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Identification of a deletion in the LDL receptor gene

A Finnish type of mutation

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A cDNA probe for the low density lipoprotein (LDL) receptor gene was used to screen DNA samples from 52 unrelated Finnish patients with the heterozygous form of familial hypercholesterolemia (FH) and 51 healthy controls. Southern blot analysis using the restriction enzyme *Pvu*II revealed an abnormal 11 kb (kilo base-pair) restriction fragment in 16 (31%) of the patients but none of the controls. A more detailed restriction enzyme analysis of the DNA from patients revealed a mutation which apparently is due to an 8 kb deletion extending from intron 15 to exon 18 of the LDL receptor gene. Co-segregation of FH with the mutated gene was demonstrated in three families. These data are consistent with a 'founder gene effect' and support the assumption that recombinant DNA methods may have great impact on the diagnostics of FH in genetically homogeneous populations.

Familial hypercholesterolemia; LDL receptor gene; Southern blotting; DNA deletion

1. INTRODUCTION

Mutations of the LDL receptor gene give rise to familial hypercholesterolemia (FH), a disease characterized by elevated levels of serum LDL, tendon xanthomata and premature atherosclerosis [1,2]. The disease is inherited in an autosomal dominant fashion and is relatively common: about one in every 500 persons is heterozygous for the mutant allele of the LDL receptor gene [1]. These patients, untreated males especially, frequently undergo myocardial infarction below 50 years of age [3], while the rare FH patients homozygous for a defective allele suffer from symptoms of coronary heart disease from childhood on.

Recombinant DNA techniques have greatly aided in the understanding of the molecular genetics of FH. Isolation of cloned DNA sequences corresponding to the LDL receptor mRNA [4] and the structural gene [5] has demonstrated that the LDL receptor gene resides in chromosome 19 [6], spanning approx. 45 kb (kilo base-pairs) with 18 exons and 17 introns. The availability of cloned gene probes has enabled the analysis of LDL receptor gene mutations at the DNA level. Russell et al. [2,6–15] have characterized some 15 mutations which include single-base substitutions, small and large insertions, exon duplications and large deletions. Heterogeneity at the DNA level undoubtedly hampers approaches for molecular genetic diagnostics of FH. The situation may be different in populations with a relatively homogenous genetic background [12,15,16]. We examined possible gross mutations of the LDL receptor gene among Finns who form a genetic isolate and have a coronary heart disease mortality rate which is among the highest in the world.

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2. MATERIALS AND METHODS

2.1. *Subjects*

A cohort of 52 patients with the heterozygous form of FH was studied. The diagnostic criteria included (i) the presence of hypercholesterolemia in at least one of the first-degree relatives, (ii) serum total cholesterol level > 8 mmol/l and/or serum LDL

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cholesterol level > 6 mmol/l and (iii) tendon xanthomas. There were 16 men (aged 32–63 years) and 36 women (aged 15–69 years). 51 normolipemic, apparently healthy subjects (25 males, 26 females) served as controls. All patients and controls studied were unrelated and of Finnish origin.

2.2. *DNA analysis*

DNA was prepared from leukocytes obtained from 20 ml of venous blood essentially as described [17]. DNA (5–10 μg) was digested with the appropriate restriction enzymes (*Bam*HI, *Eco*RV, *Nco*I, *Pvu*II, *Xba*I) using conditions recommended by the manufacturers, fractionated by gel electrophoresis on 0.6% agarose and transferred to nitrocellulose filters. Hybridization of the filter-bound DNA was carried out in a medium containing 6 × SSC (1 × SSC: 0.15 M NaCl/0.015 M trisodium citrate), 5 × Denhardt's solution, 50% deionized formamide, 0.25% SDS, 100 μg/ml salmon sperm DNA and 1–2 × 10^6 cpm/ml of ^{32}P-labelled LDL receptor gene probe. The latter, consisting of a 972 bp fragment (base pairs 1573–2544, see [4]), was prepared by double digestion of the LDL receptor cDNA clone pLDLR3 (kindly provided by Dr D.W. Russell) with the enzymes *Xho*I and *Bam*HI and subsequent recovery of the fragment from the 0.6% agarose gel. Labelling of the probe was accomplished by a random oligonucleotide priming technique using a commercial kit (Amersham). After hybridization for 20 h at 42°C, the filters were washed with 2 × SSC/0.1% SDS at room temperature and 0.2 × SSC/0.1% SDS at 50°C. Autoradiography of the dried filters was conducted by exposing them to Kodak XAR films for 2–5 days at −70°C.

