Polymorphisms of METTL3 gene and ovarian cancer susceptibility: A three-center case-control study

DONGSONG JIA[1], LIPING LAI[1], FANYUAN LI[1], HONG WANG[2] and SHANRONG SHU[1]

[1]Department of Gynecology and Obstetrics, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong 510630, P.R. China; [2]Department of Gynecology and Obstetrics, Guangzhou Nansha District Maternal and Child Health Hospital, Guangzhou, Guangdong 511466, P.R. China

Received February 11, 2025; Accepted October 1, 2025

DOI: 10.3892/ije.2025.31

Abstract. Ovarian cancer is a fatal gynecological malignancy, which leads to a high mortality rate due to its late diagnosis. Methyltransferase-like 3 (METTL3) gene polymorphisms play a crucial role in a number of malignant tumors. However, the effects of METTL3 polymorphisms on ovarian cancer susceptibility have rarely been reported, at least to the best of our knowledge. Thus, the present study aimed to determine the role of METTL3 polymorphisms in ovarian cancer. For this purpose, a three-center case-control study was conducted. Of note, four METTL3 gene polymorphisms (rs1263801G>C, rs1139130 G>A, rs1061027 C>A and rs1061026 T>G) were genotyped using TaqMan quantitative-polymerase chain reaction assay in 244 patients with ovarian cancer and 276 controls. Odds ratios and 95% confidence intervals were used as indicators to assess relation. The results revealed that the rs1263801 CC genotype was a protective factor for ovarian cancer; stratified analysis revealed that the CC genotype reduced susceptibility to ovarian cancer compared to the GG/GC genotype in women aged >51 years. The rs1061027 CA/AA genotype reduced susceptibility compared with the CC genotype. In the stratified analysis, the rs1061027 C>A mutation was a protective factor in women <51 years of age, without metastasis, clinical stage III disease, those who had been pregnant more than three times, post-menopause, with a high expression of estrogen receptor, p16, progesterone receptor, paired box 8 and WT1, and a low expression of p16 and Ki-67, wild-type p53-negative and mutant p53-positive. The rs1061026 TG genotype reduced susceptibility to ovarian cancer, compared with the TT genotype; the TG/GG genotype was a protective factor and decreased susceptibility in women with clinical stage I disease, post-menopause, with a low expression of Ki-67, those who were wild-type p53-negative and mutant p53-negative. The effects of the aforementioned three genetic polymorphisms on ovarian cancer susceptibility were independent. The rs1139130 G>A variation was not associated with susceptibility to ovarian cancer. Haplotype analysis revealed a reduced risk of developing ovarian cancer in haplotype CAT and haplotype CAG compared with haplotype GCT. On the whole, the present study demonstrates that METTL3 rs1263801 G>C, rs1061027 C>A and rs1061026 T>G polymorphisms are associated with a reduced susceptibility to ovarian cancer.

Introduction

According to statistics, ovarian cancer accounts for 2.5% of all malignant tumors, and accounts for 5% of female cancer-related deaths, mainly due to late diagnosis. Despite recent improvements in the diagnosis, ~70% of ovarian cancers are diagnosed at an advanced stage, and only 30% of patients with advanced-stage ovarian cancer survive for >5 years. Ovarian cancer is a heterogeneous group of malignant tumors that vary in etiology and molecular biology. Although the incidence and mortality rates have decreased in recent years, there is still an urgent need to explore the molecular biology of ovarian cancer in order to further identify early diagnostic and therapeutic targets (1,2).

There are numerous post-transcriptional modifications in organisms, among which N^6-methyladenosine (m^6A) is the most abundant internal modification in eukaryotes (3), which plays a key role in various biological processes, such as stem cell self-renewal and differentiation, DNA damage and heat shock. m^6A can be regulated by specific enzymes known as 'writers', 'erasers' and 'readers'. The 'writers' are methyltransferases, including methyltransferase-like (METTL)3, METTL14 and Wilms tumor 1-associated protein. The 'erasers' are demethyltransferases, including fat mass and obesity-associated and AlkB homolog 5, RNA demethylase. The 'readers' are RNA-binding proteins, including the YTH family. m^6A-related proteins play a role in modification and the regulation of the pathogenesis of various types of cancer, such as leukemia, brain tumors, breast cancer, liver cancer, cervical cancer and lung cancer (4).

Correspondence to: Dr Shanrong Shu, Department of Gynecology and Obstetrics, The First Affiliated Hospital of Jinan University, 613 Huangpu Road West, Guangzhou, Guangdong 510630, P.R. China
E-mail: butterfly1705@126.com

Key words: methyltransferase-like 3, polymorphisms, genotype, ovarian cancer

Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by variations in a single nucleotide at the genomic level, which is the most common form of genetic variation in humans (5). Some studies have found that m^6A and its polymorphisms are associated with susceptibility to bladder cancer, gastric cancer, pancreatic cancer and hepatoblastoma (6-9). Moreover, METTL3 polymorphisms have been reported to affect the susceptibility to neuroblastoma, hepatoblastoma and nephroblastoma (10-12).

The association between METTL3 polymorphisms and the development of ovarian cancer has rarely been reported, at least to the best of our knowledge. Given that m^6A and its polymorphisms are associated with tumor susceptibility, it was hypothesized that METTL3 SNPs may be associated with the risk of developing ovarian cancer. In order to verify this hypothesis, the present multicenter large sample case-control study was conducted to investigate the association between METTL3 polymorphisms and the susceptibility to ovarian cancer.

