Int J Clin Exp Pathol 2017;10(11):11169-11178
www.ijcep.com /ISSN:1936-2625/IJCEP0065713

*Original Article*

# Association between single nucleotide polymorphisms in the programmed cell death 6 gene and the risk of endometrial cancer in Chinese Han women

Mingwei Yuan[1*], Yaping Song[3*], Di You[1], Qin Li[4], Yan Zhang[2], Bin Zhou[3], Lin Zhang[4], Mingrong Xi[1]

*Departments of [1]Obstetrics and Gynecology, [2]Pathology, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, PR China; [3]Laboratory of Molecular Translational Medicine, West China Institute of Women and Children's Health, Key Laboratory of Obstetric & Gynecologic and Pediatric Diseases and Birth Defects of Ministry of Education, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, PR China; [4]Department of Immunology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan, PR China. [*]Equal contributors.*

Received September 16, 2017; Accepted October 14, 2017; Epub November 1, 2017; Published November 15, 2017

**Abstract:** The programmed cell death 6 (*PDCD6*) gene, originally identified as a pro-apoptotic gene, has recently been reported to have contradictory roles in different diseases and may promote cell proliferation. Here, we examined whether single nucleotide polymorphisms (SNPs) in *PDCD6* were associated with endometrial cancer (EC). The genotypes of these two SNPs (rs3756712 and rs4957014) in *PDCD6* were distinguished by polymerase chain reaction-restriction fragment length polymorphism in 238 patients with EC and 518 controls. Briefly, the T allele of rs3756712 was found to increase EC risk ($P$ = 0.028, odds ratio [OR] = 0.747). Moreover, EC risk was associated with these two SNPs in different genetic models ($P$ = 0.031, OR = 1.42 for rs3756712 in the dominant model; $P$ = 0.019, OR = 0.63 for rs4957014 in the codominant model; $P$ = 0.0073, OR = 0.65 for rs4957014 in the dominant model; $P$ = 0.0076, OR = 0.66 for rs4957014 in the overdominant model). Results of stratified analyses revealed that rs4957012 was linked to body mass index (BMI) and parametrial invasion and that rs4957014 was associated with BMI, although this associated was not statistically significant ($P$ = 0.065, OR = 4.42, 95% confidence interval = 1.06-18.51). Our results indicated that these two tag SNPs in *PDCD6* were associated with EC, suggesting that *PDCD6* may play a crucial role in the tumorigenesis of EC.

**Keywords:** Endometrial cancer, programmed cell death 6, single nucleotide polymorphisms, risk

## Introduction

In diseases of the female reproductive system, endometrial cancer (EC) is the most prevalent malignant tumor in the United States of America, with an estimated 61380 new cases in 2017. The annual number of deaths has increased from 6000 in 1997 to an estimated 10920 in 2017 [1, 2]. Moreover, in the last several years, obvious increases in morbidity related to EC have been observed in Asia [3-5].

According to previous research, certain external environmental factors, including obesity, nulliparity, unopposed estrogen exposure, early menarche, late menopause, and anovulation, have an impact on EC [6, 7]. Nevertheless, the great majority of patients diagnosed with EC do not have any of these risk factors. Consequently, environmental factors cannot explain all cases of EC. Indeed, other studies have demonstrated that both genetic and environmental factors promote the pathogenesis and progression of EC [8, 9].

Apoptosis, also called programmed cell death, is involved in physiological cell death. Many factors participate in the apoptotic pathway, including caspases, pro- and anti-apoptotic Bcl2 family members, and mitochondrial pro-apoptotic proteins. Previous studies have demonstrated that imbalances in apoptosis contribute to tumorigenesis in different tissues [10-16]. For example, programmed cell death 6 (PDCD6) is associated with apoptosis in the initial stage, and studies have demonstrated that

**Table 1.** Descriptive characteristics of participants

| Characteristics | Number of cases (%) |
|---|---|
| Sample size | 238 |
| Mean age ± SD (range) | 51.98±9.862 (25-81) |
| Family history of cancer | |
| Yes | 21 (8.8%) |
| No | 217 (91.2%) |
| Menopausal status | |
| Premenopausal | 112 (47.1%) |
| Postmenopausal | 126 (52.9%) |
| Uterine bleeding | |
| Yes | 218 (91.6%) |
| No | 20 (8.4%) |
| FIGO stage | |
| 0 | 2 (0.84%) |
| I | 176 (73.95%) |
| II | 22 (9.24%) |
| III | 26 (10.92%) |
| IIII | 12 (5.04%) |
| FIGO grade | |
| G1 | 68 (28.6%) |
| G2 | 87 (36.6%) |
| G3 | 81 (34.0%) |
| Histology | |
| Endometrioid adenocarcinoma | 198 (83.2%) |
| Non-endometrioid adenocarcinoma | 40 (16.8%) |
| Myometrial invasion | |
| NO | 42 (17.6%) |
| <1/2 | 135 (56.7%) |
| ≥1/2 | 53 (22.3%) |
| Parametrial invasion | |
| NO | 198 (83.2%) |
| YES | 28 (11.8%) |
| Lymph node status | |
| NO | 186 (78.2%) |
| YES | 22 (9.2%) |
| IHC | |
| ER+ | 173/191 |
| PR+ | 168/190 |
| P53+ | 117/183 |
| KI67+ | 178/178 |
| BMI | |
| <32 | 215 (95.6%) |
| >32 | 10 (4.4%) |

PDCD6 can promote the development and progression of cancer.

