Correlation between single nucleotide polymorphisms of DACH1 gene microRNA binding site and susceptibility of patients with endometrial cancer

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Abstract

Objective: To study the relationship between single nucleotide polymorphism (SNP) of the 3′ primer untranslated region (UTR) variants of the cell fate determination factor Dachshund 1 (DACH1) gene and the susceptibility of patients with endometrial cancer (EC).

Methods: Genomic DNA was extracted from the peripheral venous blood of 235 EC patients and 235 healthy controls, and the DACH1 gene rs9285274, rs9529895, rs17088351, and rs59352399 loci were analyzed by Sanger sequencing. Patients progression-free survival (PFS) was recorded after 3 years follow-up from October 2016 to October 2019.

Results: Carriers of the C allele of the DACH1 gene rs9529895 locus had a significantly lower risk for EC than T allele carriers (odds ratio = 0.56, 95% confidence interval: 0.38–0.84, \( P = .01 \)). The correlation between DACH1 gene rs9529895 locus SNP and the risk for EC was affected by age, body mass index, smoking, drinking, and diabetes. Age, and rs9285274, rs9529895, and rs59352399 locus SNP were the best models for predicting the risk for EC. The accuracy rate was 57.02%, and the Cross-validation Consistency was 10/10 (\( P = 4.33 \text{,} \ 0.01 \)). The DACH1 gene rs9529895 locus C allele (TC+CC) carriers had significantly higher PFS than the TT genotype carriers (\( P = .04 \)). The DACH1 gene was expressed in decreased amounts in the cancer tissues of EC patients, and the DACH1 mRNA expression level in the CC genotype, TC genotype, and TT genotype of rs9529895 locus was also decreased (\( P = .02 \)).

Conclusion: DACH1 gene rs9529895 locus SNP is significantly related to the risk for EC and PFS of EC patients. The possible mechanism behind this relationship is that the DACH1 gene rs9529895 locus SNP affects DACH1 expression level.

Abbreviations: BMI = body mass index, CI = confidence interval, DACH1 = dachshund family transcription factor 1, EC = endometrial cancer, ECL = enhanced chemiluminescence, MAF = minor allele frequency, MDR = dimensionality reduction method, OR = odds ratio, PFS = progression-free survival, PVDF = polyvinylidene fluoride membrane, qRT-PCR = Quantitative Real-time PCR, SNP = single nucleotide polymorphism, UTR = untranslated region.

Keywords: cell fate determination factor Dachshund 1, endometrial cancer, progression-free survival, single nucleotide polymorphisms

1. Introduction

Endometrial cancer (EC) is one of the 3 major malignant tumors that most frequently occur in female reproductive organs and is common in postmenopausal women. In recent years, clinical statistics have shown that the incidence of EC has increased worldwide, and it is increasingly being diagnosed in younger women.\textsuperscript{[1,2]} Although the 5-year survival rate of patients with EC has been significantly improved after early treatment, the prognosis of patients with advanced, poorly differentiated EC, or special types of EC is often poor.\textsuperscript{[3–5]}

The occurrence of EC is determined by a variety of factors, including endocrine factors, inflammation, family susceptibility or history, genetic factors, growth factors, dietary habits, the immune system, and external environmental factors.\textsuperscript{[6–9]} Epidemiological studies show that the risk factors for EC include long-term estrogen treatment, tamoxifen treatment, polycystic ovary syndrome, a history of infertility, irregular menstrual cycle, early age of menarche, late menopause, obesity, diabetes, hypertension, etc.\textsuperscript{[9]}

However, the occurrence and development mechanism behind the occurrence of EC has not been fully elucidated, and current early diagnosis and prognosis detection are imperfect. To find the oncogene or tumor suppressor gene that can predict malignant potential of EC will be helpful for early clinical diagnosis, treatment, and prognostic evaluation.
The dachshund family transcription factor 1 (DACH1) is a gene homologous to the mammalian Drosophila salami gene, and is a helix-turn-helix nuclear protein that determines cell fate. In recent years, DACH1 has been receiving more and more attention as a newly discovered tumor suppressor gene. For example, Wu et al. found that DACH1 in breast cancer can block the DNA synthesis of tumor epithelial cells, and inhibits the formation and growth of tumor colonies by inhibiting the activity of cyclin D1 (cyclin D1) and c-Jun, and can thus prevent tumor cell proliferation. Lee et al. found that, in addition to inhibiting cyclin D1, DACH1 can also inhibit several key embryonic stem cell regulatory factors, such as Nanog and Sox2, that can inhibit breast cancer cell proliferation. DACH1 suppresses the proliferation of prostate tumor cells by inhibiting androgen receptor signal transduction in prostate cancer. Indeed, the downregulation of DACH1 protein also affects the prognosis of tumor patients and reduces the survival rate of breast cancer patients. A study by Zhou et al. found that DACH1 is associated with progesterin resistance in EC, and DACH1 inhibits epithelial-to-mesenchymal transition through c-Jun through the Notch 1 pathway in order to retain the response to progesterin. The study by Nan et al. showed that the downregulation of DACH1 expression may be related to EC progression. This shows that DACH1 acts as a tumor suppressor gene in the occurrence and development of EC.

