Apolipoprotein E gene determines serum testosterone and dehydroepiandrosterone levels in postmenopausal women

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Abstract

Objective: Apolipoprotein E (ApoE) is believed to play an important role in lipid metabolism and has been found to be related to diseases associated with ageing, the important characteristic of which is decline in circulating sex steroids, including androgen.

Design: To find the relationships of levels of serum testosterone and its precursor, dehydroepiandrosterone (DHEA), to ApoE polymorphism in 113 postmenopausal Caucasian women.

Methods: The ApoE genotype was assessed by polymerase chain reaction and CfoI endonuclease digestion. ApoE genotype distribution was as follows: E2/3, 15%; E3/3, 71.7%; E2/4, 1.8%; E3/4, 10.6; and E4/4, 0.89%. The differences in serum androgen levels between genotypes were evaluated by ANCOVA and least significant difference (LSD) multiple comparisons test after adjustment for body mass index, age and/or years since menopause.

Results: Significant intergroup differences between the most frequent allele combination (2/3, 3/3 and 3/4) in serum DHEA levels were found (P = 0.05, ANCOVA). DHEA levels were higher in women with the E3/4 allele combination than in the E3/3 genotype (P = 0.01; LSD multiple comparisons). In serum testosterone levels, borderline intergroup differences were found (P < 0.07, ANCOVA). Higher testosterone levels were found in the E3/4 allele combination as compared with E3/3 (P < 0.05, LSD multiple comparisons). Dose effect of E4 allele analysis indicated higher serum DHEA and testosterone levels in women with the E4 allele present than in women with the E4 allele absent (P < 0.003 for DHEA, P < 0.007 for testosterone, ANCOVA).

Conclusions: Circulating testosterone and DHEA are associated with the ApoE genotype, which may render women missing the allele E4 more susceptible to the development of some diseases associated with ageing and menopause.

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Introduction

A variety of C19 steroids, including dehydroepiandrosterone (DHEA), androstenedione, testosterone and dihydrotestosterone, are produced, as well as in the adrenal, by the thecal cells and to a lesser degree by the ovarian stroma. While only testosterone and dihydrotestosterone are true androgens interacting with the functional receptor, their precursors DHEA and androstenedione have a weak androgenic activity. It is well known that production of androgen, like oestrogen, declines gradually during ageing, and markedly after the menopause, which may participate in the development of some degenerative diseases. Cholesterol is known to be a fundamental substrate for steroid synthesis in adrenals and gonads. Apolipoprotein E (ApoE), synthesis of which is controlled by the ApoE gene, is produced by a variety of cells and is available in interstitial fluids (1). This peptide has a global transport role for lipids, including cholesterol, facilitating their import and redistribution among cells within the tissues. The cells of both steroidogenic tissues can synthesise both ApoE and cholesterol de novo and mobilise intracellular cholesterol ester pools. Nevertheless, about 80% of the cholesterol precursor for steroid hormone production in the steroidogenic glands is provided by serum lipoproteins (2–4). Therefore, both circulating and tissue pools of cholesterol precursor are assumed to be controlled by the ApoE gene (5, 6). In the present study, we tested the hypothesis that levels of serum testosterone and its precursor DHEA are associated with the ApoE genotype in postmenopausal women.
Subjects and methods