Patients and methods

Study population. Tissue samples from 244 patients with ovarian cancer diagnosed by pathological analysis and blood samples from 276 normal controls were collected from the First Affiliated Hospital of Jinan University (Guangzhou, China), Guangzhou Women's and Children's Medical Center (Guangzhou, China) and Shunde Hospital of Southern Medical University (Foshan, China). The present study was approved by the ethics committees of the above three hospitals [the Ethics Committee of the First Affiliated Hospital of Jinan University (KY-2022-233), the Ethics Committee of Guangzhou Medical University Women and Children's Medical Center (117A01), and the Ethics Committee of Shunde Hospital, Southern Medical University (KYLS20220903)]. In addition, written informed consent was obtained from the subjects. The clinical data of the subjects has been permitted for public disclosure, and the personal information of the subjects has been concealed in the study results. The clinical and pathological information of all subjects in the ovarian cancer group was collected from the databases of the aforementioned hospitals, including name, age, pregnancy and delivery, tumor stage, pathological type and immunohistochemistry results. The relevant information was obtained by querying the clinical medical record system (Table SI).

SNP selection and genotyping. Genomic DNA was extracted from peripheral blood and paraffin samples using the DNA extraction kit (Tiangen Biotech Co., Ltd.) (DP304-03). SNPs with potential biological functions were screened using the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/) and SNPinfo (http://snpinfo.niehs.nih.gov/) online software. Of note, four SNPs (rs1263801 G>C, rs1139130 G>A, rs1061027 C>A and rs1061026 T>G) were selected for analysis. The sequences for these SNPs were as follows: rs1263801 G>C, CTGCCAAGAAATGACCACTACAAAA[C/G] and AGTCGTTATAACTGAGGGAACAAAG; rs1139130 G>A, ACACAACCACTACTTACCCCCAGAG[A/G] and TTTAGACATTCTCTCCCCAACTCCA; rs1061027 C>A, TTCTGTCCTTAATCATAAATAATAG[A/C] and CCCTTGAGGACTAGCCTGTTCTCTG; rs1061026 T>G, AAAACAATGTGAAGCTCTACTAAGT[G/T] and CTGTCCTTAATCATAAATAATAGCC. Genotyping of the extracted genomic DNA was performed using a TaqMan assay with the TIANtough Genotyping qPCR PreMix (Probe) (TianGen, Guangzhou Z-ZHI Biotechnology). The PCR protocol consisted of an initial denaturation at 95˚C for 10 min, followed by 45 cycles of 95˚C for 15 sec and 60˚C for 60 sec.

SNP-SNP interaction analysis. Interactions between SNP loci and their epistasis were verified using the multifactor dimensionality reduction (MDR) method using MDR software v3.0.2 (Laboratory of Computational Genetics, University of Pennsylvania, Philadelphia, PA, USA; available free of charge at http://www.epist asis.org). This method can identify correlations in studies with a small sample size and low SNP penetrance. Cross-validation consistency (CVC) and test accuracy were used to determine the optimal interaction model. The optimal model was the one with the highest CVC and test accuracy values. Values of $P<0.05$ were considered to indicate statistically significant differences.

Statistical analysis. The Chi-squared test was used to determine whether there was a statistically significant difference in age between the experimental and control groups. Logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to assess the association between METTL3 polymorphisms and susceptibility to ovarian cancer, and age was corrected to avoid the influence of confounding factors. Stratified analyses were performed according to age, clinical stage, pregnancy outcomes and immunohistochemistry results to investigate the association between genotypes and susceptibility to ovarian cancer in each sub-stratum. Haplotype analysis was performed using logistic regression analysis, which was used to comprehensively evaluate the effect of selected SNPs of the gene on susceptibility to ovarian cancer. The goodness-of-fit test was used to determine whether the frequency distribution of the genotypes of each SNP in the control group satisfied the Hardy-Weinberg equilibrium (HWE); a value of $P>0.05$ was considered to indicate statistically significant difference, which indicated that the SNP locus in the control group complied with the HWE. The Gene-Tissue Expression (GTEx) portal (https://www.gtexportal.org/home/) was also used for expression quantitative trait loci (eQTL) analysis to predict potential associations between SNPs and gene expression levels. Statistical analysis was performed using SAS 9.4 software (SAS Institute Inc.

Results

Characteristics of the study participants. Detailed information on the demographic and clinical characteristics of the patients with ovarian cancer (n=244) and the controls (n=276) is presented in Table SI. There was no statistically significant difference in age between the ovarian cancer and control groups (P=0.47).

Association of METTL3 gene polymorphisms with susceptibility to ovarian cancer. Genotyping was performed on 244 patients and 276 control subjects. The association between METTL3 polymorphisms and susceptibility to ovarian cancer is presented in Table I. None of the selected

Table I. Logistic regression analysis of associations between METTL3 polymorphisms and susceptibility to ovarian cancer.

