MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 2360-2367
DOI: 10.12659/MSM.910058

Received: 2018.03.19
Accepted: 2018.04.03
Published: 2018.04.19

Polymorphisms of the Ras-Association Domain Family 1 Isoform A (*RASSF1A*) Gene are Associated with Ovarian Cancer, and with the Prognostic Factors of Grade and Stage, in Women in Southern China

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCD 1 Wei He*
DEF 2 Pengyuan Zhang*
EF 1 Min Ye
EF 3 Yizi Wang
BDE 4 Zhikang Chen
EF 2 Jie Chen
ABC 1 Fengjuan Yao

1 Department of Ultrasound, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, P.R. China
2 Department of Endocrinology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, P.R. China
3 The Reproductive Medical Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, P.R. China
4 School of Public Health, Fudan University, Shanghai, P.R. China

* These authors contributed equally to this work

Corresponding Author: Fengjuan Yao, e-mail: guduya3@sina.com
Source of support: Departmental sources

Background: The aim of this study was to determine whether polymorphisms of the Ras-association domain family 1 isoform A (*RASSF1A*) gene were associated with ovarian cancer and with tumor grade and stage, which affect the prognosis of ovarian cancer, in women in Southern China.

Material/Methods: Women from Southern China with histologically confirmed, graded and staged ovarian cancer (n=1,375), and cancer-free controls (n=1,227), provided samples of peripheral blood. DNA was extracted from the blood samples, and five tagging single nucleotide polymorphisms (SNPs) (rs4688728G>T, rs72932987C>T, rs1989839C>T, rs2073497A>C, and rs2236947A>C) were evaluated using an online assay-by-design platform. Polymerase chain reaction (PCR) DNA amplification was performed and computational haplotyping analysis of genetic associations between the five tagging SNPs was performed to identify frequent haplotypes in women with ovarian cancer, and the associations with tumor grade and stage.

Results: In women in Southern China, the CT genotype of rs1989839 was associated with the patients with ovarian cancer (P=0.001), and was significantly correlated with tumor grade and stage (P=0.008). One of the remaining four SNPs studied, rs2073497A>C showed an association with the prognostic factors of grade and stage, but this association did not reach statistical significance.

Conclusions: Polymorphisms of the *RASSF1A* gene, most significantly the CT genotype of rs1989839, might play a role in the development and prognosis of ovarian cancer in women in Southern China. To our knowledge, this is the first study to demonstrate an association between polymorphisms in the *RASSF1A* gene in ovarian cancer.

MeSH Keywords: Polymorphism, Genetic • Prognosis • Risk

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/910058

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Worldwide, ovarian cancer is one of the most common types of cancer in women and is the leading cause of death from gynecologic cancer [1]. Despite numerous efforts in improving surgical techniques and nonsurgical treatment for ovarian cancer, patient survival has increased only slightly, and women who present with late-stage ovarian cancer or tumors that are refractory to treatment still have a poor 5-year overall survival (OS), which remains at around 45% in the USA [1].

The choice of chemotherapy regimens for women diagnosed with ovarian cancer remains controversial, and aggressive chemotherapy regimens are not always chosen as first-line treatments in many cases [2,3]. However, it is widely accepted that patients with late-stage ovarian cancer, or with high-grade tumors, may achieve an improved outcome by earlier treatment with aggressive chemotherapy regimens [3,4]. There remains a need for techniques that can be used to predict the risk of developing ovarian cancer, and in women diagnosed with ovarian cancer, to predict patient prognosis. The identification of predictive and prognostic biomarkers using routine laboratory methods, such as immunohistochemistry (IHC), polymerase chain reaction (PCR), and flow cytometry, might allow clinicians to identify high-risk individuals, and to apply more aggressive therapeutic strategies for women who have ovarian cancer with a poor prognosis.

Recently, the identification of molecular markers of cancer risk and prognosis, especially single nucleotide polymorphisms (SNPs), has attracted the attention of researchers leading to studies that have provided results that have supported a role for SNPs of different genes as being predictive in multiple cancers [5,6]. Polymorphisms in the *WWOX*, *EXO1*, and *MDM2* genes have been shown to be associated with the risk and prognosis of ovarian cancer [7–9]. Therefore, further studies on genetic polymorphisms in ovarian cancer may provide the evidence base required to develop and apply predictive strategies to evaluate patient risk and prognosis.

