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Original article

Single nucleotide polymorphism of *PIK3CA* and its interaction with the environment are risk factors for Chinese Han ovarian cancer

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ABSTRACT

Background and purpose: The PI3K pathway is an important signaling network that regulates key cell functions such as cell growth, proliferation and survival. *PIK3CA* mutations are found in a variety of human cancers. This study aimed to analyze the effect of interactions between *PIK3CA* rs2699887, rs3976507, rs6443626 single nucleotide polymorphisms (SNPs), and the environment on the risk of Chinese Han ovarian cancer.

Methods: Sanger sequencing was used to analyze the *PIK3CA* rs2699887, rs3976507, rs6443626 genotypes in 350 Chinese Han ovarian cancer patients and 350 control individuals, and the expression of PIK3CA protein was detected in 117 ovarian cancer patients.

Results: In subjects with age ≥60 years, Number liveborn ≤ 3, no smoking, no alcohol, and no family history of ovarian cancer, the risk of ovarian cancer of the rs2699887 T allele carriers were increased (all $p < 0.05$). In subjects with obesity (BMI ≥ 24 kg/m2), Number liveborn ≤3, no smoking, no alcohol, and no family history of ovarian cancer, the risk of ovarian cancer of rs3976507 T allele carriers and rs6443626 C allele carriers were increased (all $p < 0.05$). PIK3CA protein expression level in *PIK3CA* rs2699887 C > T, rs3976507 C > T, rs6443626 T > C locus homozygotes was significantly higher than that in heterozygotes ($p < 0.05$).

Conclusion: Interaction between *PIK3CA* rs2699887 SNP and age, number of liveborn, tobacco, alcohol, a family history of ovarian cancer and other factors are associated with ovarian cancer risk. Interaction between *PIK3CA* rs3976507 and rs6443626 loci, and factors such as BMI, number of liveborn, tobacco, alcohol, and family history of ovarian cancer are associated with ovarian cancer risk.

1. Introduction

Ovarian cancer is a common female reproductive genital tumor and one of the three major malignant gynecological tumors. In recent years, the incidence and mortality of ovarian cancer in Chinese women has increased significantly [1]. Ovarian cancer is a polygenic genetic disease [2,3]. The role of environmental influence in its pathogenesis cannot be ignored [4]. It is very important to prevent and treat ovarian cancer by studying key genes and their interaction with the environment.

PIK3CA encodes a catalytic subunit of phosphatidylinositol 3-kinase a (PI3Kα), which plays a key role in regulating cell proliferation, survival, and movement [5]. PIK3CA is an important functional factor in the PI3K-AKT signaling pathway and is encoded by *PIK3CA*, which is mutated in various human cancers including breast cancer [6], non-small cell lung cancer [7], and colorectal cancer [8], and ovarian cancer [9]. The PIK3CA gene mutation mainly occurs in the kinase domain (H1047R) and the helix domain (E542K or E545K) of p110α, of which H1047R is the most common mutation [10]. These tumor-associated *PIK3CA* mutations result in constitutive activation of p110α and its downstream effector AKT signaling, leading to oncogenic transformation [11]. The *PIK3CA* rs2699887 SNP site is also a sensitive site for disease, and studies have shown that this SNP is associated with a high risk of NSCLC brain metastasis at 24 months of follow-up [9]. The PIK3CA rs3976507 and rs6443626 loci are located in the 3′ UTR, which is a binding site for the regulation of gene expression by microRNAs (miRNAs) [12]. This SNP site is confirmed to affect the regulation of gene expression by miRNAs. The basic information on the PIK3CA rs2699887, rs3976507, rs6443626 loci is shown in Table 1.

This study focuses on the correlation between PIK3CA rs2699887, rs3976507, rs6443626 SNPs and the risk of Chinese Han ovarian cancer, and analyzed the effects of interaction between these SNPs and age, BMI, number of liveborn, smoking, drinking, and family history of ovarian cancer on the risk of ovarian cancer, which provided a basis for

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Table 1
PIK3CA gene SNP site information.

SNP	Chromosome	Location	MAF in Chinese Han population	Variation
rs2699887	3:179148620	Intron	0.0667	C > T
rs3976507	3:179239995	3′ UTR	0.0857	C > T
rs6443626	3:179237995	3′ UTR	0.0857	T > C

the prevention and treatment of clinical ovarian cancer.

