

Original Article

A polymorphism in the *GALNT2* gene and ovarian cancer risk in four population based case-control studies

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Received June 1, 2010; accepted July 18, 2010; available online July 26, 2010

Abstract: Recent epidemiologic evidence supports a role for MUC1 in ovarian carcinogenesis; therefore, we hypothesized that common genetic variation in the genes responsible for glycosylation of MUC1 may influence ovarian cancer risk. In a genome-wide association study of ovarian cancer, we observed an association between a non-synonymous SNP (rs2271077) in the *UDP-N-acetyl-alpha-d-galactosamine: polypeptide N-acetylglucosaminyltransferase 2* (*GALNT2*) gene and ovarian cancer risk ($p=0.005$). We sought to validate the association in four population based ovarian cancer case-control studies collaborating through the Ovarian Cancer Association Consortium. Although rs2271077 was associated with a significantly increased risk (Odds Ratio (OR) = 1.37, 95% Confidence Interval (CI) = 1.06-1.77) in one study with 961 cases and 922 controls, we observed no association in the remaining three studies including 1452 cases and 1954 controls (OR=0.83, 95% CI= 0.66-1.04). Therefore, there appears to be no strong evidence of association between *GALNT2* SNP rs2271077 and ovarian cancer risk.

Keywords: Ovarian cancer, MUC1, polymorphism

Introduction

Human mucin (MUC) family member MUC1 is a high molecular weight protein normally present in a glycosylated, membrane-bound form on the apical side of most polarized epithelial cells of the respiratory, genitourinary, and digestive tracts, as well as breast ducts [1]. With malignant transformation in those tissues, epithelial cells lose polarity and overexpress a less glycosylated form of MUC1 on their entire cell surface [2], which is recognized by the immune system [3, 4]. We recently demonstrated that anti-MUC1 antibodies are positively correlated with exposures that decrease ovarian cancer risk [5] and are found at lower levels in the pre-diagnostic serum of ovarian cancer cases than

matched controls, particularly for women less than 64 years old [6]. Thus, a natural immunity to MUC1 may offer some reduction in ovarian cancer risk. Glycosylation of MUC1, which determines how much of the antigenic MUC1 core is revealed to the immune system, is regulated by the GalNAc-transferases, specifically *GALNT1*, *GALNT2*, and *GALNT3* [7]. Consequently, genetic variation in one of these genes may influence MUC1 immunity. In an investigation of 26 glycosylation-associated genes and ovarian cancer using 829 cases and 939 controls, Sellers and colleagues observed that SNP rs17647532 in *GALNT1* was significantly associated with decreased risk ($p=0.0002$) [8].

Based on Sellers' observation we sought ge-

netic information about the GALNTs from other sources. In conjunction with the National Institute of Aging and Dr. Michael Birrer of the National Cancer Institute (Cell and Cancer Biology Branch), we completed the first phase of a small genome-wide association study, involving 317,508 SNPs evaluated by the Illumina HumanHap 300 Beadchip. The full study will be described in a subsequent publication, but one of the top non-synonymous "hits" in this study was a *GALNT2* SNP rs2271077 (per allele OR=3.6, 95% CI=1.4-9.45, $p=0.005$). Given the previous observation [8] and our prior hypothesis regarding MUC1 immunity and ovarian cancer, we sought to validate the association between rs2211077 and ovarian cancer (OMIM #167000) in four studies (New England Case Control Study, Hawaii Ovarian Cancer Study, Studies of Epidemiology and Risk Factors in Cancer Heredity Ovarian Cancer Study, United Kingdom Ovarian Cancer Population Study), collaborating through the Ovarian Cancer Association Consortium.