The Ras-association domain family 1 isoform A (*RASSF1A*) gene has been shown to have a role in the regulation of tumor cell survival and apoptosis, and the expression of *RASSF1A* has been shown to be altered in malignant disease [10]. The protein encoded by the *RASSF1A* gene plays a key role in mediating multiple cellular processes, including apoptosis, cell migration, cell survival, and microtubule stabilization [10]. The *RASSF1A* gene has also shown biological activity in arresting the cell cycle by inhibiting the accumulation of cyclin D1 [11,12]. Although genetic mutations occur less commonly in the *RASSF1A* gene when compared with other genes such as *P53*, genetic polymorphisms in the *RASSF1A* gene do occur, which might affect the cell cycle, as previous studies have shown that tagging SNPs in the *RASSF1A* gene were associated with risk, grade, and prognosis of solid malignant tumors, including lung cancer [13], osteosarcoma [14], breast cancer [15], and renal cancer [16]. Currently, an association between polymorphisms in the *RASSF1A* gene and ovarian cancer remain to be investigated. Because the *RASSF1A* gene might be involved in the development and prognosis of ovarian cancer, studies on polymorphisms in the *RASSF1A* gene in patients with and without ovarian cancer, and with the prognostic factors of tumor grade and stage, are overdue.

Therefore, the aim of this study was to determine whether polymorphisms of the *RASSF1A* gene were associated with ovarian cancer, and with the prognostic factors of tumor grade and stage, in women in Southern China. The study design chosen was a case-controlled study, in which *RASSF1A* polymorphisms were genotyped to evaluate the associations between five *RASSF1A* tagging SNPs and tumor grade and stage in histologically confirmed cases of ovarian cancer compared with healthy controls.

Material and Methods

Ethical approval, patients studied, and venous blood sampling

This study was approved by the Ethics Committees of Sun Yet-sun University and Fudan University. Signed informed consent was obtained from all study participants before the study began.

All cases included in this study were obtained from hospital-based cohorts, from two hospitals between March 2002 to September 2017, and included women who were living in Southern China. The study included 1,375 women with a histologically confirmed diagnosis of primary ovarian cancer. Women in the two study groups were matched by age, social background, and clinical history. All cases of ovarian cancer were diagnosed by experienced and specialized histopathologists, who examined formalin-fixed, paraffin-embedded, ovarian tissue sections by light microscopy. The ovarian tumors were classified and graded according to the World Health Organization (WHO) classification criteria, with low-grade tumors being Grade 1, and high-grade tumors being Grade 2 and 3. Patients were staged according to the 2013 International Federation of Gynecology and Obstetrics (FIGO) staging system, with early-stage tumors being Stage I and Stage II, and late-stage tumors being Stage III and Stage IV.

Venous blood samples were collected from all patients with ovarian cancer during hospital admission, but before any chemotherapy treatment had begun, and the blood samples were preserved. All patients with ovarian cancer were followed-up

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

for at least 36 months. Blood samples from cancer-free controls were collected from routine medical examinations. All of the clinical information was obtained from in-patient medical records (patients with ovarian cancer) and medical examination records (cancer-free controls).

DNA extraction and single nucleotide polymorphism (SNP) genotyping of the Ras-association domain family 1 isoform A (*RASSF1A*) gene

Whole DNA was extracted from whole blood cells of preserved peripheral blood samples from all study participants. Five tagging single nucleotide polymorphisms (SNPs), including rs4688728G>T, rs72932987C>T, rs1989839C>T, rs2073497A>C, and rs2236947A>C, were evaluated in this study. For SNP genotyping, 200 base pair (bp) sequences surrounding each SNP was submitted to Applied Biosystems to develop Taqman Assays using the online assay-by-design platform (Applied Biosystems, Foster City, CA, USA). Briefly, 2 µl at 5 ng/µl of total DNA was dispensed into 384-well polymerase chain reaction (PCR) plates, and each assay was performed in triplicate. The Taqman assay-by-design reagent mix (Applied Biosystems, Foster City, CA, USA) was used to run the PCR according to the manufacturer's instructions. DNA amplification was performed using the following steps: initial denaturing at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds; denaturing at 60°C for 1 minute; followed by denaturing at 72°C for 1 minute. Further analysis of the expression of given SNPs was performed using an ABI PRISM 7900HT Sequence Detection System that included two barcode readers for data entry and plate recognition (Applied Biosystems, Foster City, CA, USA).

