Variation in the Low Density Lipoprotein Receptor Gene Is Associated With Differences in Plasma Low Density Lipoprotein Cholesterol Levels in Young and Old Normal Individuals From Italy

Steve Humphries, Domenico A. Coviello, Paola Masturzo, Roberto Balestreri, Giuliana Orecchini, and Stefano Bertolini

We have used four restriction fragment length polymorphisms (RFLPs) of the human low density lipoprotein receptor (LDL-R) gene, detected by the restriction enzymes \textit{Ava} II, \textit{Pvu} II, and \textit{Apa}LI (5’ and 3’), to study the effect of variation at this gene locus in determining plasma cholesterol and LDL cholesterol levels. Two hundred eighty-nine nonmolipidemic individuals were studied from the Liguria region of Italy. The \textit{Pvu} II RFLP was significantly associated with differences in plasma total and LDL cholesterol levels, explaining 9.6% of the sample variance in LDL cholesterol levels. The other RFLPs, which are in strong linkage disequilibrium with the \textit{Pvu} II RFLP, were associated with smaller effects on LDL cholesterol. The \textit{Pvu} II allele, distinguished by the presence of the variable cutting site (\textit{P2} allele), was associated with lower levels of total and LDL cholesterol, and the frequency of the \textit{P2} allele was significantly reduced in individuals with LDL cholesterol levels higher than the 75th percentile. The frequency of the \textit{P2} allele was significantly higher in the group of individuals over 65 years old, and in this group the \textit{P2} allele was also associated with an increased reduction in LDL cholesterol levels. Because of linkage disequilibrium, only four RFLP haplotypes were common in this sample. Of these, only the haplotype \textit{P2A2VI} (relative frequency, 0.269) was associated with a reduction in LDL cholesterol (average excess, $-11.5$ mg/dl). Our data confirm, in the Italian population, the association observed with the \textit{Pvu} H RFLP by others, and the higher relative frequency of the LDL cholesterol-lowering \textit{P2} allele in individuals over the age of 65 years suggests that the allele may be associated with increased survival. (\textit{Arteriosclerosis and Thrombosis} 1991;11:509–516)

High levels of serum cholesterol and, particularly, low density lipoprotein (LDL) cholesterol are risk factors for coronary artery disease (CAD).1-3 Levels of LDL in the blood are determined by both the rate of production of LDL and the rate of its removal.4 LDL is removed both by non–receptor-mediated pathways and by interaction between the major apolipoprotein (apo) of LDL, apo B, and a specific receptor on the surface of all cells, which is called the LDL receptor (LDL-R). Defects in this receptor occur in approximately one in 500 individuals, causing elevated levels of serum LDL cholesterol and premature atherosclerosis—the disorder familial hypercholesterolemia (FH).5 The gene for the LDL-R has been cloned, sequenced, and used in many studies of patients with FH (e.g., see References 6 and 7). A number of mutations in the receptor have been characterized at the molecular level (e.g., Reference 8) and have greatly helped in our understanding of the structure–function relations of this protein.

Patients with FH and a defect in the LDL-R thus identify this gene as one with an important role in lipid metabolism. It is also possible that sequence changes in the gene may exist that have only a small effect on the function of the receptor. If such
sequence changes are common, they may make an important contribution in determining lipid levels within the normal population. Evidence to support this hypothesis comes from two types of studies. First, cholesterol levels within normal individuals overlap with cholesterol levels in patients with FH, suggesting that there may be differences in receptor function within the normal population. Even in the families of patients with FH, there is a significant overlap in the distribution of LDL cholesterol between those children who have inherited the defective gene and those who have not. Second, several studies that have measured LDL-R activity in fibroblasts from normal individuals have shown a considerable range in such activity, even in fibroblasts from normal individuals.

There have now been more than 12 restriction fragment length polymorphisms (RFLPs) identified within or near the LDL gene. Recently, an association has been shown between plasma cholesterol levels and the Pvu II RFLP in the LDL-R gene in the Norwegian population, and similar observations have been made in a sample of normolipidemic individuals in the German population. A common RFLP in the LDL-R of the baboon has also been found to be associated with differences in plasma cholesterol. We have used four RFLPs of the LDL-R detected with the restriction enzymes Ava II, Pvu II, and Apa LI to study the effect of variation at this gene locus in determining plasma lipid levels in a sample of individuals from Italy.