In this study, we investigated whether the *PDCD6* gene contributed to the development and progression of EC by genotyping of two tag single nucleotide polymorphisms (SNPs; rs3756712 and rs4957014) in the *PDCD6* gene in 238 patients with EC and 518 controls and analyzed the associations between these two SNPs and EC risk.

## Materials and methods

### *Study patients*

In this case-control study, we enrolled Han women living in Sichuan province of southwest China. Patients with lethal diseases that could influence the final results were excluded, and 238 unrelated female patients with EC plus 518 female age-matched controls met the inclusion criteria and were recruited into the study. The age of patients with EC ranged from 25 to 81 years (mean ± standard deviation [SD], 51.98±9.86 years). The diagnosis of EC was made based on the pathologists' histological examination of tissue from a biopsy at the Second University Hospital of Sichuan University from July 2013 to July 2014. We reviewed medical records of patients' basic information, including age at diagnosis, menstrual status, pathological type, clinical stage, tumor differentiation, lymph node status, and parametrial invasion. The characteristics of the cases are shown in **Table 1**. The control group was composed of 518 healthy individuals, selected randomly from a physical examination at the same hospital. All controls had no abnormal clinical symptoms of EC, and all test results were normal (including gynecologic examination, B-mode ultrasound, and cervical cytology). Ethical approval for patient and control recruitment was obtained from the Medical Ethical Review Committee of West China Second University Hospital of Sichuan University, and all participants provided written or oral informed consent.

### *DNA extraction and genotyping*

DNA was extracted from 200 μL ethylenediaminetetraacetic acid-anticoagulated peripheral blood samples using a DNA isolation kit from Bioteke (Peking, China) and stored at -20°C. All procedures were implemented according to the

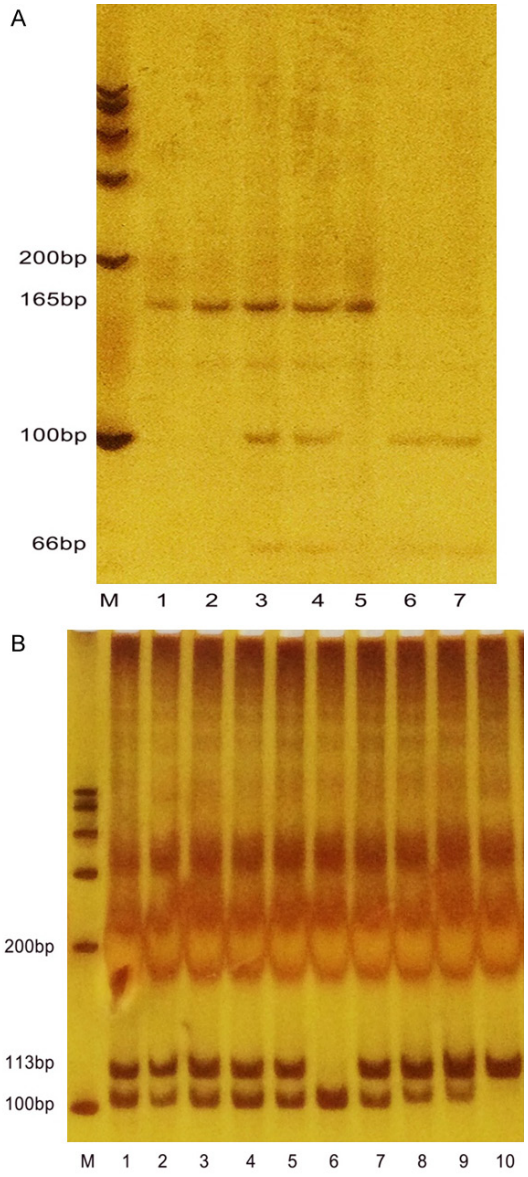


**Figure 1.** Photograph of the PDCD6 digested PCR product. A. Determination of SNP rs3756712 by PCR-polyacrylamide gel electrophoresis. M: 100-bp DNA marker; lanes 1, 2, 5 the TT homozygotes; lanes 3, 4 the GT heterozygotes; lanes 6, 7 the GG homozygote. B. Determination of SNP rs4957014 by PCR-polyacrylamide gel electrophoresis. M: 100-bp DNA marker; lanes 2, 8, 9, 10 the TT homozygotes; lanes 1, 3, 4, 5, 7 the GT heterozygotes; lane 6 the GG homozygote.