Haplotype analysis

Computational haplotyping was undertaken using the SHEsis online analysis system software platform for analysis of genetic associations at the polymorphism *loci* (*http://analysis.bio-x.cn/myAnalysis.php*). Analysis of the genetic correlations between the five included tagging SNPs and tumor grade and stage in patients with ovarian cancer were analyzed to identify potential frequent haplotypes. The SHEsis online analysis system software platform was chosen based on its previous validation in similar studies.

Statistical analysis

The SNPs were tested for Hardy–Weinberg equilibrium (HWE) using Fisher's exact test. The chi-squared (χ^2) test was used to evaluate the possible differences in the distributions of characteristics, variables, and changes in the genotypes of the *RASSF1A* gene between the patients with ovarian cancer and cancer-free healthy controls. For evaluation of the relationship among the five candidate tagging SNPs and the risk, stage, grade, and histological type of ovarian cancer, odds ratios were calculated in combination with the evaluation of 95% confidential intervals (CI). Logistic regression analysis to estimate crude odds ratios (ORs) and consequently adjusted the crude OR for age and sex. A two-sided statistical analysis was performed. A P-value <0.05 was regarded as being statistically significant. Data analysis was performed using SPSS version 22.0 (IBM, NY, USA).

Results

Clinical information of the study populations

Table 1 summarizes the clinical characteristics of all patients with ovarian cancer (n=1,375) and cancer-free controls (n=1,227) included in the study. The median age of the women with ovarian cancer was 57.32 years, and the median age of the cancer-free healthy controls was 58.02 years, with no statistical significance in the age of the two groups (P=0.245). The 2013 International Federation of Gynecology and Obstetrics (FIGO) staging system was used for all women with ovarian cancer.

Ras-association domain family 1 isoform A (*RASSF1A*) gene tagging single nucleotide polymorphisms (SNPs) were related to the presence of ovarian cancer

Table 2 summarizes the pooled data of the distribution of the five genotyped *RASSF1A* tagging single nucleotide polymorphisms (SNPs) that included rs4688728G>T, rs72932987C>T, rs1989839C>T, rs2073497A>C, and rs2236947A>C in patients with ovarian cancer and in the controls. Statistical analysis using Fisher's exact test confirmed that the genotype distributions of the five assessed tagging SNPs were all within the Hardy–Weinberg equilibrium (HWE) in the 1,227 cancer-free control cases (P=0.124, P=0.550, P=0.082, P=0.113, and P=0.328, respectively).

Among the five included tagging SNPs, rs1989839C>T was found to be associated with ovarian cancer. In rs1989839C>T, the CT genotype was found to be correlated with the presence of ovarian cancer (CT versus CC: crude OR =1.89; 95% CI, 1.28–2.41; P=0.001; adjusted OR=1.86; 95% CI, 1.26–2.39; P=0.001), when the homozygote CC genotype was defined as the reference for comparison. Also, the homozygous TT genotype was found to be significantly associated with the presence of ovarian cancer (TT versus CC: crude OR=1.67; 95% CI, 1.10–1.88; P=0.022; adjusted OR=1.70; 95% CI, 1.03–1.85; P=0.025) when compared with the homozygote CC reference group.

Statistical analysis was performed for the dominant models and recessive models. In the dominant model, there was

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Summary of patient clinical characteristics.

