Pooled analysis of the association of *PTGS2* rs5275 polymorphism and NSAID use with invasive ovarian carcinoma risk

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Abstract

Inflammation is postulated to play an important role in ovarian carcinogenesis. Prostaglandin endoperoxide synthase 2 (PTGS2) is responsible for the conversion of arachidonic acid to prostaglandins in response to inflammation. We examined the association of the *PTGS2* rs5275 polymorphism with the risk of invasive ovarian carcinoma and the joint effect of rs5275 and use of nonsteroidal antiinflammatory drugs (NSAIDs) on risk in a pooled analysis of two population-based studies, the Hawaii Ovarian Cancer Case-Control Study and the New England Case-Control Study, including 1025 women with invasive ovarian carcinoma and 1687 cancer-free controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using unconditional logistic regression. In the pooled analysis, the CC genotype was associated with a reduced risk of nonserous ovarian carcinoma (OR=0.66; CI: 0.44-0.98). In addition, the lowest risk was observed among carriers of the CC genotype who were users of nonaspirin NSAIDs (OR=0.43; CI: 0.20-0.93) in all women combined. The association of *PTGS2* rs5275 with nonserous ovarian carcinoma and possible effect modification by NSAID use needs further validation, preferably in the prospective studies.

Keywords

epithelial ovarian cancer; genetic polymorphism; prostaglandin endoperoxide synthase 2 (PTGS2) gene; nonsteroidal antiinflammatory drugs; case-control study

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Introduction

The ovarian surface epithelium contributes to ovulation by lysis and reconstruction of the ovarian cortex, and is thought to be the source of 90% of ovarian neoplasms (1). Repeated episodes of ovulation-associated injury may contribute to ovarian carcinoma pathogenesis (2). Ovulation is associated with an inflammatory response in mature follicles (3,4) that leads to the release of reactive nitrogen and oxygen species directly damaging DNA, dysregulation of cytokines associated with neoplastic progression, and overexpression of prostaglandins increasing tumor invasiveness (5,6). Hence, postovulatory tissue repair occurs in an environment that potentiates and promotes neoplastic risk. Both animal models and observational studies in humans demonstrate that some potent nonsteroidal antiinflammatory drugs (NSAIDs) can inhibit the ovulatory process (3). An inverse relation between the use of NSAIDs and the risk of ovarian cancer has been suggested (7); however, no compelling evidence was provided in further investigations (8,9).

NSAIDs interfere with prostaglandin biosynthesis by inhibiting cyclooxygenases-1 and -2 (COX1 and COX2), also known as prostaglandin endoperoxide synthases (PTGSs). Both enzymes catalyze the rate-limiting step in prostaglandin synthesis from arachidonic acid (10). PTGS1 is constitutively expressed in most tissues; in contrast, PTGS2 is induced by various stimuli including several mitogens, cytokines, growth factors, and tumor promoters (11). Increased expression of PTGS2 has been linked to inflammatory processes and ovarian cancer (12,13,14,15).

PTGS2 expression varies among individuals, and this variability may be influenced by common polymorphisms in the functional regions of the gene (16). Recent investigations suggest that *PTGS2* expression is regulated *via* the 3′untranslated region (UTR) of the gene. This evidence was further substantiated by experiments directly showing that the *PTGS2* 3′UTR confers posttranscriptional regulation through rapid mRNA turnover and translational inhibition (17). The rs5275 SNP is located in the 3′UTR of the *PTGS2* gene, and the C allele has been associated with lower steady-state PTGS2 mRNA levels (18). *PTGS2* is a small gene with only five common SNPs that are in strong linkage disequilibrium. All five common SNPs in the *PTGS2* gene described to date have been genotyped in two Nurses' Health Studies and the Harvard Women's Health Study. The rs5275 SNP was the only SNP associated with breast cancer risk in all three studies (19). In a pooled analysis (1270 cases and 1762 controls), women homozygous for the rs5275 C allele had a 20% lower risk of breast cancer than common allele homozygotes (19).