Genotype	Cases (n=244), n (%)	Controls (n=276), n (%)	P-value[a]	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value[b]
rs1263801 G>C (HWE=0.68)							
GG	124 (51.88)	128 (47.58)		1		1.00	
GC	102 (42.68)	113 (42.01)		0.945 (0.659-1.355)	0.7568	0.944 (0.658-1.354)	0.7552
CC	13 (5.44)	28 (10.41)		0.486 (0.241-0.979)	0.0435	0.480 (0.238-0.968)	**0.0402**
Additive			0.1132	0.794 (0.602-1.047)	0.1017	0.790 (0.599-1.042)	0.0949
Dominant	11 5(48.12)	141 (52.42)	0.3334	0.842 (0.594-1.193)	0.3335	0.839 (0.592-1.189)	0.3236
Recessive	226 (94.56)	241 (89.59)	0.0401	0.495 (0.250-0.980)	0.0435	0.488 (0.246-0.966)	**0.0395**
rs1139130 G>A (HWE=0.55)							
GG	102 (43.22)	206 (39.41)		1		1	
GA	101 (42.80)	122 (45.35)		0.850 (0.586-1.234)	0.3935	0.847 (0.583-1.229)	0.3812
AA	33 (13.98)	41 (15.24)		0.827 (0.488-1.402)	0.4805	0.819 (0.482-1.390)	0.4592
Additive			0.6817	0.901 (0.701-1.158)	0.4157	0.895 (0.696-1.151)	0.3875
Dominant	134 (56.78)	163 (60.59)	0.3848	0.854 (0.599-1.219)	0.3849	0.847 (0.594-1.210)	0.3615
Recessive	203 (86.02)	228 (84.76)	0.6899	0.904 (0.551-1.485)	0.6908	0.895 (0.545-1.471)	0.6622
rs1061027 C>A (HWE=0.45)							
CC	156 (71.89)	167 (61.85)		1		1	
CA	47 (21.66)	88 (32.59)		0.505 (0.335-0.761)	0.0011	0.500 (0.331-0.754)	**0.001**
AA	14 (6.45)	15 (5.56)		0.882 (0.414-1.882)	0.7460	0.894 (0.419-1.090)	0.7726
Additive			0.0276	0.771 (0.569-1.046)	0.0951	0.770 (0.567-1.045)	0.0937
Dominant	61 (28.11)	103 (38.15)	0.0198	0.634 (0.432-0.931)	0.0202	0.629 (0.428-0.925)	**0.0185**
Recessive	203 (93.55)	255 (94.44)	0.6779	1.172 (0.553-2.4850)	0.6782	1.195 (0.563-2.538)	0.6424
rs1061026 T>G (HWE=0.31)							
TT	190 (84.82)	208 (77.32)		1		1	
TG	31 (13.84)	55 (20.45)		0.577 (0.357-0.932)	0.0246	0.580 (0.359-0.937)	**0.0262**
GG	3 (1.34)	6 (2.23)		0.512 (0.126-2.074)	0.3482	0.502 (0.124-2.038)	0.3352
Additive			0.1085	0.652 (0.432-0.983)	0.0414	0.653 (0.433-0.986)	**0.0426**
Dominant	34 (15.18)	61 (22.68)	0.0356	0.610 (0.384-0.970)	0.0366	0.614 (0.386-0.977)	**0.0395**
Recessive	221 (98.66)	263 (97.77)	0.4618	0.595 (0.147-2.4070	0.4666	0.573 (0.141-2.323)	0.4351

[a]Values were obtained using the Chi-squared test for genotype distributions between patients with ovarian cancer and controls; [b]adjusted for age. Values in bold font indicate statistically significant differences (P<0.05). METTL3, methyltransferase-like 3; OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

SNPs were statistically different in HWE (P>0.05). First, single locus analysis was performed which yielded the following results: rs1263801 CC vs. GG: Adjusted OR, 0.480; 95% CI, 0.238-0.968; P=0.0402; CC vs. GG/GC: Adjusted OR, 0.48; 95% CI, 0.246-0.966; P=0.0395; rs1061027 CA vs. CC: Adjusted OR, 0.500; 95% CI, 0.331-0.754; P=0.001; CA/AA vs. CC: Adjusted OR, 0.629; 95% CI, 0.428-0.925; P=0.0185; rs1061026 TG vs. TT: Adjusted OR, 0.580; 95% CI, 0.359-0.937; P=0.0262; TG/GG vs. TT: Adjusted OR, 0.0366; 95% CI, 0.386-0.977; P=0.0395. Allelic variants reduced the risk of developing ovarian cancer. However, rs1139130 was not associated with the risk of developing ovarian cancer (Table I).

Table II. Stratification analysis of METTL3 polymorphisms with susceptibility to ovarian cancer in rs1263801 G>C.

	rs1263801 G>C (cases/controls)			
Variables	GG/GC	CC	Adjusted OR[a] (95% CI)	P-value[a]
Age, years				
≤51	108	6	0.641 (0.230-1.792)	0.3969
>51	118	7	0.398 (0.159-0.996)	**0.0489**
Metastasis				
Yes	81/241	4/28	0.403 (0.136-1.188)	0.0993
No	128/241	9/28	0.609 (0.279-1.331)	0.2141
Clinical stage				
1	46/241	5/28	0.982 (0.358-2.693)	0.9717
2	41/241	2/28	0.437 (0.100-1.915)	0.2722
3	76/241	3/28	0.325 (0.096-1.102)	0.0712
4	20/241	1/28	0.407 (0.052-3.160)	0.3897
No. of times pregnant				
≥3	92/241	5/28	0.449 (0.168-1.203)	0.1113
<3	134/241	8/28	0.511 (0.226-1.155)	0.1068
Menopause				
Post-menopause	146/241	9/28	0.476 (0.215-1.052)	0.0665
Pre-menopause	74/241	4/28	0.536 (0.176-1.632)	0.272
ER				
Low	27/241	2/28	0.587 (0.131-2.618)	0.4846
High	60/241	3/28	0.437 (0.128-1.489)	0.1859
PR				
Low	26/241	2/28	0.670 (0.150-2.990)	0.5996
High	35/242	2/28	0.507 (0.115-2.226)	0.3679
PAX8				
Low	26/241	2/28	0.654 (0.147-2.920)	0.5783
High	54/241	1/28	0.155 (0.021-1.163)	0.0698
WTI				
Low	31/241	3/28	0.752 (0.214-2.646)	0.6576
High	71/241	2/28	0.236 (0.055-1.017)	0.0526
p16				
Low	37/241	3/28	0.765 (0.218-2.686)	0.6759
High	61/241	2/28	0.276 (0.064-1.193)	0.0847
Ki-67				
Low	36/241	2/28	0.506 (0.115-2.229)	0.3683
High	69/241	3/28	0.370 (0.109-1.256)	0.1108
Wild-type p53				
Positive	51/241	1/28	0.168 (0.022-1.267)	0.0835
Negative	166/241	11/28	0.559 (0.270-1.155)	0.1164
Mutant p53				
Positive	98/241	5/28	0.436 (0.163-1.163)	0.0972
Negative	119/241	7/28	0.492 (0.208-1.161)	0.1054