Whether the DACH1 gene polymorphism is related to disease is currently less of a concern. In this study, we selected 3 primer UTR variant SNP sites with minor allele frequency (MAF) > 0.01 from the dbSNP database, namely rs9285274, rs9529895, rs17088351, and rs59352399, to study the correlation between these SNP loci and the risk and prognosis of EC. These SNP loci are found in large proportions in the Chinese Han population. It can be seen from the 1000 genomes database (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) that the MAF of rs9285274, rs9529895, rs17088351, and rs59352399 are respectively 0.02, 0.15, 0.05, and 0.05. Thus, studying this part of the population is significant for further understanding the pathogenesis of EC.

2. Materials and methods

2.1. Subjects

A total of 235 EC patients with clinical data from who underwent total abdominal hysterectomy and appendectomy in the Affiliated Hospital of Hangzhou Normal University from January 2014 to October 2016 were recruited in the study. The patients were aged between 42 and 85 years, with an average of 59.65 ± 8.32 years old. All patients were not treated with radiotherapy, chemotherapy, or hormones prior to surgery. All pathological sections from the patients were determined to be indicative of EC by 2 individual pathologists at the same time. We selected 235 healthy subjects from the health examination center according to the age matching of EC patients as the control group, aged 32 to 81 years, with an average of 59.10 ± 9.84 years. Patients with a history of tumor and systemic immune system disease were excluded. This study was conducted with the approval of the Affiliated Hospital of Hangzhou Normal University ethics committee, and both EC patients and the control subjects signed informed consent.

2.2. Genotyping of DACH1 SNP

We used the Gentra Puregene DNA extraction kit (Minneapolis, MN) to extract genomic DNA from 3 ml of venous blood extracted from EC patients and control groups. Sanger sequencing was performed using the extracted genomic DNA as a template. Primer sequence information: rs9285274: 5'-ACC TTC CTT GTG TTG TTT ATG TTT-3' (Fw); 5'-TTC ATTT CAA ACG TGG TAG TGG T-3' (Rv); rs9529895: 5'-ACC ACT ACC AGT TTG GAA TGA A-3' (Fw); 5'-TGG ATA TCT GAA AAC AGG CAT TGA-3' (Rv); rs17088351: 5'-AGC TTC TCA ACC CTA ATG A-3' (Fw); 5'-ACC ACA TGT TCC AAA ACA AGG C-3' (Rv); rs59352399: 5'-AGC TGG TTA TGT AGA TCA GTG CT-3' (Fw); 5'-AGT GGC AAC ACT ATC CAC AAC-3' (Rv). Ten percent of the samples were randomly selected for repeat verification.

2.3. Real-time PCR (qRT-PCR)

According to the manufacturers instructions, TRizol reagent (Invitrogen) was used to extract RNA from surgically excised cancerous tissue as well as normal adjacent tissues 5 cm away from the cancerous tissue. Using the extracted RNA as a template, cDNA was synthesized by reverse transcription. SYBR Green PCR Master Mix (Applied Biosystems) kit was used to detect the expression level of DACH1 mRNA relative to GAPDH on the BioRad CFX96 system (BioRad Laboratories, Inc., Berkeley, CA, USA) in triplicate. Reaction conditions: 95°C, 5 minutes, 1 cycle; 95°C, 10 seconds, 45 cycles; 68.7°C, 30 seconds, 45 cycles, 72°C, 30 seconds, 45 cycles, 30 seconds, 45 cycles; 95°C, 15 seconds; 60°C, 60 seconds; 95°C, 15 seconds; 60°C, 15 seconds; 1 cycle.

2.4. Real-time PCR (qRT-PCR)

According to the manufacturers instructions, TRizol reagent (Invitrogen) was used to extract RNA from surgically excised cancerous tissue and normal tissues located 5 cm away from the cancerous tissue. Using the extracted RNA as a template, cDNA was synthesized by reverse transcription. SYBR Green PCR Master Mix (Applied Biosystems) kit was used to detect the expression level of DACH1 mRNA relative to GAPDH on the BioRad CFX96 system (BioRad Laboratories, Inc., Berkeley, CA, USA) in triplicate. Reaction conditions: 95°C, 5 minutes, 1 cycle; 95°C, 10 seconds, 45 cycles; 68.7°C, 30 seconds, 45 cycles, 72°C, 30 seconds, 45 cycles, 30 seconds, 45 cycles; 95°C, 15 seconds; 60°C, 60 seconds; 95°C, 15 seconds; 60°C, 15 seconds; 1 cycle. Primers Information: DACH1: 5'-TAG AGC GAG ACC CAC ACA AC-3' (Fw); 5'-AGG AAC AGG TCG AAA GCC TG-3' (Rv); GAPDH primer sequence 5'-GGG TGA TGC AGG TGC TAC TT-3' (Fw); 5'-GGT GTT CTT AAC ACG AGA-3' (Rv).

