USING OF MOLECULAR BIOLOGY METHODS FOR DETECTION OF APOLIPOROTEINE E GENE POLYMORPHISM

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Summary. Apolipoprotein E (apo E) is a protein that plays an essential role in lipid metabolism and distribution. The apo E gene is polymorphic, and has three alleles code for isoforms ε2, ε3, and ε4, which differ by single-amino-acid substitutions. In the common apo ε3 polymorphism, TGC encodes for Cys^{112}, and CGC encodes for Arg^{158}. In the apo ε2 another TGC codon results in Cys^{158}, whereas in the apo ε4 a different CGC codon gives rise to Arg^{112}. The three apo E alleles determine six genotypes, i.e., three homozygotes designated ε4/ε4, ε3/ε3, and ε2/ε2 and three heterozygotes designated ε3/ε4, ε2/ε3, and ε2/ε4. Early methods for detection of apo E isoforms were based on protein isoelectrofocusing. After the identification of the apo E gene molecular methods based on PCR amplification and *Hha*I digestion were introduced and later somewhat improved. However, all PCR-based assays are difficult to interpret because the *Hha*I enzyme yields several small fragments, not all of which are specific for the apo E genotypes. In this study we used two restriction enzymes, i.e., *Afl*III and *Hae*II, that recognize the allele-specific nucleotide substitutions at codons 112 and 158, respectively, and do not recognize additional sites.

As expected, simultaneous digestion of the 218-bp amplified product yielded on 3% agarose gel electrophoresis 145-bp, 168-bp, and 195-bp fragments that were specific for apo ε3, ε2, and ε4, respectively. All six possible genotypes for apo E, i.e., ε2/ε4, ε4/ε4, ε3/ε4, ε3/ε3, ε2/ε3, and ε2/ε2, were clearly discernible. In our study of patients with cardiovascular and heart diseases the allele frequencies were 0,096, 0,692 and 0,212 for apoE ε2, ε3 ir ε4, respectively. The gene frequencies were: ε2/ε2 (0,038), ε2/ε3 (0,096), ε2/ε4 (0,019), ε3/ε3 (0,52), ε3/ε4 (0,25), ε4/ε4 (0,077).

Keywords: polymorphism, PCR, apolipoproteinE, restriction.