2. Information and methods

2.1. General information

All eligible patients (aged 32–85 years, average age 58.6 ± 9.9 years) with ovarian cancer were enrolled from March 2015 to March 2018 in Hangzhou Fuyang Women and Children hospital. All ovarian cancer cases were classified and evaluated according to the American Joint Committee on Cancer (AJCC) and the International Federation of Obstetricians and Gynecologists (FIGO) [13]. The pathological type was diagnosed as epithelial ovarian cancer, germ cell tumor, and gonadal stroma, and the tumor was surgically removed after routine pathological examination or immunohistochemistry. Patients with other organ metastatic cancers and a history of cancer were excluded. According to the age of patients with ovarian cancer (± 5 years old), 350 healthy women were enrolled as the control group, aged 30–87 years, with an average age of 58.2 ± 10.1 years. The clinical data of all subjects are shown in Table 2. The study protocol was approved by the Hangzhou Fuyang Women and Children Hospital Committee and all subjects provided signed informed consent.

Table 2
Demographic characteristics of ovarian cancer patients and the control group.

Variables	Case (n = 350)	Control (n = 350)	*p*
Age(years, mean ± SD)			
< 60	208(59.43%)	210(60.00%)	0.88
≥ 60	142(40.57%)	140(40.00%)	
BMI(kg/m^2, n(%))			
< 24	99(28.29%)	104(29.71%)	0.68
≥ 24	251(71.71%)	246(70.29%)	
Number liveborn[n(%)]			
0	21(6.00%)	24(6.86%)	0.85
1~2	228(65.14%)	230(65.71%)	
≥ 3	101(28.86%)	96(27.43%)	
Tobacco [n(%)]			
Yes	26(7.43%)	22(6.29%)	0.55
No	324(92.57%)	328(93.71%)	
Alcohol [n(%)]			
Yes	34(9.71%)	37(10.57%)	0.71
No	316(90.29%)	313(89.43%)	
Ovarian cancer family history [n(%)]			
Yes	32(9.14%)	8(2.29%)	< 0.001
No	318(90.86%)	342(97.71%)	
FIGO stage			
I	52(14.86%)		
II	66(18.86%)		
III	188(53.71%)		
IV	44(12.57%)		
Tumor grade			
G1	30(8.57%)		
G2	82(23.43%)		
G3	238(68.00%)		

BMI, body mass index. FIGO, International Federation of Gynecology and Obstetrics.

2.2. Genotyping

Peripheral blood (5 mL) from each subject was collected. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Cat no. 51104, Chatsworth, CA, USA) according to the manufacturer's instructions and stored at −80 °C. The genotypes of PIK3CA rs2699887, rs3976507, rs6443626 were analyzed by PCR and Sanger sequencing. We obtained the sequence information of the PIK3CA rs2699887, rs3976507 rs6443626 loci from the NCBI-SNP database (https://www.ncbi.nlm.nih.gov/snp/), and used Primer Blast to design the PCR amplification primers as follows: rs2699887 Fw: 5′-GGG ACC CGA TGC GGT TAG -3′; Rv: 5′-CGA CGC TGG GAG ACG AC-3′ ; rs3976507 Fw: 5′-GAA GTT TGG CCT GTG ACT GC-3′ ; Rv : 5′- CAG CGT GAT GTT ACA GAC TGC-3′ ; rs6443626 Fw : 5′- ACC CAC ATA CTC AAG AGT CCA-3′ ; Rv: 5′- AAC AAC TCC TCC CTG TTC TGC-3′. The PCR mixture contained 100 ng of genomic DNA, 4 µl of 2.5 mM dNTP, 10 µl of PCR buffer, 10 µM of upstream and downstream primers, 1 µl each, 0.5 U of PrimeSTAR HS DNA polymerase (TAKARA, DALIAN, China)in a 50-µl reaction volume. The PCR amplification conditions were: 94 °C, 5 min, 35 cycles; 98 °C, 10 s; 58 °C, 15 s; 72 °C, 2 min, final extension step, 72 °C, 5 min. The PCR products were sequenced by BioSune Biotechnology Co., Ltd (Shanghai, China) and the sequence data were analyzed using Chromas 2.31 software.

2.3. Real-time PCR

We collected a total of 117 cancer tissue samples from patients with ovarian cancer. Tissues from a single patient were divided into two parts, one of which was used to extract RNA with TRIzol Reagent (Invitrogen, Life Technologies, Grand Island, NY, USA), and reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). The relative gene expression of PIK3CA mRNA was quantified using the SYBR Green Assay on the ABI Prism 7500 sequence detection system (Applied Biosystems). The primers used were as follows: PIK3CA Fw: 5′-CCA GAG GGG AAA AAT ATG CAA-3′; Rv: 5′-ACC TGT GAC TCC ATA GAA A-3′; β-actin Fw: 5′-GGC GGC ACC ACC ATG TAC CCT-3′; Rv: 5′- AGG GGC CGG ACT CGT CAT ACT-3′. Three replicate wells were tested for each sample and $2^{-\Delta\Delta CT}$ was used to calculate the expression level of PIK3CA mRNA relative to β-actin.

