High frequency of APOB gene mutations causing familial hypobetalipoproteinaemia in patients of Dutch and Spanish descent

S W Fouchier, R R Sankatsing, J Peter, S Castillo, M Pocovi, R Alonso, J J P Kastelein, J C Defesche

INTRODUCTION

Familial hypobetalipoproteinaemia (FHBL) is an autosomal co-dominant hereditary disorder of lipoprotein metabolism characterised by decreased low density lipoprotein (LDL) cholesterol and apolipoprotein B (APOB) plasma levels. High levels of plasma APOB and LDL cholesterol are strong predictors for risk of cardiovascular disease (CVD), while individuals with low APOB and LDL cholesterol levels are thought to have lower than average risk for CVD, and in fact, heterozygous FHBL patients appear to be asymptomatic. APOB is a key structural component of triglyceride and cholesterol and apolipoprotein B (APOB) plasma levels. 12

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Methods: Rather than identifying truncated APOB proteins in plasma fractions separated by gel electrophoresis, which will most likely miss any mutations in proteins smaller than 30 kb, we analysed the APOB gene directly, using PCR.

Results: We identified nine different mutations, six of which are novel. Each mutation showed complete co-segregation with the FHBL phenotype in the families, and statistically significant differences between carriers and non-carriers were found for plasma total, LDL, and HDL cholesterol, triglycerides, and APOB levels, but not for APOA1 levels. All carriers of an APOB mutation were completely free from CVD.

Conclusions: Prolonged low levels of LDL cholesterol and elevated levels of HDL cholesterol may reduce the progression of atherosclerotic disease, but this has not been unequivocally shown that this is indeed the case in individuals with FHBL, and is the subject of a current study.

MATERIALS AND METHODS

Study subjects were selected by analysis of cholesterol levels collected during the course of a number of studies addressing several forms of genetic dyslipidaemia. Selection criterion was an LDL cholesterol level below the fifth percentile for sex and age.14 Secondary causes for low LDL cholesterol levels, such as vegetarian diet, low fat diet, or cancer, were excluded. The probands were of Dutch or Spanish descent and provided information on their own health status and the structure of their kindreds. Blood samples were obtained from probands and their relatives after an overnight fast of at least 12 hours. All study subjects provided written informed consent and the study protocol was approved by the institute’s ethics review board.

Plasma concentrations of total cholesterol, HDL cholesterol, and triglycerides were measured by commercially available kits (Boehringer Mannheim, Mannheim, Germany). LDL cholesterol concentrations were calculated by the Friedewald formula only when the triglyceride concentration was <4.5 mmol/L.16 APOB and APOAI were determined on a Behring nephelometer BN100 using standard and references supplied by the manufacturer (Behring, Marburg, Germany). Genomic DNA was prepared from 10 ml whole blood on an AutopureLS apparatus according to manufacturer’s protocol (Gentra Systems, Minneapolis, MI, USA).

ONLINE MUTATION REPORT

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To analyse the promoter region, all 29 exons and the intronic boundaries of APOB, 54 pairs of primers were designed. PCR amplification was carried out with 50 ng of genomic DNA in a 25 µl reaction volume containing 1 × Taq DNA polymerase buffer (Qiagen, Hilden, Germany), 50 µmol/l of each dNTP, 0.4 µmol/l of each primer, 5 µg bovine serum albumin, and 1 U Taq DNA polymerase. The thermal cycling conditions were as follows: 96°C for 5 minutes, then 35 cycles of 20 seconds at 96°C, 20 seconds at 55°C to 60°C (depending on primer Cg content), and 30 seconds at 74°C in a PCR apparatus (T3 Biocycler, Biometra, Germany). The reaction sequences were performed using fluorescently labelled diodeoxy chain terminations with a Big Dye Terminator ABI Prism kit (Applied Biosystems, Foster City, CA, USA) according manufacturer’s protocol and analysed on an Applied Biosystems automated DNA sequencer (model 3730). Sequences were analysed with the Sequencher package (GeneCodes Co, Ann Arbor, MI, USA).

All data were analysed using SPSS software (version 10.1; SPSS, Chicago, IL, USA) by one way analysis of variance and by multiple linear regression analyses with adjustment for age and sex. A p value <0.05 was considered to be statistically significant.

RESULTS

We identified 32 individuals meeting our inclusion criterion. After sequence analysis of APOB, we identified nine different mutations in 14 of our probands (table 1).