3. RESULTS

When DNA from healthy controls was digested with *Pvu*II, fractionated on 0.6% agarose, transferred to nitrocellulose and subsequently hybridized with the LDL receptor probe, a restriction fragment length polymorphism (RFLP) became evident (fig.1). This RFLP is due to a variable *Pvu*II site within intron 15 of the receptor gene [18,19]. The absence of the *Pvu*II site resulted in the appearance of a 16 kb restriction fragment whereas its presence produced a 14 kb fragment (fig.1). When DNA from FH patients was analyzed in the same way, the same polymorphism was

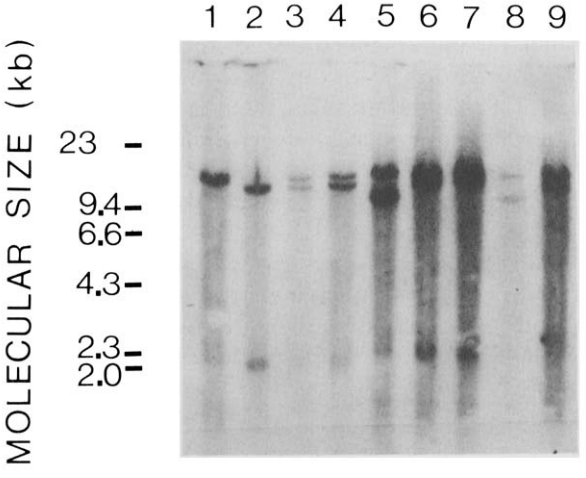

Fig.1. Autoradiogram of *Pvu*II-digested DNA samples, hybridized with the ^{32}P-labelled LDL receptor gene probe. Lanes: 1–4, samples from healthy controls; 5–9, samples from FH patients. An abnormal 11 kb *Pvu*II fragment is present in samples 5,8.

Table 1

*Pvu*II genotypes of control subjects and FH patients

	P−/P−	P−/P+	P+/P+	P−/11 kb	P+/11 kb
Controls (n = 51)	28 (55%)	18 (35%)	5 (10%)	–	–
FH (n = 52)	20 (38%)	15 (29%)	1 (2%)	13 (25%)	3 (6%)

P− indicates the absence of the *Pvu*II restriction site (16 kb fragment) and P+ its presence (14 kb fragment)

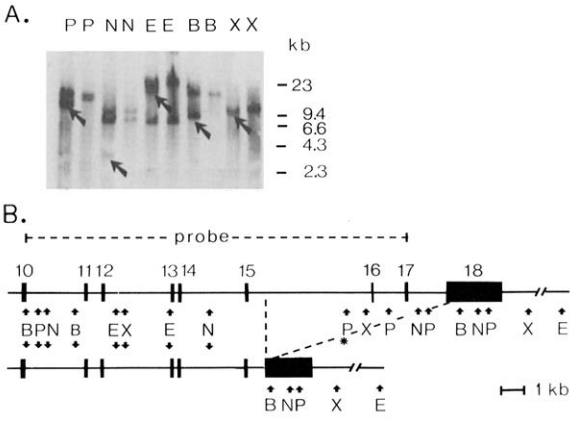

Fig.2. Restriction enzyme mapping of the LDL receptor gene mutation. (A) Autoradiogram of DNA samples from a patient with FH (left of each sample pair) and a control subject (right of each sample pair), digested with *Pvu*II (P), *Nco*I (N), *Eco*RV (E), *Bam*HI (B) and *Xba*I (X), and hybridized with the ^{32}P-labelled LDL receptor gene probe. In each case, the abnormal restriction fragment revealed in the patient's DNA is designated by an arrow. (B) Restriction map of the 3′-end of the LDL receptor gene ([15,20] and data provided by Dr D.W. Russell) from a healthy subject (above) and a Finnish patient with FH (below). Exons are denoted by numbering. The 5′- and 3′-boundaries of the cDNA probe employed are indicated uppermost. The polymorphic *Pvu*II restriction site is indicated by an asterisk. Suggested location of the deletion is indicated by broken lines.

detected, but a unique 11 kb fragment was found in DNA samples from 16 (31%) out of the 52 FH cases examined (fig.1).

The *Pvu*II genotypes of the controls and FH patients are summarized in table 1 where P+ denotes the presence of the *Pvu*II site and P− its absence. No FH patient homozygous for the 11 kb fragment was found.

To characterize further the mutation that gives rise to the abnormal 11 kb *Pvu*II fragment, DNAs from FH patients carrying the unique allele and controls were analyzed by Southern blotting using the enzymes *Bam*HI, *Eco*RV, *Nco*I and *Xba*I. Representative examples of the results are shown in fig.2. Collectively, restriction mapping data suggest that the mutation arose from a deletion of about 8 kb at the 3′-end of the LDL receptor gene. Southern blotting of genomic DNA does not permit a precise determination of the 5′- and 3′-boundaries of this deletion. The most plausible interpretation of the data is that the deletion eliminates exons 16 and 17 and probably also a portion of the 5′-end of exon 18 (fig.2).