manufacturer's instructions. The polymorphic sites were selected from the public databases (dbSNP; http://www.ncbi.nlm.nih.gov/SNP/ and http://www.hapmap.org/). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to genotype SNPs (rs3756712 and rs4957014) in the *PDCD6* gene. Primers were created in the PIRA PCR designer (http://cedar.genetics.soton.ac.uk/public_html/ primer2.html) [17]. The primer sequences were as follows: 5'-TACAGTGGCAAAGGACCACA-3' (forward), 5'-CACATTCCAGCACTCACCAC-3' (reverse) for rs3756712 and 5'-TGGTGTTTCATACCATTGACACTTGC3' (forward), 5'-CTCAGAACCAAGCAGGTTCCTTCA-3' (reverse) for rs4957014. PCR was performed using a total volume of 25 μL, including 2.5 μL 10 × PCR buffer, 1.5 mM $MgCl_2$, 0.15 mMdNTPs, 0.5 μM each primer, 100 ng genomic DNA, and 1 U Taq DNA polymerase. The PCR cycling conditions were as follows: an initial denaturing step for 5 min at 95°C; 33 cycles of 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C; and a final extension step for 10 min at 72°C. PCR products were then digested overnight with restriction enzyme, and the digested PCR products were distinguished using 6% polyacrylamide gels stained with 1.5 g/L argent nitrates to detect the amplified products. The restriction enzyme for rs3756712 was *Rsa*I, and allele G was cuttable, yielding two fragments of 66 and 99 bp; allele T was uncuttable, and the fragment was 165 bp. The restriction enzyme for rs4957014 was *Hph*I, and allele G was cuttable, yielding two fragments of 13 and 100 bp; allele T was uncuttable, and the fragment was 113 bp. Ten percent of the samples were selected randomly to repeat the procedures, and the results were 100% concordant.

### *Statistical analysis*

SPSS for Windows software package version 13.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Pearson's chi-square tests were used to test Hardy-Weinberg equilibrium among the cases and controls. $\chi^2$ tests were used to analyze the allele and genotype frequency differences between cases and controls. Genotypic association tests in a case-control pattern, assuming codominant, dominant, recessive, and overdominant genetic models, were performed using SNPstats [18]. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the differences between alleles and genotypes. Probability values of 0.05 or less were considered statistically significant.

## Results

SNPs in *PDCD6* were successfully genotyped in participants and controls (**Figure 1**), and geno-

**Table 2.** Allele frequencies of SNPs in PDCD6 among patients and controls and their association with EC risk

| SNP | Allele | Case | Control | *P* | OR (95% CI) |
|---|---|---|---|---|---|
| rs3756712 | T | 376 (79%) | 764 (74%) | 0.028 | 0.747 (0.576-0.969) |
| | G | 100 (21%) | 272 (26%) | | |
| rs4957014 | T | 297 (62%) | 699 (67%) | 0.053 | 0.8 (0.638-1.003) |
| | G | 179 (38%) | 337 (33%) | | |

type distributions in control group were in line with the Hardy-Weinberg equilibrium.

Allele frequencies of these two SNPs in patients with EC and controls are shown in **Table 2**. Significantly increased EC risk was found to be associated with the T allele of the SNP rs3756712 (*P* = 0.028, OR = 0.747, 95% CI = 0.576-0.969). As showed in **Table 3**, significant associations were observed with genotypes of rs3756712 and rs4957014 SNPs in different genetic models. For rs3756712, individuals with allele G (GT/GG genotype) in rs3756712 had a significantly decreased risk of EC compared with that of patients without allele G (TT genotypes) in the dominant model (*P* = 0.031, OR = 1.42, 95% CI = 1.03-1.94). For rs4957014, a significantly increased EC risk was associated with GT genotype carriers compared with TT homozygous carriers in the codominant model (*P* = 0.019, OR = 0.63, 95% CI = 0.45-0.87). In the dominant model, a significantly increased EC risk was associated with the G/T or G/G genotype compared with the T/T genotype (*P* = 0.0073, OR = 0.45, 95% CI = 0.47-0.89). Additionally, in the overdominant model, the G/T genotype was associated with increased EC risk compared with the T/T or G/G homozygous genotype (*P* = 0.0076, OR = 0.66, 95% CI = 0.48-0.90).

The results of stratified analyses by clinical characteristics are presented in **Tables 4** and **5**. Notably, rs3756712 was associated with body mass index (BMI) and parametrial invasion (**Table 4**), and rs4957014 was associated with BMI (**Table 5**), although this association was not statistically significant (*P* = 0.065, OR = 4.42, 95% CI = 1.06-18.51). No statistically significant differences in genotype or allele distribution were observed between the remaining clinical characteristics and these two SNPs.

## Discussion

*PDCD6*, located on chromosome 5p15.33, is also referred to as apoptosis-linked gene-2 (*ALG-2*). The structural characteristics of this 22-kDa calcium-binding protein include a penta-EF-hand domain composed of five serially similar EF-hand structures [19, 20]. Previous studies have demonstrated that PDCD6 can shuttle back and forth between the cytoplasm and nucleus, and the distribution is dependent on its interactions with distinct proteins [21]. Initially, PDCD6 was characterized as a pro-apoptotic protein and acted in T-cell receptor-, Fas-, and glucocorticoid-induced apoptosis [22, 23], endoplasmic reticulum stress-induced cell death, neuronal apoptosis during organ formation, and some other physiological processes, such as signal transduction, membrane trafficking, and post-transcriptional control of gene expression. However, when the *PDCD6* gene is knocked out in mice, apoptosis is not suppressed, indicating that the function of PDCD6 is redundant [19].

