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Genetic Polymorphisms in the *Paraoxonase 1* Gene and Risk of Ovarian Epithelial Carcinoma

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Abstract

Oxidative stress during successive ovulations increases the opportunity for DNA damage to ovarian epithelial cells and the potential for malignant transformation. Paraoxonase 1 (PON1) is an endogenous free radical scavenger that reduces oxidative stress. The association of two common functional single nucleotide polymorphisms (SNP), rs854560 T>A and rs662 A>G, with the risk of epithelial ovarian cancer was examined in a population-based case-control study in Hawaii. A personal interview and blood specimens were collected from 274 women with histologically confirmed, primary ovarian cancer and 452 controls frequency matched on age and ethnicity. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated by unconditional logistic regression. Both *PON1* SNPs were significantly associated with ovarian cancer risk. The ORs were 0.53 (95% CI, 0.35–0.79; *P* for allele-dose effect = 0.01) for women carrying the rs854560 T allele compared with women with the AA genotype and 0.65 (95% CI, 0.44–0.95; *P* for allele-dose effect = 0.03) for women carrying the rs662 A allele compared with women with the GG genotype. The association of the rs854560 T genotype with risk was stronger among smokers (OR, 0.33; 95% CI, 0.17–0.64; *P* for allele-dose effect = 0.0007) than among nonsmokers (OR, 0.68; 95% CI, 0.40–1.18; *P* for allele-dose effect = 0.53). The decreased risk associated with the rs854560 T allele was also stronger among obese women (OR, 0.19; 95% CI, 0.06–0.55; *P* for allele-dose effect = 0.007) than among nonobese women (OR, 0.62; 95% CI, 0.40–0.98; *P* for allele-dose effect = 0.16). Our study provides evidence for an association of two *PON1* SNPs with the risk of epithelial ovarian cancer. Possible effect modification of these associations by tobacco smoking and obesity needs confirmation in other studies.

Introduction

The process of ovulation is associated with inflammation at the local level, cell damage, and oxidative stress (1–3). Although oxygen radicals may play an important physiologic role within the ovary (4,5), elevated oxidative stress has been found to mediate carcinogenesis by causing metabolic malfunction and damage to biological molecules, including DNA (6–9). Oxygen radicals can oxidize DNA bases, form mutagenic lesions and chromosome aberrations, and activate chemical carcinogens into highly reactive compounds. The cyclic production of

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

oxygen radicals through repeated ovulation without long dormant periods has been hypothesized to increase susceptibility to malignant transformation of the ovarian epithelium (10), especially under conditions of reduced antioxidant status (4). Several epidemiologic studies of an association of ovarian cancer risk with polymorphisms in genes involved in oxidative stress support this hypothesis (11–13).

The antioxidant paraoxonase 1 (PON1) is a member of a family of calcium-dependent hydrolases with a variety of enzymatic properties, including lactonase, arylesterase, peroxidase, and phospholipase activities (14). It has been reported that PON1 hydrolyzes esters of phenolic compounds, including estrogen monoesters and diesters at position 3 of the steroid A-ring (15), thus increasing their antioxidant properties. High-density lipoprotein complexed to PON1 may have enhanced anti-inflammatory and antioxidant functions, protecting low-density lipoprotein against oxidation in the presence of hydrolysable estrogen esters (15,16). Such complexing would be relevant to ovarian carcinogenesis as oxidized low-density lipoprotein has been reported to have potent mitogenic properties (17) through heightened release of inflammatory cytokines and growth factors (18). High circulating levels of oxidized low-density lipoprotein among ovarian cancer patients compared with age-matched controls support this notion (19), although cause and effect relations cannot be established with this cross-sectional design.

