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# 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 ($x^2 = 4.33$, $P = .04$). 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, 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

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## 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.[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.[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.[6–8] 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.[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.[10,11] In recent years, *DACH1* has been receiving more and more attention as a newly discovered tumor suppressor gene. For example, Wu et al[12,13] 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[14] 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.[15] Indeed, the downregulation of *DACH1* protein also affects the prognosis of tumor patients and reduces the survival rate of breast cancer patients.[13] A study by Zhou et al[10] found that *DACH1* is associated with progestin resistance in EC, and *DACH1* inhibits epithelial-to-mesenchymal transition through c-Jun through the *Notch1* pathway in order to retain the response to progestin. The study by Nan et al[16] 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 $59.65 \pm 8.32$ 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 $59.10 \pm 9.84$ 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 TGT TTT ATG TTT-3′ (Fw); 5′-TTC ATT CCA AAC TGG TAG TGG T-3′ (Rv); rs9529895: 5′-ACC ACT ACC AGT TTG GAA TGA A-3′ (Fw): 5′-TGC ATA TCT GAA AAG AAG CAT TGA-3′ (Rv): rs17088351: 5′-AGC CTC TTC CAA 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 GTC 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 s; 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'-GGC AGG TTT CTC AAG ACG GA-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 dwarf normal tissues. Protein (50 ng) 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 h 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 $x^2$ 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, $P < .01$).

**Table 1**

**Comparison of general information between EC patients and control groups.**

| | EC (n = 235) | Control (n = 235) | *P* value |
|---|---|---|---|
| Age (years, mean ± SD) | 59.65 ± 8.32 | 59.10 ± 9.84 | .51 |
| BMI (kg/m$^2$, mean ± SD) | 25.99 ± 3.01 | 25.82 ± 2.16 | .48 |
| Smoking [n (%)] | | | .76 |
| Yes | 25 (10.64%) | 23 (9.79%) | |
| No | 210 (89.36%) | 212 (90.21%) | |
| Drinking [n (%)] | | | .80 |
| Yes | 34 (14.47%) | 36 (15.32%) | |
| No | 201 (85.53%) | 199 (84.68%) | |
| Diabets [n (%)] | | | <.01 |
| Yes | 28 (11.91%) | 5 (2.13%) | |
| No | 207 (88.09%) | 230 (97.87%) | |
| TNM stage [n (%)] | | | |
| I | 37 (15.74%) | | |
| II | 51 (21.70%) | | |
| III | 96 (40.85%) | | |
| IV | 51 (21.70%) | | |

BMI = body mass index, EC = endometrial cancer, SD = standard deviation, TNM = tumor node metastasis.

### 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 in this study (25.9 kg/m$^2$) which was considered as the boundary. Patients with BMI ≥ 25.9 kg/m$^2$ were considered as obese patients, and patients with BMI < 25.9 kg/m$^2$ 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* according to age, BMI, smoking and drinking habits, and diabetes in the stratified rs9285274 locus, rs17088351 locus, and rs59352399 locus ($P > .05$, Tables 3 and 5, and Table 6). This indicates that the correlation between SNPs at rs9285274, rs17088351, and rs59352399 at the *DACH1* gene and the risk for EC are not 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, nondrinking, 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 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