2.5. Western blot analysis

The RIPA buffer (Cell Signaling Technology, Inc., MA, USA) was used to isolate proteins from both cancerous and normal tissues. Protein (50ng) were boiled at high temperature and loaded onto a 12% SDS-polyacrylamide gel. The proteins were then separated and transferred to a polyvinylidene fluoride membrane (PVDF). After this, the proteins were incubated with 5% skim milk powder for 2 hours at room temperature, and then incubated with DACH1 and β-actin antibodies (anti-DACH1: ab176718; anti-β-actin, ab179467, Abcam, UK) at 4°C for 12–16 hours. After incubation with the secondary antibody for 2 hours at room temperature and color development with enhanced chemiluminescence (ECL) reagent, β-actin was used as an internal control in triplicate.
2.6. Follow-up
Follow-up was performed between October 2016 and October 2019 for 3 years after the operation and the patients survival was recorded.

2.7. Statistical analysis
In this study, GraphPad prism 7 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis. Continuous variables were presented as mean ± SD. Statistical analysis was performed using t-test and one-way analysis of variance. The x² test was used to assess whether the genotype frequency was consistent with the Hardy–Weinberg equilibrium. Binary logistic regression was used to adjust for age, body mass index (BMI), smoking, drinking, and diabetes. The odds ratio (OR) and its 95% confidence interval (CI) were used to evaluate the correlation between genotype and allele frequency of the SNP loci of DACH1 and the risk for EC. The multifactor dimensionality reduction method (MDR) 3.0.2 software was used to analyze the effects of the interactions between SACH loci of DACH1 and age, BMI, smoking, drinking, diabetes, and other factors contributing to EC risk. A P value of < .05 indicated statistically significant differences.

3. Results

3.1. General Information
Among the 235 EC patients we selected in this study, 37 had TNM stage I, 51 had stage II, 96 had stage III, 32 had stage IVa, and 19 had stage IVb EC. The results of analysis and comparison of general data of EC patients and control group showed that there was no difference between the general data from the EC group patients, such as age, BMI, smoking, and drinking status (P > .05). The proportion of patients with diabetes in EC patients was significantly higher than that in the control group (P < .01) (Table 1).

3.2. DACH1 gene SNP locus genotype and allele frequency and risk of EC disease
We analyzed 235 healthy control groups selected in this study. The results showed that the DACH1 rs9285274, rs9529895, rs17088351, and rs59352399 genotype frequency distributions in the selected control group were in accordance with the Hardy–Weinberg equilibrium (P > .05, Table 2). From the results of the analysis, it can be seen that there was no statistically significant difference in the frequency of alleles and alleles of DACH1 rs9285274, rs17088351, and rs59352399 between EC patients and the control group (P > .05). There was no significant change in the risk for EC in the dominant and recessive models of each SNP locus (P > .05). It is suggested that the SNP of rs9285274, rs17088351, and rs59352399 of the DACH1 gene has no significant correlation with the risk for EC.

However, an analysis of the different genotypes and allele frequencies at the rs9529895 locus of DACH1 revealed that, compared with the TT genotype, TC genotype carriers had a significantly reduced risk for EC (OR = 0.54, 95% CI: 0.34–0.87, P = .01). There was no significant difference in the CC genotype frequency between EC patients and the control group (P = .31). The risk for EC decreased significantly in the dominant model (OR = 0.53, 95% CI: 0.34–0.83, P < .01), but the risk for EC in the recessive model did not change significantly (OR = 0.55, 95% CI: 0.18–1.65, P = .42). Carriers of the C allele at the rs9529895 locus of the DACH1 gene had a significantly lower risk for EC than T allele carriers (OR = 0.56, 95% CI: 0.38–0.84, P < .01).

3.3. Hierarchical analysis of general clinical characteristics
Generally, the age range of patients was limited to 60 years. Patients above 60 years (≥60 years) of age were considered as elderly, and those below 60 years (<60 years) of age were considered young. BMI was based with the average BMI of subjects considered young. BMI was based with the average BMI of subjects considered elderly, and those below 60 years (<60 years) of age were considered elderly. BMI was based with the average BMI of subjects considered elderly. BMI was based with the average BMI of subjects considered elderly.