PON1 activity varies widely among individuals, which is in part related to polymorphisms in this gene (16,20). The *PON1* gene has two common missense single nucleotide polymorphisms (SNP): rs854560 T>A in exon 3 that results in a leucine-to-methionine substitution (L55M) and rs662 A>G in exon 6 that results in a glutamine-to-arginine exchange (Q192R; ref. 21). The physiologic relevance of these polymorphisms was established by their association with significant differences in serum PON1 concentration and activity (22–28). The rs662 polymorphism does not appear to influence serum PON1 levels but directly affects its enzymatic activity in relation to several substrates (24). Independently of rs662 and some newly discovered *PON1* promoter polymorphisms, the rs854560 T allele was associated with significantly higher PON1 serum concentrations (27). Decreased stability of the isoform associated with the rs854560 A allele might also contribute to variations in serum paraoxonase levels (28).

PON1 has been identified as an independent risk factor for atherosclerotic disease (29), but there have been limited investigations of PON1 activity in association with cancer risk. Serum PON1 activity was found to be significantly lower in gastric (30), pancreatic (31), and lung (32) cancer patients when compared with healthy controls. A relation of the rs854560 and rs662 genotypes with the risk of breast (33,34), prostate (35), and lung (36) cancers, non-Hodgkin's lymphoma (37), and multiple myeloma (38) has also been reported.

In this study, we examined the rs854560 and rs662 SNPs in relation to the risk of epithelial ovarian carcinoma in Caucasian, Japanese, and Native Hawaiian women in Hawaii. We also explored whether tobacco smoking and obesity modified the association of these polymorphisms with risk. Several investigations reported reduced PON1 activity among tobacco smokers [reviewed by Costa et al. (39)]. In addition, smoking may enhance oxidative stress through production of reactive oxygen radicals (40,41). Compared with nonobese women, obese women were found to have significantly lower PON1 activity (42). We hypothesized that women who carried *PON1* alleles associated with reduced PON1 activity and lower concentrations might be at higher ovarian cancer risk if they were tobacco smokers or obese.

Materials and Methods

Study Design and Population

This population-based case-control study included 274 women of Caucasian, Japanese, and Native Hawaiian ethnicity, ages ≥ 18 years, who were diagnosed with primary histologically confirmed epithelial ovarian carcinoma between 1993 and 2006. Incident cases were identified through the rapid reporting system of the Hawaii Tumor Registry, which is part of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute (43). Information on tumor histology was obtained from pathology and surgical reports. Control subjects ($n = 452$) were randomly selected from participants in an annual survey of representative households in Hawaii (44) and were frequency matched to cases based on ethnicity and 5-year age groups in $\sim 1:1.6$ ratio. Eligibility criteria for controls included ages ≥ 18 years, residency in Hawaii for a minimum of 1 year, no prior history of ovarian cancer, and having at least one intact ovary. The response rate was 64% for cases and 67% for controls. The study protocol was approved by the Institutional Review Board of the University of Hawaii. All study participants provided written informed consent.

Data Collection

Sociodemographic, lifestyle, and health-related information was collected during a 1.5- to 2-h interview using a structured pretested questionnaire that included sociodemographic, lifestyle, and health-related information (45). Interviewers were uniformly trained and supervised to standardize interviewing and coding techniques. Quality control and performance of the interviewers were monitored by the project coordinator through a repeat interview of a random sample of 15% of subjects on a random 5% of the interview questions.

Genotyping

DNA was purified from whole blood using Qiagen Midi Kits. Genotyping was done with the 5' nuclease discrimination assay using TaqMan (Applied Biosystems). Samples from cases and controls were intermixed on each plate. Each 384-well plate included 48 randomly selected blinded samples and 8 non-DNA controls to evaluate accuracy and reproducibility. The call rates were 98.6% and the concordance rates among duplicates were 100% for both SNPs. Gene and allele nomenclature was according to the National Center of Biotechnology Information (21).

