R3531C Mutation in the Apolipoprotein B Gene Is Not Sufficient to Cause Hypercholesterolemia

Jean-Pierre Rabès, Mathilde Varret, Martine Devillers, Philippe Aegerter, Ludovic Villégé, Michel Krempf, Claudine Junien, Catherine Boileau

Abstract—Familial hypercholesterolemia and familial ligand-defective apolipoprotein B-100 (FDB) are dominantly inherited disorders leading to impaired low-density lipoprotein receptor (LDLR) and apolipoprotein B-100 (APOB) interaction, plasma LDL elevation, and hypercholesterolemia. We previously identified the first French FDB-R3531C proband, a woman with very high total cholesterol, in a group of type IIa hypercholesterolemic families. We report here the investigation of her family at large that revealed the total absence of cosegregation with hypercholesterolemia. Six of the 10 subjects heterozygous for the R3531C mutation had plasma cholesterol lower than the 97.5th percentile for their age and gender, and mean cholesterol levels were not significantly different between affected and unaffected persons. Furthermore, 2 family members with similar high LDL-cholesterol levels were not carriers of the R3531C substitution, suggesting the implication of another mutation. Segregation analysis of the LDLR gene revealed statistically significant genetic linkage with hypercholesterolemia, and analysis of the proband LDLR gene led to the identification of the 664 proline to leucine defective mutation and its detection in all 6 hypercholesterolemic-related members of this family. Therefore, our results show that the family presents with familial hypercholesterolemia and give evidence that the R3531C substitution in the APOB gene is not an allelic variant leading to FDB. Furthermore, thorough analysis of our data suggests that the APOB-R3531C mutation enhances the hypercholesterolemic effect of the LDLR-P664L defect, suggesting that it is a susceptibility mutation. (Arterioscler Thromb Vasc Biol. 2000;20:e76-e82.)

Key Words: hypercholesterolemia ■ apolipoprotein B ■ familial ligand-defective apolipoprotein B ■ low-density lipoprotein ■ familial hypercholesterolemia.

D ominantly inherited type IIa dyslipoproteinemia is genetically heterogeneous and involves mutations in at least 3 genes: the gene encoding the low-density lipoprotein receptor (LDLR), the gene encoding its ligand apolipoprotein B-100 (APOB), and a recently mapped but still unidentified gene called FH3 at 1p34.1-p32.1 More than 600 mutations have been identified in the LDLR gene that lead to classical familial hypercholesterolemia (FH). Conversely, only 3 mutations have been identified in the APOB gene that induce familial ligand-defective apolipoprotein B-100 (FDB) by failure of LDL binding to its receptor and secondary plasma LDL-cholesterol elevation. The Arg3500→Gln mutation (R3500Q) was the first established cause of FDB, and its frequency has been estimated as 0.4% in Switzerland and 0.08% in North Europe and United States. Binding affinity of LDL from FDB-R3500Q subjects or FDB-R3500W subjects. This result suggested that the binding affinity of the 2 groups of mutation-carrying LDL particles was comparable. Finally, an Arg→Cys substitution was reported at residue 3531 (APOB-R3531C), and binding affinity of LDL from FDB-R3531C heterozygotes was between 63% and 70% that of normal. Although numerous R3500Q mutations have been reported in the United States and Europe, to date the R3531C substitution in the APOB gene has only been reported in 27 probands from the white population (Table I).
Sixteen probands were identified in patients with hyperli-pidemia\(^{9,18-20}\) (0.15\% to 0.5\%\(^{18}\)) or coronary artery disease (CAD)\(^{6,16,19}\) (0.1\%\(^{6,19}\) to 0.8\%\(^{16}\)). We report here the analysis of the large family of 1 hypercholesterolemic proband.\(^{19}\)

## Methods

### Subjects and Blood Samples

Blood samples were collected after an overnight fast from 22 members of the HC5 family. Informed consent was obtained from all family members. Blood was either anticoagulated with potassium-EDTA or allowed to clot and the serum separated by centrifugation.

### Serum Lipid Analysis

Serum triglycerides, total cholesterol, and high-density lipoprotein cholesterol were analyzed by standard enzymatic methods, either by direct assay or after phosphotungstate and magnesium precipitation in the case of high-density lipoprotein particles. LDL-cholesterol was indirectly determined using the Friedewald formula.\(^{21}\)

### DNA Studies

#### APOB Gene

DNA was isolated from whole blood,\(^{22}\) and the R3500Q and R3531C mutations were detected simultaneously using the PCR-mediated site-directed mutagenesis method we described previously.\(^{19}\) Ten APOB gene markers were analyzed, as reported by Loux et al\(^{23}\); 8 biallelic markers (insertion or deletion polymorphism in the signal peptide, ApaLI, HinII, PvuII, AalI, XbaI, MspI, and EcoRI) and 2 multiallelic markers (the 5\’ TGN marker and 3\’ HVR).