[a]Values were calculated using the Chi-squared test for genotype distributions between patients with ovarian cancer and controls. Values in bold font indicate statistically significant differences (P<0.05). METTL3, methyltransferase-like 3; OR, odds ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; PAX8, paired box 8.

Table III. Stratification analysis of METTL3 polymorphisms with susceptibility to ovarian cancer in rs1061027 C>A.

Variables	rs1061027 C>A (cases/contorls)		Adjusted OR[a] (95% CI)	P-value[a]
	CC	CA/AA		
Age, years				
≤51	76/86	25/52	0.544 (0.308-0.960)	**0.0357**
>51	80/81	36/51	0.715 (0.422-1.210)	0.2114
Metastasis				
Yes	55/167	24/103	0.695 (0.405-1.194)	0.1876
No	90/167	32/103	0.576 (0.359-0.924)	**0.0222**
Clinical stage				
1	24/167	14/103	0.952 (0.470-1.931)	0.8925
2	30/167	10/103	0.543 (0.255-1.157)	0.1136
3	59/167	20/103	0.542 (0.308-0.954)	**0.0338**
4	13/167	8/103	0.993 (0.397-2.482)	0.9872
No. of times pregnant				
≥3	67/167	22/103	0.525 (0.305-0.903)	**0.0200**
<3	89/167	39/103	0.709 (0.452-1.112)	0.1343
Menopause				
Post-menopause	104/167	39/103	0.578 (0.367-0.911)	**0.0182**
Pre-menopause	48/167	20/103	0.676 (0.371-1.231)	0.2002
ER				
Low	18/167	5/103	0.444 (0.160-1.237)	0.1205
High	44/167	14/103	0.517 (0.270-0.991)	**0.0470**
PR				
Low	22/167	4/103	0.297 (0.099-0.886)	0.0295
High	26/167	10/103	0.627 (0.290-1.354)	0.2345
PAX8				
Low	23/167	4/103	0.281 (0.094-0.836)	**0.0225**
High	36/167	15/103	0.668 (0.348-1.281)	0.2247
WTI				
Low	26/167	6/103	0.368 (0.146-0.928)	**0.0341**
High	48/167	18/103	0.598 (0.330-1.087)	0.0915
p16				
Low	28/167	5/103	0.284 (0.106-0.761)	**0.0123**
High	42/167	13/103	0.498 (0.255-0.972)	**0.0411**
Ki-67				
Low	25/167	3/103	0.196 (0.058-0.667)	**0.0091**
High	43/167	21/103	0.790 (0.444-1.406)	0.4227
Wild-type p53				
Positive	37/167	16/103	0.700 (0.370-1.323)	0.2723
Negative	114/167	41/103	0.583 (0.378-0.900)	**0.0148**
Mutant p53				
Positive	72/167	25/103	0.560 (0.333-0.939)	**0.0280**
Negative	79/167	32/103	0.655 (0.405-1.057)	0.0834

[a]Values were calculated using the Chi-squared test for genotype distributions between patients with ovarian cancer and controls. Values in bold font indicate statistically significant differences (P<0.05). METTL3, methyltransferase-like 3; OR, odds ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; PAX8, paired box 8.

Table IV. Stratification analysis of METTL3 polymorphisms with susceptibility to ovarian cancer in rs1061026 T>G.

Variables	rs1061026 T>G (cases/controls)		Adjusted OR[a] (95% CI)	P-value[a]
	TT	TG/GG		
Age, years				
≤51	87/104	15/34	0.527 (0.270-1.032)	0.0617
>51	103/104	19/27	0.711 (0.372-1.357)	0.3006
Metastasis				
Yes	72/208	13/61	0.624 (0.323-1.204)	0.1597
No	105/208	18/61	0.585 (0.329-1.041)	0.0684
Clinical stage				
1	39/208	3/61	0.253 (0.075-0.850)	**0.0262**
2	34/208	6/61	0.597 (0.239-1.490)	0.2693
3	66/208	13/61	0.679 (0.351-1.314)	0.2506
4	14/208	7/61	1.742 (0.671-4.527)	0.2545
No. of times pregnan				
≥3	80/208	14/61	0.602 (0.318-1.137)	0.1178
<3	110/208	20/61	0.625 (0.359-1.090)	0.0979
Menopause				
Post-menopause	127/208	20/61	0.548 (0.312-0.963)	**0.0366**
Pre-menopause	29/208	12/61	0.634 (0.313-1.284)	0.2058
ER				
Low	24/208	3/61	0.443 (0.129-1.528)	0.1976
High	48/208	10/61	0.708 (0.338-1.482)	0.3592
PR				
Low	19/208	7/61	1.252 (0.502-3.119)	0.6296
High	31/208	5/61	0.541 (0.201-1.454)	0.2233
PAX8				
Low	33/208	5/61	0.743 (0.271-2.040)	0.5646
High	44/208	10/61	0.783 (0.372-1.650)	0.5204
WTI				
Low	28/208	6/61	0.764 (0.301-1.939)	0.5707
High	57/208	11/61	0.667 (0.329-1.353)	0.2621
p16				
Low	29/208	5/61	0.610 (0.226-1.650)	0.3307
High	49/208	10/61	0.702 (0.335-1.469_	0.3473
Ki-67				
Low	32/208	1/61	0.105 (0.014-0.783)	**0.0279**
High	54/208	12/61	0.765 (0.284-1.522)	0.4447
Wild-type p53				
Positive	39/208	13/61	1.135(0.569-2.264)	0.7183
Negative	144/208	19/61	0.455(0.260-0.795)	**0.0057**
Mutant p53				
Positive	77/208	17/61	0.761 (0.418-1.384)	0.3707
Negative	106/208	15/61	0.487 (0.264-0.898)	**0.0211**