Statistical Analysis

The SAS statistical software package version 9.1 (SAS Institute) was used for the data analysis. Fisher's goodness-of-fit test was used to assess whether allele frequency distributions among controls overall and in each ethnic group were consistent with Hardy-Weinberg equilibrium. Unconditional multiple logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for the association of genotype with ovarian cancer risk. The genotype for each SNP was treated as a nonordered categorical variable to test for heterogeneity and as an ordered categorical variable (with three levels: 0, 1, and 2; one assigned to each genotype) to test for an allele-dose effect. Pair-wise linkage disequilibrium (D') and correlation coefficients (r^2) were estimated using the HAPLO-VIEW program (46). Both SNPs were in a very modest linkage disequilibrium among all studied ethnic groups (r^2 range, 0.14–0.23 among ethnic groups and 0.20 in all women combined). A test of interaction between the SNPs resulted in a P value of 0.12. Therefore, to study joint effect of both SNPs, we created combined genotypes using a dichotomous variable for each SNP (variant allele present/absent). Homozygous carriers of the common rs854560 and rs662 alleles were used as the reference group ("AA-GG combined genotype") for these models. All combinations were included in this analysis with the exception of the rare "Any T-GG genotype" ($n = 1$).

All models were adjusted for age and ethnicity to control for residual confounding by matching variables. Final models were also adjusted for parity, tubal ligation, menopausal status, use of contraceptive steroids, use of menopausal hormones, tobacco smoking (defined as daily cigarette smoking for at least 6 months), and body mass index (BMI; calculated as the ratio of self-reported usual adult weight in kilograms divided by the square of the height in meters). Association of SNP with ovarian cancer risk by histologic type was studied using polytomous logistic regression model. A Wald test was used to compare the estimated ORs among case groups defined by histologic type. The interaction of the two PON1 polymorphisms with ethnicity, tobacco smoking (current or past smoker versus nonsmoker), and obesity (BMI ≥ 30 versus BMI < 30) was examined by comparing models with and without interaction terms using the likelihood ratio test. All *P* values were based on two-tailed tests. *P* values < 0.05 were considered statistically significant.

Results

The mean age of the study participants was 55.9 years (SD, 13.6; range, 18–88). Participant characteristics are presented in Table 1. Women with a family history of ovarian cancer in first-degree relatives had a significantly increased ovarian cancer risk. Parity, use of contraceptive steroids, and use of menopausal estrogens in combination with progesterone were inversely associated with ovarian cancer risk. Tubal ligation and premenopausal status were nonsignificantly associated with decreased ovarian cancer risk. Ovarian cancer risk increased (nonsignificantly) with increased BMI. History of tobacco smoking was not associated with risk.

The distribution of *PON1* rs854560 and rs662 genotypes among controls was consistent with Hardy-Weinberg equilibrium in each ethnic group and among all subjects combined. Minor allele frequencies differed significantly by ethnic group ($P < 0.0001$ for both SNPs; Table 2). The rs854560 T allele frequency was lower in Japanese (0.07) and Hawaiian women (0.15) than in Caucasians (0.43). The rs662 G allele was a minor allele in Caucasian women (0.30) but had higher frequency in Japanese (0.61) and Hawaiians (0.66). Carriers of the rs854560 T allele (L55) and rs662 A (Q192) alleles were at significantly reduced risk of ovarian cancer compared with women who were carriers of the rs854560 AA and rs662 GG genotypes, respectively (Table 3). The trend for an allele-dose effect was statistically significant for both SNPs ($P = 0.01$ for rs854560 and 0.03 for rs662). Compared with women who were carriers of the rs854560-rs662 “AA-GG combined genotype” (most common in our population), carriers of the “Any T-Any A combined genotype” had their risk of ovarian cancer reduced by 57%. These genotype associations with risk were generally consistent among all ethnic groups studied (test for heterogeneity of among ethnic groups was not statistically significant for both SNPs).

Although the sample size was modest for histology-specific analyses, the associations of the rs854560 T and rs662 A alleles with risk were reduced among all histologic types (Table 4).

Reduction in risk reached the level of statistical significance for serous carcinoma among rs854560 T allele carriers and for clear cell and mixed/other histology carcinomas among carriers of rs662 A allele. Although effects were stronger for some histologic subtypes, there were no statistically significant differences in risk by histology ($P_{\text{interaction}} = 0.27$ and 0.70 for rs854560 and rs662, respectively).