Many studies have analyzed PDCD6 expression in tumor tissues or cell lines and shown that this protein has contradictory effects in various tumors. For example, *PDCD6* has been shown to act as an oncogene or tumor-suppressor gene in different types of cancers. PDCD6 is upregulated in lung cancer, breast cancer [24], colon cancer [25], hepatoma, and metastatic ovarian cancer cells. Moreover, in metastatic ovarian cancer cells, PDCD6 promotes the migration and invasion of cells [26, 27], and patients with epithelial ovarian cancer having intermediate or high *PDCD6* mRNA expression are at risk of contracting other diseases [26]. However, the relation between PDCD6 expression and survival is still not clear. Qin et al. observed a marked elevation in PDCD6 expression in breast cancer tissues and evaluated the relationships between PDCD6 expression and clinicpathological characteristics [24]. Moreover, PDCD6 has been shown to contribute to cancer cell viability [25, 27], and decreased PDCD6 expression has been identified in gastric cancer [25, 26], pediatric acute myelogenous leukemia [28], HeLa cells [29, 30], and Mel290 cells. In glioblastoma, Zhang et al. identified PDCD6 as a novel tumor-suppressor gene. High levels of PDCD6 expression predicted a good prognosis, and downregulation of PDCD6 promoted the proliferation and migration of glioblastoma cells [31]. In additional studies, researchers have shown that there

**Table 3.** Genotype frequencies of SNPs in PDCD6 among patients and controls in different genetic model

| SNP | Model | Genotype | Group = Ca | Group = Co | OR (95% CI) | *P*-value |
|---|---|---|---|---|---|---|
| rs3756712 | Codominant | T/T | 153 (64.3%) | 290 (56%) | 1 | 0.091 |
| | | G/T | 70 (29.4%) | 184 (35.5%) | 1.39 (0.99-1.94) | |
| | | G/G | 15 (6.3%) | 44 (8.5%) | 1.55 (0.83-2.87) | |
| | Dominant | T/T | 153 (64.3%) | 290 (56%) | 1 | 0.031 |
| | | G/T-G/G | 85 (35.7%) | 228 (44%) | 1.42 (1.03-1.94) | |
| | Recessive | T/T-G/T | 223 (93.7%) | 474 (91.5%) | 1 | 0.29 |
| | | G/G | 15 (6.3%) | 44 (8.5%) | 1.38 (0.75-2.53) | |
| | Overdominant | T/T-G/G | 168 (70.6%) | 334 (64.5%) | 1 | 0.096 |
| | | G/T | 70 (29.4%) | 184 (35.5%) | 1.32 (0.95-1.84) | |
| rs4957014 | Codominant | T/T | 83 (34.9%) | 234 (45.2%) | 1 | 0.019 |
| | | G/T | 131 (55%) | 231 (44.6%) | 0.63 (0.45-0.87) | |
| | | G/G | 24 (10.1%) | 53 (10.2%) | 0.78 (0.45-1.35) | |
| | Dominant | T/T | 83 (34.9%) | 234 (45.2%) | 1 | 0.0073 |
| | | G/T-G/G | 155 (65.1%) | 284 (54.8%) | 0.65 (0.47-0.89) | |
| | Recessive | T/T-G/T | 214 (89.9%) | 465 (89.8%) | 1 | 0.95 |
| | | G/G | 24 (10.1%) | 53 (10.2%) | 1.02 (0.61-1.69) | |
| | Overdominant | T/T-G/G | 107 (45%) | 287 (55.4%) | 1 | 0.0076 |
| | | G/T | 131 (55%) | 231 (44.6%) | 0.66 (0.48-0.90) | |

is a connection between high *miR-183* expression plus low PDCD6 expression and aggressive clinicpathological features [28], and Gu et al. showed that *miR-183* had a suppression effect on apoptosis and contributed to the proliferation and invasion of cancer cells by targeting PDCD4 [32]. Recent reports have also found decreased copy numbers and mRNA expression of *PDCD6* in gastric cancer samples, suggesting that *PDCD6* may be a significant prognostic biomarker in patients with advanced gastric cancer [33]. Additionally, compared with normal melanocytes, Mel290 cells express lower levels of PDCD6 mRNA and protein [29].

As an essential step in the development of tumors, angiogenesis plays an important role in tissue repair, tumor growth, and tumor metastasis. PDCD6 can block tumor development by suppressing angiogenesis under physiological conditions through targeting of the p70 ribosomal protein S6 kinase or phosphatidylinositol 3-kinase/mammalian target of rapamycin pathway [34]. According to a study by Rho et al., purified recombinant human PDCD6 blocked the invasion and proliferation of cells in a concentration- and time-dependent manner, and vascular endothelial growth factor-induced proliferation, invasion, and capillary-like structure tube formation in vitro were suppressed by PDCD6 overexpression. These findings demonstrated that PDCD6 is important for the modulation of cellular angiogenesis [34]. PDCD6 is also considered a novel p53-responsive gene and has been shown to accumulate in the nucleus, supporting its role in DNA damage-induced apoptosis. p53-dependent apoptosis is suppressed by PDCD6 knockdown [35], and knockdown of PDCD6 in HeLa cells increases the number of dead cells, enhances early apoptosis, and induces the accumulation of cells in the $G_2$/M phase of the cell cycle. PDCD6 also promotes passage through cell cycle checkpoints, giving it an anti-apoptotic role in HeLa cells [30]. Because PDCD6 expression promotes apoptosis through activation of the mitochondrial pathway and is closely linked to the prognosis of patients with advanced gastric cancer, PDCD6 may be a molecular biomarker for the prognosis of gastric cancer [33]. Many PDCD6-interacting proteins have been shown to function in microtubule-related activities, and HEBP2 and PDCD6 coordinately regulate spindle orientation and positioning. Additionally, depletion of PDCD6 increases cytoplasmic calcium levels in breast cancer cells, suggesting that this protein may be involved in calcium signaling under physiological conditions [36-38].

