Research Article

ESR1/SYNE1 Polymorphism and Invasive Epithelial Ovarian Cancer Risk: An Ovarian Cancer Association Consortium Study

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Abstract

We genotyped 13 single nucleotide polymorphisms (SNPs) in the estrogen receptor alpha gene (*ESR1*) region in three population-based case-control studies of epithelial ovarian cancer conducted in the United States, comprising a total of 1,128 and 1,866 non-Hispanic white invasive cases and controls, respectively. A SNP 19 kb downstream of *ESR1* (rs2295190, G-to-T change) was associated with invasive ovarian cancer risk, with a per-T-allele odds ratio (OR) of 1.24 [95% confidence interval (CI), 1.06-1.44, *P* = 0.006]. rs2295190 is a nonsynonymous coding SNP in a neighboring gene called spectrin repeat containing, nuclear envelope 1 (*SYNE1*), which is involved in nuclear organization and structural integrity, function of the Golgi apparatus, and cytokinesis. An isoform encoded by *SYNE1* has been reported to be down-regulated in ovarian and other cancers. rs2295190 was genotyped in an additional 12 studies through the Ovarian Cancer Association Consortium, with 5,279 invasive epithelial cases and 7,450 controls. The per-T-allele OR for this 12-study set was 1.09 (95% CI, 1.02-1.17; *P* = 0.017). Results for the serous subtype in the 15 combined studies were similar to those overall (*n* = 3,545; OR, 1.09; 95% CI, 1.01-1.18; *P* = 0.025), and our findings were strongest for the mucinous subtype (*n* = 447; OR, 1.32; 95% CI, 1.11-1.58; *P* = 0.002). No association was observed for the endometrioid subtype. In an additional analysis of 1,459 borderline ovarian cancer cases and 7,370 controls, rs2295190 was not associated with risk. These data provide suggestive evidence that the rs2295190 T allele, or another allele in linkage disequilibrium with it, may be associated with increased risk of invasive ovarian cancer. *Cancer Epidemiol Biomarkers Prev; 19(1); 245–50. ©2010 AACR.*

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doi: 10.1158/1055-9965.EPI-09-0729

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Introduction

Ovarian cancer is hormone-related, and it has long been hypothesized that variation in steroid hormone biosynthesis, catabolism, and response genes may be associated with ovarian cancer risk. To explore whether genetic variation in these pathways is associated with ovarian cancer risk, we genotyped tag and functional single nucleotide polymorphisms (SNP) in and around (±20,000 bp) genes in these pathways in three studies of epithelial ovarian cancer conducted in the United States. Herein, we describe both an initial suggestive finding in our three-study effort for a coding SNP (rs2295190) in the estrogen receptor-α (*ESR1*) gene region and a follow-up genotyping effort in an independent data set comprising 12 studies from the international Ovarian Cancer Association Consortium (OCAC). rs2295190 is a missense SNP located 19 kb downstream of *ESR1* in the spectrin repeat containing, nuclear envelope 1 (*SYNE1*) gene. *SYNE1* encodes several different isoforms, many of which are involved in a variety of cellular processes including cytokinesis, Golgi function, and nuclear organization and structural integrity (1-3). One isoform, Drop1, has been noted to be downregulated in ovarian and other cancers (4).

Materials and Methods

Details of each of the 15 studies that participated in this analysis have been published (5, 6). All studies were approved by the ethics review boards of their parent institutions and obtained written informed consent from study participants prior to interview and collection of blood samples. All but one study (the Mayo Clinic Ovarian Cancer Case Control Study, MAY) was population-based.

Initial analyses were based on three case-control studies in the United States: Diseases of the Ovary and their Evaluation, Washington State (DOV); the Hormones and Ovarian Cancer Prediction Study, Pennsylvania, New York, Ohio (HOP); and the University of Southern California/Los Angeles County Case-Control Studies of Ovarian Cancer, California (USC). Genotyping was done at the University of Southern California using a 1,536 Illumina Golden Gate custom array to assess steroid hormone–related polymorphisms and others. A total of 693 tag and functional SNPs were selected for 42 hormone-related candidate genes, as well as regions spanning 20,000 bp upstream and downstream of the genes, in an attempt to genotype SNPs that might influence their expression. Thirteen SNPs were genotyped in the *ESR1* gene region. A total of 4,195 samples were assayed and 3,943 had genotyping success of >90%. Because the minor allele frequency of rs2295190 is considerably lower in non-whites, we restricted analyses to the 3,315 non-Hispanic white women who were successfully genotyped for rs2295190, including 1,128 women with invasive epithelial ovarian cancer, 321 women with borderline epithelial ovarian tumors, and 1,866 controls.

