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Xenobiotic-Metabolizing Gene Polymorphisms and Ovarian Cancer Risk

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Abstract

Because selected xenobiotic-metabolizing enzymes process pro-carcinogens that could initiate ovarian carcinogenesis, we hypothesized that single-nucleotide polymorphisms (SNPs) in the genes encoding xenobiotic-metabolizing enzymes are associated with risk of ovarian cancer. Cases with invasive epithelial ovarian cancer (N = 1,571 including 956 of serous sub-type) and controls (N = 2,046) from three studies were genotyped at 11 SNPs in *EPHX1*, *ADH4*, *ADH1A*, *NQO2*, *NAT2*, *GSTP1*, *CYP1A1*, and *NQO1*, following an initial SNP screen in a subset of participants. Logistic regression analysis of genotypes obtained via Illumina GoldenGate and Sequenom iPlex technologies revealed the following age- and study-adjusted associations: *EPHX1* rs1051740 with increased serous ovarian cancer risk (per-allele odds ratio (OR) 1.17, 95% confidence interval (95% CI) 1.04–1.32, p = 0.01), *ADH4* r1042364 with decreased ovarian cancer risk (OR 0.90, 95% CI 0.81–1.00, p = 0.05), and *NQO1* rs291766 with increased ovarian cancer risk (OR 1.11, 95% CI 1.00–1.23, p = 0.04). These findings are consistent with prior studies implicating these genes in carcinogenesis and suggest that this collection of variants is worthy of follow-up in additional studies.

Keywords

Gynecologic neoplasia; carcinogenesis; epidemiology

INTRODUCTION

Ovarian cancer is the leading cause of gynecologic cancer death among women in developed countries [1]. Known risk factors for ovarian cancer overall or for particular subtypes include age, family history, smoking, fertility drug use, and postmenopausal hormone

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therapy [2]. Polymorphisms have been associated with ovarian cancer risk in the 9p22.2 chromosomal region [3] and in genes that regulate DNA repair [4].

Environmental carcinogens, such as chemicals in cigarettes, are processed by numerous xenobiotic-metabolizing enzymes including alcohol dehydrogenases (ADHs), cytochrome P450s (CYPs), epoxide hydrolases (EPHXs), glutathione S-transferases (GSTs), N-acetyltransferases (NATs), and NAD(P)H dehydrogenases (quinone) (NQOs) [5]. The CYP enzyme family is key to detoxification of the polycyclic aromatic hydrocarbons, N-nitrosamine, and aromatic amines found in cigarette smoke [6,7]. ADHs convert ethanol to acetaldehyde, a known carcinogen that interferes with DNA synthesis and repair during the first step of alcohol metabolism [8,9]. GSTs, NATs, NQOs, and EPHXs are critical to the metabolism of xenobiotics and potential pro-carcinogens that may be involved in cancer initiation [5].

Here, we hypothesize that, due to their important role in processing pro-carcinogens, inherited genetic variants in xenobiotic-metabolizing genes may be associated with risk of ovarian cancer. Following an initial screen of 163 single-nucleotide polymorphisms (SNPs) in 16 xenobiotic metabolizing genes, 11 SNPs in eight genes were evaluated in a combined analysis of three case-control study populations.

MATERIALS AND METHODS

An initial association screen was conducted within an ongoing two-site candidate gene study described in detail previously [10,11]. Briefly, 930 epithelial ovarian cancer cases and 1,037 frequency-matched (by age, race, and residence) controls from the Mayo Clinic Ovarian Cancer Study (MAY) and the North Carolina Ovarian Cancer Study (NCO) were genotyped at 163 SNPs in 16 xenobiotic-metabolizing genes using an Illumina GoldenGate assay. MAY and NCO participants were enrolled between 1999 and 2006, provided informed consent, and contributed a blood sample; risk factor information was collected through in-person interviews, and clinical data was obtained via medical record. Ascertainment of MAY participants was clinic-based and limited to a six-state catchment area that representing >85% of cases seen at the Mayo Clinic. We selected MAY controls from women seeking general medical evaluation. The NCO study was population based with a rapid case ascertainment network covering a 48-county region of North Carolina. List-assisted random digit dialing and Health Care Financing Administration roster methods were used to identify controls. Included SNPs tagged European variants ($r^2 \geq 0.8$, MAF ≥ 0.05) or were non-synonymous, within 1 kb upstream, within a 5′ UTR, or within a 3′ UTR (Supplemental Table 1) [12]. Inclusion of within-gene tagSNPs across the chromosome 4 *ADH* gene cluster enabled evaluation of inter-genic LD and suggested efficiency of the tagSNP selection strategy used (Supplemental Figure 1). Robust quality control measures were applied (Supplemental Table 2) [13]. Association-testing used logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) assuming an ordinal, recessive, dominant, or co-dominant model for risk of invasive/borderline ovarian cancer and for risk of invasive serous sub-type adjusted for race, age, study site, body mass index, hormone therapy use, oral contraceptive use, parity, and age at first birth. These covariates were included based on association with MAY+NCO case-control status following step-wise regression.

