Original Article
Interaction between use of non-steroidal anti-inflammatory drugs and selected genetic polymorphisms in ovarian cancer risk

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Abstract: Inflammation and non-steroidal anti-inflammatory agents (NSAIDs) may play important role in ovarian cancer. However, epidemiologic data are inconsistent, possibly reflecting inter-individual genetic differences affecting the metabolism of NSAIDs. We examined whether common polymorphisms affecting the metabolism of NSAIDs modify the association between NSAIDs and ovarian cancer risk. We genotyped 1,353 DNA samples from women who developed ovarian cancer and 1,823 samples from matched controls participating in the New England Case-Control study and the Nurses' Health Studies. Conditional logistic regression estimated odds ratios (ORs) and 95% confidence intervals (CIs) associated with regular use of NSAIDs and with relevant polymorphisms on ovarian cancer risk. Multivariable unconditional logistic regression estimated the association of NSAID use across stratum of each genotype. Regular use of NSAIDs was not associated with ovarian cancer risk. Multivariable OR (95% CI) associated with use NSAIDs was 0.85 (95% CI: 0.71-1.02). Associations between NSAID use and ovarian cancer risk did not differ significantly across strata of genotypes. None of the studied polymorphisms was associated with ovarian cancer risk. The multivariable ORs (95% CI) associated with *CYP2C9* and *UGT1A6* variant genotypes were 0.99 (0.90-1.08) and 0.93 (0.82-1.05), respectively. The multivariable ORs (95% CI) associated with *PPAR-γ, COX-2 -765G>C,* and *COX-2 Ex10+837T>C* polymorphisms were 1.02 (0.87-1.20), 0.87 (0.75-1.00), and 0.97 (0.87-1.09), respectively. In this relatively large study, we found no convincing evidence supporting an association between NSAIDs use and ovarian cancer risk. Furthermore, data did not suggest interaction between selected polymorphisms and use of NSAIDs in relation to ovarian cancer risk.

Keywords: Non-steroidal anti-inflammatory drugs, ovarian cancer, pharmacogenetics, interaction, CYP2C9, UGT1A6, PPAR-γ, COX, polymorphism

Introduction

Inflammation has been proposed to play an important role in ovarian cancer [1] and non-steroidal anti-inflammatory agents (NSAIDs) were hypothesized to decrease ovarian cancer risk. Accruing experimental data suggest that NSAIDs exert anti-neoplastic effects on ovarian cancer cells (reviewed in [2]), probably due to their ability to inhibit prostaglandin synthesis [3-5]. However, epidemiologic studies have pro-duced inconsistent results [6-17], which may be explained by inter-individual differences in genes affecting the metabolism of NSAIDs, their target enzymes, and the overall bioavailability of inflammatory mediators.

Most NSAIDs are oxidized by P450 cytochrome enzymes, mainly CYP2C9 [18-21], and are conjugated by phase II enzymes, mainly UGT1A6 [22]. Both CYP2C9 and UGT1A6 are encoded by highly polymorphic genes [23, 24], and their

variant alleles substantially decrease enzyme catalytic activity [25-27]. Evidence mostly from the colorectal cancer literature suggests that polymorphisms in these genes may modify the association between NSAID use and cancer risk [28, 29].

The anti-inflammatory and possibly anti-carcinogenic properties of NSAIDs are attributed primarily to their ability to inhibit cyclooxy-genases (COX) [3]. While COX-1 is constitutively expressed in many cells, expression of COX-2 is induced by a number of events and has major influence on the synthesis of prostaglandins [30], which are involved in inflammation and mitogenesis [31]. Genetic variations in the enzymes involved in the synthesis and function of prostaglandins may also influence the chemo-preventive effects of NSAIDs. Interactions between NSAIDs and polymorphisms in the regulatory region of the COX-2 gene have been suggested in colorectal [32] and breast [33] cancers.

The anti-neoplastic activities of some NSAIDs have also been attributed to their activation of peroxime proliferators-activated receptor-gamma (PPAR-γ) [34, 35], which may also suppress COX-2 expression in ovarian cancer cells [36]. The inhibitory effects of COX-inhibition and PPAR-gamma activation on ovarian cancer cells were comparable to that of cisplatin in mice models [37]. A polymorphism in PPAR-γ resulting in a Proline to Alanine amino-acid change in codon 12 was shown to reduce transcriptional activation of target genes in vitro [38] and was associated with a 50% reduction in colorectal cancer risk [39]. It is conceivable that polymorphisms in this gene may interact with the effects of NSAIDs on cancer risk.

We examined whether the association between NSAIDs and ovarian cancer risk differed according to selected genetic polymorphisms in three populations. To our knowledge, this study is the first to examine the interplay of NSAID use and genetic polymorphisms in the CYP2C9, UGT1A6, COX-2, and PPAR-γ genes in ovarian cancer risk.