Patients above 60 years (≥60 years) were considered as elderly, nonobese patients, and nonobese patients. Analyses results showed that there was no statistically significant difference in the risk for EC among different genotypes of the DACH1 gene. Patients with BMI ≥ 25.9 kg/m² were considered as obese patients, and patients with BMI < 25.9 kg/m² were considered as nonobese patients. Analyses results showed that there was no statistically significant difference in the risk for EC among different genotypes of the DACH1 gene. Patients with BMI ≥ 25.9 kg/m² were considered as obese patients, and patients with BMI < 25.9 kg/m² were considered as nonobese patients.

The results showed that the risk for EC in the carriers of C allele (TC + CC) at rs9529895 of DACH1 was significantly lower than patients who were carriers of the TT genotype (P < .05, Table 4) only in the young, obese, people without smoking and drinking history, and diabetes history. However, in elderly patients, nonobese patients, nonsmoking, non-drinking, and diabetic population, there was no significant difference in the risk for EC in carriers of the C allele (TC + CC) at rs9529895 of DACH1 compared with carriers of the TT genotype (P > .05, Table 4). The results showed that the correlation between SNP at rs9529895 of DACH1 and EC risk was affected by age, BMI, smoking and drinking habits, and the presence of diabetes.

Our analysis results showed that the risk for EC in the carriers of C allele (TC + CC) at rs9529895 of DACH1 was significantly lower than patients who were carriers of the TT genotype (P < .05, Table 4) only in the young, obese, people without smoking and drinking history, and diabetes history. However, in elderly patients, nonobese patients, nonsmoking, non-drinking, and diabetic population, there was no significant difference in the risk for EC in carriers of the C allele (TC + CC) at rs9529895 of DACH1 compared with carriers of the TT genotype (P > .05, Table 4). The results showed that the correlation between SNP at rs9529895 of DACH1 and EC risk was affected by age, BMI, smoking and drinking habits, and the presence of diabetes.

3.4. Multidimensional dimensionality reduction (MDR) analysis of the interaction between DACH1 gene SNP and general clinical characteristics
In order to further study the correlation between the DACH1 SNP and the risk for EC disease, we used MDR to analyze the effect of the interaction between the DACH1 SNP and the general clinical characteristics in the control group.
clinical characteristics of subjects of the risk for EC disease. The influence of the interaction between various factors on the risk for EC disease can be seen in the circle diagram (Fig. 1A). The dendrogram results show the strongest interactions between age, BMI, smoking, drinking, and diabetes. This shows that the PFS of rs9529895 locus C allele (TC + CC) of the DACH1 gene was significantly higher than that of patients who are TT genotype carriers. The difference was statistically significant (P = .04, Fig. 2B).

3.5. Correlation between DACH1 gene SNP and progression-free survival (PFS) in EC patients

We conducted a 3-year follow-up on EC patients and analyzed the correlation between the PFS of patients with different genotypes at rs9285274, rs9529895, and rs59352399 at DACH1. The results showed that there was no statistically significant difference in PFS between EC patients with different genotypes at rs9285274, rs17088351, and rs59352399 at DACH1 (P > .05, Fig. 2A, C, D). The results of our analysis showed that the PFS of rs9529895 locus C allele (TC + CC) of the DACH1 gene was significantly higher than that of patients who

| rs9285274 | Control | OR (95% CI) | P value | Dominant model | Recessive model |
|-----------|---------|-------------|---------|----------------|----------------|
| TT        | 209 (88.94%) 210 (89.36%) | Reference | TT       | 1.02 (0.73–1.34) | 1.44 (0.61–2.5) |
| TC        | 21 (8.94%) 23 (8.79%) | 0.96 (0.65–1.39) | 0.95 (0.60–1.92) | 1.43 (0.60–2.9) |
| CC        | 5 (2.13%) 2 (0.85%) | 0.58 (0.34–0.87) | 0.49 (0.16–1.47) | 0.55 (0.18–1.65) |

| rs9529895 | Control | OR (95% CI) | P value | Dominant model | Recessive model |
|-----------|---------|-------------|---------|----------------|----------------|
| TT        | 196 (83.40%) 171 (72.77%) | Reference | TT       | 0.99 (0.68–1.5) | 0.96 (0.66–1.39) |
| TC        | 34 (14.47%) 55 (23.40%) | 0.54 (0.34–0.87) | 0.49 (0.16–1.47) | 0.55 (0.18–1.65) |
| CC        | 5 (2.13%) 9 (3.83%) | 0.56 (0.38–0.84) | 0.34 (0.16–0.73) | 0.55 (0.18–1.65) |

We analyzed the correlation between the DACH1 rs9285274, rs9529895, and rs59352399 loci of the DACH1 gene and EC risk. Correlation between genotype frequency of rs9285274 of Dach1 gene and EC risk.

