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Rapid Diagnosis of Mitochondrial Mutation at Position 11778-Associated Leber Hereditary Optic Neuropathy

To the Editor:

Leber hereditary optic neuropathy is a maternally inherited disease associated with mutations of mitochondrial DNA (*1, 2*). A G to A substitution at position 11778 converts a highly conserved arginine to histidine in the fourth subunit of NADH dehydrogenase (EC 1.6.99.3). This mutation causes the loss of an *Sfa*NI restriction site (*1*) and creates a new *Mae* III restriction site (*3*).

We developed a rapid detection of this mutation by a simplification of DNA extraction followed by polymerase chain reaction (PCR) amplification (*4*) and digestion with restriction enzymes, *Sfa*NI or *Mae* III.

Frozen EDTA-anticoagulated blood samples (20 μL) were treated three times with 1 mL of a solution of 10 mmol of Tris · HCl and 1 mmol of EDTA per liter (pH 8.0) at 4 °C. After centrifugation at 11 000 × *g* for 2 min, the pellet was frozen at −70 °C for 5 min, then heated at 95 °C for 5 min, after which PCR amplification was performed without delay.

Primer sequences used for this purpose were 11618–11637 (light strand; 5′-GCATACTCTTCAATCAGCCA-3′) and 11893–11874 (heavy strand; 5′-TTCTCCCAGTAGGTTAATAG-3′). The following solution was prepared: per liter, 200 μmol of each deoxynucleotide triphosphate (dATP, dGTP, dCTP, and dTTP), 0.10 μmol of each oligonucleotide primer, 50 mmol of potassium chloride, 1.5 mmol of magnesium chloride, 0.1 g of gelatin, and 10 mmol of Tris · HCl (pH 8.3). We added 50 μL of this solution and 1 U of Taq DNA Polymerase (from Perkin Elmer Cetus, Norwalk, CT, or Boehringer) to the blood pellet and subjected the mixture to 25 cycles of PCR in a DNA thermal cycler (PTC-100; MJ Research Inc., Watertown, MA), with each cycle consisting of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 2 min. For the last cycle, annealing was carried out at 52 °C for 1 min and extension at 72 °C for 5 min. After PCR, 10-μL samples from the PCR mixture were digested for 2 h with 1.5 U of restriction enzyme, *Sfa*NI (New England Biolabs, Beverly, MA) or *Mae* III (Boehringer).

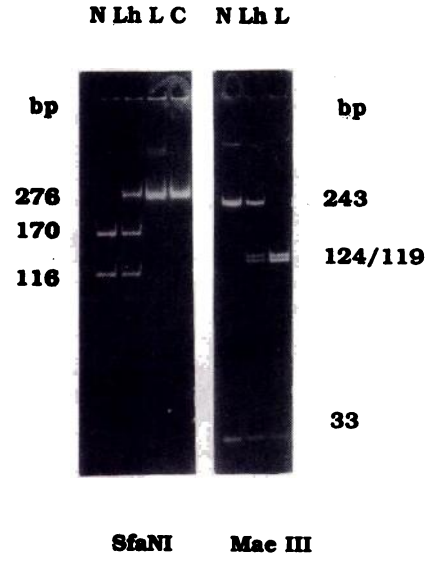

Fig. 1. Polyacrylamide gel electrophoresis of restriction digests with enzymes *Sfa*NI or *Mae* III from a normal individual (*N*) and from two patients with Leber disease, one being homoplasmic (*L*) and the other one heteroplasmic (Lh)
C is a control corresponding to undigested amplified DNA

The digested products were separated by electrophoresis, either agarose or 5% polyacrylamide gel. Agarose electrophoresis was performed as described by Wallace et al. (*1*). Polyacrylamide gel electrophoresis (PAGE) was performed in 20 mmol/L Tris, 180 mmol/L glycine buffer (pH 9.0) for 4 h at 180 V. PCR products stained with ethidium bromide were made visible by transillumination in an ultraviolet light box. Figure 1 illustrates the PAGE patterns obtained with mitochondrial DNA from two patients with Leber disease and from one normal individual. By agarose electrophoresis, the separation between the 119- and 124-bp fragments was not so sharp (data not shown), but the separation took only 1 h.

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Multiple Serum Protein Abnormalities in Carbohydrate-Deficient Glycoprotein Syndrome: Pathognomonic Finding of Two-Dimensional Electrophoresis?

To the Editor:

We wish to report that high-resolution two-dimensional polyacrylamide gel electrophoresis (2DE) and silver staining yields a potentially pathognomonic profile of multiple serum protein anomalies in carbohydrate-deficient glycoprotein syndrome (CDGS). CDGS is a recently described chronically debilitating autosomal recessive neurometabolic disorder that affects multiple organ systems (*1–5*). One of the hallmarks of this disease is the occurrence of cathodal, carbohydrate-deficient isoforms of serum transferrin (CDT) that lack between one and four sialic acid-containing terminal trisaccharides (*6*). The methods used to detect CDT have been isoelectric focusing with or without immunofixation, isocratic anion-exchange chromatography, and carbohydrate analysis after isolation of transferrin by immunoaffinity chromatography (*6*). In all cases of CDGS, the increased concentration of the cathodal CDTs exceeds that usually seen in alcoholism or severe liver disease (*7*).

We undertook the present study to determine whether these CDT isoforms could be qualitatively detected with 2DE in serum from a 24-month-old girl whose clinical findings of "flop