**Table 2**

**Correlation between genotype and allele frequency of DACH1 microRNA binding site and EC risk.**

| | EC (n = 235) | Control (n = 235) | *P* value* | OR (95%CI)# | *P* value# |
|---|---|---|---|---|---|
| rs9285274 | | | .14 | | |
| TT | 209 (88.94%) | 210 (89.36%) | | Reference | |
| TC | 21 (8.94%) | 23 (9.79%) | | 0.96 (0.65-1.29) | .91 |
| CC | 5 (2.13%) | 2 (0.85%) | | 1.43 (0.60–1.92) | .45 |
| Dominant model | | | | 1.02 (0.73–1.34) | .88 |
| Recessive model | | | | 1.44 (0.61–1.92) | .25 |
| T | 439 (93.40%) | 443 (94.26%) | | Reference | |
| C | 31 (6.60%) | 27 (5.74%) | | 1.16 (0.68–1.97) | .68 |
| rs9529895 | | | .10 | | |
| TT | 196 (83.40%) | 171 (72.77%) | | Reference | |
| TC | 34 (14.47%) | 55 (23.40%) | | 0.54 (0.34–0.87) | .01 |
| CC | 5 (2.13%) | 9 (3.83%) | | 0.49 (0.16–1.47) | .31 |
| Dominant model | | | | 0.53 (0.34–0.83) | <.01 |
| Recessive model | | | | 0.55 (0.18–1.65) | .42 |
| T | 426 (90.64%) | 397 (84.47%) | | Reference | |
| C | 44 (9.36%) | 73 (15.53%) | | 0.56 (0.38–0.84) | <.01 |
| rs17088351 | | | .21 | | |
| CC | 211 (89.79%) | 208 (88.51%) | | Reference | |
| CT | 20 (8.51%) | 25 (10.64%) | | 0.79 (0.43–1.46) | .55 |
| TT | 4 (1.70%) | 2 (0.85%) | | 1.32 (0.48–1.88) | .43 |
| Dominant model | | | | 0.88 (0.49–1.57) | .77 |
| Recessive model | | | | 1.34 (0.48–1.90) | .68 |
| C | 442 (94.04%) | 441 (93.83%) | | Reference | |
| T | 28 (5.96%) | 29 (6.17%) | | 0.96 (0.56–1.65) | .89 |
| rs59352399 | | | .08 | | |
| CC | 208 (88.51%) | 212 (90.21%) | | Reference | |
| CT | 18 (7.66%) | 21 (8.94%) | | 0.87 (0.45–1.69) | .81 |
| TT | 9 (3.83%) | 2 (0.85%) | | 1.65 (0.96–1.97) | .07 |
| Dominant model | | | | 1.09 (0.78–1.41) | .65 |
| Recessive model | | | | 1.66 (0.96–1.98) | .07 |
| C | 434 (92.34%) | 445 (94.68%) | | Reference | |
| T | 36 (7.66%) | 25 (5.32%) | | 1.48 (0.87–2.50) | .19 |

* Hardy–Weinberg equilibrium assessment results.

# Adjust age, BMI, smoking, drinking, diabetes.

CI = confidence interval, EC = endometrial cancer, OR = odds ratio.