[a]Values were calculated using the Chi-squared test for genotype distributions between patients with ovarian cancer and controls. Values in bold font indicate statistically significant differences (P<0.05). METTL3, methyltransferase-like 3; OR, odds ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; PAX8, paired box 8.

Table V. Association between inferred haplotypes of the METTL3 genes and the risk of developing ovarian cancer.

Haplotypes	Cases (n=412), n (%)	Controls (n=538), n (%)	Crude OR (95% CI)	P-value[a]	Adjusted OR (95% CI)	P-value[b]
CT	288 (69.90)	352 (65.43)	1.000		1.000	
CAT	47 (11.41)	89 (16.54)	0.645 (0.439-0.950)	**0.026**	0.638 (0.434-0.940)	**0.023**
CCG	26 (6.31)	43 (7.99)	0.739 (0.443-1.232)	0.246	0.748 (0.448-1.248)	0.266
CCT	30 (7.28)	20 (3.72)	1.833 (1.019-3.297)	**0.043**	1.796 (0.997-3.234)	0.051
GAT	12 (2.91)	10 (1.86)	1.467 (0.625-3.444)	0.379	1.454 (0.618-3.419)	0.391
CAG	4 (0.97)	17 (3.16)	0.288 (0.096-0.864)	**0.026**	0.285 (0.095-0.858)	**0.026**
GCG	5 (1.21)	5 (0.93)	1.222 (0.350-4.263)	0.753	1.141 (0.326-3.995)	0.836
GAG	0	2 (0.37)	/	0.980	/	0.981

[a]Haplotype analysis employed unconditional logistic regression, the haplotype order was rs1263801, rs1061027 and rs1061026. [b]Obtained in logistic regression models with adjustment for age. Values in bold font indicate statistically significant differences (P<0.05). METTL3, methyltransferase-like 3; OR, odds ratio; CI, confidence interval.

Table VI. Optimal multifactor dimensionality reduction interaction models.

Locus number	Testing accuracy	CVC	OR	95% CI	P-value
rs1061027	0.5242	10/10	1.5734	(0.4495-5.5072)	0.4769
rs1263801, rs1061027	0.4947	7/10	1.1874	(0.3455-4.0813)	0.785
rs1263801, rs1061027, rs1061026	0.5813	10/10	1.7637	(0.5393-5.7677)	0.346

CVC, cross-validation consistency; OR, odds ratio; CI, confidence interval.

Stratified analysis. The rs1263801 allele variant reduced the incidence of ovarian cancer in patients aged >51 years (adjusted OR, 0.398; 95% CI, 0.159-0.996; P=0.0489) (Table II).

For the rs1061027 gene polymorphism, compared with the CC genotype, the CA/AA genotype reduced the risk of developing ovarian cancer in patients aged ≤51 years (adjusted OR, 0.544; 95% CI, 0.308-0.960; P=0.0357), in those without metastases (adjusted OR, 0.576; 95% CI, 0.359-0.924; P=0.0222), those with clinical stage III disease (adjusted OR, 0.542; 95% CI, 0.308-0.954; P=0.0338), those who had been pregnant three times or more (adjusted OR, 0.525; 95% CI, 0.305-0.903 P=0.0200), those at post-menopause (adjusted OR, 0.578; 95% CI, 0.367-0.911; P=0.0182), those who were estrogen receptor (ER) strongly positive (adjusted OR, 0.517; 95% CI, 0.270-0.991; P=0.0470), progesterone receptor (PR) weakly positive (adjusted OR, 0.297; 95% CI, 0.099-0.886; P=0.0295), those who were weakly positive for paired box 8 (PAX8) (adjusted OR, 0.281; 95% CI, 0.094-0.836; P=0.0225), weakly positive for WT1 (adjusted OR, 0.3681 95% CI, 0.146-0.928; P=0.0341), strongly positive for p16 (adjusted OR, 0.498; 95% CI, 0.255-0.972; P=0.0411), weakly positive for p16 (adjusted OR, 0.284; 95% CI, 0.106-0.761; P=0.0123), weakly positive for Ki-67 (adjusted OR, 0.196; 95% CI, 0.058-0.667; P=0.0091), wild-type p53-negative (adjusted OR, 0.583; 95% CI, 0.378-0.900; P=0.0148) and mutant p53-positive (adjusted OR, 0.560; 95% CI, 0.333-0.939, P=0.0280) (Table III).

