Comprehensive Evaluation of ESR2 Variation and Ovarian Cancer Risk

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Abstract

Studies indicate that estrogen receptor β, encoded by the ESR2 gene on chromosome 14q, may play a role in ovarian carcinogenesis. Using the genetic structure data generated by the Breast and Prostate Cohort Consortium (BPC3), we have comprehensively characterized the role of haplotype diversity in ESR2 and risk of ovarian cancer. Five haplotypes with a frequency of ≥5% were observed in White subjects and five haplotype tagging SNPs (htSNPs) were selected to capture the locus diversity with a minimum R² of 0.81. The htSNPs were genotyped in 574 White controls, 417 White invasive ovarian cancer cases, and 123 White borderline ovarian cancer cases from case-control studies carried out in Los Angeles County from 1994 through 2004. No statistically significant association was observed between the five htSNPs and related haplotypes and risk of ovarian cancer overall. Haplotype D was associated with a nonstatistically significant increased risk of invasive ovarian cancer overall (odds ratio, 1.38; 95% confidence interval, 0.93-2.02; P = 0.11) relative to the most common haplotype and a statistically significant increased risk of invasive clear cell ovarian cancer (odds ratio, 3.88; 95% confidence interval, 1.28-11.73; P = 0.016). Haplotype D was also reported by the BPC3 to be associated with increased risk of breast cancer. This haplotype warrants further investigation to rule out any effect with invasive ovarian cancer risk. (Cancer Epidemiol Biomarkers Prev 2008;17(2):393–6)

Introduction

There is limited understanding of the biology of ovarian cancer but there is evidence that increasing levels of estrogen may be associated with increased risk of the disease. Estrogen replacement therapy is associated with a 30% increased risk of ovarian cancer (1) and in vitro evidence suggests that treating ovarian cancer cell lines with estrogen increases cell proliferation (2).

The effect of estrogens on the ovary is mediated by the estrogen receptor (ER) isoforms ERα and ERβ, which are encoded by the genes ESR1 and ESR2, respectively. Both ER isoforms are expressed in the human ovary (3) and in the rat ovary (4), and there is some suggestion that overexpression of ERs relative to ERβ may be an indicator of ovarian carcinogenesis (5, 6).

Whereas the specific functions of ERβ in carcinogenesis are not yet known, there is evidence that the protein may have inhibitory effects on cellular proliferation. A recent study showed that transfection of an ERα-negative ovarian cancer cell line with ERβ resulted in both decreased cellular motility and growth (7). Additionally, Rutherford et al. (8) observed a complete absence of ERβ expression in metastatic ovarian tumors. Bandera et al. (9) observed an association between ovarian cancer and deletions at 14q12-13 and 14q32, regions surrounding the ESR2 gene.

Given the possible role of ERβ in ovarian carcinogenesis, we hypothesized that variation in the ESR2 gene may be associated with risk of ovarian cancer. We report here our results of a comprehensive evaluation of the association between this locus and ovarian cancer risk.

Materials and Methods

Ethics Approval. The work presented here was approved by the University of Southern California Institutional Review Board and all subjects provided informed consent.

Study Population. The subjects included in the present analysis were recruited into ongoing ovarian cancer case-control studies being conducted in Los Angeles County. Cases included in this report were diagnosed from 1994 through 2004. The details of this study have previously been described (10, 11). Briefly, cases were identified through the Los Angeles County...
Cancer Surveillance Program at USC, which is part of the National Cancer Institute Surveillance Epidemiology and End Results program. Cases were contacted and asked to participate in an in-person interview and to provide a DNA sample. Controls, matched on age, neighborhood of residence, and ethnicity, were recruited through a well-established neighborhood walking algorithm and also interviewed in person (10). The core questions related to reproductive history, exogenous hormone use, family history, and other established ovarian cancer epidemiologic risk factors used during the in-person interview remained constant throughout the study period. Overall participation rate for this period was 73% in both cases and controls. This report is restricted to White subjects.

### Gene Characterization and Haplotype Tag Single-Nucleotide Polymorphism Selection

The genetic structure of ESR2 was characterized by the Breast and Prostate Cancer Cohort Consortium (BPC3) using a panel of subjects from the Hawaii and Los Angeles County Multiethnic Cohort Study of Diet and Cancer composed of U.S. Blacks, Japanese, Latins, Native Hawaiians, and Whites (12). Briefly, a total of 40 single-nucleotide polymorphisms (SNP) were used to characterize the region, and from those SNPs the BPC3 selected four haplotype tagging SNPs (htSNP), which resulted in a minimum $R^2$ for 0.75 for Whites (12). One additional htSNP (rs944046) was selected for the current study, resulting in a minimum $R^2$ of 0.81 for Whites. The five htSNPs are rs3020450, rs1256049, rs1626031, rs944046, and rs4986938.

### Case-Control Genotyping

DNA was isolated using either a chloroform extraction process (13) or the Qiagen Blood Kit (Qiagen) and then whole genome amplified (14).