Variables		OC cases [n (%)]	Control [n (%)]	*P*
Age	Mean (year)	57.32	58.02	0.245
FIGO stages	I	302 (21.96)		
	II	206 (14.98)		
	III	591 (42.98)		
	IV	276 (20.07)		
Tumor grade	1	286 (20.80)		
	2	387 (28.15)		
	3	605 (44.00)		
	Unclassified	97 (7.05)		
Histology	Clear cell	53 (3.85)		
	Mucinous	106 (7.71)		
	Endometrioid	343 (24.95)		
	Anaplastic	2 (0.15)		
	Serous	767 (55.78)		
	Adenocarcinoma	1 (0.01)		
	Unclassified	103 (7.49)		

a significant statistical difference (CT+TT versus CC: crude OR=1.70; 95% CI, 1.32–2.48; P=0.003; adjusted OR=1.68; 95% CI, 1.29–2.35; P=0.003). However, in the recessive model, no statistically significant differences were found.

In women in Southern China, the CT genotype of rs1989839 was associated with patients with ovarian cancer (P=0.001). When the genotype AA was regarded as the reference groups for comparison, the AC genotype showed a potential association with the presence of ovarian cancer (P=0.090), and similar results were found in the homozygous CC genotype (P=0.059) and with the AC+CC dominant model (P=0.055). There were no significant associations between the remaining three SNPs, rs4688728G>T, rs72932987C>T, and rs2073497A>C, and the presence of ovarian cancer in women in Southern China.

Polymorphisms in the *RASSF1A* gene were associated with the stage and grade of ovarian cancer

Given that polymorphisms in the *RASSF1A* gene were associated with the presence of ovarian cancer, a further investigation was undertaken to determine whether tagging SNPs were related to the grade or stage of ovarian cancer, as grade and stage are associated with patient prognosis. The clinical information on the FIGO stages, tumor grades, and histological types of ovarian cancer were documented and analyzed to determine the potential correlation between the tagging SNPs and the ovarian cancers.

Table 3 shows that among the five included SNP candidates, rs1989839C>T was shown to be associated with tumor grade and FIGO stage. On comparison of the distribution of genotypes in different FIGO stages, the frequencies of genotypes involving T (CT and CT genotypes) of rs1989839C>T in Stage III or Stage IV cases (35.96% and 11.72%, respectively) were significantly lower when compared with patients with early-stage (Stage I and Stage II) ovarian cancer (43.90% and 16.14%, respectively). A significantly increased proportion of CC genotypes were found in women with late-stage ovarian cancer compared with early-stage ovarian cancer (52.31% versus 39.96%) (P=0.008).

Also, rs1989839C>T was significantly associated with the grade of ovarian cancer (P=0.013). High-grade cases of ovarian cancer (Grade 2 or 3), when compared with low-grade cases of ovarian cancer (Grade 1), the genotype CC was significantly increased, while CT and TT genotypes had a lower frequency. As FIGO stage and the tumor grade are associated with patient prognosis of ovarian cancer, these findings supported the possibility that rs1989839C>T might be associated with prognosis in women with ovarian cancer.

The distribution of genotypes of rs1989839C>T in different histological types of ovarian cancer was studied to determine whether polymorphisms in the *RASSF1A* gene were associated with any specific histology type of tumor. However, no statistical difference was found, indicating that rs1989839C>T was not related to the histology type. As rs1989839C>T was found

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Logistic regression analyses on associations between *RASSF1A* rs4688728G>T, rs72932987C>T, rs1989839C>T, rs2073497A>C, and rs2236947A>C polymorphisms and the risk of OC.

