Effect of estrogen receptor-alpha (ESR1) gene polymorphism on high density lipoprotein levels in response to hormone replacement therapy

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Abstract

Studies have shown that estrogen replacement therapy and estrogen plus progestin replacement therapy alter serum levels of total, LDL and HDL cholesterol levels. However, HDL cholesterol levels in women vary considerably in response to hormone replacement therapy (HRT). A significant portion of the variability of these levels has been attributed to genetic factors. Therefore, we investigated the influence of estrogen receptor-alpha (ESR1) gene polymorphisms on HDL levels in response to postmenopausal HRT. We performed a prospective cohort study on 54 postmenopausal women who had not used HRT before the study and had no significant general medical illness. HRT consisted of conjugated equine estrogen and medroxyprogesterone acetate continuously for 1 year. The lipoprotein levels were measured from blood samples taken before the start of therapy and after 1 year of HRT. ESR1 polymorphism (\textit{MspI} C>T, \textit{HaeIII} C>T, \textit{PvuII} C>T, and \textit{XbaI} A>G) frequencies were assayed by restriction fragment length polymorphism. A general linear model was used to describe the relationships between HDL levels and genotypes after adjusting for age. A significant increase in HDL levels was observed after HRT (P = 0.029). Women with the ESR1 \textit{PvuII} TT genotype showed a statistically significant increase in HDL levels after HRT (P = 0.032). No association was found between other ESR1 polymorphisms and HDL levels. According to our results, the ESR1 \textit{PvuII} TT genotype was associated with increased levels of HDL after 1 year of HRT.

Key words: Hormone replacement therapy; High-density lipoprotein cholesterol levels; Estrogen receptor-alpha; Polymorphism

Introduction

Estrogen exerts beneficial systemic effects on lipoprotein and antioxidant metabolism through estrogen receptor-alpha (ESR1) \textsuperscript{(1)}. It is clear that estrogen levels decline in women during menopause and that this is one reason for the increase in plasma low-density lipoprotein (LDL) levels. LDL is one of the lipoproteins that promote endothelial damage, as well as reduction of high-density lipoprotein (HDL), a pleiotropic lipoprotein that prevents or alleviates endothelial damage \textsuperscript{(2)}. Studies have shown that estrogen replacement therapy and estrogen plus progestin replacement therapy alter these levels by decreasing LDL cholesterol levels and increasing HDL cholesterol levels \textsuperscript{(3,4)}. However, HDL cholesterol levels in women vary considerably in response to hormone replacement therapy (HRT) and a significant portion of the variability in HDL cholesterol levels can be attributed to genetic factors \textsuperscript{(5-7)}.
ESR1 is a member of a nuclear hormone receptor superfamily and acts as a ligand-activated transcription factor composed of several domains that are important for hormone and DNA binding (8). The human ESR1 gene is located on the long arm of chromosome 6 (6q25.1) and contains 8 exons separated by 7 intronic regions (9,10). Several single nucleotide polymorphisms (SNP) of the ESR1 gene have been identified, of which the MspI C>T (National Center for Biotechnology Information SNP identification, NCBI SNP ID: rs1784705) SNP in the first exon and HaeIII C>T (NCBI SNP ID: rs9322331), PvuII C>T (NCBI SNP ID: rs2234693) and XbaI A>G (NCBI SNP ID: rs9340799). SNP in the first intron have been studied the most. For example, polymorphic alterations of the ESR1 gene have been evaluated in studies of possible linkage and/or association with endometrial cancer (11), breast cancer (12), ovarian cancer (13), prostate cancer (14), leiomyoma (15,16), endometriosis (16-18), bone mineral density (19,20), and cardiovascular disease (21) with discrepant results in studies that included a very different number of patients. However, very few of them were designed to compare the same patient before and after HRT.

Data concerning an association of ESR1 genetic polymorphisms with lipid profiles in postmenopausal women have been contradictory. Some studies of postmenopausal women did not detect an association between ESR1 polymorphisms and serum lipoprotein levels after HRT (22-25). However, Herrington et al. (26) reported that postmenopausal women who were ESR1 PvuII CC carriers, as well as those with several other closely linked intron 1 polymorphisms, had an increase in HDL cholesterol levels after HRT. Later, these results were supported by the observation that postmenopausal women with an ESR1 PvuII CC genotype had an increase response of HDL, apolipoprotein A1, vascular reactivity, and myocardial perfusion to HRT (27). On the other hand, Huang et al. (28) have reported that healthy postmenopausal women with the CC or TC genotype in ESR1 PvuII restriction had higher serum total cholesterol and LDL cholesterol concentrations.

The objective of the present study was to determine if there is an association between MspI C>T, HaeIII C>T, PvuII C>T, and XbaI A>G ESR1 SNP and plasma HDL cholesterol levels in postmenopausal women who were treated with conjugated equine estrogen and medroxyprogesterone acetate continuously for 1 year.

