Detection of Apolipoprotein Gene Variants by Denaturing Gradient Gel Electrophoresis Using the DCode™ System

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Introduction

In a number of fields, techniques are required for searching candidate genes for mutations that underlie hereditary diseases. We have used the denaturing gradient gel electrophoresis method (DGGE) (Myers et al. 1987) using the Bio-Rad DCode universal mutation detection system to identify a number of new, as well as known, mutations and polymorphisms in the genes for apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB).

Methods

Genomic DNA was prepared from patients with dyslipidemia. Segments of the apoA-I and apoB genes were amplified from genomic DNA using a temperature cycler (Ericomp, Inc.). Both oligonucleotide primers had GC clamps (Myers et al. 1985). Following restriction endonuclease digestion, the samples were subjected to DGGE.

The amplification reactions were performed in 50 mM Tris-HCl, pH 9 (at 25°C), 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1 unit of Hot Tub polymerase (Amersham Life Science, Inc.), 15 pmol of each primer, and a minimum of 200 ng of DNA in a total volume of 50 µl. After initial denaturation at 96°C for 1 min, PCR was carried out for a total of 33 cycles at an annealing temperature of 62°C (apoA-I) or 58°C (apoB), in each case for 30 sec. The denaturing and elongation steps were 96°C for 30 sec and 72°C for 60 sec.

A final elongation step of 8 min at 72°C was then carried out. The temperature cycler was programmed to denature the amplification products at 96°C for 8 min and to then perform slow cooling to 40°C. Without this last step, heteroduplex DNA may not be visible on the DGGE gels.

In the case of apoA-I, a 420 bp region at the 5' end of the gene, containing the core promoter and the first exon, was amplified using two GC-clamped primers, such that the final PCR product was 532 bp in length. Similarly, 465 bp of the low-density lipoprotein (LDL) receptor binding domain of apoB was amplified with a final size of 591 bp. In each case, 10 µl of the PCR product was digested in a volume of 30 µl with either AvaII (apoA-I) or EcoRI (apoB). Figure 1 shows the melting profile graphs for the 5' and 3' fragments generated after digestion of the apoB PCR product (Lerman and Silverstein 1987). These graphs were produced using Bio-Rad MacMelt™ software and were used to predict the percentage range of the denaturing gradient to be used in the gels. Multiple aliquots of the digests were loaded onto 7.5% acrylamide (38.5:1) DGGE gels at hourly intervals. These travel gels were run in the DCode system apparatus at a constant temperature of 56°C and 200 V for a total of 5 hr to determine the optimal running time. This was found to be 3 hr for the apoA-I fragments and 4 hr for apoB. The gels were stained for 30 min in SYBR Green and photographed under epi-illumination at 254 nm (Karlsen et al. 1995). A 35–65% denaturing gradient was used for apoA-I and a 30–50% gradient for apoB.

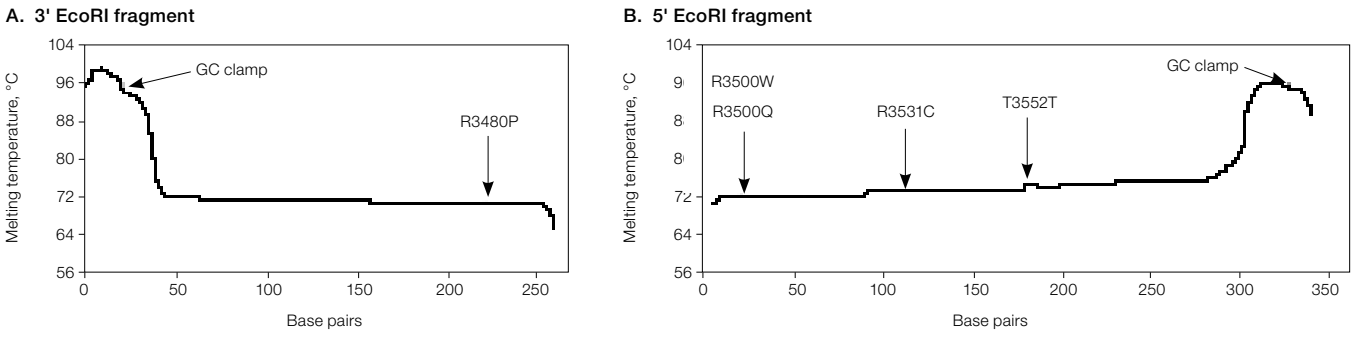

Fig. 1. Melting profiles of a portion of the receptor binding domain of the apoB gene amplified using GC-clamped primers. The graphs were generated using MacMelt software. A total of five single-base substitution mutations are shown in A and B.

