METHOD

Apolipoprotein E Polymorphism: Automated Determination of Apolipoprotein E2, E3, and E4 Isoforms

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ABSTRACT: Apolipoprotein E (apo E) plays an essential role in lipoprotein metabolism, where it is involved in the clearance of chylomicrons and very low density lipoproteins. Apart from some rare variants, apo E exists in three common isoforms (E2, E3, and E4). The different isoforms have not only been associated with different plasma lipid levels but have also been correlated with certain pathological conditions, such as lipid disorders (dysbetalipoproteinemia, hypercholesterolemia), cardiovascular diseases, and Alzheimer's disease. Here we describe a rapid, automated test for the determination of the most frequent polymorphisms (E2, E3, and E4). This polymerase chain reaction-based test allows the reliable discrimination of all six genotypes. The assay has been developed especially for the non-specialized routine clinical laboratory by employing an analyzer and chemistry often present in this type of laboratory. Because of its low costs and easy handling, the assay can be performed on a daily basis.


Apolipoprotein E (apo E) plays a central role in the receptor-mediated uptake of chylomicrons and very low density lipoprotein remnants by the liver. Apo E is one of the major proteins of these particles and acts as the ligand to the low density lipoprotein (LDL) receptor and to other receptors of this receptor family (reviewed in 1). Apo E is polymorphic; it exists in three common isoforms (E2, E3, E4) (2,3) and in some rare variants (4,5). The major isoforms differ from each other only by single amino acid substitutions at two sites. The isoforms are associated with certain diseases: apo E2 homozygosity plays an important role in the development of type III hyperlipoproteinemia (6–8) and atherosclerosis (9), because apo E2 is defective in its ability to bind to lipoprotein receptors (10,11); apo E4, apart from being associated with elevated LDL concentrations, increases—in a dose-dependent manner—the likelihood of developing Alzheimer’s disease (12,13).

Several methods have been employed for the determination of apo E isoforms. Basically, these methods can be divided into two groups: methods of the first group focus on the separation of the lipoprotein isoforms by isoelectric focusing with subsequent immunoblotting using an anti-apo E antibody (14,15) or by direct immunofixation (16). A second group of methods is based on the polymerase chain reaction (PCR) technique and therefore determines the patient’s genetic status directly. Most of these methods employ restriction endonucleases followed by the analysis of the restriction fragment length polymorphism, which can be detected by separating the fragments on agarose or polyacrylamide gels (17–19), or even by employing microplate array diagonal gel electrophoresis (20). Others employ single-strand conformation polymorphism (21) or oligonucleotide binding (22).

Although all of these methods are certainly capable of determining a patient’s apo E isoform status, most of them were developed in, and for, research laboratories and are, as such, time-consuming and laborious. It is therefore necessary to establish methods that especially meet the needs of a routine laboratory, i.e., are easy to perform, allow for a high throughput, require less hands-on time, are reliable, fast, and cost-effective.

Here we describe a rapid, automated PCR-based method for the determination of the apo E isoforms that uses electrochemiluminescence (ECL) as detection technology, employs off-the-shelf chemistry, and uses the automated detection process of an immunoassay analyzer that is present in many clinical laboratories, thus avoiding an additional investment for more specialized equipment.

MATERIAL AND METHODS

DNA isolation. Genomic DNA was isolated from 10 µL of anticoagulated whole blood (EDTA, citrate, or Li-heparin), which we obtained for routine analysis from our hospital, using the Dynabeads DNA DIRECT™ kit from DYNAL (Oslo, Norway). DNA was dissolved in 30 µL of Tris-EDTA buffer and stored at −20°C until use.

All primers were designed according to the sequence published by Hixson and Vernier (17). The currently available version of the apo E sequence from GenBank (accession number M10065) shows a few differences that are marked by underlining the respective bases.

PCR. Two fragments, separated only by 22 bp, within exon 3 of the apo E gene were amplified (Fig. 1). Each fragment covers a single, diagnostic restriction site for the enzyme 

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Abbreviations: apo, apolipoprotein; dNTP, deoxynucleotide triphosphates; ECL, electrochemiluminescence; LDL, low density lipoprotein; PCR, polymerase chain reaction.
RESULTS

The basic principle of the assay is outlined in Figures 2 and 3. Briefly, two DNA fragments, spanning either of the diagnostic restriction sites, are amplified independently, using a pair of a biotin-labeled 3′ primer and a ruthenium-labeled 5′ primer for each fragment. After amplification, each of the two samples is split into two aliquots. One aliquot is incubated with the restriction enzyme \textit{Hin} 6 I. If the restriction site is present, the ruthenium-labeled 5′ part of the fragment is removed from the biotinylated 3′ part. The other aliquot remains unaltered. Biotinylated DNA binds \textit{via} streptavidin to paramagnetic beads, which keep the biotinylated DNA in the detection chamber of the analyzer while all other components are washed away. The amount of ruthenium bound to the biotinylated DNA is determined for both samples by measuring the ECL signal generated by the ruthenium complex in the detection chamber. The genotypes are determined by calculating the ratio between the signals from the digested and the undigested aliquot for both fragments.

DNA preparation. We decided to use the Dynabeads DNA DIRECT™ kit, which allows the preparation of 10 samples within 15 min from blood samples anticoagulated with heparin, citrate, or EDTA. In almost all cases, blood samples reach the laboratory without being cooled or frozen during transport; thus DNA may degrade quite rapidly during transportation. Using the Dynabeads DNA DIRECT™ kit, we were able to obtain DNA from samples that had been stored up to 4 d at room temperature.