Mitochondrial DNA [tRNA$^{Leu(UUR)}$] Mutation in a Southern Italian Diabetic Population

Family, twin, and racial studies support the hypothesis that genetic factors play an important role in the etiology of NIDDM; however, the evidence implicating specific genes is generally controversial. Chromosomal genome has been the focus of many studies searching for mutations associated with diabetes and/or its complications (1–4). Recently, mitochondrial DNA (mtDNA) has also been studied in diabetic patients.

Eukaryotic cells usually contain one nuclear genome and up to several thousand identical copies of the mtDNA. The sequence of the human mitochondrial genome, 16,569 base pairs (bp) in length, was published in 1981 (5). The mtDNA is maternally inherited; it is circular and uses its own genetic codes for 13 subunits of the respiratory chain complexes, 22 transfer RNAs (tRNA), and 2 ribosomal RNAs (rRNA). Moreover, mtDNA is highly polymorphic, and many of the polymorphisms are related to ethnic background (6).

Preliminary reports in some NIDDM patients with maternally inherited diabetes and deafness (MIDD) have shown a G to A transition at nucleotide pair (np) 3243 in the mtDNA-encoded tRNA$^{LEU(UUR)}$ gene, the same mutation that is commonly associated with a severe neuromuscular disease called mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes (MELAS) (7).

In subsequent studies, this mutation has been detected in 1–3% of Japanese nonselected NIDDM patients (8,9) and is clearly associated with MIDD in a northern European diabetic population (10,11), but it has not been observed in 132 American diabetic patients of northern European descent (12).

Because no data are actually available concerning this mutation in Italy, we decided to screen a representative sample of our NIDDM population.

Of the patients attending our outpatient diabetes clinic, 231 consecutive unrelated NIDDM patients (123 men) were studied. They were born and were living in Sicily or Calabria (southern Italy) and were of Italian descent; their mean age was 59 ± 8 years (mean ± SD) and known disease duration was 13 ± 8 years. Of the subjects, 118 (51%) had at least one diabetic parent, 72 had a diabetic mother, 15 had a diabetic father, and 31 had both a diabetic mother and father.

None had evidence of clinically relevant deafness or neurological diseases. NIDDM diagnosis was based on World Health Organization criteria (13).

DNA was extracted from the patients' blood. To detect the 3243-bp mutation, the oligonucleotides for polymerase chain reaction (PCR) were designed to amplify the region encompassing the nucleotides 2770 to 3456.

The sequences of the PCR primers were 5′-CCCACAGGTCCTAAACTACC-3′ (nucleotide 2770–2790 of the mitochondrial tRNA$^{LEU(UUR)}$ gene) for the upstream primer and 3′-GCGAAGGGTTGGTAGTAGCC-5′ (nucleotide 3456–3437) for the downstream primer.

PCR was carried out by the standard protocol in a programmable heat block (Perkin Elmer GeneAmp PCR System 9600, Foster City, CA) with 50 ng of DNA as template. A denaturation step at 95°C for 5 min was followed by 26 cycles of denaturation (95°C, 60 s), annealing (55°C, 45 s), and primer extension at 72° C for 45 s. The PCR products were then extracted and digested with 15 units of ApaI (Life Technologies Italia Srl) for 1 h at 37°C. ApaI cleaves the mutant sequence (GGGCCC) at np 3243 but not the wild-type sequence (GAGCCC). Digestion with a standard λDNA was included as a positive digestion control to prevent digestion failures (14). Then, the digested samples were loaded onto 2.5% agarose gels and electrophoresed at 70 V for 2 h before visualization of fragments by ultraviolet transillumination.

We failed to detect any individual with the 3243 mtDNA mutation in the entire NIDDM population we have studied. Also, among patients with maternally inherited diabetes, we were unable to identify anyone with this mutation.