Methods

Study population

New England Case-Control Study: The New England Case-Control Study (NECC) is a population-based case-control study of women newly diagnosed with ovarian cancer in eastern Massachusetts and New Hampshire. Details of this study have been published elsewhere [9, 40]. Participant enrollment occurred from May 1992 to March 1997 (563 cases, 523 controls) and from July 1998 to July 2003 (668 cases, 721 controls). Over the two enrollment phases, 2,347 incident cases of ovarian cancer were identified through statewide tumor registries, of whom 502 were ineligible because they died (N=210), had moved or had no telephone (N=160), did not speak English (N=37), lived outside of the study area (N=2), or had a non-ovarian primary tumor (N=93), leaving 1,845 eligible cases. Physicians declined permission to contact 232 (13%) patients and 307 (17%) cases declined or were too ill to participate, leaving 1,306 cases. Of the eligible participants enrolled in the study, 1,231 (94%) had epithelial ovarian tumors.

Controls were identified using random digit dialing, New Hampshire driver's license records, and Massachusetts town resident lists, and were frequency-matched to cases in age (± 4 years) and state of residence. Of the eligible controls contacted in the first enrollment phase, 68% agreed to participate. During the second enrollment phase, 197 potential controls opted out; 67% agreed to participate. After written informed consent, participants responded to a detailed interviewer-administered questionnaire that evaluated demographic characteristics, medical, family and reproductive histories, and various health-related behaviors.

Over 95% of the participants provided a blood sample at enrollment. Because ethnicity differences in allele frequencies may introduce spurious associations between these genotypes and ovarian cancer, the current analyses were restricted to 1,120 cases and 1,160 controls of European ancestry. Heparinized blood samples were separated into plasma, red blood cell (RBC), and white blood cell (WBC) components. DNA was extracted from WBC using Qiagen DNA extraction (Qiagen Inc., Valencia, CA) and stored at –80ºC. This study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital and Dartmouth Medical School.

Nurses Health Study and Nurses Health Study-II: The Nurses' Health Study (NHS) was estab-

lished in 1976 among 121,700 U.S. female registered nurses, aged 30-55 at entry; and the Nurses' Health Study-II (NHS-II) was established in 1989 among 116,608 U.S. female registered nurses, aged 25-42 years at entry. Participants completed a detailed self-administered questionnaire at baseline and biennially thereafter to update information on known and suspected factors related to cancer risk and on newly diagnosed diseases. Details have been published elsewhere [41, 42].

Between 1989 and 1990 (NHS), 32,826 participants (aged 43-69) provided blood samples [43]. Between 2001 and 2004, an additional 33,040 women without blood specimens provided a buccal cell sample using a mouthwash protocol. In the NHS-II, 29,611 women (aged 32-54) who had not been pregnant, lactating, or taking hormones for at least six months provided blood samples between 1996 and 1999 [44]. Samples from both cohorts were shipped via overnight courier to our labs, where they were separated into plasma, RBC, and WBC components, extracted using *Qiagen DNA extraction* (Qiagen Inc.), and stored at -130°C. Both studies were approved by the Committee on the Use of Human Subjects in Research of Brigham and Women's Hospital.

Ovarian cancer diagnoses after sample collection and up to 4 years before sample collection were identified and confirmed as previously described [45]. Prevalent cases, comprising 24%, were included in our study, as their characteristics were similar to those of the incident cases [46]. Each case was randomly matched to 3 controls with at least one intact ovary at the time of case diagnosis on age (±1 year), date (±1 month), and time (±2 hours) of blood collection, fasting status, menopause status, and postmenopausal hormone use. We excluded 2 control participants who subsequently developed ovarian cancer, as they were included as cases and were matched to 3 other controls; 31 controls were excluded due to unavailability of genotyping data, leaving 233 cases and 663 controls of European ancestry.

Exposure assessment

The NECC collected information on use of over-the-counter analgesics (including mainly aspirin, ibuprofen, and acetaminophen) by questionnaires administered in person. Information on demographic characteristics, reproductive and family histories, and various health habits was collected at the same time. Participants were asked about exposures at least one year prior to date of ovarian cancer diagnosis (or interview date for controls), including analgesic use, dose, duration, and age at first regular analgesic use. Both cases and controls were asked to report long-term habits. For the current analyses, we considered participants to be regular users of aspirin, ibuprofen, and/or acetaminophen if they reported using at least 2 tablets per week for at least 6 months.

In the NHS and NHS-II cohorts, information on aspirin use was first collected in 1980 (NHS)/1989 (NHS-II), and updated approximately biennially. Use of other NSAIDs (*e.g.* ibuprofen, naprosyn) and acetaminophen was first queried in 1990 (NHS)/1989 (NHS-II), and updated approximately biennially. Information on intake of COX-2 inhibitors (e.g. Vioxx or Celebrex) was first queried in 2000 (NHS)/2001 (NHS-II). Information on individual formulations of NSAIDs was not collected. Participants who reported consuming aspirin, non-aspirin NSAIDs, and/or acetaminophen at least twice a week were considered regular users. We considered analgesic use, demographic characteristics, reproductive and family histories, and lifestyle factors reported two questionnaire cycles prior to the date of the diagnosis of the cases.