Results

While no new mutations were found in the apoA-I promoter region, we were able to detect, using DGGE, a common polymorphism at nucleotide –75 (Figure 2), which had been previously shown to be associated with differences in plasma high-density lipoprotein (HDL) levels.

Three mutations in the LDL receptor binding domain of the apoB gene that cause the disorder familial ligand-defective apoB (FDB) (Soria et al. 1989, Pullinger et al. 1995, Gaffney et al. 1995) were detected by DGGE (Figure 3, lanes 3, 4, and 5). In addition, a rare missense mutation at codon 3480 (Figure 3, lane 1) and a new silent mutation (Figure 3, lane 2) were detected.

Discussion

Detection of naturally mutant alleles of candidate genes and a study of their consequent effects can be expected to provide important clues to the precise biochemical roles of these gene products. For example, new causes of atherogenic dyslipidemia will be revealed in the search for mutations in proteins involved in lipid metabolism. We have shown that the DGGE approach is an excellent method to detect these mutations.

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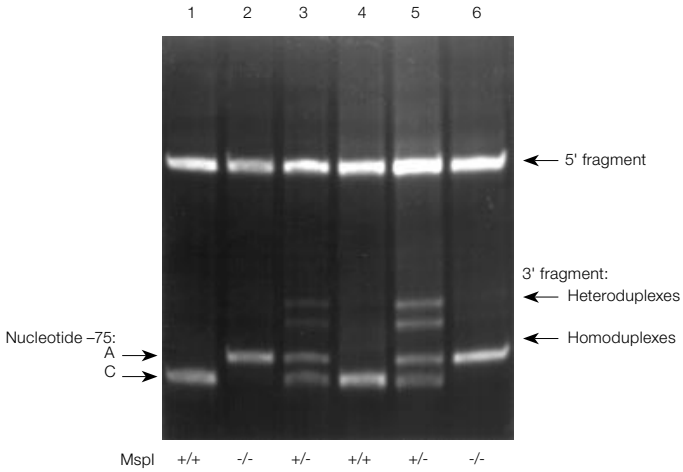

Fig. 2. DGGE gel of the apoA-I gene promoter showing an MspI polymorphism. Lanes 1 and 4 are homozygous for a C residue, and lanes 2 and 6 are homozygous for an A residue, at position –75. Lanes 3 and 5 are heterozygous subjects with the low-melting heteroduplexes clearly visible.

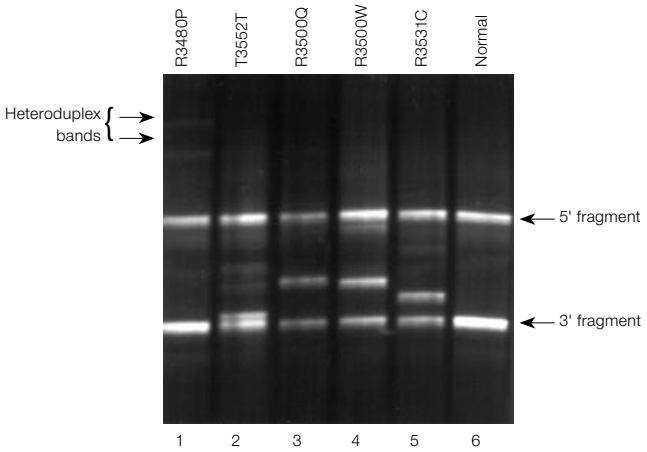

Fig. 3. DGGE gel of part of the receptor binding domain of the apoB gene showing five separate single-base substitutions. Four of the mutations, lanes 2 through 5, are on the 5' fragment (following EcoRI digestion) and, in each case, this band shows splitting. The fifth mutation (R3480P, lane 1) is on the 3' fragment and is only detectable because of the presence of heteroduplex bands.

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