2.4. Western blot

The remaining tissue samples were lysed in RIPA buffer (1x PBS, 0.1% sodium dodecyl sulfate, 1% NP40, 5 mM EDTA, 1 mM sodium orthovanadate, 0.5% sodium deoxycholate and protease inhibitors). 40 µg of protein was electrophoresed on 15% SDS-PAGE and transferred to PVDF (Millipore) using a Mini Trans-Blot Cell (Bio-Rad Laboratories, Hercules, CA, USA). Immunoblotting was performed using a primary antibody against PIK3CA (ab40776, Abcam, Cambridge, MA, USA) and β-actin (ab8226, Abeam, Cambridge, MA, USA). The secondary antibody (KPL, Gaithersburg, MD, USA) was labeled with HRP (horseradish peroxidase). Membranes were visualized using an ECL kit (Merck, Darmstadt, Germany) with β-actin as the control.

2.5. Statistical analysis

We used SPSS 22.0 (SPSS, Chicago, Illinois, USA) to analyze the statistical data, using mean ± SD to represent continuous variables, and analyzed statistical differences between groups using the *t*-test. We used [n(%)] to represent categorical variables and performed statistical analysis using the chi-square test. The genotype and allele distribution in the ovarian cancer group and the control group were analyzed using the chi-square test, and Fisher's exact test was used with a genotype count < 5. The Hardy-Weinberg equilibrium (HWE) was tested using the goodness of fit $\chi 2$ test. The odds ratio (OR) and 95% confidence

interval (CI) of logistic regression analysis was calculated in two genetic models (dominant and recessive) to estimate the association between variation and ovarian cancer risk, corrected age, BMI, number of live-born, tobacco, alcohol, ovarian cancer family history, and other factors. Gene-gene interactions were analyzed using the multi-factor dimensionality reduction (MDR). All tests were two-tailed and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Demographic characteristics

The demographic characteristics of 350 ovarian cancer patients and 350 control subjects are shown in Table 2. Among the 350 ovarian cancer patients included in the study, 52 were in stage I, 66 in stage II, 188 in stage III, and 44 in stage IV. There was no significant difference in age, BMI, number of liveborn, smoking, and drinking between the ovarian cancer patients and control subjects ($p > 0.05$). The proportion of ovarian cancer family history in ovarian cancer patients was significantly higher than that in the control group ($p < 0.001$).

3.2. Analysis of the relationship between genotype and ovarian cancer risk

The PIK3CA rs2699887, rs3976507, rs6443626 locus genotype was assessed in association with ovarian cancer risk from 350 ovarian cancer patients and 350 control subjects (Table 3). The genotype frequencies of each SNP locus were consistent with the Hardy-Weinberg equilibrium ($p > 0.05$). PIK3CA rs2699887 T-allele (adjusted OR = 1.522, 95% CI: 1.348–1.683, $p < 0.001$), rs3976507 T allele (adjusted OR = 1.312, 95% CI: 1.135–1.483, $p < 0.001$) and the rs6443626 locus C allele (adjusted OR = 1.321, 95% CI: 1.150–1.488, $p < 0.001$) are risk factors for ovarian cancer.

3.3. Stratification analysis of demographic factors

We divided the age into < 60 years old and ≥60 years old, BMI was divided into < 24 kg/m^2 and ≥24 kg/m^2, number of liveborn was divided into ≤3 and > 3, whether smoking (Tobacco), whether drinking, whether there is family history of ovarian cancer, to analyze the relationship between different genotypes of *PIK3CA* rs2699887, rs3976507, rs6443626 and ovarian cancer risk. The results showed that rs2699887 mutation (CT + TT) was only found in subjects aged ≥ 60 years, number liveborn ≤3, non-smoking subjects, subjects with no history of drinking, and subjects without a family history of ovarian cancer. It is a risk factor for ovarian cancer (adjusted OR = 1.341, 95%CI: 1.008–1.681, $p < 0.001$/ adjusted OR = 1.412, 95%CI: 1.190–1.635, $p < 0.001$/ adjusted OR = 1.464, 95%CI: 1.236–1.691, $p < 0.001$/ adjusted OR = 1.494, 95% CI: 1.265–1.721, $p < 0.001$ / adjusted OR = 1.413, 95% CI: 1.181–1.649, $p < 0.001$). Subjects with mutations (CT + TT) in different BMI subjects (< 24 kg/m^2, ≥24 kg/

Table 3
Genotypes of *PIK3CA* rs2699887, rs3976507, rs6443626 loci and risk of ovarian cancer.