Pooled analysis included MAY, NCO, and an additional collection of 904 invasive cases and 1,105 controls from the Australian Ovarian Cancer Study and the Australian Cancer Study, Ovarian (AUS) [14] genotyped using the Sequenom iPlex (Supplemental Table 3). AUS cases were diagnosed from 2002 to 2007; recruited through surgical treatment centers throughout Australia & cancer registries of Queensland, South Australia, West Australia,

New South Wales, and Victoria. AUS controls were randomly selected from Commonwealth electoral rolls and frequency matched for age & geographical region. Eleven initially-screened SNPs which had $p < 0.10$ in at least one genetic model and which could be genotyped by Sequenom iPlex technology were assessed. A pooled approach was used because it has been shown to be more powerful than separate replication studies [15], and a simplified series of analyses was conducted to reduce multiple testing issues. Association testing used logistic regression to estimate ORs and 95% CIs assuming an ordinal model for risk of invasive ovarian cancer and for risk of invasive serous sub-type among white non-Hispanic women (97% of the pooled study population), adjusted for study site and age. Interactions between genotype and alcohol intake (never, monthly, daily/weekly) and between genotype and two characterizations of tobacco use (never smokers, former smokers, current smokers; 0 pack-years, ≤ 20 pack-years, > 20 pack-years) were explored for SNPs in relevant genes and evaluated using likelihood-ratio testing. Differences in risk by histological sub-type (serous, endometrioid, clear cell, mucinous) were examined using polytomous logistic regression. All analyses were conducted in SAS (SAS Institute, Cary, NC, Version 8, 1999), and, because of the *a priori* nature of the candidate gene hypotheses being tested, no corrections were made for multiple-testing.

RESULTS

Characteristics of 1,571 white non-Hispanic invasive epithelial ovarian cancer cases and 2,046 controls are shown by study site in Table 1. Approximately 60% of the cases had serous histology, and trends in known risk factors were as expected; in addition, the prevalence of alcohol and tobacco use was sufficiently high to permit assessment of interactions with the candidate genes of interest. Eleven SNPs in *EPHX1*, *ADH4*, *ADH1A*, *NQO2*, *NAT2*, *GSTP1*, *CYPA1A*, and *NQO1* were evaluated for association with risk of invasive ovarian cancer and for serous sub-type (Table 2), following initial screening (Supplemental Table 2). We found evidence that a non-synonymous SNP rs1051740 in *EPHX1* was associated with increased invasive ovarian cancer risk ($p = 0.03$), particularly for serous sub-type ($p = 0.01$), that a 3′ UTR SNP rs1042364 in *ADH4* was associated with decreased invasive ovarian cancer risk ($p = 0.05$), and that an upstream, possibly promoter-related, SNP rs2917666 in *NQO1* was associated with increased invasive ovarian cancer risk ($p = 0.04$) (Table 2). These results were consistent across studies (Figure 1, Supplemental Table 4).

No compelling trends in risk were observed in analyses stratified by alcohol use (examined for *ADH4* and *AHD1A*) or tobacco use (examined for *EPHX1*, *NQO2*, *NAT2*, *GSTP1*, *CYPA1A*, and *NQO1*; Supplemental Table 5). Finally, no significant ($p < 0.05$) heterogeneity of risks was observed across histological sub-types (Supplemental Table 6), including at *EPHX1* rs1051740.

