APOLIPOPROTEIN E POLYMORPHISM IN NORTHWESTERN GREECE: FREQUENCY AND EFFECT ON LIPID PARAMETERS

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Running title: Apolipoprotein E in Greek population

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ABSTRACT

Purpose: Apolipoprotein (apo) E gene polymorphism and its effect on serum lipid parameters were examined in a Greek population originating from northwestern Greece (N=555).

Results: The allele frequencies were \( \varepsilon 2: 6.3\% \), \( \varepsilon 3: 80.7\% \) and \( \varepsilon 4: 13\% \). The \( \varepsilon 4 \) allele frequency was higher in our population compared to that previously reported in individuals from other parts of Greece. ApoE polymorphism was associated with significant differences in serum lipid and lipoprotein levels. Particularly, individuals with the \( \varepsilon 2 \) allele had higher serum triglyceride (TRG) and apoE levels and lower levels of total cholesterol (TCHOL), low-density lipoprotein cholesterol (LDL-C) and apoB compared to those with the alleles \( \varepsilon 3 \) and \( \varepsilon 4 \). However, the impact of the \( \varepsilon 4 \) allele on lipid parameters seen in other populations was not observed in our population. Furthermore, the combination of both apoE polymorphism and serum apoE concentration explained a larger percentage of serum lipid variability than the polymorphism alone.

Conclusion: The results of our study suggest that ethnic differences, as well as alterations in serum apoE levels can significantly modify the relationship between apoE gene polymorphism and serum lipid variability.

Key words: Apolipoprotein E, Greece, Lipids, Polymorphism, Levels, Lipoproteins
INTRODUCTION

Apolipoprotein (apo) E is one of the protein constituents of the chylomicrons, the very low density lipoproteins (VLDL), the remnant particles and the high density lipoproteins (HDL) (1). On these lipoprotein particles, apoE serves as a ligand for the uptake by cellular receptors [the low density lipoprotein (LDL) receptor and the apoE receptor] (2,3).

The human apoE gene is located in chromosome 19 and is polymorphic (4,5). Two point mutations in the exon 4 of the apoE gene account for the three common alleles known as ε2, ε3 and ε4 allele, which code for six protein isoforms: the predominant E3 isoform and its mutant variants E2 and E4 (6). The E2 isoform shows defective, whereas the E4 isoform shows higher receptor binding ability compared to the E3 isoform (7,8). The ε3 allele is the most common allele in all populations studied (9). Furthermore, within Europe there is a clear gradient with higher ε4 allele frequencies in the north and lower ε4 allele frequencies in the south (10). The average allele frequencies in the Caucasian race are ε2: 8.0%, ε3: 76.9% and ε4: 15% (9).

A number of studies have demonstrated a relationship between the apoE polymorphism and serum lipid and apolipoprotein levels (9,11-14). ApoE2 homozygosity is sometimes associated with type III hyperlipoproteinemia, a condition that involves an additional genetic or environmental factor for full clinical expression (15). Studies of lipoprotein levels in a large number of individuals have demonstrated that the ε2 allele is associated with decreased and ε4 allele is associated with increased levels of total cholesterol (TCHOL), LDL cholesterol (LDL-C) and apoB levels compared to the ε3 allele (9,11,13). Additionally, alleles ε2 and ε4 are associated with increased and decreased serum apoE levels, respectively (13). Furthermore, according to a meta-analysis, serum triglyceride (TRG) and HDL cholesterol (HDL-C) levels are
also modulated by the apoE genotype (14). Moreover, de Knijff et al have reported an association between serum Lp(a) levels and apoE allele polymorphism (16). Finally, the ε4 allele was associated with higher Lp(a) levels compared to ε2 and ε3 alleles in normolipidemic individuals (16).