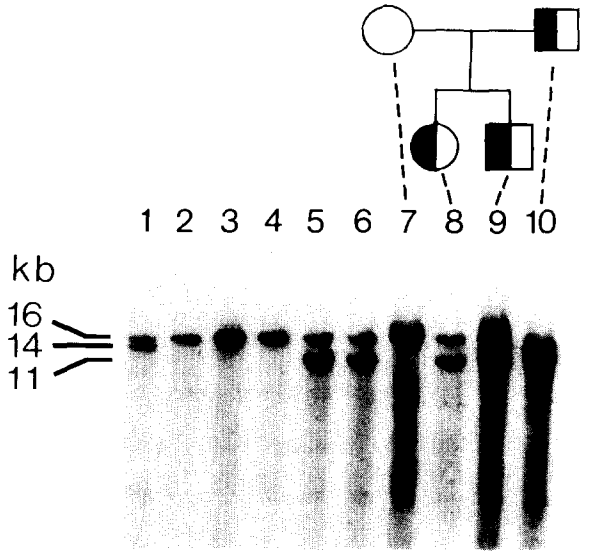

Fig.3. Demonstration of co-segregation of the FH phenotype with the deleted allele in a family. DNA was digested with *Pvu*II and hybridized with the ^{32}P-labelled LDL receptor gene probe. Lanes: 1 (genotype P+/P−) and 2–4 (genotype P−/P−), DNA from healthy controls; 5,6 (genotype P−/11 kb), DNA from two unrelated patients with heterozygous FH. Family with FH − lanes: 7, healthy mother (genotype P−/P−); 8, child with FH (genotype P−/11 kb); 9, child with FH (genotype P−/11 kb); 10, father with FH (genotype P+/11 kb).

The inheritance of the deleted LDL receptor gene was followed in three informative families. An example of these studies is shown in fig.3. An unequivocal co-segregation of hypercholesterolemia with the mutant allele was found in each family investigated.

4. DISCUSSION

This study demonstrates that a specific type of mutation of the LDL receptor gene is involved in about one-third of Finns with the heterozygous form of familial hypercholesterolemia. The mutation deletes about 8000 base pairs from the 3′-end of the LDL receptor gene. The mutated allele may be expected to encode a truncated receptor protein lacking the membrane spanning region and the cytoplasmic tail of the receptor [5].

Large deletions between exons 12 and 18 have been described in occasional patients with FH [7,10,13,21,22]. Whether the Finnish type of mutation represents one of those reported previously awaits further studies in which the mutated gene is cloned and sequenced.

Characterization of the mutation by haplotype studies with the aid of a published database [23] is hampered by the fact that no Finnish patient homozygous for the deleted allele was found. Very recently, we were able to investigate DNA from a Finnish patient with homozygous familial hypercholesterolemia. This patient proved to have one deleted allele, similar to that found in the heterozygotes in the present study, and one allele with no gross abnormalities, thus representing a genetic compound (Aalto-Setälä, K. et al., unpublished).

With the exception of a few populations, mutations from families to families are different. The 'Lebanese allele' is responsible for the extraordinarily high incidence of FH in Lebanon and is biochemically attributable to a single nucleotide substitution that creates a premature termination codon at amino acid 660, eliminating 180 residues from the carboxyl end of the mature protein [12]. A large deletion eliminating the promoter region and the first exon of the LDL receptor gene occurs in 63% of French Canadians with heterozygous FH, providing an effective diagnostic means in this population [15]. Haplotype analysis of DNA from FH patients among South African Afrikaners

similarly demonstrates a founder gene effect in this population; the predominant mutation involved awaits, however, more detailed characterization [16]. It is of note that in other populations gross mutations, such as large deletions, of the LDL receptor gene represent only a minority (3–6%) of the spectrum of mutations (Langlois, P. and Humphries, S., papers presented at the International Symposium on Familial Hypercholesterolemia, Oslo, August 28–29, 1987). Our preliminary findings suggest that, with the exception of the mutation described here, no other type of major LDL receptor gene alterations occurs in Finns.

An explanation for the enrichment of a specific LDL receptor gene mutation in Finland may be sought via historical, geographical and linguistic arguments [24]. Relatively few ancestor Finns originally inhabited the south-western part of the country during the first centuries AD. The increase in population was very slow up to the 16th and 17th century when effective inhabitation of other parts of the country commenced. At no time did any significant immigration from Sweden or Russia occur. A major barrier against both west and east has been the Finnish language, bearing no philological resemblance to any major class of languages. It is thus not surprising that Finland has its own peculiar panel of inherited diseases and virtually lacks other inherited diseases (e.g. phenylketonuria, cystic fibrosis and thalassemias) which are rather common in other parts of the world [24]. The exact prevalence of FH in Finland is not known.

The practical benefits of the present data are obvious. A simple and definitive diagnosis by DNA methods can be offered to about one-third of Finnish patients with FH. In the affected families, inheritance of the disease can be easily traced from generation to generation. It remains to be investigated whether the same type of LDL receptor gene mutation occurs in other European populations.

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