I405V polymorphism of the cholesteryl ester transfer protein (CETP) gene in young and very old people

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Abstract

This study was designed to analyse the prevalence of I405V polymorphism in the cholesteryl ester transfer protein (CETP) gene, the CETP serum concentration, the lipoprotein profile, and certain clinical end-points in two populations, one young and another of very old people. We recruited 100 healthy young people (median age 31 years) and 100 very old people (median age 89 years) and analysed their DNA for the presence of I405V polymorphism. The frequency of the VV genotype in very old people was more than double that in the young population. Subjects with this genotype had lower serum concentrations of CETP. Young people with the V/V genotype had a less atherogenic lipoprotein profile (lower total cholesterol, LDL cholesterol, Apo B, and Apo B/Apo A-I ratio) than those with the I/V or I/I genotypes. The older subjects, particularly the older women with the V/V genotype, had larger LDL than the young people. The prevalence of clinical endpoints was much lower among the very old people with the V/V genotype. In conclusion, the V/V genotype of the I405V CETP polymorphism is more frequent among very old people than young ones, and is associated with a lower incidence of vascular damage.

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1. Introduction

Cardiovascular diseases (CVD) are the main cause of death in Western countries. In Italy 46% of people over the age of 60 die of vascular diseases (Istituto Nazionale di Statistica, 2004), which cause disability and loss of quality of life among the elderly.

Dyslipidemia is among the main risk factors for CVD. The pathogenic role of cholesterol does not depend solely on its levels, but also on the lipoprotein composition. It is estimated that a 1 mg/dl rise in HDL cholesterol (HDL-C) lowers the cardiovascular risk by 2–3% (Gordon et al., 1989; Brewer, 2004). High HDL-C levels are associated with longer life (Glueck et al., 1975).

Various factors play vital roles in the HDL metabolism: the ATP binding cassette transporter A1 protein (ABC A1) (Hayden et al., 2000; Brewer and Santamaria-Fojo, 2003), lecithin: cholesterol acyltransferase (LCAT) (Glomset, 1968) and lipoprotein lipase (LPL) (Merkel et al., 2002) are involved in its production while cholesteryl ester transfer protein (CETP) (Barter and Rye, 1996; Inazu et al., 2000) and hepatic lipase (LH) (Perret et al., 2002; Gotto and Brinton, 2004) are involved in its catabolism.

CETP mediates the transfer of cholesterol esters (CE) from HDL to lipoproteins containing Apo B. The CETP gene, on chromosome 16 (Drayna et al., 1987), is highly polymorphous (Kuivenhoven et al., 1997) and some of its variants influence lipoprotein levels and composition (Inazu et al., 1990; Tall et al., 1999; Okumura et al., 2002). Knoblauch et al. (2004) worked out that polymorphisms of the CETP gene accounted for 10% of individual differences in HDL-C levels.

We investigated the I405V polymorphism of this gene due to A → G transition in position +20206 of exon 14 which leads to a missense mutation with the substitution of valine for isoleucine in position 405 of the protein (Boekholdt and Thompson, 2003). In the homozygous form for the rarest allele (V/V genotype) the I405V polymorphism is associated with a reduction in CETP activity, and with changes in the levels of HDL-C and the composition of HDL and LDL (Okumura et al., 2002; Barzilai et al., 2003; Boekholdt et al., 2004; Brousseau et al., 2004).

This study investigated the distribution of I405V polymorphism of the CETP gene and the lipoprotein profile in very old people and in selected healthy young subjects without cardiovascular risk factors.