Serum apoE concentration significantly modulates lipoprotein levels in an isoform independent manner by its effects on clearance rate, lipolytic conversion and very-low density (VLDL) production (17). For example, apoE levels accounted for 34.6% of the triglycerides variance, independently of apoE polymorphisms, in the Stanislas cohort study (18), while apoE concentration was independently associated with the levels of total cholesterol, triglycerides, apoB and apoAI in another study (19). Consequently, the association between apoE polymorphism and serum lipid parameters may be confounded or masked by the apoE concentration. In fact, apoE concentration together with apoE polymorphisms is associated with a much larger percentage of cholesterol and triglyceride variability than apoE polymorphism alone (20).

Apart from its impact on serum lipid and apolipoprotein levels, apoE gene polymorphism has been associated with longevity, coronary artery disease, Alzheimer’s disease (21), as well as with the pathogenesis and the progression of a variety of renal diseases (22). The apoE polymorphism also affects the lipid response to hypolipidemic drugs (statins or fibrates), tamoxifen or to dietary intervention, the response to head trauma, the cognitive decline upon ageing and several other disorders (21,23-25).

In this study we examine the apoE allele frequencies and the effect of both apoE gene polymorphism and serum apoE concentration on serum lipid and apolipoprotein levels in a Greek population originating from the northwestern part of Greece.
MATERIAL AND METHODS

Study Population

The study population consisted of consecutive unrelated individuals aged 18 to 85 years old who underwent a regular check-up in our outpatient internal medicine clinic between September and December 2003. Exclusion criteria were hypothyroidism [thyroid-stimulating hormone (TSH)>5µU/ml], diabetes mellitus (fasting blood glucose>126mg/dl), alcoholism, renal failure (serum creatinine>1.6mg/dl), proteinuria (24hour urinary protein>300mg), hepatic disease (aspartate or alanine aminotransferase values> 3 times the upper normal limits), history of receiving drugs affecting lipid profile (lipid lowering drugs, β-blockers, diuretics, contraceptives, etc). Sixty hundred individuals were screened, 45 were excluded (at least one exclusion criteria was present) and thus 555 finally participated in the study. All participants gave informed consent for genetic analysis, and the ethic committee of our university hospital approved the study protocol.

Laboratory methods

In all individuals blood samples were obtained after a 14 hour overnight fast for gene genotype detection as well as for the determination of lipid parameters. Blood samples were centrifuged for 30 min (3600g) and then the serum was separated and stored at 4°C for analysis of lipid parameters. Serum for the assay of lipoprotein (a) [Lp(a)] was frozen and stored at -70°C.

Concentrations of total cholesterol and triglycerides were determined enzymatically on the Olympus AU600 Clinical Chemistry analyzer (Olympus Diagnostica, Hamburg, Germany). HDL-C was determined in the supernatant, after precipitation of the apoB-containing lipoproteins with dextran sulphate-Mg^{++} (Sigma Diagnostics, St. Louis, MO, USA). Our laboratory is currently participating in the Murex Clinical Chemistry
Quality Assessment Program. Our CV values in this program for the past years have ranged between 0.7 and 1.1% for cholesterol, and between 0.9 and 2.5% for triglycerides. LDL-C was calculated using the Friedewald formula if serum TRG levels were <400 mg/dl (26). If serum TRG levels were >400 mg/dl, LDL-C was determined directly in serum by the Direct LDL$^\text{TM}$ method (Sigma Diagnostics, St Louis, MO, USA), which is based on the immunoseparation of LDL particles from chylomicrons, VLDL, and HDL using antibodies against apolipoproteins A-I and E. (27). Apolipoproteins A1, B and E were measured with a Behring Nephelometer BN100 using reagents (antibodies and calibrators) from Dade Behring Holding GmbH (Liederbach, Germany). These assays were calibrated according to the International Federation of Clinical Chemistry (IFCC) standards. Lp(a) levels were determined by the enzyme immunoassay Macra Lp(a) (Trinity Biotech, Jamestown, NY, USA). The lower limit of detectability was 0.8 mg/dl.

