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.4-3 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 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/L 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 Xba I 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 ApaLI 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 (y)</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</td>
<td>1+</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>2−</td>
<td>F</td>
<td>60</td>
<td>6.2</td>
<td>1.1</td>
<td>4.3*</td>
<td>3/3</td>
<td>20.3</td>
</tr>
<tr>
<td>II</td>
<td>1+</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></td>
<td>2−</td>
<td>F</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></td>
<td>3†</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</td>
<td>1†</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></td>
<td>2−</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
Genotypes at the AvaII and HindII polymorphic sites in the LDL receptor gene were determined as described.† Haplotypes at the apoB and LDL receptor loci were deducible from the genotypes in all seven individuals.

### Results

**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.† 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. 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-
ence in environmental and genetic factors that influence the phenotype. In heterozygous familial hypercholesterolemia (FH), eg, interindividual variation in plasma LDL levels and in clinical signs is probably due to a combination of environmental effects, such as habitual diet, and genetic effects, which may include differences in the nature of the mutation in the mutant LDL receptor allele.3 Hobbs et al10 described an FH family in which some individuals appear to have inherited a dominant gene that suppresses hypercholesterolemia, resulting in normal plasma LDL levels in FH heterozygotes who have inherited the suppressor gene.

In contrast to FH, all FDB heterozygotes have an identical mutation in the mutant allele that gives rise to their disorder. Apart from this difference from FH, factors analogous to those responsible for variability in expression of the FH gene are likely to be responsible for variability in the plasma LDL concentration in FDB heterozygotes. Nongenetic factors may well make a major contribution to variability in the severity of hypercholesterolemia in the FDB population as a whole. However, a predominantly genetic basis for variability seems more likely in cases in which one or two FDB heterozygotes within a family are normocholesterolemic while other affected members are hypercholesterolemic. In the AE family, the two heterozygotes II 1 and II 3 were both men in their mid-30s, both ate a modified low-fat diet, and their body mass indexes were similar. Nevertheless, 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 III 1, who had FDB, was similar to that in his unaffected normocholesterolemic sister, although both ate the same diet and had similar body mass indexes. The absence of hypercholesterolemia in this 8-year-old boy is unlikely to have been due to a normally delayed expression of the FDB mutation, since we have noted marked hypercholesterolemia in an FDB heterozygote at age 2 years.4 These findings strongly suggest a major genetic contribution both to the difference in plasma LDL levels in the sibling pair II 1 and II 3 and to the similarity in the levels in III 1 and III 2.

In the FDB family described by Friedl et al,6 one of six heterozygotes (J.H.) was normocholesterolemic. J.H. had no siblings, so that a sibling-pair comparison was not possible. However, the haplotype of his non-FDB allele at the apoB locus was different from that of the non-FDB alleles in his five hypercholesterolemic FDB relatives. Friedl et al6 suggested that J.H. had inherited a rare mutant non-FDB allele at the apoB locus, whose product was underrepresented in his plasma. In support of this, they showed that the IC50 of J.H.’s LDL (determined by a competitive binding assay) was two to three times the IC50 of the LDLs obtained from his heterozygous relatives, and that this anomaly was due to the presence of an abnormally high proportion of LDL particles in his plasma containing apoB encoded by the FDB allele.

Taken by itself, our observation that AE II 1 and II 3 inherited different non-FDB alleles from their unaffected mother is consistent with the possibility that the apoB allele specified by haplotype c suppressed the hypercholesterolemic effect of the FDB allele in II 3. However, 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 IC50 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

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### Table 2. Haplotypes Based on Six Variable Sites in or Near the ApoB Locus in a Family With FDB

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>I/D</th>
<th>ApoLI</th>
<th>Xba I</th>
<th>3500</th>
<th>EcoRI</th>
<th>HVR</th>
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<tbody>
<tr>
<td>x</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Gln</td>
<td>-</td>
<td>49</td>
</tr>
<tr>
<td>a</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Arg</td>
<td>-</td>
<td>47</td>
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<tr>
<td>b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Arg</td>
<td>+</td>
<td>41</td>
</tr>
<tr>
<td>c</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Arg</td>
<td>+</td>
<td>29</td>
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<tr>
<td>d</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Arg</td>
<td>-</td>
<td>43</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Arg</td>
<td>+</td>
<td>35</td>
</tr>
</tbody>
</table>

Apo, apolipoprotein; FDB, familial defective apoB-100; I/D, insertion or deletion in exon 1; HVR, 3' hypervariable region (number of 15-base repeats); +, insertion or restriction site present or (-) absent. x is the haplotype in which the 3500 mutation in the apoB gene is present. E is the haplotype of the normal apoB gene in the normocholesterolemic FDB heterozygote referred to in Reference 5.
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

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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