**Table 4.** Association between rs3756712 and patient's characteristics

| Characteristics | Genotype | | | Genetic model | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | T/T | G/T | G/G | Dominant (TT VS. GT/GG) | | Recessive (GG VS. TT/GT) | | Overdominant (TT/GG VS. GT) | |
| | | | | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Age | | | | | | | | | |
| <52 year | 73 (66.4%) | 29 (26.4%) | 8 (7.3%) | 1.18 (0.69-2.02) | 0.53 | 0.74 (0.26-2.10) | 0.57 | 1.32 (0.75-2.31) | 0.34 |
| ≥52 year | 80 (62.5%) | 41 (32%) | 7 (5.5%) | | | | | | |
| Figo stage | | | | | | | | | |
| I | 117 (65.7%) | 49 (27.5%) | 12 (6.7%) | 1.28 (0.70-2.33) | 0.43 | 0.73 (0.20-2.67) | 0.62 | 1.42 (0.76-2.65) | 0.28 |
| II-IV | 36 (60%) | 21 (35%) | 3 (5%) | | | | | | |
| Grade | | | | | | | | | |
| G1 | 39 (57.4%) | 22 (32.4%) | 7 (10.3%) | 0.65 (0.37-1.17) | 0.15 | 0.44 (0.15-1.25) | 0.13 | 0.81 (0.44-1.49) | 0.51 |
| G2-G3 | 113 (67.3%) | 47 (28%) | 8 (4.8%) | | | | | | |
| BMI | | | | | | | | | |
| <32 | 139 (64.7%) | 66 (30.7%) | 10 (4.7%) | 1.22 (0.33-4.45) | 0.77 | 8.79 (1.97-39.14) | 0.012 | 0.25 (0.03-2.02) | 0.12 |
| >32 | 6 (60%) | 1 (10%) | 3 (30%) | | | | | | |
| Pathological type | | | | | | | | | |
| Endometrioid adenocarcinoma | 126 (63.6%) | 59 (29.8%) | 13 (6.6%) | 0.84 (0.41-1.73) | 0.64 | 0.75 (0.16-3.46) | 0.7 | 0.89 (0.42-1.91) | 0.77 |
| Non-endometrioid adenocarcinoma | 27 (67.5%) | 11 (27.5%) | 2 (5%) | | | | | | |
| Parametrial invasion | | | | | | | | | |
| NO | 126 (63.6%) | 57 (28.8%) | 15 (7.6%) | 0.97 (0.43-2.22) | 0.95 | 0.00 (0.00-NA) | 0.042 | 1.37 (0.60-3.16) | 0.46 |
| YES | 18 (64.3%) | 10 (35.7%) | 0 (0%) | | | | | | |
| Menopausal status | | | | | | | | | |
| Premenopausal | 72 (64.3%) | 31 (27.7%) | 9 (8%) | 1.00 (0.59-1.70) | 1 | 0.57 (0.20-1.66) | 0.3 | 1.17 (0.67-2.05) | 0.58 |
| Postmenopausal | 81 (64.3%) | 39 (30.9%) | 6 (4.8%) | | | | | | |
| Uterine bleeding | | | | | | | | | |
| NO | 14 (70%) | 3 (15%) | 3 (15%) | 1.33 (0.49-3.59) | 0.57 | 0.33 (0.08-1.28) | 0.14 | 2.51 (0.71-8.87) | 0.12 |
| YES | 139 (63.8%) | 67 (30.7%) | 12 (5.5%) | | | | | | |