#### LDLR Gene

Two LDLR locus polymorphisms were studied: D19S394 and D19S221.\(^{24}\) These multiallelic markers lie, respectively, 250-kilobase telomeric and 1-megabase centromeric to the LDLR gene. Their heterozygosity indices are 0.9 and 0.8, respectively. For the proband, each exon of the LDLR gene was amplified using specific oligonucleotides, as described by Leitersdof et al,\(^{25}\) and sequenced directly by theideoxyme method. The detection of the P664-L mutation in family members included amplification of exon 14, digestion with PsI, electrophoresis through a 6\% acrylamide gel, and visualization with ethidium bromide.\(^{26}\)

#### Apolipoprotein E Gene

Apolipoprotein E genotype was determined using the INNO-LiPA Apo E kit (Innogenetics) as recommended by the manufacturer.

### Linkage Analysis

Pairwise and multipoint linkage analyses were performed using the SLINK and MLINK programs of the LINKAGE package.\(^{27}\) Lod scores were computed with a complete and incomplete (0.9) penetrance of the trait defined as plasma cholesterol above the 97.5th percentile\(^{28}\) for heterozygous individuals and with a gene frequency of 0.002. Linkage was investigated assuming equal female to male recombination rates.

### Statistical Analysis

Plasma levels of total cholesterol were expressed as multiple of median (MoM) for age and gender using the reference for the French population.\(^{29}\) Plasma levels of LDL-cholesterol were not adjusted for age and gender, because no French population data have been published for this parameter. Data are expressed as mean±95\% confidence interval. The Statistical Package for the Social Sciences, version 8.0 for Windows, was used for the statistical analysis. We performed a 2-way ANOVA to test the effect of the APOB-R3531C and LDLR-P664L mutations on the phenotype in carriers identified in the family and tested the interaction between the 2 factors. \(P<0.05\) was considered significant.

### Results

#### Nuclear Family Study

The family (Figure I) was investigated through the mother (II-2), who presented bilateral corneal arcus associated with highly elevated total and LDL-cholesterol levels poorly lowered by treatment with 3-hydroxy 3-methylglutaryl coenzyme A reductase inhibitors. Ultrasound investigation revealed atherosclerosis in the carotid arteries. Her 13-year-old son (III-1) also displayed highly elevated total and LDL-cholesterol levels requiring lipid-lowering therapy with bile-acid sequestrants. Serum cholesterol levels (Table II) were not high (clearly below the 97.5th percentile) for her daughter (III-2) and husband (II-1) when compared with gender- and age-matched levels established in the French population.\(^{28}\)

To identify the molecular defect associated with the hypercholesterolemic phenotype observed in the family, we first screened for the 2 APOB mutations in the white population (OMIM *107730.0017 and 0009) leading to FDB.\(^{17}\) No R3500Q mutation was found, but the R3531C mutation of the APOB gene was found to be heterozygous in the mother and her 2 children. Surprisingly, the same mutation was also identified in the father. Family members were separately

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**Table I. Summary of Published APOB-R3531C Probands**

<table>
<thead>
<tr>
<th>Population</th>
<th>APOB-R3531C Probands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>Sample Size</td>
</tr>
<tr>
<td>Hyperlipidemic subjects</td>
<td>1368</td>
</tr>
<tr>
<td>Patients with CAD</td>
<td>516</td>
</tr>
<tr>
<td>Hypercholesterolemic subjects</td>
<td>412</td>
</tr>
<tr>
<td>Hypercholesterolemic probands*</td>
<td>49</td>
</tr>
<tr>
<td>Subjects suspected of CAD</td>
<td>991</td>
</tr>
<tr>
<td>Subjects from the general population</td>
<td>9255</td>
</tr>
<tr>
<td>Patients with CAD</td>
<td>948</td>
</tr>
<tr>
<td>Patients at lipid clinics</td>
<td>†</td>
</tr>
<tr>
<td>Unselected volunteers</td>
<td>†</td>
</tr>
</tbody>
</table>

*Belonging to families who displayed autosomal dominant hypercholesterolemia.