In this analysis, data from the Hawaii Ovarian Cancer Case-Control and the New England Case-Control Study of Ovarian Cancer (NECC) were pooled to test the hypothesis that the *PTGS2* rs5275 C allele is associated with decreased ovarian cancer risk, and that this association is modified by NSAID use. Both studies are part of the Ovarian Cancer Association Consortium, a forum for researchers to evaluate genetic associations with ovarian cancer with increased power (20).

Materials and methods

The Hawaii Ovarian Cancer Case-Control Study (HAW) is a population-based study that includes women 18 years of age or older who were diagnosed with primary histologically-confirmed epithelial ovarian cancer between 1993 and 2008. 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. Control subjects were randomly selected from participants in an annual survey of representative households, conducted by the Hawaii Department of Health under statutory

provision resulting in almost 100% participation rates. Only invasive ovarian carcinoma cases (n=302) were included in this analysis. Controls (n=592) were frequency-matched to cases based on ethnicity and 5-year age groups in an approximate 1:2 ratio. Eligibility criteria for controls included age 18 years or older, residency in Hawaii for a minimum of one year, no prior history of ovarian cancer, and having at least one intact ovary. The response rate was 65% for cases and 68% for controls. Socio-demographic, life style, and health-related information was collected during a ~2.5-hour interview, using a structured pre-tested questionnaire (21). Detailed history of NSAID use was available for 217 cases and 419 controls who completed a questionnaire revised in 2001 that included NSAID use. To distinguish occasional versus long-term users of NSAIDs, participants were asked whether they ever used NSAIDs 12 or more times during a single year. Those who answered 'yes' were asked to provide detailed information on frequency of specific medications used, numbers of episodes of use, and duration of each episode. Women who used NSAIDs continuously for 6 months or longer were classified as long-term NSAID users. Interviewers were uniformly trained and supervised to standardize interviewing and coding techniques.

The New-England Case-Control Study of Ovarian Cancer (NECC) is a population-based study in New Hampshire and eastern Massachusetts that began in 1998 (22). Histologically-confirmed incident ovarian cancer cases are identified through hospital tumor boards and statewide cancer registries. Controls were selected through a combination of random digit dialing, town books, and drivers' license lists, and were matched to the distribution of cases by age and study center. Epidemiological data was collected by in-person administered questionnaires that included information about demographics, menstrual and reproductive history, medical and family history, and personal habits. Women were asked whether they had continuously (at least once a week) used NSAIDs for at least 6 months. Detailed history on the specific NSAID medication, frequency, and length of use was collected (23). The NECC study contributed information from 723 women with invasive ovarian carcinoma and 1095 controls for the replication analysis of the *PTGS2* rs5275 association with ovarian cancer risk.

Clinical and questionnaire data from both studies were merged into a common data set at the Ovarian Cancer Association Consortium (OCAC) coordinating center at Duke University. The combined data set used in the pooled analysis included 1025 cases and 1687 controls. The following characteristics were available for all participants: case-control status, age at diagnosis/interview, race/ethnicity, education, tumor behavior and histologic subtype, family history of breast and/or ovarian cancer among first-degree female relatives (mothers and sisters) menopausal status, use of contraceptive steroids and menopausal hormones (estrogen alone or in combination with progestin), history of tubal ligation, and hysterectomy. Information on NSAID use was available for 940 cases and 1514 controls that included all women interviewed after 2001 (217 cases and 419 controls) from the Hawaii study and all NECC study participants.

The Hawaii study protocol was approved by the Institutional Review Board of the University of Hawaii. The NECC study protocol was approved by the Human Subjects Review Committees at both Brigham and Women's Hospital and Dartmouth Medical School, and each participant provided signed informed consent. In addition, Duke University has Institutional Review Board approval as a data coordinating center.

Genotyping

DNA was purified from whole blood using Qiagen Midi Kits (Qiagen, Valencia, CA). At each site, genotyping was performed using 5′ nuclease TaqMan allelic discrimination assay (TaqMan, Applied Biosystems, Foster City, CA, USA). Samples from cases and controls were intermixed on each plate, and laboratory personnel were blinded to the case-control

status of the study participants. We used the following criteria to measure the acceptability of the genotyping results: (1) >3% sample duplicates included, (2) concordance rate for duplicate samples ≥ 98%, (3) overall call rate (by study) >95% and (4) call rate >90% for each 384-well plate and (5) cases and controls intermixed on each plate. Both studies met each of the criteria. Gene and allele nomenclature was according to the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

