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MUTATION IN BRIEF

Identification of the Haplotype Associated With the APOB-3500 Mutation in a French Hypercholesterolemic Subject: Further Support for a Unique European Ancestral Mutation

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INTRODUCTION

Two autosomal dominant disorders causing hypercholesterolemia are now identified at the molecular level: low-density lipoprotein receptor (LDLR) and apolipoprotein B (APOB) defects (Goldstein and Brown, 1989; Soria et al., 1989). Mutations in the LDL receptor gene are known to be responsible for familial hypercholesterolemia (FH), which is characterized by increased serum LDL cholesterol levels, tendon xanthomas, and premature coronary heart disease (Goldstein and Brown, 1989). More recently, a defect in the apo B gene that leads to familial defective apolipoprotein B100 (FDB), has been characterized. Clinical features of FDB heterozygotes are strikingly similar to those observed in FH heterozygotes (Rauh et al., 1992a). The mutation, which interferes with the normal binding of apo B to the LDL receptor, is located in the codon for aminoacid 3500, and substitutes an arginine to a glutamine (Soria et al., 1989). This mutation has been reported in populations from Europe and North America (reviewed by Rauh et al., 1992b), with a frequency above 1/500 in the general population (Innerarity et al., 1990). An identical haplotype, constructed with 10 markers of the APOB gene, and associated with the mutation has been described in unrelated Caucasians from North America and Austria (Ludwig and McCarthy, 1990). The same haplotype was subsequently reported in FDB subjects from Germany and the United Kingdom (Rauh et al., 1991; Myant et al., 1991) thus indicating a common ancestral chromosome of European origin.

In this study, we screened a French population of 101 unrelated hypercholesterolemic individuals for the APOB-3500 mutation. We used the polymerase chain reaction (PCR) and allele-specific oligonucleotide (ASO) hybridization, to evaluate the impact of this disorder in this French hypercholesterolemic population. The associated haplotype was characterized using the same 10 APOB markers as those used in previous reports.

PATIENTS AND METHODS

One hundred and one unrelated subjects were recruited from different areas of France (Nantes, Montpellier, Paris, Toulouse). Most of the subjects were severely hypercholesterolemic and many were considered as FH heterozygotes on pedigree analysis. Subjects, on the basis of their clinical features, plasma lipid levels and lipoprotein profiles, belonged to two groups: 93 patients were type IIa (triglycerides < 150 mg/dl) and 8 were type IIb (triglycerides > 175 mg/dl). All patients had a positive family history of hypercholesterolemia in at least one first degree relative.

The analysis was subsequently extended to include members of the family of the FDB proband.

The APOB-3500 mutation was detected by PCR amplification and hybridization with allele-specific oligonucleotide (Soria et al., 1989). The members of the family of the FDB proband were

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tested for the APOB-3500 mutation, by *Msp*I cleavage following gene amplification as reported by Hansen et al. (1991). The PCR primers used were derived from those previously described by the authors: 5′-CTTACTTGAATTCCAAGAG-CACCC-3′ for the upstream primer (the mismatched nucleotide is underlined) and 5′-TC-CCAGAGGGAATATATGCGTTGG-3′ for the downstream primer. In the normal allele, the *Msp*I site is present and the resulting restriction fragment sizes are 113 and 23 bp, whereas the absence of the *Msp*I site in the mutant allele results in a 136 bp fragment.

Genotypes associated with the mutation were determined for 7 diallelic RFLPs by PCR amplification followed by appropriate enzyme digestion (*Apa*LI, *Hinc*II, *Pvu*II, *Alu*I, *Xba*I, *Eco*RI, and *Msp*I), for 2 multiallelic markers [5′(*TG*)*n* and 3′*HVR*: hypervariable repeat] and one insertion/deletion polymorphism (*SP*: signal peptide) by direct size analysis of the products of PCR amplification. All polymorphisms were assayed using primers and PCR conditions described by Ludwig and McCarthy (1990). For the 5′(TG)n and the 3′HVR markers, PCR amplifications were electrophoresed on standard denaturing polyacrylamide DNA sequencing gels. DNA was subsequently transferred to Hybond-N+ membranes (Amersham). Hybridization was performed with one of the primers used in the amplification step, labeled at its 3′ end with [α-^{32}P]dCTP by the terminal transferase (Boehringer, Mannheim) according to Hazan et al. (1992). Autoradiography was carried out at −80°C after two washes in 2× SSC, 0.1% SDS.

