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DNA VARIANTS

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A new restriction-site polymorphism in exon 18 of the low density lipoprotein receptor (LDLR) gene

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Abstract A new restriction fragment length polymorphism (RFLP) in exon 18 of the low density lipoprotein receptor (LDLR) gene is described. It should be a useful marker in linkage to familial hypercholesterolemia.

Source/description. Primers, based on the nucleotide sequence of the low density lipoprotein receptor (LDLR) gene exon 18 (Yamamoto et al. 1984) 3′ untranslated region, that flank three *Alu* (one truncated) sequences were used to amplify a 1011-bp fragment by polymerase chain reaction (PCR).

PCR conditions. Genomic DNA (500 ng) was added to a 50-μl volume reaction mix containing 1 × *Taq* buffer (Promega), 3.0 m*M* $MgCl_2$, 200 μ*M* dNTPs, 250 n*M* each primer [1019 (5′-ACTTCAAAGCCGTGATCGTGA-3′) and 2029 (5′-TGCAACAGTAACACGGCGATT-3′)] and 1 unit *Taq* polymerase (Promega) and amplified using Hybaid (Omnigene) thermal cycler (block control) under the following conditions: 94°C 3 min 1 ×; 94°C 1:30 min., 52°C 1:30 min, 72°C 3 min, 30 ×; 72°C 3 min. An 8-μl sample of the PCR reaction mix was digested in a 20-μl reaction using excess enzyme and 1 × buffer (NEB) and examined following electrophoresis on ethidium bromide stained 1.5% agarose gels.

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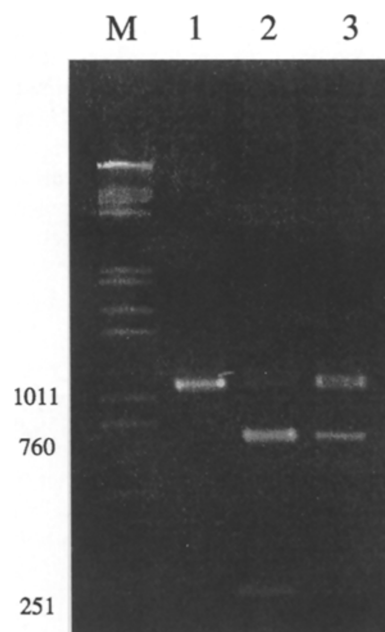

Fig. 1 Identification of low density lipoprotein receptor (LDLR) polymorphisms. Individuals are either homozygous for the absence (*lane 1*), or presence (*lane 2*) or heterozygous (*lane 3*) for the *Afl*III restriction site. Molecular weight marker (*lane M*) is λ DNA cut with *Hind*III and *Eco*RI restriction enzymes

Polymorphism. DNA sequence analysis of amplified human DNA revealed a variant that involves a transversion and a single nucleotide deletion that is distinguished by *Afl*III restriction enzyme. The alleles are represented by an uncut (1011 bp) fragment (A1) or two (760 bp, 251 bp) fragments (A2) obtained by restriction digestion (Fig. 1).

Frequency. In 20 unrelated caucasians A1 = 0.70, A2 = 0.30, observed heterozygosity = 0.50; in 22 unrelated African Americans A1 = 0.30, A2 = 0.70, observed heterozygosity = 0.41.

Chromosomal location. LDLR maps to chromosome 19p13.2 by physical and genetic mapping (Sistonen et al. 1993; Ropers et al. 1992).

Mendelian inheritance. Codominant segregation was observed in two three-generation Utah families.

Other comments. The polymorphism was easily analyzed using a small amount of DNA and no radioactivity. Additionally, we can analyze the previously described *Nco*I polymorphism (Miserez et al. 1993) with the same PCR products, by obtaining RFLPs of either the uncut 1011-bp fragment or fragments of 486 bp and 525 bp following restriction digestion. Upon examination of the same unrelated individuals, and the two Utah families, we could not confirm the existence of one of the four possible haplotypes, suggesting that the two polymorphisms form a triallelic system with the *Nco*I variant historically preceding the *Afl*III variant. We found that although the faint nonspecific PCR products do not interfere with the analysis, Perfect Match Enhancer (Strategene) helped reduce this effect. The LDLR locus is associated with familial hypercholesterolemia (FH), a common inborn error of metabolism inherited by autosomal dominant transmission. The haplotypes attainable from single PCR products of various individuals may prove useful as genetic markers for linkage analysis of LDLR with FH.

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References

Miserez AR, Schuster H, Chiodetti N, Keller U (1993) Polymorphic haplotypes and recombination rates at the LDL receptor locus in subjects with and without familial hypercholesterolemia who are from different populations. Am J Hum Genet 52: 808–826

Ropers HH, Pericak-Vance MA, Siciliano MJ, Mohrenweiser HW (1992) Report of the second international workshop on human chromosome 19 mapping. Cytogenet Cell Genet 60:88–95

Sistonen P, Traskelin A-L, Lehvaslaiho H, Chapelle A de la (1993) Genetic mapping of the erythropoietin receptor gene. Hum Genet 92:299–301

Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW (1984) The human LDL receptor: a cysteine-rich protein with multiple *Alu* sequences in its mRNA. Cell 39:27–38