RASSF1A genotype	Cases (n=1375)		Controls (n=1227)		Crude OR (95%CI)	*P*	Adjusted OR (95%CI)	*P*
	n	%	n	%				
rs4688728G>T								
GG	211	15.35	172	14.01	1.00		1.00	
GT	566	41.16	523	42.62	0.67 (0.31–1.54)	0.667	0.70 (0.32–1.53)	0.682
TT	598	43.49	532	43.36	0.92 (0.50–1.57)	0.762	0.93 (0.52–1.55)	0.763
GT+TT	1164	84.65	1055	85.98	0.83 (0.50–1.33)	0.454	0.82 (0.50–1.32)	0.439
GG+GT	777	56.51	695	56.63	1.00		1.00	
TT	598	43.49	532	43.36	1.17 (0.67–1.79)	0.255	1.19 (0.66–1.78)	0.356
rs72932987C>T								
CC	551	40.07	515	41.97	1.00		1.00	
CT	509	37.02	490	39.93	0.77 (0.55–1.32)	0.520	0.75 (0.56–1.31)	0.521
TT	315	22.91	222	18.10	1.14 (0.71–1.83)	0.671	1.15 (0.72–1.84)	0.657
CT+TT	824	59.93	712	58.03	0.91 (0.56–1.25)	0.501	0.92 (0.56–1.25)	0.500
CC+CT	1060	77.09	1005	81.90	1.00		1.00	
TT	315	22.91	222	18.10	1.15 (0.71–1.43)	0.209	1.16 (0.71–1.44)	0.207
rs1989839C>T								
CC	481	34.98	639	52.78	1.00		1.00	
CT	733	53.31	417	33.99	1.89 (1.28–2.41)	0.001*	1.86 (1.26–2.39)	0.001*
TT	161	11.71	171	13.93	1.67 (1.10–1.88)	0.022*	1.70 (1.03–1.85)	0.025*
CT+TT	894	65.02	588	47.92	1.70 (1.32–2.48)	0.003*	1.68 (1.29–2.35)	0.003*
CC+CT	1214	88.29	1058	86.87	1.00		1.00	
TT	894	65.02	588	47.92	1.47 (0.83–2.24)	0.210	1.45 (0.83–2.21)	0.208
rs2236947A>C								
AA	83	6.04	39	3.18	1.00		1.00	
AC	240	17.45	235	19.15	0.40 (0.15–1.07)	0.090	0.43 (0.16–1.13)	0.088
CC	1052	76.51	953	77.67	0.52 (0.17–1.03)	0.059	0.53 (0.17–1.06)	0.057
AC+CC	1292	93.96	1188	96.82	0.41 (0.17–1.02)	0.055	0.43 (0.17–1.06)	0.066
AA+AC	323	23.49	274	22.33	1.00		1.00	
CC	1052	76.51	953	77.67	0.92 (0.47–1.55)	0.582	0.94 (0.48–1.59)	0.580
rs2073497A>C								
AA	166	12.07	154	12.55	1.00		1.00	
AC	522	37.96	448	36.51	0.96 (0.57–1.63)	0.862	0.98 (0.58–1.67)	0.865
CC	687	49.96	625	50.94	1.19 (0.70–2.01)	0.510	1.18 (0.70–2.01)	0.512
AC+CC	1209	87.92	1073	87.45	1.07 (0.65–1.76)	0.788	1.08 (0.65–1.78)	0.768
AA+AC	688	50.03	602	49.06	1.00		1.00	
CC	1209	87.92	1073	87.45	1.22 (0.88–1.71)	0.202	1.20 (0.86–1.69)	0.208

* Statistically significant ($P<0.05$).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. The genotype frequencies of *RASSF1A* rs1989839C>T and the clinical features in OC patients.

Variables		n	CC n (%)	CT n (%)	TT n (%)	*P*
FIGO stages	I–II	508	203 (39.96)	223 (43.90)	82 (16.14)	0.008*
	III–IV	887	464 (52.31)	319 (35.96)	104 (11.72)	
Tumor grade	1	286	143 (50.00)	101 (35.31)	42 (14.69)	0.013*
	2–3	992	609 (61.39)	277 (27.92)	4 (10.68)	
Histology	Clear cell	53	22 (41.51)	23 (43.40)	8 (15.09)	0.552
	Mucinous	106	41 (38.68)	43 (40.57)	22 (20.75)	
	Endometrioid	343	137 (39.94)	141 (41.11)	65 (18.95)	
	Anaplastic	2	N/A	N/A	N/A	
	Serous	767	289 (37.68)	331 (43.16)	147 (19.16)	
	Adenocarcinoma	1	N/A	N/A	N/A	
	Unclassified	103	N/A	N/A	N/A	

* Statistically significant ($P<0.05$). N/A – the comparison was not performed as limited number of cases or unclassified histology type.