Genotyping

Genotyping was conducted at the Dana-Farber/ Harvard Cancer Center High Throughput Genotyping Core. The polymorphisms in *CYP2C9* (rs1799853 and rs1057910), *UGT1A6* (rs2070959 and rs1105879), *PTGS2* (rs20417 and rs5275), and PPAR-γ (rs1801282) were genotyped in 384-well plates using the 5' nuclease assay (Taqman) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Cases and matched controls were assayed in the same plate; blinded replicate samples were included in each plate for quality control. Laboratory personnel were blinded to the case-control status of the samples. The replicates were above 98% concordant for all genotypes.

Over 94% of the samples were successfully genotyped for each of the seven polymorphisms in both the NECC and NHS. We considered

genotyping failure as missing data; therefore, the total number of observations varied between polymorphisms. Taqman primers, probes, and assay conditions are available upon request.

Statistical analyses

We conducted chi-square tests to assess whether genotypes were in Hardy-Weinberg equilibrium. Linkage disequilibrium between pairwise combination of SNPs were estimated using Lewontin's D' statistics [47]. For each SNP, the most common allele served as the reference group. Odds ratios (ORs; as estimates of the relative risks) and 95% confidence intervals (CI) for the main effects of NSAIDs and of each polymorphism were estimated using conditional logistic regression stratified on matching factors (NHS/NHS-II) and unconditional logistic regression adjusted for matching factors (NECC). Study-specific estimates were combined using DerSimonian and Laird random effects; heterogeneity by study was assessed via Cochran's Q statistics [48]. Multivariable analyses were additionally adjusted for the main risk factors for ovarian cancer, including duration of oral contraceptive use in months (continuous), parity (continuous), history of tubal ligation (yes/no), menopausal status (premenopausal/postmenopausal), and use of post-menopausal hormones (PMH) (never/past/current). Further adjustments for breastfeeding, age at menopause, age at menarche, physical activity, and smoking history did not substantially change the results and were not included in the final models. Wald statistics were used to test for linear trend across categorical variables modeled linearly.

In both the NHS/NHS-II and NECC, unconditional logistic regression adjusting for matching factors was used to estimate the association between NSAIDs use and ovarian cancer risk within strata of each genotype. To increase power of the interaction analyses, heterozygotes and homozygotes variants were combined. Likelihood ratio tests were used to compare nested models, including NSAIDs/genotype interaction terms against models with main effects only. *A priori* power estimates indicated sufficient power to detect RR ≤ 0.70 for the NSAIDs main effect analyses. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Results

We identified 1,353 (NHS/NHSII:233; NECC:1,120) ovarian cancer cases and 1,823 (NHS/NHSII:663; NECC:1,160) control participants who were of European ancestry and had genotyping data available. Compared to controls, women who developed ovarian cancer were more likely to be nulliparous and to have a family history of ovarian cancer, while less likely to have a history of oral contraceptive use, breastfeeding, and tubal sterilization. The distributions of the *CYP2C9*, *UGT1A6*, *COX-2*, and *PPAR-γ* polymorphic variants in cases and controls were not significantly different (**Table 1**).

Regular use of NSAIDs was not significantly associated with ovarian cancer risk among NHS participants, which is consistent with our previous analysis of the full NHS and NHSII cohorts [14]. The ORs (95% CI) for the association with regular use of aspirin, non-aspirin NSAIDs, and acetaminophen were 0.91 (0.64,1.31), 0.95 (0.58,1.56), and 0.55 (0.26,1.13), respectively. Similar results were observed among NECC participants for aspirin and acetaminophen (0.80 (0.62,1.05) and 0.90 (0.65,1.23), respectively). However, regular use of non-aspirin NSAIDs (mostly ibuprofen) was associated with a borderline significant decrease in ovarian cancer risk (OR and 95% CI: 0.75 (0.57, 0.99)) (**Table 2**). No clear dose-response was observed with increasing number of tablets consumed per week or duration of use. For example, the multivariable OR (95% CI) associated with intake of 5+ tablets of ibuprofen per week *versus* fewer than 2 tablets/week was 0.76 (0.56, 1.03), with a non-significant trend across number of tablets/week (*p*-trend=0.43). The multivariable OR (95% CI) for 5 or more years of regular use compared to 0 years was 0.84 (0.56, 1.27); the *p*-value for a trend across number of years of use was 0.84 (data not shown). In estimates pooled across all studies, aspirin, non-aspirin NSAIDs, and acetaminophen were not significantly associated with ovarian cancer risk 0.84 (0.68, 1.04), 0.79 (0.62, 1.01), and 0.78 (0.51, 1.21), respectively. No statistically significant heterogeneity by study population was found (all *p*-heterogeneity >0.22) (**Table 2**).

