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

Clive R. Pullinger, Arghavan K. Shahidi, Andrea L. Verhagen and John P. Kane*‡
Cardiovascular Research Institute, *Department of Medicine, ‡Department of Biochemistry and Biophysics, University of California, San Francisco, California

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)1 using the Bio-Rad DCode 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).

Materials And 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., San Diego, CA). Both oligonucleotide primers had GC clamps.2 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 (NH4)2SO4, 1.5 mM MgCl2, 200 µM of each dNTP, 1 unit of Hot Tub™ polymerase (Amersham Life Science Inc., Cleveland, OH), 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 minute, 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 s. The denaturing and elongation steps were 96 °C for 30 s and 72 °C for 60 s. A final elongation step of 8 minutes at 72 °C was then carried out. The temperature cycler was programmed to denature the amplification products at 96 °C for 8 minutes 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 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.3

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 hours to determine the optimal running time. This was found to be 3 h for the apoA-I fragments and 4h for apoB. The gels were stained for 30 minutes in SYBR® Green and photographed under epi-illumination at 254 nm.4 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 apolipoprotein B gene amplified using GC clamped primers. The graphs were generated using MacMelt software. The positions of five single-base substitution mutations are shown.](image-url)
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) 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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References


* The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffmann-LaRoche. Use of the PCR process requires a license.