Follicle-Stimulating Hormone Receptor Polymorphisms and Its Association with Ovarian Carcinoma

Amal MH Mackawy, Wafaa A Emam
Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Zagazig University

ABSTRACT

Objectives: Follicle stimulating hormone (FSH) and its receptor (FSHR) are important in ovarian follicular development and can influence the growth of ovarian epithelial cells. It seems to implicate in ovarian carcinogenesis. Thr307Ala and Asn680Ser are two single nucleotide polymorphisms (SNPs) of the FSHR gene which have effects on FSH efficacy. Our aim was to examine the association between these two SNPs of FSHR gene and the risk of epithelial ovarian carcinoma in Egyptian females. Subjects and Methods: Genomic DNA was extracted from 40 histopathologically confirmed ovarian cancer patients and 20 cancer-free control subjects using Polymerase chain reaction (PCR) assays with restriction fragment length polymorphism (RFLP). Results: showed a non-significant association between the genotypes with tumor stage for SNPs Ala307Thr and Ser680Asn ($X^2=3.935$, $P=0.047$, OR =2.81, 95% CI =0.99–8.02; and $X^2=5.26$, $P=0.022$, OR =3.491, 95% CI =1.158–10.526, respectively). The 307Ala and 680Ser carriers had higher risk to develop ovarian cancer when compared with the controls ($X^2=15.597$, $P=0.000$ and $X^2=19.858$, $P = 0.000$, respectively), with non-significant associations in endometrioid (EC) and clear cell (CC) subtypes ($P>0.05$). The two SNPs were found to be in modest linkage disequilibrium, $D^\prime =0.182$ and 0.1, $r^2 = 0.553$ and 0.333 for the cancer and control groups, respectively. Haplotype Ala307-Ser680 was shown to be associated with higher risk of ovarian cancer ($X^2=5.79$, $P=0.026$, OR =0.303, 95% CI =0.111–0.825), with more association with SC and MC subtypes ($X^2=0.213$, $P= 0.002$, OR = 0.184, 95% CI =0.062–0.543), in the EC and CC subtypes this haplotype showed no significant correlation ($P>0.05$). Conclusion: SNPs at these two sites of FSHR may influence FSHR function and enhance the probability to specific subtypes of ovarian cancer. They may be useful as a DNA-based diagnostic biomarker for identifying high-risk Egyptian females susceptible to ovarian cancer. SC and MC ovarian cancer may have different carcinogenetic pathways when compared with EC and CC carcinomas in Egyptian females.

Key words: FSHR, single nucleotide polymorphism, ovarian carcinoma predisposition.
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\(^1\). The risk of ovarian cancer increases from the age of 35 and decreases after the age of 59\(^2\). The probability that a woman will be diagnosed with ovarian cancer before the age of 80 years is estimated at 1.5\(^%\)\(^3\). The majority of ovarian cancer cases are sporadic. Familial cancer (about 10\(^%\)) may develop due to inherited mutations of highly Penetrant \(BRCA1\) and \(BRCA2\) genes.\(^4\) Much more common are low-penetrance genes that may account for a larger proportion of ovarian cancers in the general population\(^5\). Unfortunately, both high- and low-penetrance genes are difficult to identify.

The most well-known hypotheses of ovarian cancers development is the incessant ovulation hypothesis that promote cellular proliferation and genetic instability, the gonadotropin hypothesis with direct involvement of endogenous hormones\(^6\) and the androgen hypothesis\(^7\), which postulates that ovarian cancer risk is enhanced by excessive androgen stimulation, while it is decreased by progesterone stimulation. Conditions associated with increase the number of ovulation such as pregnancy, oral contraceptive use and breastfeeding would significantly elevate the risk of developing ovarian cancer\(^8\). Ovulation was implicated as a risk factor for ovarian cancer\(^9\). Follicle-stimulating hormone (FSH) is essential for development of ovarian follicular. 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 and the FSH receptor (FSHR) expression levels were found to be significantly higher in the peritoneal fluids of the ovarian cancer patients\(^10\). It is thus likely that both ligand and receptor play a vital role in ovarian epithelial cancer development in a synergistic manner.