Although analyses of the steroid hormone pathway genes and gene-environment interactions within our three-study collaboration are still ongoing, the OCAC provides an early opportunity for independent fast-track evaluation of initial positive findings of participating studies, and rs2295190 was nominated because it was the SNP most strongly associated with invasive ovarian cancer risk among the hormone-related genes (additive model *P* = 0.006). Thus, this SNP was examined in 12 additional case-control studies that are members of the OCAC (six from the United States [the Hawaiian Ovarian Cancer Study (HAW), MAY, the North Carolina Ovarian Cancer Study (NCO), the New England–based Case-Control Study (NEC), Genetic Epidemiology of Ovarian Cancer Study, Stanford University (STA), and the UC Irvine Ovarian Cancer Study (UCI)], one from Australia [the Australian Cancer Study/Australian Ovarian Cancer Study (AUS)], and five from Europe [the German Ovarian Cancer Case-Control Study (GER), MALOVA Ovarian Cancer Case-Control Study, Danish Cancer Society, Denmark (MAL), the Polish Ovarian Cancer Study (POL), Studies of Epidemiology and Risk Factors in Cancer Heredity, UK (SEA), and the UK Ovarian Cancer Population Study (UKO)]. Genotyping for these latter studies was done using the TaqMan allelic discrimination assay (TaqMan; Applied Biosystems) with the exception of AUS which used the Sequenom iPlex gold genotyping platform (Sequenom, Inc.). After excluding 65 women with missing tumor behavior, these 12 studies provided genotype data for 5,279 non-Hispanic white women with invasive and 1,138 women with borderline epithelial ovarian cancer, and 7,450 controls. Information regarding Hispanic ethnicity was not collected in some studies conducted in populations with few Hispanics; in those instances, all white women were considered to be non-Hispanic.

The OCAC has developed a set of genotyping quality control procedures, described in Ramus et al. (7). All laboratories providing data for these analyses genotyped a common set of DNA samples; the concordance across laboratories was 100% and genotyping success was >90%. All studies had concordance for duplicate samples of ≥98%, with ≥95% sample call success overall, and call rates for every 384-well plate of 90% or above.

Statistical Analyses

Data were compiled centrally at the OCAC data coordinating centers at Duke University and the University of Southern California. Genotype frequencies in (non-Hispanic white) controls were assessed for departure from Hardy-Weinberg Equilibrium using the Fisher's exact test. Per allele odds ratios (OR) and their 95% confidence intervals (CI) were estimated using unconditional logistic regression. Our analyses examined risk of invasive and borderline epithelial tumors separately, and we assessed risk within histologic subgroups. All analyses were adjusted for age and study site and were done using Stata v. 10 (STATA Corporation). The "meta" command, which

Table 1. Distribution of rs2295190 genotypes by case-control status, and Hardy-Weinberg equilibrium *P* values for controls, by study population

Study	Case/control genotype*			T allele frequency, controls	P_{HWE}
	GG	GT	TT		
Initial three studies					
DOV	329/483	141/162	16/12	0.142	0.87
HOP	214/497	77/166	3/8	0.136	0.19
USC	258/417	80/117	10/4	0.116	0.21
Twelve additional studies					
AUS	617/789	236/295	23/16	0.149	0.06
GER	148/302	52/111	3/14	0.163	0.37
HAW	45/110	23/46	1/2	0.158	0.37
MAL	281/546	143/223	15/25	0.172	0.71
MAY	193/262	74/99	7/5	0.149	0.30
NCO	446/559	155/169	9/10	0.128	0.62
NEC	449/682	145/200	11/19	0.132	0.31
POL	160/408	87/164	7/17	0.168	0.88
SEA	675/893	244/291	18/29	0.144	0.35
STA	191/129	72/48	3/4	0.155	1.00
UCI	203/331	64/79	11/10	0.118	0.06
UKO	336/411	122/133	10/19	0.152	0.05

NOTE: Restricted to non-Hispanic white women.
*Invasive cases only.