For the rs1061026 gene polymorphism, the TG/GG genotype reduced the risk of developing ovarian cancer compared with the TT genotype in those with clinical staged stage I disease (adjusted OR, 0.253; 95% CI, 0.075-0.850; P=0.0262), those at post-menopause (adjusted OR, 0.548; 95% CI, 0.312-0.963; P=0.0366), those who were weakly positive for Ki-67 (adjusted OR, 0.105; 95% CI, 0.014-0.783; P=0.0279), those who were wild-type p53-negative (adjusted OR, 0.455; 95% CI, 0.260-0.795; P=0.0057) and mutant p53-negative (OR, 0.487; 95% CI, 0.264-0.898; P=0.0211) (Table IV).

METTL3 haplotype analysis. Polymorphisms of rs1263801, rs1061027 and rs1061026 were selected for haplotype analysis, as demonstrated in Table V; haplotype GCT was used as a control. It was found that the risk of developing ovarian cancer was significantly reduced in subjects with haplotype CAT (adjusted OR, 0.638; 95% CI, 0.434-0.940; P=0.023) and haplotype CAG (adjusted OR, 0.285; 95% CI, 0.095-0.858; P=0.026).

SNP-SNP interactions. The MDR analysis revealed that the CVC value of the rs1061027 polymorphism as a single factor model in the METTL3 gene was 10/10, with a testing accuracy of 0.5242, 95% CI, 0.4495-5.5072 and OR, 1.5734. The interaction models rs1263801 x rs1061027 and rs1263801 x rs1061027 x rs1061026 were not statistically significant (Table VI). The interaction map revealed rs1061026 x rs1263801>rs1061026 x rs1061027>rs1061027 x rs1263801 with negative entropy or independence (0.36, 0.33 and 0.07%, respectively, indicated in blue and yellow) (Fig. 1).

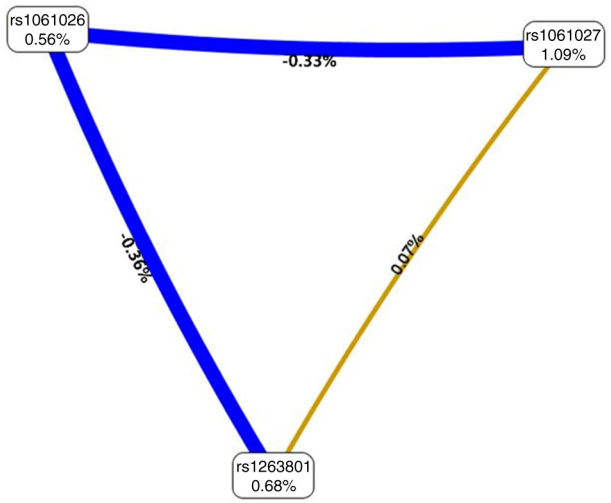

Figure 1. Interaction diagram for the risk of developing ovarian cancer. The interaction model describes the percentage of entropy (information gain) explained by the two-way interaction of each factor. Positive entropy indicates synergistic or non-additive relationships (plotted in yellow), while negative entropy indicates independent or additive (redundancy) relationships (plotted in blue).

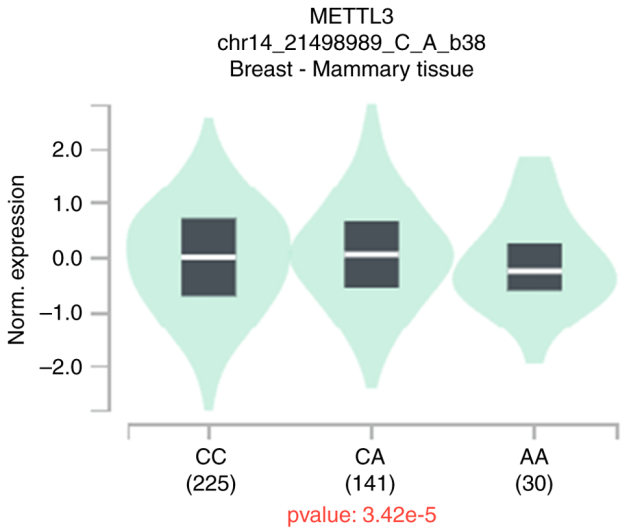

Figure 2. Functional implication of the rs1061027 polymorphism in the METTL3 gene in breast tissue. The expression of rs1061027 genotype and WTAP gene in breast tissue was studied based on the public database GTEx portal. GTEx, Gene-Tissue Expression.

eQTL analysis. To further analyze the functional relevance of rs1263801 G>C, rs1061027 C>A and rs1061026 T>G, eQTL analysis was performed using data published by GTEx. The expression of the appeal locus was not found in the patients with ovarian cancer; however, it was found that patients with breast cancer who carry the rs1061027 A genotype have a decreased expression of METTL3 (Fig. 2).

Discussion

m^{6}A has been reported to be involved in the regulation of specific developmental processes in eukaryotes. METTL3 with 580 amino acids is composed of a zinc finger structural domain and a methyltransferase structural domain. When combined with METTL14, METTL3 exerts methyltransferase activity and plays a key role in cancer development as an oncogene or an oncogene suppressor (13,14). It has been reported that mice transplanted with ovarian cancer cells accompanied by myeloid-specific METTL3 knockout exhibited increased tumor growth (15). Moreover, it has been reported that METTL3 targeting miR-1246 promotes the proliferation and migration, and inhibits the apoptosis of ovarian cancer cells (16). The silencing of METTL3 inhibits miR-126-5p to block the PI3K/Akt/mTOR pathway and inhibit the development of ovarian cancer (17).