Statistical Analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC). A goodness of fit chi-square 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 (ORs) and 95% confidence intervals (CIs) for the association of the *PTGS2* rs5275 genotype with ovarian carcinoma risk. ORs and CIs were estimated separately for heterozygous and homozygous variant C allele carriers, using women with the TT genotype as the reference group. We also performed genetic analyses testing a log-additive model in which genotype was categorized by three levels (0, 1 and 2) representing combinations of alleles. In addition, we compared risk among heterozygotes and homozygote C allele carriers combined (testing a dominant genetic model) (data not shown) and among women with the CC genotype compared to the TT and TC genotypes combined (testing a recessive genetic model). Based on the Akaike Information Criterion (AIC), the recessive model provided better fit for the data.

Using data available for the duration of NSAID use, we categorized women who never used NSAID for 6 months or longer as 'nonusers' and women who reported long-term use of NSAIDs were classified as 'users'. Among NSAID users, separate analyses were performed for women who used 1/only acetylsalicylic acid or other salicylates (further referred as 'aspirin'); 2/ other NSAIDs; 3/ used both; 4/ used any NSAID.

To evaluate potential confounders, the distributions of genotype and NSAID use were examined by factors associated with ovarian cancer risk in a multiple logistic regression model. The following covariates were included into all models: age, ethnicity, education, family history of breast and/or ovarian cancer, menopausal status, use of contraceptive and menopausal hormones (estrogen alone and in combination with progestin), and, in combined analyses, study. Heterogeneity of effects by study, NSAID use and other covariates was examined using a Wald test of the genotype-covariate interaction term. Heterogeneity of associations of the rs5275 genenotype with risk by histological type (serous, mucinous, endometrioid, clear cell, and other), was evaluated using Wald tests comparing ORs across strata by tumor behavior and histology in polytomous logistic regression models. Analyses were conducted for each study separately and for both studies combined. All p-values were based on two-tailed tests. Statistical significance was considered at a *p* value less than 0.05.

Results

Participant characteristics are presented in Table 1. Cases (age: 54.6; SD, 12.0) were slightly (nonsignificantly) older than controls (age: 52.2; SD, 13.4).The distribution of *PTGS2* rs5275 genotypes among control subjects was consistent with Hardy-Weinberg equilibrium in each stratum by ethnicity and by study and in all strata combined. The rare (C) allele frequency was higher among white non-Hispanic women (0.34) than among Asian women (0.22) and among women of mixed/other ethnicity (0.25) (p=0.06), but was similar among white non-Hispanic women in Hawaii and NECC studies (p for heterogeneity among genotypes=0.62).

No significant associations of rs5275 genotype with age, ethnicity, parity, menopausal status, tubal ligation, hysterectomy, use of contraceptive or menopausal hormones, or NSAID use were observed (p > 0.05). Use of any NSAIDs was significantly positively associated with education (p=0.001), and use of contraceptive (p=0.0001) and menopausal hormones (p <0.0001). Postmenopausal women were significantly more likely to use aspirin and other salicylates (p<0.0001), and less likely to use nonaspirin NSAIDs (p<0.0001) than premenopausal women. Among Hawaii participants, women with family history of breast and/or ovarian cancer were more likely to use both aspirin and nonaspirin NSAIDs than women who did not report ovarian cancer among first-degree relatives. NSAID use was significantly associated with decreased ovarian cancer risk (OR=0.79; CI:0.67-0.95). Significant inverse associations with risk were observed among women who used both aspirin and nonaspirin NSAIDs, but not among women who used one of these types exclusively (Table 1).

We did not observe significant associations of *PTGS2* genotype with ovarian cancer risk among all women combined (OR=0.86; CI:0.66-1.12; p for heterogeneity between studies=0.10) or in the analyses restricted to white women only (Table 2). In sub-group analyses by study site, we found that homozygous rs5275 C allele carriers had significantly reduced ovarian cancer risk when compared to carriers of any T allele (recessive genetic model) (OR=0.51; CI: 0.26-0.98; p=0.04) in the Hawaii study, but not in the NECC study. No heterogeneity in the association of rs5275 with risk was observed by ethnicity (p for trend in all models tested ranged from 0.60-0.99; data not shown).

