DNA analysis on previously collected routine specimens should facilitate retrospective studies of genetic pedigrees or infectious agents, postmortem and forensic studies, and should obviate the need to collect specimens from patients.

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References

Apolipoprotein E Genotyping on Agarose Gels, Paul W.A. Reymers, Björn E. Groenemeyers, Remco van de Burg, and John J.P. Kastelein (Dept. of Vascular Med., Academic Med. Centre, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands; author for correspondence: fax + 31-205664440, e-mail lansberg@uva.amc.nl)

Apolipoprotein E (apo E), one of the major proteins of very-low-, intermediate-, and high-density lipoprotein particles, can be present as three isoforms (apo E2, E3, and E4) in human plasma, each with a different affinity for the apo-E/B receptor. By serving as a ligand for the uptake of lipoproteins from the circulation by the liver, apo E protein plays a significant role in the pathogenesis of atherosclerosis (1); recently, a strong association between the apo E4 allele and late-onset Alzheimer disease was also demonstrated (2–7). The demand for apo E genotype analysis will therefore increase, making a fast, reliable assay for apo E genotyping essential.

Formerly, two-dimensional gel electrophoresis (8) or isoelectric focusing (9) were commonly used for apo E phenotyping in laboratory medicine. Another approach is to determine apo E genotypes by amplifying the area around the polymorphic sites in the apo E gene by using polymerase chain reaction (PCR), followed by hybridization with allele-specific probes or digestion with restriction enzymes (10–13).

Apo E phenotyping does not always identify changes at the molecular level, especially when a nucleotide substitution does not result in an alteration of protein charge. Thus, phenotyping can be erroneous in comparison with DNA-based apo E genotyping. However, the genotyping described by Hixson and Vernier (12) is not favored by most laboratories because acrylamide is a neurotoxic substance and only a few samples can be analyzed in 1 day.

To improve the apo E genotype assay for routine use, we modified the method by elongating the 3'-primer with a 23-nucleotide AT-tail, which obviates the use of dimethyl sulfoxide and acrylamide and allows genotyping of ~300 samples on agarose gels by one technician in one working day.

Genomic DNA isolated from leukocytes as described previously (13) was dissolved in 10 mmol/L Tris, pH 8.0, containing 1 mmol/L EDTA and then stored at 4 °C. The primers used have the following sequences:

Apo E-AT3'5'-ATAAAATATAAATATAAATACAGATT CGCCCCGGCTGGTAC-3'
Apo E-5'5'-TAAGCTGGCAAGGCTGTCACAGGA-3'

The PCR was carried out in standard PCR buffer (per liter, 50 mmol of KCl, 10 mmol of Tris, pH 9.0, 0.1 g of gelatin, 1.5 mmol of MgCl₂, and 1 mL of Triton X-100) with 0.5 μmol/L of each primer, 100 μmol/L of each dNTP (Pharmacia, Sollentuna, Sweden), 0.2 g/L bovine serum albumin (Bosera1; Organon, Eppelheim, Germany), and 100–500 ng of genomic DNA in a total volume of 50 μL. We used a DNA thermal cycler (PTC-100; M.J. Research, Watertown, MA) for amplification (30 cycles each of 30 s at 94 °C, 1 min at 60 °C, and 1 min at 72 °C) with 0.5 U of Supertaq (Ht-Biotechnology, Cambridge, UK).

After amplification, the samples were digested with restriction enzyme CfoI (Fromega, Madison, WI). We mixed PCR product (55 μL), restriction enzyme buffer (3 μL), and CfoI (4 U) in a total volume of 30 μL, and incubated this for 1.5 h at 37 °C. Hixson and Vernier (12) used HhaI instead of CfoI. The two enzymes are isochimomeres and therefore recognize the same restriction sites.

To discern the different genotypes, we added loading buffer (3 μL), followed by the samples (30 μL), onto a 5% agarose gel (agarose MP; Boehringer Mannheim, Tutzing, Germany). Electrophoresis was performed at room temperature in TBE buffer (90 mmol/L Tris-borate, 2 mmol/L EDTA), pH 8.0, for 2 h at 150 V. Afterwards, we stained the gel with ethidium bromide (5 g/L) to visualize the different genotypes.

The human apo E gene exhibits three common variants (E2, E3, and E4), which differ from each other at residue positions 112 and 158 in the mature protein. Partial amplification of exon 4 of the apo E gene generates a DNA fragment of 267 bp. This fragment contains four constant and two polymorphic CfoI sites. Restriction enzyme digestion of the fragment and electrophoresis allow the products of the six different apo E genotypes to be visualized (Fig. 1). The apo E2 allele is characterized by the presence of the 104- and 91-bp bands and the absence of the 56- and 48-bp bands. The absence of both the 104- and 72-bp bands is typical for the presence of the apo E3 allele. The apo E4 allele is characterized by the absence of both the 104- and 91-bp bands and the presence of a 72-bp band.

Because the use of the extended primer results in a more specific and easier amplification of exon 4 of the apo E gene than does the method of Hixson and Vernier, the use of dimethyl sulfoxide is no longer necessary. The easier separation of the longer fragments produced in our method allows the use of agarose instead of polyacrylamide electrophoresis. With the PTC-100 DNA thermal cycler the apo E gene in as many as 96 samples can be amplified within 2 h. By loading the samples onto one agarose gel, the genotypes of 50 patients can be visualized within 5.5 h after starting the PCR.

1046 CLINICAL CHEMISTRY, Vol. 41, No. 7, 1995