Table 4. Confounding variable (FIGO stages).

Confounding variables		I or II cases [n (%)]	III or IV cases [n (%)]	*P*
Age	Mean ±SD (year)	55.32±10.66	57.02±9.66	0.330

Table 5. Confounding variable (tumor grade).

Confounding variables		Grade 1 cases [n (%)]	Grade 2 or 3 cases [n (%)]	*P*
Age	Mean ±SD (year)	59.20±9.35	58.89±10.10	0.266

to be associated with the FIGO stage and the grade of ovarian cancer, the confounding variables were analyzed to verify the study findings. Further analysis of the confounding variable, of patient age, did not show statistical significance (Tables 4, 5).

Haplotype analysis of patients with ovarian cancer and healthy controls

Haplotype analysis was undertaken to detect any potential frequent haplotypes in patients with ovarian cancer. In total, 11 highly frequent haplotypes (frequency >3%) were selected: CACTT, CATCT, CACCG, CATTT, CCTTT, CCCCT, CCTCT, CCCTG, CCCTT, CCCCT and CCCCG (Table 6). Among them, CACCT, CCTTT, and CCTCT showed statistically significant differences between women with ovarian cancer when compared with controls (P=0.017; 95% CI, 1.376–4.555; P=0.041; 95% CI, 1.036–2.726; P=0.028; 95% CI, 1.098–2.648, respectively).

Discussion

Single nucleotide polymorphisms (SNPs) are one of the most common types of genetic variations [17]. Although the vast majority of SNPs have minimal influences on the function of genes, some specific SNPs have been identified as functional variants which have been shown to participate in the process of carcinogenesis [18]. Therefore, functional variants in SNPs might drive somatic mutations in the cancer genome. There are increasing numbers of studies that have focused on predicting ovarian cancer risk and on identifying prognosis-related factors, including molecular markers such as SNPs, including studies on genes including *WWOX* [8] , *MDM2* [9], and *EXO1* [7].

The findings of the current study showed that Ras-association domain family 1 isoform A (*RASSF1A*) gene SNPs were associated with the occurrence, stage, and grade of ovarian cancer in women in Southern China, but not on the histological type of tumor. In the present study, after preliminary evaluation, five candidate SNPs were chosen for analysis in women with ovarian cancer and a healthy control population, which

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 6. Haplotype analysis.

Haplotype	OC cases (n=1375)		Controls (n=1227)		p	OR (95% CI)
	n (frequency)		n (frequency)			
CACTT	24.87	(0.045)	39.91	(0.070)	0.017*	2.181 (1.376–4.555)
CATCT	29.49	(0.053)	25.92	(0.045)	0.514	1.199 (0.696–2.066)
CACCG	33.19	(0.059)	30.28	(0.053)	0.583	1.154 (0.692–1.922)
CACCT	25.48	(0.046)	30.45	(0.053)	0.598	0.864 (0.502–1.488)
CATTT	20.16	(0.036)	29.36	(0.051)	0.232	0.702 (0.392–1.257)
CCTTT	49.92	(0.089)	31.84	(0.056)	0.041*	1.613 (1.036–2.726)
CCTCT	29.12	(0.052)	16.03	(0.028)	0.028*	1.659 (1.098–2.648)
CCCTG	39.73	(0.071)	16.34	(0.029)	0.336	1.682 (0.485–3.845)
CCCTT	40.85	(0.073)	46.19	(0.081)	0.690	0.914 (0.587–1.422)
CCCCT	123.39	(0.221)	149.76	(0.262)	0.135	0.805 (0.606–1.070)
CCCCG	9.65	(0.017)	25.88	(0.045)	0.058	0.575 (0.177–1.325)

* Statistically significant ($P<0.05$).

showed that rs1989839C>T was significantly associated with the presence of ovarian cancer. Further analysis on the association between this SNP and the stage and grade of the cases of ovarian cancer indicated that this SNP was associated with late-stage and a higher grade of ovarian cancer, which are known to be prognostic indicators. Also, the findings of the present study showed that another SNP, rs2236947A>C showed some potential in evaluating the presence, stage, and the grade of ovarian cancer, although the association did not reach statistical significance. Although this study included more than one thousand patients with ovarian cancer, the recruitment of more cases might further identify the potential of the polymorphisms in the *RASSF1A* gene in predicting the risk of developing ovarian cancer, and patient prognosis in terms of the tumor stage, and tumor grade.