The association of the rs854560 genotype with risk was stronger among smokers than among nonsmokers, although evidence for a gene-environment interaction was weak ($P = 0.07$; Table 5). Among smokers, women who were carriers of the rs854560 T allele had a significant 67% reduction in ovarian cancer risk compared with women who were homozygous for the A allele;

the allele-dose effect was statistically significant ($P = 0.0007$). Smokers who were carriers of the combined rs854560-rs662 “Any T-Any A genotype” had a significantly reduced ovarian cancer risk compared with women with an AA-GG genotype. Among nonsmokers, the association of the rs854560 and rs854560-rs662 genotypes with risk were not statistically significant. The relation of the rs854560 T allele to risk was also stronger among obese women than among nonobese women, although both associations were statistically significant (Table 5) and no evidence for gene-environment interaction was observed. By contrast, the association of the rs662 genotype with risk did not vary by smoking status or obesity.

Discussion

We examined the association of two common functional polymorphisms in the *PON1* gene in relation to ovarian epithelial cancer risk in a multiethnic population of women in Hawaii. Allele distributions in control subjects varied significantly by ethnic group and were similar to those reported by the National Center of Biotechnology Information (21) for Caucasian and Japanese women and by SNP500CANCER³ for Hawaiian participants. We found that the rs854560 T (L55) and rs662 A (Q192) alleles were associated with a reduced risk of ovarian cancer compared with carriers of the common AA and GG genotypes in an allele-dose-dependent manner. Although both SNPs were associated with ovarian cancer risk, the linkage disequilibrium between them was modest. Assessment of their joint effect showed that the rs854560-rs662 combined genotype including variant alleles of both SNPs (“Any T-Any A”) was also associated with a decreased ovarian cancer risk. Results were generally consistent across all ethnic groups studied.

Tobacco smoking and obesity appeared to strengthen the association of the rs854560 genotype with risk, although we did not find significant gene-environment interactions. Cigarette smoking increases oxidative stress not only through the production of reactive oxygen radicals (41) but also through inhibition of PON1 enzymatic activity (39). Carriers of the rs854560 AA genotype associated with reduced PON1 concentrations might be more vulnerable to oxidative stress through tobacco smoking, thereby increasing their risk of ovarian cancer. Indeed, risk among nonsmokers did not differ significantly by rs854560 genotype. Increased ovarian cancer risk among obese women with rs854560 AA genotype is also biologically plausible, as PON1 activity is known to be reduced among obese individuals (42).

Findings from several investigations indicate that the *PON1* polymorphism might influence cancer risk. Investigators of the rs854560 SNP in relation to breast (33) and prostate (35) cancers reported higher risk associated with the AA (M55M) genotype, which is consistent with our findings. In contrast to our findings, carriers of the rs662 AA (Q192Q) genotype have been observed to have an increased risk of lung (36), breast (34), and prostate (35) cancers. However, the higher ovarian cancer risk found among women with the GG (R192R) genotype in our investigation is consistent with the nearly 3-fold increased risk of non-Hodgkin’s lymphoma (37) and with an elevated risk of multiple myeloma (38) found in relation to this genotype in other studies. No relation of either rs854560 or rs662 genotype was found in a case-control study of colorectal cancer (47). The rs662 G (192R) allele has been associated with an increased risk of coronary heart disease (OR, 1.12; 95% CI, 1.07–1.16) in a meta-analysis of 43 studies (48), including studies in Japanese populations.

The biological plausibility of an rs854560 T (L55) allele association with higher ovarian cancer risk is supported by experimental studies. Carriers of the rs854560 T (L55) allele have significantly higher serum PON1 concentrations than A (55M) allele carriers (23,26) resulting in differences in enzyme activities with all substrate types (26). The rs662 polymorphism, on

³<http://www.genecards.org/cgi-bin/carddisp.pl?gene=PON1&search=PON1>

the other hand, determines substrate-dependent alloenzymes. The alloenzyme coded by the G (192R) allele hydrolyzes various substrates, such as paraoxone, more rapidly than the enzyme associated with the A (Q192) allele (20). However, the Q-type isozyme is more efficient in protecting against low-density lipoprotein oxidation than the R-type (49,50).