Materials and methods

Study populations

The New England Case-Control Study (NEC), Hawaii Ovarian Cancer Study (HAW), Studies of Epidemiology and Risk Factors in Cancer Heredity Ovarian Cancer Study (SEA), and United Kingdom Ovarian Cancer Population Study (UKO) have been described previously [9-11] and are summarized in (Table 1). Briefly, NEC is a population-based case control study with cases identified through hospital tumor boards and cancer registries and controls identified through town-books (population lists) since 1992 in eastern Massachusetts and driver license lists in New Hampshire [11]. HAW is a population-based study with ovarian cancer cases 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, and controls randomly selected from an annual survey conducted by the Hawaii Department of Health since 1993 [9]. SEA is a population-based study with cases less than 70 years old identified from the East Anglian, West Midlands, and Trent regions of England starting in 1991. Controls were selected from the EPIC-Norfolk cohort of 25,000 individuals aged 45-74, based in the same geographical region as the cases [10]. For the UKO study, cases were identified from 2006 onwards through ten major Gynecologic Oncology National Health Service centers in England, Wales, and Northern Ireland. Controls are women ages 50 to 74 from the general population participating in the United Kingdom Collaborative Trial of Ovarian Cancer Screening [10]. Investigators for each study obtained Institutional Review Board (IRB) approval from their institution. In addition, investigators from University of Southern California and Duke obtained approval from their IRBs to serve as data coordinating centers for Ovarian Cancer Association Consortium (OCAC). All study participants signed informed consent.

Gentotyping and quality control

GALNT2 SNP rs2271077 was gentotyped in 1175 cases and 1201 controls from NEC and subsequently gentotyped in 4285 samples from HAW (365 cases, 602 controls), SEA (1178 cases, 1235 controls) and UKO (302 cases, 603 controls), using the Taqman allelic discrimination assay (Taqman; Applied Biosystems, Foster City, CA) at each study site. For quality assurance, a standardized plate by Coriell (HAPMAPPT01 panel of CEPH-Utah trios) that includes 90 unique DNA samples and five duplicate samples, and a negative template control was genotyped for the rs2271077 SNP

Table 1. Characteristics of participating studies

Study abbreviation	Study name	Cases	Controls	Source population	Participation %
NEC	New England Case Control Study	1175	1201	Population-based; New England, USA	Cases: 72% Controls: 69%
HAW	Hawaii Ovarian Cancer Study	365	602	Population-based; Hawaii, USA	Cases: 66% Controls: 69%
SEA	Studies of Epidemiology and Risk Factors in Cancer Heredity	1178	1235	Population-based; England	Cases: 67% Controls: 84%
UKO	United Kingdom Ovarian Cancer Population Study	302	603	Population-based; England	Cases: 86% Controls: 97%

Table 2. Association between GALNT2 SNP rs2271077 and ovarian cancer in NEC, HAW, SEA, UKO¹

Study	CC		CT/TT		MAF ²	OR (95% CI) ³
	cases n (%)	controls n (%)	cases n (%)	controls n (%)		
Discovery						
NEC	810 (84)	873 (88)	151 (16)	119 (12)	0.06	1.37 (1.06, 1.77)
Replication						
HAW	77 (88)	133 (86)	11 (13)	22 (14)	0.07	0.84 (0.38, 1.83)
SEA	989 (91)	1091 (90)	99 (9)	125 (10)	0.05	0.87 (0.66, 1.15)
UKO	250 (91)	516 (89)	26 (9)	67 (11)	0.06	0.70 (0.43, 1.15)

¹ Restricted to white non-Hispanics

² Minor allele frequencies among white non-Hispanic controls.

³ Age adjusted odd ratio and 95% confidence interval comparing variant carriers to wild type.

at all laboratories. Based on these plates, concordance across studies was >99%. In addition, each study included its own duplicate samples. Within study concordance of duplicate samples was >97% for all studies. Observed genotype frequencies did not differ significantly from expected frequencies under Hardy-Weinberg Equilibrium (HWE). One NEC plate (n=282 samples) with <95% genotyping success was excluded from the analyses. Otherwise, genotyping success was $\geq 97\%$.

ELISA assay for anti-MUC1 antibodies

Previously, anti-MUC1 antibodies were measured in plasma specimens from 705 controls from the NEC study [5]. Antibodies were measured against a synthetic 100-mer MUC1 peptide corresponding to five tandem repeats of the MUC1 polypeptide core, according to a previously published protocol [12]. The absorbance at 405 to 410 nm was measured using the MRX Revelation plate reader (Thermo Labsystems, Chantilly, VA). Non-specific binding was identified by comparing absorbance values in the MUC1-coated plate to values in the antigen-negative plates. Absorbance reactions at the 1:20 dilution less than 0.6 were scored as negative. Twenty blinded replicates had a strong correlation in absorbance values ($r=0.93$, $p<0.0001$).

