tice. New York: Churchill Livingstone, 1991:274–90.

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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).

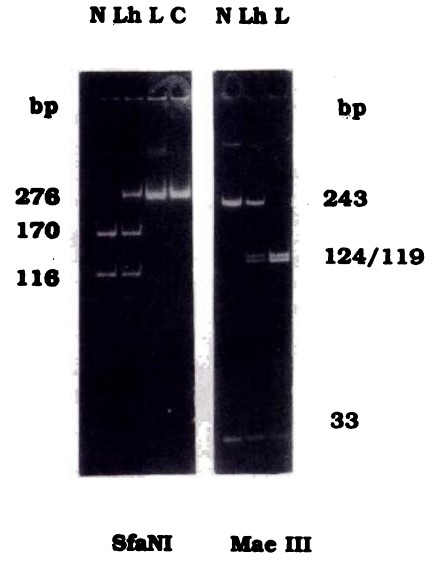

Fig. 1. Polyacrylamide gel electrophoresis of restriction digests with enzymes *Sfa*NI or *Mae* III from a normal individual (*N*) and from two patients with Leber disease, one being homoplasmic (*L*) and the other one heteroplasmic (Lh)
C is a control corresponding to undigested amplified DNA

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.

References

1. Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 1988;242:1427–30.
2. Huoponen K, Vilkki J, Aula P, Nikoskelainen EK, Savontaus ML. A new mt DNA mutation associated with Leber hereditary optic neuroretinopathy. Am J Hum Genet 1991;48:1147–53.
3. Stone ME, Coppinger JM, Kardon RH, Donelson J. *Mae* III positively detects the mitochondrial mutation associated with Type I Leber's hereditary optic neuropathy. Arch Ophthalmol 1990;108:1417–20.
4. Saiki RK, Gelfand DH, Stoffel S, et al. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988;239:487–91.

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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

piness," delayed development, cerebellar hypoplasia, and metabolic crises were consistent with the diagnosis of CDGS. We used the 180 × 180 × 1.5 mm Anderson ISO-DALT® 2DE system (*8*, *9*) with silver staining and slight procedural modifications for improved quality assurance (*10*). Our findings (Figure 1) revealed not only CDTs but also abnormalities in the spot patterns for α_1-acid glycoprotein (AAG), α_1-antichymotrypsin (ACT), α_1-antitrypsin (A1AT), α_1-B glycoprotein (A1BG), ceruloplasmin (Cp), complement C1 esterase inhibitor (C1INH), complement C3a (C3α) and C4a (C4α), and the serum peptide PLS:34 (*11*). In contrast to the observation of isoforms that had both charge and mass changes, as was seen for transferrin, some of the abnormal isoforms observed for other proteins, though also lower in mass, were isoelectric only, e.g., for A1AT and PLS:34. The lower-mass isoforms were generally most prominent at the basic end (right-most in Figure 1) of each of the spot series. Such neutral, low-mass isoforms were also seen for the A1AT dimer series. This finding implies that for some proteins the putative carbohydrate transferase deficiency of CDGS results in the (mis)-transfer of neutral carbohydrate side chains. In addition, no stainable haptoglobin was subsequently detected with nephelometry. The series of three spots for A1B exhibited a discrete charge and mass saltation to a lower-mass, more-basic series of three spots, consistent with an apparent mass reduction of ~5000 Da and a +2 esu charge shift. The amount of protein in the abnormal isoforms varied among proteins but qualitatively was usually <50%. The 2DE pattern of serum from the subject's mother was normal; the father was unavailable for study.

The observation of multiple serum protein anomalies is consistent with other CDGS-related biochemical abnormalities, e.g., low concentrations of serum albumin, haptoglobin, apolipoprotein B, thyroxine-binding globulin, transcortin, and serum cholesterol, and with fluctuant concentrations of hormones such as prolactin, follicle-stimulating hormone, and growth hormone (*1*). The prominence of the CDT finding in CDGS is due to the relatively high concentration of transferrin in serum, the fact that the terminal trisaccharide-depleted isoforms have altered chemical charge, and the availability of the CDT isoform assays that were developed for other clinical indications (*7*). However, because increased CDT may also occur in ethanolism, primary biliary cirrhosis, and the D genetic type, the specificity of the CDT assay is <100% for CDGS (*6*, *7*). In contrast, the 2DE assay allows the simultaneous identification of a constellation of protein alterations characteristic for CDGS. Furthermore, with 2DE the CDT can be distinguished from the D genetic type of transferrin because the D allele product, although more cathodal, is glycosylated normally and does not show a decrease in mass (*8*, *12*, *13*). The serum 2DE pattern of CDGS is also distinct from the 2DE pattern of serum proteins in severe liver disease, where there is occasionally evidence for incomplete sialylation of AAT and haptoglobin, but not the smaller isoelectric mass isoforms seen in this CDGS patient (H. Harrison, unpublished). We do not yet know how many of the (mis)modified serum proteins, for which CDGS-related structural changes were observed with 2DE, have altered function. Because the primary enzyme defect in CDGS is not clearly defined, it is possible that the pattern reported here contains secondary anabolic or catabolic defects in protein–carbohydrate structure. Examination of the proteins of additional CDGS families and longitudinal studies in individual patients will help to clarify this.