Results

The distribution of the rs2295190 GG, GT, and TT genotypes among women with invasive ovarian cancer and controls by study site is presented in Table 1. We found no evidence of deviation from Hardy-Weinberg equilibrium ($P > 0.05$ for each study), and the frequency of the T allele among controls ranged from 12% to 17% (Table 1). The minor allele was associated with an increased risk of invasive epithelial ovarian cancer both in the initial 3-study collaboration and in the 12-study data [per-T-allele ORs and 95% CIs were, respectively: 1.24 (1.06-1.44, $P = 0.006$) and 1.09 (1.02-1.17, $P = 0.017$; Table 2)]. Results from unconditional logistic regression models are presented in Table 2 and were essentially identical for the random- and fixed-effects models. No heterogeneity between studies was observed ($P_{heterogeneity}$ for 15 studies = 0.73; Fig. 1). Among invasive cancers, the association was present for serous tumors ($n = 3,545$; OR in 15 studies, 1.09; 95% CI, 1.01-1.18; $P = 0.025$) and strongest for the mucinous subtype ($n = 447$; OR in 15 studies, 1.32; 95% CI, 1.11-1.58; $P = 0.002$). No association was observed for endometrioid tumors (Table 2). The SNP was not associated with risk of borderline tumors overall (Table 2), or

with histologic subtypes of serous ($n = 802$; OR in 15 studies, 0.94; 95% CI, 0.80-1.10; $P = 0.415$) or mucinous borderline tumors ($n = 580$; OR in 15 studies, 1.06; 95% CI, 0.89-1.26; $P = 0.510$; data not shown).

Discussion

The T allele of rs2295190 was associated with increased invasive ovarian cancer risk in our initial three-study investigation, and an association (although smaller in magnitude) subsequently was observed in an independent set of 12 additional studies. Although rs2295190 was selected because it is located in the *ESR1* gene region, it is also a missense SNP in an adjacent gene, *SYNE1*.

A recent genome-wide association study reported that a SNP 180 bp upstream of *ESR1* (rs2046210) was associated with breast cancer risk (8). This SNP is located nearly 296 kb away from rs2295190 and is not in linkage disequilibrium with it. rs2295190 is located 19 kb downstream of *ESR1* in the COOH terminus region of *SYNE1*, and is a G-to-T change that results in a conservative amino acid substitution from leucine to methionine. Based on a >1,300 kb region encompassing 250 kb upstream of *ESR1* to 250 kb upstream of *SYNE1* in the International Haplotype Map Project (HapMap, build 24) Caucasian data, three SNPs were in linkage disequilibrium with rs2295190 ($r^2 > 80\%$; rs6934016, rs13211987, and rs17082180); all are noncoding SNPs in *SYNE1*. The T allele of rs2295190 is predicted to be damaging to *SYNE1*

Table 2. Per allele ORs and 95% CIs for borderline and invasive ovarian cancer by histologic subtype

	Initial three studies*				Twelve additional studies[†]				All			
	Cases	OR[‡]	(95% CI)	P	Cases	OR[‡]	(95% CI)	P	Cases	OR[‡]	(95% CI)	P
All invasive	1,128	1.24	(1.06-1.44)	0.006	5,279	1.09	(1.02-1.17)	0.017	6,407	1.11	(1.04-1.19)	0.001
Serous	644	1.31	(1.09-1.57)	0.004	2,901	1.05	(0.97-1.15)	0.248	3,545	1.09	(1.01-1.18)	0.025
Mucinous	61	1.65	(1.01-2.68)	0.043	386	1.28	(1.06-1.55)	0.011	447	1.32	(1.11-1.58)	0.002
Endometrioid	172	0.93	(0.66-1.31)	0.675	854	1.00	(0.87-1.15)	0.986	1,026	0.99	(0.87-1.13)	0.852
Clear cell	72	1.37	(0.87-2.16)	0.176	462	1.06	(0.88-1.28)	0.532	534	1.10	(0.93-1.31)	0.276
All other histologies	179	1.21	(0.89-1.64)	0.229	676	1.23	(1.06-1.43)	0.006	855	1.23	(1.08-1.41)	0.002
All borderline[§]	321	1.01	(0.78-1.32)	0.913	1,138	0.99	(0.86-1.13)	0.852	1,459	1.00	(0.88-1.12)	0.936

*DOV, HOP, and USC; 1,866 controls.