The five selected htSNPs were genotyped in the ovarian cancer cases and controls using the 5' nuclease TaqMan allelic discrimination assay (TaqMan, Applied Biosystems). The cases and controls were spread across the plates randomly and concordance was 100% between the 5% replicate samples. Hardy-Weinberg equilibrium was evaluated among control subjects and no deviations were observed.

### Statistical Analysis

Unconditional logistic regression (SAS Institute, version 9) was used to examine the association between the five htSNPs and risk of all epithelial ovarian cancer, and separately for invasive and borderline tumor cases. Analyses by histologic subtype were also conducted for serous, clear cell, endometrioid, and mucinous tumors separately. All analyses were adjusted for age group (<40, 40-49, 50-59, 60-69, and 70+). Log additive models were fitted and odds ratios (OR) were expressed per copy of the minor allele carried.

Haplotypes were reconstructed from genotype data using the TagSNPs program and the expected haplotypes for each individual were modeled. Haplotype risk was modeled using unconditional logistic regression (SAS Institute, version 9) by simultaneously modeling all haplotypes relative to the most common haplotype.

### Results

A total of 578 White controls, 417 invasive ovarian cancer cases, and 123 borderline ovarian cancer cases were included in this analysis. The mean ages were 55.7, 57.4, and 47.4 years for controls, invasive cases, and borderline cases, respectively.

There was no association between the five htSNPs and risk of ovarian cancer overall (Table 1). Neither invasive nor borderline tumors separately showed an association (Table 1). In addition, there was no association between the htSNPs and any histologic subtype (results not shown).

Five haplotypes with a frequency of ≥5% were observed (Table 2). Haplotype analysis revealed no statistically significant association between ovarian cancer and the common ESR2 haplotypes for either invasive cases or borderline cases separately or combined (Table 2). No statistically significant associations were observed with the haplotypes by histologic subtype with the exception of haplotype D, which showed an increased risk of clear cell invasive ovarian cancer [OR, 3.88; 95% confidence interval (95% CI), 1.28-11.73; $P = 0.016$; Table 3].

### Discussion

We conducted a comprehensive evaluation of variation at the ESR2 locus with ovarian cancer risk using a haplotype tagging framework and observed no significant association with either the individual htSNPs.

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9 http://www-rcf.usc.edu/%7Estram/tagSNPs.html
Table 2. Haplotype frequencies in controls (n = 578) and ORs for all cases (n = 540), invasive cases (n = 417), and borderline cases (n = 123) per copy of haplotype carried

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Haplotype frequency (%)</th>
<th>All cases</th>
<th>Invasive cases</th>
<th>Borderline cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All cases</td>
<td>Invasive</td>
<td>Borderline</td>
<td>All cases</td>
</tr>
<tr>
<td>CGCCG (haplotype A)</td>
<td>41.5</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TACAT (haplotype B)</td>
<td>26.0</td>
<td>0.98 (0.80-1.21)</td>
<td>0.88</td>
<td>1.02 (0.81-1.28)</td>
</tr>
<tr>
<td>TACAC (haplotype C)</td>
<td>10.7</td>
<td>1.05 (0.78-1.42)</td>
<td>0.76</td>
<td>1.14 (0.82-1.57)</td>
</tr>
<tr>
<td>CACAC (haplotype D)</td>
<td>7.1</td>
<td>1.23 (0.85-1.77)</td>
<td>0.27</td>
<td>1.38 (0.93-2.02)</td>
</tr>
<tr>
<td>CACAT (haplotype E)</td>
<td>5.9</td>
<td>0.94 (0.63-1.39)</td>
<td>0.74</td>
<td>0.99 (0.65-1.51)</td>
</tr>
</tbody>
</table>

*All analyses adjusted for age.

Table 3. Haplotype frequencies in controls (n = 578) and ORs for risk of invasive ovarian cancer by histologic subtype per copy of each haplotype carried

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Haplotype frequency (%)</th>
<th>Clear cell (n = 22)</th>
<th>Endometrioid (n = 55)</th>
<th>Mucinous (n = 34)</th>
<th>Serous (n = 262)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR* (95% CI)</td>
<td>P</td>
<td>OR* (95% CI)</td>
<td>P</td>
<td>OR* (95% CI)</td>
</tr>
<tr>
<td>CGCCG (haplotype A)</td>
<td>41.5</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TACAT (haplotype B)</td>
<td>26.0</td>
<td>1.06 (0.43-2.63)</td>
<td>0.90</td>
<td>0.86 (0.50-1.45)</td>
<td>0.56</td>
</tr>
<tr>
<td>TACAC (haplotype C)</td>
<td>10.7</td>
<td>1.05 (0.32-3.51)</td>
<td>0.93</td>
<td>0.94 (0.44-2.00)</td>
<td>0.86</td>
</tr>
<tr>
<td>CACAC (haplotype D)</td>
<td>7.1</td>
<td>1.38 (1.26-11.73)</td>
<td>0.016</td>
<td>1.01 (0.39-2.59)</td>
<td>0.99</td>
</tr>
<tr>
<td>CACAT (haplotype E)</td>
<td>5.9</td>
<td>1.20 (0.24-4.95)</td>
<td>0.91</td>
<td>1.20 (0.51-2.78)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

*All analyses adjusted for age.
References

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