All genotypes among control participants were in Hardy-Weinberg equilibrium within each study population, and the minor allele frequencies were similar to reports in other populations of

Table 1. Characteristics of study participants *

Characteristic ‡	Nurses Health Studies †			NECC		
	Cases	Controls	P §	Cases	Controls	P §
N	233	663		1120	1160	
	Mean (SD)			*Mean (SD)*		
Age (yrs) § §	59.4 (9.9)	59.2 (10.0)		51.7 (12.7)	51.1 (12.9)	
BMI (Kg/m2)	26.0 (5.5)	25.6 (4.7)		26.3 (6.3)	25.7 (5.5)	**
Parity ‖	3.0 (1.3)	3.2 (1.5)	**	2.6 (1.3)	2.8 (1.5)	**
Duration of OC (mos) ††	47.8 (41.6)	53.9 (48.9)		53.1 (54.7)	60.9 (55.3)	**
Age at menarche (yrs)	12.7 (1.5)	12.5 (1.4)		12.6 (1.5)	12.7 (1.6)	
Age at first birth (yrs) ‖	25.1 (3.8)	24.9 (3.3)		24.7 (5.0)	25.1 (5.0)	
	%			*%*		
Nulliparous	11.2	7.5		32.0	18.7	**
Breastfeeding (18+ mos) ‖	11.8	15.3		8.7	13.6	**
Postmenopausal § §	73.8	70.9		52.1	49.9	
Current smoker	11.2	10.3		22.0	19.7	
History of tubal ligation	15.5	21.4	**	13.8	18.2	**
Hystory of hysterectomy	19.7	16.7		8.2	8.5	
Family history of ovarian cancer	7.3	3.3	**	5.0	2.9	**
Family history of breast cancer	13.3	13.7		14.2	12.0	
Regular use any NSAIDs ‡‡	39.1	37.3		18.4	21.8	**
CYP2C9 R144C / I359L ‖‖	37.0	36.1		34.4	34.6	
UGT1A6 T181A / R184S ‖‖	56.5	60.0		57.6	58.1	
COX-2 765G>C / Ex10+837T>C ‖‖	59.0	56.0		54.0	56.2	

* Restricted to Caucasians and to those with genotyping data.

† Includes epithelial ovarian cancer cases (incident and prevalent cases diagnosed ≤4years of blood collection) and their matched controls

‡ Characteristics ascertained 2-4 years (NHS/NHS-II) and 1 year (NECC) prior to ovarian cancer diagnosis

§ P-values adjusted for matching factors

§ § Matching factor (only NHS/NHSII matched on menopausal status)

** P-value<0.05

‖ Among parous participants

†† Among partipants who *ever* used oral contraceptives (OC)

‡‡ Regular user if 2+ times a week (NHS/NHS-II) or 2+ tablets/week (NECC)

‖‖ Any variant allele

European ancestry. In our population, *CYP2C9* SNPs (rs179853 and rs1057910) were in strong linkage disequilibrium (D':0.90), as were the *UGT1A6* SNPs (rs2070959 and rs1105879) (D':0.96) and the *COX-2* SNPs (rs5275 and rs20417) (D':0.88). There was no association between *CYP2C9* and *UGT1A6* genotypes and ovarian cancer risk. The ORs (95% CI) for the association with each variant allele were 0.99 (0.90, 1.08) and 0.93 (0.82, 1.05), respectively. There was also no association between -

765G>C and Ex10+837T>C COX-2 polymorphisms and ovarian cancer risk across the study populations (ORs and 95% CIs for each variant allele were 0.87 (0.75, 1.00) and 0.97 (0.87, 1.09), respectively) or with the *Ala12Pro* polymorphism in the *PPAR-γ* gene (ORs and 95% CIs associated with each variant allele were 1.02 (0.87, 1.20)) (**Table 3**). There was no evidence of a gene-gene interaction between polymorphisms in the *CYP2C9* and *UGT1A6* genes (data not shown).

Table 2. Regular use of analgesics and ovarian cancer risk in the Nurses Health Studies (NHS/NHS-II) and in the New England Ovarian Cancer Case-Control Study (NECC)*

	Nurses Health Studies (NHS/NHS-II)			NECC			Pooled
	cases N(%)	controls N(%)	OR (95% CI)†	cases N(%)	controls N(%)	OR (95% CI)†	OR (95% CI)†‡
N §	233	663		1120	1160		
Regular use of Aspirin ‡							
No	148(68)	426 (68)	1.00	1000(89)	1013(87)	1.00	1.00
Yes	69(32)	202 (32)	0.91 (0.64, 1.31)	120(11)	147(13)	0.80 (0.62, 1.05)	0.84 (0.68, 1.04)
Regular use of non-aspirin NSAIDs ‖ ¶							
No	153(85)	422 (85)	1.00	1018(91)	1020(88)	1.00	1.00
Yes	26(15)	72 (15)	0.95 (0.58, 1.56)	102(9)	140(12)	0.75 (0.57, 0.99)	0.79 (0.62, 1.01)
Regular use *any* NSAIDs ‖¶							
No	137(61)	410 (63)	1.00	914(82)	907(78)	1.00	1.00
Yes	88(39)	244 (37)	0.99 (0.70, 1.39)	206(18)	253(22)	0.80 (0.65, 0.99)	0.85 (0.71, 1.02)
Regular use of Acetaminophen ‖							
No	180 (95)	486 (91)	1.00	1041(93)	1063(92)	1.00	1.00
Yes	10 (5)	49 (9)	0.55 (0.26, 1.13)	79(7)	97(8)	0.90 (0.65, 1.23)	0.78 (0.51, 1.21)

