DNA repair gene (XRCC1 and XPD) polymorphism and risk of primary ovarian failure

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Abstract. – OBJECTIVE: DNA repair genes may be related with the onset of primary ovarian failure (POF). The study was planned to investigate whether the polymorphisms in the DNA repair genes modulate the risk of POF.

PATIENTS AND METHODS: This prospective study included 25 women diagnosed with POF and 25 healthy controls. The genotyping and allele of XRCC1 and XPD genes were determined by using Polymerase Chain Reaction and fluorescence melting curve analysis.

RESULTS: The genotype and allele distribution of the Arg194Trp and Arg399Lys polymorphism of the XRCC1 gene did not differ statistically between those of the POF and control groups. The frequency of the C (Gln) allele was significantly lower in patients diagnosed with POF when compared to that in healthy controls [48% vs. 76%, p=0.040, OR: 3.43 (CI: 1.03-11.48)]. The Lys/Lys genotype for XPD-Lys751Gln polymorphism in patients diagnosed with POF was more common than in healthy controls (p=0.028, 52% vs. 24%).

CONCLUSIONS: The genotype distribution and allele frequency of XRCC1-Arg194Trp and XPD-Lys751Gln polymorphism may have a possible protective role against the development of POF.

Key Words:
DNA repair gene, XPD, XRCC1, Premature ovarian failure, Primary ovarian insufficiency.

Introduction

Premature Ovarian Failure (POI) is the loss of normal ovarian function before the age of 40 where there is still the possibility of pregnancy for many years due to the intermittent ovarian function[13]. The prevalence of POF ranges from 1.1% to 3.7%[1,15]. The incidence of POF is approximately 1% in women under 40 years of age and 0.1% in women under 30 years of age[1].

FSH receptor mutation, autoimmune, numerical chromosome abnormalities, and FMR1 premutation are known possible causes of POF. However, the main causes of POF more than 90% of women with non-syndromic POF are still unknown. Unknown etiology with genetic basis is the most common cause of POF[1]. The role of other genes and their mutations, including NR5A1, BMPR1B, FOXL2, LH receptor, GALT and TIMP2 genes, are still under research in the pathogenesis of POF[1,2]. Proper division of primordial germ cells to form primordial follicles and their eventual transition to meiosis requires efficient DNA repair mechanisms[2]. DNA repair genes play an important role in meiosis integrity, hence, identifying specific genes helps identifying women who may be predisposed to cancer and leads to better genetic counselling[1]. Several DNA repair genes have been proposed to be involved in the initiation mechanism of POF[2]. In accordance, proteins encoded by genes involved in DNA repair mechanisms, such as CSB-PGDBD3 fusion protein, DNA excision repair protein ERCC-6, Franconia anemia, complementation group A, Mini-chromosome maintenance component-8/9, MutS protein homolog 4/5, Nibrin, and the scaffolding proteins have been the subject of considerable research in the literature[16-11].

X-ray repair cross-complementary 1 (XRCC1) is one of the key enzymes of the base excision repair pathway[2]. Xeroderma pigmentosum D (XPD) is one of the major proteins of the nucleotide excision repair pathway and also known as excision-repair cross-complementing group 2[10]. The polymorphisms of XRCC1 and XPD DNA repair genes have been widely studied[13,14]. We hypothesized that XRCC1 and XPD DNA repair genes might be related to the development of POF. This study was planned to investigate whether polymorphisms in DNA repair genes, including XPD-Lys751Gln, XRCC1-Arg194Trp, and XRCC1-Arg399Gln, alter the risk of having POF.
Patients and Methods

This prospective thesis study was conducted at a University Hospital between 2011 and 2012. A total of 50 women were divided into two groups. The study group included 25 women diagnosed with POF. The control group included 20 premenopausal women with regular menstruation cycles and five postmenopausal women. None of the patients in the control group used hormonal drugs in the last six months. Women with abnormal karyotype, previous cancer treatment with chemotherapy or radiotherapy, previous ovarian surgery and a history of any metabolic or autoimmune disease were excluded from the study.