In this report, we have screened 231 southern Italian NIDDM patients for a mutation at np 3243 in the mitochondrial tRNA$^{LEU(UUR)}$ gene. To screen a reasonably large number of patients, DNA from peripheral leukocytes was studied. Overall, estimated prevalence of NIDDM in Italy is 4.5% (15), and people living in the nearby area of our clinic are about 250,000; consequently, it seems reasonable to calculate that we have studied 2% of all NIDDM patients living in this area.

Family history of NIDDM is often incomplete because many relatives are never tested or die before they develop the disease. We therefore chose not to limit our search to subjects with a definite maternal history of the disease. However, of the 118 subjects with affected siblings that we have studied, 103 were aware of an affected mother. In our population, PCR-restriction digestion failed to detect the point mutation at np 3243 in any patient.

Recent studies have demonstrated that this mutation co-segregates with diabetes in about 2% of Japanese and northern European NIDDM patients with a positive family history (6) and presumably in <1% of white Americans of northern European descent (12). Our results indicate that also in southern Italian diabetic patients, the tRNA^{LEU} mutation is at least extremely rare and does not suggest a major role for this mutation in our NIDDM population.

This discrepancy with previous reports can be explained by ethnic differences between this and other studied populations or by different recruitment methods. Selected families with NIDDM or a larger number of patients could be necessary to definitely assess the role of this mutation in our diabetic population.

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Are Young Age and Insulin Treatment Enough to Diagnose IDDM?

In a paper recently published in *Diabetes Care*, V.A. Koivisto et al. (1) addressed the issue of cardiovascular disease in IDDM patients in Europe. The study was based on cross-sectional data from 16 European countries (EURODIAB IDDM Complications Study) (2). The target population was all individuals with IDDM in the participating centers. The eligibility criteria were an age range of 15–59 years, a diagnosis of diabetes before 36 years of age, and unbroken record of insulin treatment.

We have published in a recent issue of *Diabetes Care* (3) our experience with IDDM in adults in a community-based study in Israel. To determine the clinical characteristics of insulin-treated NIDDM and IDDM diabetic patients, we examined all known insulin-treated diabetic patients from three community clinics (registered population, 9,573 adults). Fasting plasma C-peptide levels were measured in all insulin-treated patients; insulinopenia was diagnosed when plasma C-peptide was <0.132 nmol/l. A total of 588 diabetic patients were found, 100 of whom were insulin-treated; of those, only 25 were insulinopenic. The mean ages (range) of the IDDM and NIDDM groups were 49.5 (24–75) and 62.0 (29–86) years, respectively; the mean ages at diagnosis of diabetes were 26.9 (4–60) and 46.9 (17–73) years, respectively. Moreover, 43% of the IDDM patients were diagnosed at ≥36 years of age, whereas 22% of the insulin-treated NIDDM patients were diagnosed at ≤36 years of age. Interestingly, only 66% of our insulinopenic patients received insulin as a first treatment, while 21% of insulin-treated NIDDM patients received insulin as a first treatment.

If our patients were to be classified according to the EURODIAB inclusion criteria, 43% of the insulinopenic IDDM patients would be excluded, and 22% of the insulin-treated NIDDM patients would be included in the IDDM group. Since most insulin-treated patients, at least in our study, actually had NIDDM (3), it is possible that a large number of patients included in the EURODIAB study were insulin-treated NIDDM patients. Also, Laakso and Pyörälä (4), who studied insulin-treated patients in Scandinavia, found that 50% of insulin-treated patients were positive for endogenous insulin secretion.

Thus, the measurement of endogenous insulin secretion capacity in insulin-treated patients is of great value to differentiate between IDDM and insulin-treated NIDDM individuals. Despite the added technical burden and the increase in cost, fasting plasma C-peptide measurements should be included in large epidemiology studies dealing with IDDM.

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Reply to Maislos and Weitzman

Maislos and Weitzman (1) criticize the definition of IDDM used in the EURODIAB study, suggesting that this could result in diagnostic misclassification. It is clear that there is no one universally accepted diagnostic test that will completely differentiate between IDDM