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

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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 11,7% of them were found to be heterozygous for the polymorphism. Mendelian segregation of the RFLP was found in 3 informative families. The possible use of the RFLP in the diagnosis of familial hypercholesterolaemia in South Africa is discussed.

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Familial hypercholesterolaemia (FH) is a relatively common dominant genetic disease with a heterozygote frequency of about 1 in 500.[1] 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 ischaemic heart disease in heterozygous middle-aged subjects, while in homozygotes myocardial infarction may occur before the age of 20 years.

Goldstein and Brown[2] 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.*[3] 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 at least 1 in 100.[4,5] A predominance of the receptor-defective type has been found in Afrikaner families with FH, suggesting a founder gene effect in this population.[6]

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.[7-9] 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[10] and made available to study defects of this gene. We have used this probe to identify a new restriction fragment length 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.

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Material and methods

Blood samples were obtained mainly from laboratory staff who are of European descent and Afrikaans-speaking and the DNA was prepared by a Triton X100 lysis method.[11] Aliquots of DNA (10 μg) from 9 healthy individuals were each digested with the following restriction endonucleases: *Eco* RI, *Bam* HI, *Hind* III, *Pst* I, *Taq* I, *Rsa* I, *Sst* I, *Sst* II, *Sph* I, *Msp* I and *Stu* I (Boehringer Mannheim). DNA fragments were separated by size on a 0,6% agarose gel and transferred to nitrocellulose filters (Schleicher & Schnell, BA85, 0,45 μm) by the Southern blotting technique.[12]

The human DNA probe for the LDL-receptor gene, pLDLR-2HH1, was a kind gift from Dr D. 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 *Bam* HI site of the vector pSP64.[10] The insert was excised with *Bam* HI, 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 10^8 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 *Stu* I, 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).

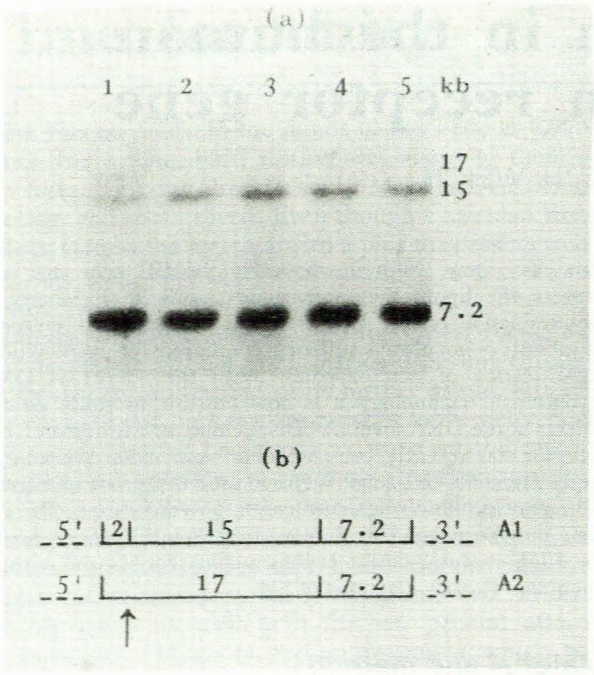

Fig. 1. (a) Southern blot analysis of hybridization patterns obtained from a *Stu* I digest and the LDL-receptor probe pLDLR-2HH1. Lanes 1 - 5 contain DNA from 5 normal individuals. Fragment sizes are indicated in kb. (b) Diagram showing how genomic DNA fragments detected with the probe can be produced in an *A1A2* heterozygote. Vertical lines indicate binding sites for *Stu* I. The absence of a *Stu* I site (arrow) within the DNA sequence of the gene produces the 17 kb fragment of the *A2* allele.

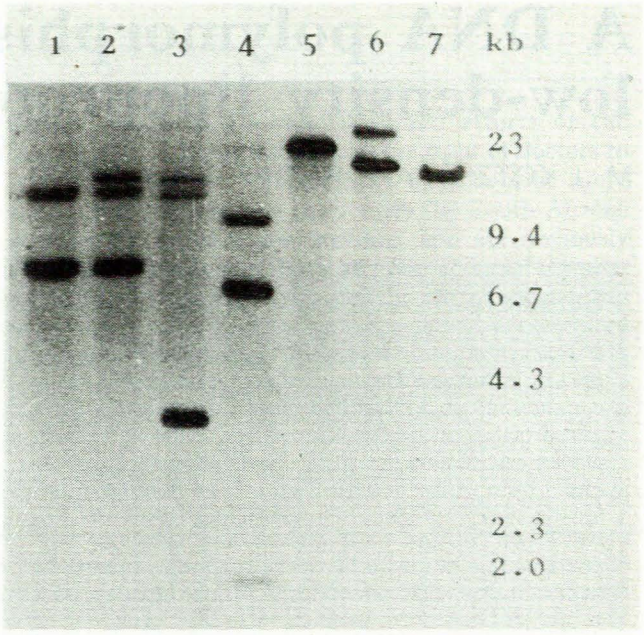

Fig. 2. Southern blot analysis of hybridization patterns obtained from six restriction endonuclease digests using DNA probe pLDLR-2HH1. The enzymes used are: lane 1 — *Stu* I (DNA from a *A1A1* homozygote); lane 2 — *Stu* I (DNA from a *A1A2* heterozygote); lane 3 — *Pvu* II; lane 4 — *Xba* I; lane 5 — *Eco* RI; lane 6 —*Hind* III and lane 7 — *Bam* HI. Fragment sizes from marker DNA are indicated in kb.