DISCUSSION

Xenobiotic-metabolizing enzymes are clearly important in processing of pro-carcinogens, and several studies have observed associations and interactions with non-genetic factors in the etiology of cancer. Here, we used a multi-site study to examine the hypothesis that inherited variation which may alter xenobiotic metabolism relates to ovarian cancer risk. We found that rs1051740 in *EPHX1* was associated with increased invasive ovarian cancer risk (particularly serous sub-type); this SNPs results in an amino acid change at position 113 from the polar hydrophilic tyrosine to the electrically-charged (positive) histidine. This change is predicted to be damaging to the function of epoxide hydrolase [16] and has been shown to alter epoxide hydrolase's processing of several carcinogens [17–19]. Genotypes at this SNP have been studied previously in relation to ovarian cancer risk in two smaller study

populations [20,21], and association with risk was observed in one study (OR 2.6, 95% CI: 1.3 – 5.0) [20]. The modest *EPHX1* rs1051740 risk estimate we observed may only be apparent with large sample size. Associations between genotypes at this SNP and increased risk of squamous cell esophageal cancer [22] and colorectal cancer [23] have also been reported; notably the "slow" phenotype was associated with increased risk of colorectal adenomatous and hyperplastic polyps in individuals exposed to cigarette smoke and high red meat consumption [24]. It is of particular interest that, in the current study, this SNP was selected for pooled analysis over other *EPHX1* SNPs based on initial screening results regardless of its functional or tagging status.

We also report that an *ADH4* SNP was associated with decreased ovarian cancer risk and that an *NQO1* SNP was associated with increased risk. Alcohol dehydrogenase 4 (class II), pi polypeptide (ADH4) appears to be important to the initial metabolism of ethanol and also in the synthesis of retinoic acid [25]. Our findings in *ADH4* are consistent with prior evidence indicating that the retinoic acid pathway plays a role in ovarian carcinogenesis [26,27]. NAD(P)H:quinone oxidoreductase 1 (NQO1) metabolizes quinones, aromatic compounds found in benzene and chemotherapeutics, and acts as an antioxidant that protects against the production of DNA- damaging and protein-damaging reactive oxygen species. Additionally, NQO1 expression is up-regulated in tumor tissues as a result of cancer-induced hypoxia [28] which could also be involved in ovarian carcinogenesis. Our observation of increased risk is consistent with the minor allele conferring a decrease in NQO1 function.

Strengths of the current approach include the use of three ovarian cancer study populations and examination of risk by alcohol and tobacco use and across histological subtypes. This study is limited by the inclusion of only 11 variants in pooled analysis, the inability to assess generalizability of associations across multiple ethnicities, multiple tests performed, and reduced power in subset and interaction analyses. Although only modest differences in relative risk are conferred by the risk alleles found here, they are consistent with risk estimates at other confirmed loci studied in over 8,000 cases and 8,000 controls [3,12,29]. As we note that a Bonferroni correction for multiple testing would suggest that no association is significant at α=0.05, follow-up in a larger sample will rule out both false positives and false negatives. In conclusion, further examination of *EPHX1* rs1051740, *ADH4* r1042364, and *NQO1* rs291766 in additional study populations and an evaluation of interactions with relevant carcinogenic exposures in larger collections are needed to make more definitive conclusion on the role of inherited variation in xenobiotic metabolism in ovarian cancer etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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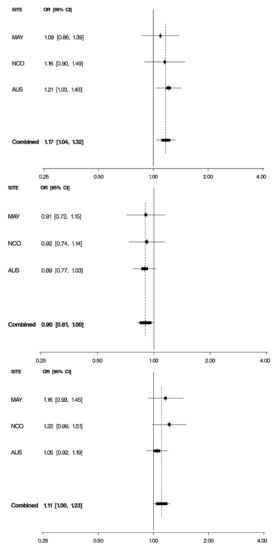

Figure 1. Odds Ratios by Study and Combined

Per-allele odds ratios (ORs) and 95% confidence intervals (CIs) by study and combined, adjusted for age and study site. Boxes indicate ORs and are proportionally sized relative to the number of particpants; horizontal bars represent 95% CIs. A vertical dashed line indicates the combined OR estimate. A. *EPHX1* rs1051740 and risk of invasive serous ovarian cancer. B. *ADH4* rs1042364 and risk of invasive ovarian cancer. C. *NQO1* rs2917666 and risk of invasive ovarian cancer.