The *RASSF1A* gene is now recognized to act as a tumor suppressor gene, which is involved in the process of cell survival and proliferation [19,20]. The *RASSF1A* gene has previously been studied in several types of malignancy, to demonstrate its role in regulating tumorigenesis, tumor progression, and patient prognosis. Genetic studies, including studies of the SNPs of the *RASSF1A* gene, have shown that hypermethylation of the promoter of the *RASSF1A* gene was associated with carcinogenesis and progression of lung cancer [21], prostate cancer [22], esophageal cancer [23], and breast cancer [24].

Previous studies in East Asia have shown an association between the RASSF1A genotype and haplotype and the progression of renal cell carcinoma in patients in Japan, and the association of SNPs in RASSF1A in lung cancer patients in Korea [16,25]. In these previously published studies, rs1989839C>T was also shown to be associated with an increased risk of developing cancer and with poor clinical prognosis [16,25]. Also, in a recently published study, the SNP rs1989839C>T was also shown be associated with the risk and clinical outcome of osteosarcoma [14]. Therefore, it might be possible to propose that rs1989839C>T plays a role in several types of malignancy in populations in East Asia. Even though the design of the present study did not include an investigation into the mechanism by which rs1989839C>T might affect tumorigenesis or tumor progression, the findings of this study support the view that abnormal expression of polymorphisms of the *RASSF1A* gene might facilitate tumor progression. Also, from the findings of this study, haplotype analysis showed that some haplotypes were frequently present in patients with ovarian cancer.

However, this study had several limitations. There was inherent and inevitable bias in this study, as genotyping was performed based on blood samples, which were all collected in our hospital. Also, for analysis of ovarian cancer, even with the patient sample size of 1,375, this was too small to reach solid conclusions. The current datasets for individuals having homozygotic genotypes might not have been reliable. This study did not include a detailed analysis of the different histological types of ovarian cancer in terms of their genetic polymorphisms. More patients with ovarian cancer continue to be recruited from additional clinical sites, with follow-up studies currently being planned.

Conclusions

This study was the first to show that polymorphisms in the Ras-association domain family 1 isoform A (*RASSF1A*) gene were

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

associated with the presence, stage, and grade of ovarian cancer. This case-control study included 1,375 women with histologically confirmed, graded, and clinically staged ovarian cancer, and 1,227 women who were free from malignancy, from Southern China, who underwent genotyping of single nucleotide polymorphisms (SNPs) from blood samples. The *RASSF1A* rs1989839C>T SNP was shown to be significantly associated with the presence of ovarian cancer (P=0.001 for CT genotype, and P=0.025 for TT genotype), and with increased stage and grade of ovarian cancer in women in Southern China.

Conflicts of interest

None.

References:

