New Variants in the Apolipoprotein AV Gene in Individuals with Extreme Triglyceride Levels

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Summary
Animal studies (on transgenic and knock-out mice) and human association analysis assessed the importance of APOAV gene for plasma triglyceride determination. New APOAV missense variants (Val153 → Met and Cys185 → Gly) have been detected recently. We have analyzed these variants in 83 unrelated patients with extreme lipid parameters (triglycerides of 20.4±12.8 mmol/l and total cholesterol of 10.4±3.7 mmol/l) and in a control population group consisting of 2,559 unrelated Caucasians. In patients, the frequency of the Met153 carriers was slightly but not significantly higher (9.64 % vs. 6.49 %) compared to the population sample. This suggested that Val153 → Met polymorphism in the APOAV gene does not represent an important risk factor for developing the extreme levels of plasma triglycerides. We did not detect carriers of the Gly185 allele among patients or 420 healthy individuals. We suppose that this variant is probably not present in Caucasian populations.

Key words
APOAV • Triglyceride • Polymorphism

Cardiovascular disease (CVD) is the most common cause of death in industrialized countries. Elevated plasma triglycerides (TG) have been shown to be an independent risk factor for CVD (Forester 2001). APOAV gene has been identified in the APOAI/APOCIII/APOAIV gene cluster by comparative sequencing (Pennacchio et al. 2001, Pennacchio and Rubin 2003, Šeda and Šedová 2003). The human APOAV gene consists of 4 exons and codes for a 369-amino acid protein, which is expressed in the liver only. Generation of transgenic and knock-out mice assessed the importance of this gene for plasma triglyceride determination. The transgenic mice exhibit decreased, and the knock-out mice show elevated levels of plasma triglycerides, while the plasma cholesterol levels are not influenced significantly.

In the human APOAV gene, four common variants (T-1131 → C, Ser19 → Trp, Val153 → Met and Cys185 → Gly) have been detected so far (Pennacchio et al. 2002, Talmud et al. 2003, Cohen and Hobbs, personal communication).
communication, Kao et al. 2003). An association between T-1131→C and Ser19→Trp polymorphisms with TG levels has been found in studies with different designs, and some race-specific associations were observed (Pennacchio et al. 2002, Nabika et al. 2002, Talmud et al. 2003, Baum et al. 2003, Hubáček et al. 2004a, b). Additionally, rare alleles of both polymorphisms (C-1131 and Trp19) were associated with extreme TG levels (Ribalta et al. 2002, Hofinek et al. 2003, Vráblík et al. 2003).

The aim of the present study was to evaluate the putative association of two newly described APOAV variations (Val153→Met and Cys185→Gly) with extreme plasma TG concentrations.

The patients were selected from the database of Prague Lipid Clinic of the Third Internal Department, which actively follows almost 2500 patients and has more than a 30-year tradition in the diagnosis and treatment of lipid metabolism disorders. The group of patients consisted of 83 unrelated individuals (67 males and 16 females) aged 50.2±9.3 years with extreme lipid parameters (triglycerides of 20.4±12.8 mmol/l and total cholesterol of 10.4±3.7 mmol/l). For inclusion to the study, initial lipid levels measured without any lipid-lowering medication were considered.

A control group consisted of 2559 unrelated Caucasians (1191 males and 1368 females, aged 28-67 years) selected as a 1 % representative Czech population sample recruited as a follow-up to the MONICA study (Multinational Monitoring of Trends and Determinants in Cardiovascular Diseases: “MONICA Project”. Manual of operations WHO/MNC 82.2, Nov 1983). Their plasma lipid levels were as follows: triglycerides 2.0±1.3 mmol/l (males) and 1.5±0.8 mmol/l (females); total cholesterol 5.8±1.0 mmol/l (males) and 5.8±1.2 mmol/l (females). Body mass index and smoking prevalence in control group was comparable with the patient group, but there were more diabetics among the patients (39.0 % versus 5.2 %).