**Table 5.** Association between rs4957014 and patient's characteristics

| Characteristics | Genotype | | | Genetic model | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | T/T | G/T | G/G | Dominant (TT VS. GT/GG) | | Recessive (GG VS. TT/GT) | | Overdominant (TT/GG VS. GT) | |
| | | | | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Age | | | | | | | | | |
| <52 year | 37 (33.6%) | 60 (54.5%) | 13 (11.8%) | 0.90 (0.53-1.54) | 0.71 | 0.70 (0.30-1.64) | 0.41 | 1.04 (0.62-1.73) | 0.89 |
| ≥52 year | 46 (35.9%) | 71 (55.5%) | 11 (8.6%) | | | | | | |
| Figo stage | | | | | | | | | |
| I | 63 (35.4%) | 97 (54.5%) | 18 (10.1%) | 1.10 (0.59-2.03) | 0.77 | 0.99 (0.37-2.62) | 0.98 | 1.09 (0.61-1.97) | 0.77 |
| II-IV | 20 (33.3%) | 34 (56.7%) | 6 (10%) | | | | | | |
| Grade | | | | | | | | | |
| G1 | 24 (35.3%) | 35 (51.5%) | 9 (13.2%) | 1.01 (0.56-1.82) | 0.98 | 0.60 (0.24-1.45) | 0.26 | 1.23 (0.70-2.16) | 0.48 |
| G2-G3 | 59 (35.1%) | 95 (56.5%) | 14 (8.3%) | | | | | | |
| BMI | | | | | | | | | |
| <32 | 78 (36.3%) | 118 (54.9%) | 19 (8.8%) | 2.28 (0.47-10.99) | 0.27 | 4.42 (1.06-18.51) | 0.065 | 0.82 (0.23-2.92) | 0.76 |
| >32 | 2 (20%) | 5 (50%) | 3 (30%) | | | | | | |
| Pathological type | | | | | | | | | |
| Endometrioid adenocarcinoma | 69 (34.9%) | 107 (54%) | 22 (11.1%) | 0.99 (0.49-2.03) | 0.99 | 0.42 (0.10-1.87) | 0.21 | 1.28 (0.64-2.55) | 0.49 |
| Non-endometrioid adenocarcinoma | 14 (35%) | 24 (60%) | 2 (5%) | | | | | | |
| Parametrial invasion | | | | | | | | | |
| NO | 70 (35.4%) | 106 (53.5%) | 22 (11.1%) | 1.15 (0.50-2.69) | 0.74 | 0.30 (0.04-2.29) | 0.17 | 1.56 (0.69-3.55) | 0.28 |
| YES | 9 (32.1%) | 18 (64.3%) | 1 (3.6%) | | | | | | |
| Menopausal status | | | | | | | | | |
| Premenopausal | 37 (33%) | 61 (54.5%) | 14 (12.5%) | 0.86 (0.50-1.47) | 0.57 | 0.60 (0.26-1.42) | 0.24 | 1.05 (0.63-1.74) | 0.87 |
| Postmenopausal | 46 (36.5%) | 70 (55.6%) | 10 (7.9%) | | | | | | |
| Uterine bleeding | | | | | | | | | |
| NO | 7 (35%) | 11 (55%) | 2 (10%) | 1.01 (0.39-2.63) | 0.99 | 1.01 (0.22-4.65) | 0.99 | 1.00 (0.40-2.52) | 1 |
| YES | 76 (34.9%) | 120 (55%) | 22 (10.1%) | | | | | | |

According to a study by Li et al., PDCD6 acts as a lysosome calcium sensor, physically associating with the minus-end-directed dynes-dynasty motor for regulation of lysosome positioning and motility [39].

Centrosome aberrations, including functional and structural defects, have been observed in tumorigenesis [40]. According to Qin et al., the localization of γ-tubulin and patricentric is disrupted by PDCD6 overexpression in breast cancer cells, leading to the formation of centrosome protein aggregates. Additionally, PDCD6 regulates the rearrangement of the cytoskeleton to drive migration and polarization in breast cancer cells [24]. Montaville et al. found a link between alternative splicing regulation and PDCD6, showing effects on basic cellular processes during cancer development [21]. Moreover, Maki reported that PDCD6 serves as a bridge and functions as an adaptor molecule [19, 20].

To the best of our knowledge, this is the first report discussing the relationship between the polymorphisms in *PDCD6* and EC in China. Our results demonstrated that the T allele of rs3756712 may increase EC risk and provided evidence that PDCD6 may serve as a biomarker for EC susceptibility.

However, our study had some limitations. First, we only enrolled 238 patients with EC and 518 controls, which may limit the statistical power of the analysis. Further studies genotyping more polymorphisms in the *PDCD6* gene using a large, diverse population could contribute to identification of the true association between these SNPs and EC. Furthermore, the expression of PDCD6 in tumor tissues and cell lines, the influence of SNPs on EC tumorigenesis, and the molecular mechanisms through which PDCD6 is involved in the development of EC should be studied to further improve our understanding of the relationship between PDCD6 and EC risk.

## Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81572573) and the Applied Basic Research Programs of Science and Technology Commission Foundation of Sichuan Province (No. 2016SZ0013).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Mingrong Xi, Department of Obstetrics & Gynecology, West China Second University Hospital, Sichuan University, Chengdu 610041, Sichuan, PR China. Tel: +86 28 85501633; Fax: +86 28 85501633; E-mail: xmrjzz@126.com

## References

[1] Parker SL, Tong T, Bolden S and Wingo PA. Cancer statistics, 1997. CA Cancer J Clin 1997; 47: 5-27.

[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.

[3] Ushijima K. Current status of gynecologic cancer in Japan. J Gynecol Oncol 2009; 20: 67-71.

[4] Huang CY, Chen CA, Chen YL, Chiang CJ, Hsu TH, Lin MC, Lai MS, Chen CJ, You SL and Cheng WF. Nationwide surveillance in uterine cancer: survival analysis and the importance of birth cohort: 30-year population-based registry in Taiwan. PLoS One 2012; 7: e51372.

[5] Jung KW, Won YJ, Oh CM, Kong HJ, Lee DH and Lee KH. Prediction of cancer incidence and mortality in Korea, 2017. Cancer Res Treat 2017; 49: 306-312.