| Table 2 Correlation between genotype and allele frequency of DACH1 microRNA binding site and EC risk. |
|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| rs9285274 | Control | OR (95% CI) | P value | Dominant model | Recessive model |
|-----------|---------|-------------|---------|----------------|----------------|
| TT        | 209 (88.94%) 210 (89.36%) | Reference | TT       | 1.02 (0.73–1.34) | 1.44 (0.61–2.5) |
| TC        | 21 (8.94%) 23 (8.79%) | 0.96 (0.65–1.39) | 0.95 (0.60–1.92) | 1.43 (0.60–2.9) |
| CC        | 5 (2.13%) 2 (0.85%) | 0.58 (0.34–0.87) | 0.49 (0.16–1.47) | 0.55 (0.18–1.65) |

3.6. Correlation between DACH1 gene SNP and general information of EC patients

We analyzed the correlation between the DACH1 rs9285274, rs9529895, and rs59352399 loci genotype and general clinical data of EC patients. The results showed that there were no significant differences in age and BMI between patients with different genotypes at the rs9285274, rs9529895, and rs59352399 loci of the DACH1 gene (P > .05, Fig. 3A–H). This shows that the DACH1 rs9285274, rs9529895, and rs59352399 loci of the DACH1 gene have no significant correlation with age and BMI.

| Table 3 Correlation between genotype frequency of rs9285274 of Dach1 gene and EC risk. |
|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| rs9285274 | Control | OR (95% CI) | P value | Dominant model | Recessive model |
|-----------|---------|-------------|---------|----------------|----------------|
| TT        | 145 (91.19%) 158 (88.76%) | Reference | TT       | 1.02 (0.73–1.34) | 1.44 (0.61–2.5) |
| TC+CC     | 14 (8.81%) 20 (11.24%) | 0.76 (0.37–1.57) | 0.49 (0.16–1.47) | 0.55 (0.18–1.65) |
| TT        | 64 (84.21%) 52 (91.23%) | Reference | TT       | 1.02 (0.73–1.34) | 1.44 (0.61–2.5) |
| TC+CC     | 12 (15.79%) 22 (8.77%) | 1.95 (0.65–5.89) | 0.56 (0.38–0.84) | 0.96 (0.66–1.39) |

We extracted total RNA from 235 patients with surgically resected cancer tissues and adjacent normal tissues, and detected clinical characteristics of subjects of the risk for EC disease. The influence of the interaction between various factors on the risk for EC disease can be seen in the circle diagram (Fig. 1A). The dendrogram results show the strongest interactions between age, BMI, smoking, drinking, and diabetes. This shows that the PFS of rs9529895 locus C allele (TC + CC) of the DACH1 gene was significantly higher than that of patients who are TT genotype carriers. The difference was statistically significant (P = .04, Fig. 2B).
Diabetes TC genotype, and TT genotype of rs9529895 loci decreased in expression level in tumor tissues of patients with CC genotype, univariate analysis of variance showed that the of EC patients revealed that the patients with different genotypes of rs9285274, rs17088351, and (Fig. 4B). Further analysis of the correlation between the was lower in EC patients with advanced tumor progression was underexpressed in cancer tissues, and the Western Blot test for age, BMI, smoking, drinking, diabetes. Although the specific etiology of EC has not yet been fully elucidated, studies have shown that there are some factors that increase the risk associated with the incidence of EC, such as obesity, diabetes, hypertension, the use of exogenous estrogen, menstrual history, birth history, the effects of oral contraceptives, and genetic family history. We know that

| Table 4 | Correlation between genotype frequency of rs9529895 of DACH1 and risk of EC disease. |
|---------|---------------------------------|
|          | TC (n=235) | CC (n=235) | OR (95%CI) | P value* |
| Age (years) |          |
| <60       | TT 135 (84.91%) 131 (73.60%) Reference |
|           | TC+CC 24 (15.09%) 47 (26.40%) 0.50 (0.29–0.86) .02 |
| ≥60       | TT 61 (80.26%) 40 (70.18%) Reference |
|           | TC+CC 15 (19.74%) 17 (29.82%) 0.58 (0.26–1.29) .25 |
| BMI (kg/m²) |          |
| <25.9     | TT 115 (82.73%) 100 (74.07%) Reference |
|           | TC+CC 24 (17.27%) 35 (25.93%) 0.60 (0.33–1.07) .11 |
| ≥25.9     | TT 81 (84.38%) 71 (71.00%) Reference |
|           | TC+CC 15 (15.63%) 29 (29.00%) 0.45 (0.23–0.91) .04 |
| Smoking   |          |
| Yes       | TT 23 (92.00%) 16 (69.57%) Reference |
|           | TC+CC 2 (8.00%) 7 (30.43%) 0.20 (0.04–1.08) .11 |
| No        | TT 173 (82.38%) 155 (73.11%) Reference |
|           | TC+CC 37 (17.62%) 57 (26.89%) 0.58 (0.37–0.93) .03 |
| Drinking  |          |
| Yes       | TT 31 (91.18%) 23 (63.89%) Reference |
|           | TC+CC 3 (8.82%) 13 (36.11%) 0.17 (0.04–0.67) .02 |
| No        | TT 165 (82.00%) 148 (74.37%) Reference |
|           | TC+CC 36 (17.91%) 51 (25.63%) 0.63 (0.39–1.02) .08 |
| Diabetes  |          |
| Yes       | TT 26 (92.86%) 5 (100.00%) Reference |
|           | TC+CC 2 (7.14%) 0 (0%) 1.19 (0.24–1.26) .54 |
| No        | TT 170 (82.13%) 166 (72.71%) Reference |
|           | TC+CC 37 (17.87%) 64 (27.29%) 0.57 (0.36–0.89) .02 |