**Methods**

**Subjects**

As part of an investigation of defects in the LDL-R gene causing FH, DNA samples from more than 124 families have been collected for study. From these families, 289 unrelated normocholesterolemic individuals, selected as having cholesterol levels within the 95th percentile of the normal distribution, have been genotyped for four RFLPs in the LDL-R gene. Blood samples were taken after the last meal. Lipoprotein levels were determined by preparative ultracentrifugation Blood cells were stored at −20°C for later DNA analysis.

**Methods**

Genomic DNA was extracted from 10 ml of fresh whole blood by a sodium dodecyl sulfate (SDS) lysis method. DNA was digested with Pvu II, Apa LI, and Ava II/Xba I restriction enzymes (following the manufacturer's conditions; Amersham International, Buckinghamshire, U.K.). DNA fragments were separated according to size on a 0.8–1% agarose gel and transferred to Hybond-N filters (Amersham) by the Southern blotting technique. The LDL-R probe pLDLR-2HH1 is a 1.9-kb fragment (bp 1,573–3,485) of the 3' end of the LDL-R cDNA clone, which contains sequences of exons 10–18. The fragment was excised from a 1% low-melting agarose gel and labeled by the Multiprime DNA Labeling Kit with 32P(a) at 3,000 Ci/nmol (Amersham). Aliquots (5×105 cpm/ml) of the probe were incubated with filters for 36 hours at 65°C in a 6x standard saline citrate (SSC), 1% SDS, 5x Denhardt's solution, and 100 g/ml herring DNA solution. Filters were washed with 0.2x SSC and 1% SDS at 65°C and exposed to x-ray film (Kodak X-Omat AR) for 2–4 days at −80°C. The Ava II/Xba I polymorphism was detected using a 581-bp BamHI/Pst I fragment of the LDL-R probe 2HHI cDNA.

**Statistical Methods**

Differences among lipid and lipoprotein concentrations in different groups of individuals were compared using the Tukey–Kramer method. Lipid and lipoprotein levels were adjusted using multiple regression for age, age squared, and body mass index in males and females separately, and residuals were added to the grand mean of the whole sample; the adjusted values were used in subsequent analyses. Genotype distribution for each RFLP was determined by gene counting. Analysis was used to test for Hardy–Weinberg equilibrium and to compare allele frequencies. Contingency analysis was used to compare the observed genotype distributions and

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**TABLE 1. Unadjusted Lipid Values and Characteristics of 289 Italian Normocholesterolemic Individuals With Amount of Variance in Each Lipid Trait Explained by Age, Body Mass Index, and Gender**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Males (n = 136)</th>
<th>Females (n = 153)</th>
<th>All subjects (n = 289)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>R²×100</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.1±18.2</td>
<td>...</td>
<td>48.4±19.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9±2.3</td>
<td>...</td>
<td>22.4±2.4</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194.0±36.9</td>
<td>17.5</td>
<td>195.7±31.0</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>123.7±31.7</td>
<td>18.1</td>
<td>119.5±28.6</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>47.1±10.2</td>
<td>4.9</td>
<td>57.3±12.5</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>116.6±62.1</td>
<td>11.5</td>
<td>95.6±53</td>
</tr>
</tbody>
</table>

Variance was calculated as R²×100.

BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.
allele numbers in individuals divided according to different quartiles of LDL cholesterol or to different ages. The association between LDL-R genotype and lipoprotein levels was tested by analysis of variance (ANOVA). Significance was considered to be at the 5% level. The effect on traits associated with the RFLPs was estimated by linear regression. The linkage disequilibrium coefficient delta was estimated as described. Average excess values associated with RFLP alleles or haplotypes were calculated as described by Templeton.

