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Association between SNPs in Wnt signaling pathway genes and ovarian cancer risk in Northern Chinese population

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Summary

Objective: Wnt signaling pathway is important for tumorigenesis, due to its regulation of many critical biological processes, while the association between SNPs in Wnt pathway genes and ovarian cancer risk has not been established in Chinese, the authors performed a large case control study to analyze this association. *Materials and Methods:* A case control study was designed including 732 ovarian cancer cases and 765 controls. A total of six SNPs in five core genes in Wnt pathway were genotyped in all the samples. Logistic regression analysis was performed to evaluate the association between SNPs and ovarian cancer risk, odds ratios (ORs), and 95% confidence intervals (CIs) were estimated. *Results:* In the univariate analysis, among the six SNPs, the authors found two SNPs significantly associated with ovarian cancer risk. One is SNP rs4135385 in β-catenin gene, compared with AA genotype, GG genotype was associated with a significant lower risk of ovarian cancer, OR=0.62; 95% CI (0.45, 0.87). The other is SNP rs6485350 in DKK3 gene, compared with GG genotype, AA genotype was associated with a significant lower risk of ovarian cancer, OR=0.73; 95% CI (0.55, 0.98). In the multivariate analysis adjusting for common demographic variable, the authors found SNP rs4135385 in β-catenin gene significantly associated with ovarian cancer risk, Compared with AA genotype, GG genotype was associated with a significant lower risk of ovarian cancer, OR=0.57; 95% CI (0.40, 0.79), $p = 0.006$. After Bonferroni's correction for six SNPs, this SNP rs4135385 was still significantly associated with ovarian cancer risk. *Conclusion:* In this case control study, the authors found significant association between SNP in β-catenin gene and ovarian cancer risk in Northern Chinese and further studies are warranted to validate their finding and investigate the mechanism for the association.

Key words: Ovarian cancer; Case control study; Wnt pathway; β-catenin.

Introduction

Ovarian cancer is one of the most common cancers among women and the leading cause of deaths from gynecological malignancies in the world [1]. In addition to BRCA1 and BRCA2 mutations, there are other kinds of genetic risk factors including common genetic variants of lower penetrance [2, 3]. Molecular epidemiological studies have been conducted with the candidate gene approach to identify low penetrance susceptibility genes for ovarian cancer, many of which have showed important results [4-11].

The Wnt pathway participates in many physiologic events in embryogenesis and adult homeostasis including cell fate specification, control of proliferation, and migration. Wnt signaling plays a key role in the embryonic development of the ovary [12, 13] and is also involved in normal follicular development and ovarian function [14]. The Wnt signaling pathway has been well-studied in a number of cancers, in the majority of cancers, the mutational inactivation of adenomatous polyposis coli (APC), an important component in the Wnt pathway, is an early event [15]. This leads to the stabilization of the cytoplasmic pool of β-catenin leading to its accumulation and translocation to the nucleus where it associates with T-cell factor/lymphoid enhancer factor-1 (TCF/LEF1) and promotes transcription of target genes. Some cancers exhibit constitutive β-catenin/TCF transcriptional activity despite the lack of an inactivating APC mutation. This has been shown to result from activating β-catenin gene mutations [16]. Thus, an inactivating APC gene mutation approximates an activating β-catenin gene mutation: both lesions finally lead to the initiation of constitutive β-catenin/TCF-mediated transcription and cancer progression.