A variety of risk factors for ovarian cancer including long-term ovulation, endometriosis, and pelvic inflammatory disease support the hypothesis that persistent inflammation of the ovarian epithelium may play a role in the carcinogenic process (51). Inflammation involves oxidative stress at the local level, enhancing the opportunity for mutagenesis during repair of the damaged ovarian epithelium. PON1, which has been found in human ovarian follicles, prevents the oxidation of low-density lipoproteins and may influence oxidative events in the cyclooxygenase and lipoxygenase pathways (52). An association of PON1 with the development of ovarian cancer is consistent with the anti-inflammatory role of this enzyme. Circulating PON1 levels were significantly lower among women with endometriosis (53), the presence of endometrial tissue outside of the uterus, which causes a marked local inflammatory reaction. PON1 activity is also reduced among women with polycystic ovary syndrome (54), a condition associated with low-grade chronic inflammation (55) and possibly ovarian cancer (56).

The PON gene family consists of three members, PON1, PON2, and PON3, located adjacent to each other on chromosome 7 (57). All three gene products share an ability to protect against low-density lipoprotein oxidation and to hydrolyze aromatic and longchain aliphatic lactones, although PON1 is the most abundant in human serum. As all these genes are located in close proximity, haplotype block analysis involving all three genes may be informative.

Strengths of this study include its population-based, histologic confirmation of all case diagnoses and stringent laboratory quality-control procedures. This investigation included a modest number of study subjects, limiting our ability to examine gene-environment interactions or to stratify by histologic subtype. Based on our case-control design, we were unable to measure prediagnostic levels of PON1, although we think that such an assessment would be informative using a prospective study design where the issue of reverse causality is not a concern (39). We acknowledge that *PON1* SNPs do not account for all of the variability in PON1 protein level or activity, but PON1 activity is influenced by rs854560 and rs662 genotypes to an important degree (27). In conclusion, our data suggest that *PON1* genetic variants may alter the risk of epithelial ovarian cancer among women in a multiethnic population.

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Table 1

Participant characteristics

Characteristics	Participants, <i>n</i> (%)		OR (95% CI) [*]
	Cases (<i>n</i> = 274)	Controls (<i>n</i> = 452)	
Age (y)			
<45	52 (19)	75 (17)	Matching factor
45–54	79 (28)	128 (28)	
55–64	75 (27)	134 (30)	
>64	68 (25)	115 (25)	
Ethnicity			
Caucasian	90 (33)	158 (35)	Matching factor
Japanese	102 (37)	175 (39)	
Hawaiian	82 (30)	119 (26)	
Family history of ovarian cancer			
No	259 (95)	445 (98)	1.00 (reference)
Yes	15 (5)	7 (2)	4.08 (1.53–10.87)
Parity			
Nulliparous	85 (31)	72 (16)	1.00 (reference)
1	41 (15)	66 (15)	0.61 (0.36–1.04)
2	66 (24)	120 (27)	0.55 (0.34–0.88)
3	41 (15)	99 (22)	0.41 (0.24–0.71)
≥4	41 (15)	95 (21)	0.36 (0.20–0.64)
Used oral contraceptives			
No	141 (51)	144 (32)	1.00 (reference)
Yes	133 (49)	308 (68)	0.39 (0.26–0.58)
Had tubal ligation			
No	225 (82)	313 (69)	1.00 (reference)
Yes	49 (18)	139 (31)	0.69 (0.45–1.07)
Menopausal status			
Postmenopausal	185 (68)	303 (67)	1.00 (reference)
Premenopausal	89 (32)	149 (33)	0.52 (0.23–1.22)
Type of menopause			
Natural menopause	161 (87)	262 (86)	1.00 (reference)
Hysterectomy	24 (13)	41 (14)	0.97 (0.51–1.85)
Use of menopausal hormones			
Never used	106 (57)	135 (45)	1.00 (reference)
Estrogen only	23 (13)	38 (12)	0.79 (0.40–1.55)
Progesterone only	11 (6)	15 (5)	0.69 (0.28–1.68)
Combined estrogen and progesterone	45 (24)	115 (38)	0.58 (0.36–0.93)
BMI			
Underweight (BMI ≤ 18.5)	6 (2)	18 (4)	1.00 (reference)
Normal (18.5 < BMI < 25)	141 (52)	218 (48)	1.72 (0.63–4.75)
Overweight (25 ≤ BMI < 30)	63 (23)	126 (28)	1.44 (0.50–4.09)