Material and Methods

One hundred non-hysterectomized and postmenopausal women were enrolled in the study, which was approved by the Ethics Committee of the Federal University of São Paulo (Brazil). All patients gave written informed consent to participate. No patient had been previously treated with HRT and all had a maximum of 3 years of menopause. Other exclusion criteria were a history of breast or endometrial cancer, deep vein thrombosis, uncontrolled hypertension, valvular or congenital heart disease, heart failure, cardiomyopathy, diabetes, and renal or thyroid disease. Fifty-four women were initially included, after 46 had been excluded due to thyroid, kidney, liver, and/or diabetes problems.

HRT consisted of conjugated equine estrogen 0.625 mg/day and medroxyprogesterone acetate 2.5 mg/day continuously for 1 year, with a medical visit every 3 months. Blood samples were obtained between 8 and 9 am after overnight fasting before and after 1 year of treatment for the determination of HDL levels on day zero (HDLpre) and after 1 year of treatment (HDLpost). All samples were analyzed immediately after collection. Routine chemical methods were used to determine lipoprotein levels (Bayer Healthcare, USA).

Genomic DNA was extracted from peripheral blood leukocytes using the Easy-DNA™ Kit (Invitrogen Corporation, USA) according to manufacturer instructions. The DNA (0.2 μg) was amplified in 50 μL PCR Master Mix (Promega Corporation, USA). Five micromoles per microliter of oligonucleotide primer set was used for each reaction. The polymerase chain reaction (PCR) was performed for 35 cycles to amplify each specific fragment of the ESR1 gene. The PCR conditions, oligonucleotide primers, and genotype sizing were described elsewhere (26,29,30).

After PCR amplification, the polymorphisms were assayed using restriction fragment length polymorphism (RFLP). Each PCR product was incubated with 3 units of a specific restriction enzyme at 37°C for 4 h and the resulting fragments were visualized on a 3% ethidium bromide-stained agarose gel using UV light and documented with a digital camera (Eastman Kodak Co., USA). Genotypes for MspI, HaeIII, and PvuII polymorphisms were termed CC/CT/TT, and XbaI polymorphisms were AA/AG/GG and CC/CT/TT, respectively.

The chi-square test was used to test for significant departures from Hardy-Weinberg equilibrium. Pairwise linkage disequilibrium (D’) was calculated using the linkage disequilibrium pairs program from the GENECONTOUng utilities (31) and the WHAP software (http://pngu.mgh.harvard.edu/~purcell/whap/) was used to estimate haplotype frequencies. HDL levels were calculated during the trial as mean follow-up measurements obtained before (HDLpre) and after (HDLpost) treatment. A general linear model was used to describe the relationships between HDL levels and various genotypes after adjusting for age. A paired sample t-test and one-way ANOVA were employed for within- and between-group comparisons, respectively.
Results

Fifty-four women started the treatment. Mean age (± SD) was 51.7 ± 3.6 years. Plasma HDL levels were 55.33 ± 14.49 mg/dL before and 58.24 ± 14.36 mg/dL after HRT. A significant difference in HDL levels was observed before and after HRT (P = 0.029). After HRT, HDL levels were maintained or decreased in 35.2% (N = 19) of these postmenopausal patients, but were increased in the remaining 64.8% (N = 35).

The genotype frequencies of the estrogen receptor-alpha gene polymorphisms ESR1 MspI C>T, ESR1 HaeIII C>T, ESR1 PvuII C>T, and ESR1 XbaI A>G are shown in Table 1. The four ESR1 SNP were in Hardy-Weinberg equilibrium (P > 0.05). The three ESR1 SNP in intron 1 were in linkage disequilibrium with each other (HaeIII C>T with PvuII T>C; HaeIII C>T with XbaI A>G and XbaI A>G with PvuII C>T; D’ = 1.000; P < 0.0001). No linkage disequilibrium was observed between the SNP in exon 1 and the SNP in intron 1.

We analyzed the genotype of the ESR1 gene together with plasma HDL levels before and after HRT (Table 1). After 1 year of HRT, we observed an increase in plasma HDL levels in all ESR1 genotypes. There was no significant difference in HDLpre or HDLpost levels in ESR1 MspI genotypes (MspI CC: P = 0.786; MspI CT: P = 0.069; MspI TT: P = 0.191), ESR1 HaeIII genotypes (HaeIII CC: P = 0.074; HaeIII CT: P = 0.518; HaeIII TT: P = 0.241), or ESR1 XbaI genotypes (XbaI AA: P = 0.142; XbaI AG: P = 0.417; XbaI GG: P = 0.101). However, in ESR1 PvuII genotypes, PvuII TT carriers showed a statistically significant increase in HDL levels after HRT (P = 0.032). In the other PvuII genotypes, the increase of HDL levels was not significantly different after HRT (PvuII CC: P = 0.246; PvuII CT: P = 0.592).

Analyses using the mean difference in HDL levels as a quantitative trait did not provide evidence of an association for the following haplotypes: i) alleles of the four markers (likelihood ratio test, LRT = 0.370; P = 0.831), ii), the strongly linked combination of ESR1 HaeI, ESR1 PvuII and ESR1 XbaI (LRT = 3.11; P = 0.370), iii) the ESR1 MspI/ESR1 HaeII combination (LRT = 0.563; P = 0.755), iv), the ESR1 HaeII/ESR1 PvuII combination (LRT = 1.22; P = 0.544), and v) the ESR1 PvuII and ESR1 XbaI combination (LRT = 3.10; P = 0.210).