* Restricted to Caucasian participants with genotyping data

† NHS/NHS-II: Conditional logistic regression stratified on matching factors (*i.e* .age, menopause status at blood collection and diagnosis, PMH use at blood collection, date, time and fasting status at blood collection, DNA type); NECC: Unconditional logistic regression adjusted for age (cont), study center (MA/NH). NHS/NHS-II and NECC: All models additionally adjusted for parity, duration of OC use (months), history of tubal ligation (y/n), PMH use (never/past/current), and menopausal status (pre/post)

‡ Estimates pooled using DerSimonian and Laird random effects models; all *P*-het >0.22

§ Numbers may not add up to total N due to missing information on analgesic use.

‖ In NHS/NHS-II, ≥2 days/week in the 2-4 years preceding ovarian cancer diagnosis. In NECC, ≥2 tablets/week for 6 months in the year preceding

¶ In the NECC, "non-aspirin NSAIDs" refers primarily to ibuprofen

We also evaluated whether these associations differed by tumor histology. While results were similar for serous, endometrioid, and clear-cell tumors, possession of *CYP2C9 *1/*2* and *CYP2C9 *1/*3* variant alleles was associated with a borderline significant 42% increase in risk of mucinous ovarian tumors (*p*-value=0.06). This increase was more pronounced among participants with a smoking history (OR and 95% CI associated with any of the *CYP2C9* variant alleles was 1.58 (0.98, 2.56)). The associations between each genotype/polymorphism and ovarian cancer risk did not differ by BMI, OC use, parity, menopause status, or age at diagnosis (data not shown).

Table 4 shows the OR and 95% CI associated with regular use of NSAIDs within each genotype stratum. We observed no differences in the NSAIDs/ovarian cancer association between individuals who were wild-type and carriers of the variant alleles for the polymorphisms in the *CYP2C9, UGT1A6, COX-2,* and *PPAR-γ* genes. ORs were compatible with unity; all *p*-interactions were greater than 0.06.

Discussion

In this population, comprised of 1,353 cases and 1,823 controls, we found no strong evidence to support an association between regular use of aspirin, non-aspirin NSAIDs, and acetaminophen and ovarian cancer risk. Further, the associations between analgesic use and ovarian cancer risk did not differ significantly according to polymorphisms in selected genes. We also found no compelling evidence linking these polymorphisms with risk of ovarian cancer independently of analgesic use.

Other than oral contraceptive use and tubal ligation, few modifiable risk factors are known to reduce ovarian cancer risk. Experimental data suggest that COX inhibition by NSAIDs exerts anti-neoplastic properties in ovarian cancer cells [2], but epidemiological data have been less consistent, particularly for non-aspirin NSAIDs. While most population-based studies do not support an association with aspirin [6-10, 14, 15, 49], intake of non-aspirin NSAIDs

TABLE 3. Main effects of genes involved in the metabolism of NSAIDs and risk of epithelial ovarian tumors