DNA was extracted from the whole blood specimens according to standard procedures as previously described (28). ApoE genotyping was performed as described by Hixson and Vernier (29). Polymerase chain reaction was used to amplify a 244-bp sequence of the apoE gene, including the two polymorphic sites. The PCR products were then digested with the restriction enzyme Hha I and the different genotypes were detected after electrophoresis on 8% non-denaturing polyacrylamide gels, treated with ethidium bromide and visualized in ultra-violet radiation.

**Statistical Analysis**

Statistical analysis was performed with SAS statistical software. Values are expressed as mean±SD, except for Lp(a), which is expected as median (range). A chi square-test was used to compare apoE allele frequencies. Values between hyperlipidemic and normolipidemic subjects were compared using the Student’s t-test.
for independent samples, except for Lp(a), TRG and Apo E where the Mann-Whitney U test was used. Values among the three hyperlipidemic groups, as well as the effect of the apoE gene polymorphism on lipid parameters were tested using analysis of variance (one-way ANOVA) followed, in case of significant results, by the LSD (Least Significant Differences) test for multiple pairwise comparisons, except for TRG and apoE where the Kruskal-Wallis ANOVA median test was used followed by the Mann-Whitney U test for multiple pairwise comparisons in case of significant results. Bonferroni’s correction was also used to establish a new alpha level for determining statistical significance. Since serum triglycerides were not normally distributed, they were log-transformed. Analysis of co-variance (ANCOVA) was performed to test the overall effect on lipid parameters of apoE gene polymorphism taking as covariates the sex, age, BMI (Model A), and apoE concentration (Model B). The percentage (%) of the explained variability of each studied lipid parameter by the above mentioned independent variables in the general linear models is defined as $R^2 \times 100$.

**RESULTS**

The apoE allele frequencies were $\varepsilon2$: 6.3%, $\varepsilon3$: 80.7% and $\varepsilon4$: 13%. All allele frequencies were in Hardy Weinberg equilibrium. Three individuals were $\varepsilon2/\varepsilon2$ homozygotes, nine were $\varepsilon4/\varepsilon4$ homozygotes and six were $\varepsilon2/\varepsilon4$ heterozygotes. The study population was then divided into two groups: individuals with hyperlipidemia ($n=455$) and normolipidemic individuals (LDL-C<160mg/dl and TRG<200mg/dl) ($n=100$). The characteristics of both groups are shown in table 1. The apoE allele frequencies were not statistically different between the hyperlipidemic and the normolipidemic individuals (table 1). The allele frequencies were $\varepsilon2$: 7.1%, $\varepsilon3$: 81.2% and $\varepsilon4$: 11.7% for the hyperlipidemic individuals and $\varepsilon2$: 5.5%, $\varepsilon3$: 80.3% and $\varepsilon4$: 14.2%
for the normolipidemic individuals. When the hyperlipidemic population was divided into three subgroups according to serum lipid levels [individuals with LDL-C>160mg/dl and TRG<200mg/dl (primary hypercholesterolemia), individuals with LDL-C>160mg/dl and TRG>200mg/dl (combined hyperlipidemia), and individuals with LDL-C<160mg/dl and TRG>200mg/dl (primary hypertriglyceridemia)], there were no significant apoE allele frequency differences between the three groups. However, individuals with primary hypertriglyceridemia tended to have higher ε2 allele and lower ε4 allele frequency compared to the other groups (table 2).