SNP	Case (n = 350)	Control (n = 350)	*HWE p*	*p*	Crude OR(95%CI)	*p*	Adjusted OR(95%CI)
rs2699887							
CC	256 (73.14%)	301 (86.00%)	0.127		1.000(reference)		
CT	57 (16.29%)	45 (12.86%)		0.065	1.489(0.953-2.328)	0.082	1.216(0.975-1.466)
TT	37 (10.57%)	4 (1.14%)		**< 0.001**	10.876(3.643-36.437)	**< 0.001**	1.964(1.625-2.129)
Dominant model				**< 0.001**	2.256(1.511-3.372)	**< 0.001**	1.430(1.214-1.647)
Recessive model				**< 0.001**	10.225(3.435-34.175)	**< 0.001**	1.900(1.577-2.056)
C	569 (81.29%)	647 (92.43%)			1.000(reference)		
T	131 (18.71%)	53 (7.57%)		**< 0.001**	2.811(1.978-4.000)	**< 0.001**	1.522(1.348-1.683)
rs3976507							
CC	265 (75.71%)	294 (84.00%)	0.116		1.000(reference)		
CT	65 (18.57%)	51 (14.57%)		0.091	1.414(0.928-2.157)	0.112	1.182(0.962-1.411)
TT	20 (5.71%)	5 (1.43%)		**0.001**	4.438(1.546-13.697)	**0.003**	1.688(1.223-1.971)
Dominant model				**0.006**	1.684(1.137-2.496)	**0.008**	1.272(1.065-1.484)
Recessive model				**0.002**	4.182(1.462-12.871)	**0.004**	1.636(1.189-1.907)
C	595 (85.00%)	639 (91.29%)			1.000(reference)		
T	105 (15.00%)	61 (8.71%)		**< 0.001**	1.849(1.306-2.620)	**< 0.001**	1.312(1.135-1.483)
rs6443626							
TT	265 (75.71%)	289 (82.57%)	0.084		1.000(reference)		
TC	54 (15.43%)	55 (15.71%)		0.744	1.071(0.696-1.648)	0.825	1.036(0.819-1.269)
CC	31 (8.86%)	6 (1.71%)		**< 0.001**	5.635(2.200-15.295)	**< 0.001**	1.752(1.386-1.974)
Dominant model				**0.026**	1.520(1.034-2.235)	**0.032**	1.217(1.017-1.426)
Recessive model				**< 0.001**	5.572(2.183-15.079)	**< 0.001**	1.741(1.382-1.958)
T	584 (83.43%)	633 (90.43%)			1.000(reference)		
C	116 (16.57%)	67 (9.57%)		**< 0.001**	1.877(1.345-2.621)	**< 0.001**	1.321(1.150-1.488)

HWE, Hardy-Weinberg equilibrium. OD, odds ratio. CI, Confidence interval.

Table 4
Correlation between *PIK3CA* rs2699887 locus and demographic characteristics.

Variables	Case (n = 350)	Control (n = 350)	*p*	Crude OR(95%CI)	*p*	Adjusted OR(95%CI)
Age(years, mean ± SD)						
< 60						
CC	150 (72.12%)	182 (86.67%)		1.000(reference)		
CT + TT	58 (27.88%)	82 (39.05%)	0.453	0.858(0.564-1.305)	0.517	0.917(0.714-1.157)
≥ 60						
CC	106 (74.65%)	119 (85.00%)		1.000(reference)		
CT + TT	36 (25.35%)	21 (15.00%)	**0.030**	1.925(1.017-3.659)	**0.044**	1.341(1.008-1.681)
BMI[kg/m2, n(%)]						
< 24						
CC	70 (70.71%)	88 (84.62%)		1.000(reference)		
CT + TT	29 (29.29%)	16 (15.38%)	**0.017**	2.279(1.090-4.797)	**0.027**	1.455(1.045-1.882)
≥ 24						
CC	186 (74.10%)	213 (86.59%)		1.000(reference)		
CT + TT	65 (25.90%)	33 (13.41%)	< **0.001**	2.256(1.386-3.681)	**0.001**	1.423(1.166-1.677)
Number liveborn[n(%)]						
≤ 3						
CC	237 (72.70%)	278 (85.28%)		1.000(reference)		
CT + TT	89 (27.30%)	48 (14.72%)	< **0.001**	2.175(1.444-3.280)	< **0.001**	1.412(1.190-1.635)
> 3						
CC	19 (79.17%)	23 (95.83%)		1.000(reference)		
CT + TT	5 (20.83%)	1 (4.17%)	0.081	6.053(0.585-149.440)	0.190	1.842(0.745-2.306)
Tobacco [n(%)]						
Yes						
CC	21 (80.77%)	18 (81.82%)		1.000(reference)		
CT + TT	5 (19.23%)	4 (18.18%)	0.926	1.071(0.204-5.757)	0.999	1.032(0.396-1.771)
No						
CC	235 (72.53%)	283 (86.28%)		1.000(reference)		
CT + TT	89 (27.47%)	45 (13.72%)	< **0.001**	2.382(1.571-3.618)	< **0.001**	1.464(1.236-1.691)
Alcohol [n(%)]						
Yes						
CC	31 (91.18%)	31 (83.78%)		1.000(reference)		
CT + TT	3 (8.82%)	6 (16.22%)	0.350	0.500(0.089-2.555)	0.563	0.667(0.173-1.503)
No						
CC	225 (71.20%)	270 (86.26%)		1.000(reference)		
CT + TT	91 (28.80%)	43 (13.74%)	< **0.001**	2.540(1.665-3.881)	< **0.001**	1.494(1.265-1.721)
Ovarian cancer family history [n(%)]						
Yes						
CC	21 (65.63%)	8 (100%)	\	\	\	\
CT + TT	11 (34.38%)	0 (0%)	\	\	\	\
No						
CC	235 (73.90%)	293 (85.67%)		1.000(reference)		
CT + TT	83 (26.10%)	49 (14.33%)	< **0.001**	2.112(1.401-3.188)	< **0.001**	1.413(1.181-1.649)