Characteristics	Participants, <i>n</i> (%)		OR (95% CI) [*]
	Cases (<i>n</i> = 274)	Controls (<i>n</i> = 452)	
Obese (BMI ≥ 30)	64 (23)	90 (20)	1.63 (0.57–4.71)
Smoking			
Never smoked	114 (42)	198 (44)	1.00 (reference)
Past or current smoker	160 (58)	254 (56)	0.92 (0.66–1.30)

* OR and 95% CI based on unconditional logistic regression models including all listed variables.

Table 2
Minor allele frequency distribution among control subjects by ethnic group

PON1	Minor allele *	Protein residue	All	Caucasians	Japanese	Hawaiians	P for the χ^2 test
No. for rs854560/rs662			445/444	155/155	174/173	117/117	
rs854560 (L55M)	T	L (Leu)	0.18	0.43	0.07	0.15	<0.0001
rs662 (Q192R)	G	R (Arg)	0.52	0.30	0.61	0.66	<0.0001

NOTE: Nomenclature is based on National Center of Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>), accessed January 10, 2008.

* Minor allele in Caucasian women.

Table 3
PON1 rs854560 T>A (L55M) and rs662 A>G (Q192R) genotype association with epithelial ovarian cancer risk

Genotype	All			Caucasian			Japanese			Hawaiian		
	Cases (<i>n</i> = 274)	Controls (<i>n</i> = 452)	OR (95% CI) *	Cases (<i>n</i> = 90)	Controls (<i>n</i> = 158)	OR (95% CI) *	Cases (<i>n</i> = 102)	Controls (<i>n</i> = 175)	OR (95% CI) *	Cases (<i>n</i> = 82)	Controls (<i>n</i> = 119)	OR (95% CI) *
rs854560												
AA	192	276	1.00 [†]	33	46	1.00 [†]	87	148	1.00 [†]	72	82	1.00 [†]
TA	65	145	0.51 (0.34–0.77)	43	86	0.58 (0.30–1.09)	12	26	0.58 (0.26–1.28)	10	33	0.29 (0.12–0.74)
TT	14	24	0.67 (0.31–1.47)	12	23	0.56 (0.22–1.39)	2	0	N/A	0	1	N/A
<i>P</i> _{trend}			0.01			0.12			0.42			0.01
Any T	79	169	0.53 (0.35–0.79)	55	109	0.57 (0.31–1.06)	14	26	0.64 (0.30–1.39)	10	34	0.28 (0.11–0.74)
Undetermined	3	7		2	3		1	1		0	3	
rs662												
GG	86	111	1.00 [†]	7	11	1.00 [†]	44	66	1.00 [†]	35	34	1.00 [†]
AG	120	211	0.67 (0.45–0.99)	38	71	0.86 (0.27–2.72)	45	80	0.74 (0.41–1.32)	37	60	0.62 (0.29–1.36)
AA	66	122	0.60 (0.37–0.97)	45	73	0.92 (0.30–2.84)	11	27	0.47 (0.10–1.12)	10	22	0.46 (0.16–1.34)
<i>P</i> _{trend}			0.03			0.37			0.08			0.06
Any A	186	333	0.65 (0.44–0.95)	83	144	0.90 (0.30–2.69)	56	107	0.67 (0.38–1.16)	47	88	0.58 (0.28–1.21)
Undetermined	2	8		0	3		2	2		0	3	
rs854560-rs662 combined genotype												
AA-GG [†]	86	109	1.00	7	10	1.00	44	66	1.00	35	33	1.00
AA-Any A	106	165	0.74 (0.50–1.12)	26	36	1.03 (0.31–3.42)	43	81	0.72 (0.40–1.29)	37	48	0.73 (0.34–1.58)
Any T-Any A	78	167	0.43 (0.27–0.70)	55	107	0.74 (0.13–2.37)	13	26	0.52 (0.22–1.21)	10	34	0.20 (0.08–0.53)
Undetermined	4	10		2	4		2	2		0	4	