Discussion

Endogenous and exogenous estrogens have been reported to affect the hemostatic system and to exert effects on lipoprotein metabolism (2,3). In the present study, we determined whether estrogen receptor-alpha (ESR1 MspI C>T, HaeIII C>T, PvuII C>T, and XbaI A>G) gene polymorphisms are associated with high HDL levels in women undergoing postmenopausal hormone replacement therapy. ESR1 PvuII TT carriers showed a statistically significant increase in HDL levels after HRT (HDLpre = 53.25 ± 15.70; HDLpost = 57.60 ± 14.35; P = 0.032). No association was found between the three other polymorphisms and HDL levels.

The previous in vitro observations of allele-dependent differences of ESR1 PvuII polymorphisms and HDL levels are contradictory. Herrington et al. (6) showed that the ESR1 PvuII T allele eliminates a functional binding site for the transcription factor Myb, which suggests that the presence of this allele may result in lower ESR1 transcription, and postmenopausal PvuII CC carriers showed increased HDL cholesterol levels in response to HRT (26). However, Figgtree et al. (32) showed that the variant T allele of ESR1 PvuII abolished the negative transcriptional regulation by an adjacent glucocorticoid receptor binding sequence, and was strongly associated with HDL levels in a large cohort of postmenopausal women. Moreover, they suggested that the functional promoter variant associated with increased HDL might act by increasing ESR1 expression and that the ESR1 PvuII polymorphism may also alter both beneficial and detrimental responses to exogenous estrogen administered in the form of HRT.

Importantly, we observed a significant increase of HDL levels in this postmenopausal group after HRT. In this regard, it would be interesting to suggest that for patients with low HDL levels the genotyping procedure could define more accurately the type of patient who would benefit from HRT. Moreover, only PvuII TT carriers, but not PvuII CC carriers, had a significant increase in HDL levels after 1 year of hormone replacement. The limitation of our study is the relatively small number of patients included. However, a strength of our study is that it is a prospective study of the Brazilian population, comparing the same women before and after HRT to address the question of whether or not HDL levels are associated with the ESR1 genotype.

We showed here that in our sample of Brazilian postmenopausal women with the ESR1 PvuII TT genotype, there is a significantly higher serum HDL lipoprotein concentration after HRT than in women with the PvuII CC or CT genotype.

Acknowledgments

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References


Table 1. Genotype frequencies and high-density lipoprotein levels before (HDLpre) and after (HDLpost) hormone replacement therapy in each estrogen receptor-alpha polymorphism.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency (%)</th>
<th>HDLpre</th>
<th>HDLpost</th>
</tr>
</thead>
<tbody>
<tr>
<td>MspI CC</td>
<td>7 (13)</td>
<td>57.29 ± 9.99</td>
<td>58.57 ± 13.08</td>
</tr>
<tr>
<td>MspI CT</td>
<td>25 (46)</td>
<td>52.44 ± 14.24</td>
<td>55.96 ± 15.78</td>
</tr>
<tr>
<td>MspI TT</td>
<td>22 (41)</td>
<td>58.00 ± 15.84</td>
<td>60.73 ± 13.19</td>
</tr>
<tr>
<td>HaeIII CC</td>
<td>38 (70)</td>
<td>55.58 ± 15.06</td>
<td>58.16 ± 13.46</td>
</tr>
<tr>
<td>HaeIII CT</td>
<td>12 (22)</td>
<td>53.92 ± 12.66</td>
<td>56.25 ± 15.33</td>
</tr>
<tr>
<td>HaeIII TT</td>
<td>4 (8)</td>
<td>57.25 ± 17.67</td>
<td>65.00 ± 21.67</td>
</tr>
<tr>
<td>PvuII CC</td>
<td>11 (20)</td>
<td>57.64 ± 18.08</td>
<td>61.64 ± 17.40</td>
</tr>
<tr>
<td>PvuII CT</td>
<td>23 (43)</td>
<td>56.04 ± 11.72</td>
<td>57.17 ± 13.19</td>
</tr>
<tr>
<td>PvuII TT</td>
<td>20 (37)</td>
<td>53.25 ± 15.70</td>
<td>57.60 ± 14.35*</td>
</tr>
<tr>
<td>Xbal AA</td>
<td>28 (52)</td>
<td>56.36 ± 14.49</td>
<td>58.93 ± 14.34</td>
</tr>
<tr>
<td>Xbal AG</td>
<td>21 (39)</td>
<td>54.14 ± 12.05</td>
<td>55.95 ± 13.51</td>
</tr>
<tr>
<td>Xbal GG</td>
<td>5 (9)</td>
<td>54.60 ± 16.41</td>
<td>64.00 ± 18.90</td>
</tr>
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</table>

Data are reported as means ± SD in mg/dL. *P < 0.05, HDLpost compared to HDLpre (t-test).