OD, odds ratio. CI, Confidence interval. BMI, body mass index. SD, Standard deviation.

Table 5
Correlation between *PIK3CA* rs3976507 locus and demographic characteristics.

Variables	Case (n = 350)	Control (n = 350)	*p*	Crude OR(95%CI)	*p*	Adjusted OR(95%CI)
Age(years, mean ± SD)						
< 60						
CC	160 (76.92%)	178 (84.76%)		1.000(reference)		
CT + TT	48 (23.08%)	32 (15.24%)	0.042	1.669(0.988-2.823)	0.056	1.268(0.994-1.551)
≥ 60						
CC	105 (73.94%)	116 (82.86%)		1.000(reference)		
CT + TT	37 (26.06%)	24 (17.14%)	0.069	1.703(0.920-3.161)	0.094	1.277(0.959-1.612)
BMI(kg/m^2, n(%))						
< 24						
CC	77 (77.78%)	87 (83.65%)		1.000(reference)		
CT + TT	22 (22.22%)	17 (16.35%)	0.288	1.462(0.685-3.132)	0.377	1.201(0.815-1.624)
≥ 24						
CC	188 (74.90%)	207 (84.15%)		1.000(reference)		
CT + TT	63 (25.10%)	39 (15.85%)	**0.011**	1.779(1.113-2.846)	**0.015**	1.298(1.054-1.549)
Number liveborn[n(%)]						
≤ 3						
CC	248 (76.07%)	277 (84.97%)		1.000(reference)		
CT + TT	78 (23.93%)	49 (15.03%)	**0.004**	1.778(1.174-2.695)	**0.006**	1.300(1.082-1.523)
> 3						
CC	17 (70.83%)	17 (70.83%)		1.000(reference)		
CT + TT	7 (29.17%)	7 (29.17%)	1.000	1.000(0.242-4.127)	1.000	1.000(0.443-1.826)
Tobacco [n(%)]						
Yes						
CC	22 (84.62%)	19 (86.36%)		1.000(reference)		
CT + TT	4 (15.38%)	3 (13.64%)	0.864	1.152(0.182-7.617)	1.000	1.065(0.357-1.811)
No						
CC	243 (75.00%)	275 (83.84%)		1.000(reference)		
CT + TT	81 (25.00%)	53 (16.16%)	**0.005**	1.730(1.154-2.595)	**0.007**	1.289(1.073-1.511)
Alcohol [n(%)]						
Yes						
CC	27 (79.41%)	30 (81.08%)		1.000(reference)		
CT + TT	7 (20.59%)	7 (18.92%)	0.860	1.111(0.299-4.139)	1.000	1.056(0.477-1.801)
No						
CC	238 (75.32%)	264 (84.35%)		1.000(reference)		
CT + TT	78 (24.68%)	49 (15.65%)	**0.005**	1.766(1.164-2.681)	**0.007**	1.295(1.077-1.520)
Ovarian cancer family history [n(%)]						
Yes						
CC	26 (81.25%)	8 (100%)	\	\	\	\
CT + TT	6 (18.75%)	0 (0%)	\	\	\	\
No						
CC	239 (75.16%)	286 (83.63%)		1.000(reference)		
CT + TT	79 (24.84%)	56 (16.37%)	**0.007**	1.688(1.131-2.522)	**0.009**	1.285(1.065-1.516)

OD, odds ratio. CI, Confidence interval. BMI, body mass index. SD, Standard deviation.

Table 6
Correlation between *PIK3CA* rs6443626 locus and demographic characteristics.