In the present multicenter large sample case-control study, it was investigated whether METTL3 polymorphisms are associated with the development of ovarian cancer. First, four SNPs were screened, among which rs1263801 may affect the binding force of transcription factors, rs1139130 is located at the splicing site, and rs1061026 and rs1061027 are the binding sites of miRNAs (12,18). The present study revealed that the CC genotype of rs1263801 was a protective factor against ovarian cancer and was closely related to the risk of developing ovarian cancer in women aged >51 years. The CA genotype of rs1061027 was also a protective factor for ovarian cancer, and the results revealed that compared with the CC genotype, the prevalence of the CA/AA genotype was lower in women aged <51 years, those who were pregnant three times or more and those at post-menopause. The same findings were found in patients with clinical stage III disease and without metastasis. For rs1061026, it was found that the TG genotype was associated with a reduced risk of developing ovarian cancer. Further stratified analysis demonstrated that compared with the TT genotype, the TG/GG genotype reduced the risk of ovarian cancer in patients with clinical stage I disease and in post-menopausal women.

Ki-67 suggests that proliferation is associated with the prognosis of ovarian cancer (19). It has been reported that Ki-67 and p53 expression are significantly elevated in ovarian cancer stages III and IV compared with stages I and II (20). p53 mutations are the most common mutated genes in ovarian cancer, the majority of which are missense mutations, resulting in the loss of tumor suppressor function and enhancing oncogenic function (21,22).

The present study demonstrated that the rs1061027 A allele and rs1061026 G allele were protective factors against ovarian cancer in women with a low Ki-67 expression and in wild-type p53-negative women. However, as regards rs1061027, the CA/AA genotype reduced the risk of ovarian cancer in mutant p53-positive women compared with the CC genotype. As regards rs1061026, the TG/GG genotype reduced the risk of developing ovarian cancer in mutant p53-negative women compared with the TT genotype.

PAX8 belongs to the paired-box gene family and plays a role in tumor growth and participates in multiple oncogenic pathways (23-25). It has been reported PAX8 expression is higher in primary ovarian cancer than in metastatic ovarian cancer; the downregulation of PAX8 can decrease ovarian cancer cell migration and invasion, leading to apoptosis (26). The present study identified rs1061027A as a protective factor for ovarian cancer in women with a low PAX8 expression.

The present study did not find an association between rs1263801 G>C, rs1061027C>A and rs1061026 T>G with METTL3 expression in ovarian tissues by analyzing the data released by GTEx. Considering that ovarian and breast cancer share the same cancer-related genes, such as BRCA1/2, p53, Ki-67, PKP3, CHEK2, PALB2, and PVRL4 (27-29) Furthermore, it was found that patients with breast cancer who carry the rs1061027A genotype have a decreased expression of METTL3; it was thus inferred that rs1061027 affected ovarian cancer development by influencing the expression of METTL3. However, further studies are required to confirm these finidngs.

