Human LDL receptor gene: HincII polymorphism detected by gene amplification

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Source/Description: Synthetic Oligonucleotides SP76 ('5-TCTCCTTATCCACTTGTGT GTCTAG-3') and SP77 ('5-CTTCGATCTCGTACGTAAGCCACAC-3') were prepared on a DNA synthesizer (Applied Biosystems). These oligonucleotides flank exon 12 of the LDL receptor gene (see figure).

Polymorphism: There is a HincII polymorphic site located within exon 12 of the human LDL receptor gene. DNA sequence analysis revealed that the polymorphism is caused by a single base substitution (T to C). The polymorphism is detected by digesting amplified genomic DNA with HincII (New England Biolabs).

Protocol: Human genomic DNA is subjected to gene amplification (1) using SP76 and ^{32}P end-labeled SP77 (2). The amplified DNA is then size fractionated on a 6% polyacrylamide nondenaturing gel and subjected to autoradiography. The 190 bp DNA fragment encompassing EXON 12 and the sequence corresponding to the two flanking oligonucleotides is excised from the gel and purified (see right lane of figure). The purified DNA is then digested with HincII and fractionated by gel electrophoresis on a 6% acrylamide gel. Autoradiography reveals 98 bp or 133 bp bands corresponding to the presence or absence of the polymorphic site (see figure). The difference between the sizes of these two fragments (35bp) requires the use of gene amplification.

Frequency: In 10 unrelated American caucasian individuals: 98 bp - 0.45, 133 bp - 0.55.

Chromosomal localisation: 19p13.1-13.3

Mendelian inheritance: Co-dominant segregation is shown in one family.

References:

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