Genes encoding hormone receptors are among candidate genes modulating the risk of ovarian cancer. Several single nucleotide polymorphisms (SNPs) and microsatellite polymorphic variants were detected in hormone receptor genes; some of them are common and may influence the receptor activity\(^11\). With regard to FSH receptor (FSHR) gene, two out of five currently known exonic polymorphic sites are under extensive investigations. Both are SNPs located in exon 10, which is fundamental for signal transduction. G919A results in Ala or Thr at position 307, while G2039A results in Ser or Asn at position 680 in the protein. It was shown in patients undergoing IVF procedure that ovarian response to FSH stimulation was more efficient in individuals with AsnFSHR\(^680\)Asn than with Ser FSHR \(^680\) Ser genotype\(^12\).

The single nucleotide polymorphism (SNP) Ala307Thr situated at the extracellular domain of FSHR, the site responsible for high affinity hormone binding\(^13\), 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.14 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.15

The purpose of the current study was to examine possible associations between genetic polymorphisms of the FSHR (FSHRAla307Thr, FSHRSer680Asn) in ovarian carcinogenesis. Moreover, we aimed to analyze the relation of haplotype of the two FSHR SNPs with the different epithelial ovarian carcinoma stages and types.

SUBJECTS & METHODS

A total of 60 individuals were genotyped. 40 women patients diagnosed with epithelial ovarian cancer were enrolled in this study. This study was conducted from June 2011 to May 2012 at Medical Biochemistry and Pathology Departments, Zagazig University.

Hematoxylin and eosin-stained section of each tissue blocks was assessed to diagnose and ensure the absence of tumor before performed DNA extraction. Forty ovarian cancer cases with available paraffin embedded non-tumor tissue were retrieved with successful DNA extraction was performed in 40 cancer cases and 20 control subjects. The mean age of control subjects was 45.35 ± 11.08 years (23–63 years). The mean age of these 40 cancer patients at diagnosis was 47.25 ± 10.69 years (range 23–83 years). Among the patients, 18 (45%) were diagnosed with a serous carcinoma (SC), 6 (15%) with mucinous one (MC), 11 (27.5%) with endometroid one (EC) and 5 (12.5) clear cell one (CC). The histological subtypes, were reviewed by the pathologist. Metastatic tumors, germ cell tumors and sex-cord stromal tumors were excluded.

The mean age ±SD for all cancer subtypes SC, MC, EC and CC were: 50.83±10.01, 44.66±11.96, 43.90 ±9.56 and 45.76 ±12.61 years respectively. ANOVA (F-test) recorded non-significant different among cancer subtypes regarding to age (F= 1.017, P= 0.407) (Figure1).

Twenty randomly selected control subjects were included in this study. They had undergone salpingo-oophorectomy for benign conditions and all had exclusion criteria from ovarian carcinoma. The paraffin embedded tissues were retrieved from the Department of Pathology, Zagazig University.

Extraction of DNA:

Dissection was performed to the tissue and prepared for DNA extraction after deparaffinization. After delivering tissues from Pathology Department, tissues were carved with surgical blade into 1 mm pieces and between 25 and 75 mg of each paraffin embedded tissue block was transferred to a microtube. Genomic DNA was then extracted from the deparaffinized tissue using the conventional phenol/ chloroform method following the proteinase K digestion.16 Equal volume of tris-saturated phenol (pH=8) was added to
the tubes and centrifuged at 12000 rpm for 2 min. 300 µl was transferred to equal volume of phenol – chloroform mixture (1:1) and after centrifugation 2.5 volume of ethanol was added for DNA precipitation.

The DNA pellet was washed gently with 70% ethanol and then the pellet was dried and dissolved in 70 µl distilled water to be ready for PCR run.