Genotype	All		Caucasian		Japanese		Hawaiian					
	Cases (<i>n</i> = 274)	Controls (<i>n</i> = 452)	OR (95% CI) *	Cases (<i>n</i> = 90)	Controls (<i>n</i> = 158)	OR (95% CI) *	Cases (<i>n</i> = 102)	Controls (<i>n</i> = 175)	OR (95% CI) *	Cases (<i>n</i> = 82)	Controls (<i>n</i> = 119)	OR (95% CI) *

NOTE: Rare combined genotype “Any T/GG” (*n* = 1) was not included in the analysis.

* OR and 95% CI from logistic regression adjusted for age, ethnicity, parity, tubal ligation, menopausal status, use of contraceptive and menopausal hormones, BMI, and tobacco smoking.

† Homozygous carriers of the common allele among the majority of the controls were used as the reference group.

Table 4
PON1 rs854560 T>A (L55M) and rs662 A>G (Q192R) genotype associations with epithelial ovarian cancer risk by histology

Genotype	Controls		Serous		Endometrioid		Clear cell		Mucinous		Mixed/other	
	<i>n</i>		<i>n</i>	OR (95% CI)*	<i>n</i>	OR (95% CI)*	<i>n</i>	OR (95% CI)*	<i>n</i>	OR (95% CI)*	<i>n</i>	OR (95% CI)*
rs854560												
AA [†]	276	82	1.00		31	1.00	16	1.00	40	1.00	23	1.00
Any T	169	34	0.46 (0.26–.83)		11	0.52 (0.21–.29)	8	0.85 (0.29–2.48)	10	0.65 (0.18–2.57)	16	0.95 (0.42–2.11)
Undetermined	7	2			0		0		1		0	
rs662												
GG [†]	111	35	1.00		13	1.00	10	1.00	19	1.00	9	1.00
Any A	333	82	0.62 (0.36–1.07)		29	0.75 (0.34–1.66)	13	0.30 (0.11–0.87)	32	0.69 (0.25–1.86)	30	0.04 (0.01–0.92)
Undetermined	8	1			0		1		0		0	
rs854560-rs662 combined genotypes												
AA-GG [†]	109	35	1.00		13	1.00	10	1.00	19	1.00	9	1.00
AA-Any A	165	47	0.78 (0.46–1.32)		18	0.94 (0.42–2.11)	6	0.32 (0.11–0.98)	21	0.70 (0.34–1.43)	14	0.58 (0.35–2.14)
Any T-Any A	169	36	0.39 (0.21–0.74)		11	0.55 (0.20–1.54)	7	0.41 (0.12–1.38)	10	0.65 (0.19–1.66)	16	0.56 (0.32–2.42)
Undetermined	11	2			0		1		1		0	

NOTE: Rare combined genotype “Any T/GG” (*n* = 1) was not included in the analysis.

* OR and 95% CI from polytomous logistic regression adjusted for age, ethnicity, parity, tubal ligation, menopausal status, use of contraceptive menopausal hormones, BMI, and tobacco smoking.

[†] Homozygous carriers of the common allele(s) among the majority of the controls were used as the reference group.