As shown in table 3, hyperlipidemic individuals with the ε2 allele (ε2/ε2 and ε2/ε3 genotypes) had lower serum TCHOL, LDL-C and apoB levels and higher serum TRG and apoE levels compared to those with the ε3 allele (ε3/ε3 genotype) and the ε4 allele (ε3/ε4 and ε4/ε4 genotypes). Serum HDL-C levels were higher in individuals with the ε3/ε3 genotype compared to those with the ε2/ε2, ε2/ε3, ε3/ε4 and ε4/ε4 genotypes. Also, individuals with the ε3/ε3 genotype had higher serum apoA1 levels compared to those with the ε3/ε4 and ε4/ε4 genotypes. The effect of apoE polymorphism still had statistically significant effect on serum lipid parameters after adjusting for age, sex and the BMI among hyperlipidemic individuals (table 4). Furthermore, apoE polymorphism could explain a high variability of serum TRG, apoA1 and HDL-C levels (22.9, 20.2 and 17%, respectively) (table 4).

When the serum apoE concentration was included in the analysis of co-variance (ANCOVA, Model B) in addition to age, sex and BMI, the percentage of explained variance (R^2 X100) of the serum lipid parameters was significantly increased (table 5).

As shown in table 4, serum Lp(a) levels were not affected by apoE allele polymorphism.
Among normolipidemic individuals, apoE polymorphism had almost the same effect on the serum lipid parameters, though the results did not reach statistical significance (data not shown).

**DISCUSSION**

Although the impact of apoE allelic variation on serum lipid parameters has been studied in many populations (9,11-14), no such study in the Greek population has been reported. Therefore, we examined the effect of apoE polymorphism on serum lipid parameters in a Greek population originating from northwestern Greece. Our study showed that the ε2 allele is associated with lower TCHOL, LDL-C and apoB levels and higher TRG and apoE levels compared to the ε3 allele at least in hyperlipidemic individuals. Furthermore, the combination of apoE polymorphism and serum apoE concentration could explain a larger percentage of serum lipid variability than did the polymorphism alone.

To our knowledge, two previous studies have reported the apoE allele frequencies in healthy Greek individuals (table 6). Sklavounou et al have reported an underexpression of apoE ε4 allele in Greek blood donors (30). However, Kolovou et al have reported a frequency of 10.2% for ε4 allele in healthy individuals, a frequency closer enough to that reported in southern European populations (France, Italy) (10,31). The frequency for the ε4 allele reported in our study is even higher (13%), which could be attributed to the high degree of close breeding/inbreeding in our population favored by the remoteness of this part of Greece. Even though individuals with hypertriglyceridemia tended to have a higher frequency of the ε2 allele and a lower frequency of the ε4 allele, apoE allele distribution was not significantly different between the three
subgroups of hyperlipidemic subjects (table 2). These findings are in accordance with
most of the previously published data (32-35).

Many studies have shown that the ε2 allele is actually associated with decreased
levels of TCHOL, LDL-C and apoB, whereas the ε4 allele is associated with increased
levels of the above parameters, mainly due to up-regulation and down-regulation of
LDL receptors by each allele respectively (9,11-14). In our population, the association
of the apoE gene polymorphism with TCHOL, LDL-C and apoB levels was only seen
with the ε2 allele. It has been postulated that the hypolipidemic effect of the ε2 allele is
larger than the hyperlipidemic effect of the ε4 allele in some populations and that the
effect of the ε4 allele on lipid parameters is not independent from cultural and/or ethnic
background (36). The above observations may account for the lack of association
between TCHOL, LDL-C and apoB levels and the ε4 allele in Greek individuals.

A meta-analysis by Dallongeville et al has demonstrated that TRG concentrations
were significantly lower in individuals homozygous for the ε3 allele compared to those
with the alleles ε2 or ε4 (14). As suggested by the above authors, the presence of the ε2
allele is associated with slower plasma clearance of chylomicrons and VLDL and the
ε4 allele is associated with delayed lipolysis of serum TRG levels compared to the ε3
allele. In agreement, hyperlipidemic individuals with the ε3 allele in our study had
lower serum TRG levels compared to those with ε2 and ε4 alleles (table 3).

