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Detection of ApoE E2, E3 and E4 Alleles Using Denaturing Gradient Gel Electrophoresis (DGGE).

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ABSTRACT

Background: Genetic polymorphisms can affect drug response. Apolipoprotein E (ApoE) genotype is associated with the effect of statins and is likely the most important polymorphism concerning LDL-cholesterol reduction by statins. The use of statins has showed a reduction in cholesterol levels in individuals with alleles ε2 and ε3 but not to ε4. **Methods:** We This study aims to describe an assay for the simultaneous genotyping of the ApoE variants as support in the treatment of patients with hypercholesterolemia. A total of 30 anonymous genomic DNA samples were tested, and the amplification reaction was performed using the primers designed by Hixon and Vernier. The ApoE alleles were determined by DGGE technique whose principle is the mobility shifts. **Result:** The sequences of each band observed in DGGE technique confirmed the nucleotide differences among the alleles of the ApoE gene. **Conclusion**: we have developed a method easy and effective for the detection of different alleles of the ApoE gene, and with potential clinical application.

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INTRODUCTION

Apoprotein E (ApoE) is a 299 amino acid protein that is synthesized in the liver, its role in the organism is the lipoprotein metabolism specifically the hepatic clearance of triglyceride-rich lipoproteins and its remnants. ApoE is a polymorphic protein with three isoform ε2, ε3 and ε4, coded for three alleles and generating six differences genotype. The differences in these SNPs are resuming in the Scheme 1. Inter-individual differences in blood measures of lipid metabolism are repeatedly associated with the allele 4 (Weisgraber *et al*., 1981; Hanis *et al*., 1991; Siest *et al*., 1995).

ε2 ..... 5’´-CTG$_{(Leu97)}$ ..... TGC$_{(Cys112)}$ GGC .....AAG TGC$_{(Cys158)}$ ..... GCA-3’

ε3 ..... 5’-CTG$_{(Leu97)}$ ..... TGC$_{(Cys112)}$ GGC .....AAG CGC$_{(Arg158)}$ .....GCA-3’

ε4 ..... 5’-CTG$_{(Leu97)}$ ..... CGC$_{(Arg112)}$ GGC .....AAG CGC$_{(Arg158)}$ ..... GCA-3’

Scheme 1: ApoE Polimorphims.

Several approaches were used previously to type ApoE alleles (Dallinga-Thie *et al*., 1995; Ghebranious *et al*., 2005; Hixson *et al*., 1990; Havekes *et al*., 1987). The most commonly used approaches are based on PCR–restriction fragment length polymorphism. These approaches are not suitable for the high-throughput analysis and alleles are sometimes difficult to be discerned (Hixson *et al*., 1990).

And other hands DGGE- finding polymorphisms with PCR amplified DNA should provide an excellent method of finding polymorphisms the principle of this method is the mobility shifts of the amplified selected fragments and can be use for determined the ApoE genotypes. For this reason we development the DGGE technical for determined the ApoE allele we the objective that this technical can be uses such as clinical supported.

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MATERIAL AND METHODS

DNA extraction: The DNA was obtained from the blood of patients treated with pravastatin, extraction was performed with the DNA FlexiGene mark Qiagen kit.

PCR: The PCR reaction was performed using the primers designed for Hixon and Vernier, 1990, by attaching a tail of 40 bp of G and C at the 5' primer. We used 3mM MgCl, 0.2 mM dNTP's, 0.4 μM each of the primers, 80 ng DNA and 1 unit of enzyme brand pfx Invitrogen. The reaction was subjected to the next program in the thermocycler at 94°C for 2.5 minute denaturation, then 30 cycles of amplification, using 94°C for 30 seconds for denaturation, 63.8°C for annealing and 68°C for 1 minute for polymerization. In each PCR reaction were obtained 200 ng of amplified DNA.

DGGE: The PCR products were run in an acrylamide gel denaturing gradient using team D-Code universal mutation detection system (Bio-Rad, USA). 10 μL were placed in each of the PCR products on each gel well. PCR: Products were loaded on a gel containing 10% acrylamide denaturing gradient of 60 to 65% of urea and formamide at 60°C with an initial voltage of 200 volts for 10 minutes and then 85 volts for 7 h using TAE running buffer to 0.5X. After the shift the gel was stained with silver nitrate according to protocol Sanguinetty, *et al*. 1994. Each band was cut from the gel and sent for sequencing.

RESULTS AND DISCUSSION

The PCR products of the fragment amplified were analyzed by DGGE gel and founded three differences displacement this which were amplified again, in the figure 1 can be show this differences displacement.

These bands (Figure 1) was sequences and confirmed the three differences alleles scheme 1, this which the DGGE method can be difference the polymorphism in the position 112 and 158 (Scheme 1).

Is important to mention that the displacement of the fragment in the DGGE has correlation with the G/C container, this correlation is due at the chain conformation.

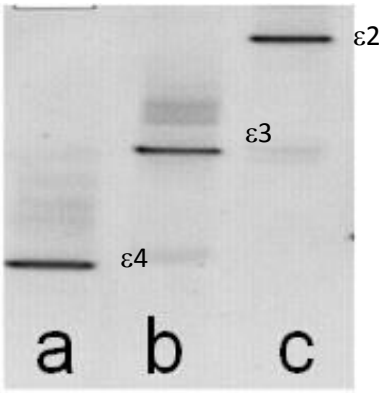

Fig. 1: DGGE gel. Lane A shows the allele ε4, lane B allele ε3 and C allele ε2 of the ApoE gene.

Several approaches were used previously to type ApoE alleles. The most commonly used approaches used for the determination of the alleles of ApoE has three steps: 1 amplified, 2 digestion whit *HhaI*, 3 separation use Meta-Phor agarose instead of polyacrylamide, the pattern of unique DNA fragments obtained unequivocally characterizes the different ApoE alleles, however this method sometimes difficult to be discerned because of the incomplete digestion of the PCR product that is often seen with the use of restriction enzymes (Dallinga-Thie *et al*., 1995; Ghebranious *et al*., 2005; Hixson *et al*., 1990), other method is the MALDI-TOF mass spectrometry, this which is accuracy but expensive (Ghebranious *et al*., 2005). In contras whit the other method describe above the DGGE technical combines accuracy whit cost and time of laboratory.

In summary, we have developed a highly accurate genotyping assay for ApoE 112 and 158 polymorphisms using DGGE method.

Conclusion:

Was possible the detection of the ApoE alleles using denaturing gradient gel electrophoresis (DGGE). This method is a new alternative for the polymorphism determination.

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