**Table 3**

**Correlation between genotype frequency of rs9285274 of Dach1 gene and EC risk.**

| | EC (n = 235) | Control (n = 235) | OR (95%CI) | *P* value* |
|---|---|---|---|---|
| Age (year) | | | | |
| <60 | | | | |
| TT | 145 (91.19%) | 158 (88.76%) | Reference | |
| TC+CC | 14 (8.81%) | 20 (11.24%) | 0.76 (0.37–1.57) | .58 |
| ≥60 | | | | |
| TT | 64 (84.21%) | 52 (91.23%) | Reference | |
| TC+CC | 12 (15.79%) | 5 (8.77%) | 1.95 (0.65–5.89) | .35 |
| BMI (kg/m$^2$) | | | | |
| <25.9 | | | | |
| TT | 124 (89.21%) | 121 (89.63%) | Reference | |
| TC+CC | 15 (10.79%) | 14 (10.37%) | 1.05 (0.48–2.26) | .91 |
| ≥25.9 | | | | |
| TT | 85 (88.54%) | 89 (89.00%) | Reference | |
| TC+CC | 11 (11.46%) | 11 (11.00%) | 1.05 (0.43–2.54) | .92 |
| Smoking | | | | |
| Yes | | | | |
| TT | 20 (80.00%) | 20 (86.96%) | Reference | |
| TC+CC | 5 (20.00%) | 3 (13.04%) | 1.67 (0.35–7.93) | .52 |
| No | | | | |
| TT | 189 (90.00%) | 190 (89.62%) | Reference | |
| TC+CC | 21 (10.00%) | 22 (10.38%) | 0.96 (0.51–1.80) | .90 |
| Drinking | | | | |
| Yes | | | | |
| TT | 26 (76.47%) | 32 (88.89%) | Reference | |
| TC+CC | 8 (23.53%) | 4 (11.11%) | 2.46 (0.67–9.10) | .17 |
| No | | | | |
| TT | 183 (91.04%) | 178 (89.45%) | Reference | |
| TC+CC | 18 (8.96%) | 21 (10.55%) | 0.83 (0.43–1.62) | .59 |
| Diabetes | | | | |
| Yes | | | | |
| TT | 22 (78.57%) | 5 (100.00%) | Reference | |
| TC+CC | 6 (21.43%) | 0 (0%) | 1.23 (0.67–1.42) | .61 |
| No | | | | |
| TT | 187 (90.34%) | 205 (89.13%) | Reference | |
| TC+CC | 20 (9.66%) | 25 (10.87%) | 0.88 (0.47–1.63) | .38 |

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

BMI = body mass index, CI = confidence interval, EC = endometrial cancer, OR = odds ratio.

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, rs9285274, rs9529895, and rs59352399 (Figure 1B). Age, rs9285274, rs9529895, rs59352399, and 4 other factors are the best models for determining the risk for EC. The accuracy rate is 57.02% and the Cross-validation Consistency is 10/10. ($x^2 = 4.33$, $P = .04$, Table 7).

### 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, rs17088351, and rs59352399 at *DACH1*. The results showed that there was no statistically significant difference in PFS between EC patients with different genotypes of 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 are TT genotype carriers. The difference was statistically significant ($P = .04$, Fig. 2B).

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

We analyzed the correlation between the *DACH1* rs9285274, rs9529895, rs17088351, 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, rs17088351, and rs59352399 loci of the *DACH1* gene ($P > .05$, Fig. 3A–H). This shows that the *DACH1* rs9285274, rs9529895, rs17088351, and rs59352399 loci SNP have no significant correlation with age and BMI.

### 3.7. DACH1 SNP and DACH1 gene expression in tumor tissues of EC patients

We extracted total RNA from 235 patients with surgically resected cancer tissues and adjacent normal tissues, and detected

**Table 4**

**Correlation between genotype frequency of rs9529895 of *DACH1* and risk of EC disease.**

| | EC (n = 235) | Control (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/m$^2$) | | | | |
| <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 (72.17%) | Reference | |
| TC+CC | 37 (17.87%) | 64 (27.83%) | 0.57 (0.36–0.89) | .02 |

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

BMI = body mass index, CI = confidence interval, EC = endometrial cancer, OR = odds ratio.

**Table 5**

**Correlation between genotype frequency of rs17088351 of *DACH1* and risk of EC disease.**

| | EC (n = 235) | Control (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 | 8 (10.53%) | 8 (14.04%) | 0.72 (0.25–2.05) | .73 |
| BMI (kg/m$^2$) | | | | |
| <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%) | 28 (77.78%) | Reference | |
| CT+TT | 5 (14.71%) | 8 (22.22%) | 0.60 (0.18–2.07) | .42 |
| No | | | | |
| CC | 182 (90.55%) | 180 (90.45%) | 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 (0.04–2.49) | .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.