1. Torre L A, Bray F, Siegel RL et al: Global cancer statistics, 2012. Cancer J Clin, 2015; 65(2): 87–108.
2. Sundar S, Khetrapal-Singh P, Frampton J et al: Harnessing genomics to improve outcomes for women with cancer in India: Key priorities for research. Lancet Oncol, 2018; 19(2): e102–12
3. Overbeek A, van den Berg MH, van Leeuwen FE et al: Chemotherapy-related late adverse effects on ovarian function in female survivors of childhood and young adult cancer: A systematic review. Cancer Treat Rev, 2017; 53: 10–24
4. George A, Kaye S, Banerjee S: Delivering widespread *BRCA* testing and *PARP* inhibition to patients with ovarian cancer. Nat Rev Clin Oncol, 2017; 14(5): 284–96
5. Martinez E, Silvy F, Fina F et al: Rs488087 single nucleotide polymorphism as a predictive risk factor for pancreatic cancers. Oncotarget, 2015; 6(37): 39855–64
6. Jiang Y, Sun S, Wei W et al: Association of *FGFR3* and *FGFR4* gene polymorphisms with breast cancer in Chinese women of Heilongjiang province. Oncotarget, 2015; 6(32): 34023–29
7. Shi T, Jiang R, Wang P et al: Significant association of the *EXO1* rs851797 polymorphism with clinical outcome of ovarian cancer. Onco Targets Ther, 2017; 10: 4841–51
8. Paige AJ, Zucknick M, Janczar S et al: *WWOX* tumour suppressor gene polymorphisms and ovarian cancer pathology and prognosis. Eur J Cancer, 2010; 46(4): 818–25
9. Gansmo LB, Bjornslett M, Halle MK et al: *MDM2* promoter polymorphism del1518 (rs3730485) and its impact on endometrial and ovarian cancer risk. BMC Cancer, 2017; 17(1): 97
10. Agathanggelou A, Cooper WN, Latif F: Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. Cancer Res, 2005; 65(9): 3497–508
11. Grawenda AM, O'Neill E: Clinical utility of *RASSF1A* methylation in human malignancies. Br J Cancer, 2015; 113(3): 372–81
12. Li B, Ye Z: Epigenetic alterations in osteosarcoma: Promising targets. Mol Biol Rep, 2014; 41(5): 3303–15
13. Kanzaki H, Hanafusa H, Yamamoto H et al: Single nucleotide polymorphism at codon 133 of the *RASSF1* gene is preferentially associated with human lung adenocarcinoma risk. Cancer Lett, 2006; 238(1): 128–34
14. Xu H, Zhan W, Chen Z: Ras-association domain family 1 isoform A (*RASSF1A*) gene polymorphism rs1989839 is associated with risk and metastatic potential of osteosarcoma in young Chinese individuals: a multi-center, case-control study. Med Sci Monit, 2016; 22: 4529–35
15. Gao B, Xie XJ, Huang C et al: *RASSF1A* polymorphism A133S is associated with early-onset breast cancer in *BRCA1/2* mutation carriers. Cancer Res, 2008; 68(1): 22–25
16. Kawai Y, Sakano S, Okayama N et al: Association of *RASSF1A* genotype and haplotype with the progression of clear cell renal cell carcinoma in Japanese patients. BJU Int, 2012; 110(7): 1070–75
17. Song K, Nam Y J, Luo X et al: Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature, 2012; 485(7400): 599–604
18. Brown JS, O'Carrigan B, Jackson SP et al: Targeting DNA repair in cancer: Beyond PARP inhibitors. Cancer Discov, 2017; 7(1): 20–37
19. Zhang C, Li Z, Cheng Y et al: CpG island methylator phenotype association with elevated serum alpha-fetoprotein level in hepatocellular carcinoma. Clin Cancer Res, 2007; 13(3): 944–52
20. Lo KW, Chung GT, To KF: Deciphering the molecular genetic basis of NPC through molecular, cytogenetic, and epigenetic approaches. Semin Cancer Biol, 2012; 22(2): 79–86
21. Kim DH, Kim JS, Ji YI et al: Hypermethylation of *RASSF1A* promoter is associated with the age at starting smoking and a poor prognosis in primary non-small cell lung cancer. Cancer Res, 2003; 63(13): 3743–46
22. Liu L, Kron KJ, Pethe VV et al: Association of tissue promoter methylation levels of *APC*, *TGFbeta2*, *HOXD3* and *RASSF1A* with prostate cancer progression. Int J Cancer, 2011; 129(10): 2454–62
23. Guo Q, Wang HB, Li YH et al: Correlations of promoter methylation in *WIF-1*, *RASSF1A*, and *CDH13* genes with the risk and prognosis of esophageal cancer. Med Sci Monit, 2016; 22: 2816–24
24. Muller HM, Widschwendter A, Fiegl H et al: DNA methylation in serum of breast cancer patients: An independent prognostic marker. Cancer Res, 2003; 63(22): 7641–45
25. Sung JS, Han SG, Whang YM et al: Putative association of the single nucleotide polymorphisms in *RASSF1A* promoter with Korean lung cancer. Lung Cancer, 2008; 61(3): 301–8

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]