The R412X, 11712delC, and 20507delX mutations, resulting in truncated APOB-9 and APOB-86 proteins, respectively, and the missense mutation, R463W, 18 have been described previously. Additionally, we identified six novel APOB mutations resulting in truncated APOB of different sizes. The frameshift causative deletion of nucleotides AT at base pair 1718 (1718delAT) in exon 13 resulted in a stop codon at amino acid position 547, which leads to a predicted APOB-12 protein. Deletion of an adenosine at nucleotide 2534 (2534delA) in exon 17 causes a frameshift resulting in amino acid changes running from amino acid 835 to 834, and finally a stop codon at amino acid 925, which leads to a truncated APOB-20 protein. A nonsense mutation comprising a single C→T transition of nucleotide 4006 in exon 25, thereby creating a Δdel restriction site, changes the codon for glutamine at amino acid 1309 into a stop codon (Q1309X), leading to a predicted APOB-29 protein. The C→T substitution at position 6700 in exon 26, converting an arginine at amino acid 2507 into a stop codon (R2507X). The deletion of a cystidine at base pair 2783 (2783delC), resulted in amino acid changes running from amino acid 818 to 834, and finally a stop codon at amino acid 925, leading to a truncated APOB-18 protein. The deletion of an adenosine at nucleotide 2534 (2534delA) in exon 17 causes a frameshift resulting in amino acid changes running from amino acid 835 to 834, and finally a stop codon at amino acid 925, which leads to a truncated APOB-20 protein. A nonsense mutation comprising a single C→T transition of nucleotide 4006 in exon 25, thereby creating a Δdel restriction site, changes the codon for glutamine at amino acid 1309 into a stop codon (Q1309X), leading to a predicted APOB-29 protein. The C→T substitution at position 6700 in exon 26, converting an arginine at amino acid 2507 into a stop codon (R2507X). The deletion of a cystidine at base pair 2783 (2783delC), resulted in amino acid changes running from amino acid 818 to 834, and finally a stop codon at amino acid 925, leading to a truncated APOB-18 protein. The deletion of an adenosine at nucleotide 2534 (2534delA) in exon 17 causes a frameshift resulting in amino acid changes running from amino acid 835 to 834, and finally a stop codon at amino acid 925, which leads to a truncated APOB-20 protein. A nonsense mutation comprising a single C→T transition of nucleotide 4006 in exon 25, thereby creating a Δdel restriction site, changes the codon for glutamine at amino acid 1309 into a stop codon (Q1309X), leading to a predicted APOB-29 protein. The C→T substitution at position 6700 in exon 26, converting an arginine at amino acid 2507 into a stop codon (R2507X). The deletion of a cystidine at base pair 2783 (2783delC), resulted in amino acid changes running from amino acid 818 to 834, and finally a stop codon at amino acid 925, leading to a truncated APOB-18 protein. The deletion of an adenosine at nucleotide 2534 (2534delA) in exon 17 causes a frameshift resulting in amino acid changes running from amino acid 835 to 834, and finally a stop codon at amino acid 925, which leads to a truncated APOB-20 protein. A nonsense mutation comprising a single C→T transition of nucleotide 4006 in exon 25, thereby creating a Δdel restriction site, changes the codon for glutamine at amino acid 1309 into a stop codon (Q1309X), leading to a predicted APOB-29 protein. The C→T substitution at position 6700 in exon 26, converting an arginine at amino acid 2507 into a stop codon (R2507X).

Table 1  Apolipoprotein B mutations identified

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutation</th>
<th>WT</th>
<th>MT</th>
<th>Pos (bp)</th>
<th>Predicted size</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>R412X</td>
<td>CGA</td>
<td>TGA</td>
<td>1315</td>
<td>APOB-9</td>
<td>SP809</td>
</tr>
<tr>
<td>11</td>
<td>R463W</td>
<td>CGG</td>
<td>TGG</td>
<td>1468</td>
<td>NA</td>
<td>SP810</td>
</tr>
<tr>
<td>13</td>
<td>1718delAT</td>
<td>AT</td>
<td>delAT</td>
<td>1718</td>
<td>APOB-12</td>
<td>SP807</td>
</tr>
<tr>
<td>17</td>
<td>2534delA</td>
<td>A</td>
<td>delA</td>
<td>2534</td>
<td>APOB-18</td>
<td>NL804</td>
</tr>
<tr>
<td>18</td>
<td>2783delC</td>
<td>C</td>
<td>delC</td>
<td>2783</td>
<td>APOB-20</td>
<td>NL826, NL827</td>
</tr>
<tr>
<td>25</td>
<td>Q1309X</td>
<td>CAA</td>
<td>AAA</td>
<td>4006</td>
<td>APOB-29</td>
<td>NL808, NL822</td>
</tr>
<tr>
<td>26</td>
<td>R2507X</td>
<td>CGA</td>
<td>TGA</td>
<td>7600</td>
<td>APOB-55</td>
<td>NL809, SP812</td>
</tr>
<tr>
<td>26</td>
<td>11548delTT</td>
<td>TT</td>
<td>delTT</td>
<td>11548</td>
<td>APOB-84</td>
<td>NL825</td>
</tr>
<tr>
<td>26</td>
<td>11712delC</td>
<td>C</td>
<td>delC</td>
<td>11712</td>
<td>APOB-86</td>
<td>NL801, NL802</td>
</tr>
</tbody>
</table>

Pos: position. The reference sequence used was NM_000384, with the A of the ATG translation initiation codon numbered nucleotide +1 and the methionine numbered as amino acid –27.

