Comprehensive Evaluation of ESR2 Variation and Ovarian Cancer Risk

Celeste Leigh Pearce,[1] Aimee M. Near,[5] Johannah L. Butler,[6,8] David Van Den Berg,[2] Philip Bretsky,[3] David V. Conti,[4] Daniel O. Stram,[1] Malcolm C. Pike,[1] Joel N. Hirschhorn,[6,8,7] and Anna H. Wu[1]

[1]USC/Norris Comprehensive Cancer Center and Department of Preventive Medicine, Keck School of Medicine of the University of Southern California; [2]Department of Urology, Norris Comprehensive Cancer Center, University of Southern California; [3]Cedars Sinai Medical Center; [4]Zilkha Neurogenetic Institute, University of Southern California, Los Angeles, California; [5]Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan; [6]Program in Genomics and Divisions of Genetics and Endocrinology, Children's Hospital; [7]Departments of Genetics and Pediatrics, Harvard Medical School, Boston, Massachusetts; and [8]Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, Massachusetts

Downloaded from http://aacrjournals.org/cebp/article-pdf/17/2/393/2266899/393.pdf by guest on 15 September 2024

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 (htSNP) were selected to capture the locus diversity with a minimum R_h^2 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 ERα 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

Received 8/24/07; revised 11/15/07; accepted 11/28/07.

Grant support: California Cancer Research Program grants 00-01389V-20170 and 2110200; USPHS grants CA14089, CA17054, CA61132, CA63464, N01-PC-67010, and R03-CA113148; and California Department of Health Services subcontract 050-E8709 as part of its statewide cancer reporting program.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Celeste Leigh Pearce, USC Norris Comprehensive Cancer Center, Room 4415A, 1441 Eastlake Avenue, Los Angeles, CA 90089. E-mail: cpearce@usc.edu

Copyright © 2008 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-07-2512

Table 1. Association between the five ESR2 htSNPs and ovarian cancer risk (per copy of minor allele carried) in Whites, displayed for all epithelial ovarian cancer cases, and invasive and borderline cases

SNP ID	Major/minor allele	MAF*	Controls (*n*)	All cases			Invasive			LMP		
				Cases (*n*)	OR† (95% CI)	*P*	Cases (*n*)	OR† (95% CI)	*P*	Cases (*n*)	OR† (95% CI)	*P*
rs4986938 (G1730A)	C/T	0.38	563	510	0.97 (0.82-1.16)	0.76	397	0.99 (0.82-1.19)	0.91	113	0.90 (0.67-1.23)	0.51
rs944046	A/G	0.44	556	523	0.95 (0.80-1.12)	0.54	403	0.88 (0.73-1.06)	0.16	120	1.24 (0.93-1.67)	0.15
rs1256049	C/T	0.02	576	533	1.17 (0.69-1.98)	0.57	412	1.37 (0.79-2.37)	0.26	121	0.53 (0.15-1.79)	0.30
rs1256031	A/G	0.47	560	531	1.00 (0.85-1.18)	1.00	410	0.95 (0.79-1.14)	0.55	121	1.22 (0.91-1.64)	0.18
rs3020450	C/T	0.33	572	534	0.95 (0.79-1.13)	0.55	412	0.94 (0.77-1.14)	0.50	122	0.98 (0.73-1.33)	0.91

*Minor allele frequency in controls.
† Adjusted for age group (five groups).

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, Latinas, 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_h^2 of 0.75 for Whites (12). One additional htSNP (rs944046) was selected for the current study, resulting in a minimum R_h^2 of 0.81 for Whites. The five htSNPs are rs3020450, rs1256031, rs1256049, rs994046, 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[9] 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

[9] http://www-rcf.usc.edu/%7Estram/tagSNPs.html

Downloaded from http://aacrjournals.org/cebp/article-pdf/17/2/393/2266899/393.pdf by guest on 15 September 2024

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 haploptype carried

Haplotype	Haplotype frequency (%)	All cases		Invasive		Borderline	
		OR* (95% CI)	*P*	OR* (95% CI)	*P*	OR* (95% CI)	*P*
CGCGC (haplotype A)	41.5	1.00		1.00		1.00	
TACAT (haplotype B)	26.0	0.98 (0.80-1.21)	0.88	1.02 (0.81-1.28)	0.89	0.86 (0.60-1.23)	0.41
TACAC (haplotype C)	10.7	1.05 (0.78-1.42)	0.76	1.14 (0.82-1.57)	0.44	0.76 (0.45-1.30)	0.31
CACAC (haplotype D)	7.1	1.23 (0.85-1.77)	0.27	1.38 (0.93-2.02)	0.11	0.76 (0.38-1.52)	0.44
CACAT (haplotype E)	5.9	0.94 (0.63-1.39)	0.74	0.99 (0.65-1.51)	0.96	0.80 (0.40-1.60)	0.53
Global *P*			0.87		0.56		0.80

*All analyses adjusted for age.