Serum ApoE levels were also affected by the apoE polymorphism in our population.
Specifically, the presence of the ε2 allele was associated with higher apoE levels
compared to the presence of the alleles ε3 and ε4, in accordance with other studies
(13,37,38). The low binding affinity to cellular receptors of the ε2 allele compared to
alleles ε3 and ε4 may account for the lower catabolic rate of the apoE and therefore for
the higher serum apoE levels (7,8).
James et al have reported lower levels of HDL-C in individuals with the ε4 allele compared to those with alleles ε2 and ε3 in an Italian population (39). Moreover, we have previously reported lower HDL-C levels in patients with familial hypercholesterolemia carrying the ε4 allele compared to patients not carrying this allele (40). In the present study, hyperlipidemic individuals with the ε4 allele also had significantly lower levels of HDL-C and ApoAI compared to individuals with the ε3 allele, suggesting a putative role of apoE in HDL metabolism (40).

Previously published data by de Knijff et al showed that serum Lp(a) levels were influenced by the apoE polymorphism in a manner similar to that of the LDL-C levels in normolipidemic individuals (16). However, this observation was not confirmed by this and other studies (41).

Serum apoE concentration has been shown to significantly influence lipoprotein metabolism independently of the apoE polymorphism in a number of ways (17-20). In detail, apoE directly stimulates hepatic VLDL and triglyceride production in an isoform independent manner (42), inhibits lipoprotein lipase-mediated lipolysis of triglyceride-rich lipoproteins, possibly by displacing or masking apoC-II, which is the necessary cofactor for the lipoprotein lipase activity (43), and modulates the clearance of VLDL particles through modification of the receptor binding properties of VLDL along with the apoE specific isoform effect (44). There seems to be an optimal level for serum apoE in maintaining normolipidemia; in cases of excess apoE levels, the lipid raising effects of inhibited lipolysis and increased VLDL production could offset the benefits of increased lipoprotein clearance rate, resulting in hyperlipidemia (17). Consequently, as shown in table 5, when apoE levels are taken into account, the apoE polymorphism-lipid interactions become more pronounced and a much larger percentage of the studied parameters could be explained. These data suggest that the different effect of apoE
polymorphism on serum lipid parameters reported in several populations may be related to differences in serum apoE concentration, which may confound or mask the pure effect of apoE polymorphism.

Although the effect of apoE polymorphism on serum lipid parameters had the same trend in the normolipidemic individuals as in the hyperlipidemic ones, the results did not reach statistical significance, possibly due to the limited number of individuals in the normolipidemic group (n=100) (data not shown).

In conclusion, ε2 allele exhibits a hypocholesterolemic along with a hypertriglyceridemic effect on Greek healthy subjects, whereas the previously reported hypercholesterolemic impact of the ε4 allele is not seen in our population. Apart from apoE gene polymorphism, serum apoE concentration was also proven to be a major determinant of serum lipid variability in our population. Therefore, our data clearly show that ethnic differences, as well as alterations in serum apoE levels can significantly modify the relationship between apoE gene polymorphism and serum lipid variability.