Results

Lipid and lipoprotein levels were available from 289 unrelated normolipidemic individuals from the Liguria region of Italy (Table 1), who were the unaffected relatives of patients with FH. For all subjects, genotype was determined for RFLPs detected with the restriction enzymes AvaII, PvuII, and ApaI, and Figure 1 shows the map of the human LDL-R gene and the location of the four variable restriction sites. For all four polymorphisms, genotype distribution did not differ from that expected assuming Hardy-Weinberg equilibrium (Table 2). The frequency of the rare alleles of the RFLPs was similar to that previously reported, with a frequency of the rare allele of the PvuII polymorphism, individuals with one or two V alleles (absence of cutting site) had levels of LDL cholesterol that were lower by 8.0% and 12.4%, respectively. Variation associated with this RFLP explained 4.2% of the sample variance. For both of these RFLPs and the two ApaI polymorphisms, the effect on LDL cholesterol associated with the alleles (calculated as the average excess value) is presented in Table 3. For the ApaI polymorphism, the effect associated with the A1 and A3 alleles was to raise LDL cholesterol by 4.3-4.2 mg/dl and that associated with the A2 allele was to lower LDL cholesterol by 9.2 mg/dl. Variation associated with this RFLP explained 9.2% of the sample variance in LDL cholesterol.

The distribution of RFLP genotypes and alleles was also determined in individuals divided into quartiles for LDL cholesterol. The results for the PvuII RFLP are presented in Table 3. As would be predicted from the ANOVA, the frequency of the P2 allele was higher in individuals in the lowest quartile compared with those in the highest quartile (relative frequency of 0.48 versus 0.17, \( p<0.001 \)). Similar significant differences were observed for the distribution of the alleles of the AvaII and ApaI RFLPs (not shown).

Sixty-one of the individuals in the sample were over the age of 65 years, and the characteristics of individuals above and below this age are shown in Table 4. Total, LDL, and HDL cholesterol levels but not triglyceride levels were lower in the older group, but the relative distribution of males and females was

### Table 2. Mean Adjusted Low Density Lipoprotein Cholesterol Levels in Individuals With Different Low Density Lipoprotein Receptor Restriction Fragment Length Polymorphism Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Adj LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1P1</td>
<td>134</td>
</tr>
<tr>
<td>P1P2</td>
<td>125</td>
</tr>
<tr>
<td>P2P2</td>
<td>30</td>
</tr>
</tbody>
</table>

### Results

Lipid and lipoprotein levels were available from 289 unrelated normolipidemic individuals from the Liguria region of Italy (Table 1), who were the unaffected relatives of patients with FH. For all subjects, genotype was determined for RFLPs detected with the restriction enzymes AvaII, PvuII, and ApaI, and Figure 1 shows the map of the human LDL-R gene and the location of the four variable restriction sites. For all four polymorphisms, genotype distribution did not differ from that expected assuming Hardy-Weinberg equilibrium (Table 2). The frequency of the rare alleles of the RFLPs was similar to that previously reported, with a frequency of the rare allele of the PvuII polymorphism, individuals with one or two V alleles (absence of cutting site) had levels of LDL cholesterol that were lower by 8.0% and 12.4%, respectively. Variation associated with this RFLP explained 4.2% of the sample variance. For both of these RFLPs and the two ApaI polymorphisms, the effect on LDL cholesterol associated with the alleles (calculated as the average excess value) is presented in Table 3. For the ApaI polymorphism, the effect associated with the A1 and A3 alleles was to raise LDL cholesterol by 4.3-4.2 mg/dl and that associated with the A2 allele was to lower LDL cholesterol by 9.2 mg/dl. Variation associated with this RFLP explained 9.2% of the sample variance in LDL cholesterol.

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similar in both groups. The distribution of genotypes and allele frequencies for all RFLPs was determined in the two groups. Only for the Pvu II RFLP was the difference in allele frequencies statistically significant, with the P2 allele being more frequent in the older group (0.410 versus 0.296, p<0.05). In both groups, the effect of LDL cholesterol lowering associated with the P2 allele was apparent and was of similar size in the older age group (compared with individuals homozygous for the P1 allele; mean levels of LDL cholesterol in young and old individuals homozygous for the P2 allele were 18.9% and 23.8% lower, respectively).

In the whole sample of 289 individuals, the P2, A2, and V1 alleles are all associated with lower levels of LDL cholesterol. To examine the possibility that the effect associated with the RFLPs might be additive, multiple linear regression was used to estimate the percentage of the sample variance associated with pairs of RFLPs. For Ava II and Pvu II genotypes and Apal I and Pvu II genotypes, this was 9.9% and 10.4%, respectively (not significantly different from Pvu II alone).