BMI = body mass index, CI = confidence interval, EC = endometrial cancer, OR = odds ratio.

the *DACH1* mRNA expression level using Quantitative Real-time PCR (qRT-PCR). Our results showed that *DACH1* mRNA was underexpressed in cancer tissues, and the Western Blot test results also showed that the *DACH1* protein was underexpressed in cancer tissues (Fig. 4A). Further analysis of the correlation between the *DACH1* mRNA expression level and the TNM stage of EC patients revealed that the *DACH1* mRNA expression level was lower in EC patients with advanced tumor progression (Fig. 4B). Further analysis of the correlation between the *DACH1* gene SNP and *DACH1* mRNA expression levels in tumor tissues of EC patients showed that there were no differences in the expression levels of *DACH1* mRNA in tumor tissues of EC patients with different genotypes of rs9285274, rs17088351, and rs59352399 at *DACH1* ($P > .05$, Fig. 4C, E, F). However, univariate analysis of variance showed that the *DACH1* mRNA expression level in tumor tissues of patients with CC genotype, TC genotype, and TT genotype of rs9529895 loci decreased in that order ($P = .02$, Fig. 4D).

## 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.[17–19] 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.[20–22] We know that

**Table 6**

**Correlation between genotype frequency of DACH1 gene rs59352399 and EC risk.**

| | 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/m$^2$) | | | | |
| <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 (11.46%) | 10 (10.00%) | 1.17 (0.47–2.88) | .74 |
| Smoking | | | | |
| Yes | | | | |
| CC | 23 (92.00%) | 22 (95.65%) | Reference | |
| CT+TT | 2 (8.00%) | 1 (4.35%) | 1.30 (0.23–2.00) | .60 |
| No | | | | |
| CC | 185 (88.10%) | 190 (89.62%) | Reference | |
| CT+TT | 25 (11.90%) | 22 (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 (88.56%) | 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 (88.41%) | 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.

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

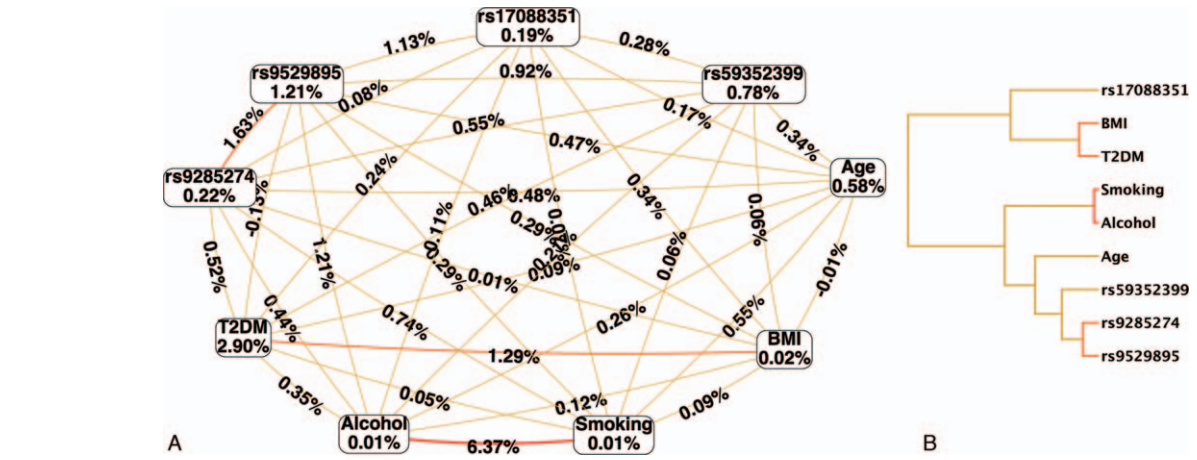


**Figure 1.** Multifactor dimensionality reduction (MDR) analysis of the interaction between *DACH1* gene SNPs and general clinical features. A, Age, BMI, Smoking, Alcohol, T2DM, rs9285274, rs9529895, rs17088351, rs59352399. The data at the apex represent the influence of this factor on the risk for 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.

**Table 7**

**Prediction model analysis of the interaction between the *DACH1* SNP and general clinical characteristics by multidimensional dimension reduction.**

| 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, rs9285274, rs9529895 | 56.38% | 0.78 | .38 | 6/10 |
| Smoking, Alcohol, T2DM, rs9285274, 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 | 52.98% | 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.