Table 5
PON1 rs854560 T>A (L55M) and rs662 A>G (Q192R) genotype associations with ovarian cancer risk stratified by cigarette smoking and obesity

Genotype	Smoking status						Obesity					
	Smokers			Nonsmokers			BMI ≥ 30 (obese)			BMI < 30		
	Cases (n = 114)	Controls (n = 198)	OR (95% CI) *	Cases (n = 160)	Controls (n = 254)	OR (95% CI) *	Cases (n = 64)	Controls (n = 90)	OR (95% CI) †	Cases (n = 210)	Controls (n = 362)	OR (95% CI) †
rs854560												
AA ‡	80	108	1.00	112	168	1.00	49	52	1.00	143	224	1.00
TA	30	74	0.36 (0.18–0.69)	35	71	0.61 (0.35–1.07)	12	29	0.19 (0.06–0.57)	53	116	0.58 (0.36–0.93)
TT	2	11	0.16 (0.03–0.84)	12	13	1.32 (0.48–3.63)	3	7	0.17 (0.03–1.17)	11	17	1.00 (0.41–2.45)
Undetermined	2	5		1	2		0	2		3	5	
<i>P</i> _{trend}			0.0007*			0.53*			0.007 ‡			0.16 ‡
Any T allele	32	85	0.33 (0.17–0.64)	47	84	0.68 (0.40–1.18)	15	36	0.19 (0.06–0.55)	64	133	0.62 (0.40–0.98)
<i>p</i> [§]	0.07*						0.43 ‡					
rs662												
GG ‡	33	44	1.00	53	67	1.00	21	21	1.00	65	90	1.00
AG ‡	51	91	0.69	69	120	0.63 (0.37–1.07)	27	39	0.69 (0.27–1.76)	93	172	0.67 (0.43–1.05)
AA	29	59	0.56 (0.26–1.19)	37	63	0.58 (0.30–1.12)	16	28	0.51 (0.16–1.61)	50	94	0.61 (0.35–1.05)
Undetermined	2	4		1	4		0	2		2	6	
<i>P</i> _{trend}			0.14*			0.09*			0.28 ‡			0.07 ‡
Any A allele	80	150	0.65 (0.36–1.20)	106	183	0.62 (0.37–1.02)	43	67	0.63 (0.26–1.57)	143	266	0.65 (0.42–1.01)
<i>p</i> [§]	0.72*						0.89 ‡					
rs854560-rs662 combined genotypes												
AA-GG ‡	33	42	1.00	53	67	1.00	21	20	1.00	65	89	1.00
AA-Any A	47	65	0.86	59	100	0.66	28	51	1.02	78	134	0.50

Genotype	Smoking status				Obesity							
	Smokers		Nonsmokers		BMI ≥ 30 (obese)		BMI < 30					
	Cases (n = 114)	Controls (n = 198)	OR (95% CI) [*]	Cases (n = 160)	Controls (n = 254)	OR (95% CI) [*]	Cases (n = 64)	Controls (n = 90)	OR (95% CI) [†]	Cases (n = 210)	Controls (n = 362)	OR (95% CI) [‡]
Any T-Any A	32	84	(0.45–1.66)	46	83	(0.39–1.13)	15	36	0.18 (0.05–0.64)	63	131	0.71 (0.44–1.12)
Undetermined	2	6		2	4		0	3		4	7	
<i>P</i> [§]	0.56 [*]						0.95 [†]					

NOTE: Rare combined genotype “Any T/GG” (*n* = 1) was not included in the analysis.

^{*} Adjusted for age, ethnicity, parity, tubal ligation, menopausal status, use of contraceptive steroids, use of menopausal hormones, and BMI.

[†] Adjusted for age, ethnicity, parity, tubal ligation, menopausal status, use of contraceptive and menopausal hormones, and tobacco smoking.

[‡] Homozygous carriers of the common allele among the majority of the controls were used as the reference group.

[§] *P* for the likelihood ratio test comparing models with and without interaction terms.