**Genotyping:**

The Ala307Thr and Ser680Asn SNPs were determined using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) method described by Loutradis et al. (2006).17

The two SNPs, Ala307Thr and Ser680Asn introduced restriction sites that could be investigated using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique.18,19

The primers used for Ala307Thr were as follows: forward 5’-GCT CTG AGC TTC ATC CAA TTT G-3’ and reverse 5’-CTC TGC TGT AGC TGG ACT CAT T-3’, and for Ser680Asn :forward 5’-CCC AAA TTT ATA GGA CAG-3’ and reverse 5’-GAG GGACAA GTA TGT AAG TG-3’. The PCR was carried out in 20 µl containing 1x PCR buffer, 3 mM MgCl2, 200 mM dNTP and 0.6 U of AmpliTaq polymerase (Hoffman-LaRoche, Branchburg, NJ) ,and 300nM each forward and reverse primers. After heating and denaturation for 5 min at 95°C for 5 min, the PCR was performed for 40 cycles of 20 sec at 95°C, 1 min at 55°C (for Ala307Thr) or 50 °C (for Ser680Asn) for 30 sec, and 72°C for 30 sec with final extension of 72°C for 5 min in a thermal cycler (a Perkin Elmer 4800 thermal cycler (PTC-100 machine, MJ Research, Inc., Watertown, Mass. USA).

The amplified PCR products (120 and 114 bp, respectively) were digested by restriction enzymes of AhdI (for SNP Ala307Thr) and BsrI (for SNP Ser680Asn) ( Hoffman-LaRoche) at the optimized conditions for 18 h and then separated by 2.5 % polyacrylamide gel electrophoresis (Pharmacia Biotech by SEMKO AB, Sweden) using submarine chamber (Maxicell, EC 360 M-E-C apparatus Cooperation ST. Petersburg. Florida USA) including 5 mg/ml ethidium bromide. After electrophoresis, the gel was visualized under UV illuminator and the length of resulted fragments were detected using 100 Base-Pair Ladder (Bioron) was 0.2 mg/ml in 10 Mm Tris (pH 8.0), 1mM EDTA19.

**Statistical analysis:**

All statistical tests were performed using the SPSS software (Version 11.0). Numerical data were expressed as Mean ±S D. Qualitative data were expressed as frequency and percentage. Chi-square (x^2) test was used to examine the relation between qualitative variables to evaluate the association of the FSHR genotypes in the diseased –control populations. Odds ratio (OR) and 95% confidence interval (CI) were used to measure the strength of the association. Histological subtypes, tumor stages and grades of the cancer, had also been analyzed independently for their risk association with the FSHR SNPs. Linkage disequilibrium (D’) analysis among the SNPs and the correlation coefficient, r^2 were analyzed between two SNPs. A probability value (p-
value) less than 0.05 was considered significant.

RESULTS

This study included 40 female patients with ovarian carcinoma age (Mean ± SD) 47.25 ± 10.69 years (range 23–83 years) and 20 normal controls. The sizes of amplified products for the SNPs Ala307Thr and Ser680Asn were 120 and 114 bp, respectively. After digestion of the SNP Ala307Thr by AhdI, the Thr/Thr genotype produced three fragments, 70, 31 and 19 bp. While the Ala/Ala genotype produced two fragments, 101 and 19 bp.

Digestion of SNP Ser680Asn by BsrI produced two fragments, 86 and 28 bp for the Ser/Ser genotype whereas the Asn/Asn genotype 114 bp. The heterozygote Asn/Ser was represented by a combination of the fragments presented in either genotype. The association between the FSHR Genotypes with tumor stages is demonstrated in table 1.

There was no statistical significant association between the genotypes with tumor stage ($X^2=4.54$, $P=0.60$ and $X^2=2.3$ $P=0.898$ for SNPs Ala307Thr and Ser680Asn, respectively) (Table 1) and patients’ age ($F=0.021$, $P=0.979$ and $F=0.454$ $P=0.637$ for SNPs Ala307Thr and Ser680Asn, respectively).

The distribution of the SNPs Ala307 Thr and Ser680Asn genotypes in patients & controls are shown in table 2.