The present study collected cases from three hospitals; however, as a genetic polymorphism study, the sample size remains relatively small. Ovarian cancer exhibits multiple pathological subtypes, each with distinct mechanisms of onset and prognosis. However, the present study did not standardize for pathological subtypes. Despite the limitations of the present study, it was found that METTL3 gene polymorphisms are associated with susceptibility to ovarian cancer, and the three SNP loci of the METTL3 gene are independent risk factors. The findings may provide new insight for the early diagnosis of ovarian cancer. Further studies are warranted however, to include more cases meeting the inclusion criteria, classify pathological types and conduct external validation.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National College Students Innovation and Entrepreneurship Training Program (grant no. CX18024) and the Science and Technology Projects in Guangzhou (grant no. 202102010134).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SS and DJ designed the study. FL performed the statistical analysis. LL and HW collected the clinical samples. SS and DJ confirm the authenticity of all the raw data. DJ edited the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Jinan University (KY-2022-233), the Ethics Committee of Guangzhou Medical University Women and Children's Medical Center (117A01), and the Ethics Committee of Shunde Hospital, Southern Medical University (KYLS20220903) and written informed consent was obtained by all enrolled patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A and Siegel RL: Ovarian cancer statistics, 2018. CA Cancer J Clin 68: 284-296, 2018.
2. Cho KR and Shih I: Ovarian cancer. Annu Rev Pathol 4: 287-313, 2009.
3. Zaccara S, Ries RJ and Jaffrey SR: Reading, writing and erasing mRNA methylation. Nat Rev Mol Cell Biol 20: 608-624, 2019.
4. Deng X, Su R, Weng H, Huang H, Li Z and Chen J: RNA N^6-methyladenosine modification in cancers: Current status and perspectives. Cell Res 28: 507-517, 2018.
5. Zheng Q, Ma C, Ullah I, Hu K, Ma RJ, Zhang N and Sun ZG: Roles of N6-methyladenosine demethylase FTO in malignant tumors progression. Onco Targets Ther 14: 4837-4846, 2021.
6. Zhuo Z, Hua R, Chen Z, Zhu J, Wang M, Yang Z, Zhang J, Li Y, Li L, Li S, *et al*: WTAP gene variants confer hepatoblastoma susceptibility: A Seven-center Case-control study. Mol Ther Oncolytics 18: 118-125, 2020.
7. Wang X, Guan D, Wang D, Liu H, Wu Y, Gong W, Du M, Chu H, Qian J and Zhang Z: Genetic variants in m^6A regulators are associated with gastric cancer risk. Arch Toxicol 95: 1081-1098, 2021.
8. Ying P, Li Y, Yang N, Wang X, Wang H, He H, Li B, Peng X, Zou D, Zhu Y, *et al*: Identification of genetic variants in m^6A modification genes associated with pancreatic cancer risk in the Chinese population. Arch Toxicol 95: 1117-1128, 2021.
9. Lv J, Song Q, Bai K, Han J, Yu H, Li K, Zhuang J, Yang X, Yang H and Lu Q: N6-methyladenosine-related single-nucleotide polymorphism analyses identify oncogene RNFT2 in bladder cancer. Cancer Cell Int 22: 301, 2022.
10. Bian J, Zhuo Z, Zhu J, Yang Z, Jiao Z, Li Y, Cheng J, Zhou H, Li S, Li L, *et al*: Association between METTL3 gene polymorphisms and neuroblastoma susceptibility: A nine-centre case-control study. J Cell Mol Med 24: 9280-9286, 2020.
11. Lin A, Zhou M, Hua RX, Zhang J, Zhou H, Li S, Cheng J, Xia H, Fu W and He J: METTL3 polymorphisms and Wilms tumor susceptibility in Chinese children: A five-center case-control study. J Gene Med 22: e3255, 2020.
12. Chen H, Duan F, Wang M, Zhu J, Zhang J, Cheng J, Li L, Li S, Li Y, Yang Z, *et al*: Polymorphisms in METTL3 gene and hepatoblastoma risk in Chinese children: A seven-center case-control study. Gene 800: 145834, 2021.
13. Zeng C, Huang W, Li Y and Weng H: Roles of METTL3 in cancer: Mechanisms and therapeutic targeting. J Hematol Oncol 13: 117, 2020.
14. Xu Y, Song M, Hong Z, Chen W, Zhang Q, Zhou J, Yang C, He Z, Yu J, Peng X, *et al*: The N6-methyladenosine METTL3 regulates tumorigenesis and glycolysis by mediating m6A methylation of the tumor suppressor LATS1 in breast cancer. J Exp Clin Cancer Res 42: 10, 2023.
15. Wang J, Ling D, Shi L, Li H, Peng M, Wen H, Liu T, Liang R, Lin Y, Wei L, *et al*: METTL3-mediated m6A methylation regulates ovarian cancer progression by recruiting myeloid-derived suppressor cells. Cell Biosci 13: 202, 2023.
16. Bi X, Lv X, Liu D, Guo H, Yao G, Wang L, Liang X and Yang Y: METTL3 promotes the initiation and metastasis of ovarian cancer by inhibiting CCNG2 expression via promoting the maturation of pri-microRNA-1246. Cell Death Discov 7: 237, 2021.
17. Bi X, Lv X, Liu D, Guo H, Yao G, Wang L, Liang X and Yang Y: METTL3-mediated maturation of miR-126-5p promotes ovarian cancer progression via PTEN-mediated PI3K/Akt/mTOR pathway. Cancer Gene Ther 28: 335-349, 2021.
18. Xu Z and Taylor JA: SNPinfo: Integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res 37: W600-W605, 2009.

19. Kucukgoz Gulec U, Gumurdulu D, Guzel AB, Paydas S, Seydaoglu G, Acikalin A, Khatib G, Zeren H, Vardar MA and Altintas A: Prognostic importance of survivin, Ki-67, and topoisomerase IIα in ovarian carcinoma. Arch Gynecol Obstet 289: 393-398, 2014.
20. Harlozinska A, Bar JK, Sedlaczek P and Gerber J: Expression of p53 protein and Ki-67 reactivity in ovarian neoplasms. Correlation with histopathology. Am J Clin Pathol 105: 334-340, 1996.
21. Walerych D, Napoli M, Collavin L and Del Sal G: The rebel angel: Mutant p53 as the driving oncogene in breast cancer. Carcinogenesis 33: 2007-2017, 2012.
22. Walerych D, Lisek K and Del Sal G: Multi-omics reveals global effects of mutant p53 gain-of-function. Cell Cycle 15: 3009-3010, 2016.
23. Chaves-Moreira D, Morin PJ and Drapkin R: Unraveling the mysteries of PAX8 in reproductive tract cancers. Cancer Res 81: 806-810, 2021.
24. Di Palma T and Zannini M: PAX8 as a potential target for ovarian cancer: What We Know so Far. Onco Targets Ther 15: 1273-1280, 2022.
25. Zhou Q, Li H, Cheng Y, Ma X, Tang S and Tang C: Pax-8: Molecular biology, pathophysiology, and potential pathogenesis. Biofactors 50: 408-421, 2024.
26. Kim J, Kim NY, Pyo J, Min K and Kang D: Diagnostic roles of PAX8 immunohistochemistry in ovarian tumors. Pathol Res Pract 250: 154822, 2023.
27. Zhang Y, Chen J, Tian J, Zhou Y and Liu Y: Role and function of plakophilin 3 in cancer progression and skin disease. Cancer Sci 115: 17-23, 2024.
28. Infante M, Arranz-Ledo M, Lastra E, Olaverri A, Ferreira R, Orozco M, Hernández L, Martínez N and Durán M: Profiling of the genetic features of patients with breast, ovarian, colorectal and extracolonic cancers: Association to CHEK2 and PALB2 germline mutations. Clin Chim Acta 552: 117695, 2024.
29. Nanamiya T, Takane K, Yamaguchi K, Okawara Y, Arakawa M, Saku A, Ikenoue T, Fujiyuki T, Yoneda M, Kai C and Furukawa Y: Expression of PVRL4, a molecular target for cancer treatment, is transcriptionally regulated by FOS. Oncol Rep 51: 17, 2024.