(Table 1) or the related haplotypes (Tables 2 and 3) for all cases or for invasive or borderline tumors separately.

One statistically significant association was observed for invasive clear cell tumors with haplotype D (OR, 3.88; 95% CI, 1.28-11.73; P = 0.016); however, this result is based on 22 cases and did not survive correction for multiple comparisons based on the Bonferroni or other criteria. Of interest, however, is that the BPC3 (12) has reported an association between breast cancer risk and this haplotype. Additionally, risk of invasive serous ovarian cancer was also elevated with this haplotype (OR, 1.36; 95% CI, 0.86-2.15; P = 0.18), and therefore haplotype D warrants further investigation.

This study was powered to detect effect sizes for the common htSNPs of 1.38 for all cases and 1.41 for invasive cases with 80% power, a two-sided α of 0.005 (to allow for the correction for multiple tests), and a log additive genetic model; we cannot rule out effect sizes of a lower magnitude. The minimum detectable ORs for the haplotypes ranged from 1.86 for the haplotypes with 6% frequency (haplotype E) to 1.41 for those with 41% frequency (haplotype A) under the same assumptions for invasive cases. The OR for haplotype D and risk of invasive ovarian cancer was 1.38 (95% CI 0.93-2.02; P = 0.11), which we were not powered to detect because the frequency of this haplotype was only 7.1%.

In the last 2 years, it has become common to evaluate the role of genetic variation using a SNP tagging framework, in addition to examining haplotype associations. For 72 SNPs downloaded (January 27, 2007) from the HapMap for the CEU (White) subjects, the multiallelic r^2 (r_s^2 from ref. 15) with the four htSNPs available in these data (rs3020450, rs1256031, rs1256049, and rs4986938) was at least 0.50 for 62 (86%) and at least 0.80 for 42 (58%) of the 72 SNPs. Thus, we seem to be capturing the majority of the known SNP variation in our haplotype analysis. Only a relatively few quite rare SNPs were very poorly tagged ($r^2 < 0.30$).

The biological rationale for examining *ESR2* as an ovarian cancer susceptibility locus is strong; however, we found limited evidence of an association between *ESR2* haplotype variation and ovarian cancer risk. Observations from Rutherford et al. (8) of loss of ERβ in metastatic ovarian cancer and the deletions in the *ESR2* regions in ovarian cancer (6) suggest that the gene may play a role in disease progression, but not necessarily susceptibility to disease.

Identifying genetic risk factors for ovarian cancer has thus far proved challenging with no unequivocally convincing findings to date. This is not surprising given our modest understanding of the etiology of ovarian cancer and the differences in underlying molecular characteristics of histologic subtypes of ovarian tumors (16-18), which limits our ability to select candidate genes and pathways. The most highly associated SNPs from the first breast cancer genome-wide scan study (19) have not fallen in the genes expected to be related to breast cancer based on our understanding of both the epidemiology and biology of the disease, which is far advanced compared with that of ovarian cancer. It is likely that genome-wide scans or larger-scale candidate gene studies in consortia-based efforts are critical to identifying the variants related to risk of this rare disease. Such studies are currently under way by multiple groups and will likely shed light on genetic susceptibility to ovarian cancer (11).

Acknowledgments

We thank all the study participants who contributed to this research.