The association between SNPs and development of carcinoma was first assessed as a whole patient group. Furthermore, owing to the different carcinogenetic pathways of EC+CC and SC+MC, their correlation with these two SNPs were separately analyzed.20

The genotype frequencies of the SNP Ala307Thr and Ser680Asn were significantly different between the cancer and control groups ($X^2=12.919$, $P=0.002$ and $X^2=17.468$, $P=0.000$, respectively) (Table 2). The 307Ala and 680Ser carriers had higher risk to develop ovarian cancer when compared with the controls ($X^2=3.935$, $P=0.047$, OR = 2.81, 95% CI = 0.99–8.02; and $X^2=5.26$, $P=0.022$, OR = 3.491, 95% CI = 1.158–10.526, respectively). Furthermore, the genotypes of the two SNPs were shown to have significant association with the SC and MC subtypes ($X^2=15.597$, $P=0.000$ and $X^2=19.838$ $P=0.000$, respectively). (Table 2), while the 307Ala and 680Ser carriers were shown to have higher risk association in SC and MC ($X^2=4.286$, $P=0.038$, OR = 3.15, 95% CI = 1.04–9.54; and $X^2=5.421$, $P=0.02$, OR = 3.764, 95% CI = 1.19–11.89, respectively). However, this study recorded non-significant associations in EC and CC subtypes ($X^2=3.821$, $P=0.148$, $X^2=5.3$, $P=0.07$, respectively) (Table 2).

The two FSHR SNPs were modestly in linkage disequilibrium ($D'$) in the cancer and control groups as $D'=0.182$ and $r^2=0.553$, and $D'=0.1$ and $r^2=0.333$, respectively (Table 3).

Thr307-Asn680 and Ala307-Ser680 were the major haplotypes whereas Thr307-Ser680 and Ala307-Asn680 were the minor haplotypes in patients and controls (Table 3).
Haplotype Ala307-Ser680 was shown to be associated with higher risk of ovarian cancer ($X^2=5.79$, $P=0.026$, OR=0.303, 95% CI =0.111–0.825) (Table 3). This haplotype showed significant correlation regarding the SC and MC subtypes ($X^2=0.213$, $P=0.002$, OR = 0.184, 95% CI =0.062–0.543). In the EC and CC subtypes, this haplotype showed non significant correlation ($X^2 =0.639$, $P =0.549$) (Table 4).

**Figure 1:** Mean ages of controls and ovarian carcinoma subtypes.

**Figure 2:** Agarose gel electrophoresis pattern of FSH SNP Ala307Thr showing the genotypes of homozygous 307Ala, homozygous 307Thr and heterozygous Ala307Thr. The bands corresponding to 31 and 19 bp were not shown.
Table 1: The association between the FSHR Genotypes with tumor stages.

<table>
<thead>
<tr>
<th>FSHR Genotypes in all patients =40</th>
<th>Stage 1 n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Ala307Thr</td>
<td>Thr/Thr</td>
</tr>
<tr>
<td></td>
<td>Ala/Thr</td>
</tr>
<tr>
<td></td>
<td>Ala/Ala</td>
</tr>
</tbody>
</table>

X² 4.54
P 0.604

Ser680Asn

<table>
<thead>
<tr>
<th></th>
<th>Stage 1 n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Asn/Asn</td>
<td>3</td>
</tr>
<tr>
<td>Ser/Asn</td>
<td>8</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>1</td>
</tr>
</tbody>
</table>

X² 2.23
P 0.898

Figure 3: Electrophoresis gel pattern of FSH Ser680Asn SNP showing the genotypes of homozygous 680Asn, homozygous 680Ser and heterozygous Ser680Asn. The bands corresponding to 28 bp in lower panel (B) was not shown.
Table 2: The distribution of the SNPs Ala307Thr and Ser680Asn genotypes in patients and controls.

