Polymorphism detection £2, £3 and £4 of the *Apo*E gene by denaturing gradient electrophoresis (DGGE)

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Introduction. Genetic polymorphisms can affect drug response. There are genetic polymorphisms that have an effect on the response to statin therapy (Guttmacher and Collins, 2002).

A polymorphism associated with the effect of statins is the apolipoprotein E (ApoE). ApoE genotype is likelly the most important polymorphish concerning LDL cholesterol reduction by statins. The use of statins has showed a reduction in cholesterol levels in individuals with alleles $\epsilon 2$ and $\epsilon 3$ but not to $\epsilon 4$ (Pena, *et al.* 2002)

This study aims to describe an assay for the simultaneous genotyping of the ApoE variants as support in the treatment of patients with hypercholesterolemia

Methods. A total of 30 anonymized genomic DNA samples were tested. Most samples came from Mexican mestizos. The amplification reaction was performed using the primers F4 and F6 designer by Hixon and Vernier, 1990. The alleles $\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$ of the ApoE gene were determined by DGGE technique whose principle is the mobility shifts in the agarose gel. Finally, the PCR product was sequenced to verify the allele type and relation with displacement in the gel.

Results. The PCR products were analyzed by DGGE gel, and the nucleotide differences among each allele of *Apo*E gene were clear (Fig. 1).

The sequences of each band confirmed the nucleotide differences among the alleles of *Apo*E gene (Fig. 2).



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

	Cys	Cys		
	112	158		
£2 5'-CTG	TGC GGC	AAG TOC	GCA-3	
£3 5'-CTG	TGC GGC	AAG CGC GCA-3"		
±4 5'-CTG	CGC GGC	AAG CGC	GCA-3	
Leu	Arg	Arg	Ala	
97	112	158	160	

Fig 2. Sequence analysis. ApoE polymorphism alleles differ from each other by two amino acid substitutions at codons 112 and 158.

Conclusions. The DGGE method is effective in the detection of different alleles of *Apo*E gene.

References

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