Each group of women underwent a full gynecological examination and ultrasonographic examination. POF was defined as an amenorrhea longer than 4 months before the age of 40 years and accompanying low estrogen and elevated serum gonadotropin levels. Primary amenorrhea was defined as the absence of menstruation in women with normal karyotype and was defined by two serum FSH levels greater than 40 mIU/mL. Secondary amenorrhea was diagnosed as the cessation of menstruation for a period of more than 6 months, in those younger than 40 years of age and with two serum FSH levels greater than 40 mIU/mL. This study was approved by the Local Institutional Ethical Board (No.2011/107) and the cost of the used kits was funded by the Inonu University Scientific Research Projects Coordination Unit (Protocol No. 2011/165). Written consent was obtained from all participants.

DNA Isolation

Two milliliters of peripheral blood in EDTA were collected from all participants and centrifuged plasma was stored at -80°C. Subsequently, genomic DNA was isolated using a DNA isolation kit (High Pure PCR Template Preparation Kit, Roche Diagnostic, Germany) and was stored at -20°C.

Genotyping Based on PCR and Fluorescence Melting Curve Analysis

Detection of polymorphisms was performed by rapid capillary PCR with melting curve analysis using fluorescence-labelled hybridization probes in a LightCycler-System (Roche Diagnostics, Mannheim, Germany): the XPD-Lys751Gln polymorphism, the XRCC1-Arg194Trp polymorphism and the XRCC1-Arg399Gln polymorphism. The amplification of PCR products was carried out in a volume of 20 µl using 5 µl of DNA, 1× buffer, 10.8 µl H2O, 2 µl of FastStartDNA Master and 1 µl reagent mix. Cycling conditions were as follows: initial denaturation at 95°C for 20 seconds, follow-up at 40°C for 20 seconds, slow heating to 85°C with a ramping rate of 0.3°C/s and continuous fluorescence detection. Melting curves were obtained by plotting the negative derivative of the fluorescence intensity with respect to temperature (-dF/dT) vs. temperature (T). The Tm value for each probe was automatically obtained by identifying the peak of the corresponding melting curve. Each amplification set included a negative control consisting of water instead of DNA and all other agents. Melting curves were evaluated by two independent observers. Genotyping was performed in optimized conditions of LightCycler 2.0 Real-Time Polymerase Chain Reaction (Roche Applied Science, Mannheim, Germany) using the LightSNiP (TIB-MolBiol, Berlin, Germany) typing measurement kit.

Statistical Analysis

The SPSS 25.0 (IBM Corp., Armonk, NY, USA) program was used to analyze the variables. Pearson Chi-Square, Fisher Exact and Fisher-Freeman-Halton tests with Monte Carlo Simulation were used in a comparison of POF and control groups with regard to XPD-Lys751Gln, XRCC1-Arg194Trp and XRCC1-Arg399Gln. The comparison of the column rates was expressed with Benjamini-Hochberg corrected p-values. The results were expressed with the Odds ratio at 95% confidence intervals. Categorical variables were expressed with n (%). A p-value below 0.05 was accepted as statistically significant.

Results

Twenty-five women were enrolled in the study group and 25 in the control group, respectively. Out of 25 women diagnosed with POF, 3 had primary and 22 had secondary amenorrhea. The mean age at the onset of menopause was 48 (SD: ±4.6, ranging between 43-54 years) years in five women in the control group. The mean age of patients with POF was 27.8 years (SD: ±6.8, ranging between 17-39 years) and 36.6 years (SD: ±11.3, ranging between 21-64 years) in the control group.

The mean age of patients with POF was 27.8 years (SD: ±6.8, ranging between 17-39 years) and 36.6 years (SD: ±11.3, ranging between 21-64 years) in the control group. The mean FSH level of patients with POF was 65.6 mIU/mL (SD: ±27.4, ranging between 146.1-42.1 mIU/mL). The mean age and mean FSH level of premenopausal women
with regular menstrual cycles was 32.1 years (SD: ±7.5, ranging between 21 and 48 years) and 7.6 mIU/mL (SD: ±2.2, ranging between 2.6-6.9 mIU/mL). Peripheral blood karyotype analyses of all patients younger than 30 years of age diagnosed with POF were found to be compatible with 46,XX.