<table>
<thead>
<tr>
<th>FSHR Genotypes</th>
<th>Cancer</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients</td>
<td>SC+MC=24</td>
</tr>
<tr>
<td>Ala307Thr</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>9</td>
<td>22.5%</td>
</tr>
<tr>
<td>Ala/Thr</td>
<td>24</td>
<td>60%</td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>7</td>
<td>17.5%</td>
</tr>
</tbody>
</table>

\[X^2\]

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cancer</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>p</td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>12.919</td>
<td>0.002</td>
</tr>
<tr>
<td>Ala/Thr</td>
<td>3.935</td>
<td>0.047</td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>17.468</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ser680Asn</th>
<th>Cancer</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Asn/Asn</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Ser/Asn</td>
<td>25</td>
<td>62.5%</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>7</td>
<td>17.5%</td>
</tr>
</tbody>
</table>

\[X^2\]

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cancer</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>p</td>
</tr>
<tr>
<td>Asn/Asn</td>
<td>17.468</td>
<td>0.000</td>
</tr>
<tr>
<td>Ser/Asn</td>
<td>3.26</td>
<td>0.022</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>5.26</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3: FSHR SNPs haplotypes distribution in all patients and controls.

<table>
<thead>
<tr>
<th>FSHR SNPs haplotypes</th>
<th>Ovarian cancer Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Thr307-Asn680</td>
<td>41</td>
<td>51.25%</td>
</tr>
<tr>
<td>Thr307-Ser680</td>
<td>3</td>
<td>3.75%</td>
</tr>
<tr>
<td>Ala307Asn680</td>
<td>7</td>
<td>8.75%</td>
</tr>
<tr>
<td>Ala307-Ser680</td>
<td>29</td>
<td>36.25%</td>
</tr>
</tbody>
</table>

\[D^2\] and \[r^2\]

|                | 0.182 and 0.553 | 0.1 and 0.333 |

198
Table 4: FSHR SNPs haplotypes comparison in all patients according to their subtype of ovarian carcinoma and controls

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>SC+MC n</th>
<th>OR (95% CI)</th>
<th>X² P-value</th>
<th>EC+CC n</th>
<th>OR (95% CI)</th>
<th>X² P-value</th>
<th>n %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr307-Asn680</td>
<td>18</td>
<td>0.643 (0.083-4.98)</td>
<td>0.181 &gt;0.05</td>
<td>23</td>
<td>1.643 (0.14-19.287)</td>
<td>0.159 &gt;0.05</td>
<td>28 70%</td>
</tr>
<tr>
<td>Thr307-Ser680</td>
<td>2</td>
<td>0.643 (0.142-2.902)</td>
<td>0.333 &gt;0.05</td>
<td>1</td>
<td>1.095 (0.222-5.399)</td>
<td>0.012 &gt;0.05</td>
<td>2 5%</td>
</tr>
<tr>
<td>Ala307Asn680</td>
<td>4</td>
<td>0.184 (0.062-0.543)</td>
<td>0.213 0.002</td>
<td>8</td>
<td>0.616 (0.187-2.032)</td>
<td>0.639 &gt;0.05</td>
<td>6 15%</td>
</tr>
<tr>
<td>Ala307-Ser680</td>
<td>21</td>
<td>0.184 (0.096 and 0.708)</td>
<td>(0.096 and 0.156)</td>
<td>0.1 and 0.333</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Ovarian cancer is the eighth leading cancer in women, as it accounts for 4% of all malignant tumors in females. It is the fourth to fifth leading cause of death in U.S.A. The incidence of ovarian cancer is up to 10 times higher in western countries than in rural Asian and African ones. Studies have documented FSHR expression in normal surface epithelium of the ovary and the fallopian tube and at a higher level in ovarian cancers.

FSHR over expression was also found to stimulate proliferation in pre-neoplastic ovarian epithelial cells. Mutation screening of the FSHR gene revealed various SNPs in the core promoter and in the coding region.

The two most common SNPs in the coding region occur at nucleotides 919 and 2039 in exon 10, in which A/G transitions cause amino acid exchange from threonine to alanine at codon 307 and from asparagine to serine at codon 680 respectively. The most studied SNP in the core promoter occurs at position −29.