Statistical analysis

We calculated odds ratios using unconditional logistic regression adjusted for age at diagnosis or interview. We used a dominant model for genotypes since there were no homozygous variant cases or controls in some studies.

Twelve women (4 from SEA, 8 from UKO) were excluded from the analyses due to missing age. We restricted the analysis to non-Hispanic Whites, because we observed significant differences in the genotype distribution by race ($p<0.0001$). Pooled estimates were calculated using inverse-variance weighted random effects models. Heterogeneity between studies was assessed using the Q statistic. In the subset of NEC controls with anti-MUC1 antibody measurements, we used logistic regression to compare those with an antibody reaction at any level against those considered negative for MUC1 (<0.6), while adjusting for age. Statistical analyses were performed using SAS Version 9.1 (SAS Inc., Cary, NC) and Intercooled Stata 9.0 (StataCorp LP, College Station, TX).

Results

The initial replication of the GALNT2 rs2271077 association with ovarian cancer was performed in the NEC study including successful genotype data on 961 non-Hispanic White ovarian cancer cases (532 serous, 116 mucinous, 135 endometrioid, 121 clear cell, 57 other/undifferentiated) and 922 non-Hispanic White controls. The mean ages were 51.7 years in cases and 50.9 years in controls. We observed a higher frequency of women carrying the rs2271077 variant in cases (16%) than controls (12%), resulting in a 37% increase in ovarian cancer risk (Table 2). The risk associated with rs2271077 appeared to be restricted to women with no family history of breast or ovarian cancer (OR=1.58, 95% CI: 1.17-2.13 in women with no family history; OR=0.85, 95% CI: 0.51-1.43 in women with a family history; $p_{\text{heterogeneity}}=0.04$). We observed no association between

Table 3. Genotype distribution and minor allele frequency of *GALNT2* SNP rs2271077 by study*

GALNT2 (rs2271077)	NEC		HAW		SEA		UKO	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
CC	810 (84)	873 (88)	77 (88)	133 (86)	989 (91)	1091 (90)	250 (91)	516 (89)
CT	141 (15)	119 (12)	9 (10)	21 (14)	98 (9)	124 (10)	26 (9)	64 (11)
TT	10 (1)	0 (0)	2 (2)	1 (1)	1 (<1)	1 (<1)	0 (0)	3 (<1)

rs2271077 and presence of anti-MUC1 antibodies among the 608 NEC controls with both genotype data and antibody measurements (OR=1.05, 95% CI=0.62-1.79).

Our validation set included successful genotype data on 1452 non-Hispanic White ovarian cancer cases (606 serous, 242 mucinous, 213 endometrioid, 115 clear cell, and 276 other/undifferentiated) and 1954 non-Hispanic White controls from three studies (HAW, SEA, UKO). The mean ages were 56.2 in cases and 58.1 in controls. Minor allele frequencies among controls in the validation set were similar to the NEC study (NEC 6%, HAW 7%, SEA 5%, UKO 6%) but we observed no significant associations (Table 3) between rs2271077 and ovarian cancer risk in these replication studies (Table 2). We observed no significant heterogeneity between the estimates from HAW, SEA, and UKO (pooled OR=0.82, 95 % CI=0.63-1.02, $p_{\text{heterogeneity}}=0.75$). However, the inclusion of NEC data in led to significant heterogeneity (pooled OR=0.95, 95% CI= 0.78-1.12, $p_{\text{heterogeneity}}=0.046$) (Figure 1). We observed no associations with specific ovarian cancer histologic categories or interactions with age, parity, oral contraceptive use, or tubal ligation in the pooled analysis (data not shown).

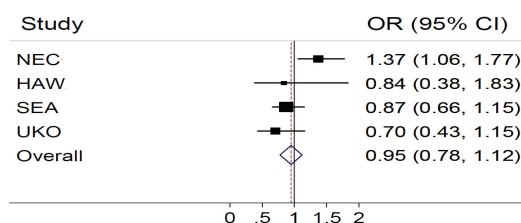


Figure 1. Forest plot for study specific odds ratios and 95% confidence intervals for the association between *GALNT2* SNP rs2271077 (dominant model) and ovarian cancer risk in four studies, restricted to white non-Hispanics.