The genotype of patients with POF and that of the healthy controls for XPD-Lys751Gln, XRCC1-Arg194Trp and XRCC1-Arg399Gln polymorphisms are shown in Table I. The genotype and allele distribution of the Lys751Gln polymorphisms of the XPD gene are given in Table II. The genotype distribution \((p=0.028)\) was found to be statistically significant, and the frequency of A (Lys) and C (Gln) alleles \((p=0.133)\) did not statistically differ between POF and control groups. The frequency of C (Gln) allele (irrespective of being homozygote or heterozygote) was significantly lower in patients diagnosed with POF when compared to healthy controls (48% vs. 76%, \(p=0.040)\). The Lys/Lys genotype for XPD-Lys751Gln polymorphism in patients diagnosed with POF was 3.43-fold more frequent than that for the healthy controls [n=13 (52%) vs. n=6 (24%)]; \(p=0.028)\).

The genotype and allele distribution of the Arg194Trp polymorphism of the XRCC1 gene is given in Table II. The genotype distribution \((p=0.345)\), the frequency of C (Arg) and T (Trp) alleles \((p=0.388)\) and the frequency of C (Arg) allele (irrespective of whether it was homozygote or heterozygote) \((p=0.345)\) did not statistically differ between POF and control groups. The genotype and allele distribution of the Arg399Lys polymorphism of the XRCC1 gene is given in Table II. The genotype distribution \((p=0.500)\), the frequency of A (Gln) and G (Arg) alleles \((p=0.999)\) and the frequency of A (Gln) and G (Arg) alleles (irrespective of whether they were homozygote or heterozygote) \((p=0.999 and p=0.490, respectively)\) did not statistically differ between POF and control groups.

### Table I. The genotype distribution of all women diagnosed with POF and healthy controls for polymorphism of XPD-Lys751Gln, XRCC1-Arg194Trp, and XRCC1-Arg399Gln.

<table>
<thead>
<tr>
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<th>POF</th>
<th>Control</th>
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<tr>
<td></td>
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<td>XRCC1 Arg194Trp</td>
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Discussion

This study showed that the genotype distribution and the allele frequency in DNA repair gene polymorphisms of XPD-Lys751Gln, XRCC1-Arg194Trp and XRCC1-Arg399Gln were found to be similar in patients diagnosed with POF and in the healthy controls. The frequency of the Gln/Gln+Lys/Gln genotype for XPD-Lys751Gln polymorphism was found to be significantly lower in patients diagnosed with POF when compared to healthy controls, suggesting a possible protective role for the 751Gln allele against POF. The Lys/Lys genotype for XPD-Lys751Gln polymorphism in patients diagnosed with POF was 3.43-fold more frequent than that in healthy controls suggesting possible role of the Lys/Lys genotype in the etiology of the POF.
The genes involved in DNA repair pathways and genomic stability play a critical role in the protection against mutations. Several polymorphisms in DNA repair genes have been reported to be associated with different pathologies, including lung cancer, polycystic ovary syndrome (PCOS), ovarian cancer, breast cancer and myeloid leukemia. Polymorphisms in DNA repair genes are well known to affect the function of the protein products of those genes. The base excision repair, nucleotide excision repair, and double strand break repair are known to be the most important DNA repair pathways. Mutations are early events in carcinogenesis and impaired DNA repair might be a risk factor for many cancers.

DNA damage repair pathways are important for removing different types of DNA damage. The base excision repair (BER), nucleotide excision repair (NER), and double strand break repair (DSB repair) are the most important DNA repair pathways. Mutations are considered to be early events in serious pathological conditions, and thus, we believe that the impaired DNA repair pathway may be a risk factor for ovarian failure. Polymorphisms of X-ray repair cross complementing group 1 (XRCC1) and X-ray repair cross complementing group 3 (XRCC3) have been studied extensively.

The data in the literature about the possible role of the polymorphism of DNA repair genes are inconsistent. The XPD Lys751Gln variant genotype was found to increase the risk of developing chronic myeloid leukemia, but not XRCC1 Arg399Gln, Arg280His, Arg194Trp and XRCC3 Thr241Met polymorphisms. On the other hand, polymorphic variants of XRCC1 Arg399Gln and XPD Lys751Gln were found not to be associated with the risk of developing gastric cancer. The polymorphisms of XPD 751Gln/Lys and Gln/Gln genotypes (Lys/Lys), XRCC1 399Arg/Gln and Gln/Gln genotypes (Arg/Arg) or the XRCC1 Arg/Trp and Trp/Trp genotypes (Arg/Arg) in XPD and XRCC1 genes were shown to be only weakly associated with breast cancer in a case-control study with potential bias. The XPD Lys751Gln polymorphism was shown to have a prognostic value in patients with colorectal cancer, who underwent oxaliplatin-based chemotherapy in a recent systemic review and meta-analysis.