The current study investigated the possible association between ovarian cancer and the FSHR non-synonymous SNPs in Egyptian female patients. The results revealed that patients with the 307Ala and 680Ser carriers were more at risk to be afflicted with ovarian cancers when compared with the non-307Ala carriers and non-680Ser carriers (OR = 2.81, 95% CI:0.99-8.02; p = 0.047) and (OR =3.49, 95% CI:1.158-10.526; p = 0.022), respectively. In particular, these results showed that homozygous carriers of Ala at position 307 or Ser at position 680 of FSHR protein had significantly higher risk of developing SC and MC subtypes of ovarian cancer. This study recorded a modest linkage...
disequilibrium between these two SNPs. Haplotype analysis results showed that the haplotype of 307Ala-680Ser had significant association with ovarian cancer risk with significant association with SC and MC subtypes when compared with the other haplotypes.

Similarly, in studies done by Sonya et al., Agnieszka et al., and Yang et al.17, and Choi et al.19 who showed that Ala 307 or Ser 680 carriers of FSHR had increased risk incidence of having SC and MC ovarian tumors. They also agreed with these current results, reporting no difference in the frequencies of the genotypes of the two SNPs in the EC and CC ovarian tumor subtypes.

FSHR polymorphisms at positions 307 and 680 influence the serum FSH levels in women and the sensitivity of the FSHR to FSH in vivo. In particular, the Ser680 variant is associated with a less active receptor and the Asn 680 variant results in a higher active receptor.15

It was suggested that FSH may be an important growth-promoting factor in ovarian cancer cells. A number of studies have shown that FSH acting on FSH receptor in the cell surface play a role by activating of intracellular signal transduction pathways. Fuller et al.16 in their study focused on granulosa cell tumor, they studied the SNP Ser680Asn in seven mucinous cystadenocarcinoma and suggested the tendency for 680Ser carriers to have higher risk of developing this cancer.

Some SNPs in the FSHR promoter region were found to alter FSHR expression in vitro through changes in transcription factor binding sites although no correlation with basal FSH serum levels or ovarian response in women undergoing controlled ovarian stimulation for IVF treatment could be detected.17

Gaber et al.18 investigated the association between FSHR gene polymorphism at position 680 and the outcomes of controlled ovarian stimulation for in vitro fertilization (IVF) in Egyptian women. They stated that the homozygous Asn/Asn genotype of FSHR polymorphism at position 680 may be associated with a reduced ovarian response to controlled ovarian hyper-stimulation for IVF.

Another study of Griswold and Kim39 performed on mouse Sertoli cells also suggested that hypermethylation of some CpG sites in the FSHR promoter was associated with downregulation of FSHR expression. Methylation of these CpG sites would hinder the binding of the transcription factors and repressed FSHR expression.

Localization of FSHR gene SNPs corresponds well with the degree of receptor inactivation. Mutations in the extracellular region of the FSHR protein have been associated with abolished ligand binding and signaling, while mutations in the trans-membrane region impair signal transduction.23

Agnieszka et al.27 suggested that FSHR Ala/Ala genotype at position 307 may diminish chances of cancer recurrence in patients treated with taxane–platinum agents, while two serines at position 680 of the FSHR protein may increase the risk of recurrence and death.

In previous studies done by Sudo et al.19 reported that the haplotype
307Ala-680Ser had significantly higher basal level of serum FSH, which might enhance proliferation and malignant transformation of the ovarian epithelium and thus contributed to the higher risk of ovarian cancer. This was concurred with our findings regarding risk association with the 307Ala and 680Ser carriers and the haplotype 307Ala-680Ser.

In opposite, Heubner et al. could not find any association between FSHR Polymorphisms and ovarian cancer risk.

Our results proved that different histological subtypes of ovarian carcinoma showed different association patterns with the FSHR SNPs. While the FSHR 307Ala and 680Ser allele carriers were associated with increased risk of developing SC and MC, no significant association was found in EC and CC types of ovarian cancers. FSH and its receptor may play distinct roles regarding carcinogenesis of different subtypes of ovarian cancers. However, the genotyping of two SNPs (307Ala and 680Ser) results demonstrated no statistical significant association with other clinical parameters such as tumor stages and patients’ age.