Genotypes	Nurses Health Studies *			NECC *			Pooled
	Cases(%)	Controls(%)	OR (95% CI) †	Cases(%)	Controls(%)	OR (95% CI) ‡	OR (95% CI) §
N ‖	233	663		1120	1160		
CYP2C9 genotypes							
rs1799853/rs105791							
*1/*1	145 (63)	419 (64)	1.0	724 (66)	756 (65)	1.0	1.0
*1/*2	51 (22)	142 (22)	1.12 (0.76, 1.65)	212 (19)	231 (20)	0.95 (0.76, 1.18)	0.99 (0.82, 1.20)
*1/*3	22 (10)	77 (12)	0.79 (0.46, 1.36)	130 (12)	122 (11)	1.09 (0.83, 1.43)	1.01 (0.77, 1.32)
*2/*2, *3/*3, *2/*3	12 (5)	18 (3)	1.81 (0.82, 3.99)	37 (3)	46 (4)	0.73 (0.46, 1.15)	1.08 (0.45, 2.61)
P-trend ¶			0.61			0.59	0.82
Per variant genotype			1.05 (0.87, 1.27)			0.97 (0.88, 1.08)	0.99 (0.90, 1.08)
UGT1A6 genotypes							
rs2070959/rs110587							
Wild type	100 (43)	262 (40)	1.0	471 (42)	485 (42)	1.0	1.0
181 T->A/184R->S	114 (50)	329 (50)	0.91 (0.66, 1.26)	561 (51)	583 (50)	0.99 (0.83, 1.18)	0.97 (0.83, 1.14)
184 R->S var only	16 (7)	64 (10)	0.59 (0.31, 1.11)	79 (7)	90 (8)	0.88 (0.63, 1.24)	0.79 (0.56, 1.12)
P-trend ¶			0.15			0.59	0.24
Per variant genotype			0.83 (0.65, 1.07)			0.96 (0.84, 1.11)	0.93 (0.82, 1.05)
COX-2 -765G>C							
rs 20417							
G/G	164 (73)	465 (72)	1.0	748 (71)	753 (68)	1.0	1.0
C/G	53 (24)	167 (26)	0.86 (0.59, 1.25)	272 (26)	314 (28)	0.86 (0.71, 1.05)	0.86 (0.72, 1.03)
C/C	7 (3)	13 (2)	1.43 (0.55, 3.73)	30 (3)	44 (4)	0.65 (0.40, 1.06)	0.87 (0.41, 1.81)
P-trend ¶			0.83			0.04	0.05
Per variant			0.97 (0.71, 1.32)			0.84 (0.72, 0.99)	0.87 (0.75, 1.00)
COX-2 Ex10+837T>C							
rs 5275							
T/T	87 (40)	264 (43)	1.0	486 (46)	494 (45)	1.0	1.0
C/T	107 (49)	281 (46)	1.18 (0.84, 1.66)	437 (42)	471 (43)	0.95 (0.79, 1.14)	1.00 (0.83, 1.22)
T/T	23 (11)	67 (11)	0.96 (0.55, 1.68)	123 (12)	138 (13)	0.91 (0.68, 1.20)	0.92 (0.71, 1.18)
P-trend ¶			0.73			0.43	0.59
Per variant			1.04 (0.82, 1.33)			0.95 (0.84, 1.08)	0.97 (0.87, 1.09)
PPAR-γ Ala12Pro							
rs 1801282							
CC	166 (74)	487 (76)	1.0	831 (77)	882 (78)	1.0	1.0
CG	56 (25)	144 (22)	1.15 (0.80, 1.66)	228 (21)	241 (21)	1.02 (0.83, 1.26)	1.05 (0.88, 1.26)
CC	2 (1)	13 (2)	0.37 (0.08, 1.71)	16 (2)	13 (1)	1.12 (0.52, 2.38)	0.79 (0.29, 2.15)
P-trend ¶			0.96			0.76	0.81
Per variant			0.99 (0.72, 1.36)			1.03 (0.85, 1.24)	1.02 (0.87, 1.20)

* Restricted to participants of European ancestry with genotyping data available. In the NHS/NHS-II, both incident (N=177) and prevalent (N=56) cases diagnosed <4 years before blood collection are included.

† Conditional logistic regression stratified by matching factors and adjusted for duration of oc use (months), parity, tubal ligation (yes/no), PMH use (past/never/current), and menopausal status (pre/post)

‡ Unconditional logistic regression adjusted for age (years), study center (MA/NH), duration of oc use (months), parity, tubal ligation (yes/no), menopausal status (pre/post), PMH use (never/past/current)

§ Multivariate estimates were pooled using DerSimonian and Laird random effects [47]. P-values for tests for heterogeneity comparing NECC and NHS/NHS-II results were all >0.05 .

‖ Numbers may not add up to total N due to missing genotpying data

¶ P-values for trend calculated using Wald test with genotype categories modeled linearly

has been inversely associated with ovarian cancer risk in some studies [12-14, 17].

In our study, regular use of aspirin or of non-aspirin NSAIDs was not significantly associated with ovarian cancer risk. A 25% decrease in risk was suggested for regular users of non-aspirin NSAIDs (primarily ibuprofen) in the NECC only. However, no dose response was observed with number of tablets or duration of use. Although it

is unclear why prospective and the retrospective estimates differed for use of non-aspirin NSAIDs, it is possible that reporting among cases in the NECC was influenced by symptoms of subclinical disease (abdominal discomfort discouraging their use). The inverse association between non-aspirin NSAIDs and ovarian cancer risk across all three study populations was not statistically significant. These findings are consistent with the associations we observed

TABLE 4. Regular use of *any* (aspirin and non-aspirin) NSAIDs* and risk of epithelial ovarian cancer according to genotype among participants of the Nurses' Health Studies (NHS/NHS-II) and the New England Ovarian Cancer Case Control (NECC)