Testing of two other CDGS-affected individuals, a 19-year-old female and

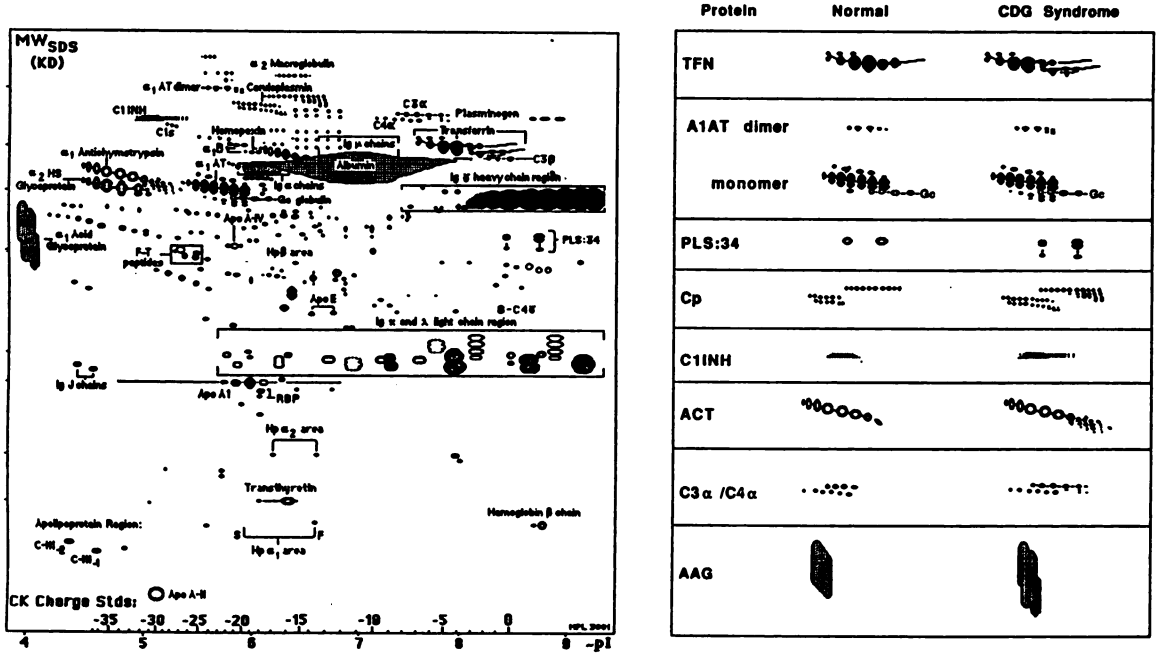

Fig. 1. (*Left*) Detailed schematic of silver-stained ISO-DALT 2DE gel run of serum proteins from patient; (*right*) schematic vignettes of normal and CDG-related polypeptide spot groups for eight affected serum proteins

ISO-DALT 2DE was performed as described (*9–11*). The serum sample was mixed with four volumes of sodium dodecyl sulfate–mix denaturant (*9*), and 6 μL (1.2 μL serum vol. equivalent) were loaded onto the ISO gel. For a normal reference electrophoretogram or schematic map of microheterogeneity patterns and major genetic types, see refs. *8* and *10–14*. Apo, apolipoprotein; α2HS, α2 Haupt-Schwick glycoprotein; C, complement; CK, creatine kinase; F-T peptides, freeze-thaw peptides; Gc, group-specific component (vitamin D binding globulin); Hp, haptoglobin; Ig, immunoglobulin; MWSDS, apparent mass (molecular weight) in sodium dodecyl sulfate-polyacrylamide gel electrophoresis; pI, isoelectric point; PLS, unnamed plasma/serum protein; RBP, retinol binding protein; and TFN, transferrin. Other abbreviations given in text

a 21-year-old male, in an unrelated family yielded identically abnormal results in the two-dimensional electrophoresis procedure. We also noticed a marked diminution of PLS:29. Both parents (obligate carriers) in this family were studied and found to have normal serum protein 2DE patterns.