This was in agreement of Yang et al. and Tung et al. who stated that different histological subtypes of ovarian cancers displayed different association patterns with various reproductive risk factors. Other studies, demonstrated differences in genotype and allelic frequencies among different populations and ethnic groups.

In conclusion, this study showing the involvement of the mutant 307Ala and 680Ser alleles as independent risk factors for ovarian cancer, especially in the SC and MC subtypes but not the EC and CC subtypes in our population. SC and MC subtypes might arise from ovarian epithelium responsive to stimulation of FSH while the EC and CC subtypes might develop from ectopic endometrium in endometriosis.

However, these findings should be considered as preliminary results, although, promising, but warrant further investigation with larger sample size. Future studies also needed to clarify the functional aspect of these SNPs in ovarian cancer development and investigate the effects of these SNPs on the binding affinity to the FSH hormone. These studies are important, as understanding the interplay of FSH hormones with FSHR SNPs will facilitate knowledge about cancer etiology and the identification of the individuals who are at increased risk of developing ovarian cancer and may be important in selecting patients for ovulation induction therapy.

ACKNOWLEDGEMENT
I would like to thank Dr. Ola Abdel-Rahman Megahed, Assistant professor of Pathology, Faculty of Medicine, Zagazig University for her effort in providing the control and patient samples and determining the stages and subtypes of ovarian carcinoma.

REFERENCES
14- Hipkin RW, Liu X and Ascoli M (1995): Truncation of the C-terminal tail of the follitropin receptor does not impair the agonist- or phorbol ester-induced receptor phosphorylation and


36- Fuller PJ, Verity K, Shen Y, Mamers P, Jobling Tand Burger HG (1998): No evidence of a role for mutations or polymorphisms of the follicle-


الطفرات الجينية لمستقبلات الهرمون المنبه للتبويض وارتباطه بخطر الإصابة بسرطان المبيض

د. أمحمد حسين مكاوي - د. وفاء إمام
قسم الكيمياء الحيوية الطبية
كلية الطب البشري، جامعة الزقازيق

الهرمون المحفز (FSHR) ومستقبلاته (FSH) لعملية التبويض على نحو الخلايا القشرية للمبيض وينبغي أن لهما تأثير سلبي على نمو سرطان البظر. التحالات الجينية (لا تبدأ في طور سرطان) (أسرامينين 680Ser) و (ASA680Ser) من التحالات الجينية لمستقبلات الهرمون المحفز للتبويض وقد يؤثر على كفاءة هذا الهرمون.

كان العرض الرسمي لهذه الدراسة هو فحص العلاقة بين هاتين الطفرتين وخطر الإصابة بسرطان المبيض وقد تم تحديد مدى ارتباطهما بمرحلة الور. وجد النساء المصابات بحمض الديوكسوسفانتي فورامل (OR=2.81, 95% CI =0.99–8.02) و P=0.026، OR=3.491, 95% CI =1.158–10.526

وقد أوضحت النتائج أيضا وجود تغير ذات دلالة معينة بين المجموعة المختلطة (SC) والمجموعة (MC) المختلطة مع اتصال هذه العلاقة مع نوعي الورم البشري (clear cell).

الخلايا (A) مرتبطة بزيادة خطور حدوث مرضا Alas07-Ser680 في البظر. وصلت هذه النتائج إلى أن الطفرات الجينية في الخلايا المختلطة (OR=0.026, OR=0.303, 95% CI =0.111–0.825) مع ارتفاع نسبة (X2=3.79). P=0.026, OR=0.303, 95% CI =0.111–0.825. في الورم البشري (OR=0.026, OR=0.303, 95% CI =0.111–0.825).

وبينما أنه يمكن اعتبار هذه النتائج كعوامل محددة على حدث بعض أعراض سرطان البظر، إذ أن هذه النتائج قد تكون من النتائج الأولية لعدة سنوات للدراسة. فإن هذه النتائج قد تكون ضرورية لدراسة الفروق الجينية في البظر، إذ أن هذه النتائج قد تكون ضرورية لدراسة الفروق الجينية في البظر.