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Table 1

Characteristics of Study Participants

		Mayo Clinic Ovarian Cancer Study (MAY)			North Carolina Ovarian Cancer Study (NCO)			Australian Ovarian Cancer Study and Australian Cancer Study, Ovarian (AUS)		
		Cases (N=328)			Cases (N=339)			Cases (N=904)		
Histology	Serous	201 (61.3%)			201 (59.3%)			554 (62.0%)		
	Endometrioid	62 (18.9%)			54 (15.9%)			109 (12.2%)		
	Clear Cell	22 (6.7%)			28 (8.3%)			64 (7.2%)		
	Mucinous	10 (3.0%)			20 (5.9%)			29 (3.2%)		
	Mixed/Other	33 (10.1%)			36 (10.6%)			138 (15.4%)		
	Unknown	0			0			10		
Stage	I	56 (17.2%)			81 (24.3%)			151 (18.4%)		
	II	22 (6.7%)			31 (9.3%)			72 (8.8%)		
	III	192 (58.9%)			207 (62.2%)			523 (63.7%)		
	IV	56 (17.2%)			14 (4.2%)			75 (9.1%)		
	Unknown	2			6			83		
		Cases (N=328)	**Controls (N=462)**	**p-value**	**Cases (N=339)**	**Controls (N=479)**	**p-value**	**Cases (N=904)**	**Controls (N=1,105)**	**p-value**
Age	Mean (S.D.)	61 (12.61)	60 (13.02)	0.31	56.8 (10.55)	54.8 (11.97)	**0.01**	59.2 (10.69)	57.3 (11.64)	**<0.001**
Age at Menarche	<12	45 (18.3%)	67 (15.8%)	0.67	84 (24.9%)	85 (17.7%)	0.07	136 (15.0%)	193 (17.5%)	0.29
	12	62 (25.2%)	97 (22.9%)		99 (29.3%)	143 (29.9%)		207 (22.9%)	229 (20.7%)	
	13	65 (26.4%)	124 (29.2%)		81 (24.0%)	139 (29.0%)		227 (25.1%)	294 (26.6%)	
	≥14	74 (30.1%)	136 (32.1%)		74 (21.9%)	112 (23.4%)		334 (36.9%)	389 (35.2%)	
	Unknown	82	38		1	0		0	0	
Oral contraceptive use	Never	154 (49.7%)	164 (38.6%)	**<0.001**	115 (34.6%)	147 (30.9%)	0.15	288 (33.1%)	219 (19.9%)	**<0.001**
	1–48 months	78 (25.2%)	90 (21.2%)		104 (31.3%)	134 (28.2%)		239 (27.4%)	254 (23.1%)	
	>48 months	78 (25.2%)	171 (40.2%)		113 (34.0%)	194 (40.8%)		344 (39.5%)	627 (57.0%)	
	Unknown	18	37		7	4		33	5	
Number of live births	Nulliparous	55 (17.2%)	64 (14.7%)	0.61	69 (20.4%)	62 (12.9%)	**0.02**	156 (17.7%)	118 (10.7%)	**<0.001**
	1–2	112 (35.1%)	153 (35.2%)		170 (50.1%)	268 (55.9%)		351 (39.9%)	459 (41.5%)	

