Variable Expression of the Mutation in Familial Defective Apolipoprotein B-100

J.J. Gallagher and N.B. Myant

Although most subjects with familial defective apolipoprotein B-100 (FDB) have raised plasma low-density lipoprotein (LDL) levels, a few have LDL levels within the normal range. We have previously identified two normocholesterolemic FDB heterozygotes in an affected family. Results obtained from a study of this family are compatible with a major genetic contribution to the normocholesterolemia in the two heterozygotes. However, our findings are not compatible with inheritance of a variant normal allele at the apolipoprotein B locus in this family that neutralizes the effect of an FDB allele on the plasma LDL level. Polymorphic variations at the apolipoprotein E and LDL receptor loci did not explain the presence of normal LDL levels in the two heterozygous FDB subjects. (Arteriosclerosis and Thrombosis 1993;13:973-976)

KEY WORDS • familial defective apolipoprotein B-100 • plasma LDL level • variable gene expression

Familial defective apolipoprotein (apo) B-100 (FDB) is a rare disorder of lipoprotein metabolism caused by a mutation in the apoB gene.1 The mutation gives rise to the substitution of arginine for glutamine at residue 3500 in apoB-100, the protein of low-density lipoprotein (LDL). The effect of the Arg->Gln substitution is to reduce the binding of LDL by the LDL receptor by more than 90%.2 In most FDB heterozygotes, this gives rise to hypercholesterolemia caused by the accumulation of LDL particles containing mutant apoB-100.3 However, the plasma LDL concentrations in age-matched heterozygotes vary over a wide range, and in some affected families the serum LDL cholesterol (LDL-C) and total cholesterol levels are normal in one or more heterozygous carriers of the mutation.4,5 We have described a family with a total of nine FDB heterozygotes, two of whom had normal LDL levels on repeated measurement in the absence of treatment/ and Friedl et al5 report a heterozygous man whose plasma LDL-C level was 3.5 mmol/L. Friedl et al5 suggest that the low LDL level in their FDB patient was due to the presence of a mutation in the normal ("non-FDB") allele at the apoB locus that leads to underrepresentation of its product in the plasma. In the present work we tested the hypothesis that the normal cholesterol levels in our two atypical FDB heterozygotes are also caused by the presence of a variant normal allele at the apoB locus that counteracts the hypercholesterolemic effect of the mutant allele. Our results were not consistent with this hypothesis.

Methods

Subjects

Four FDB heterozygotes from an FDB family, together with three of their unaffected relatives, were examined. The characteristics of the seven subjects are shown in Table 1. The members of the AE family shown in Table 1 and the Figure are a branch of the more extended family described in Reference 4. The upper limit of the normal range for plasma LDL-C concentration in men and women was taken as 5.0 mmol/L6 and 3.3 mmol/L for children aged 2 through 14 years.7 FDB was diagnosed by analysis of the subject’s genomic DNA, as described.8 High-affinity binding of LDL by normal LDL receptors on fibroblasts in culture was determined by a competitive binding assay, as previously described.9 The test sample of LDL was added in triplicate, at increasing concentrations, in the presence of 125I-labeled normal LDL (3 μg protein/mL medium), to wells containing monolayers of fibroblasts after induction of receptors by growth in the presence of lipoprotein-deficient serum for 48 hours. The concentration of test sample required to displace 50% of the 125I-LDL from the fibroblasts (IC50) was calculated from the competition curves, as described.9

Plasma Lipids and Lipoproteins

Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), and total triglyceride concentrations were determined as described elsewhere.4 Plasma LDL-C concentration was estimated by using the Friedewald equation.10

Haplotype Analysis

Genotypes were determined at five polymorphic sites in the apoB gene and at two polymorphic sites in the LDL receptor gene. For the apoB gene, genotypes at the insertion/deletion site in exon 1, at the restriction sites for XbaI and EcoRI, and at the 3' hypervariable region (HVR) were determined as described.4 The sizes of the HVR fragments were determined by comparison with DNA markers of known lengths, kindly supplied by Alison Dunning. The genotype at the ApaL1 site in the apoB gene was determined by the method of Young and
TABLE 1. Characteristics of AE Family FDB Heterozygotes and Their Relatives

<table>
<thead>
<tr>
<th>Subject</th>
<th>FDB</th>
<th>Sex</th>
<th>Age</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>ApoE phenotype</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 1</td>
<td>+</td>
<td>M</td>
<td>61</td>
<td>8.7</td>
<td>0.8</td>
<td>7.3</td>
<td>3/3</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>6.2</td>
<td>4.3*</td>
<td>3/3</td>
<td>20.3</td>
</tr>
<tr>
<td>II 1</td>
<td>+</td>
<td>M</td>
<td>35</td>
<td>7.2</td>
<td>1.0</td>
<td>5.6†</td>
<td>3/3</td>
<td>23.4</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>M</td>
<td>34</td>
<td>3.8</td>
<td>0.8</td>
<td>2.6</td>
<td>3/3</td>
<td>23.6</td>
</tr>
<tr>
<td>3†</td>
<td>+</td>
<td>M</td>
<td>37</td>
<td>5.0</td>
<td>0.9</td>
<td>3.4†</td>
<td>3/3</td>
<td>22.3</td>
</tr>
<tr>
<td>III 1†</td>
<td>+</td>
<td>M</td>
<td>8</td>
<td>3.6</td>
<td>0.5</td>
<td>2.7†</td>
<td>3/3</td>
<td>18.7</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>F</td>
<td>6</td>
<td>3.6</td>
<td>0.9</td>
<td>2.6</td>
<td>3/3</td>
<td>18.7</td>
</tr>
</tbody>
</table>

FDB, familial defective apolipoprotein (apo) B-100; TC, plasma total cholesterol; TG, plasma triglyceride concentration; LDL-C, low-density lipoprotein cholesterol; BMI, body mass index. Subject 1 is III 5 in Reference 4.