	NHS/NHS-II †‡			NECC †			Pooled					
							Cases (N)		Controls (N)			
	NSAIDs			NSAIDs			NSAIDs		NSAIDs		NSAIDs	
	No	Yes	Cases/Controls	No	Yes	Cases/Controls	No	Yes	No	Yes	No	Yes
		OR (95% CI) §			OR (95% CI) §							OR (95% CI)
CYP2C9 genotype												
Wild-type	1.0	0.94 (0.62, 1.43)	139/423	1.0	0.95 (0.73, 1.25)	724/756	673	190	865	314	1.0	0.95 (0.76, 1.19)
Any variants	1.0	1.31 (0.74, 2.34)	83/239	1.0	0.62 (0.44, 0.89)	379/399	360	102	453	185	1.0	0.87 (0.42, 1.80) ‖
P-int ¶		*0.31*			*0.06*							*0.83*
UGT1A6 genotype												
Wild-type	1.0	0.85 (0.51, 1.43)	95/267	1.0	0.73 (0.52, 1.02)	471/485	452	114	547	205	1.0	0.76 (0.58, 1.01)
Any variants	1.0	1.24 (0.79, 1.95)	127/394	1.0	0.86 (0.65, 1.13)	640/673	590	177	770	297	1.0	0.98 (0.70, 1.39)
P int ¶		*0.36*			*0.46*							*0.26*
COX-2 -765G>C												
Wild-type	1.0	0.97 (0.65, 1.44)	161/465	1.0	0.86 (0.66, 1.11)	748/753	702	207	873	345	1.0	0.89 (0.71, 1.10)
Any variants	1.0	1.13 (0.59, 2.18)	56/185	1.0	0.79 (0.53, 1.18)	302/358	279	79	400	143	1.0	0.87 (0.62, 1.23)
P-int ¶		*0.34*			*0.75*							*0.80*
COX-2 Ex10+837T>C												
Wild-type	1.0	1.12 (0.63, 1.97)	85/265	1.0	0.76 (0.55, 1.05)	486/494	456	115	556	203	1.0	0.86 (0.60, 1.21)
Any variants	1.0	0.93 (0.60, 1.46)	127/351	1.0	0.91 (0.67, 1.22)	560/609	525	162	693	267	1.0	0.91 (0.71, 1.17)
P-int ¶		*0.84*			*0.45*							*0.60*
PPAR-γ Ala12Pro												
Wild-type	1.0	1.17 (0.79, 1.75)	160/487	1.0	0.75 (0.58, 0.97)	831/882	791	200	1012	357	1.0	0.91 (0.59, 1.41)
Any variants	1.0	0.78 (0.40, 1.55)	56/162	1.0	1.06 (0.69, 1.61)	244/254	215	85	284	132	1.0	0.97 (0.68, 1.39)
P-int ¶		*0.13*			*0.15*							*0.89*

* In NHS/NHS-II, ≥2 days/week in the 2-4 years preceding ovarian cancer diagnosis. In NECC, ≥2 tablets/week for 6 months in the year preceding diagnosis.

† Includes participants of European ancestry with genotyping information available.

‡ Includes incident and prevalent (≤4 years of blood collection) cases and matched controls

§ Unconditional logistic regression models adjusted for age (months), duration of oral contraceptive use (months), parity (continuous), tubal ligation (yes/no), PMH use (never/past/current) and menopausal status (pre/post). Additionally adjusted for menopause status at diagnosis (pre/post/dubious) and DNA type (WBC/cheek) in NHS/NHS-II, and for study center in NECC

‖ *P*-value for heterogeneity by study population =0.03; all others *p*-values for heterogeneity >=0.06

¶ *P*-values comparing the likelihood of models with and without interaction terms for NSAIDs use and each genotype

between aspirin and non-aspirin NSAIDs use and ovarian cancer risk in the entire NHS and NHS-II cohorts [14].

Use of acetaminophen has been inversely associated with ovarian cancer risk in some retrospective studies [8, 12, 13, 50], including a previous report among NECC participants [9]. However, in our current analysis, which includes more than twice the number of cases included in the previous NECC study [9], regular use of acetaminophen was not associated with risk of ovarian cancer either in study-specific or pooled analyses.

NSAIDs are a structurally diverse group of compounds that act through similar pathways. Beyond the variation in pharmacokinetics observed across different formulations [51], there also appears to be large inter-individual variability, as mean serum concentrations were considerably different among patients who received the same therapeutic dosages [52]. Several different formulations of NSAIDs are known to

be at least partially metabolized via CYP2C9 [18-21] and UGT1A6 [22]. In colorectal cancer, where the chemopreventive properties of NSAIDs are consistently documented [53], interactions between NSAIDs and genetic polymorphisms in *CYP2C9* and *UGT1A6* have been reported. Two studies reported greater risk reduction of colorectal adenoma risk with aspirin use among carriers of *UGT1A6* variant alleles [28, 29]. Interactions between NSAIDs and *CYP2C9* are more difficult to interpret. In one study, risk reduction associated with aspirin was observed only among *CYP2C9* wild-type individuals [28]; in another, risk reduction associated with ibuprofen use was restricted to individuals with *CYP2C9* variant alleles [54]. In our population, the association between (aspirin and non-aspirin) NSAIDs and ovarian cancer risk did not differ according to *CYP2C9* and *UGT1A6* variant alleles.