2. Subjects and methods

2.1. Study population

We studied Caucasian 100 young people (42 men and 58 women), median age 31 years (range 21–55), and Caucasian 100 very old people (39 men and 61 women), median age 89 years (range 85–100) community-dwelling in Northern Italy. Healthy young subjects were recruited bearing in mind the NCEP Adult Treatment Panel (ATP III) criteria (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), 2002). Therefore, we excluded anyone with coronary disease (history of myocardial infarction,
stable and unstable angina, coronary angioplasty or bypass surgery), with equivalents of coronary risk (diabetes mellitus, peripheral arterial disease, abdominal aortic aneurysm, transient ischemic attacks (TIA) or stroke of carotid origin or >50% obstruction of a carotid artery), with hypertension (>140/90 mm Hg or antihypertensive therapy), hyperlipidemia (total cholesterol (TC) ≥240 mg/dl, triglycerides (TG) ≥200 mg/dl, lipid-lowering drugs), or obesity (body mass index (BMI) >30 and or waist circumference >88 cm for women and >102 cm for men). We also excluded anyone with liver disease, chronic renal insufficiency, or thyroid disorders assessed by laboratory data. Subjects were not to be taking any drugs, including hormone replacement therapy for post-menopausal women that might affect the lipoprotein profile, and were not to drink more than 30 g alcohol/day. For the group of old people we consecutively recruited all outpatients aged more than 85. The only exclusion criterion was use of lipid-lowering drugs. We took their clinical history to check for ischemic heart disease, peripheral vascular disease, abdominal aortic aneurysm, TIA, or stroke. Color-flow Doppler ultrasonography of the carotid artery was done to check for subclinical atherosclerosis. Blood pressure was measured and blood glucose metabolism tested.

Informed consent was obtained from all the subjects or their relatives. The study protocol was approved by the Ethics Committee of the University Hospital.

2.2. Materials

Genomic DNA was extracted using the salting-out procedure (Miller et al., 1988). As the I405V polymorphism is not associated with a restriction site, we used a mutagenized primer to introduce a forced MspI restriction site (Gudnason et al., 1999). Polymerase chain reaction (PCR) was done with 2 μg DNA and a solution of 0.2 mM dNTP, 0.2 mM of the mutagenized primer and the complementary primer, 1.5 mM MgCl2, 1× reaction buffer without MgCl2, and 1 U Taq DNA polymerase (Promega Corporation, Italy). The PCR conditions were 95 °C for 5 min, 60 °C for 1 min, and 72 °C for 1 min followed by 35 cycles at 95 °C for 15 s, then 60 °C for 30 s and 72 °C for 30 s, and finally 72 °C for 5 min. We obtained a 142 bp amplification fragment (allele I), which in the presence of the restriction site for the enzyme MspI gives rise to one 121 bp and one 21 bp fragment (allele V). The PCR product was digested with 10 U MspI at 37 °C overnight. Samples were then electrophoresed in 4% agarose gel.

Serum levels of TC, TG, HDL-C and LDL cholesterol (LDL-C) were determined using standard procedures (Roche Diagnostics S.p.A., Italy). Serum levels of apolipoproteins Apo A-I and Apo B were measured by turbidimetric immunoassay (Immucor Gamma S.p.A., Italy). CETP was assayed by ELISA (Wako Pure Chemical Industries Ltd., Japan). Data were analyzed statistically using the χ² test to check the genotype distribution, and ANOVA and the Bonferroni test for biochemical findings. A value of p <0.05 was considered significant.