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

Haplotype	Haplotype frequency (%)	Clear cell (n = 22)		Endometrioid (n = 55)		Mucinous (n = 34)		Serous (n = 262)	
		OR* (95% CI)	*P*	OR* (95% CI)	*P*	OR* (95% CI)	*P*	OR* (95% CI)	*P*
CGCGC (haplotype A)	41.5	1.00		1.00		1.00		1.00	
TACAT (haplotype B)	26.0	1.06 (0.43-2.63)	0.90	0.86 (0.50-1.45)	0.56	0.81 (0.42-1.57)	0.54	1.05 (0.80-1.37)	0.74
TACAC (haplotype C)	10.7	1.05 (0.32-3.51)	0.93	0.94 (0.44-2.00)	0.86	0.83 (0.32-2.14)	0.70	1.32 (0.92-1.89)	0.13
CACAC (haplotype D)	7.1	3.88 (1.28-11.73)	0.016	1.01 (0.39-2.59)	0.99	0.80 (0.24-2.67)	0.71	1.36 (0.86-2.15)	0.18
CACAT (haplotype E)	5.9	1.09 (0.24-4.95)	0.91	1.20 (0.51-2.78)	0.68	0.93 (0.30-2.92)	0.90	0.89 (0.53-1.49)	0.64

*All analyses adjusted for age.

Downloaded from http://aacrjournals.org/cebp/article-pdf/17/2/393/2266899/393.pdf by guest on 15 September 2024

References

1. Beral V, Bull D, Green J, Reeves G. Ovarian cancer and hormone replacement therapy in the Million Women Study. Lancet 2007;369: 1703 – 10.
2. Zhou H, Luo MP, Schonthal AH, et al. Effect of reproductive hormones on ovarian epithelial tumors: I. Effect on cell cycle activity. Cancer Biol Ther 2002;1:300 – 6.
3. Enmark E, Pelto-Huikko M, Grandien K, et al. Human estrogen receptor β-gene structure, chromosomal localization, and expression pattern. J Clin Endocrinol Metab 1997;82:4258 – 65.
4. Byers M, Kuiper GG, Gustafsson JA, Park-Sarge OK. Estrogen receptor-β mRNA expression in rat ovary: down-regulation by gonadotropins. Mol Endocrinol 1997;11:172 – 82.
5. Pujol P, Rey JM, Nirde P, et al. Differential expression of estrogen receptor-α and -β messenger RNAs as a potential marker of ovarian carcinogenesis. Cancer Res 1998;58:5367 – 73.
6. Bardin A, Boulle N, Lazennec G, Vignon F, Pujol P. Loss of ERβ expression as a common step in estrogen-dependent tumor progression. Endocr Relat Cancer 2004;11:537 – 51.
7. Treeck O, Pfeiler G, Mitter D, Lattrich C, Piendl G, Ortmann O. Estrogen receptor β1 exerts antitumoral effects on SK-OV-3 ovarian cancer cells. J Endocrinol 2007;193:421 – 33.
8. Rutherford T, Brown WD, Sapi E, Aschkenazi S, Munoz A, Mor G. Absence of estrogen receptor-β expression in metastatic ovarian cancer. Obstet Gynecol 2000;96:417 – 21.
9. Bandera CA, Takahashi H, Behbakht K, et al. Deletion mapping of two potential chromosome 14 tumor suppressor gene loci in ovarian carcinoma. Cancer Res 1997;57:513 – 5.
10. Pearce CL, Hirschhorn JN, Wu AH, et al. Clarifying the PROGINS allele association in ovarian and breast cancer risk: a haplotype-based analysis. J Natl Cancer Inst 2005;97:51 – 9.
11. Gayther SA, Song H, Ramus SJ, et al. Tagging single nucleotide polymorphisms in cell cycle control genes and susceptibility to invasive epithelial ovarian cancer. Cancer Res 2007;67: 3027 – 35.
12. Consortium TBaPCC. Haplotypes of the estrogen receptor β (ESR2) gene and breast cancer risk. Int J Cancer 2008;122:387 – 92.
13. Sambrook J, Russell DW. Molecular cloning: a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2001.
14. Dean FB, Hosono S, Fang L, et al. Comprehensive human genome amplification using multiple displacement amplification. Proc Natl Acad Sci U S A 2002;99:5261 – 6.
15. Stram DO. Tag SNP selection for association studies. Genet Epidemiol 2004;27:365 – 74.
16. Gemignani ML, Schlaerth AC, Bogomolniy F, et al. Role of KRAS and BRAF gene mutations in mucinous ovarian carcinoma. Gynecol Oncol 2003;90:378 – 81.
17. Obata K, Morland SJ, Watson RH, et al. Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. Cancer Res 1998;58:2095 – 7.
18. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer 2005;104:2807 – 16.
19. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007; 447:1087 – 93.

Downloaded from http://aacrjournals.org/cebp/article-pdf/17/2/393/2266899/393.pdf by guest on 15 September 2024