* Adjusted for age, BMI, smoking, drinking, diabetes.

| Table 5 | Correlation between genotype frequency of rs17088351 of DACH1 and risk of EC disease. |
|---------|---------------------------------|
|          | TC (n=235) | CC (n=235) | OR (95%CI) | P value* |
| Age (years) |          |
| <60       | CC 143 (89.94%) 159 (89.33%) Reference |
|           | CT+TT 16 (10.06%) 19 (10.67%) 0.94 (0.46–1.89) .85 |
| ≥60       | CC 68 (89.47%) 49 (85.96%) Reference |
|           | CT+TT 6 (10.53%) 8 (14.04%) 0.72 (0.25–2.05) .73 |
| BMI (kg/m²) |          |
| <25.9     | CC 126 (90.65%) 119 (88.15%) Reference |
|           | CT+TT 13 (9.35%) 16 (11.85%) 0.77 (0.35–1.66) .63 |
| ≥25.9     | CC 85 (88.54%) 89 (89.00%) Reference |
|           | CT+TT 11 (11.46%) 11 (11.00%) 1.05 (0.43–2.54) .92 |
| Smoking   |          |
| Yes       | CC 21 (84.00%) 19 (82.61%) Reference |
|           | CT+TT 4 (16.00%) 4 (17.39%) 0.91 (0.20–4.13) .90 |
| No        | CC 190 (90.48%) 189 (89.15%) Reference |
|           | CT+TT 20 (9.52%) 23 (10.85%) 0.87 (0.46–1.63) .65 |
| Drinking  |          |
| Yes       | CC 29 (85.29%) 26 (77.78%) Reference |
|           | CT+TT 5 (14.71%) 8 (22.22%) 0.60 (0.18–2.07) .42 |
| No        | CC 182 (90.55%) 180 (89.45%) Reference |
|           | CT+TT 19 (9.45%) 19 (9.55%) 0.99 (0.51–1.93) .97 |
| Diabetes  |          |
| Yes       | CC 23 (82.14%) 3 (60.00%) Reference |
|           | CT+TT 5 (17.86%) 2 (40.00%) 0.33 (0.04–2.40) .27 |
| No        | CC 188 (90.82%) 205 (89.13%) Reference |
|           | CT+TT 19 (9.18%) 25 (10.87%) 0.83 (0.44–1.55) .56 |

* Adjusted for age, BMI, smoking, drinking, diabetes.

4. Discussion

In this case-control study, we found that the SNP at rs9529895 at the 3 primer UTR region of the DACH1 gene was significantly correlated with the risk of EC and PFS. This correlation was related to the subjects age, BMI, smoking and drinking habits, and the presence of diabetes. From the results of the analysis, the DACH1 rs9529895 locus C allele is a protective factor for the occurrence of EC, and EC patients with the C allele have better prognoses than patients who are T allele carriers.

Due to the combined effects of environmental and lifestyle factors, the incidence of EC has recently been increasing and patients who develop EC are becoming younger as well. An in-depth study of the pathogenesis of EC is urgently needed for the prevention and treatment of EC. Although the specific etiology of EC has not yet been fully elucidated, studies have shown that there are some factors that increase the risk associated with the incidence of EC, such as obesity, diabetes, hypertension, the use of exogenous estrogen, menstrual history, birth history, the effects of oral contraceptives, and genetic family history.
The occurrence of malignant tumors is related to oncogene activation and tumor suppressor gene inactivation. With the rapid developments in molecular biology and immune technology, new oncogenes, and tumor suppressor genes are being continuously discovered, providing new indicators for the early diagnosis and treatment of EC.