3. Results

The genotypes were distributed differently in the young and old subjects (p = 0.02). The V/V genotype was significantly (p < 0.05) more frequent in the very old people (17%)
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Young subjects (n = 100)</th>
<th>Very old subjects (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V/V ~ I/V ~ I/I</td>
<td>V/V ~ I/V ~ I/I</td>
</tr>
<tr>
<td>Number</td>
<td>7 ~ 61 ~ 32</td>
<td>17* ~ 44** ~ 39</td>
</tr>
<tr>
<td>CETP concentration (µg/ml)</td>
<td>1.03 ± 0.51 ~ 1.46 ± 0.52* ~ 1.47 ± 0.44†</td>
<td>1.22 ± 0.42 ~ 1.52 ± 0.44 ~ 1.55 ± 0.49</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>156 ± 22 ~ 175 ± 33 ~ 179 ± 29</td>
<td>205 ± 64 ~ 213 ± 39 ~ 216 ± 46</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>84 ± 28 ~ 103 ± 33 ~ 105 ± 26</td>
<td>134 ± 54 ~ 139 ± 33 ~ 146 ± 44</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>61 ± 20 ~ 60 ± 14 ~ 63 ± 14</td>
<td>61 ± 18 ~ 64 ± 17 ~ 61 ± 13</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>91 ± 67 ~ 75 ± 26 ~ 79 ± 35</td>
<td>109 ± 52 ~ 112 ± 47 ~ 116 ± 52</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dl)</td>
<td>187 ± 24 ~ 175 ± 21 ~ 182 ± 23</td>
<td>173 ± 23 ~ 178 ± 21 ~ 177 ± 24</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dl)</td>
<td>68 ± 20 ~ 83 ± 32 ~ 84 ± 25</td>
<td>85 ± 20 ~ 97 ± 29 ~ 99 ± 33</td>
</tr>
<tr>
<td>Apo B/Apo A-I</td>
<td>0.37 ± 0.12 ~ 0.48 ± 0.17 ~ 0.47 ± 0.16</td>
<td>0.50 ± 0.13 ~ 0.56 ± 0.20 ~ 0.57 ± 0.23</td>
</tr>
<tr>
<td>LDL-C/Apo B</td>
<td>1.21 ± 0.22 ~ 1.35 ± 0.59 ~ 1.32 ± 0.43</td>
<td>1.60 ± 0.64 ~ 1.53 ± 0.50 ~ 1.55 ± 0.42</td>
</tr>
<tr>
<td>HDL-C/Apo A-I</td>
<td>0.30 ± 0.09 ~ 0.35 ± 0.08 ~ 0.35 ± 0.07</td>
<td>0.35 ± 0.09 ~ 0.36 ± 0.09 ~ 0.35 ± 0.08</td>
</tr>
</tbody>
</table>

SI conversion factor: to convert cholesterol to mmol/l multiply by 0.0259, to convert TG to mmol/l multiply by 0.01129. Values are mean ± S.D.

* p < 0.05 vs. V/V young subjects.

** p = 0.02 vs. I/V young subjects.
than in young people (7%) while the genotype I/V was less frequent, 44 versus 61% ($p = 0.02$) (Table 1 and Fig. 1). The young population is out of Hardy–Weinberg equilibrium. This may be due to the selection criteria applied.

Serum levels of CETP were lower in V/V subjects than in those with I/V and I/I genotypes; the difference was significant in young people ($p < 0.05$) (Table 1). Taking the two groups as a whole, CETP concentrations varied significantly with the genotype

![Fig. 1](image1.png)

Fig. 1. Frequency of different genotypes of the I405V polymorphism in young and very old people. Distribution of the genotypes: $\chi^2 = 7.609$, degrees of freedom = 2, $p = 0.02$. (*) Frequency of the V/V genotype in young vs. very old people $p < 0.05$. (***) Frequency of the I/V genotype in young vs. very old people $p = 0.02$.

![Fig. 2](image2.png)

Fig. 2. CETP concentration and polymorphism I405V genotypes in young and very old people. (*) $p < 0.05$ vs. genotype V/V.
Table 2
Clinical end-points and obstruction of carotid artery in very old people with different genotypes of the I405V polymorphism

<table>
<thead>
<tr>
<th>Genotype</th>
<th>V/V</th>
<th>I/V</th>
<th>I/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>17</td>
<td>44</td>
<td>39</td>
</tr>
<tr>
<td>Carotid obstruction (&gt;50%) (n)</td>
<td>0 (0.0%)</td>
<td>2 (4.5%)</td>
<td>5 (12.8%)</td>
</tr>
<tr>
<td>Coronary heart disease (n)</td>
<td>3 (17.6%)</td>
<td>15 (34.1%)</td>
<td>10 (25.6%)</td>
</tr>
<tr>
<td>TIA/ictus (n)</td>
<td>1 (5.9%)</td>
<td>9 (20.5%)</td>
<td>7 (17.9%)</td>
</tr>
</tbody>
</table>

(\(p = 0.007\)), being substantially lower in V/V subjects than in people with other genotypes (\(p < 0.05\)) (Fig. 2).