Variables	Case (n = 350)	Control (n = 350)	*p*	Crude OR(95%CI)	*p*	Adjusted OR(95%CI)
Age(years, mean ± SD)						
< 60						
TT	164 (78.85%)	176 (83.81%)		1.000(reference)		
TC + CC	44 (21.15%)	34 (16.19%)	0.193	1.389(0.822-2.349)	0.239	1.169(0.904-1.451)
≥ 60						
TT	101 (71.13%)	113 (80.71%)		1.000(reference)		
TC + CC	41 (28.87%)	27 (19.29%)	0.060	1.699(0.941-3.071)	0.081	1.278(0.970-1.609)
BMI(kg/m^2, n(%))						
< 24						
CC	76 (76.77%)	80 (76.92%)		1.000(reference)		
CT + TT	23 (23.23%)	24 (23.08%)	0.979	1.009(0.500-2.036)	1.000	1.004(0.678-1.391)
≥ 24						
TT	189 (75.30%)	209 (84.96%)		1.000(reference)		
TC + CC	62 (24.70%)	37 (15.04%)	**0.007**	1.853(1.151-2.988)	**0.010**	1.319(1.071-1.571)
Number liveborn[n(%)]						
≤ 3						
TT	243 (74.54%)	269 (82.52%)		1.000(reference)		
TC + CC	83 (25.46%)	57 (17.48%)	**0.013**	1.612(1.084-2.398)	**0.017**	1.249(1.041-1.466)
> 3						
TT	22 (91.67%)	20 (83.33%)		1.000(reference)		
TC + CC	2 (8.33%)	4 (16.67%)	0.383	0.455(0.051-3.402)	0.663	0.636(0.109-1.608)
Tobacco [n(%)]						
Yes						
TT	22 (84.62%)	19 (86.36%)		1.000(reference)		
TC + CC	4 (15.38%)	3 (13.64%)	0.864	1.152(0.182-7.617)	1.000	1.065(0.357-1.811)
No						
TT	243 (75.00%)	270 (82.32%)		1.000(reference)		
TC + CC	81 (25.00%)	58 (17.68%)	**0.023**	1.552(1.044-2.308)	**0.029**	1.230(1.022-1.449)
Alcohol [n(%)]						
Yes						
TT	28 (82.35%)	30 (81.08%)		1.000(reference)		
TC + CC	6 (17.65%)	7 (18.92%)	0.890	0.918(0.235-3.554)	1.000	0.956(0.400-1.706)
No						
TT	237 (75.00%)	259 (82.75%)		1.000(reference)		
TC + CC	79 (25.00%)	54 (17.25%)	**0.017**	1.599(1.065-2.402)	**0.023**	1.243(1.032-1.463)
Ovarian cancer family history [n(%)]						
Yes						
TT	25 (78.13%)	6 (75.00%)		1.000(reference)		
TC + CC	7 (21.88%)	2 (25.00%)	0.850	0.840(0.108-7.644)	1.000	0.964(0.533-1.272)
No						
TT	240 (75.47%)	283 (82.75%)		1.000(reference)		
TC + CC	78 (24.53%)	59 (17.25%)	**0.021**	1.559(1.048-2.319)	**0.027**	1.241(1.025-1.469)

OD, odds ratio. CI, Confidence interval. BMI, body mass index. SD, Standard deviation.

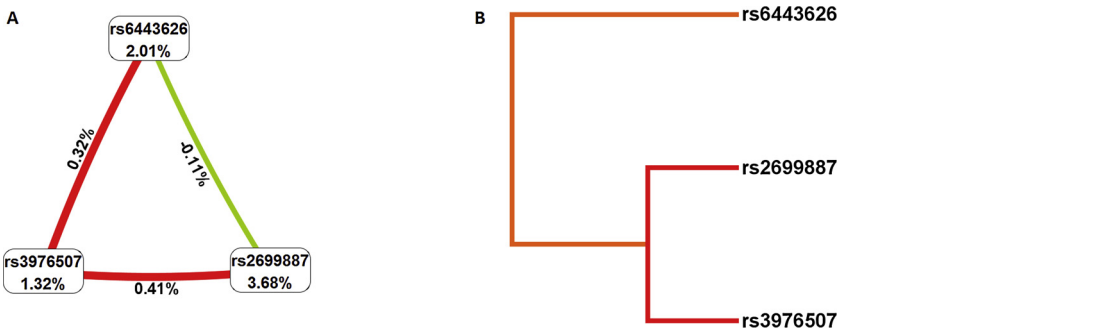

Fig. 1. MDR analysis of the interaction between SNPs. A is an interactive ring diagram, the data at the top corner represents the impact on ovarian cancer, and the values on the line represent the magnitude of the synergy interaction/redundancy interaction. B is the interaction tree.

m^2) had a significantly increased risk of ovarian cancer (adjusted OR = 1.455, 95% CI: 1.045–1.882, p = 0.027; adjusted OR = 1.423, 95% CI: 1.166–1.677, p = 0.001) (Table 4).

