tissues. For example, in blood cells of the diabetic grandmother in the second generation (II_2) restriction analysis using *ApaI* digested PCR fragments does not provide any evidence for the 3,243 mutation. Furthermore, allele specific mismatch priming yields only a very faint band in the grandmother's blood. In contrast, the healthy boy (15 years) in the fourth generation carries a relatively high amount of mutant mtDNA in his blood cells. Thus it seems likely that other epigenic factors, mitochondrial or nuclear in origin, influence penetrance and expression of the syndrome. Recent cell culture studies with cell lines containing in heteroplasmic form the 3,243 $tRNA^{Leu(UUR)}$ mutation, that were constructed by introducing mutant mtDNA into mtDNA-depleted cells, have demonstrated a marked replicative advantage of mutant over wild-type mtDNA [12]. The reason for this phenomenon, which can result in a rapid genetic drift towards high degree of mutant mtDNA, is unclear so far.

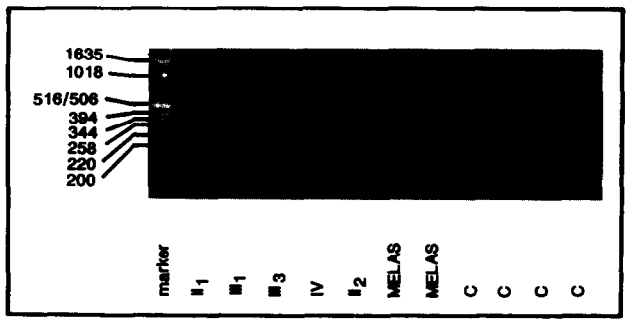

Fig. 3. PCR fragments amplified by use of allele specific mismatch priming. Numbers represent pedigree members in Fig. 1. C, controls; MELAS, two index patients from another family with typical MELAS syndrome but without diabetes.

MtDNA deletions [3] and point mutations [4,5] are not only found in maternally transmitted familial diabetes, but also in occasional patients as well as in single pedigree members suffering from both mitochondrial encephalomyopathy and diabetes. In none of the reported cases is diabetes the only symptom; there is always an association with other clinical symptoms such as deafness, ataxia, renal tubulopathy, etc., which are usually not combined with diabetes [11,13]. Thus it seems unlikely that those forms of diabetes which are associated with mtDNA mutations belong to the classical idiopathic type 1 or type 2 diabetes. They may represent subclassifications which are part of syndromic disorders. This assumption is supported by the fact that even in insulin-dependent subjects with mtDNA mutations testing for islet cell antibodies has revealed negative results in any case [5].

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