Single nucleotide polymorphisms of follicle-stimulating hormone receptor are associated with ovarian cancer susceptibility

C.Q.Yang1, K.Y.K.Chan1,2,†, H.Y.S.Ngan2, U.S.Khoo1, P.M.Chiu1, Q.K.Y.Chan1, W.C.Xue1 and A.N.Y.Cheung1,*

1Department of Pathology, 2Department of Obstetrics and Gynecology, Jockey Club Clinical Research Centre, The University of Hong Kong, Hong Kong, China

Abstract

Epidemiological studies suggested that ovulation was associated with ovarian carcinogenesis. Follicle-stimulating hormone (FSH) played an important role in follicular development and was recently found to affect growth of ovarian epithelial cells. Single nucleotide polymorphisms (SNPs) Thr307Ala and Asn680Ser were two non-synonymous variations in the coding region of the FSH receptor (FSHR) gene. This hitherto first case–control study investigating the association between these two FSHR SNPs and the risk of ovarian cancer involved 202 histopathologically confirmed ovarian cancer patients and 266 age-matched cancer-free control subjects using restriction fragment length polymorphism assay and direct sequencing. Our results demonstrated that the 307Ala and 680Ser carriers were associated with significantly increased risk of developing serous and mucinous types of ovarian cancers ($P < 0.0005$, OR = 2.60, 95% CI = 1.56–4.34; and $P < 0.0005$, OR = 2.89, 95% CI = 1.73–4.84, adjusted for age, respectively) but not endometrioid and clear cell types. The two SNPs were found to be in modest linkage disequilibrium, $D' = 0.804$ and $r^2 = 0.581$ and 0.406 for the cancer and control groups, respectively. The major haplotype of 307Ala-680Ser was also associated with higher cancer risk ($P = 0.033$, OR = 1.39, 95% CI = 1.03–1.88), especially for the serous and mucinous carcinomas ($P = 0.001$, OR = 1.82, 95% CI = 1.27–2.60). Our results suggested that the two FSHR SNPs might affect the susceptibility of women to specific subtypes of ovarian cancer. Different types of ovarian cancer might adopt distinct carcinogenic pathways. Such understanding may be important in selecting patients for ovulation induction therapy.

Introduction

Ovarian cancer contributes to the highest mortality among all gynecological cancers and difference in incidence of ovarian cancer in different ethnic groups was reported (1). Important hypotheses regarding ovarian carcinogenesis include stimulation due to incessant ovulation, gonadotropins and sex-steroid hormones (2). Epidemiological studies suggested that events that would increase the number of ovulation such as pregnancy, oral contraceptive use and breastfeeding would significantly elevate the risk of developing ovarian cancer (3). Ovulation was implicated as a risk factor for ovarian cancer (4,5).

Follicle-stimulating hormone (FSH) is essential for ovarian follicular development. FSH evokes its biological effects by interacting with high affinity receptor located on the plasma membrane of its target cells in the gonads. The FSH levels were found to be significantly higher in the peritoneal fluids of the ovarian cancer patients (6,19). Moreover, the FSH receptor (FSHR) expression levels were demonstrated to be higher in the peritoneal implants and in ovarian epithelial tumors (7,8) when compared with its expression in normal surface epithelium of human ovary and the fallopian tube (7–9). It is thus likely that both ligand and receptor play important role in ovarian epithelial cancer development in a synergistic manner. FSH has also been found to be an important growth-promoting factor for ovarian cancer cells (8,10). Moreover, gene profiling data revealed that FSH could exert biological actions on ovarian cancer and on immortalized normal human ovarian surface epithelial cell lines (11). Furthermore, the ovarian response to FSH stimulation is apparently affected by and associated with the FSHR genotype (12–15).

The single nucleotide polymorphism (SNP) Ala307Thr situated at the extracellular domain of FSHR, the site responsible for high affinity hormone binding (16), has been reported to affect hormone trafficking and signal transduction. Phosphorylation of the Ser and Thr residues within the intracellular regions of FSHR, which harbors SNP Ser680Asn, may influence the uncoupling from adenylyl cyclase (17). As a result, amino acid alteration related to the corresponding SNPs might affect the post-translational modifications of the FSHR protein, and hence the function of the receptor including FSH efficacy (18). Haplotype analysis on these two FSHR non-synonymous SNPs also suggested that different haplotypes are significantly related to different basal level of serum FSH (19). Such observations have promoted our interest in investigating the role of FSHR polymorphism in ovarian carcinogenesis.

In this study, we postulated that two non-synonymous SNPs of the FSHR gene, Ala307Thr and Ser680Asn located at the nucleotide positions of 919 and 2039 of the FSHR coding sequence (GeneBank accession no. NM_000145), may affect the susceptibility of ovarian epithelium to development of cancer.