Young subjects with the V/V genotype had lower TC, LDL-C, Apo B levels, and a lower Apo B/Apo A-I ratio than those with the I/V and I/I genotypes; the very old subjects had lower Apo B (Table 1). Very old people had higher levels of TC, LDL-C, TG, and Apo B, and a higher ratio of LDL-C/Apo B than young people. The ratio was notably higher among very elderly V/V genotype women, in comparison with the I/V and I/I genotypes (1.89 ± 0.75, 1.52 ± 0.51, and 1.55 ± 0.47, respectively). Levels of HDL-C, Apo A-I, and the HDL-C/Apo A-I ratio did not differ in relation to the genotype and were virtually the same in the young and old populations.

Table 2 sets out the prevalence of clinical end-points and carotid occlusion in the very old people with different genotypes. Coronary disease and cerebrovascular events were less prevalent among the very old people with the V/V genotype, who also did not have a more than 50% obstruction of the carotid artery.

4. Discussion

Atherosclerosis is the result of synergism between variants of candidate genes, interacting with the environment and life styles (Smith et al., 2004). Polymorphism of the CETP gene is particularly important in the lipoprotein metabolism. If CETP is totally absent the lipoprotein profile is atherogenic (Berard et al., 1997; Tall et al., 1999) though some studies found that low CETP activity correlated with an anti-atherogenic state (Barter and Rye, 2001; Barzilai et al., 2003; Boekholdt et al., 2004). In animal models inhibition of CETP attenuates the onset of atherosclerosis (Okamoto et al., 2000) and in humans torcetrapib, a CETP inhibitor, improves the lipoprotein pattern (Brousseau et al., 2004). People with low CETP activity because of Taq1B polymorphism (Barter and Rye, 2001; Okumura et al., 2002; Boekholdt et al., 2004) treated with pravastatin show the same changes in the lipoprotein pattern and in the incidence of clinical events as subjects without the polymorphism (De Grooth et al., 2004). In men of the Honolulu Heart Program, CETP deficiency due to the D442G gene polymorphism was associated with an increased risk of coronary heart disease (Zhong et al., 1996). The relationship between CETP activity and coronary risk is, therefore, still open to debate (Kuivenhoven et al., 1998; Barter et al., 2003) but it appears to be influenced by the person’s overall metabolic state (Inazu et al., 2000; Barter and Rye, 2001).
In the very old people examined here, with median age 89 years, the V/V genotype of I405V polymorphism was more than twice as frequent as in the group of young people, with median age 31 years. Barzilai et al. (2003) reported similar findings in people of Ashkenazi Jewish origin. In our series, those with the V/V genotype had 23% lower serum CETP concentrations; other studies report reductions ranging from 9 to 23% (Boekholdt et al., 2004).

In our study the low CETP was not related to any rise in HDL-C or any change in the ratio of HDL-C/Apo A-I, but there was a less atherogenic lipid pattern. In young subjects with the V/V genotype, levels of TC, LDL-C, and Apo B were lower than in I/V and I/I genotypes, and the Apo B/A-I ratio was also lower. These differences were less evident in the very old people, where the environment and lifestyle play a larger part (Ljungquist et al., 1998; Cooper, 2003). The high LDL-C/Apo B ratio in the very old subjects suggests they have larger, less atherogenic circulating LDL (Austin et al., 1990; Lamarche et al., 1997; Scanu, 2003; Sniderman et al., 2003). This was particularly evident in the old women, in line with the report by Barzilai et al. (2003). This finding of large LDL in very old people contrasts with other studies (Katzel and Goldberg, 2003), which found smaller, more atherogenic LDL predominant during aging. In the presence of the same cholesterol level, therefore, the lipoprotein composition influences the lipid’s distinctive pathogenic significance in each individual (Harris, 2004; Karlamangla et al., 2004).

The favorable lipoprotein pattern in people with the V/V genotype, especially young people, might explain the lower prevalence of vascular damage, longer lifespan, and higher frequency of this genotype in older age. In our series, the four very old V/V subjects with CVD were hypertensive or diabetic, or both. It is worth noting that the higher levels of HDL in women in the Copenhagen Heart Study with the I405V polymorphism were not associated with any reduction in cardiovascular risk (Agerholm-Larsen et al., 2000). The antiatherogenic potential of HDL, therefore, also appears to depend on their composition (Nofer et al., 2002).

The question of CETP’s role in the lipoprotein metabolism and the pathogenesis of atherosclerosis is, therefore, still open to investigation.

References