The **DACH1** gene is a newly discovered tumor suppressor gene that participates in cell signaling pathways such as Ey/Pax6, So/Six, eya/Eya. It plays an important role in the differentiation of *Drosophila* eyes as well as a decisive role in the direction of cell differentiation in mammals.[23] DACH1 binds to cyclin D1 and prevents tumor cell proliferation by blocking tumor epithelial cell DNA synthesis and inhibiting the formation and growth of tumor colonies in the tumor stroma.[12] Xu et al.[24] conducted a meta-analysis of a comprehensive database of 19 breast cancer gene expressions. The results showed that the higher the expression of **DACH1**, the longer was the time for death, recurrence, and metastasis in breast cancer patients. Zhao et al.[25] has shown that **DACH1** can inhibit SNAI1-mediated epithelial-to-mesenchymal transition and inhibit breast cancer metastasis. In the present study, we observed that the **DACH1** protein was underexpressed in the cancer tissues of EC patients and overexpressed in normal tissues adjacent to the cancerous tissue, indicating that **DACH1** may play a role as a tumor suppressor gene in the occurrence of EC. Study has shown that the low expression of **DACH1** in EC is related to the methylation of CpG islands in the promoter region of **DACH1**. The methylation of **DACH1** is one of the characteristics of tumor suppressor genes.[26]

However, there is a dearth of research on whether the **DACH1** gene SNP affects the risk for EC. We know that differences in genetic background may be related to different rates of EC occurrence. From the results of the present study, the **DACH1** gene rs9285274 locus, rs9529895 locus, rs17088351 locus, and rs59352399 locus are common in the Chinese Han population. In the control group selected in this study, the MAF of rs9285274 was 5.74%, the MAF of rs9529895 was 15.53%, the MAF of rs17088351 was 6.17%, and the MAF of rs59352399 was 5.32%, which are similar to the results found in the 1000 genomes database, which shows that the population selected in this study is sufficiently representative. From the results of the

### Table 6

|                  | EC (n = 235) | Control (n = 235) | OR (95%CI) | P value* |
|------------------|--------------|------------------|------------|----------|
| **Age (year)**   |              |                  |            |          |
| <60              |              |                  |            |          |
| CC               | 143 (89.94%) | 159 (89.33%)     | Reference  |          |
| CT+TT            | 16 (10.06%)  | 19 (10.67%)      | 0.94 (0.46–1.89) | .85      |
| ≥60              |              |                  |            |          |
| CC               | 65 (85.53%)  | 53 (92.98%)      | Reference  |          |
| CT+TT            | 11 (14.47%)  | 4 (7.02%)        | 2.24 (0.68–7.45) | .18      |
| **BMI (kg/m²)**  |              |                  |            |          |
| <25.9            |              |                  |            |          |
| CC               | 123 (88.49%) | 122 (90.37%)     | Reference  |          |
| CT+TT            | 16 (11.51%)  | 13 (9.63%)       | 1.22 (0.56–2.65) | .61      |
| ≥25.9            |              |                  |            |          |
| CC               | 85 (88.54%)  | 90 (90.00%)      | Reference  |          |
| CT+TT            | 11 (14.46%)  | 10 (10.00%)      | 1.17 (0.47–2.88) | .74      |
| **Smoking**      |              |                  |            |          |
| Yes              |              |                  |            |          |
| CC               | 23 (92.00%)  | 22 (95.65%)      | Reference  |          |
| CT+TT            | 8 (8.00%)    | 1 (4.35%)        | 1.30 (0.23–2.00) | .60      |
| No               |              |                  |            |          |
| CC               | 185 (88.10%) | 190 (89.62%)     | Reference  |          |
| CT+TT            | 15 (10.90%)  | 10 (10.38%)      | 1.17 (0.64–2.14) | .62      |
| **Drinking**     |              |                  |            |          |
| Yes              |              |                  |            |          |
| CC               | 30 (88.24%)  | 32 (88.89%)      | Reference  |          |
| CT+TT            | 4 (11.76%)   | 4 (11.11%)       | 1.07 (0.25–4.65) | .93      |
| No               |              |                  |            |          |
| CC               | 178 (85.56%) | 180 (90.45%)     | Reference  |          |
| CT+TT            | 23 (11.44%)  | 19 (9.55%)       | 1.22 (0.64–2.33) | .54      |
| **Diabetes**     |              |                  |            |          |
| Yes              |              |                  |            |          |
| CC               | 25 (89.29%)  | 3 (60.00%)       | Reference  |          |
| CT+TT            | 3 (10.71%)   | 2 (40.00%)       | 0.67 (0.24–1.11) | .32      |
| No               |              |                  |            |          |
| CC               | 183 (84.41%) | 209 (90.87%)     | Reference  |          |
| CT+TT            | 24 (11.59%)  | 21 (9.13%)       | 1.31 (0.70–2.42) | .40      |

*Adjusted for age, BMI, smoking and drinking habits, and presence of diabetes. BMI = body mass index, CI = confidence interval, EC = endometrial cancer, OR = odds ratio.