REFERENCES


Table 1. Clinical and laboratory characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hyperlipidemic individuals (n=455)</th>
<th>Normolipidemic individuals (n=100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>233/222</td>
<td>54/46</td>
<td>NS</td>
</tr>
<tr>
<td>Age, years</td>
<td>55±15</td>
<td>51±13</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.3±3.7</td>
<td>26±3</td>
<td>NS</td>
</tr>
<tr>
<td>TCHOL, mg/dl</td>
<td>285±73</td>
<td>207±46</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>TRG, mg/dl</td>
<td>289±145</td>
<td>120±109</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>203±71</td>
<td>137±45</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>46±14</td>
<td>45±12</td>
<td>NS</td>
</tr>
<tr>
<td>Apo A1, mg/dl</td>
<td>146±26</td>
<td>113±22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Apo B, mg/dl</td>
<td>143±43</td>
<td>100±26</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Apo E, mg/dl</td>
<td>5.0±1.8</td>
<td>3.8±1.1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Lp(a), mg/dl</td>
<td>11.1 (0.8-67)</td>
<td>8 (0.8-56)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Frequencies of apoE alleles % ε2/ε3/ε4</td>
<td>7.1/81.2/11.7</td>
<td>5.5/80.3/14.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD, except for Lp(a), which is expressed as median (range). BMI; body-mass index, TCHOL; total cholesterol, TRG; triglycerides, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, Apo; apolipoprotein, Lp(a); lipoprotein (a). Values were compared using the Student’s t-test for independent samples, except for Lp(a), TRG and Apo E where the Mann-Whitney U test was used. Apo E allele frequencies were compared with the chi-square test. NS; not significant.
Table 2. Characteristics of the hyperlipidemic individuals.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Primary hypercholesterolemia n=273</th>
<th>Combined hyperlipidemia n=125</th>
<th>Primary hypertriglyceridemia n=57</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCHOL, mg/dl</td>
<td>296±70</td>
<td>309±60</td>
<td>239±80</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TRG, mg/dl</td>
<td>105±67</td>
<td>295±88</td>
<td>467±200</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>246±35</td>
<td>224±65</td>
<td>135±21</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>54±10</td>
<td>45±15</td>
<td>36±8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Frequencies of apoE alleles %</td>
<td>ε2/ε3/ε4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.3/86.4/10.3</td>
<td>5.8/77.1/17.1</td>
<td>12.2/80/7.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. TCHOL; total cholesterol, TRG; triglycerides, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol. Values were compared by the one-way analysis of variance (ANOVA) followed by the LSD test for multiple pairwise comparisons in case of significant results, except for TRG where the Kruskal-Wallis ANOVA median test was used followed by the Mann-Whitney U test for multiple pairwise comparisons in case of significant results. Apo E allele frequencies were compared with the chi-square test. NS; not significant.
Table 3. Effect of the ApoE polymorphisms on serum lipid parameters in the hyperlipidemic patients (N=449, the 6 patients with the ApoE2/4 genotype were excluded from the analysis).

<table>
<thead>
<tr>
<th></th>
<th>ε2 allele (N=59)</th>
<th>ε3 allele (N=294)</th>
<th>ε4 allele (N=96)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE2/3, E2/2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-CHOL (mg/dl)</td>
<td>238±55&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>293±60</td>
<td>275±78</td>
<td>0.000</td>
</tr>
<tr>
<td>LogTRG (mg/dl)</td>
<td>2.27±0.27&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.16±0.20</td>
<td>2.22±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>42±13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48±13</td>
<td>40±21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>155±56&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>211±56</td>
<td>194±75</td>
<td>0.001</td>
</tr>
<tr>
<td>ApoAI (mg/dl)</td>
<td>140±23</td>
<td>149±24</td>
<td>137±35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>114±35&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>147±40</td>
<td>139±41</td>
<td>0.000</td>
</tr>
<tr>
<td>ApoE (mg/dl)</td>
<td>6.1±0.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>5.0±1.7</td>
<td>4.9±1.9</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. TCHOL; total cholesterol, TRG; triglycerides, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, Apo; apolipoprotein. Values were compared by the one-way ANOVA followed by LSD test for multiple pairwise comparisons in case of significant results except for TRG and ApoE where the Kruskal-Wallis ANOVA median test was used followed by the Mann-Whitney U test for multiple pairwise comparisons in case of significant results. TRG levels were log-transformed because of the skewed distribution. NS; not significant. <sup>a</sup>p<0.01 for comparison between ε2 vs ε3 allele, <sup>b</sup>p<0.01 for comparison between ε2 vs ε4 allele, <sup>c</sup>p<0.01 for comparison between ε4 vs ε3 allele.
Table 4. Effect of apoE gene polymorphism on lipid parameters of the hyperlipidemic individuals.