The data were further examined by histological subtype of ovarian cancer (Table 3). Women with the *CC* genotype were at significantly reduced risk of nonserous tumors compared to women with the *TT* genotype or any *T* allele carriers (OR=0.66; CI:0.44-0.98; p=0.04) (recessive genetic model). No association of the *PTGS2* rs5275 genotype with risk of serous cancer was observed.

The joint association of the *PTGS2* rs5275 genotype and NSAID use on ovarian carcinoma risk was examined (Table 4). Women who were *CC* allele carriers and who were users of nonaspirin NSAIDs had the lowest risk of ovarian carcinoma compared to women who were *TT* genotype carriers and who did not use NSAIDs (OR=0.43; CI:0.20-0.93; p=0.03).

Discussion

In this large pooled analysis of two population-based studies, we explored the association of the *PTGS2* rs5275 polymorphism with invasive ovarian carcinoma risk and the possible effect modification of the genetic association by NSAID use. We observed a significant 36% reduction in the risk of nonserous carcinoma among women with the rs5275 *CC* genotype compared to women homozygous for the common allele. Interestingly, the most pronounced decrease in ovarian cancer risk was observed among women who were NSAID users and homozygous for the rs5275 C allele, although the interaction term for this association was not statistically significant.

Given its location in the 3′UTR, the rs5275 SNP is a likely candidate to influence *PTGS2*RNA half-life, which is controlled by sequence-specific elements in the region of the mRNA (17). Previous studies have reported that in the proximal upstream region of this SNP there is a conserved AU-rich sequence element which mediates posttranscriptional degradation of PTGS2 mRNA (17). A functional analysis measuring *PTGS2* mRNA suggests that the decreased ovarian carcinoma risk associated with the rs5275 C allele may be attributed to lower *PTGS2* expression (18).

It is biologically plausible that genetic variation in *PTGS2* that alters expression levels or the biochemical function of prostaglandin endoperoxide synthase 2 may influence a woman's risk of ovarian carcinoma. PTGS2 converts arachidonic acid to prostaglandin H2, which is a precursor to all other prostaglandins. Prostaglandins are integral components in the cellular response to inflammation, promoting cellular proliferation and angiogenesis (24).

NSAID use and *PTGS2* genotype may be more strongly associated with endometrioid and clear cell histologic types of ovarian cancer that are more likely to arise from endometriotic foci (25). Endometriosis, the presence of endometrial tissue outside the endometrium, causes a marked local inflammatory reaction (26). In this study, nonserous histological types were more strongly associated with NSAID use and *PTGS2*.

Strengths of this study include the population-based nature of both Hawaii and NECC studies, histologic confirmation of all case diagnoses, stringent genotyping quality control procedures, and the completeness of epidemiological data related to ovarian cancer risk. Although the pooled analysis included a relatively large number of cases and controls, the statistical power was adequate (>90%) to detect ORs of 0.69 and lower at a critical level of 5% (two-sided), using a recessive genetic model. This low risk was present among nonserous carcinoma cases. Due to the retrospective manner of collecting data on NSAID use in case-control studies, there is the possibility of recall bias, although participants were likely unaware of the potential association of NSAID use with ovarian cancer risk.

In summary, we observed an inverse association between a potentially functional SNP in the 3′UTR of the *PTGS2* gene and nonserous ovarian carcinoma risk. The potential for a reduced risk of ovarian cancer among women with the *CC* genotype who use NSAIDs needs to be examined further in larger studies, preferably with prospective collection of risk factor information.

