A DNA polymorphism in the human low-density lipoprotein receptor gene

M. J. KOTZE, A. E. RETIEF, P. A. BRINK, H. F. H. WEICH

Summary

A new restriction fragment length polymorphism (RFLP) in the low-density lipoprotein receptor gene is described using the Stu I restriction endonuclease and a cDNA probe. The frequency of the two RFLP alleles was determined in 60 unrelated white subjects and 11 children, and was found to be heterozygous for the polymorphism. Mendelian segregation of the RFLP was found in three informative families. The possible use of the RFLP in the diagnosis of familial hypercholesterolaemia in South Africa is discussed.

Familial hypercholesterolemia (FH) is a relatively common dominant genetic disease with a heterozygote frequency of about 1 in 500. Mutations in the gene for the low-density lipoprotein (LDL) receptor lead to high levels of LDL in the plasma. This receptor defect is associated with premature atherosclerosis and ischemic heart disease in heterozygous middle-aged subjects, while in homozygotes myocardial infarction may occur before the age of 20 years.

Goldstein and Brown proposed a broad classification of three LDL-receptor mutations based on measurements of LDL-receptor activity found on the surface of cultured fibroblasts. Homozygotes are classified as: (i) receptor-negative, when cells fail to express any functional LDL receptors (<2% of the normal number); (ii) receptor-defective, when cells contain 2-30% of the normal number of active receptors; or (iii) internalization-defective, when cells produce receptors that bind LDL but do not mediate LDL internalization. Tolleshaug et al. presented a more detailed classification of LDL-receptor defects and described seven mutations that disrupt synthesis, processing and transport of the receptor in fibroblasts.

The prevalence of FH in South Africa is unusually high in the Afrikaner population, with a heterozygote frequency of 1 in 100. A predominance of the receptor-defective type has been found in Afrikaner families with FH, suggesting a founder gene effect in this population.

FH can be diagnosed in early childhood on the basis of family history and raised serum cholesterol concentrations.

However, some children who carry the FH gene may not express the lipoprotein abnormality until after puberty. Determination of the LDL-receptor molecules on cultured fibroblasts is not always sufficiently accurate for unambiguous identification of heterozygotes. With the advent of DNA recombinant technology it is now possible to study genetic defects at the DNA level. A cDNA clone for the human LDL receptor has recently been isolated and made available to investigators for the polymorphism (RFLP).

This polymorphism can be used to follow the inheritance of the LDL-receptor defect in informative families and permits unequivocal, early diagnosis of FH.

Material and methods

Blood samples were obtained mainly from laboratory staff who are of European descent and Afrikaners; the DNA was prepared by a Triton X100 lysis method. Aliquots of DNA (10 μg) from 9 healthy individuals were each digested with the following restriction endonucleases: EcoRI, BamHI, HindIII, PstI, TagI, KsaI, SstI, SstII, SphI, MspI and StuI (Boehringer Mannheim). DNA fragments were separated on a 0.6% agarose gel and transferred to nitrocellulose filters (Schleicher & Schuell, BA85, 0.45 μm) by the Southern blotting technique.

The human DNA probe for the LDL-receptor gene, pLDLR-2HH1, was a kind gift from Dr. W. Russell of Dallas. It consists of a 1.9 kilobase (kb) fragment of the 3' end of the LDL-receptor cDNA clone and subcloned into the BamHI site of the vector pSP64. The insert was excised with BamHI, separated from the vector on a 1% agarose gel and recovered. The probe DNA was labelled in vitro by nick translation to a specific activity of 10^6 cpm/μg (BRL Kit, Amersham International).

Filters were prehybridized for 3 hours at 65°C in 3 x SSC (1 x SSC: 0.15M NaCl, 0.015M sodium citrate), 0.1% sodium dodecyl sulphate (SDS), 10 x Denhardt’s solution, 50 μg/ml denatured salmon sperm DNA (Sigma) and 10 μg/ml poly A (Sigma). The probe was then added at a concentration of 50 ng/ml and hybridizations were carried out for 24 hours at 65°C. Filters were finally washed in 1 x SSC, 0.1% SDS and exposed to Kodak XAR film for 1-3 days.

