Variants in *DENND1A* and *LHCGR* are associated with endometrioid adenocarcinoma

Zhenyan Wang, Tao Li, Wei Zhang, Li You, Yueran Zhao, Mingdi Xia, Han Zhao, Zi-Jiang Chen

**Objective.** The aim of this study was to explore the polycystic ovary syndrome (PCOS) related single nucleotide polymorphisms (SNPs) rs13405728 (in gene *LHCGR*), rs13429458 (in gene *THADA*) and rs2479106 (in gene *DENND1A*) in women with endometrial carcinoma.

**Methods.** We conducted a case–control study comprising 96 Han Chinese women with endometrial carcinoma, and 192 healthy controls. SNPs rs13405728, rs13429458 and rs2479106 were genotyped by polymerase chain reaction (PCR) and direct sequencing. The effects of body mass index (BMI) and age were evaluated using an unconditional logistic regression model adjusted for potential confounders.

**Results.** The allele frequencies of SNPs rs2479106 and rs13405728 were significantly different (P < 0.05) between endometrial carcinoma group and control group, and the difference was especially significant in the subgroup of endometrioid adenocarcinoma. Genotyping analysis showed that allele G in rs2479106 and allele A in rs13405728 could confer risk to endometrioid adenocarcinoma.

**Conclusions.** Our results suggest that SNPs rs2479106 in gene *DENND1A* and rs13405728 in gene *LHCGR* are associated with endometrioid adenocarcinoma.

© 2012 Elsevier Inc. All rights reserved.
Materials and methods

Subjects

A total of 96 EC patients and 192 healthy controls were recruited from the Affiliated Hospital of Qingdao University and Shandong Provincial Hospital Affiliated to Shandong University from May 2008 to August 2011. The study was approved by the Institutional Review Board for Reproductive Medicine of both Qingdao University and Shandong University. Informed consent was obtained from all participants. All the female participants had complete clinical and biochemical data. Diagnosis of EC, 76 of which were EA and 20 were Non-EA, was based on postoperative pathology by two pathological experts. All EC slides were re-confirmed before experiments and no atypical endometrial hyperplasia cases included. Control subjects were healthy females referred for routine physical examination. They had no clinical manifestation of endometrial carcinoma (such as vaginal bleeding, vaginal discharge, abdominal pain and soft and enlarged uterine), and gynecologic examination, B ultrasound and thinprep cytologic test excluded their uterus and ovary pathological changes. They had regular menstrual cycles, no infertile and PCOS history, no hysterectomy or bilateral oophorectomy, or other tumor history. All EC cases and controls had no diabetes and hypertension. Age and BMI of both cases and controls were listed in Table 1, and unconditional logistic regression was performed for further analysis to minimize the impacts of age and BMI.

Genotyping methods

Genomic DNA was extracted from EDTA anti-coagulated peripheral whole blood with standard techniques following the manufacturer’s instructions (Tiangen Biotech Co. Ltd, Beijing, China). Genotyping of SNPs rs2479106, rs13429458 and rs13405728 was performed with a polymerase chain reaction Tm-shift genotyping method [7]. The PCR was performed as follows: 10 min denaturation at 95 °C and then extension for 45 s at 72 °C, and at last extension for 10 min at 72 °C.

Statistical analysis

The numerical data were displayed as mean±SD. Analysis of allele and genotype frequencies between women with EC and controls was compared using Pearson’s Chi-square. Hardy-Weinberg equilibrium was assessed using the Haploview software. Univariate logistic regression was applied to adjust BMI and age. Genetic models were compared by one-way ANOVA. The SNPs rs2479106, rs13429458 and rs13405728 were presented in Table 2. The minor allele frequencies (MAF) of rs2479106 and rs13405728 were different in the two groups (P=0.007, OR=1.464, \(P_{\text{adjusted}}=0.095\)). However, there was no difference of the three SNPs between Non-EA and control group (data not shown), which indicated that rs2479106 and rs13405728 were related to EA.