<table>
<thead>
<tr>
<th></th>
<th>$R^2 \times 100^*$</th>
<th>ApoE polymorphism $^*$</th>
<th>age $^*$</th>
<th>sex $^*$</th>
<th>BMI $^*$</th>
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<tbody>
<tr>
<td>TCHOL</td>
<td>3.8</td>
<td>0.001</td>
<td>0.02</td>
<td>NS</td>
<td>0.05</td>
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<tr>
<td>LogTRG</td>
<td>22.9</td>
<td>0.006</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>LDL-C</td>
<td>3</td>
<td>0.002</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>HDL-C</td>
<td>17</td>
<td>0.002</td>
<td>0.03</td>
<td>0.03</td>
<td>0.000</td>
</tr>
<tr>
<td>Apo A1</td>
<td>20.2</td>
<td>0.000</td>
<td>0.02</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B</td>
<td>6</td>
<td>0.000</td>
<td>NS</td>
<td>NS</td>
<td>0.003</td>
</tr>
<tr>
<td>Apo E</td>
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<td>0.01</td>
<td>NS</td>
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<td>0.000</td>
</tr>
<tr>
<td>LogLp(a)</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
</tr>
</tbody>
</table>

TCHOL; total cholesterol, TRG; triglycerides, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, Apo; apolipoprotein, Lp(a); lipoprotein (a), BMI; body-mass index. Levels of TRG and Lp(a) were log-transformed because of their skewed distribution. $^*$Percentage (%) of the explained variability of the studied parameter by the apoE gene polymorphism in the analysis of co-variance (ANCOVA) taking as covariates the age, sex and BMI. $^{**}$P-value by the analysis of co-variance (ANCOVA).
Table 5. Combined effect of both apoE gene polymorphism and apoE concentration on the serum lipid parameters of the hyperlipidemic subjects.

<table>
<thead>
<tr>
<th></th>
<th>Model A</th>
<th></th>
<th>Model B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2 \times 100^*$</td>
<td>$p$-value*</td>
<td>$R^2 \times 100^{**}$</td>
<td>$p$-value‡</td>
</tr>
<tr>
<td>TCHOL</td>
<td>3.8</td>
<td>0.001</td>
<td>24.3</td>
<td>0.000</td>
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<tr>
<td>LogTRG</td>
<td>22.9</td>
<td>0.006</td>
<td>43.1</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL-C</td>
<td>3</td>
<td>0.002</td>
<td>20.5</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL-C</td>
<td>17</td>
<td>0.002</td>
<td>21</td>
<td>0.000</td>
</tr>
<tr>
<td>Apo A1</td>
<td>20.2</td>
<td>0.000</td>
<td>20.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Apo B</td>
<td>6</td>
<td>0.000</td>
<td>26.3</td>
<td>0.000</td>
</tr>
</tbody>
</table>

TCHOL; total cholesterol, TRG; triglycerides, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, Apo; apolipoprotein, BMI; body-mass index. *Percentage (%) of the explained variability of the studied parameter by the apoE gene polymorphism in the analysis of co-variance (ANCOVA) taking as covariates the age, sex and BMI (Model A). **Percentage (%) of the explained variability of the studied parameter when adding apoE concentration as covariate in the Model A (Model B). †P-value by ANCOVA in the Model A. ‡P-value by ANCOVA in the Model B.
Table 6. Reported apoE allele frequencies in healthy Greek populations

<table>
<thead>
<tr>
<th>Study</th>
<th>ε2 (%)</th>
<th>ε3 (%)</th>
<th>ε4 (%)</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sklavounou, 1997</td>
<td>5.3</td>
<td>88.2</td>
<td>6.5</td>
<td>216</td>
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<tr>
<td>Kolovou, 2002</td>
<td>8.1</td>
<td>81.7</td>
<td>10.2</td>
<td>240</td>
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<tr>
<td>Present study</td>
<td>6.3</td>
<td>80.7</td>
<td>13</td>
<td>555</td>
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</tbody>
</table>