Südhof *et al.*[13] published data on the genomic map of the human LDL-receptor gene indicating the cleavage sites for selected restriction endonucleases. Samples of DNA from normal individuals were digested with four of the enzymes used by Südhof *et al.*[13] to compare and determine the exact fragment sizes after hybridization with probe pLDLR-2HH1. Fig. 2 shows the autoradiographs of fragments that correlate with those published by Südhof *et al.*,[13] as well as the fragments produced with *Stu* I and *Hind* III.

Lanes 1 and 2 show fragment sizes with enzyme *Stu* I: 15 kb + 7,2 kb (*A1A1* homozygote) and 17 kb + 15 kb + 7,2 kb (*A1A2* heterozygote), respectively. Lanes 3 - 7 show the fragment sizes generated by each enzyme; *Pvu* II: 16,5 kb + 14 kb + 3,6 kb + 2,6 kb; *Xba* I: 23 kb + 10 kb + 6,6 kb + 1,7 kb; *Eco* RI: 23 kb; *Hind* III: 30 kb + 19 kb; *Bam* HI: 17 kb.

These results indicate that the probe received from Dr Russell hybridizes to the same fragments of the LDL-receptor gene as indicated by Südhof *et al.*[13] after digestion with enzymes *Pvu* II, *Xba* I and *Eco* RI. These known fragment sizes were used to determine the exact sizes generated by enzyme *Stu* I. Comparing the fragment sizes found with *Stu* I in this study with those described in a table by Südhof *et al.*,[13] an extra binding site was found between exons 15 and 16 to produce the common 15 kb and 7,2 kb fragments. The *Stu* I site which produces the polymorphism when absent (Fig. 1(b)) is situated in exon 8, according to the genomic map of Südhof *et al.*[13]

Frequency of *Stu* I polymorphism in the Afrikaner population

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.

Segregation of the LDL-receptor gene *Stu* I polymorphism in 3 informative families showing the inheritance of all three genotypes, *A1A1*, *A1A2* and *A2A2*, is illustrated in Fig. 3. Studies are currently underway to follow the inheritance of the LDL-receptor gene in families with FH to determine possible linkage with the *Stu* I polymorphism.

Discussion

Hypercholesterolaemia is a major risk factor in the development of atherosclerosis and coronary heart disease.[1] 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.[14] Horsthemke *et al.*[15] 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[16] 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.*[17] described the first RFLP with the enzyme *Pvu* II using the same probe. Berg *et al.*[18] 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-

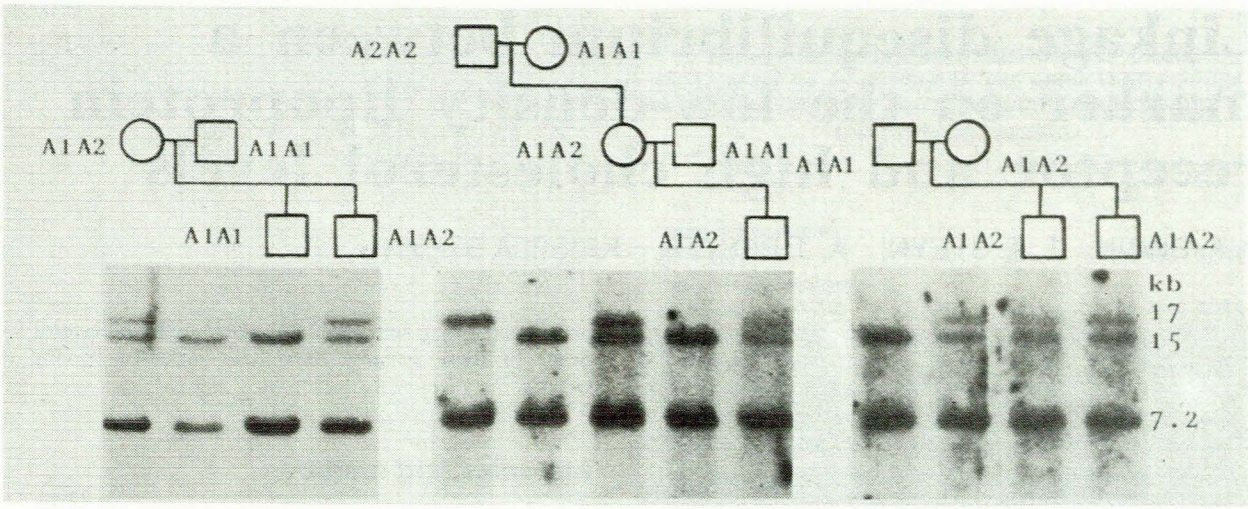

Fig. 3. Segregation of the LDL-receptor gene *Stu* I polymorphism in 3 informative families, showing inheritance of all three genotypes, *A1A1, A1A2, A2A2.*

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.*[19] found the heterozygote frequency of *Pvu* II polymorphism in the normal Afrikaner population to be 41% compared with the 30% found by Humphries *et al.*[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.*[19] described a significant linkage disequilibrium of the common *Pvu* II polymorphism and high cholesterol levels in Afrikaner individuals. This finding supports other evidence for a founder origin of FH in South Africa.[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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