In the late 1990s, several authors began to detect the presence of β-catenin gene mutations in ovarian cancer, mostly confined to the endometrioid subtype [17-20]. These findings led to interest in whether Wnt signaling played a role in ovarian tumorigenesis. Since then, compelling data have emerged implicating components of the Wnt signaling pathway in the molecular events that lead to ovarian cancer development, despite the fact that gene mutations are uncommon. Unlike colorectal cancer, a clear-cut, obvious etiologic role such as that involving APC has, to date, not been discovered. Instead, the evidence suggests that Wnt signaling is probably involved via multiple and diverse mecha-

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Table 1. — *SNPs in the Wnt signaling pathway genes included in this study.*

Gene	SNP	Chromosome	Location	Variation	MAF
APC	rs454886	5	112146117	C>T	0.35
β-catenin	rs4135385	3	41237949	A>G	0.23
AXIN2	rs4791171	17	63541497	A>G	0.45
AXIN2	rs11079571	17	63548681	G>A	0.27
DKK3	rs6485350	11	12013617	G>A	0.46
LRP6	rs2284396	12	12274935	C>T	0.47

nisms. In this case-control study, the authors genotyped the SNPs in Wnt signaling pathway genes, and analyzed their association with ovarian cancer risk in Northern Chinese population.

Materials and Methods

Study population

A total of 1,497 blood samples were obtained from Tianjin Medical University Cancer Institute and Hospital, Mudanjiang Medical School, and Haerbin Medical University. These encompassed 732 patients with ovarian cancer and 765 controls with no history of cancer. Ovarian cancer cases included patients of all age (median age = 50 years) and stages of the disease. The patient and control population were from Northern China. All controls were age-matched and recruited from physical examinations after diagnostic exclusion of cancer and cancer related diseases. Blood samples of the cases were obtained before the start of any treatment.

DNA extraction

Approximately five ml of blood samples were collected in vacutainers containing ethylenediaminetetraacetic acid (EDTA) from all subjects enrolled in the study. Genomic DNA was isolated from blood samples using DNA blood mini kit following the manufacturer's instructions. After extraction and purification, the DNA was quantitated spectrophotometrically on NanoDrop 8000, and its purity examined using standard A260/A280 and A260/A230 ratios.

SNP selection and genotyping

Common SNPs in important genes in the Wnt signaling pathway were selected from SNP500 cancer project and from previous literature. A total of six SNPs in Wnt pathway genes were genotyped using TaqMan allelic discrimination assay (Table 1). For each sample, 20 ng DNA per reaction was used with 5.6 ml of 2X Universal Master Mix and 200 nM primers. All genotypes were determined by endpoint reading on a 7500 real time PCR instrument. Primers and probe mix were purchased directly through the assays-on-demand service. Five percent of the samples were randomly selected and subjected to repeat analysis as a quality control measure for verification of genotyping procedures. The results were reproducible without any discrepancies.

Statistical analysis

Genotype and allelic frequencies were computed and checked for deviation from Hardy-Weinberg equilibrium in the control group. Case-control genotype comparisons were performed using the chi-square test and odds ratios (OR), and 95% confidence intervals (CI) were calculated by Logistic regression. The authors considered *p*-value of 0.05 as significant. Additionally, Bonferroni's correction was applied for multiple comparison of the six SNPs with an a = 0.0083 considered as significant. All the statistical analysis was done using SPSS 17.0.

Table 2. — *Demographic factors in ovarian cancer cases and controls.*

Variables	N (%)		*p*
	Cases (732)	Controls (765)	
Age (years)			0.83
≤ 50	354 (48.39)	373 (48.74)	
> 50	378 (51.61)	392 (51.26)	
BMI (kg/m^2)			0.21)
< 24	321 (43.89)	299 (39.03)	
≥ 24	411 (56.11)	466 (61.97)	
Age at menarche (years)			0.002
≤ 14	345 (47.14)	256 (33.52)	
> 14	387 (52.86)	509 (66.48)	
Number of live birth			0.001
≤ 2	636 (86.82)	571 (74.66)	
> 2	96 (13.18)	194 (25.34)	
Breast feeding			0.12
No	70 (9.58)	49 (6.46)	
Yes	662 (90.42)	716 (93.54)	
Menopause			0.11
No	345 (47.11)	310 (40.49)	
Yes	387 (52.89)	455 (59.51)	
Smoking			0.001
Never	643 (87.86)	716 (93.61)	
Ever	89 (12.14)	49 (6.39)	
Family history of cancer			0.001
No	514 (70.19)	678 (88.60)	
Yes	218 (29.81)	87 (11.40)	

Results

A total of 732 ovarian cancer cases and 765 controls were included in this analysis, the demographic characteristics of cases and controls are shown in Table 2. Age was matched well between cases and controls, and BMI has no difference between the two groups. We found younger age at menarche, less live birth, higher proportion of smokers, and higher proportion of family history of cancer in ovarian cancer cases than in controls (Table 2). All these variables were used in the multivariate analysis for the association between SNPs and ovarian cancer risk.