It should be possible to identify the haplotypes of the LDL-R that are associated with the largest effect on lowering LDL cholesterol levels. Since genotype information was available on the relatives of these individuals, four-RFLP haplotypes could be determined unambiguously in 162 individuals by inspection of the inheritance of the alleles (assuming no recombination). Nine haplotypes were identified, and the number and relative frequency of these

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**TABLE 3. Distribution of Subjects According to Low Density Lipoprotein Cholesterol Quartile and Pvu II Restriction Fragment Length Polymorphism Genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>&lt;25 (&lt;103.9)</th>
<th>25–50 (104.0–120.8)</th>
<th>50–75 (120.9–140.2)</th>
<th>&gt;75 (&gt;140.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1P1</td>
<td>18</td>
<td>30</td>
<td>38</td>
<td>48</td>
</tr>
<tr>
<td>P1P2</td>
<td>38</td>
<td>31</td>
<td>33</td>
<td>23*</td>
</tr>
<tr>
<td>P2P2</td>
<td>16</td>
<td>11</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>72</td>
<td>73</td>
<td>72</td>
</tr>
</tbody>
</table>

**P2 alleles, n (frequency)**

- <25 (LDL-C, mg/dl)
  - 18 (0.48)
  - 16 (0.70)
  - 72 (0.70)
  - Total: 72

- 25–50 (104.0–120.8)
  - 30 (0.43)
  - 31 (0.43)
  - 11 (0.37)
  - Total: 72

- 50–75 (120.9–140.2)
  - 38 (0.52)
  - 33 (0.52)
  - 2 (0.03)
  - Total: 73

- >75 (>140.2)
  - 48 (0.65)
  - 23* (0.32)
  - 1 (0.02)
  - Total: 72

*χ² genotypes=39.15 (6 df), p<0.001.
†χ² alleles=36.93 (3 df), p<0.001.
haplotypes are presented in Table 5. The four variable sites are in strong linkage disequilibrium \(15,25\) (Table 6), with allelic association apparent between \(P2A2V1\) and \(P1A3V2\). The RFLPs are therefore not acting independently, and this raises the possibility that the effect on LDL cholesterol associated with these RFLPs may be explained by linkage disequilibrium with only a single functionally important sequence change at the LDL-R locus. To investigate this, the average excess value on LDL cholesterol associated with the haplotypes was estimated (Table 5). The \(P2A2V1\) haplotype was associated with a lowering of LDL cholesterol values, and the \(P1A3V1\), \(P1A3V2\), and \(P1A1V1\) haplotypes were associated, in decreasing order, with an effect on raising LDL cholesterol values. All other haplotypes are too rare to be able to make an accurate estimate of effect.

**Discussion**

In individuals in the general population, plasma and LDL cholesterol levels are determined both by environmental factors, such as dietary fat intake, and genetic factors. The search for these genetic factors was originally performed using protein polymor-

### Table 4. Characteristics in Individuals Older and Younger Than 65 Years, Genotype Distribution, and Relative Frequency of \(Pvu\) II Restriction Fragment Length Polymorphism

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>(\leq 65) yr</th>
<th>(&gt;65) yr</th>
<th>Statistic</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n) (M/F)</td>
<td>228 (105/123)</td>
<td>61 (31/30)</td>
<td>(\chi^2=0.26)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>23.0±2.5</td>
<td>23.4±2.2</td>
<td>(t=1.18)</td>
<td>NS</td>
</tr>
<tr>
<td>Total C (mg/dl)</td>
<td>197.7±33.7</td>
<td>184.6±32.7</td>
<td>(t=2.76)</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>124±30.1</td>
<td>111.3±28.2</td>
<td>(t=3.11)</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>53.6±12.8</td>
<td>48.3±10.2</td>
<td>(t=3.37)</td>
<td>0.001</td>
</tr>
<tr>
<td>Trig (mg/dl)</td>
<td>100.4±56.3</td>
<td>124.9±62.0</td>
<td>(t=2.79)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Age group**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>(n)</th>
<th>LDL-C (mg/dl)</th>
<th>(\leq 65) yr</th>
<th>(&gt;65) yr</th>
<th>Statistic</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P1P1)</td>
<td>112</td>
<td>132.4±28.5</td>
<td>22†</td>
<td>122.0±20.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P1P2)</td>
<td>97</td>
<td>117.8±24.7</td>
<td>28</td>
<td>109.9±31.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P2P2)</td>
<td>19</td>
<td>107.3±23.5</td>
<td>11</td>
<td>92.9±12.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Frequency**