*Mean of two determinations separated by 3 years. All lipid measurements were made before treatment with lipid-lowering drugs. For pedigree see Figure.
†Mean of three or more determinations made at intervals of at least 6 months.
‡FDB with normal plasma LDL-C concentration.

Hubl.1 Genotypes at the AvaII and HinClI polymorphic sites in the LDL receptor gene were determined as described.4 Haplotypes at the apoB and LDL receptor loci were deducible from the genotypes in all seven individuals.

ApoB Haplotypes

Five different apoB haplotypes, based on six variable sites at or near the apoB locus, were identified in the seven members of the AE family (Table 2). These included the haplotype (designated x in Reference 4 and Table 2 and A in Reference 5) that specifies the apoB allele bearing the mutation at codon 3500 in most white FDB heterozygotes reported from Europe and North America.4-5-12-13

The AE Family

The branch of the AE family investigated in this study comprised seven individuals and included two heterozygous brothers (II 1 and II 3), one hypercholesterolemic and the other normocholesterolemic, and a heterozygous boy (III 1) who was normocholesterolemic. The affected father of the two brothers (I 1) was hypercholesterolemic. Their unaffected mother was normocholesterolemic, but her age-adjusted LDL-C level was close to the 90th percentile for women.14 The two brothers were of similar age and body mass index, both had apoE phenotype 3/3, and both were nonsmokers. The plasma LDL-C and total cholesterol levels in the 8-year-old heterozygous boy were similar to those of his normal 6-year-old sister.

The inheritance of haplotypes at the apoB and receptor loci is shown in the Figure. The two FDB brothers (II 1 and II 3) inherited different normal (non-FDB) apoB alleles from their mother. The two brothers also inherited different LDL receptor alleles from their mother, II 1 receiving B and II 3 receiving A. III 1 inherited one of the two receptor alleles with haplotype B present in his father. Since his father was hypercholesterolemic, neither of these alleles is likely to have had a significant cholesterol-lowering effect. From his mother, III 1 inherited a receptor allele with haplotype C.

Receptor binding of LDLs from II 1 and II 3 was defective, and the defect was equally marked in the two samples. The IC₅₀ for LDL from II 1 was 12.2 μg/mL and from II 3 was 13.9 μg/mL compared with a mean value of 3.4±0.24 μg/mL obtained from LDLs from seven normal donors.

Discussion

Variation between different individuals in the expression of a harmful mutation may be explained by differ-
we have noted marked hypercholesterolemia in an FDB heterozygote. II 1 was consistently hypercholesterolemic, whereas II 3 has remained normocholesterolemic in the absence of drug treatment throughout an almost 3-year period of observation. In addition, the plasma LDL level in II 3 was near the upper limit of the normal range. It should also be noted that if the apoB allele with haplotype c were responsible for the normal LDL level in III 1, the presence of this allele in the AE family would not explain the normal plasma LDL level in III 1, who inherited from his mother a non-FDB allele (with haplotype a) that was different from the non-FDB allele in his heterozygous uncle (II 3). Moreover, the IC\textsubscript{50} of LDL from II 1 and II 3 were increased to the same extent. This is not compatible with the presence of an abnormally high proportion of defective LDL particles in the plasma of II 3, as observed in J.H. It should also be noted that if the apoB allele with haplotype c were responsible for the normal LDL level in II 3, the presence of this allele in I 2 should have caused her to have an abnormally low LDL level. In fact, her LDL level was near the upper limit of the normal range. These findings argue against the presence of a mutation in the non-FDB allele at the apoB locus in the AE family that leads to underrepresentation of its product in the plasma.

The inheritance of maternal LDL receptor alleles by the two brothers II 1 and II 3 is consistent with the possibility that receptor allele A lowered the plasma LDL level in II 3. However, if the presence of this allele is a sufficient cause of the normal level in II 3, its presence in I 2 should have caused her to be hypercholesterolemic. Subject III 1 inherited receptor allele B from his hypercholesterolemic father and receptor allele C from his unaffected mother. Since his mother had low plasma total cholesterol and LDL-C levels, it is possible that the maternal receptor allele with haplotype C contributed to the low plasma LDL level in II 2 and III 1.

Although our findings are hard to reconcile with the hypothesis that the normal LDL levels in two heterozygotes resulted from the inheritance of a variant normal
apoB allele, they do not exclude the possibility that the normal levels in these individuals were caused by the combined effects of several genes, each having a small effect on the plasma LDL when acting alone. Possible components of a polygenic system acting on the plasma LDL include polymorphic variants at the apoB and LDL receptor loci and at other gene loci that influence the metabolism of LDL or the rate of production or catabolism of its lipoprotein precursors. Variation at the apoE locus cannot have contributed to variability in plasma LDL level in this family because all subjects investigated had the same apoE phenotype.

References
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doi: 10.1161/01.ATV.13.7.973

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