[6] Oh H, Arem H, Matthews CE, Wentzensen N, Reding KW, Brinton LA, Anderson GL, Coburn SB, Cauley JA, Chen C, Goodman D, Pfeiffer RM, Falk RT, Xu X and Trabert B. Sitting, physical activity, and serum oestrogen metabolism in postmenopausal women: the women's health initiative observational study. Br J Cancer 2017; 117: 1070-1078.

[7] Merritt MA, Tzoulaki I, Tworoger SS, De Vivo I, Hankinson SE, Fernandes J, Tsilidis KK, Weiderpass E, Tjonneland A, Petersen KE, Dahm CC, Overvad K, Dossus L, Boutron-Ruault MC, Fagherazzi G, Fortner RT, Kaaks R, Aleksandrova K, Boeing H, Trichopoulou A, Bamia C, Trichopoulos D, Palli D, Grioni S, Tumino R, Sacerdote C, Mattiello A, Bueno-de-Mesquita HB, Onland-Moret NC, Peeters PH, Gram IT, Skeie G, Quiros JR, Duell EJ, Sanchez MJ, Salmeron D, Barricarte A, Chamosa S, Ericson U, Sonestedt E, Nilsson LM, Idahl A, Khaw KT, Wareham N, Travis RC, Rinaldi S, Romieu I, Patel CJ, Riboli E and Gunter MJ. Investigation of dietary factors and endometrial cancer risk using a nutrient-wide association study approach in the EPIC and Nurses' Health Study (NHS) and NHSII. Cancer Epidemiol Biomarkers Prev 2015; 24: 466-471.

[8] Meyer LA, Westin SN, Lu KH and Milam MR. Genetic polymorphisms and endometrial cancer risk. Expert Rev Anticancer Ther 2008; 8: 1159-1167.

[9] Deming SL, Zheng W, Xu WH, Cai Q, Ruan Z, Xiang YB and Shu XO. UGT1A1 genetic polymorphisms, endogenous estrogen exposure, soy food intake, and endometrial cancer risk. Cancer Epidemiol Biomarkers Prev 2008; 17: 563-570.

[10] Ghavami S, Kerkhoff C, Los M, Hashemi M, Sorg C and Karami-Tehrani F. Mechanism of apoptosis induced by S100A8/A9 in colon cancer cell lines: the role of ROS and the effect of metal ions. J Leukoc Biol 2004; 76: 169-175.

[11] Rashedi I, Panigrahi S, Ezzati P, Ghavami S and Los M. Autoimmunity and apoptosis--therapeutic implications. Curr Med Chem 2007; 14: 3139-3151.

[12] Ghavami S, Hashemi M, Ande SR, Yeganeh B, Xiao W, Eshraghi M, Bus CJ, Kadkhoda K, Wiechec E, Halayko AJ and Los M. Apoptosis and cancer: mutations within caspase genes. J Med Genet 2009; 46: 497-510.

[13] Ghavami S, Mutawe MM, Hauff K, Stelmack GL, Schaafsma D, Sharma P, McNeill KD, Hynes TS, Kung SK, Unruh H, Klonisch T, Hatch GM, Los M and Halayko AJ. Statin-triggered cell death in primary human lung mesenchymal cells involves p53-PUMA and release of Smac and Omi but not cytochrome c. Biochim Biophys Acta 2010; 1803: 452-467.

[14] Ghavami S, Mutawe MM, Sharma P, Yeganeh B, McNeill KD, Klonisch T, Unruh H, Kashani HH, Schaafsma D, Los M and Halayko AJ. Mevalonate cascade regulation of airway mesenchymal cell autophagy and apoptosis: a dual role for p53. PLoS One 2011; 6: e16523.

[15] Chaabane W, Cieslar-Pobuda A, El-Gazzah M, Jain MV, Rzeszowska-Wolny J, Rafat M, Stetefeld J, Ghavami S and Los MJ. Human-gyrovirus-Apoptin triggers mitochondrial death pathway--Nur77 is required for apoptosis triggering. Neoplasia 2014; 16: 679-693.

[16] Rollano Penaloza OM, Lewandowska M, Stetefeld J, Ossysek K, Madej M, Bereta J, Sobczak M, Shojaei S, Ghavami S and Los MJ. Apoptins: selective anticancer agents. Trends Mol Med 2014; 20: 519-528.

[17] Ke X, Collins A and Ye S. PIRA PCR designer for restriction analysis of single nucleotide polymorphisms. Bioinformatics 2001; 17: 838-839.

[18] Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics 2006; 22: 1928-9.

[19] Maki M, Suzuki H and Shibata H. Structure and function of ALG-2, a penta-EF-hand calcium-dependent adaptor protein. Sci China Life Sci 2011; 54: 770-779.

[20] Maki M, Yamaguchi K, Kitaura Y, Satoh H and Hitomi K. Calcium-induced exposure of a hydrophobic surface of mouse ALG-2, which is a member of the penta-EF-hand protein family. J Biochem 1998; 124: 1170-1177.

[21] Montaville P, Dai Y, Cheung CY, Giller K, Becker S, Michalak M, Webb SE, Miller AL and Krebs J. Nuclear translocation of the calcium-binding protein ALG-2 induced by the RNA-binding protein RBM22. Biochim Biophys Acta 2006; 1763: 1335-1343.