RESULTS AND DISCUSSION

One hundred and one hypercholesterolemic French subjects were screened for the presence of the APOB-3500 mutation. PCR amplification of the APOB region surrounding amino acid 3500 was performed on genomic DNA from all subjects, and the amplified DNA was dotted onto duplicate filters. The filters were hybridized with radiolabeled oligonucleotide probes, specific for the normal or mutant sequence. Only one subject among the 101 was heterozygous for the APOB-3500 mutation. These results indicate that the APOB-3500 mutation occurs at a frequency of approximately 1% in our population of hypercholesterolemic subjects: 1/101 in the whole population and 1/93 in the type IIa patients. Although the number of subjects tested is still small, this frequency is similar to that reported in other populations with hypercholesterolemia and/or coronary artery disease (reviewed by Rauh et al., 1992b).

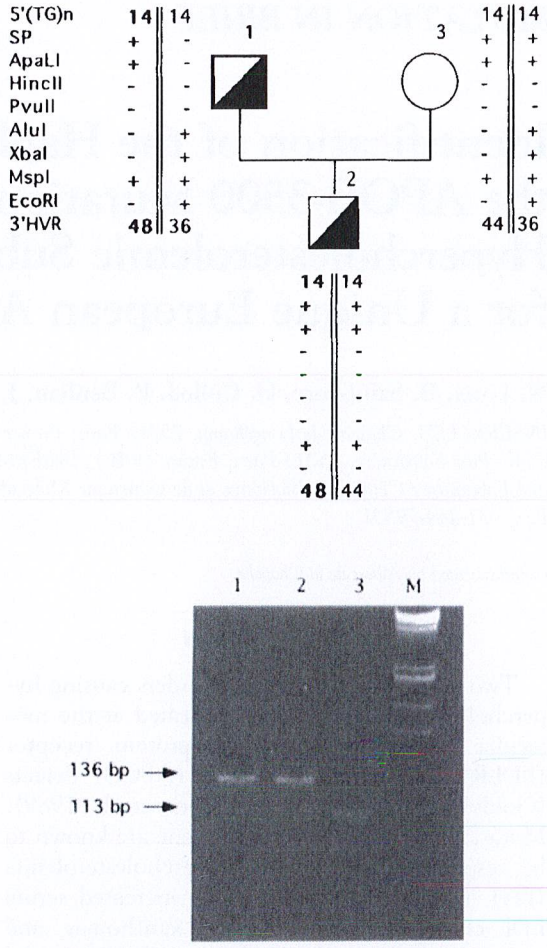

FIGURE 1. Detection of the APOB-3500 mutation and haplotype analysis in the FDB family. PCR amplification of the region around the 3500 mutation (product 136 bp) was followed by *Msp*I digestion yielding a 113 bp fragment in the nonaffected mother (subject 3). In the proband and his son (subjects 1 and 2) two fragments (113 and 136 bp) were generated. "M" is the molecular weight marker. The haplotype associated with the APOB-3500 mutation is in bold on the left side. For the SP insertion/deletion, + and − refer to the presence and the absence of the 9 bp fragment. For the 7 diallelic RFLPs, + and − refer to the presence and the absence of the restriction site. The 5′(TG)n and 3′HVR alleles are presented as numbers of TG dinucleotide or 15 bp repeats, respectively.

The family of the FDB proband was studied to identify members carrying the codon 3500 mutation, by gene amplification and *Msp*I cleavage (Hansen et al., 1991). One additional FDB subject was found, the son of the proband (Fig. 1). The 45-year-old FDB heterozygote proband had overt hypercholesterolemia (total cholesterol = 313 mg/

dl) and tendon xanthomas. No coronary heart disease was observed. His son, carrying the codon 3500 mutation, had a blood cholesterol of 246 mg/dl at 18 years. The plasma lipid levels and clinical features of the proband are similar to those observed in FDB patients from Germany and the United Kingdom (Rauh et al., 1992a; Myant et al., 1991), as opposed to the FDB patients from North America and Austria who had moderate hypercholesterolemia (Innerarity et al., 1990). We performed haplotype analysis using the same 10 markers as those used in a previous report (Ludwig and McCarthy, 1990) in which the haplotype associated with the APOB-3500 mutation had been determined in unrelated patients. The haplotype associated with the mutation was unambiguously determined in the FDB proband and in his son. It is strictly identical to the previously reported haplotype (Ludwig and McCarthy, 1990). (Fig. 1).

In conclusion, this is the first identification of the APOB-3500 mutation in a French subject. Moreover, the associated haplotype is strictly identical to the common 10 marker-haplotype described in subjects from North America, Austria, Germany, and United Kingdom (Ludwig and McCarthy, 1990; Rauh et al., 1991; Myant et al., 1991). The proband was born in the Southwest of France, he belongs to a family of recognized French ancestry that has lived in this part of the country for over three generations and with no notion of a possible Germanic origin. Therefore, this finding supports the hypothesis that the APOB-3500 mutation is a unique European ancestral mutation and is not related to a founder effect limited to people of Germanic origin.

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