†In this paper, 2570 subjects were studied, but the sample size for each category (patients and volunteers) was not given.
resampled and retested to eliminate the possibility of incorrect sample assignment. The presence of the mutation was confirmed in all subjects. Genotypes were determined for 10 APOB polymorphic markers, and complete haplotypes could be deduced unequivocally for the proband, her husband, and their 2 children (Figure I). The mutation in the proband (II-2) and her husband (II-1) was associated with haplotype E as in the Celtic and Native American kindred originally reported by Pullinger et al18 and in the 2 index cases of Celtic origin reported by Wenham et al.18 This result suggested that the parents (II-1 and II-2), both from the western part of France, were of hitherto unknown common Celtic descent. The APOB haplotype analysis in this nuclear family also revealed that the asymptomatic daughter had inherited the mutation from her hypercholesterolemic brother, whereas her hypercholesterolemic brother had inherited the mutation from his normocholesterolemic father. Because the discrepancy between the molecular and laboratory data could be attributed either to a lack of penetrance of the R3531C mutation or the existence of a defect in another major gene, investigation of the family at large was undertaken.

Study of the Family at Large
Eighteen additional subjects were available for study (Figure II). Serum lipid levels were determined for all subjects (Table II), and DNA analysis was performed. Six additional heterozygous carriers of the R3531C mutation were identified (Figure II). The proband’s husband (II-1) inherited the mutation from his asymptomatic mother (I-2). Furthermore, his family history revealed no instances of hypercholesterolemia or CAD. Conversely, the father (I-3) of the proband (II-2) had documented hypercholesterolemia, as did 2 of the proband’s siblings (II-6 and II-9) and 2 of her nephews (III-6 and III-8). Despite the presence in her family of elevated lipid values and the R3531C mutation, there was no segregation of the mutation with the very high total cholesterol levels (Figure II). Linkage was excluded on the basis of lod scores equal to −2.64 and −2.61 with complete and incomplete penetrance, respectively. Furthermore, there was no significant difference in lipid levels between carriers of the R3531C mutation (mean value and 95% confidence interval for total cholesterol and LDL-cholesterol were 6.77 [5.48 to 8.06] and 4.34 [3.21 to 5.48] mmol/L, respectively) and family members lacking the mutation (6.02 [5.31 to 6.72] and 4.13 [3.47 to 4.8] mmol/L, respectively). Variations between different individuals in the expression of an identical mutation is well documented in dominantly inherited disorders and has been reported for the common FDB mutation R3500Q.29,30 These variations may be explained by differences in environmental factors but are unlikely in a single family. Furthermore, 2 subjects, a woman (II-9) and her son (III-8), with severe hypercholesterolemia did not carry the R3531C mutation, suggesting the existence of a second mutation in the maternal family.

Using the SLINK program and assuming a marker with a heterozygosity index of 0.83 (very close to the heterozygosity index of the 2 LDLR markers used in the study), we estimated expected maximum lod scores of 2.36 with complete penetrance and 2.24 with incomplete penetrance. These scores are above the threshold level (lod score = 2) that is statistically significant for linkage when analyzing a candidate gene.31 Therefore, because the family was well suited to linkage analysis, we tested the possible involvement of the LDLR gene. Two polymorphic markers were studied: D19S394 and D19S221, which lie 250-kilobase telomeric and 1-megabase centromeric, respectively, to the LDLR gene on chromosome 19.24 Using the MLINK program, the highest 2-point lod scores were obtained for marker D19S394 at 2.28 and 2.14 (θ = 0, complete and incomplete penetrance, respectively), therefore establishing linkage between 1 allele of the LDLR gene and hypercholesterolemia in the family (Figure II). Sequencing each exon of the proband LDLR gene led to the identification of a 664 proline to leucine defective mutation (LDLR-P664L) and its detection in all 6 hypercholesteremic-linked members of the family. This recurrent mutation has already been reported. It results in a receptor with a reduced binding affinity for LDL and in delayed processing of the precursor form of the protein in cultured cells (2B+3 functional classes).32 Informative marker (5’ TGn and 3’ HVR) analysis showed no cosegregation of a particular APOB haplotype with hypercholesterolemia, excluding implication of an APOB mutation different from R3500Q and R3531C (data not shown).