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References

1. Jaeken J, Vanderschueren-Lodewyck M, Caesar P, et al. Familial psychomotor retardation with markedly fluctuating serum prolactin, FSH and GH levels, partial TBG deficiency, increased serum arylsulfatase A and increased CSF protein: a new syndrome? Pediatr Res 1980;14:170.
2. Jaeken J, Eggermont E, Stibler H. An apparent homozygous X-linked disorder with carbohydrate-deficient serum glycoproteins. Lancet 1987;ii:1398.
3. Jaeken J, Stibler H. A newly-recognized inherited neurological disease with carbohydrate-deficient secretory glycoproteins. In: Wetterberg I, ed. Wenner-Gren Intl Symp Ser Vol. 51, Genetics of neuropsychiatric diseases. London: Macmillan Press, 1989:69–80.
4. Jaeken J, Hagberg B, Strømme P. Clinical presentation and natural course of the carbohydrate-deficient glycoprotein syndrome. Acta Paediatr Scand Suppl 1991; 375:6–13.
5. Stibler H, Jaeken J, Kristiansson B. Biochemical characteristics and diagnosis of the carbohydrate-deficient glycoprotein syndrome. Acta Paediatr Scand Suppl 1991; 375:21–31.
6. Stibler H, Jaeken J. Carbohydrate deficient serum transferrin in a new systemic hereditary syndrome. Arch Dis Child 1990; 65:107–11.
7. Stibler H. Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed [Review]. Clin Chem 1991;37:2029–37.
8. Anderson NL, Anderson NG. High resolution two-dimensional electrophoresis of human plasma proteins. Proc Natl Acad Sci USA 1977;74:5421–5.
9. Tollaksen SL, Anderson NL, Anderson NG. Operation of the ISO-DALT system, 7th ed. US Dept of Energy Publ ANL-BIM-84-1. Argonne, IL: Argonne Natl Lab, 1984.
10. Harrison HH, Miller KL, Dickinson C, Daufeldt JA. Quality assurance and reproducibility of high-resolution two-dimensional electrophoresis and silver staining in polyacrylamide gels. Am J Clin Pathol 1992;97:97–105.
11. Anderson NL, Tracy RP, Anderson NG. High-resolution two-dimensional electrophoretic mapping of plasma proteins. In: Putnam FW, ed., The plasma proteins. Vol. 4. Orlando and London: Academic Press, 1984:221–70.
12. Anderson NL, Anderson NG. Microheterogeneity of serum transferrin, haptoglobin, and alpha-2-HS glycoprotein examined by high resolution two-dimensional electrophoresis. Biochem Biophys Res Commun 1979;88:258–64.
13. Harrison HH, Ober C, Miller KL, Elias S. A high resolution two-dimensional electrophoretic survey of serum protein genetic types in Schmiedeleut Hutterites. Am J Hum Biol 1991;3:639–46.
14. Harrison HH. The "ladder light chain" or "pseudo-ologoclonal" pattern in urinary immunofixation electrophoresis (IFE) studies: a distinctive IFE pattern and an explanatory hypothesis relating it to free polyclonal light chains. Clin Chem 1991; 37:1559–64.

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Pitfalls of Restriction Endonuclease Digestion for Direct Detection of Point Mutations

To the Editor:

The necessity of heeding subtle anomalies frequently encountered upon electrophoretic analyses of restriction endonuclease digestion products of genomic and polymerase chain reaction (PCR)-generated DNA templates became obvious to us recently. We wish to alert clinicians and molecular pathologists to such cues because, without such vigilance, we would have drawn an erroneous conclusion as to the cause of one patient's genetic impairment.

While routinely mapping point mutations in exon 8 of the arginase locus of a collection of hyperargininemic patients, we discovered a single base change immediately outside a restriction enzyme recognition site (TaqI), which severely inhibited cleavage of this site (*1*).

The consistent and reproducible results of both standard Southern mappings and endonuclease digestions of PCR-generated DNA templates of exon 8 had predicted that a point mutation, present in heterozygosity, was located directly within a unique TaqI site of exon 8 of patient 1 (Figure 1). Because of a minor aberration we noticed in the gels used to analyze the digestion products (Figure 1*B*), we proceeded to sequence this PCR product. We would not have done so if we had not noticed that the upper "uncut" band looked slightly less intense than the lower "cut" band of patient 1. The results of this gel suggested that this allele from patient 1 had been partially digested by TaqI. The anomaly resulted because the point mutation was actually in a base adjacent to the tetranucleotide recognition site, not directly within it. This flanking base inhibited cleavage by TaqI by ~80% on the mutated chromosome of patient 1, resulting in bands that were of unequal intensity by ethidium bromide staining.

Factors affecting the efficiency of cleavage of DNA templates by restriction endonucleases have been addressed (*1–6*). However, this is the first instance of inhibition of cleavage of genomic and PCR-generated DNA templates (the latter of which rules out methylation as a cause of inhibition of cleavage) and in the clinical setting of the diagnosis of a human genetic disorder.

Publications in this and other journals tout direct DNA analysis for direct detection of point mutations and polymorphisms (*7–11*). Generally, these methods are inexpensive, precise, simple, rapid, and reliable for diagnostic characterization of heritable diseases (*7–9*). Additionally, these methods yield far more information about genotype than do methods that rely on protein analyses, such as isoelectric focusing (as for apolipoprotein E polymorphism), or microvillar enzyme analysis for the prenatal diagnosis of genetic diseases such as cystic fibrosis (*8, 9*). The advantages of the detection of rare mutant alleles via direct DNA analysis over detection with site-specific monoclonal antibodies are well documented, particularly for the diagnosis and prognosis of α_1-antitrypsin deficiency (*10*).

Indeed, the precision of genotypic characterization allows more accurate predictions of the clinical outcome of many inherited diseases that are caused by single base changes in specific loci (*12, 13*). For example, in the diagnosis of cystic fibrosis, prenatal and pediatric genotype characterization has begun to predict whether pan-