Discussion

Given our interest in MUC1[5, 13] and a recent report of a significant decreased ovarian cancer risk with SNP rs17647532 in *GALNT1* (OR=0.07, 95% CI 0.01-0.53) [8] which subsequently failed to be replicated in a larger dataset [14], we sought to validate a “hit” in the *GALNT2* gene from the initial phase of genome-wide association study (manuscript in preparation) in 2413 cases and 2876 controls.

Although the association between *GALNT2* SNP rs2271077 and ovarian cancer was initially validated in a single population-based case-control study (NEC), we were unable to replicate this result in three additional case-control studies (HAW, SEA, UKO). We restricted our analyses to non-Hispanic whites to minimize differences in study populations; however, differences may remain that could potentially lead to variable results between studies. For instance, in the NEC study we observed that the association between rs2271077 was not relevant to women with a family history of breast or ovarian cancer. The evaluation of this polymorphism by reproductive characteristics and age showed no significant differences in the pooled data but other not yet identified population characteristics may be relevant. Furthermore, differences in ovarian carcinogenesis by histologic categories may obscure genetic associations. For instance, the most significant SNP (rs3814113, located near *BNC2*) in a recent GWAS study of ovarian cancer was strongly associated with serous ($p_{\text{trend}} = 4.1 \times 10^{-21}$) and less so with endometrioid ($p_{\text{trend}} = 0.001$) ovarian cancer but not associated with mucinous or clear cell ovarian cancer [15]. Although we observed no significant associations between rs2271077 and histologic categories of ovarian cancer, power to detect a significant association is diminished for any given subgroup. Given the small size of the

original genome-wide study used to identify the GALNT2 SNP rs2271077 and the low frequency of the variant, the initial observation was likely due to chance. Initial false positive results are common in polymorphism studies [16-18], and studies like this one highlight the value of validating promising results in independent study populations.

The GALNT2 gene, along with GALNT1 and GALNT3, is responsible for glycosylation of the human tumor antigen MUC1 [7]. The degree of glycosylation determines how much of the antigenic MUC1 core is exposed to the immune system and may be important in the generation of anti-MUC1 antibodies. Interestingly, lifetime events which predict anti-MUC1 antibodies, like oral contraceptive use, breast mastitis, and bone fracture, have been associated with decreased ovarian cancer risk [5], suggesting an immune response to MUC1 may play a role in ovarian carcinogenesis. Furthermore, serum measurements of anti-MUC1 antibodies from prospectively collected blood samples are inversely correlated with ovarian cancer risk. Among women less than 64 years of age, those with antibodies above the 25th percentile had a relative risk of 0.48 (95% CI= 0.28-0.81), suggesting the development of anti-MUC1 antibodies reduces ovarian cancer risk [6].

Sellers and colleagues evaluated genetic variation in 26 genes involved in the glycosylation process, including GALNT2 [8]. In the GALNT2 gene, only rs3213495 was significantly associated with ovarian cancer risk ($OR_{\text{additive}} = 0.62$, 95% CI = 0.44-0.87). The GALNT2 SNP rs2270177 was not genotyped as a part of this effort (personal communication, T. Sellers, March 17, 2010). Our observations suggest that rs2270177 is not associated with ovarian cancer risk. However, other common variants and rare variants (with minor allele frequency less than 5%) may be associated with ovarian cancer risk. Even genome-wide association studies that include thousands of participants are limited to the detection of common variants and can miss causal genetic associations [19]. Deep sequencing efforts, like the 1000 Genomes Project, are required to identify rarer SNPs [20].

In conclusion, we were not able to validate the original observation of an increased risk of ovarian cancer associated with GALNT2 SNP

rs2271077, suggesting that it is not a key determinant of MUC1 immunity; however, other genetic and environmental exposures potentially relevant to mucin immunity warrant further research.

Acknowledgements

This research was supported by National Institutes of Health grants R01 CA54419, P50 105009, R01-CA-58598 and by contracts N01-CN-67001 and N01-CN-25403. Some of the work in this study was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. We are grateful to the family and friends of Kathryn Sladek Smith for their generous support of OCAC through their donations to the Ovarian Cancer Research Fund.

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