Acknowledgments

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Table 1

Description of the studies and participant baseline characteristics by case-control status

Characteristics	HAW (Hawaii Ovarian Cancer Study)		NECC (New England Case Control Study of Ovarian Cancer)		All		
Source	Hawaii, Population-based		New England, Population-based		USA, Population-based		
	Cases	Controls	Cases	Controls	Cases	Controls	OR (95% CI)*
Participation rate	65%	68%	72%	69%			
No. (%) of participants	302 (34)	592 (66)	723 (40)	1095 (60)	1025 (38)	1687 (62)	
No. (%) of participants with NSAID data available	217 (34)	419 (66)	723 (40)	1095 (60)	940 (38)	1514 (62)	
Age (SD; range in years)	56.2 (12.9; 22-87)	55.2 (13.9; 19-88)	53.9 (11.5; 20-76)	51.0 (13.0; 16-77)	54.6 (12.0; 20-87)	52.5 (13.4; 16-88)	
Ethnicity							
White non-Hispanic	70 (23)	154 (26)	723 (100)	1095 (100)	793 (77)	1249 (74)	
Asian	144 (48)	282 (48)	-	-	144 (14)	282 (17)	
Mixed/Other	88 (29)	156 (26)	-	-	88 (9)	156 (9)	
Education							
High school or less	123 (41)	177 (30)	246 (34)	330 (30)	369 (36)	507 (30)	1.00 (reference)
Some college	94 (31)	196 (33)	184 (25)	320 (29)	278 (27)	516 (31)	0.84 (0.61-1.17)
College/graduate school	85 (28)	219 (37)	293 (41)	445 (41)	378 (37)	664 (39)	0.96 (0.78-1.17)
Family history of breast and/or ovarian cancer							
Yes	58 (19)	74 (13)	133 (18)	150 (14)	191 (19)	224 (13)	1.40 (1.12-1.75)
No	244 (81)	518 (87)	590 (82)	945 (86)	834 (81)	1463 (87)	1.00 (reference)
Hormonal contraceptive use							
Yes	131 (43)	402 (68)	342 (47)	686 (63)	473 (46)	1088 (64)	0.54 (0.45-0.65)
No	171 (57)	190 (32)	381 (53)	409 (37)	552 (54)	599 (36)	1.00 (reference)
Menopausal status							
Premenopausal	93 (31)	224 (38)	269 (37)	510 (47)	367 (36)	736 (44)	1.00 (reference)
Postmenopausal	209 (69)	368 (62)	454 (63)	585 (53)	658 (64)	951 (56)	1.25 (0.94-1.66)
Menopausal hormone use							
None	119 (57)	163 (44)	311 (69)	372 (63)	430 (65)	535 (56)	1.00 (reference)

Characteristics	HAW (Hawaii Ovarian Cancer Study)		NECC (New England Case Control Study of Ovarian Cancer)		All		
Source	Hawaii, Population-based		New England, Population-based		USA, Population-based		
	Cases	Controls	Cases	Controls	Cases	Controls	OR (95% CI)*
Estrogen only	52 (25)	88 (24)	73 (16)	92 (16)	125 (19)	180 (19)	1.03 (0.77-1.36)
Estrogen and progestin	38 (18)	117 (32)	70 (15)	121 (21)	108 (16)	238 (25)	0.74 (0.56-0.99)
Rs5275 C allele frequency	0.31	0.32	0.33	0.34	0.33	0.34	
NSAID use by type							
Nonusers	101 (47)	148 (35)	481 (67)	679 (62)	582 (62)	827 (55)	1.00 (reference)
Aspirin	31 (14)	87 (21)	105 (14)	140 (13)	136 (14)	227 (15)	0.84 (0.66-1.08)
Nonaspirin NSAIDs	46 (21)	91 (22)	94 (13)	201 (18)	140 (15)	292 (19)	0.79 (0.62-1.01)
Both	39 (18)	93 (22)	43 (6)	75 (7)	82 (9)	168 (11)	0.72 (0.53-0.98)
Any NSAID	116 (53)	271 (65)	242 (33)	416 (38)	358 (38)	687 (45)	0.79 (0.67-0.95)

*
Odds ratios (OR) and 95% confidence intervals (CI) from a multivariate logistic regression model that included age, ethnicity, education, family history of breast and/or ovarian cancer, and use of contraceptive and menopausal hormones, when applicable. P for heterogeneity of genotype distribution among white control individuals by study=0.62

‡
P for the goodness of fit chi-square test testing for consistency of genotype with Hardy-Weinberg equilibrium.