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 primer 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.

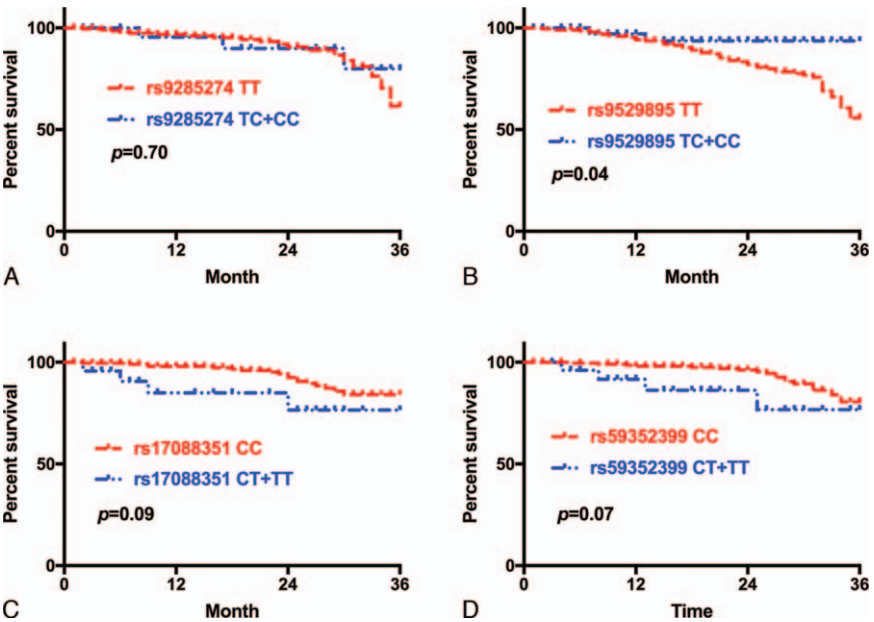


**Figure 2.** Correlation between SNPs at rs9285274, rs9529895, rs17088351, and rs59352399 of *DACH1* gene and progression-free survival (PFS) in EC patients A. Comparison of PFS of EC patients with different genotypes of rs9285274 at *DACH1*. B, Comparison of PFS of EC patients with different genotypes of rs9529895 at *DACH1*. C, Comparison of PFS of EC patients with different genotypes of rs17088351 at *DACH1*. D, Comparison of PFS of EC patients with different genotypes of rs59352399 at *DACH1*.

