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Approach to identification of a point mutation in apo B100 gene by means of a PCR-mediated site-directed mutagenesis

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Submitted December 13, 1990

We have devised a simple approach to the identification of an earlier described (1) mutation in codon 3500 of apo B100 gene (G→A transition leading to Arg→Gln substitution), determining predisposition to myocardial infarction in case of familial hypercholesterolemia (for a review see (2)). To that goal we amplified *in vitro* a 245-bp segment of apo B100 gene comprising the mutation. The downstream primer 24-mer dAACAAGAGC-TGACATTTGCCATGG was complementary to codons 3566–3573. As upstream primer we used a 24-mer dCTTA-CTTGAATTCAAAGAGCACCC, which is homologous to codons 3492–3500 (first letter), ends up one nucleotide short of the mutation site, and contains, as the 3′-penultimate nucleotide, a C rather than the native A residue (Figure 1). Thus, elongation of this primer must lead through a site-directed mutagenesis (A→C in the coding strand) to the *Msp*I-site CCGG formation in case of the wild-type DNA (ACGG→CCGG) but not in case of the codon 3500 mutation (ACAG→CCAG) (cf. (3)). *Msp*I-t treatment of the 245- bp amplification product would therefore lead to 222 and 23-bp subfragments (wtDNA) or not affect the 245-bp fragment.

Using this approach, we analysed 120 DNA speciments from myocardial infarction patients and 130 DNA specimens from the control group. None of these analyses revealed the above mutation in any of the alleles studied (Figure 2, lanes 2 and 3). Similar results were obtained on a large group of myocardial infarction patients by means of the allele-specific hybridisation (5).

It thus follows that the transition G→A in codon 3500 of apo B100 gene is very rare, at least in some populations. At the same time, a few cases of the mutation have recently been described (6, 7); the results obtained by us on kindly supplied samples of DNA heterozygous in the codon 3500's second nucleotide are shown in Figure 2, lanes 4 and 5. The method suggested in the present paper might be valuable for the fast screening of larger groups of patients.

ACKNOWLEDGEMENTS

The authors are very grateful to Dr P.Talmud (The Charing Cross Sunley Research Centre, London) and Dr H.Schuster (Medizinische Poliklinik der Universität München) for kind gifts of DNA bearing the codon 3500 mutation.

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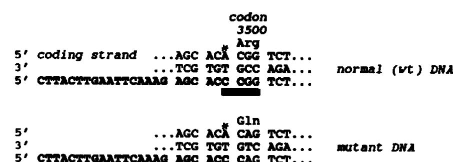

Figure 1. Scheme of the site-directed mutagenesis in apo B100 gene (*A→C) by means of PCR with a modified upstream primer (bold letters), leading to *Msp*I site (closed bar) only in case of normal (CGG) but not mutant (CAG) codon 3500.

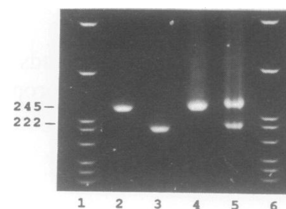

Figure 2. Human genomic DNA from leukocytes was amplified *in vitro* with two primers encompassing codon 3500 of apo B100 gene 1, aliquots were treated with *Msp*I and analysed by 10% PAGE with the ethidium bromide detection. Lanes 1 and 6: pBR322 DNA/*Msp*I size markers; Lanes 2 and 3: the PCR product on a patient's DNA, having the wild-type codon 3500, before and after the *Msp*I – treatment, respectively; Lanes 4 and 5: the same with a patient's DNA bearing the codon 3500 mutation in one allele (a control genomic DNA sample gave a pattern identical to Lanes 2 and 3, not shown). Only 222-bp digestion product is seen, the smaller subfragment being out of the gel.

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