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Table 2

Association of the *PTGS2* rs5275 polymorphism with invasive ovarian carcinoma risk by study and ethnicity and in all women combined

Study and ethnicity	No. cases (%) by genotype			No. controls (%) by genotype			Heterozygotes and rare allele homozygotes [*]			Log-additive model		Recessive model	
	TT	TC	CC	TT	TC	CC	TC OR (95% CI) [†]	CC OR (95% CI) [†]	P 2 d.f.	Per C allele OR (95% CI) [†]	P 1 d.f.	CC vs TT+TC OR (95% CI) [†]	P 1 d.f.
HAW/All	169 (56)	120 (40)	13 (4)	338 (57)	207 (35)	47 (8)	1.08 (0.79-1.46)	0.52 (0.26-1.02)	0.12	0.89 (0.70-1.13)	0.34	**0.51 (0.26-0.98)**	**0.04**
HAW/ with NSAID data available	117 (54)	88 (41)	12 (5)	233 (56)	150 (36)	36 (8)	1.12 (0.78-1.61)	0.65 (0.32-1.35)	0.35	0.95 (0.72-1.24)	0.68	0.62 (0.31-1.16)	0.19
HAW/White	34 (49)	33 (47)	3 (4)	75 (49)	60 (39)	19 (12)	1.16 (0.62-2.19)	0.38 (0.10-1.42)	0.26	0.83 (0.52-1.32)	0.42	0.35 (0.10-1.29)	0.11
HAW/Asian	88 (61)	49 (34)	7 (5)	173 (61)	92 (33)	17 (6)	1.01 (0.64-1.60)	0.85 (0.32-2.27)	0.95	0.97 (0.68-1.39)	0.86	0.85 (0.32-2.23)	0.74
HAW/Other	47 (53)	38 (43)	3 (4)	90 (58)	55 (35)	11 (7)	1.05 (0.57-1.92)	0.31 (0.07-1.35)	0.27	0.80 (0.49-1.31)	0.37	0.30 (0.07-1.29)	0.11
NECC/White	333 (46)	304 (42)	86 (12)	490 (45)	469 (43)	136 (12)	0.94 (0.77-1.16)	0.93 (0.68-1.25)	0.82	0.96 (0.83-1.11)	0.82	0.96 (0.71-1.28)	0.77
All White	367 (46)	337 (43)	89 (11)	565 (45)	529 (42)	155 (13)	0.98 (0.80-1.18)	0.89 (0.66-1.20)	0.75	0.95 (0.83-1.09)	0.48	0.90 (0.68-1.20)	0.47
All combined	502 (49)	424 (41)	99 (9)	828 (49)	676 (40)	183 (11)	1.01 (0.85-1.19)	0.86 (0.65-1.14)	0.44	0.95 (0.85-1.08)	0.44	0.86 (0.66-1.12)	0.26
[‡]P for heterogeneity among ethnic groups							0.80	0.68		0.99		0.60	
[‡]P for heterogeneity between studies							0.36	0.15		0.73		0.10	

Note: statistically significant estimates (p<0.05) are presented in bold font

[*] TT genotype was used as the reference group

[†] Odds ratios (OR) and 95% confidence intervals (OC) adjusted for age, ethnicity, education, family history of breast and/or ovarian cancer, menopausal status, use of contraceptive and menopausal hormones and, in the combined analysis, study.

[‡] P for heterogeneity of the association of the rs5275 with risk by ethnicity and study strata was estimated using a Wald test of the genotype-stratum interaction term

Table 3

Association of *PTGS2* rs5275 SNP with invasive ovarian carcinoma risk by histology