Table 2

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>MAF</th>
<th>P</th>
<th>OR</th>
<th>(P_{\text{adjusted}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2479106</td>
<td>G/A</td>
<td>0.240</td>
<td>0.076</td>
<td>1.464(0.960–2.234)</td>
<td>0.008</td>
</tr>
<tr>
<td>rs13405728</td>
<td>G/A</td>
<td>0.188</td>
<td>0.384</td>
<td>0.824(0.533–1.274)</td>
<td>0.036</td>
</tr>
<tr>
<td>rs13429458</td>
<td>G/A</td>
<td>0.156</td>
<td>0.284</td>
<td>0.824(0.533–1.274)</td>
<td>0.702</td>
</tr>
</tbody>
</table>

Notes: EA = endometrioid adenocarcinoma; allele = minor allele/major allele; MAF = minor allele frequency; OR = odds ratio between case and control group.

Results

Characteristics of EC and control subjects were expressed in Table 1. The EC group was older than the control group (P=0.009), and had significantly higher Body Mass Index (BMI) than the control group (P<0.001).

No significant deviation of allele frequencies from the Hardy-Weinberg equilibrium was found in the EC and control groups (all \(P>0.05\)). The allele frequencies of rs2479106, rs13429458 and rs13405728 were different in the two groups after age and BMI adjustment (rs2479106: \(P=0.076\), OR=1.464, \(P_{\text{adjusted}}=0.095\)); rs13405728: \(P=0.384\), OR=0.824, \(P_{\text{adjusted}}=0.036\)). There was no statistical difference for rs13429458. The results indicated that rs2479106 and rs13405728 were related to EA.

To explore whether the correlation of the three SNPs and EC was related to histological type, we further analyzed the sub-group of EA and Non-EA, respectively (Table 3). Between EA and controls, MAF of rs2479106 and rs13405728 was still notably different after age and BMI adjustment. It was not statistically different in rs13429458 though the direction of association was consistent with that in PCOS (\(P=0.192\), \(P_{\text{adjusted}}=0.095\)). However, there was no difference of the three SNPs between Non-EA and control group (data not shown), which indicated that rs2479106 and rs13405728 were related to EA rather than Non-EA.

Table 3

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>MAF</th>
<th>P</th>
<th>(P_{\text{adjusted}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2479106</td>
<td>G/A</td>
<td>0.263</td>
<td>0.025</td>
<td>0.031</td>
</tr>
<tr>
<td>rs13405728</td>
<td>G/A</td>
<td>0.171</td>
<td>0.218</td>
<td>0.030</td>
</tr>
<tr>
<td>rs13429458</td>
<td>C/A</td>
<td>0.145</td>
<td>0.192</td>
<td>0.095</td>
</tr>
</tbody>
</table>

Notes: EA = endometrioid adenocarcinoma; allele = minor allele/major allele; MAF = minor allele frequency.

Discussion

The association between PCOS and EC was first suggested in the year 1949, fourteen years after the original description of PCOS [8]. Women with PCOS have been thought to have increased risk of EC because of chronic anovulation and unopposed estrogen exposure of the endometrium. This study showed new evidence of the relationship between EC and PCOS in genetic background. The MAF of rs2479106 and rs13405728 in EC was consistent with what we reported in PCOS [6].

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>EC</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>96</td>
<td>192</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.66±6.62</td>
<td>56.39±5.81</td>
</tr>
<tr>
<td>BMI</td>
<td>24.26±2.02</td>
<td>23.26±2.74</td>
</tr>
</tbody>
</table>

EC = endometrioid carcinoma; data represent as mean±SD.
We excluded EA patients with irregular menstrual cycles (some extent excluding PCOS), and found that MAF of all three SNPs showed significance in EA patients who had regular menses (data not shown), which indicated that rs2479106 and rs13405728 might have risk to EA independent of PCOS. Moreover, this is the first research in Han Chinese with EA reporting the genotype–phenotype correlation in DENND1A and LHCGR genes. Further functional studies are requested to gain substantial insights on the etiological action of these genes contributing to the EA phenotypes.

In conclusion, the current study provides new evidence of the genetic relationship between EA and PCOS. PCOS associated SNPs rs2479106 in gene DENND1A and rs13405728 in gene LHCGR could be new risk markers for EA.

Conflict of interest statement
The authors declare they have no conflicts of interest.

Acknowledgments
The authors thank Changming Zhang, Di Wu, Zhao Wang, Lei Cheng and Jianfeng Wang from the Center for Reproductive Medicine, Provincial Hospital affiliated with Shandong University for their contribution of patients’ recruitment. We thank all of the participants involved in this study. This work was supported by the National Basic Research Program of China (973 program) (2012CB944700), the National Natural Science Foundation of China (81000238, 30973170).

References