Statistical analysis revealed significant differences (P < 0.001) in total and LDL-cholesterol between carriers of the P664L mutation (mean value and 95% confidence interval for total cholesterol and LDL-cholesterol were 8.68 [8.05 to

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**Figure I.** APOB gene haplotype analysis in the proband’s nuclear family. Subjects are numbered with respect to the complete family pedigree shown in Figure 2. Half-linked symbols denote individuals heterozygous for the R3531C mutation. Arrows show the transmission of the boxed haplotype associated with the mutation. Polymorphic markers tested were, from top to bottom, 5’ (TG)n, SP, ApalI, HincII, PvuII, Alul, XbaI, MspI, EcoRI, and 3’ HVR. For the SP insertion and deletion, + and − refer to the presence and absence, respectively, of the 9-base pair fragment. For the 7 diallelic restriction fragment length polymorphisms, + and − refer to the presence and absence, respectively, of the restriction site. 5’ (TG)n, and 3’ HVR alleles are presented as numbers of TG dinucleotides or 15-base pair repeats, respectively.
9.32] and 6.20 [5.64 to 6.76] mmol/L, respectively) and family members lacking the mutation (mean value and 95% confidence interval for total cholesterol and LDL-cholesterol were 5.49 [5.06 to 5.92] and 3.60 [3.17 to 4.05] mmol/L, respectively). Mean total cholesterol expressed as MoM for age and gender was 1.67 for the 2 subjects carrying only the LDLR-P664L mutation and 1.12 for the group without the 2 mutations screened (Figure III). On the other hand, an effect of the isolated APOB-R3531C mutation on plasma cholesterol levels was not detectable. Subjects carrying only the APOB-R3531C mutation (6 subjects) showed lower mean lipid levels (5.22 mmol/L or 1.06 MoM for total cholesterol and 3.27 mmol/L for LDL-cholesterol) than the 10 subjects without the 2 mutations screened (5.65 mmol/L or 1.12 MoM and 3.81 mmol/L, respectively) (Figure III). Furthermore, we did not detect significant interaction between the 2 mutations per se. 