		Mayo Clinic Ovarian Cancer Study (MAY)			North Carolina Ovarian Cancer Study (NCO)			Australian Ovarian Cancer Study and Australian Cancer Study, Ovarian (AUS)		
		Cases (N=328)			Cases (N=339)			Cases (N=904)		
	≥3	152 (47.6%)	218 (50.1%)		100 (29.5%)	149 (31.1%)		372 (42.3%)	528 (47.8%)	
	Unknown	9	27		0	0		25	0	
Alcohol Use	Never	51 (27.9%)	55 (12.6%)	**<0.001**	137 (40.4%)	171 (35.7%)	0.35	188 (25.3%)	195 (17.8%)	**<0.001**
	Monthly	87 (47.5%)	172 (39.5%)		102 (30.1%)	149 (31.1%)		49 (6.6%)	66 (6.0%)	
	Weekly/Daily	45 (24.6%)	208 (47.8%)		100 (29.5%)	159 (33.2%)		507 (68.1%)	837 (76.2%)	
	Unknown	145	27		0	0		160	7	
Smoking status	Never	200 (64.7%)	279 (64.3%)	0.23	185 (54.6%)	239 (49.9%)	**<0.001**	474 (58.9%)	659 (59.7%)	**0.02**
	Former	83 (26.9%)	131 (30.2%)		125 (36.9%)	153 (31.9%)		214 (26.6%)	330 (29.9%)	
	Current	26 (8.4%)	24 (5.5%)		29 (8.6%)	87 (18.2%)		117 (14.5%)	115 (10.4%)	
	Unknown	19	28		0	0		99	1	
Smoking, pack- Years	None	200 (65.8%)	279 (68.0%)	0.34	188 (57.8%)	248 (54.0%)	0.56	474 (59%)	659 (59.7%)	0.45
	≤20 yrs	57 (18.8%)	83 (20.2%)		79 (24.3%)	120 (26.1%)		214 (26.7%)	308 (27.9%)	
	>20 yrs	47 (15.5%)	48 (11.7%)		58 (17.8%)	91 (19.8%)		115 (14.3%)	137 (12.4%)	
	Unknown	24	52		14	20		101	1	

Includes data from white non-Hispanics and invasive ovarian cancer cases only; data are counts (percentage) unless otherwise indicated; p-values are from within-sites tests of case-control differences; continuous variables (t-test) and categorical variables (Chi square test); bold indicates site-specific case-control p-value < 0.05; covariates used in adjusted logistic regression models included age and study site for combined analysis of MAY+NCO+AUS and race, age, study site, body mass index, hormone therapy use, oral contraceptive use, parity, and age at first birth for initial MAY+NCO analyses..

Table 2

SNPs and Risk of Epithelial Ovarian Cancer

				All Sub-types (1,571 cases, 2,046 controls)		Serous Sub-type (951 cases, 2,046 controls)	
Gene	**SNP**	**Location**	**Alleles**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
EPHX1	rs1051740	ns Y113H	T>C	**1.12 (1.01,1.24)**	**0.03**	**1.17 (1.04,1.32)**	**0.01**
ADH4	rs1042364	3′ UTR	G>A	**0.90 (0.81,1.00)**	**0.05**	0.91 (0.81,1.04)	0.16
ADH1A	rs2276332	intron	T>G	1.14 (0.96,1.36)	0.12	1.11 (0.91,1.36)	0.30
	rs13134764	5′ (31)	T>A	0.96 (0.88,1.05)	0.37	1.00 (0.90,1.11)	0.98
NQO2	rs927340	3′ (3,724)	G>A	0.90 (0.80,1.02)	0.10	0.92 (0.80,1.06)	0.27
NAT2	rs2410556	intron	T>C	1.00 (0.89,1.14)	0.95	0.98 (0.85,1.14)	0.80
GSTP1	rs17593068	5′ (354)	C>A	0.96 (0.86,1.07)	0.45	1.03 (0.90,1.16)	0.69
CYP1A1	rs4646421	intron	G>A	0.96 (0.82,1.13)	0.64	0.96 (0.80,1.15)	0.67
	rs2470893	5′ (1,572)	C>T	1.06 (0.96,1.18)	0.23	1.01 (0.89,1.13)	0.91
NQO1	rs1800566	ns P187S	G>A	1.08 (0.96,1.22)	0.22	1.02 (0.88,1.18)	0.80
	rs2917666	5′ (3,427)	C>G	**1.11 (1.00,1.23)**	**0.04**	1.06 (0.94,1.19)	0.32

White non-Hispanic participants from MAY, NCO, and AUS; position from genome build 36.3; Refseq release 29 (May 4, 2008); ns indicates non-synonymous SNP, other indications represent location and distance in base-pairs from gene; alleles represents major>minor; per-allele (ordinal) odds ratio and 95% confidence intervals adjusted for age and study site; *GSTP1* rs17593068 failed for NCO samples; bold indicates p-value < 0.05.