The authors selected six common SNPs from five core Wnt signaling pathway genes; they were rs454886 in APC gene, rs4135385 in β-catenin gene, rs4791171 and rs11079571 in AXIN2 gene, rs6485350 in DKK3 gene, and rs2284396 in LRP6 gene. All the six SNPs were in Hardy-Weinberg equilibrium in controls with *p*-values of 0.32, 0.41, 0.77, 0.18, 0.25, and 0.86, respectively. In the univariate analysis, the authors identified two SNPs significantly associated with ovarian cancer risk. One is SNP rs4135385 in β-catenin gene, compared with AA genotype, GG genotype was associated with a significant lower risk of ovarian cancer, OR=0.62; 95% CI (0.45, 0.87). The other

Table 3. — *Logistic Regression analysis for SNPs in the Wnt signaling pathway genes and ovarian cancer risk.*

SNP/gene	Variation	N (%)		OR (95%CI)	OR (95%CI)[a]	*p*
		Control (765)	Case (732)			
rs454886	CC	217 (28.4)	200 (27.4)	1.00	1.00	
APC	CT	383 (50.1)	368 (50.4)	1.04 (0.82, 1.33)	1.09 (0.85, 1.43)	0.733
	TT	165 (21.6)	162 (22.2)	1.07 (0.80, 1.42)	1.11 (0.84, 1.47)	0.669
rs4135385	AA	361 (47.2)	381 (52.0)	1.00	1.00	
β-catenin	AG	299 (39.1)	282 (38.5)	0.89 (0.72, 1.11)	0.88 (0.71, 1.09)	0.310
	GG	105 (13.7)	69 (9.4)	0.62 (0.45, 0.87)	0.57 (0.40, 0.79)	0.006
rs4791171	AA	295 (38.6)	255 (34.8)	1.00	1.00	
AXIN2	AG	357 (46.7)	359 (49.0)	1.16 (0.93, 1.45)	1.12 (0.90, 1.37)	0.183
	GG	113 (14.8)	118 (16.1)	1.21 (0.89, 1.64)	1.18 (0.84, 1.55)	0.229
rs11079571	GG	544 (71.1)	496 (68.0)	1.00	1.00	
AXIN2	GA	194 (25.4)	210 (28.8)	1.19 (0.94, 1.49)	1.23 (0.97, 1.59)	0.144
	AA	27 (3.5)	23 (3.2)	0.93 (0.53, 1.65)	0.96 (0.55, 1.69)	0.815
rs6485350	GG	200 (26.1)	228 (31.1)	1.00	1.00	
DKK3	GA	394 (51.4)	360 (49.2)	0.80 (0.63, 1.02)	0.86 (0.71, 1.10)	0.068
	AA	172 (22.5)	144 (19.7)	0.73 (0.55, 0.98)	0.77 (0.59, 1.01)	0.052
rs2284396	CC	208 (28.0)	197 (28.1)	1.00	1.00	
LRP6	CT	377 (50.7)	346 (49.3)	0.97 (0.76, 1.24)	0.96 (0.74, 1.21)	0.800
	TT	159 (21.4)	159 (22.6)	1.06 (0.79, 1.42)	1.07 (0.81, 1.45)	0.717

[a] Multivariate analysis including age, BMI, age at menarche, number of live birth, breast feeding, menopause, smoking, and family history of cancer.

is SNP rs6485350 in DKK3 gene, compared with GG genotype, AA genotype was associated with a significant lower risk of ovarian cancer, OR=0.73; 95% CI (0.55, 0.98). In the multivariate analysis adjusting for common demographic variable, the authors found SNP rs4135385 in β-catenin gene significantly associated with ovarian cancer risk. Compared with AA genotype, GG genotype was associated with a significant lower risk of ovarian cancer, OR=0.57; 95% CI (0.40, 0.79), $p = 0.006$. After Bonferroni's correction for six SNPs, this SNP rs4135385 was still significantly associated with ovarian cancer risk (Table 3).