Subjects

The cross-sectional study was conducted on 114 Czech postmenopausal female subjects of Caucasian origin (62.4±9.8 years of age and 13.0±7.8 years since menopause, means±S.D.). The women were screened for the risk of diseases associated with menopause evaluation, but only subjects with normal clinical, biochemical and hormone indices were included in the study group. Their mean body mass index (BMI) was 25.4 (±3.6). None of the investigated women had a history of early or late menarche or premature menopause (before 45 years of age). Six subjects had less than 2 years, but not less than 18 months, since menopause. Postmenopausal status was documented by high serum follicle-stimulating hormone (FSH; 86.8±35.8 mU/l) and luteinising hormone (LH; 19.0±9.1 mU/l) and low serum oestradiol levels (0.080±0.08 nmol/l). Subjects with unclear menopausal status were not included in the study. The prior menstrual history was regular (11–13 cycles/year). The study group did not include any alcoholics, heavy smokers, women with psychosis, active endocrinopathy or other serious internal disease. No woman was markedly underweight or obese and they all had normal calcium, caloric and/or protein intake. The daily life of the women was usual in physical activity. None of the subjects had been treated with oestrogen or calcitropic drugs, including vitamin D. Informed consent was obtained from all of the women, and all procedures were approved by the Ethical Committee of the Institute of Endocrinology, Prague.

Protocol

Blood for measurement of serum DHEA, testosterone, oestradiol, FSH and LH, insulin, cholesterol and triglycerol was drawn in the morning, after overnight fasting. Samples for hormonal estimation were stored at −80°C until analysed.

Analytical methods

Circulating DHEA, oestradiol and FSH and LH were measured by a commercial kit from Immunotech (Backman Coulter Company, Prague, Czech Republic). Serum total testosterone levels were determined by a modified method using 125I-tyrosyl methyl ester as a tracer. Serum insulin levels were assessed by RIA, using an RIA-SAX-Insulin kit (Laboratorium Saxonian, GmGH, Sebnitz, Germany), serum cholesterol and triglycerol enzymatically using the kits and semi-automatic analyser (Vitalab Eclipse) from Merck KGaH (Darmstadt, Germany). The interassay coefficients of variation were as follows: 11.9% for DHEA, 6.1% for oestradiol, 9.9% for testosterone, 4.5% for FSH, 2.7% for LH and 5.3% for insulin. Duplicate measurements were used to form mean values.

Genotyping of ApoE

Genomic DNA was isolated from peripheral blood leukocytes by a standard salt extraction procedure (7). After PCR amplification, with previously described primers (8, 9), PCR products were cleaved with restriction endonuclease CfoI (Promega, Madison, WI, USA), an isoschizomer of HhaI, at 37°C overnight. Digested fragments were separated on 3% agarose gel and ApoE genotypes were identified.

Figure 1 Intergroup differences between the most frequent ApoE allele combinations E2/3, E3/3 and E3/4 with adjustment to constant age and BMI in all explanatory variables. LSD multiple comparisons followed an ANCOVA test. Circles represent the levels of steroid in individual subjects, while the bars denote re-transformed group mean values with their 95% confidence intervals adjusted to constant covariates.
Statistical analysis

First, circulating levels of DHEA and testosterone were compared among the most frequent 2/3, 3/3 and 3/4 allele combinations using a one-way ANCOVA test with adjustment to constant age and BMI after transformation of explained variable by power transformation to minimum skewness of normalised residuals. Differences in the levels of substances between individual genotype subgroups were further tested by least significant difference (LSD) multiple comparisons test.

Secondly, the dose effect of allele ApoE 4 was determined using a one-way ANCOVA test with adjustment to constant age and BMI. Residual analysis was used as follows: the skewness of normalised residuals was tested in fractions between $2^{-2}$ and $2^{+2}$, ensuring the elimination of influence of outliers. Similarly, the experimental points with absolute values of normalised residuals greater than 2 were excluded from the ANCOVA test as outliers. The homogeneity of the group variances was tested using Bartlett’s and Cochrane’s tests. Statistical software Statgraphics Plus 3.3 was used for calculations. The level of significance for the analyses was set at 0.05.

Results

The ApoE genotype frequencies in our cohort of women were as follows: 15% for E2/3, 71.7% for E3/3, 1.8% for E2/4, 10.6% for E3/4 and 0.89% for E4/4. These genotype frequencies were in Hardy–Weinberg equilibrium. Distribution of allele combinations in our cohort is comparable with genotype frequencies in other Caucasian populations (10, 11). The absence of the ApoE2/2 genotype is explained by its extremely low frequency in Caucasians (6, 11).