Subgroups by histology	No. (%) cases by histology Total N=987			Heterozygotes and rare allele homozygotes [*]			Log-additive model		Recessive model	
	TT	TC	CC	TC OR (95% CI)[†]	CC OR (95% CI)[†]	P (2 d.f.)	Per allele OR (95% CI)[†]	P (1 d.f.)	CC vs. TT+TC OR (95% CI)[†]	P (1 d.f.)
Serous (n=533)	247 (46)	225 (42)	61 (12)	1.04 (0.83-1.29)	1.05 (0.75-1.47)	0.93	1.03 (0.88-1.20)	0.72	1.03 (0.75-1.42)	0.86
Endometrioid (n=201)	104 (52)	80 (40)	17 (8)	0.92 (0.67-1.27)	0.70 (0.40-1.21)	0.43	0.87 (0.69-1.09)	0.23	0.72 (0.43-1.23)	0.23
Clear cell (n=130)	64 (49)	56 (43)	10 (8)	1.03 (0.71-1.52)	0.72 (0.36-1.44)	0.59	0.92 (0.69-1.72)	0.56	0.71 (0.36-1.39)	0.31
Mucinous (n=79)	45 (57)	28 (35)	6 (8)	0.81 (0.50-1.33)	0.68 (0.28-1.66)	0.56	0.82 (0.57-1.18)	0.29	0.75 (0.32-1.77)	0.51
Other (n=44)	21 (48)	22 (50)	1 (2)	1.45 (0.78-2.69)	0.23 (0.03-1.75)	0.13	0.92 (0.72-1.18)	0.52	0.57 (0.30-1.09)	0.09
All nonserous (n=454)	234 (52)	186 (41)	34 (7)	0.99 (0.79-1.24)	**0.66 (0.44-0.99)**	0.12	0.88 (0.75-1.04)	0.13	**0.66 (0.44-0.98)**	0.04
P[‡] heterogeneity: serous versus nonserous tumors				0.91	0.07		0.12		0.07	
P[‡] heterogeneity: among nonserous tumors				0.96	1.00		0.96		1.00	

Note: histology was not available for 38 cases

Note: statistically significant estimates (p < 0.05) are presented in bold font

[*] TT genotype was used as the reference group

[†] ORs and 95% CIs adjusted for age, ethnicity, education, family history of breast and/or ovarian cancer, menopausal status, use of contraceptive and menopausal hormones, and study

[‡] Heterogeneity of associations of the rs5275 genenotype with risk by histological type was evaluated using the Wald test comparing ORs across subgroups by histology in polytomous logistic regression models

Table 4

Joint association of NSAID use and *Ptgs2* rs5275 genotype with invasive ovarian carcinoma risk

NSAID use	No. (%) cases by genotype			No. (%) controls by genotype			OR (95% CI)[*]			P	P[§]
	TT	TC	CC	TT	TC	CC	TT	TC	CC		
				HAWAII and NECC studies combined (940 cases/1514 controls)							
Nonusers	282 (30)	235 (25)	65 (7)	375 (25)	354 (23)	98 (6)	1.00 (reference)	0.86 (0.68-1.08)	0.85 (0.60-1.22)		
Aspirin	65(7)	54 (6)	17 (2)	118 (8)	92 (6)	17 (1)	0.72 (0.50-1.02)	0.74 (0.51-1.08)	1.21 (0.60-2.44)		
Nonaspirin	64 (7)	67 (7)	9 (1)	157 (10)	103 (7)	32 (2)	**0.61 (0.43-0.85)**	0.97 (0.68-1.38)	**0.43 (0.20-0.93)**		
Both groups	39 (4)	36 (4)	7 (1)	73 (5)	70 (5)	25 (2)	**0.70 (0.45-1.07)**	0.68 (0.43-1.07)	0.42 (0.18-1.01)	*0.05*[†]	*0.09*
Any NSAID	172 (18)	160 (17)	34 (4)	361 (24)	269 (18)	75 (5)	**0.67 (0.52-0.86)**	0.81 (0.63-1.05)	**0.63 (0.40-0.99)**	*0.04*[‡]	*0.16*

¶P for heterogeneity of the effects of genotype and NSAID use by type between studies *0.37*

¶P for heterogeneity of the effects of genotype and any NSAID use between studies *0.51*

Note: statistically significant estimates (p<0.05) are presented in bold font

[*] ORs and 95% CIs adjusted for age, ethnicity, education, family history of breast and/or ovarian cancer, menopausal status, use of contraceptive and menopausal hormones and, in combined analysis, study

[†] P global (11 d.f) from the multivariate logistic regression models comparing joint effect of rs5275 genotype and NSAID use by group

[‡] P global (5 d.f.) from the multivariate logistic regression models comparing joint effect of rs5275 genotype and any NSAID

[§] P for interaction of rs5275 with risk by strata of NSAID use was calculated using Wald test for the genotype-stratum interaction terms

¶P for heterogeneity of the effects of NSAID use and genotype by study was calculated using Wald test for the genotype-study interaction terms