Results

To find a common DNA polymorphism of the LDL-receptor gene, DNA samples from 9 unrelated white subjects were digested with eleven different restriction endonucleases. Southern blot filters of these samples were incubated with the LDL-receptor probe (pLDLR-2HH1), washed, and exposed to X-ray film. Autoradiographs showed the same fragment pattern generated by each enzyme, except for StuI, which generated an extra fragment in one of the subjects. In 4 of 5 samples shown in Fig. 1 two fragments were seen after hybridization: one of 15 kb and one of 7.2 kb (Fig. 1(a)). In the fifth, a normal control subject, an extra 17 kb fragment was detected on the autoradiograph. These patterns were interpreted as being the product of two alleles, which we designate A1 (15 kb + 7.2 kb) and A2 (17 kb + 7.2 kb). The individual (lane 1) showing all three fragments has both alleles and is heterozygous for the polymorphism (A1A2). The genomic DNA of a heterozygote showing the recognition sites of Stu I enzyme is illustrated diagrammatically in Fig. 1(b).

Reprints requests to: Prof. A. E. Retief, Dept of Human Genetics, University of Stellenbosch Medical School, PO Box 63, Tygerberg, 7505 RSA.
The frequency of the RFLP alleles was determined in 60 normal, unrelated Afrikaner individuals. The frequency of the rare allele A2 in this population was 5.8% and 11.7% (7/60) of the individuals were found to be heterozygous for the polymorphism. The distribution of genotypes is close to the expected value if the population is in Hardy-Weinberg equilibrium.

**Discussion**

Hypercholesterolaemia is a major risk factor in the development of atherosclerosis and coronary heart disease. Defects in the LDL-receptor gene have been shown to be of considerable importance in the development of hypercholesterolaemia. One defect described in a FH homozygote is a deletion of 5 kb DNA coding for the membrane-spanning and cytoplasmic domains of the receptor. Horsthemke et al. recently described a 2 kb deletion in the 3' part of the LDL gene. Although there are probably several genes involved in the development of hypercholesterolaemia, these are the only LDL-receptor gene deletions that have as yet been elucidated at the DNA level. Indications are that most defects are likely to be point mutations, which will be more difficult to identify with present DNA techniques.

The need for early diagnosis of FH has recently been stressed and linkage studies of FH and RFLPs provide a means of diagnosing FH with complete accuracy. We have used a cDNA clone for the LDL-receptor gene, developed by Russell et al., to identify a RFLP of the gene, detected with the enzyme Stu I. This polymorphism was shown to be present in the normal Afrikaner population, with a heterozygote frequency of 12%. Recently Humphries et al. described the first RFLP with the enzyme Pvu II using the same probe. Berg et al. firmly established a linkage between this polymorphism and FH, with no evidence of recombination. The Pvu II polymorphism detected with the probe for the LDL-
receptor gene can therefore be used as a marker for the FH locus. As we have used the same probe but a different enzyme, the same conclusion is valid for the Stu I polymorphism described in this study.

Brink et al.\textsuperscript{19} found the heterozygote frequency of Peu II polymorphism in the normal Afrikaner population to be 41\% compared with the 30\% found by Humphries et al.\textsuperscript{17}. Combining the use of both polymorphisms as markers for the LDL-receptor gene, 53\% (12\% + 41\%) of the Afrikaner population will theoretically be informative for the use of these markers in the diagnosis of FH. This naturally excludes the possibility of a founder origin for FH among the Afrikaners. There is, however, evidence for a founder origin in South Africa. Brink et al.\textsuperscript{19} described a significant linkage disequilibrium of the common Peu II polymorphism and high cholesterol levels in Afrikaner individuals. This finding supports other evidence for a founder origin of FH in South Africa.\textsuperscript{4,5} Studies are currently in progress to determine the linkage status in Afrikaners with FH and the Stu I polymorphism.

The discovery of a new RFLP associated with the LDL-receptor gene permits screening of family members, and accurate diagnosis and effective counselling. The information obtained might answer the question whether one or more defective LDL-receptor genes are present in the Afrikaner population. Prenatal studies to identify homozygotes are now a possibility in informative families.

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REFERENCES