DNA was isolated by standard salting-out method (Miller et al. 1988). To genotype the Val153→Met polymorphism of the APOAV gene, oppositely-oriented oligonucleotides AV153-F 5’ TGA TGG AGC AGG TGG CCC TGC GAG TGC AG and AV153-R 5’ TCA CCA GGC TCT CGG CGT ATG GGT GG were used. A 10 µl of PCR product was digested in a total volume of 25 µl with 1 U of Bsh1236I (Fermentas) at 37°C overnight in the buffer provided by the manufacturer. DNA fragment containing the Cys185→Gly variant was amplified with oligonucleotides AV185-F 5’ AGA CAC CAA GGC CCA GTT GCT GGG and AV185-R 5’ ATG CCG CTC ACC AGG CTC TCG GCG. A 10 µl of PCR product was digested in a total volume of 25 µl with 5 U of HaeIII (Fermentas) at 37°C overnight in the buffer provided by the manufacturer. The restriction fragments were analyzed by 10 % polyacrylamide microtiter array diagonal gel electrophoresis (Day and Humphries 1994), stained with ethidium bromide and visualized on a UV transilluminator.

The lipoprotein parameters were measured enzymatically in the Regional Lipid Reference Center (IKEM, Prague) with a Roche COBAS MIRA autoanalyzer, using conventional enzymatic methods. Body mass index was calculated as weight in kg divided by height in meters squared. Statistical analysis was performed using the chi-square test with Yates correction.

In 83 hypertriglyceridemic individuals and 420 healthy controls, we did not find carriers of the Gly185 allele. We suppose, that this variant is not present in Caucasians so we did not analyzed all control DNAs. Originally this variant was found in Chinese (Kao et al. 2003) and its presence is probably race-specific.

Table 1. Allele and genotype distributions of the Val153→ Met polymorphism in the APOAV gene in hypertriglyceridemic patients and in controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>%</th>
<th>Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val</td>
<td>4951</td>
<td>96.74</td>
<td>158</td>
<td>95.18</td>
</tr>
<tr>
<td>Met</td>
<td>167</td>
<td>3.26</td>
<td>8</td>
<td>4.82</td>
</tr>
<tr>
<td>Val/Val</td>
<td>2393</td>
<td>93.51</td>
<td>75</td>
<td>90.36</td>
</tr>
<tr>
<td>Val/Met</td>
<td>165</td>
<td>6.45</td>
<td>8</td>
<td>9.64</td>
</tr>
<tr>
<td>Met/Met</td>
<td>1</td>
<td>0.04</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>
The pattern of distribution of APOAV genotypes of the Val153 → Met polymorphism is summarized in Table 1. The frequency of carriers of the Met153 allele was slightly higher (9.64 % vs. 6.49 %, n.s.) in patients with extreme TG levels compared to the population sample.

Previously, rare alleles of APOAV polymorphisms have been associated with high plasma TG levels. Pennacchio et al. (2001) have found a higher frequency (22.3 % vs. 6.7 %) of C-1131 carriers in 169 males with plasma TG>90 % of the population compared to 298 males from the opposite end of the distribution curve (TG<10 %). The same authors (Pennacchio et al. 2002) described a higher frequency (22.7 % vs. 6.0 %) of Trp19 carriers in 132 individuals with plasma TG > 90 % compared to 132 individuals from the opposite end of the distribution curve (TG<10 %).

Ribalta et al. (2002) have reported a higher frequency of C-1131 carriers in 42 hypertriglyceridemic individuals from 16 families with familiar combined hyperlipidemia. However, part of the probands were first-degree relatives, a fact limiting the power of this study. The same results were described in 83 unrelated hypertriglyceridemic individuals and 2559 representatively selected controls from the same population, suggested a lack of association between Val153 → Met polymorphism in the APOAV gene and extreme levels of plasma triglycerides. Cys185 → Gly variant, discovered in Chinese population, was not detected in more than 500 Caucasians at all.

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