### TABLE II. Clinical and Biochemical Features of Family Members

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age (y)</th>
<th>Clinical Findings</th>
<th>Serum Lipid Levels (mmol/L)</th>
<th>LDL-Cholesterol</th>
<th>TG</th>
<th>APOB-R3531C</th>
<th>LDLR-P664L</th>
<th>ApoE Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-2</td>
<td>F</td>
<td>70</td>
<td>—</td>
<td>4.7 (0.77)</td>
<td>3.4</td>
<td>0.5</td>
<td>+</td>
<td>–</td>
<td>3/3</td>
</tr>
<tr>
<td>I-6</td>
<td>F</td>
<td>71</td>
<td>—</td>
<td>5.7 (0.93)</td>
<td>4.1</td>
<td>1.5</td>
<td>–</td>
<td>–</td>
<td>3/4</td>
</tr>
<tr>
<td>II-1</td>
<td>M</td>
<td>47</td>
<td>—</td>
<td>6.3 (1.13)</td>
<td>4.4</td>
<td>0.8</td>
<td>+</td>
<td>–</td>
<td>3/4</td>
</tr>
<tr>
<td>II-2*</td>
<td>F</td>
<td>41</td>
<td>PAD, Arc</td>
<td>8.4 (1.68)</td>
<td>5.8</td>
<td>1.4</td>
<td>+</td>
<td>+</td>
<td>2/3</td>
</tr>
<tr>
<td>II-3</td>
<td>M</td>
<td>46</td>
<td>—</td>
<td>5.9 (1.05)</td>
<td>4.3</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>3/4</td>
</tr>
<tr>
<td>II-4</td>
<td>F</td>
<td>44</td>
<td>—</td>
<td>4.8 (0.96)</td>
<td>3.0</td>
<td>0.5</td>
<td>+</td>
<td>–</td>
<td>2/3</td>
</tr>
<tr>
<td>II-5</td>
<td>M</td>
<td>45</td>
<td>—</td>
<td>5.9 (1.05)</td>
<td>3.8</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
<td>3/3</td>
</tr>
<tr>
<td>II-6</td>
<td>M</td>
<td>40</td>
<td>Arc</td>
<td>9.3 (1.69)</td>
<td>7.1</td>
<td>1.6</td>
<td>+</td>
<td>+</td>
<td>2/3</td>
</tr>
<tr>
<td>II-8</td>
<td>M</td>
<td>39</td>
<td>—</td>
<td>7.7 (1.40)</td>
<td>5.7</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
<td>3/3</td>
</tr>
<tr>
<td>II-9</td>
<td>F</td>
<td>37</td>
<td>Arc</td>
<td>7.9 (1.58)</td>
<td>6.0</td>
<td>0.8</td>
<td>–</td>
<td>+</td>
<td>2/3</td>
</tr>
<tr>
<td>II-10</td>
<td>M</td>
<td>42</td>
<td>—</td>
<td>6.2 (1.13)</td>
<td>4.2</td>
<td>1.6</td>
<td>–</td>
<td>–</td>
<td>3/3</td>
</tr>
<tr>
<td>II-11</td>
<td>F</td>
<td>39</td>
<td>—</td>
<td>5.1 (1.02)</td>
<td>3.3</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
<td>3/4</td>
</tr>
<tr>
<td>III-1</td>
<td>M</td>
<td>17</td>
<td>—</td>
<td>8.9 (2.23)</td>
<td>6.6</td>
<td>0.9</td>
<td>+</td>
<td>+</td>
<td>3/4</td>
</tr>
<tr>
<td>III-2</td>
<td>F</td>
<td>21</td>
<td>—</td>
<td>5.5 (1.25)</td>
<td>3.5</td>
<td>0.8</td>
<td>+</td>
<td>–</td>
<td>3/3</td>
</tr>
<tr>
<td>III-3</td>
<td>F</td>
<td>18</td>
<td>—</td>
<td>5.2 (1.18)</td>
<td>3.0</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
<td>2/4</td>
</tr>
<tr>
<td>III-4</td>
<td>F</td>
<td>11</td>
<td>—</td>
<td>5.1 (1.15)</td>
<td>2.8</td>
<td>0.4</td>
<td>–</td>
<td>+</td>
<td>2/3</td>
</tr>
<tr>
<td>III-5</td>
<td>M</td>
<td>8</td>
<td>—</td>
<td>4.9 (1.13)</td>
<td>2.5</td>
<td>0.4</td>
<td>+</td>
<td>–</td>
<td>2/3</td>
</tr>
<tr>
<td>III-6</td>
<td>M</td>
<td>5</td>
<td>—</td>
<td>9.8 (2.25)</td>
<td>0.8</td>
<td>+</td>
<td>+</td>
<td>3/3</td>
<td></td>
</tr>
<tr>
<td>III-7</td>
<td>M</td>
<td>15</td>
<td>—</td>
<td>5.1 (1.28)</td>
<td>3.6</td>
<td>0.3</td>
<td>–</td>
<td>–</td>
<td>2/3</td>
</tr>
<tr>
<td>III-8</td>
<td>M</td>
<td>12</td>
<td>—</td>
<td>7.8 (1.75)</td>
<td>5.5</td>
<td>0.5</td>
<td>+</td>
<td>2/3</td>
<td></td>
</tr>
<tr>
<td>III-9</td>
<td>F</td>
<td>14</td>
<td>—</td>
<td>3.8 (0.85)</td>
<td>1.9</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>3/4</td>
</tr>
<tr>
<td>III-10</td>
<td>F</td>
<td>10</td>
<td>—</td>
<td>5.9 (1.31)</td>
<td>4.2</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
<td>3/3</td>
</tr>
</tbody>
</table>

*PAD, peripheral artery disease; Arc, arcus corneae; TC, total cholesterol; TG, triglycerides.

*proband.
on total and LDL-cholesterol levels. Using raw total cholesterol concentration values, the interaction test was barely significant (P < 0.05), but using these data expressed as MoM for age and gender, the test was no longer significant (P < 0.09) (Figure III). However, thorough analysis of our data indicates that carriers of the 2 mutations all have higher total cholesterol levels than LDLR-P664L heterozygotes (Table II) and that the mean adjusted total cholesterol of the first group of patients is higher than that of the second group (Figure III).

**Discussion**

There is no doubt that the R3531C mutation causes reduced binding of LDL to the LDL receptor in vitro.9,16,17 However, our results additionally support that this reduction is not sufficient to cause hypercholesterolemia in vivo in heterozygotes. The evidence that the R3531C mutation alone does not cause hypercholesterolemia stems from functional, epidemiological, and linkage analysis.

