Study of the sequence tagged site (STS) in the beginning of human apo A4 gene region

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The co-translational translocation of most secretory proteins, including the apolipoproteins, across the endoplasmic reticular requires a 15–30 residue NH₂-terminal extension: the signal peptide. Signal peptides containing mutations that decrease the efficiency of export have been characterized for some exported proteins (1). We have recently found that the signal peptide of human apolipoproteins (apo) B is of variable length among individuals (2, 3). To our knowledge this insertion/deletion mutation is the only such mutation described in humans.

In this communication we report amplification conditions for the 5' end of the human apo A4 gene and results of a search for polymorphisms in its signal peptide. Apo A4 is a major component of post-prandial chylomicrons and high density lipoprotein particles. It is known to stimulate LCAT activity as well as to be a ligand for a high-affinity hepatic receptor (4). The gene for human apo A4 has been cloned, sequenced and localized to the region 11q13→qter on the long arm of chromosome 11.

Our analyses were carried out on 59 unrelated caucasian individuals taking part in systematic health examinations at the Center for Preventive Medicine in Nancy, France. Blood was collected into ethylenediamine tetraacetate (EDTA) vacutainers. After spinning the buffy coat was separated and genomic DNA purified by salt extraction (5).

The two oligonucleotide primers for the polymerase chain reaction (PCR) (6) were 20 nucleotides in length and were chosen from the sequence published by Elshourbagy *et al.* (7). The 5' oligonucleotide was 5'-AG CCC AGC AAGC AGCTC AGG-3' and the 3' oligonucleotide was 5'-ACT GAC CTC AGC CCT GG CTC-3'. PCR was performed using a Perkin-Elmer DNA Thermal Cycler. The reaction was carried out in a final volume of 100 μ l containing 0.66 μ g of each oligonucleotide, approximately 0.5 μ g of genomic DNA, 10 μ l of reaction buffer [500 mM KCl/100 mM Tris-HCl, pH 8.3/15 mM MgCl₂/0.01% (w/v) gelatin, (Perkin Elmer Cetus 10 $\times$)], 5 μ l of DMSO. The 4 dNTPs were present in the 100 μ l reaction mixture in a concentration of 150 μ M. Prior to adding 0.5 U of thermostable Taq Polymerase (Perkin Elmer Cetus), the reaction mixture was heated at 99°C for 6 min and then allowed to cool briefly. The denaturation step was carried out at 94°C for 1 min, annealing and extension were carried out simultaneously for 2 min at 66°C, for 30 cycles. The extension time per cycle was 3 sec. Amplified DNA was subjected to non-denaturing electrophoresis in 8% polyacrylamide gels at 90 V for 4 to 5 hours. After electrophoresis, PCR products were directly visualized under UV after ethidium bromide staining of the acrylamide gels (figure 1). A single band of 453 bp is visible corresponding to the expected STS including the 60 bp signal

Figure 1. PCR products electrophoresed on 8% acrylamide gels at 90 V for 5 hours photographed after EtBr staining (0.0002%, ethidium bromide solution). Lanes 1 to 8 contain 10 μ l of amplified products from 8 unrelated individuals; lane 9 contains 400 ng of ϕ X174 DNA/HaeIII digest. A single band of 453 bp is visible in all individuals studied corresponding to the expected STS.

peptide. No length variation was found in the apo A4 signal peptide region.

Restriction site analysis of the amplification product with BglI, PstI and PvuII was studied. The restriction enzyme digestion was carried out at 37°C, overnight, in a final volume of 30 μ l with 3 μ l of the appropriate 10 $\times$ buffer [Tris-HCl 50 mmol/l, NaCl 100 mmol/l, MgCl₂ 10 mmol/l, dithioerythritol 1 mmol/l, pH 7.5 (at 37°C), (Boehringer Mannheim Incubation Buffer H)], 15 μ l of the amplification product, 7.5 μ l of water and 6 U of PvuII or 11 U of PstI, or 10 U of BglI. Restriction enzyme digestions revealed no polymorphism. These restriction digests were also used to confirm that we had amplified the correct product.

Direct sequencing of PCR products is underway in order to detect eventual variations which could not be detected by the methods used here.

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