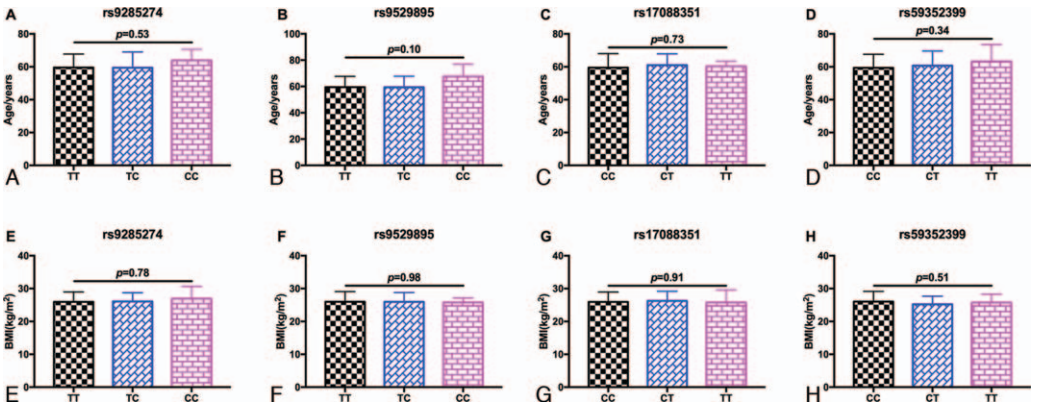


**Figure 3.** Age and BMI of EC patients with different genotypes at rs9285274, rs9529895, rs17088351, and rs59352399 of *DACH1*. A. Age comparison of EC patients with different genotypes at rs9285274 of *DACH1*. B. Age comparison of EC patients with different genotypes at rs9529895 of *DACH1*. C. Age comparison of EC patients with different genotypes at rs17088351 of *DACH11*. D. Age comparison of EC patients with different genotypes at rs59352399 of *DACH1*. E. BMI comparison of EC patients with different genotypes at rs9285274 of *DACH1*. F. BMI comparison of EC patients with different genotypes at rs9529895 of *DACH1*. G. BMI comparison of EC patients with different genotypes at rs17088351 of *DACH1*. H. BMI comparison of EC patients with different genotypes at rs59352399 of *DACH1*.

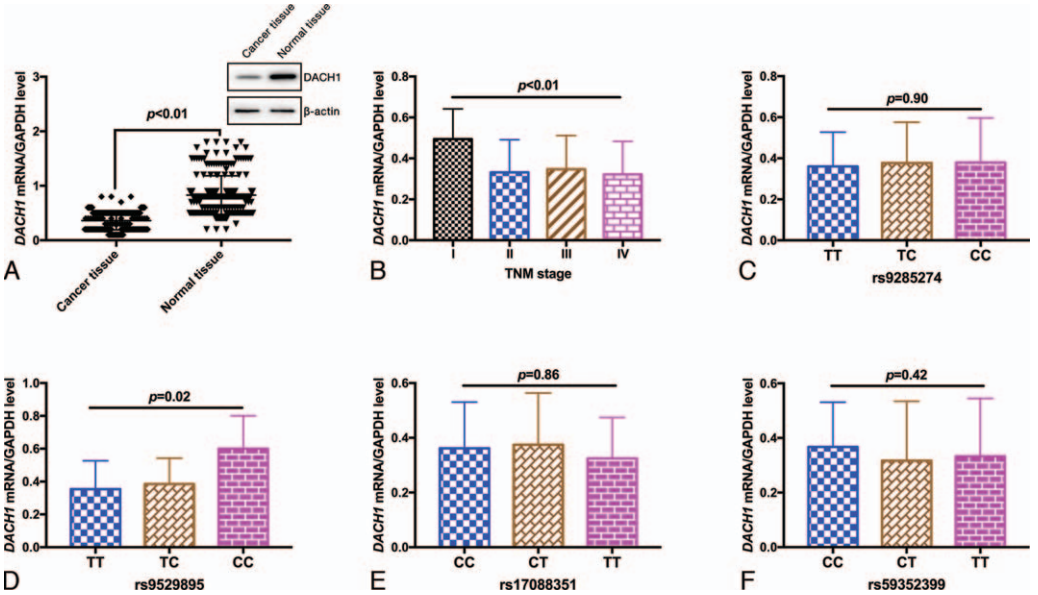


**Figure 4.** *DACH1* expression in tumor tissue from EC patients. A. Comparison of *DACH1* expression levels in cancer tissues and normal tissues adjacent from EC patients. B. Correlation between *DACH1* mRNA expression level and TNM stage in EC patients. C, Comparison of *DACH1* mRNA expression levels in tumor tissues of EC patients with different genotypes of rs9285274 at *DACH1*. D, Comparison of *DACH1* mRNA expression levels in tumor tissues of EC patients with different genotypes at rs9529895 locus of *DACH1*. E, *DAHC1* mRNA expression levels in tumor tissues of patients with different genotypes with EC at *DACH1* rs17088351 loci. F, *DACH1* mRNA expression levels in tumor tissues of patients with different genotype with EC at the *DACH1* rs59352399 locus.

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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