DISCUSSION

In 14 of 32 probands with low cholesterol levels, we were able to identify an APOB gene mutation, resulting mainly in truncated forms of APOB. Although the functionality of these mutations was not validated in a strictest sense, it is well established that truncated APOB proteins are the cause of FHBL. Moreover, all mutations co-segregated with the FHBL phenotype, and therefore it seems likely that these variants are the cause of the FHBL phenotype in our families.
In one case, occasional episodes of diarrhoea were noted, which disappeared after introduction of a diet low in carbohydrates and triglycerides. Different composition in terms of cholesterol and triglyceride levels. The normal level of cholesterol, triglycerides, and APOB levels, but not for APOA1 levels. The low levels of LDL cholesterol are the result of failure to produce normal amounts of VLDL from truncated APOB. The assessment of lipoprotein fractions would have been helpful in the diagnosis of FHBL, with or without neurological complaints, has been described before in five cases and our patients do not seem to differ clinically from those examined by others.20–23 However, additionally, this difference could be explained by the fact that FHBL probands differ clinically from those examined by others.20–23

Some of the FHBL patients presented with a mild clinical phenotype not definitely linked to their lipoprotein disorder. In one case, occasional episodes of diarrhoea were noted, consistent with FHBL, and in another case mild neurological symptoms were found, which diminished after supplementation with vitamin E. The two cases of diabetes were in all likelihood not related to FHBL. The combination of DM and FHBL, with or without neurological complaints, has been described before in five cases and our patients do not seem to differ clinically from those examined by others.20–23

One interesting case was an 8-year-old girl, initially diagnosed with familial defective apolipoprotein B (FDB) by the R350Q mutation that she inherited from her father. However, her lipid profile (TC 5.71 mmol/l; LDL cholesterol 3.25 mmol/l; HDL cholesterol 0.81 mmol/l; triglycerides 1.04 mmol/l; apoB 1.20 g/l) did not match the phenotypic characteristic of FDB. The subsequent identification of the 11712delC mutation, inherited from her father, explained her normal cholesterol level. As the girl had no complaints associated with neither FDB nor FHBL, we could assume that the resulting phenotypic expression is a consequence of the compensation of one disorder by the other.

The precise prevalence of APOB gene mutations causing truncated APOB and FHBL is not known. However, several larger studies in individuals with persistent low levels of total and LDL cholesterol show an estimated frequency between 1.4% and 2.7%.28–31 From these studies, it appears that truncated APOB is rare in healthy subjects with low LDL cholesterol levels. However, these data are very different from the frequency of 52% for the mutations we identified in our study population. This discrepancy might be explained by a number of different factors. Firstly, we only included individuals free from any secondary causes of hypocholesterolaemia, secondly, we applied very strict inclusion and exclusion criteria for enrolment, and lastly, our cohort was substantially larger than any other previously studied. Additionally, this difference could be explained by the approach used, as we choose to analyse the APOB gene by direct sequencing, rather than through analysing the APOB protein. The assessment of lipoprotein fractions would have been helpful in the diagnosis of FHBL, with or without neurological complaints, has been described before in five cases and our patients do not seem to differ clinically from those examined by others.20–23

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1. Analysis of the proband cohort studied after DNA analysis of the APOB gene.

2. Clinical characteristics of FHBL carriers and their unaffected relatives.

3. Low cholesterol probands.
failed to detect most of our mutations, as they represent truncated APOB proteins smaller than APOB-30.

Interestingly, we were not able to identify a causal APOB gene mutation in all FHBL patients. Although we sequenced at least 50 bp into each intron and analysed up to 600 bp upstream of the promoter region, the presence of mutations outside these regions could not be ruled out, nor the presence of a large deletion or insertion. Additionally, as yet unidentified genes could be the cause of the FHBL phenotype in these kindreds, as evidence is accumulating that other genetic factors besides the APOB gene may lead to a FHBL trait, such as loci identified on chromosome 3p21.1–22 and chromosome 13q. Identification of these putative genes would provide novel insights into the mechanisms operating in APOB metabolism.

It is well established that high levels of plasma APOB are strong predictors for risk of cardiovascular disease, but less is known about this risk in individuals with a FHBL phenotype. In our study, all carriers of an APOB mutation were completely free from CVD. It can be hypothesised that prolonged low levels of LDL cholesterol and elevated levels of HDL cholesterol will reduce the progression of atherosclerotic disease. Nevertheless, it has not been unequivocally shown that this is indeed the case in individuals with FHBL. Assessment of the thickness of the intima–media complex in individuals with FHBL, compared with non-affected siblings, could be used to test this hypothesis. Such a study is currently under way in our centre and is the subject of a future report.

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Competing interest: none declared

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