[†]ACS, GER, HAW, MAL, MAY, NCO, NEC, POL, SEA, STA, UCI, and UKO; 7,450 controls.

[‡]Adjusted for age and study site.

[§]Sites MAL, POL, and UKO had no borderline ovarian cancer cases and their 1,946 controls were excluded from this analysis.

protein function based on the Sorting Intolerant from Tolerant algorithm, which aligns related protein sequences and predicts the functional effect of SNPs based on the concept that an amino acid change in a highly conserved area across protein sequences would be damaging to protein function, whereas a poorly conserved region is more likely to be tolerant to such changes (9). Although rs2295190 is a coding SNP in the *SYNE1* gene, it is also possible that this SNP, or one in linkage disequilibrium with it, could lie in a region encoding an as-of-yet uncharacterized enhancer or repressor of *SYNE1*, or less plausibly, *ESR1*.

SYNE1 is an unusually large gene (0.5 Mb) on chromosome 6q25.1-25.2 (10) that encodes several protein isoforms through alternative transcriptional initiation, termination, and splicing. Many of the isoforms localize to the nuclear envelope, Golgi apparatus, and cytoskeleton and are involved in a variety of cellular processes including cytokinesis and Golgi function as well as organization, structural integrity, and positioning of the nucleus (1-3). Mutations towards the 3′ end of this gene are associated with cerebellar ataxia and Emery-Dreifuss muscular dystrophy (10, 11).

One of the shorter transcripts, Drop1, is encoded in the 5′ end of the *SYNE1* gene and has been observed to be markedly downregulated in ovarian tumors compared with normal ovarian surface epithelium from the same individual. These results were supported by expression profiling, *in situ* hybridization, and immunostaining for Drop1 protein. Estrogen receptor-α expression was not correlated with Drop1 expression in tumor samples, although their expression was correlated in ovarian carcinoma cell lines (4). In the same study, Drop1 mRNA was also observed to be downregulated in carcinomas of the uterus, cervix, kidney, thyroid, pancreas, and lung (4). *SYNE1* is frequently methylated in lung cancer cell lines, lung adenocarcinoma (12), and colorectal cancer (13), and has been observed to be mutated in colorectal cancer (14).

Among invasive ovarian cancers, the association with rs2295190 was observed for the serous subtype and was strongest for the mucinous subtype, with no association observed for endometrioid tumors. These results should be interpreted cautiously as differences may exist between studies in defining histology. Although some evidence suggests that mucinous invasive and borderline ovarian tumors may share a common origin (15), we noted no clear association of rs2295190 with risk of mucinous borderline tumors.

Even with this relatively large sample set, because the frequency of the T allele is low (12-17%), the statistical power for some subgroup analyses is limited, and it is possible that our findings are due to chance. It is also possible that residual confounding due to population
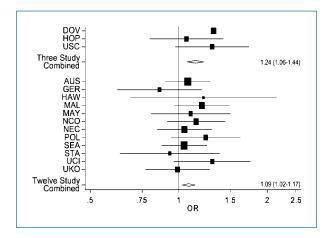

Figure 1. Per-allele age-adjusted ORs (boxes) and 95% CIs (lines) for rs2295190 and invasive ovarian cancer risk by study, and combined ORs and 95% CIs for the initial three studies and the additional 12 studies.

stratification may influence our results, although it is unlikely because analyses were restricted to non-Hispanic white women and adjusted for study site, and results were similar across studies with different populations.

In conclusion, our study, which is based on the international OCAC, provides suggestive evidence that the T allele of rs2295190, or another allele in linkage disequilibrium with it, may be associated with an increased risk of invasive ovarian cancer. Efforts to further characterize this region around *ESR1/SYNE1* are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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