Only in subjects with BMI (> 24 kg/m^2), number of liveborn (< 3), non-smokers, non-alcoholic drinkers, and no family history of ovarian cancer, the rs3976507 mutation (CT + TT) was a risk factor for ovarian cancer (adjusted OR = 1.298, 95%CI: 1.054–1.549, p = 0.015/ adjusted OR = 1.300, 95%CI: 1.082–1.523, p = 0.006/ adjusted OR = 1.289, 95%CI: 1.073–1.511, p = 0.007/ adjusted OR = 1.295, 95%CI: 1.077–1.520, p = 0.007/ adjusted OR = 1.285, 95%CI: 1.065–1.516, p = 0.009) (Table 5).

Only in subjects with BMI (> 24 kg/m^2), number of liveborn (< 3), non-smokers, non-alcoholic drinkers and no family history of ovarian cancer, rs6443626 mutation (TC + CC) was a risk factor for ovarian cancer (adjusted OR = 1.319, 95%CI: 1.071–1.571, p = 0.010/ adjusted OR = 1.249, 95%CI: 1.041–1.466, p = 0.017/ adjusted OR = 1.230, 95%CI: 1.022–1.449, p = 0.029/ adjusted OR = 1.243, 95%CI: 1.032–1.463, p = 0.023/ adjusted OR = 1.241, 95%CI: 1.025–1.469, p = 0.027) (Tables 6).

3.4. MDR analysis of gene-gene interactions

There is synergy between rs2699887, rs3976507 SNP and rs3976507, rs6443626, and redundant interaction exists between rs2699887 and rs6443626 (Fig. 1).

3.5. PIK3CA rs2699887, rs3976507, rs6443626 gene polymorphism and PIK3CA protein expression

We further analyzed the correlation between PIK3CA protein expression levels in tissues and PIK3CA rs2699887, rs3976507, rs6443626 locus polymorphisms. RT-PCR results are shown in Fig. 2. We found PIK3CA rs2699887, rs3976507, and rs6443626 locus mutations. The PIK3CA mRNA level in homozygous ovarian cancer tissue samples was significantly higher than that in the heterozygotes, and that in the wild type was the lowest. One-way ANOVA showed statistically significant differences (all p < 0.001). We further used western blotting to detect the expression level of PIK3CA protein in ovarian cancer tissue samples. The results showed that PIK3CA rs2699887, rs3976507, rs6443626 mutations in homozygous samples had the highest PIK3CA protein level, followed by the heterozygotes and wild type (all p < 0.001) (Fig. 3).

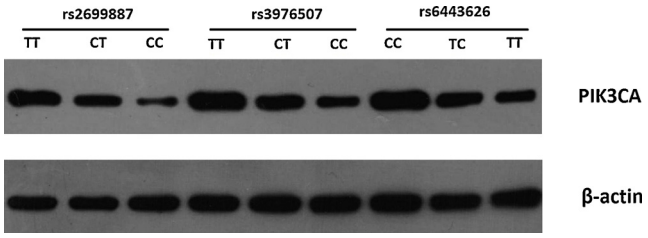

Fig. 3. PIK3CA rs2699887, rs3976507, rs6443626 PIK3CA protein expression in different genotypes.

4. Discussion

In this study, we analyzed the effects of interactions between environmental factors and the risk factors of ovarian cancer in 350 Chinese Han ovarian cancer patients and 350 healthy controls (PIK3CA rs2699887, rs3976507, rs6443626). Our study found that interaction between PIK3CA rs2699887 SNP and age, number of liveborn, smoking, alcohol consumption, family history of ovarian cancer and other factors is associated with ovarian cancer risk. Interaction between PIK3CA rs3976507, rs6443626 loci and factors such as BMI, number of liveborn, smoking, alcohol consumption, and a family history of ovarian cancer is also associated with ovarian cancer risk.

Recent studies have shown that genetic polymorphisms play an important role in the pathogenesis of ovarian cancer [14–16]. Some researchers have found that mutations in the PIK3CA gene play a carcinogenic role in ovarian cancer [17]. Activation of the phosphatidylinositol 3-kinase (PI3K)-AKT pathway leads to increased cell proliferation, survival and metastasis, and is carcinogenic in many cancer types [18,19]. The *PIK3CA* gene encodes a p110alpha catalytic subunit of PI3K that is amplified in some ovarian cancers [20]. Increased mitogenic signaling by receptor tyrosine kinases has been shown to play a major role in human tumorigenesis [21]. One of the major downstream