The potential anti-neoplastic properties of NSAIDs are attributed to their inhibition of cyclooxygenases (COX), enzymes that catalyze

the synthesis of prostaglandins [4, 5], which contribute to carcinogenesis by promoting cellular proliferation, stimulating angiogenesis, and inhibiting apoptosis [55, 56]. Therefore, it is biologically plausible that the putative protective effects of NSAIDs are influenced by the amount or activity of COX in cells, both partially determined by specific polymorphisms. A common polymorphism in the regulatory region of COX-2 (-765G>C, known as -898C>G) has been linked to both reduced gene expression and lower serum concentrations of C-reactive protein, suggesting a reduced inflammatory response among carriers of the variant alleles [57]. In colorectal cancer, reduced risk was reported in individuals with lower COX-2 expression/activity either due to NSAID use the COX -765C>G polymorphism [32]. Interestingly, no added risk reduction was reported among participants who both used NSAIDs and had the variant genotype, suggesting that the NSAIDs may not offer additional benefits to those with already-low levels of COX-2. Another COX-2 polymorphism (Ex10+837T>C, known as T8473C) in the 3'UTR region of the gene was associated with higher prostaglandin E_2 levels [58]. Use of NSAIDs was found to decrease hormone receptor–positive breast cancer risk only among women with the Ex10+837T>C variant alleles [33]. However, our findings do not support modification of the NSAIDs/ovarian cancer association by polymorphisms in the COX-2 gene.

Prostaglandins may also function through proliferator-activated receptors (particularly PPAR-g), which are thought to attenuate the inflammatory response. A number of NSAIDs, including ibuprofen, are known to activate PPAR-g [34, 35]. A common polymorphism produces a Proline to Alanine substitution at codon 12 (Pro12Ala) in the PPAR-g domain, encoding for a less active transcription activator [59]. The chemopreventive effects of NSAIDs on colorectal adenoma risk were more pronounced among participants with the Pro12Ala PPAR-g variant alleles [60]. Here we found no evidence for an interaction between analgesic use and the PPAR-g genotype.

We found polymorphisms in the CYP2C9, UGT1A6, COX-2, and PPAR-g genes to be unassociated with risk in our study population. To our knowledge, this is the first study to examine the association between these polymorphisms and ovarian cancer risk. Our findings were similar across different histological subtypes of ovarian

tumors (serous, endometrioid, clear cell) except for the association between CYP2C9 and mucinous tumors, where CYP2C9 variant alleles were associated with a 42% (p-value=0.06) increase in risk of mucinous tumors. This finding may not be related to the differential effects of NSAIDs but to environmental carcinogens, as CYP2C9 may be also involved in detoxification of polycyclic aromatic hydrocarbons produced by cigarette smoking [61], a risk factor for colorectal neoplasia [62] and mucinous ovarian tumors [63]. In the current analysis, the association between CYP2C9 and mucinous ovarian tumors was more pronounced among participants with a history of smoking, although not statistically significant. Due to the limited power of these analyses (n=167 mucinous cases), these findings warrant replication in larger studies.

In one of our study populations (NECC), information on analgesic use was collected after diagnosis of ovarian cancer, making our study susceptible to recall bias. However, intake of NSAIDs is not a well-known risk factor for ovarian cancer, making its self-report less likely to be influenced by disease status. Additionally, to reduce the possibility of influence of subclinical disease on analgesic intake, NECC participants were asked to report intake at least one year prior to diagnosis. Analgesic data also differed slightly between study populations. In the NHS, use of other NSAIDs referred to a variety of formulations, while in the NECC it referred mostly to ibuprofen. However, ibuprofen was also the most commonly reported NSAID among a subset of NHS/NHS-II participants (64%) [64, 65]. Additionally, because information on Cox-2 inhibitors was unavailable in the NECC and in the NHS prior to 2000, it was not possible to evaluate the relationship between Cox-2 and other NSAIDs separately in this study.

The current analyses were restricted to women of European ancestry. While this avoids population stratification by ethnicity, it may limit the generalizability of our results. Moreover, while we focused our analyses on polymorphisms suggested to have functional significance (CYP2C9, UGT1A6, PPAR-γ), it is possible that other, less studied polymorphisms or polymorphisms involved in different pathways may influence the relation between NSAIDs and ovarian cancer risk. Moreover, the COX-2 SNPs included in this study offer limited coverage of the COX-2 gene. According to Hapmap data [66], six more

SNPs (rs689470, rs2745557, rs4648261, rs4648262, rs5277, and rs2206593) are needed to capture common genetic variation (MAF>0.05) among individuals of European ancestry. Finally, our study may have had limited power to detect interactions between use of NSAIDs and certain polymorphisms and larger studies are warranted to confirm these null findings.

A notable strength of our study was its relatively large sample size of over 1,350 ovarian cancer cases, with data from one retrospective and two prospective studies. Genotyping error in this study was low, as evidenced by high genotyping success rates and high inter-assay concordance rates. Also, we had detailed data on analgesic intake and on known and potential confounders.

This is the first study to explore interactions between use of NSAIDs and genetic polymorphisms affecting the metabolism of NSAIDs or the expression of their target enzymes in the context of ovarian cancer. Our findings do not suggest an association between regular use of aspirin or non-aspirin NSAIDs and ovarian cancer risk. We also found no evidence that the associations between analgesic intake and ovarian cancer risk differ according to presence of selected genetic polymorphisms. Larger studies that capture a greater variability in the genes involved in the metabolism of NSAIDs are needed to further elucidate the interplay between genetic polymorphisms and NSAIDs in ovarian cancer risk.

Acknowledgements

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