[22] Vito P, Lacana E and D'Adamio L. Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. Science 1996; 271: 521-525.

[23] Jung YS, Kim KS, Kim KD, Lim JS, Kim JW and Kim E. Apoptosis-linked gene 2 binds to the death domain of Fas and dissociates from Fas during Fas-mediated apoptosis in Jurkat cells. Biochem Biophys Res Commun 2001; 288: 420-426.

[24] Qin J, Li D, Zhou Y, Xie S, Du X, Hao Z, Liu R, Liu X, Liu M and Zhou J. Apoptosis-linked gene 2 promotes breast cancer growth and metastasis by regulating the cytoskeleton. Oncotarget 2017; 8: 2745-2757.

[25] la Cour JM, Hoj BR, Mollerup J, Simon R, Sauter G and Berchtold MW. The apoptosis linked gene ALG-2 is dysregulated in tumors of various origin and contributes to cancer cell viability. Mol Oncol 2008; 1: 431-439.

[26] Su D, Xu H, Feng J, Gao Y, Gu L, Ying L, Katsaros D, Yu H, Xu S and Qi M. PDCD6 is an independent predictor of progression free survival in epithelial ovarian cancer. J Transl Med 2012; 10: 31.

[27] la Cour JM, Mollerup J, Winding P, Tarabykina S, Sehested M and Berchtold MW. Up-regulation of ALG-2 in hepatomas and lung cancer tissue. Am J Pathol 2003; 163: 81-89.

[28] Wang X, Zuo D, Yuan Y, Yang X, Hong Z and Zhang R. MicroRNA-183 promotes cell proliferation via regulating programmed cell death 6 in pediatric acute myeloid leukemia. J Cancer Res Clin Oncol 2017; 143: 169-180.

[29] Yamada Y, Arao T, Gotoda T, Taniguchi H, Oda I, Shirao K, Shimada Y, Hamaguchi T, Kato K, Hamano T, Koizumi F, Tamura T, Saito D, Shimoda T, Saka M, Fukagawa T, Katai H, Sano T, Sasako M and Nishio K. Identification of prognostic biomarkers in gastric cancer using endoscopic biopsy samples. Cancer Sci 2008; 99: 2193-2199.

[30] Hoj BR, la Cour JM, Mollerup J and Berchtold MW. ALG-2 knockdown in HeLa cells results in G2/M cell cycle phase accumulation and cell death. Biochem Biophys Res Commun 2009; 378: 145-148.
[31] Zhang D, Wang F, Pang Y, Zhao E, Zhu S, Chen F and Cui H. ALG2 regulates glioblastoma cell proliferation, migration and tumorigenicity. Biochem Biophys Res Commun 2017; 486: 300-306.
[32] Gu W, Gao T, Shen J, Sun Y, Zheng X, Wang J, Ma J, Hu XY, Li J and Hu MJ. MicroRNA-183 inhibits apoptosis and promotes proliferation and invasion of gastric cancer cells by targeting PDCD4. Int J Clin Exp Med 2014; 7: 2519-2529.
[33] Yoon JH, Choi YJ, Kim SG, Nam SW, Lee JY and Park WS. Programmed cell death 6 (PDCD6) as a prognostic marker for gastric cancers. Tumour Biol 2012; 33: 485-494.
[34] Rho SB, Song YJ, Lim MC, Lee SH, Kim BR and Park SY. Programmed cell death 6 (PDCD6) inhibits angiogenesis through PI3K/mTOR/p70S6K pathway by interacting of VEGFR-2. Cell Signal 2012; 24: 131-139.
[35] Suzuki K, Dashzeveg N, Lu ZG, Taira N, Miki Y and Yoshida K. Programmed cell death 6, a novel p53-responsive gene, targets to the nucleus in the apoptotic response to DNA damage. Cancer Sci 2012; 103: 1788-1794.
[36] Lawo S, Bashkurov M, Mullin M, Ferreria MG, Kittler R, Habermann B, Tagliaferro A, Poser I, Hutchins JR, Hegemann B, Pinchev D, Buchholz F, Peters JM, Hyman AA, Gingras AC and Pelletier L. HAUS, the 8-subunit human Augmin complex, regulates centrosome and spindle integrity. Curr Biol 2009; 19: 816-826.
[37] Nikonova AS, Astsaturov I, Serebriiskii IG, Dunbrack RL Jr and Golemis EA. Aurora A kinase (AURKA) in normal and pathological cell division. Cell Mol Life Sci 2013; 70: 661-687.
[38] Mohan R and John A. Microtubule-associated proteins as direct crosslinkers of actin filaments and microtubules. IUBMB Life 2015; 67: 395-403.
[39] Li X, Rydzewski N, Hider A, Zhang X, Yang J, Wang W, Gao Q, Cheng X and Xu H. A molecular mechanism to regulate lysosome motility for lysosome positioning and tubulation. Nat Cell Biol 2016; 18: 404-417.
[40] Godinho SA and Pellman D. Causes and consequences of centrosome abnormalities in cancer. Philos Trans R Soc Lond B Biol Sci 2014; 369.