Materials and methods

Study population and data
Archival paraffin embedded tissue blocks were retrieved from the files (1985–2002) of the Department of Pathology, Queen Mary Hospital, a major referral center in Hong Kong for patients with gynecologic
malignancies. Hematoxylin and eosin-stained section of each tissue blocks was assessed to review the diagnosis and ensure the absence of tumor before performed DNA extraction. Two hundred and twenty ovarian cancer cases with available paraffin embedded non-tumor tissue were retrieved with success and DNA extraction performed in 202 cases. The mean age of these 202 cancer patients at diagnosis was 51.40 ± 12.6 years (range 23–83 years). The histological subtypes, which included serous (SC, n = 68), mucinous (MC, n = 36), endometrioid (EC, n = 68) and clear cell carcinoma (CC, n = 30), were reviewed by histopathologists. Ovarian borderline tumors, sex-cord stromal tumors, germ cell tumors and metastatic tumors were excluded. The mean ages for serous, mucinous, endometrioid and clear cell types of cancer cases were similar: 54.7, 50.1, 49.6 and 49.8, respectively. Two hundred and sixty-six randomly selected control subjects who had undergone salpingectomy for benign conditions and known not to have ovarian carcinoma were included in this study. Their paraffin embedded tissues were retrieved from the Department of Pathology, Queen Mary Hospital. The mean age of control subjects was 49.26 ± 11.60 years (range 20–81 years). These control subjects were diagnosed to have leiomyomas (n = 238), tubal ectopic pregnancy (n = 6), endometrial polyp (n = 2) and uterine prolapse (n = 20). All of the studied cancer and control subjects were of Chinese origin, while three of the cancer patients were born in Vietnam and Philippines. Out of the 202 cancer patients, data regarding previous hormone replacement, ovulation or contraceptive drug history was available in 177 patients. Six have received birth control pills while one has received hormonal replacement therapy.

**Extraction of DNA**

Microdissection was performed if necessary so that only tissue without tumor contamination was used for DNA extraction. Ten consecutive 10 μm sections were cut from each paraffin embedded tissue block. Genomic DNA was then extracted from the deparaffinized tissue using the conventional phenol/chloroform method following the proteinase K digestion (20). DNA was then ethanol precipitated, vacuum dried and then suspended in 1× TE buffer.

**Genotyping**

The two SNPs, Ala307Thr and Ser680Asn (rs6165 and rs6166 in dbSNP, respectively), introduced restriction sites that could be investigated using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique (13,19,21) with the primers which were designed based on the published sequence of the human FSHR gene (GenBank accession no. NM_000145): forward 5’-GCT CTG AGC TTC ATC CAA TTT G-3’ and reverse 5’-CCC AAA TTT ATA GGA CAG-3’ for Ala307Thr, forward 5’-CCC AAA TTT ATA GGA CAG-3’ and reverse 5’-GAG GGA CAA GTA TGT AAG TG-3’ for Ser680Asn. The PCR was carried out in 20 μl containing 1× PCR buffer, 3 mM MgCl2, 200 mM dNTP and 0.6 U of AmpliTaq polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA) at the optimized conditions for 18 h and then separated by 1.5% agarose gel. The sizes of amplified products for the SNPs Ala307Thr and Ser680Asn were 120 and 114 bp, respectively. After digestion by HhaI and BsrI, two fragments, 86 and 28 bp in upper panel (A) were not shown.

The association between SNPs and development of carcinoma was first assessed as a group. Since the EC and CC carcinomas may have different carcinogenetic pathways distinct from MC and SC, owing to their strong association with endometriosis (23), their correlation with these two SNPs were separately analyzed in addition.

**Results**

The sizes of amplified products for the SNPs Ala307Thr and Ser680Asn were 120 and 114 bp, respectively. For the SNP Ala307Thr, after digestion by HhaI, three fragments, 70, 31 and 19 bp (Figure 1A), were produced for the Thr/Thr genotype while two fragments, 101 and 19 bp (Figure 1A) were produced for the Ala/Ala genotype. The amplified product harbored one more restriction site for the enzyme resulting in the 19 bp fragment, and that served as an internal digestion control. For SNP Ser680Asn, after digestion by BsrI, two fragments, 86 and 28 bp (Figure 1B) would stand for the Ser/Ser genotype whereas the Asn/Asn genotype remained as the size of 114 bp. The heterozygote was represented by a combination of the fragments found in either genotype. Twenty percent of the total studied cases were duplicated, all had reproducible results. Five percent of cases from each studied population were directly sequenced, and the sequencing results confirmed the PCR–RFLP findings (Figure 2).

The genotypes of the control subjects were in HWE for both SNPs (χ²-test, df = 1, P = 0.286 and 0.731 for SNPs Ala307Thr and Ser680Asn, respectively) while the genotypes in the cancer group was deviated from HWE (P = 0.028 and 0.001 for SNPs Ala307Thr and Ser680Asn, respectively).

The association between SNPs and development of carcinoma was first assessed as a group. Since the EC and CC carcinomas may have different carcinogenetic pathways distinct from MC and SC, owing to their strong association with endometriosis (23), their correlation with these two SNPs were separately analyzed in addition.

The genotype frequencies of the SNP Ala307Thr and Ser680Asn were significantly different between the cancer and control groups (P = 0.023 and 0.010, respectively) (Table I). The 307Ala and 680Ser carriers had higher risk to develop ovarian cancer when compared with the studied controls (P = 0.018, OR = 1.58, 95% CI = 1.08–2.30; and P = 0.004, OR = 1.74, 95% CI = 1.19–2.54, adjusted for age, respectively).