First, R3531C functional consequences measured in vitro are only half of the reduction observed with the R3500Q mutation. In the total of 12 R3531C heterozygous subjects who have now been reported, the overall binding affinity of LDL particles compared with reference was 57%,20 whereas it was 34% for the R3500Q mutation.9 LDL particles from individuals with the R3531C mutation were 74% as effective at promoting growth as normal LDL in a U937 cell assay,20 whereas the relative growth rates using LDL particles from heterozygous R3500Q and R3500W subjects were about 50% that of normal LDL particles.13 Ligand-defective Cys3531 LDL particles accumulated and comprised a mean of 58% of the total particles present, whereas the mass ratio of Gln3500 to Arg3500 LDL was 73:27.20 Defective apoB Cys3531 LDL itself has been calculated to have 27% of normal affinity compared with <10% for Gln3500 particles.9 LDLR mutations and the R3500Q mutation in APOB each lead to impaired LDL receptor and apo B-100 interaction, but FDB-R3500Q patients tend to have a milder phenotype (significantly lower plasma total and LDL-cholesterol and subsequent lower incidence of clinical atherosclerosis).10–12 Because the R3531C mutation in vitro causes only 50% of the reduction for the R3500Q mutation, in vivo consequences are expected to be reduced or undetectable.

Second, APOB-R3531C proband screening suffered from ascertainment bias attributable to patient status. Sixteen of 27 probands reported (Table I) were identified among either patients with hyperlipidemia who were attending lipid clinics9,18–20 or patients with CAD6,16,19 among whom patients with hypercholesterolemia are naturally overrepresented. Therefore, the presence of hyperlipidemia could be a consequence of the study design and not attributable to any mutation. Despite this bias, only 15 of 27 probands heterozygous for the R3531C mutation displayed hypercholesterolemia.6,9,16,18–20 For example, of the 4 CAD patients identified by Ludwig et al,16 only 2 had hypercholesterolemia and 1 had the rare E4/E4 apolipoprotein E genotype that is associated with increased plasma cholesterol. Recently, Pullinger et al20 reported no statistically significant difference in lipids between 24 affected and 18 unaffected individuals from 9 kindreds. This difference became significant only when all published data were combined. However, the possible coexistence of another mutation, as in the proband we report, and evident ascertainment bias could explain how the level of significance was reached. This hypothesis is in agreement with data published by Tybjaerg-Hansen et al6 reporting the first 7 R3531C probands identified in the general population. This was a very large study (9255 women and men) without ascertainment bias attributable to patient status. None of the R3531C probands identified in the general population had plasma total or LDL-cholesterol elevation, suggesting that this mutation is not sufficient to cause high cholesterol levels, ie, FDB. Tybjaerg-Hansen et al6 also clearly showed that the frequency of the R3531C mutation was not increased in patients with hypercholesterolemia or ischemic heart disease compared with the general population. In contrast, the frequencies of the R3500Q mutation identified in exactly the same populations were clearly increased.6 Although the frequencies of these 2 APOB mutations are the same in the general population, only
APOB-R3500Q causes sufficient hypercholesterolemia to be a risk factor for ischemic heart disease. Furthermore, all studies except 1,8 that have examined the presence of either of these mutations in the same patient population consistently showed a lower frequency of R3531C versus R3500Q in hyperlipidemic or CAD patients.6,9,18,19

Finally, in support of this theory is the fact that there was an absence of simple cosegregation of this mutation with hypercholesterolemia in 11 of 12 families studied.9,18–20 The description of our informative French family, the largest studied so far, shows no cosegregation of the R3531C mutation with hypercholesterolemia that is linked to a defective LDLR mutation. Contrary to our investigation, most of the family studies did not definitely exclude the involvement of an LDLR gene defect18,20 for the implication of the third locus (FH3) associated with autosomal dominant hypercholesterolemia.1,33 Of the 8 R3531C probands reported by Tybjaerg-Hansen et al, a,1 had ischemic heart disease and hypercholesterolemia, but a family history of these traits was absent.

However, when associated with the LDLR-P664L mutation, the APOB-R3531C mutation seems to enhance hypercholesterolemia. Heterozygote carriers of the APOB-R3531C mutation alone have mean adjusted total cholesterol levels lower than subjects without the 2 mutations screened, whereas carriers of the APOB-R3531C mutation and the LDLR-P664L mutation have mean adjusted total cholesterol levels higher than the carriers of the LDLR-P664L mutation alone (Figure III). This inversion of influence, although not statistically significant, suggests that the APOB-R3531C mutation enhances the hypercholesterolemic effect of the LDLR-P664L defect. This is characteristic of a susceptibility mutation and fits well with the in vitro effects of this mutation.

In conclusion, the APOB-R3531C substitution, in view of its in vitro effects and our family study, is possibly a susceptibility mutation that, when present with other factors (genetic or environmental), slightly increases cholesterolemia. However, it is not sufficient in itself to cause hypercholesterolemia and should not be considered as an allelic variant leading to FDB.

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