In DHEA, significant intergroup differences between 2/3, 3/3 and 3/4 genotypes were found ($P < 0.05$, ANCOVA). DHEA was higher in E3/4 than in E3/3 allele combination ($P < 0.01$, LSD multiple comparisons). A highly significant positive relation was found with age ($P < 0.00001$). In testosterone, borderline intergroup differences between 2/3, 3/3 and 3/4 genotypes were found ($P < 0.07$, ANCOVA). LSD multiple comparisons indicated significantly higher serum testosterone levels in women with E3/4 allele combination as compared with E3/3 genotype ($P < 0.05$) (Fig. 1).

The dose effect of allele ApoE4 is indicated in Table 1. Serum DHEA levels were higher in women with the E4 allele present as compared with those with E4 absent ($P < 0.003$). Similarly, testosterone levels were higher in the genotype with the E4 allele present than in the genotype with the E4 allele missing ($P < 0.007$). No differences in age, years since menopause, BMI, serum insulin, cholesterol and triglycerol levels were found between subjects with or without the E4 allele.

Discussion

The present study showed that women with an E3/4 allele combination of the ApoE genotype had higher serum testosterone and DHEA levels than those with an E3/3 allele combination. Moreover, the dose effect of the E4 allele indicated higher values of both androgens in women with E4 present as compared with those without this allele. Considering the results, the effect of the E4 allele on androgen synthesis may be located in the transport of cholesterol via ApoE into the steroidogenic organs, its local synthesis and hydroxylation of DHEA.

Genetic control of steroidogenesis is not completely understood. Sex steroid synthesis in humans is related to the P450 polymorphism. The transport of free

<table>
<thead>
<tr>
<th>ApoE allele</th>
<th>Absent</th>
<th>Present</th>
<th>$P$ value</th>
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<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td>ANCOVA</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.38 (0.87–1.91)</td>
<td>2.03 (1.42–2.42)</td>
<td>$P &lt; 0.007$</td>
</tr>
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<td>DHEA (nmol/l)</td>
<td>6.2 (4.0–10.3)</td>
<td>11.0 (6.7–13.8)</td>
<td>$P &lt; 0.003$</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 (54–66)</td>
<td>63.5 (55–70)</td>
<td></td>
</tr>
<tr>
<td>YSM (years)</td>
<td>12 (7–18)</td>
<td>12 (6–21)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>25.1 (22.7–27.4)</td>
<td>25.7 (24.2–27.0)</td>
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</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.41 (5.37–6.84)</td>
<td>6.41 (5.56–7.01)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerol (mmol/l)</td>
<td>1.18 (0.85–1.46)</td>
<td>1.26 (0.91–1.58)</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>8.5 (6.8–12.7)</td>
<td>10.3 (7.3–13.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

YSM, years since menopause.
cholesterol through the cytosol to the inner mitochondrial membrane, as well as cholesterol side chain cleavage are coded by the CYP11A1 gene, while conversions of pregnenolone to 17-OH pregnenolone and DHEA are determined by the CYP17 gene (12). Synthesis of progesterone, 17-OH progesterone and androstenedione (activated by 17β-hydroxysteroid dehydrogenase) is encoded by HSD3B2 and HSD17B2 genes, the latter being localised on chromosome 16q24.1–q24.2 (13). The present results are consistent with the hypothesis that the ApoE gene is the next member of a polygenic system regulating androgen production in postmenopausal subjects.

Women with hyperandrogenism have been found to have an insulin resistance syndrome (14). Although in this preliminary study no differences in serum insulin levels were observed between probands with and without E4 allele, a pathogenetic importance of insulin levels were observed between probands with androgen production in postmenopausal subjects.

In conclusion, this is the first study suggesting an association between postmenopausal serum androgen levels and ApoE genotype, which could contribute to the development of diseases associated with menopause and/or ageing.

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References

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