By further stratification, the genotypes of the two SNPs were shown to have significant association with the serous and mucinous subtypes (P = 0.001 and P < 0.0005, respectively) (Table I), while the 307Ala and 680Ser carriers were shown among the SNPs to be completed to obtain the linkage disequilibrium coefficient, D’, and the correlation coefficient, r².
to have higher risk association \( (P < 0.0005, \text{OR} = 2.60, 95\% \text{ CI} = 1.56–4.34) \); and \( P < 0.0005, \text{OR} = 2.89, 95\% \text{ CI} = 1.73–4.84 \), respectively). However, such associations were not significant in endometrioid and clear cell subtypes \( (P > 0.05) \) (Table I).

The two FSHR SNPs were modestly in linkage disequilibrium in the cancer and control groups as \( D' = 0.80 \) and \( r^2 = 0.58 \), and \( D' = 0.70 \) and \( r^2 = 0.41 \), respectively (Table II). 307Thr-680Asn and 307Ala-680Ser were the major haplotypes, whereas 307Thr-680Ser and 307Ala-680Asn were the minor haplotypes in both populations (Table II). The haplotypes distribution in the cancer were significantly different from that in the control group \( (P = 0.024) \). The haplotype 307Ala-680Ser was shown to be associated with higher risk of ovarian cancer \( (P\text{-value} = 0.033, \text{OR} = 1.39, 95\% \text{ CI} = 1.03–1.88) \), particularly for the serous and mucinous subtypes \( (P\text{-value} = 0.001, \text{OR} = 1.82, 95\% \text{ CI} = 1.27–2.60) \). No correlation with this haplotype was demonstrated in the endometrioid and clear cell types \( (P > 0.05) \) (Table II).

Discussion

Recent studies have documented FSHR expression in normal surface epithelium of the ovary and the fallopian tube (9) and at a higher level in ovarian cancers (7–9,24). FSHR over-expression was also found to stimulate proliferation in preneoplastic ovarian epithelial cells (25). It was suggested that FSH may be an important growth-promoting factor in ovarian cancer cells (8,10). Fuller et al. (26) in their study focused on granulosa cell tumor, had studied the SNP Ser680Asn in seven mucinous cystadenocarcinoma. Six heterozygous Ser680Asn and one homozygous 680Ser were found suggesting the tendency for 680Ser carriers to have higher risk of developing this cancer. The number of cases, though too few to draw conclusions, concurred with our findings.

Some SNPs in the FSHR promoter region were recently found to alter FSHR expression \( \text{in vitro} \) through changes in transcription factor binding sites although no correlation...
Table II. FSHR SNPs haplotypes comparison in the cancer and control groups

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Overall ovarian cancer</th>
<th>SC + MC</th>
<th>EC + CC</th>
<th>Normal control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of alleles (%)</td>
<td>OR (95% CI)</td>
<td>P-values</td>
<td>No. of alleles (%)</td>
</tr>
<tr>
<td>Thr307-Asn680</td>
<td>57.55</td>
<td>233</td>
<td></td>
<td>Reference</td>
</tr>
<tr>
<td>Thr307-Ser680</td>
<td>4.33</td>
<td>17</td>
<td>0.76 (0.41–1.40)</td>
<td>0.446</td>
</tr>
<tr>
<td>Ala307-Asn680</td>
<td>6.81</td>
<td>27</td>
<td>0.70 (0.43–1.15)</td>
<td>0.182</td>
</tr>
<tr>
<td>Ala307-Ser680</td>
<td>31.31</td>
<td>127</td>
<td>1.39 (1.03–1.88)</td>
<td>0.033</td>
</tr>
<tr>
<td>D' and r²</td>
<td>0.803 and 0.581</td>
<td>0.775 and 0.567</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ²-test on overall difference among haplotypes in the cancer and normal control populations. SC, serous type of ovarian cancer; MC, mucinous type of ovarian cancer; EC, endometrioid type of ovarian cancer; CC, clear cell type of ovarian cancer; OR, odds ratio; CI, confidence interval; D', standard linkage disequilibrium coefficient; r², correlation coefficient. Boldface indicates χ²-values less than 0.05.

To our best knowledge, this is the first systematic study on the possible association between ovarian cancer and the FSHR polymorphisms. The results of this study suggested that the FSHR polymorphisms could be used as markers for ovarian cancer risk prediction. Further studies are needed to confirm these findings.
and clear cell subtypes. Serous and mucinous subtypes might arise from ovarian epithelium responsive to stimulation of FSH while the endometrioid and clear cell subtypes might develop from ectopic endometrium in endometriosis. These findings need to be confirmed in a much larger series of cases. Such knowledge may be important in selecting patients for ovulation induction therapy. The functional aspect of these SNPs in ovarian cancer development will be investigated in the future, especially to elaborate the effects of these SNPs on the binding affinity to the FSH hormone.

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Conflict of Interest Statement: None declared.

References