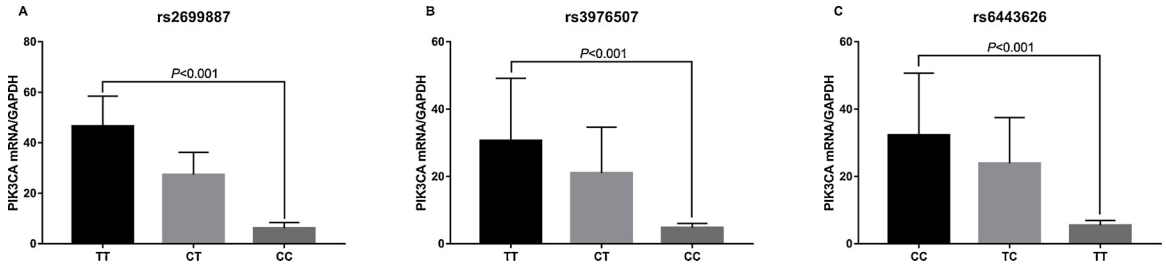

Fig. 2. PIK3CA rs2699887, rs3976507, rs6443626 PIK3CA mRNA levels in different genotypes.

mediators of signaling triggered by these receptors is the PI3K-AKT pathway, several components of which are dysregulated in a variety of cancer types, including amplification and/or overactivation of AKT2 and PIK3CA, PIK3R1 mutations [22–24]. Typically, mutations in the PIK3CA gene occur in the p85, C2, helix or kinase functional domains, and most mutations occur in exon 9 and exon 20, which respectively encode the helix and kinase domains [17].

All the above mutations in *PIK3CA* structurally alter the expression of the PIK3CA protein. The SNPs selected in this study were located in the non-coding region, in which the PIK3CA rs2699887 locus was located in the intron, and the rs3976507 and rs6443626 loci were located in the 3′ UTR, and the mutations did not involve amino acid sequence changes. Our results showed that PIK3CA rs2699887 T allele carriers showed increased risk of ovarian cancer by 1.522 times compared to the C allele carriers. However, Li et al [25] found that this site mutation was not a risk factor for breast cancer in 880 breast cancer patients and 910 control subjcts. Because the mechanism involving the PI3K-AKT pathway in the occurrence of breast cancer and ovarian cancer is similar, we believe that the reasons for different outcomes may be inconsistent with the choice of the population. The MAF of the rs2699887 locus that Li et al. chose was 0.1324, and according to the 1000genomes database (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/), the MAF of the Chinese Han population was 0.0667. In this study, it was 0.0757, which is close to that in the 1000genomes database. At present, there is no research to explore the relationship between rs3976507 and rs6443626 loci and risk of ovarian cancer. This study aims to explore the relationship between the change in PIK3CA protein expression levels and ovarian cancer risk from the perspective of genetic factors. The results indicate that the T allele of rs3976507 and the C allele of rs6443626 are risk factors for ovarian cancer. In another study where the expression of PIK3CA was analyzed after collecting tissue samples from 117 patients with ovarian cancer, the expression level of PIK3CA protein was significantly increased after *PIK3CA* rs2699887, rs3976507, rs6443626 mutation. Activation of the PI3K/AKT pathway inhibited apoptosis and promoted the development of ovarian cancer [26,27], which may be the reason for the increased risk of ovarian cancer after *PIK3CA* rs2699887, rs3976507, rs6443626 mutation.

The influence of genetic factors often differs in different populations. The author believes that this may be related to the role of environmental factors [28]. Therefore, further analysis was performed on the effect of interaction between *PIK3CA* rs2699887, rs3976507, rs6443626 SNPs and environmental factors on the occurrence of ovarian cancer. The T allele of PIK3CA rs2699887 was a risk factor for ovarian cancer in elderly subjects (age ≥60 years), and there was no synergy between age and the rs3976507 and rs6443626 SNPs. In addition, there was synergy between BMI and rs3976507, rs6443626 SNPs, but not with rs2699887. Apparently, when carrying the rs3976507, rs6443626 locus mutant allele, proper weight control may be important to reduce the incidence of ovarian cancer. Factors such as number of liveborn, smoking, drinking, and family history of ovarian cancer were synergistic with PIK3CA rs2699887, rs3976507, and rs6443626 SNPs. From this result, we can suggest that such subjects are at high risk of ovarian cancer, and measures such as reducing fertility, smoking cessation, and alcohol withdrawal may be necessary.

However, this study also has some shortcomings. First, more SNP sites should be chosen to study the effects of multi-gene-gene interactions and gene-environment interactions on ovarian cancer risk, which may be more clinically useful. Secondly, because the sample size is small and the number of homozygotes is small, the error of statistical analysis results may be enlarged and the objectivity of the results may be affected. In addition, whether SNPs located in the 3′ UTR region of *PIK3CA* and miRNAs have an influence on gene expression regulation is an interesting direction and deserves further study.

5. Conclusion

From this study, we observed that PIK3CA rs2699887, rs3976507, rs6443626 SNPs are associated with Chinese Han ovarian cancer risk, and the influence of environmental factors such as age, BMI, number of liveborn, smoking, drinking, and a family history of ovarian cancer is also worthy of attention. We need to further expand the sample size to study more sensitive SNP sites and design experiments in order to explore the role of miRNAs.

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