<table>
<thead>
<tr>
<th>Allele</th>
<th>(n)</th>
<th>Frequency</th>
<th>Statistical</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P1)</td>
<td>321</td>
<td>72</td>
<td>0.590</td>
</tr>
<tr>
<td>(P2)</td>
<td>135</td>
<td>50</td>
<td>0.410</td>
</tr>
</tbody>
</table>

The mean age±SD for those \(<65\) yr is 42.8±13.8 yr; for those \(>65\) yr, 77.4±8.8 yr.

BMI, body mass index; C, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; Trig, triglycerides.

*Adjusted for sex and BMI.

†Distribution of genotypes not significantly different from Hardy-Weinberg proportions.

### Table 5. Haplotype Distribution of 324 Normal Low Density Lipoprotein Receptor Genes for Four Restriction Fragment Length Polymorphisms Determined by Family Analysis

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>(Pvu) II</th>
<th>(Ape)LI (5')</th>
<th>(Ape)LI (3')</th>
<th>(Ava) II</th>
<th>(n)</th>
<th>Frequency</th>
<th>Average excess (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P1A1V1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>58</td>
<td>0.179</td>
<td>3.05</td>
</tr>
<tr>
<td>(P1A1V2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>3</td>
<td>0.009</td>
<td>-12.80</td>
</tr>
<tr>
<td>(P1A2V1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>4</td>
<td>0.012</td>
<td>-12.27</td>
</tr>
<tr>
<td>(P1A2V2)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>20</td>
<td>0.062</td>
<td>8.35</td>
</tr>
<tr>
<td>(P1A3V1)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>0.435</td>
<td>4.55</td>
<td></td>
</tr>
<tr>
<td>(P1A3V2)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>141</td>
<td>0.003</td>
<td>-13.97</td>
</tr>
<tr>
<td>(P2A1V1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.025</td>
<td>13.49</td>
</tr>
<tr>
<td>(P2A2V1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>0.269</td>
<td>-11.49</td>
</tr>
<tr>
<td>(P2A2V2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>0.006</td>
<td>3.68</td>
</tr>
</tbody>
</table>

The 324 low density lipoprotein (LDL) receptor genes were studied in 162 unrelated individuals. A total of 522 individuals from 100 families have been studied.

The effect associated with haplotypes on adjusted LDL cholesterol levels (average excess) was calculated according to Templeton.24
phisms such as haptoglobin and secretor, and associations between these protein markers and differences in cholesterol levels have been reported. The identification of candidate genes such as enzymes of lipid metabolism and apolipoproteins genetically linked to some of these protein markers suggests mechanism for these associations. Variation in the amino acid sequence and function of some of these candidate genes, such as apo E, has led to a greater understanding of the impact of common polymorphisms in determining interindividual differences in plasma cholesterol. There are three common isoforms of the apo E protein, designated E2, E3, and E4. The E2 allele is associated with a lowering of serum cholesterol and the E4 with an elevation of serum cholesterol. Taken together, this polymorphism explains 5–8% of the variance in serum cholesterol levels in populations from several different countries.

More recently, neutral variation at candidate gene loci detected by RFLPs has been used to look for an association with plasma and LDL cholesterol levels. The most consistent finding has been an association between the common Xba I variable site in the apo B gene (which does not alter an amino acid) and levels of plasma cholesterol. The mechanism of this association is unknown, but it is presumed that the RFLP is in population association with a DNA sequence change that alters a functionally important amino acid in apo B and thus alters the affinity of the LDL particle for the normal LDL-R. This causes alterations in the clearance rate of LDL from the plasma and, thus, genotype-associated differences in plasma LDL cholesterol. Similar associations between RFLPs in other apoprotein genes and lipid, lipoprotein, and apoprotein levels have been reported, but the mechanism of these associations is also unknown.