Discussion

In this case control study, the authors identified a SNP rs4135385 in the Wnt signaling pathway gene β-catenin significantly associated with ovarian cancer risk in Northern Chinese women. Since its discovery as a protein associated with the cytoplasmic region of E-cadherin, beta-catenin has been shown to perform two apparently unrelated functions: it has a crucial role in cell-cell adhesion in addition to a signaling role as a component of the Wnt/wg pathway. Wnt/wg signaling results in beta-catenin accumulation and transcriptional activation of specific target genes during development. It is now apparent that deregulation of beta-catenin signaling is an important event in the genesis of a number of malignancies, such as colon cancer, melanoma, hepatocellular carcinoma, ovarian cancer, endometrial cancer, medulloblastoma pilomatricomas, and prostate cancer. Beta-catenin mutations appear to be a crucial step in the progression of a subset of these cancers, suggesting an important role in the control of cellular proliferation or cell death.

When β-catenin gene mutations were initially discovered in ovarian cancer and were thought to be limited to the endometrioid subtype [21]. A study by Wu *et al.* carried out a comprehensive molecular analysis of 45 tumor specimens of primary ovarian endometrioid adenocarcinomas and two ovarian endometrioid adenocarcinoma-derived cell lines. They found Wnt/β-catenin pathway defects in both the cell lines, and in nearly half of the primary ovarian endometrioid adenocarcinomas analyzed. β-catenin deregulation was most often attributable to a mutation of the β-catenin gene (CTNNB1) itself, although less frequently β-catenin deregulation may have resulted from inactive mutations in the APC, AXIN1, or AXIN2 genes [22]. Depending on the study, a wide range (3-59%) of serous ovarian cancers have also been reported to stain positive for cytoplasmic and nuclear β-catenin by immunohistochemistry, even in the absence of mutations in APC, Axin or β-catenin, which are more common in the endometrioid subtype [23-25]. More recent data have shown that although gene mutations in the Wnt/β-catenin pathway are relatively uncommon in ovarian cancer in general, especially in serous ovarian cancer, components of the pathway are still important in the molecular events that lead to ovarian cancer development [26]. There are three main Wnt signaling pathways: 1) the canonical Wnt/β-catenin pathway, 2) the non-canonical planar cell polarity pathway, and 3) the non-canonical Wnt-Ca2+ pathway. These pathways belong to one of two categories: canonical or non-canonical. The difference between these two categories is the presence or absence of β-catenin. The canonical Wnt/β-catenin pathway involves this protein and the non-canonical pathway operates independently of it.

Wnt signaling is activated in epithelial ovarian cancer,

both directly through ligand activated upregulation of the pathway and through a ligand independent increase in nuclear β-catenin through cross-talk with other pathways. Recently, Yo *et al.* reported that niclosamide, which has been shown to have anti-Wnt activity, inhibits growth in ovarian tumor-initiating cells [27]. More pre-clinical studies, specifically animal studies and mechanistic studies, are warranted to further investigate other Wnt inhibitors in ovarian cancer. The Wnt pathway is very complex, and further studies with targeted agents need to be performed to assess if inhibition of a single component of the pathway will be clinically useful.

In conclusion, the authors found from this case control, epidemiological evidence for the association between Wnt signaling pathway gene β-catenin and the risk of ovarian cancer. Further studies are warranted to validate their findings and investigate the mechanism of the association.

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