Figure 1. Multifactor dimensionality reduction (MDR) analysis of the interaction between **DACH1** gene SNPs and general clinical features. A, Age, BMI, Smoking, Alcohol, T2DM, rs9285274, rs9529895, rs17088351, rs59352399. The data at the apex represent the influence of this factor on the risk for EC disease, and the larger the value represents the greater the influence of this factor on the risk for EC disease. The magnitude of the value on the line between the 2 factors represents the magnitude of the interaction between the 2 factors. B, Age, BMI, Smoking, Alcohol, T2DM, rs9285274, rs9529895, rs17088351, rs59352399 factors. The strength of the interaction depends on the proximity of these 2 factors.
analysis in this study, we found that only the rs9529895 locus SNP was associated with the risk for EC and PFS, reflecting the importance of this SNP locus analysis for further studies of EC.

We know that the genetic background of different populations and different living environments may have a certain impact on the risk for EC, so we further analyzed the effects of factors, such as age, BMI, smoking and drinking habits, and history of diabetes. The results showed that the correlation between SNP at rs9529895 locus of DACH1 and EC risk was affected by age, BMI, smoking and drinking habits, and the presence of diabetes. Further analysis of MDR revealed that the interaction model between age, rs9285274, rs9529895, rs59352399, and 4 other factors is the best model for judging the risk for the development of EC. This shows that when analyzing the correlation between the SNP of rs9529895 locus of the DACH1 gene and the risk for EC, the analysis may be more accurate in different populations. It also shows that multifactor interactions play an important role in the pathogenesis of EC, and the impact of a single factor may be limited.

There are some deficiencies in this study that require further investigation. First, the analysis of the correlation between the DACH1 SNP and the risk for EC needs to be verified in larger samples, which can reduce the impact of large errors caused by small samples and increase the accuracy of the results. In addition, whether different alleles of the rs9529895 locus of DACH1 affect the expression of the DACH1 protein is lacking in evidence from in vitro studies. Further feedback on the occurrence and progress of EC also needs to be verified in in vivo models. It is predicted that the DACH1 rs9529895 locus is located in the DACH1 gene 3 prime UTR region. The MirSNP tool (http://bioinfo.bjmu.edu.cn/mirsnp/search/) predicted that the rs9529895 site was located at the target binding site of hsa-miR-570–3p and DACH1. Further investigations are required to determine whether the rs9529895 locus affects the regulation of DACH1 expression by hsa-miR-570–3p.

| Model | Accuracy | $x^2$ | $P$ | CVC |
|-------|----------|-------|-----|-----|
| rs9529895 | 53.83% | 0.30 | .58 | 8/10 |
| Smoking, Alcohol | 54.89% | 0.49 | .48 | 6/10 |
| Smoking, Alcohol, rs9529895 | 55.74% | 0.70 | .40 | 7/10 |
| Age, rs9285274, rs9529895, rs59352399 | 57.02% | 4.33 | .04 | 10/10 |
| Smoking, Alcohol, T2DM, rs9529895 | 56.38% | 0.78 | .38 | 6/10 |
| Smoking, Alcohol, T2DM, rs9529895, rs17088351 | 54.47% | 0.41 | .52 | 4/10 |
| Age, BMI, Smoking, Alcohol, rs9285274, rs9529895, rs59352399 | 53.62% | 0.27 | .60 | 5/10 |
| Age, BMI, Smoking, Alcohol, rs9285274, rs9529895, rs17088351, rs59352399 | 52.38% | 0.18 | .67 | 6/10 |
| Age, BMI, Smoking, Alcohol, T2DM, rs9285274, rs9529895, rs17088351, rs59352399 | 56.17% | 0.77 | .38 | 8/10 |

BMI = body mass index, CVC = cross-validation consistency, T2DM = type 2 diabetes mellitus.

Table 7. Prediction model analysis of the interaction between the DACH1 SNP and general clinical characteristics by multidimensional dimension reduction.
In summary, it can be seen from this study that the SNP at the rs9529895 locus of the DACH1 gene is significantly related to the risk for EC and PFS in EC patients. The possible mechanism is that the SNP at the rs9529895 locus of DACH1 affects the expression level of DACH1, but these results still need to be verified in both in vivo and in vitro models.

**Author contributions**

Liyan Xu: Data curation, Methodology, Formal analysis, Software, Writing – original draft Yafen Qiu and Ling Feng: Formal analysis, Software, Data curation, Visualization Li Zhou and Xufeng Chen: Software, Data curation, Visualization Dongqi Yu: Funding acquisition, Resources, Supervision, Writing – review & editing.

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