The level of LDL in the plasma is the result of the balance between production and metabolism of apo B–containing lipoproteins from the liver and their rate of removal via receptor-independent and receptor-dependent pathways. For apo B–containing lipoproteins, removal by the LDL-R makes a major contribution, either by direct interaction with apo B in LDL particles or by the apo E–mediated removal of the very low density lipoprotein remnants, which are precursors of the LDL particles. Common variation in the LDL-R gene that affects either the affinity of the receptor for apo B on LDL or that affects expression of the gene and thus the number of receptors on the surface could contribute to interindividual differences in plasma LDL cholesterol levels.

Although an earlier report failed to detect a significant association between LDL-R genotype and plasma lipid levels, a study of men in Norway and of men and women in Germany have both reported a significant association between the Pvu II RFLP and plasma total and LDL cholesterol levels. Our results confirm this association, and in all three studies the affect associated with the Pvu II rare allele (cutting site) is to lower total and LDL cholesterol levels. The correspondence of these three studies can leave no doubt that variation at the LDL-R locus, as detected by the Pvu II RFLP, contributes to the determination of plasma cholesterol levels in normolipidemic healthy individuals. It is likely that in all three studies, an association has been detected between the same functionally important sequence change and the neutral Pvu II site in intron 15. Recently, a similar association between an LDL-R RFLP and plasma LDL cholesterol and apo B levels was observed in a study in baboons. It is interesting that in this study, the variable site used to detect the association (Avn II in intron 17) is very close to the variable Pvu II site in the human LDL-R gene that is most strongly associated with the effect.

The magnitude of the effect associated with the Pvu II RFLP is larger in this sample of Italian individuals than that reported in Germany (about 3% sample variance) and in Norway. In the German study, the average excess value associated with the Pvu II cutting allele was –3 mg/dl, considerably smaller than the value we estimate in the Italian sample (–9 mg/dl). These differences may be due to chance and small sample size, differences in genetic background, or differences in the environmental factors that contribute to the total sample variance.

The effect on cholesterol lowering associated with the Pvu II cutting site was evident in both young and old individuals. Moreover, it appears that the effect on cholesterol lowering associated with the allele may be one factor that promotes increased survival, since the allele is present in a significantly higher frequency in the sample of individuals over 65 years old. In a similar way, differences in frequencies of the apo E alleles have been reported in octogenarians and in survivors of heart attacks, presumably acting

<table>
<thead>
<tr>
<th>RFLP</th>
<th>Chromosomes (n)</th>
<th>Delta value</th>
<th>95% confidence limits</th>
<th>$\chi^2$ (1 df)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pvu II–AvnLI (3')</td>
<td>378</td>
<td>–0.639</td>
<td>0.575–0.694</td>
<td>154.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pvu II–AvnLI (5')</td>
<td>378</td>
<td>–0.297</td>
<td>0.202–0.385</td>
<td>33.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ava II–AvnLI (3')</td>
<td>324</td>
<td>–0.807</td>
<td>0.767–0.843</td>
<td>212.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ava II–AvnLI (5')</td>
<td>324</td>
<td>–0.416</td>
<td>0.320–0.501</td>
<td>56.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pvu II–Ava II</td>
<td>324</td>
<td>–0.497</td>
<td>0.405–0.570</td>
<td>78.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

RFLP, restriction fragment length polymorphism.
through the documented effect of the apo E alleles on plasma lipid levels and, thus, atherosclerosis.

In the sample we have studied, the Pvu II cutting site occurs on four different haplotypes determined by the other three variable sites. We have attempted to use additional RFLP information to identify the particular haplotype that is most strongly associated with the lowering of LDL cholesterol levels. Because of linkage disequilibrium and the small number of individuals, this analysis has not been conclusive, but our data are compatible with the hypothesis that a single functionally important sequence change has occurred at the LDL-R locus and that this can be detected through variable disequilibrium with all three RFLP enzyme sites. Identification of this sequence change would allow the mechanism of the association to be understood and allow a more accurate estimate of the impact of variation at the locus in determining plasma cholesterol levels in healthy individuals.

References


**KEY WORDS** • low density lipoprotein receptor • restriction fragment length polymorphisms • low density lipoprotein cholesterol
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