Data about those relationships are more limited for gynecological diseases. Another well-designed case-control study has evaluated the role of the Lys751Gln (rs13181) ERCC2 gene polymorphism in the risk of the development of ovarian cancer. The genotype Gln/Gln was found to be associated with a 5-fold increase in the risk of ovarian cancer. More interestingly, an increase in ERCC2 Gln/Gln homozygote frequencies in Stage I ovarian cancer and Gln allele frequencies in SI was observed. The genetic variants of XRCC1, XRCC3, XPD, XPG, APE1 and HOGG1 may be associated with endometrial cancer. A similar study conducted for PCOS showed that there was no association between the polymorphism in several DNA repair genes including XRCC1 Arg194Trp, XRCC1 Arg399Gln, and XPD Lys751Gln, but that Asp148Glu was related with the risk of developing PCOS. The current study, the frequency of the Lys/Lys genotype of XPD-Lys751Gln polymorphism indicated relationship with ovarian failure. The Lys/Lys genotype was observed four times more in patients diagnosed with POF. In addition, the presence of the C (Gln) allele was significantly reduced in patients with ovarian failure.

Management of endometrial cancer is rapidly changing with the integration of molecular markers into treatment. These markers not only predict the prognosis of patients, but they also predict the response to conservative treatment. However, the effectiveness of these markers in fertility-preserving approaches has not yet been clearly evaluated. Adding POI-related genes to biomolecular and genetic prognostic factors used to make fertility-preserving treatment decisions in early-stage endometrial cancers may facilitate the treatment decision-making process. There are promising results of oral hormone treatment, LNG-IUD and GnRHα and metformin treatment especially for obese women in fertility sparing approaches. Oocyte vitrification may be required in patients who have the Lys/Lys genotype for XPD-Lys751Gln polymorphism. When the protocols were compared, open and closed vitrification protocols were found to be equally effective for sibling-oocyte cycles. The male factor should also be taken into account while giving consultancy regarding the POI. If necessary, endocrine evaluation, genetic testing using next-generation sequencing (NGS) and testicular histology may also be added to the examinations.

As far as we know, this is the first study to evaluate polymorphism in the DNA repair genes in women with premature ovarian failure. We believe that the current conflicting findings may actually be due to the interaction of genetic and environmental factors. In addition, our results...
should be approached with caution in terms of their generalizability, because we know that several candidate genes and their variants may be specific for a certain population. The greatest strength of this study is its case-controlled. The major limitation is its relatively small sample size. However, we believe that our results are clinically relevant as this study was a pilot study.

Data on the combined effects of variant alleles are still lacking in the literature and should be the focus of future larger population-based studies. Specific combinations of variant alleles could play a more important role than single DNA repair gene variants alone. Linkage analysis and genome-wide association studies are required to identify rare variants. In addition, pharmacokinetic studies are needed to identify patients who may benefit from additional reproductive treatments.

Conclusions

The genotype distribution and the allele frequency in DNA repair gene polymorphisms of XPD-Lys751Gln, XRCC1-Arg194Trp and XRCC1-Arg399Gln did not modulate the risk of developing POF. The Gln/Gln+Lys/Gln genotype for XPD-Lys751Gln polymorphism may play a protective role against POF. The Lys/Lys genotype for XPD-Lys751Gln polymorphism may play a role in the POF etiology.

Authors’ Contributions

Study design and idea was made by Prof. Dr. Önder Çelik. All authors contributed to the study conception and design. Material preparation was performed by Cagdas Dogan and Zercan Kali. Data were collected by Fatma Cagiran, Zercan Kali and Pinar Kirici. All authors contributed to statistical analysis. The first draft was written by Cagdas Dogan. The article was edited and finalized by Onder Celik. All authors approved the final version of the manuscript.

Conflict of Interests

The Authors declare that they have no conflict of interests.

Informed Consent

Informed consent was obtained from all participants.

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References